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GYMNOXANTHELLA RADIOLARIAE GEN. ET SP. NOV. (DINOPHYCEAE), A DINOFLAGELLATE SYMBIONT FROM SOLITARY POLYCYSTINE RADIOLARIANS[1]

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The symbiotic dinoflagellate *Gymnoxanthella radiolariae* T. Yuasa et T. Horiguchi gen. et sp. nov. isolated from polycystine radiolarians is described herein based on light, scanning and transmission electron microscopy as well as molecular phylogenetic analyses of SSU and LSU rDNA sequences. Motile cells of *G. radiolariae* were obtained in culture, and appeared to be unarmored. The cells were 9.1–11.4 μm long and 5.7–9.4 μm wide, and oval to elongate oval in the ventral view. They possessed an counterclockwise horseshoe-shaped apical groove, a nuclear envelope with vesicular chambers, cingulum displacement with one cingulum width, and the nuclear fibrous connective; all of these are characteristics of *Gymnodinium* sensu stricto (*Gymnodinium* s.s.). Molecular phylogenetic analyses also indicated that *G. radiolariae* belongs to the clade of *Gymnodinium* s.s. However, in our molecular phylogenetic trees, *G. radiolariae* was distantly related to *Gymnodinium fuscum*, the type species of *Gymnodinium*. Based on the consistent morphological, genetic, and ecological divergence of our species with the other genera and species of *Gymnodinium* s.s., we considered it justified to erect a new, separate genus and species *G. radiolariae* gen. et sp. nov. As for the peridinioid symbiont of radiolarians, *Brandtodinium* has been erected as a new genus instead of *Zooxanthella*, but the name *Zooxanthella* is still valid. *Brandtodinium* is a junior synonym of *Zooxanthella*. Our results suggest that at least two dinoflagellate symbiont species, peridinioid *Zooxanthella nutricula* and gymnodinioid *G. radiolariae*, exist in radiolarians, and that they may have been mixed and reported as "*Z. nutricula*" since the 19th century.

Key index words: **dinoflagellate; *Gymnoxanthella radiolariae* gen. et sp. nov.; molecular phylogeny; radiolarians; symbiont; *Zooxanthella nutricula***

***Abbreviations*: BV, bootstrap proportion values; ML, maximum-likelihood; NFC, nuclear fibrous connective; PP, posterior probabilities; TB, transverse basal body**

Dinoflagellate symbionts within radiolarians are generally yellow-brown, spherical, minute cells, measuring several micrometers in diameter. The morphological features of typical dinoflagellates are lost or modified, and they have seldom been observed as free-living forms (e.g., Hollande and Enjumet 1953, Anderson 1983, Probert et al. 2014). The host-algal relationship between radiolarians and symbiotic dinoflagellates has been studied since the late 19th century. However, because of their appearance in the host cytoplasm during the symbiotic state, dinoflagellate symbionts were first thought to be a component of the radiolarians known as "yellow cells" (Huxley 1851). Brandt (1881) examined the role of the yellow cells of the colonial radiolarian *Collozoum inerme* collected from the Mediterranean Sea, and he concluded that they were algal symbionts. He named them *Zooxanthella nutricula* Brandt 1881 and erected the monotypic genus *Zooxanthella* Brandt. In 1885, Brandt provided figures of the isolated and bi-flagellated cells and tentatively assigned the organisms to the dinoflagellates. However, the original description of *Z. nutricula* by Brandt (1881) was rather ambiguous, and the poor morphological features of the dinoflagellates in their symbiotic state limited the ability to resolve taxonomic affiliations. Therefore, the taxonomy of *Z. nutricula* was confused until recently (see also Blank and Trench 1986), and the taxonomic revisions of *Z. nutricula* have been considered for use

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with the generic name, specific name or both (Geddes 1882, Pascher 1911, Taylor 1974, 1984, Hollande and Carré 1975, Blank and Trench 1986, Banaszak et al. 1993, Gast and Caron 1996, 2001, Probert et al. 2014).

Geddes (1882) invalidly described the genus *Philozoon* with four symbiont species for which no figures or descriptions were given, resulting in a taxonomy as confusing as that for *Zooxanthella* Brandt. Pascher (1911) regarded the algae previously called zooxanthellae as cryptomonads and renamed all the groups of the zooxanthellae *Chrysidella nutricola* (=*nutricula*) (Brandt). However, the genus name is superfluous as the genus *Chrysidella* is a later homotypic synonym of *Zooxanthella* Brandt. In 1992, and again in more detail in 1923, Hovasse described the monotypic genus *Endodinium* Hovasse with the species *E. chattoni* (=*chattonii*) Hovasse, an intracellular symbiont in the cnidarian *Velella velella*. Subsequently, Hovasse (1924) transferred the species *E. chattoni* to the genus *Zooxanthella* Brandt, introducing the combination *Zooxanthella chattoni* (Hovasse) Hovasse. By this nomenclatural act, *Endodinium* becomes a later heterotypic synonym of *Zooxanthella* Brandt. On the other hand, Hollande and Carré (1975) assigned the symbiotic dinoflagellates observed in the radiolarian *Collozoum* sp., *Collosphaera* sp., and *Thallassicolla nucleata* to the genus *Endodinium* instead of the genus *Zooxanthella* Brandt, based on Hollande and Enjumet's (1953) illustration of the unarmored *Gymnodinium*-like symbiont isolated from the radiolarian *Thalassophysa sanguinolenta*, and they proposed a new combination *Endodinium nutricola* (=*nutricula*) (Brandt). Namely, they regarded *Z. nutricula* Brandt as a basionym of *E. nutricola* (Brandt). As *Zooxanthella* is an accepted genus name, this combination is illegitimate. At almost the same time, Taylor (1971) introduced a combination *Amphidinium chattonii* (Hovasse) Taylor, citing it as basionym *Endodinium chattonii* Hovasse. Thus, *Endodinium* becomes a later heterotypic synonym of *Amphidinium* Claparède et Lachmann, and subsequently Taylor (1974) reported *Amphidinium* sp. from the type host and type locality of *Z. nutricula* Brandt that had an obvious *Amphidinium* morphology in the motile stage. Loeblich and Sherley (1979) commented on this confusion and concluded that *Endodinium* and *Zooxanthella* are synonymous and, because *Z. nutricula* had been validly published, the latter has priority.

In 1986, Blank and Trench, building on the work of Hollande and Carré (1975) and Taylor (1974), proposed *Amphidinium nutricula* (Brandt) Blank et Trench as the appropriate name for the symbiotic dinoflagellates found in radiolarians. This generic assignment was based on the assumption by Taylor (1971) that *Endodinium* took precedence over *Zooxanthella*, and that *Endodinium* Hovasse was a younger synonym of *Amphidinium* Claparède et Lachmann. Although Blank and Trench (1986) proposed that the name *Zooxanthella* be rejected, this proposal was rejected by the Nomenclatural Committee (Nicolson 1993). In 1993, Banaszak et al. isolated and cultured a dinoflagellate symbiont from the cnidarian *V. velella* collected from the Pacific Ocean. Based on ultrastructural observations they described this species as *Scrippsiella velellae* Banaszak, Iglesias-Prieto, et Trench and they suggested that *S. velellae* morphologically resembles (but is distinguishable from) the radiolarian symbiont, which was originally reported as *Z. nutricula* Brandt. They assumed *Z. nutricula* as a peridinioid species and proposed a new combination *Scrippsiella nutricula* (Brandt) Banaszak, Iglesias-Prieto, et Trench – this combination is not validly published. Banaszak et al. (1993) then raised the possibility that *Zooxanthella* Brandt, *Endodinium* Hovasse, and *Scrippsiella* Balech are synonyms. If this view is accepted, *Zooxanthella* will have priority, however, their paper did not present new morphological data for *Z. nutricula* to confirm the morphological similarities needed to assign the symbiont to the genus *Scrippsiella*. This was critical because the dinoflagellate symbionts of radiolarians had not previously been described from cultured specimens. Thus, there was no direct way to resolve how the previously described species were related to *Scrippsiella nutricula* based on morphology.

In an effort to overcome some of these morphological limitations, molecular techniques have been applied to the taxonomic identification of the radiolarian symbionts (e.g., Gast and Caron 1996, 2001, Dolven et al. 2007, Decelle et al. 2012, Gottschling and McLean 2013). Some of the first sequences were obtained by Gast and Caron (1996), who analyzed the SSU rDNA isolated from the symbionts of radiolarians *Collozoum caudatum*, *Spongostaurus* sp., unidentified radiolarian species, and the symbiont of cnidarian *V. velella*. The dinoflagellate sequences, which were obtained from the symbionts of both radiolarians and cnidarian *V. velella* collected in the Sargasso Sea, were very similar, with a difference of 0.2%, or four bases out of 1,802 bp (Gast and Caron 1996, 2001). Because of the similarity of the SSU rDNA sequences and the similarity of the restriction fragment length polymorphism patterns, Gast and Caron (1996) contended that there was no distinction between the radiolarian symbiont *S. nutricula* and the cnidarian symbiont, and they proposed that these symbionts were synonymous. Therefore, the sequence of the symbiont from cnidarian *V. velella* has been deposited as "*S. nutricula*" (recently replaced to *Brandtodinium nutricula*: accession number U52357) in GenBank.

Probert et al. 2014 cultured dinoflagellate symbiont specimens of three polycystine orders (Spumellaria, Nassellaria, and Collodaria) from three different oceans: the Mediterranean Sea, the East China Sea, and the South Pacific Ocean. Their SEM observations and SSU and LSU rDNA phylogenetic analyses revealed the symbionts with

an obvious peridinioid morphology in the motile stage, and they showed that the sequences formed a distinct clade within the Peridiniales together with the sequence of *S. nutricula* in Gast and Caron (1996, 2001). Probert et al. (2014) identified those peridinioid dinoflagellate symbionts of radiolarians as Brandt's (1881) *Z. nutricula*, and they proposed a new combination, *Brandtodinium nutricula* (Brandt) Probert et Siano. Although Blank and Trench (1986), Banaszak et al. (1993), and Probert et al. (2014) all proposed the rejection of *Zooxanthella* Brandt, the name *Zooxanthella* still remains valid as a correct genus name in the dinophytes (see Nicolson 1993). The generic name *Brandtodinium* is therefore superfluous and thus illegitimate.

As mentioned above, several researchers have reported dinoflagellate symbionts isolated from different radiolarian species in different areas; moreover, Ishitani et al. (2014) recently obtained a *Gymnodinium* sequence from polycystine radiolarian *Spongotrocus glacialis* in the Pacific Ocean. These findings implied that radiolarians might have at least two or three types of symbiotic dinoflagellate: the peridinioid taxon identified by Probert et al. (2014) as *Z. nutricula* Brandt, the gymnodinioid species, and the amphidinioid species.

In this study, we detected an unarmored *Gymnodinium*-like symbiont from solitary polycystine radiolarians in the East China Sea, and we here propose *Gymnoxanthella radiolariae* T. Yuasa et T. Horiguchi gen. et sp. nov., based on light, scanning and transmission electron microscopy as well as on molecular phylogenetic analyses using SSU and LSU rDNA sequences from the symbiotic dinoflagellates.

MATERIALS AND METHODS

Sampling and culture conditions. Radiolarians were collected from the surface seawater on July 2006 and March 2009, using a plankton net (60 cm circle opening with 37 μm mesh net) at the Site 990528 (26°37′ N, 127°47′ E) located approximately 5 km off the northwest coast of Okinawa Island, Japan. The collected samples were put in jars, stored at about 25°C, and they were immediately brought back to the laboratory at the Tropical Biosphere Research Center, University of the Ryukyus. Radiolarian specimens were isolated from the samples into six-well culturing dishes containing filtered seawater. Single cell of the radiolarians was transferred to the slide glass with a Pasteur pipette and was subsequently rinsed three times in sterile seawater, and then it was microdissected on the slide glass under inverted light microscope with a sterile razor blade, which was used to tear open the organic layers of the extracytoplasm of radiolarians and release the symbionts. We examined three polycystine species: *Acanthodesmia vinculata* (Müller), *Euchitonia elegans* (Ehrenberg), and *Pterocanium praetextum* (Ehrenberg) (Fig. 1). The symbionts both from *A. vinculata* and *P. praetextum* were directly used for PCR amplifications only, while approximately half of each symbiont sample from *E. elegans* was directly used for PCR amplifications and the other half was used for culture. The culture was maintained in Daigo's IMK medium for Marine Microalgae (Nihon Pharmaceutical, Tokyo, Japan; Appendix S1 in the Supporting Information), and incubated at 26°C with a 14:10 h light:dark cycle.

Light microscopy. Cultured cells were examined using an Olympus BX53 light microscope (Olympus, Tokyo, Japan) equipped with a COOLPIX 950 digital camera (Nikon, Tokyo, Japan). For differential interference contrast light and autofluorescence microscopy, cells were observed with a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany), and images were taken with an Olympus DP71 digital camera. To detect the autofluorescence of the plastid, we used a filter set consisted of excitation filter, bandpass 369 nm and suppression filter, longpass 397 nm.

Transmission and scanning electron microscopy. A cell of *E. elegans* was embedded in 1.5% low-temperature-gelling agarose (Merck, Darmstadt, Germany) that made up with seawater, while cultured symbiont cells were accumulated in 1.5 mL Eppendorf tubes and centrifuged at 2,200 ×g for 5 min. Each piece of the agarose gel within single radiolarian cell and the pellet of the cultured symbiont cells was fixed in 2.0% glutaraldehyde made up with 0.1 M sodium cacodylate buffer (pH 7.0) with 0.1 M sucrose. After that, they were rinsed three times in 0.1 M sodium cacodylate buffer (pH 7.0) with 0.1 M sucrose before postfixation in 1.0% OsO_4 at room temperature for 2 h. After dehydration through an acetone series (30, 50, 80, 90, and 100%), they were embedded in Spurr's resin (Agar Scientific, Essex, UK) and sectioned. Sections were picked up onto the Formvar-coated grids and then double stained with uranyl acetate and lead citrate. These sections were examined with a JEM-100S transmission electron microscope (JEOL, Tokyo, Japan).

For SEM, cultured symbiont cells were fixed in 4% OsO_4 on a 0.1% poly-L-lysine-coated glass plate for 10 min and rinsed in sterilized filtered seawater followed by dehydration through an ethanol series (30, 50, 70, 90, 95, and 100%), and then the dehydrated cells were dried by a JCRD-5 critical point dryer (JEOL). The dried cells were coated with platinum–palladium in a JFC-1100 ion-sputter (JEOL) and examined with an S-4500 field emission scanning electron microscope (Hitachi, Tokyo, Japan).

PCR amplification, cloning, and sequencing. The symbiont cells directly obtained from the radiolarian extracytoplasm were used as a template for the amplification of a part of SSU rDNA. On the other hand, the cultured symbiont cells were transferred into 0.2 mL Eppendorf tubes and centrifuged at 1,000 ×g for 3 min and the pellet of the cultured cells was rinsed twice in distilled water, and then, it was used as a template for the amplification of full length of SSU rDNA and a part of LSU rDNA-coding regions. The SSU rDNA was amplified using eukaryotic forward primers A (Hendriks et al. 1989): 5′-ACCTGGTTGATCCTGCCAGT-3′ or 90F (Hendriks et al. 1989): 5′-GAAACTGCGAATGGCTCATT-3′ and the reverse primer B (Medlin et al. 1988): 5′-CCTTCTGCAGGTTCACCTAC-3′. The part of the LSU rDNA was amplified using primers SR12c (Takano and Horiguchi 2006): 5′-TAGAGGAAGGAGAAGTCGTAA-3′ and LSU R2 (Takano and Horiguchi 2006): 5′-ATTCGGCAGGTGAGT TGTTAC-3′. The PCR amplifications were performed in a 50 μL reaction volume using KOD FX (Toyobo, Osaka, Japan) and used by following method: initial denaturation step at 95°C for 3 min, followed by 35 cycles of 95°C for 10 s, 54°C for 30 s, and 68°C for 1 min in a MiniCycler (Bio-Rad, Hercules, CA, USA). The PCR products were purified by the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Only the PCR products of the symbionts directly obtained from the radiolarian extracytoplasm were cloned in the pGEM-T Easy Vector System (Promega) using *E. coli* JM109 Competent Cells (Promega). All sequences were performed with the ABI-PRISM Big Dye Terminator Cycle

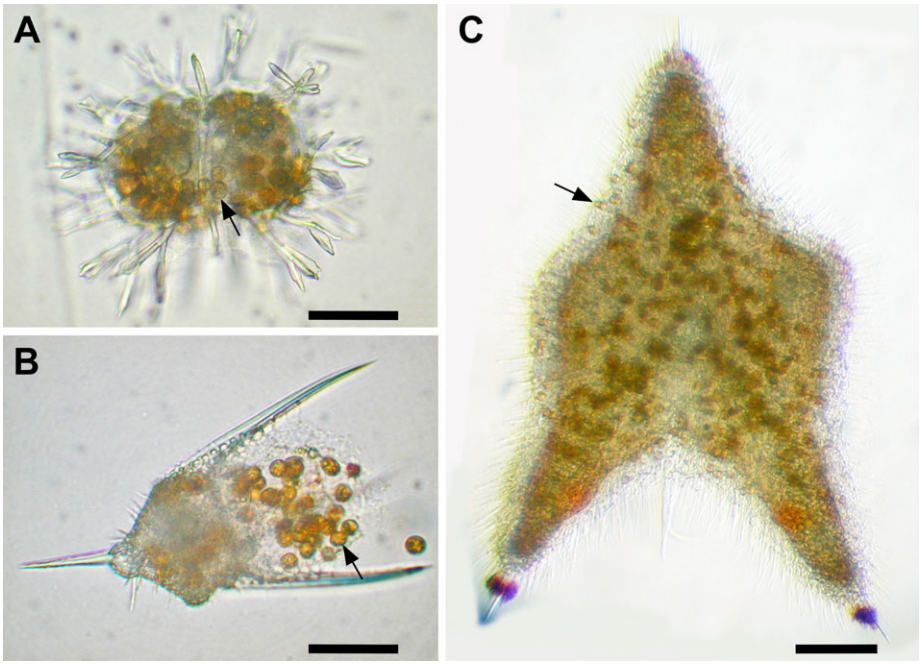

FIG. 1. Light micrographs of polycystine radiolarians. (A) *Acanthodesmia vinculata*; (B) *Pterocanium praetextum*; (C) *Euchitonia elegans* with yellow-brown algal symbionts. Arrows indicate the symbiotic algae, scale bars = 50 μm.

Sequencing Kit and analyzed with an ABI 3130 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Alignment and phylogenetic analysis. The determined sequences of the SSU and LSU rDNAs were aligned by ClustalW version 1.81 (Thompson et al. 1994) with the other dinoflagellate sequences obtained from GenBank. Subsequently, the alignment was manually refined using the nucleotide sequence editor Se-Al v1.0a1 (Rambaut 1996). The accession numbers of the SSU and LSU rDNA sequences used in this study are indicated in figures 8 and 9. Total of 52 taxa (1,164 bp) for SSU rDNA and 37 taxa (485 bp) for LSU rDNA sequences were used in the phylogenetic analyses for the datasets. The perkinsozoan *Perkinsus marinus* (SSU: X75762, LSU: AY876328) was used as outgroup for both SSU and LSU phylogenetic analyses. To determine the best-fit model of DNA evolution, the alignment was subjected to hierarchical likelihood ratio tests in Modeltest v3.06 (Posada and Crandall 1998), indicated that GTR + I + G model were the best-fit substitution models for both SSU and LSU rDNA datasets. ML analyses were performed with PAUP* version 4.0b10 (Swofford 2002). The ML trees were then analyzed using a heuristic search method with a TBR branch-swapping option and random taxon addition. The relative levels of support for nodes were assessed by calculating full heuristic bootstrap proportion values (BV; Felsenstein 1985) based on 100 replicates. Bayesian analyses were carried out with MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Trees were generated from two runs with one heated and three cold chains in the Markov chain Monte Carlo. 5,700,000 generations for SSU rDNA dataset and 3,500,000 generations for LSU rDNA dataset were run, and trees were sampled every 100 generations after a burn-in of 25%. The remaining trees were used to construct both the majority-rule consensus tree and the posterior probabilities (PP) of the nodes.

RESULTS

Symbiotic state of algal symbiont in radiolarians. The solitary polycystine radiolarian *E. elegans* (Ehrenberg) (order Spumellaria, family Spongodiscidae) was used to observe the symbiotic state of the algal symbionts under light and transmission electron microscopy. *E. elegans* (Fig. 1C) had a triangular and flattened shell with a maximum shell length of ~400 μm in the adult stage. The surface of the shell was a spongy meshwork with veil-like ornamentation. The cytoplasm was generally colorless or, rarely, brownish-red with radiating pseudopodia. Many algal symbionts, more than 50, were observed under light microscopy. The algal symbionts were yellow-brown and ranged from 5 to 8 μm in diameter.

Under the TEM observation, the nucleus with condensed chromosomes and chloroplasts were typical of dinoflagellates (Fig. 2, A–E), however, the features of amphiesma, flagella, and typical cell shape of dinoflagellates were lost in the host radiolarians. The coccoid cells of the dinoflagellate symbionts were surrounded by perialgal envelope of radiolarian cytoplasm (Fig. 2, A and C). The nucleus was offset to one side of the cell, and the nuclear envelope contained vesicular chambers (Fig. 2, B and D). The chloroplasts were situated at the periphery of the cell (Fig. 2, A and B) and the lamella consisted of two or three thylakoids (Fig. 2E). Each chloroplast possessed a projecting spherical pyrenoid enclosed by a starch sheath, and its matrix was partly invaded by a few lamellae (Fig. 2B). The mitochondria had tubular cristae (Fig. 2F).

Light microscopy of motile cells. In culture, the symbiont produced motile cells 9.1–11.4 μm (mean = 10.5 μm, $n = 10$) long and 5.7–9.4 μm (mean = 7.4 μm, $n = 10$) wide, and oval to elongate oval (Fig. 3). The epicone was longer than the hypocone, constituting about 0.6–0.7 of the cell

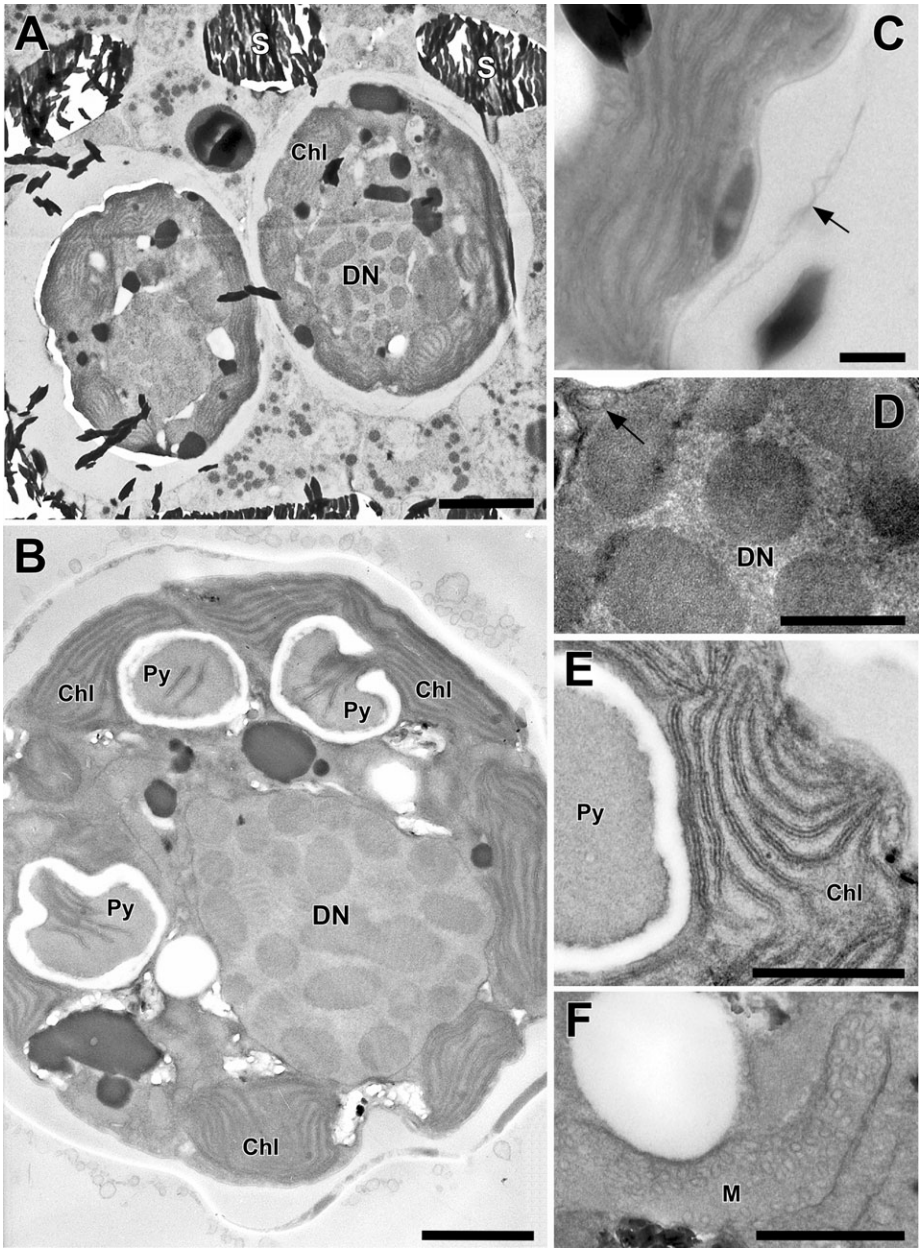

FIG. 2. Transmission electron micrographs of *Euchitonia elegans* with algal symbionts. (A) A section of *E. elegans* showing the algal symbionts within the ectocytoplasm, scale bar = 2 μm. (B) A section of an algal symbiont associated with *E. elegans* showing the nucleus with condensed chromosomes, the peripheral chloroplasts, and the pyrenoids in the cell cytoplasm, scale bar = 1 μm. (C) Enlargement of membrane surrounding the algal symbionts. An envelope of radiolarian extracytoplasm (arrow) surrounds the algal symbiont, scale bar = 0.5 μm. (D) Enlargement of a nucleus showing the nuclear envelope with the nuclear chamber (arrow), scale bar = 0.5 μm. (E) Enlargement of a chloroplast showing several lamellae consisting of two or three thylakoids, scale bar = 0.5 μm. (F) Enlargement of mitochondrion with tubular cristae, scale bar = 0.5 μm. Chl, chloroplast; DN, dinoflagellate nucleus; Py, pyrenoid; M, mitochondrion; S, siliceous shell of *E. elegans*.

length. The epicone was conical in shape with a slightly spheroidal or somewhat raised apex, while the hypocone was hemispheroidal and slightly convex medially (Fig. 3A). The sulcus was narrow, and extended from the antapex onto the epicone and continued around the apex (Fig. 3, A and B). The cingulum was relatively wide, and the displacement was one cingulum width (Fig. 3B). The dinokaryon was situated in the middle part of the cell (Fig. 3, C and D). They possessed spherical pyrenoids surrounded by a starch sheath (Fig. 3C). Yellow-brown chloroplasts were distributed along the periphery of the cell (Fig. 3, D and E).

Scanning electron microscopy of motile cells. The cingulum of the motile cell was wide and relatively shallow, and the distal end of the cingulum was displaced one cingulum width (Fig. 4A). The sulcus was also shallow, relatively narrow, running straight to the apex and connected to the onset of the apical groove (Fig. 4, A, C and F). The longitudinal flagellum arose at the middle portion of the sulcus, and the transverse flagellum arose at the anterior portion of the sulcus (Fig. 4, A and C). The cells were covered with tetragonal, pentagonal, or hexagonal amphiesmal vesicles (Fig. 4). The amphiesmal vesicles were arranged in a latitudinal series, four on the epicone, three in the cingular groove, and three on the hypocone (Figs. 4–6). The sulcal extension consisted of six amphiesmal vesicles (Figs. 5A and 6A). The apical groove,

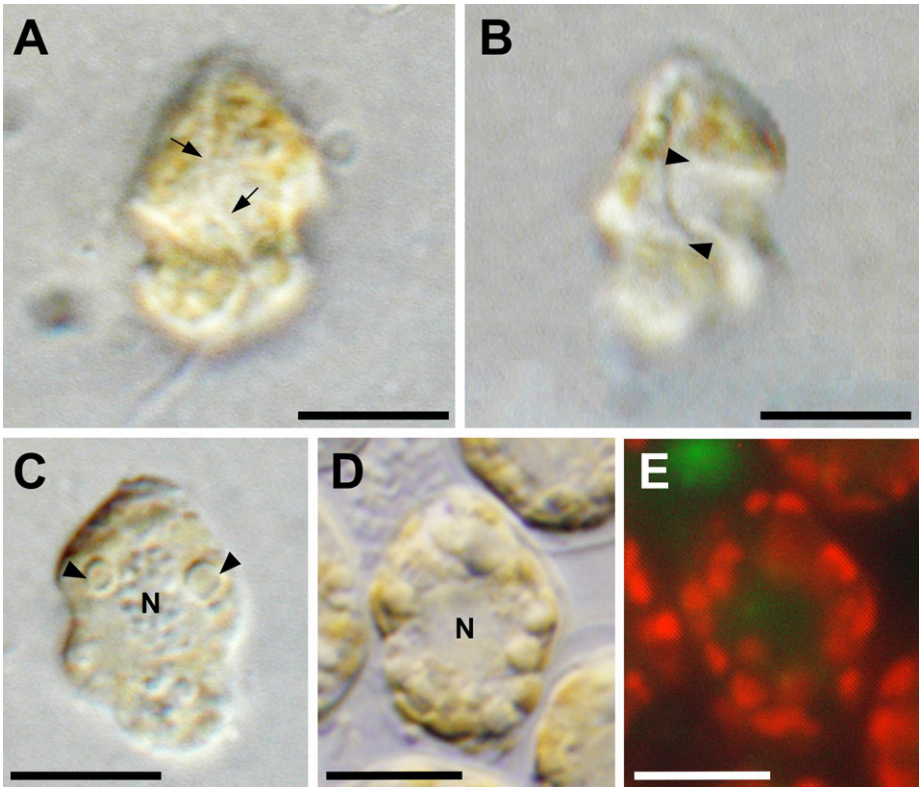

FIG. 3. Light micrographs of *Gymnoxanthella radiolariae* gen. et sp. nov. (A) Ventral view of the motile cell showing sulcal extension (arrows) onto the epicone and the yellow-brown chloroplasts located in the cell periphery. (B) Ventral view of the motile cell showing the cingulum displacement (arrowheads). (C) Dorsal view of the motile cell showing the large nucleus (N) and two pyrenoids (arrowheads). (D) Motile cell showing the large nucleus (N) and yellow-brown chloroplasts. (E) The same cell of "D" showing chloroplasts with red autofluorescence, scale bars = 5 μm.

horseshoe-shaped, ran from the end of the sulcal extension and formed an counterclockwise loop around the apex (Figs. 4, E and F; 6). Six tetragonal amphiesmal vesicles (E1) were arranged around the apical groove (Figs. 4E and 6C). A furrow continued from the sulcus (Figs. 4F; 6, A and C). On the outer side of the furrow, a row of six elongated amphiesmal vesicles was observed and it was ornamented by many small knobs that were regularly arranged (Figs. 4, E and F; 6C). On the inner portion of the apex enclosed by the apical groove, eight amphiesmal vesicles were observed (Figs. 4F and 6C). A peduncle was observed in several specimens (Fig. 5D), however, most specimens had only a slit where the peduncle probably had been retrieved (Fig. 5E).

Transmission electron microscopy of motile cells. The ultrastructure of the cultured motile cells showed typical dinoflagellate organelles, such as a dinokaryon, mitochondria with tubular cristae, and trichocysts (Fig. 7A). The chloroplast profiles were located peripherally of the cell and were bounded by three membranes (Fig. 7, A and B). Several lamellae (4–6), each consisting of two or three thylakoids, were contained in the chloroplast (Fig. 7B), and there were less lamellae than under the symbiotic state in radiolarians (8–10; Fig. 2E). Each chloroplast had a branched pyrenoid enclosed by a starch sheath, and its matrix was partly invaded by a few lamellae (Fig. 7C). The nucleus that was located in the central part of the cell was a typical dinokaryon with condensed chromosomes (Fig. 7A). The nuclear envelope contained vesicular chambers, in which nuclear pores were situated (Fig. 7D). The nuclear fibrous connective (NFC) linked the proximal parts of the transverse basal body (TB) with the nucleus (Fig. 7E). The mitochondria had tubular cristae and were scattered throughout the cell (Fig. 7, A, C and F). The trichocysts were situated at the periphery of the cell (Fig. 7F). The amphiesmal vesicles contained thin, plate-like structures (Fig. 7G).

Phylogenetic analyses. The SSU rDNA sequences of the symbiotic algae from three polycystine species, *A. vinculata*, *E. elegans*, and *P. praetextum*, were determined from the cells in both the radiolarian extracytoplasm and the culture strain. With regard to the SSU rDNA sequences of the symbionts from the radiolarian extracytoplasm, we analyzed the sequences of five colonies obtained from the cloning. All the sequences from the colonies were the same, and they were also the same as those from the culture strain. The LSU rDNA sequences of the symbiont were determined from the cells of the culture strain. The maximum-likelihood (ML) trees based on the SSU and LSU sequences are shown in Figures 8 and 9. In both the SSU and LSU phylogenetic trees, our symbiont branched in the *Gymnodinium* sensu stricto (*Gymnodinium* s.s.) clade that included *Gymnodinium fuscum*. The BV and PP of the clade were 65% and <0.50 in the SSU rDNA tree, and 91% and 1.00 in the LSU rDNA tree,

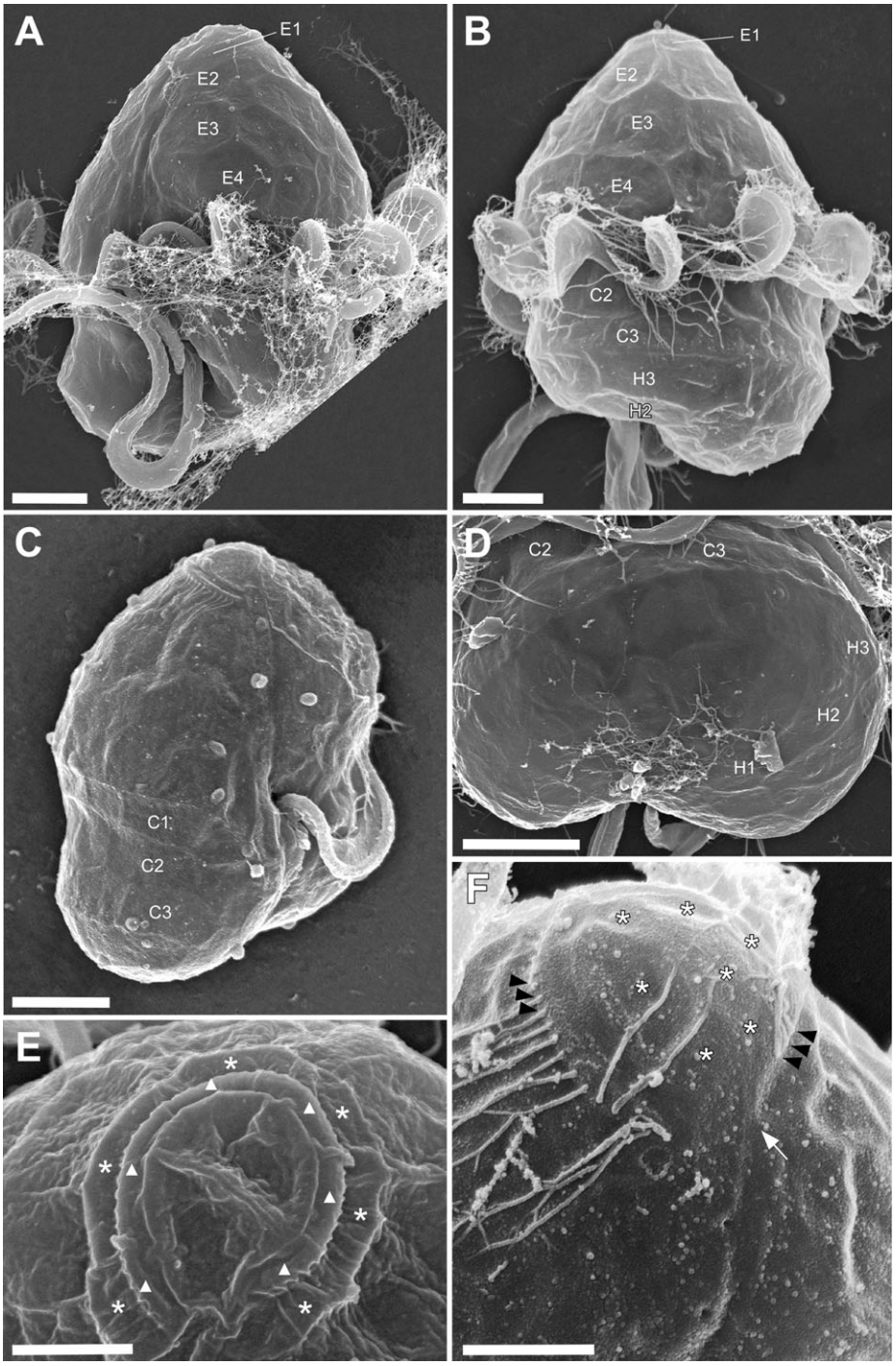

FIG. 4. Scanning electron micrographs of *Gymnoxanthella radiolariae* gen. et sp. nov. (A) Ventral view showing four rows (E1–E4) of amphiesmal vesicles on the epicone, the apical groove and the sulcal extension onto the epicone. Holotype, scale bar = 2 μm. (B) Dorsal view showing four rows (E1–E4) of amphiesmal vesicles on the epicone, two rows (C2, C3) of amphiesmal vesicles on the cingulum, and two rows (H2, H3) of amphiesmal vesicles on the hypocone, scale bar = 2 μm. (C) Right lateral view showing three rows (C1–C3) of amphiesmal vesicles on the cingulum, scale bar = 2 μm. (D) Antapical view showing two rows (C2, C3) of amphiesmal vesicles on the cingulum, three rows (H1–H3) of amphiesmal vesicles on the hypocone, scale bar = 2 μm. (E) Apical view showing six amphiesmal vesicles (E1) (asterisks) around the apical groove and a row of six elongated amphiesmal vesicles (white triangles) with many small knobs on the apical groove, scale bar = 2 μm. (F) Apical view showing the horseshoe-shaped apical groove and sulcal extension (white arrow) on the epicone, and eight amphiesmal vesicles (asterisks) on the inner portion of the apex enclosed by the apical groove. Notice the small knobs (arrowheads) on the amphiesmal vesicles of the horseshoe-shaped apical groove, scale bar = 1 μm.

respectively. The branching patterns in the clade of *Gymnodinium* s.s., which were obtained from the SSU and LSU rDNA trees, were different from each other.

The SSU rDNA phylogenetic tree (Fig. 8) showed that our symbiont formed a clade together with the following species in the *Gymnodinium* s.s.: *Gymnodinium catenatum*, *G. dorsalisulcum*, *G.* cf. *nolleri*, *G. impudicum*, *G. microreticulatum*, *G. palustre*, *G. smaydae*, *Nusuttodinium acidotum*, *N. amphidinioides*, *N. desymbiontum*, *N. myriopyrenoides*, *N. poecilochroum*, *Pellucidodinium psammophilum*, *Spiniferodinium galeiforme*, *S. palauense*, *Gymnodinium* sp. from radiolarian *Spongotrochus glacialis*, and three sequences from environmental samples in the clade of *Gymnodinium* s.s. In this clade, our symbiont was most closely related to *Gymnodinium* sp. from radiolarian *S. glacialis* in the Pacific Ocean, this relationship was supported by the high BV and PP (BV: 89%, PP: 1.00). Subsequently, the group was shown to be a sister to the uncultured

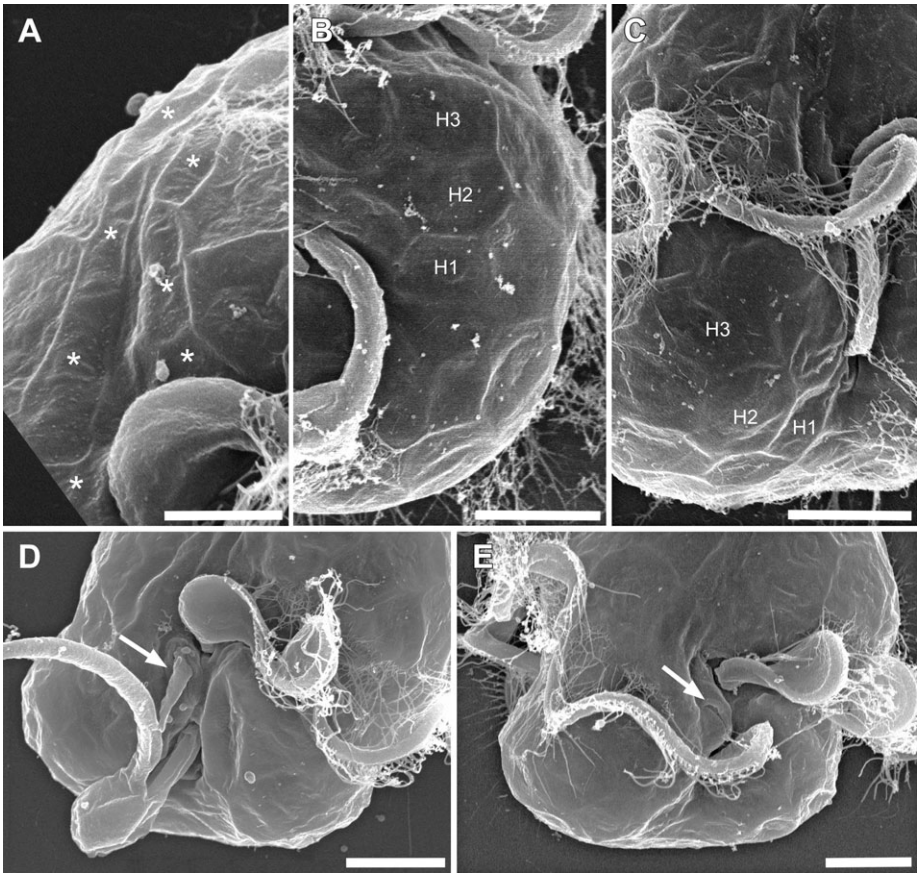

FIG. 5. Scanning electron micrographs of *Gymnoxanthella radiolariae* gen. et sp. nov. (A) Left apical view showing two rows of the amphiesmal vesicles (asterisks) along the sulcal extension, scale bar = 1 μm. (B) Left antapical view showing three rows (H1–H3) of amphiesmal vesicles on the hypocone, scale bar = 2 μm. (C) Right antapical view showing three rows (H1–H3) of amphiesmal vesicles on the hypocone, scale bar = 2 μm. (D) Antapical view showing a deployed peduncle (white arrow), scale bar = 2 μm. (E) Antapical view showing a slit (white arrow) where the peduncle is probably retrieved, scale bar = 2 μm.

eukaryote clone CC02A105.081 from the South China Sea; this clade was also supported by high BV and PP (BV: 88%, PP: 1.00).

In the LSU rDNA phylogenetic tree (Fig. 9), our species formed a monophyletic clade with *Gymnodinium* sp. from radiolarian *S. glacialis* with high BV and PP support (BV: 100%, PP: 1.00), and the clade was shown to be a sister to *G. smaydae*. The sister relationship was supported by the moderate to high BV and PP (BV: 71%, PP: 1.00). In the *Gymnodinium* s.s., the clade consisting of the radiolarian symbionts and *G. smaydae* was constructed of a monophyletic group with the species of *G. palustre*, *N. acidotum*, *N. desymbiontum*, *N. myriopyrenoides*, *N. poecilochroum*, *P. psammophilum*, and *S. galeiforme*, and the branching support of the clade was BV: 51% and PP: 0.98, respectively.

The differences between the sequences of *G. radiolariae* and *Gymnodinium* sp. from radiolarian *S. glacialis* were two bases out of 1,792 bp for SSU rDNA, or one base out of 1,321 bp for LSU rDNA.

Gymnoxanthella T. Yuasa et T. Horiguchi **gen. nov.**

Description. Dinoflagellate, marine, photosynthetic, and symbiotic with solitary polycystine radiolarians. In the host, the cells were spherical and non-motile. In culture, the dinoflagellate produces motile cells covered by ten latitudinal series of amphiesmal vesicles. A horseshoe-shaped apical groove running in an counterclockwise loop around the apex. Cingulum displaced one cingulum width. Nuclear envelope with vesicular chambers. NFC linked the proximal parts of transverse and longitudinal basal body with the nucleus. Chloroplasts with pyrenoids, trichocysts, and peduncle present.

Type species. G. radiolariae T. Yuasa et T. Horiguchi sp. nov.

Etymology. Greek *Gymno* (naked), *xanthos* (yellow), and *ella* (diminutive), in reference to the small, naked and yellow organism.

Gymnoxanthella radiolariae T. Yuasa et T. Horiguchi **sp. nov.** (Figs. 2–7)

Description. Non-motile cells spherical, 5–8 μm in diameter. Flagella, even reduced ones, not observed. Dinokaryon located in the middle or offset to one side of the cell. Free-living motile cells 9.1–11.4 μm long and 5.7–9.4 μm wide. Motile cells unarmored, oval to elongate oval from the ventral side, slightly flattened dorsoventrally, covered with tetragonal, pentagonal, or hexagonal amphiesmal vesicles. Amphiesmal vesicles arranged in a latitudinal series, four on the epicone, three in the cingular groove, and three on the hypocone. Horseshoe-shaped apical groove running in an counterclockwise loop around the apex. A row of six amphiesmal vesicles along the apical groove displays many small knobs in a regular arrangement. Cingulum wide and

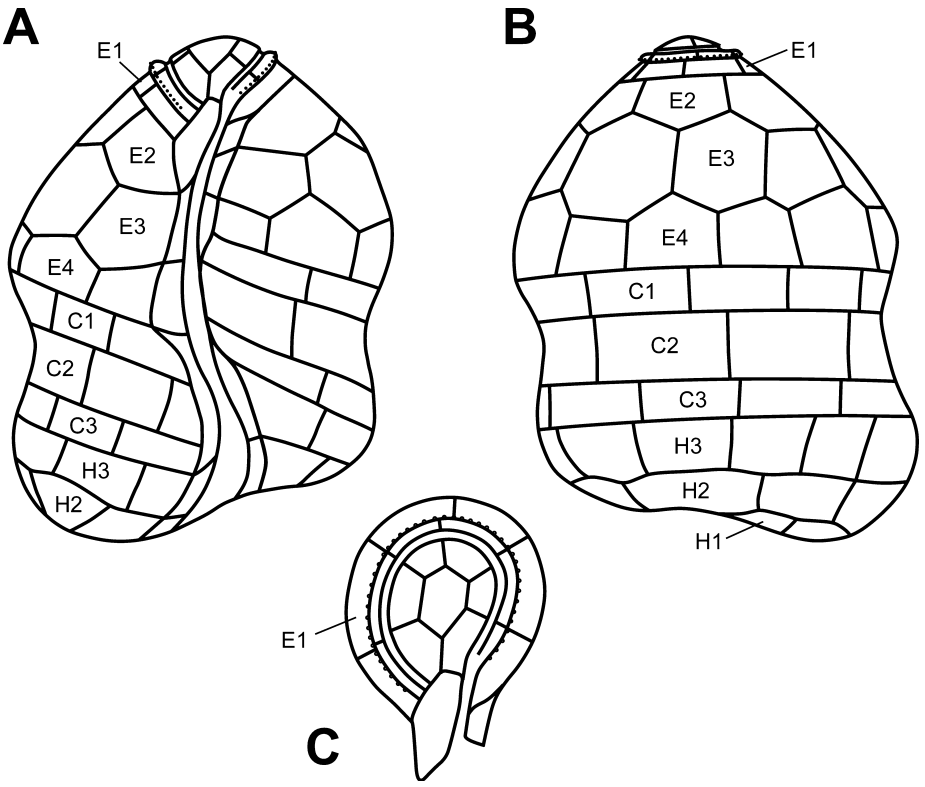

FIG. 6. Schematic drawings showing the amphiesmal vesicles of the motile cells of *Gymnoxanthella radiolariae* gen. et sp. nov. (A) ventral view; (B) dorsal view; (C) apical view.

relatively shallow, descending and displaced one cingulum width. Sulcus narrow and shallow, running straight to the apex and connected to the onset of the apical groove. A typical dinokaryon located in the middle of the cell. Nuclear envelope with vesicular chambers. NFC linked the proximal parts of transverse and longitudinal basal body with the nucleus. Chloroplasts yellow-brown, located peripherally, with pyrenoid. Trichocysts and peduncle present.

Holotype. Figure 4A. A fixed and dried specimen on a SEM stub has been deposited in the Department of Botany, National Museum of Nature and Science, Japan, as MPC-26753.

Type locality. Site 990528 (26°37′ N, 127°47′ E), East China Sea, off the northwest coast of Okinawa Island, Japan, collected on March 31, 2009.

Type host. *E. elegans* (Spumellarida, Spongodiscidae)

Etymology. The specific epithet *radiolariae*, in reference to the symbiotic nature in radiolarians.

Authentic culture strain. Culture strain has been deposited in the National Institute for Environmental Studies, Japan, as NIES-3649.

DISCUSSION

Comparisons with related Gymnodinium *species*. The molecular phylogenetic position of *G. radiolariae* indicated that this alga is related to the clade referred to as *Gymnodinium* s.s. (Hansen and Moestrup in Daugbjerg et al. 2000; Figs. 8 and 9). *Gymnodinium* is one of the genera of unarmored or naked dinoflagellates, and it has traditionally been distinguished from other unarmored genera such as *Gyrodinium*, *Amphidinium*, and *Katodinium* on the basis of the relative sizes of the epicone and hypocone and the degree of cingular displacement (Kofoid and Swezy 1921). However, several ultrastructural studies combined with molecular phylogeny have revealed that the traditional taxonomy of the gymnodinioid genera did not reflect their phylogenetic relationships (e.g., Daugbjerg et al. 2000). On the basis of several ultrastructural features and LSU rDNA sequence data, Daugbjerg et al. (2000) emended the traditional definition of the genus *Gymnodinium* and divided it into *Gymnodinium* s.s. and three other new genera: *Akashiwo*, *Karenia*, and *Karlodinium*. According to Daugbjerg et al. (2000), the members of *Gymnodinium* s.s. are defined as possessing the following four characters: (i) the horseshoe-shaped apical groove running in the counterclockwise direction, (ii) the nuclear envelope with vesicular chambers, (iii) cingulum displacement with its own or more width, and (iv) the nuclear or dorsal fibrous connective. In the present study, SEM and TEM observations revealed that *G. radiolariae* possesses all four of these key characters. These morphological features combined with the SSU and LSU rDNA molecular phylogenetic data indicate that *G. radiolariae* is a member of *Gymnodinium* s.s.

In comparison with the related *Gymnodinium* species, *G. radiolariae* has some unique morphological

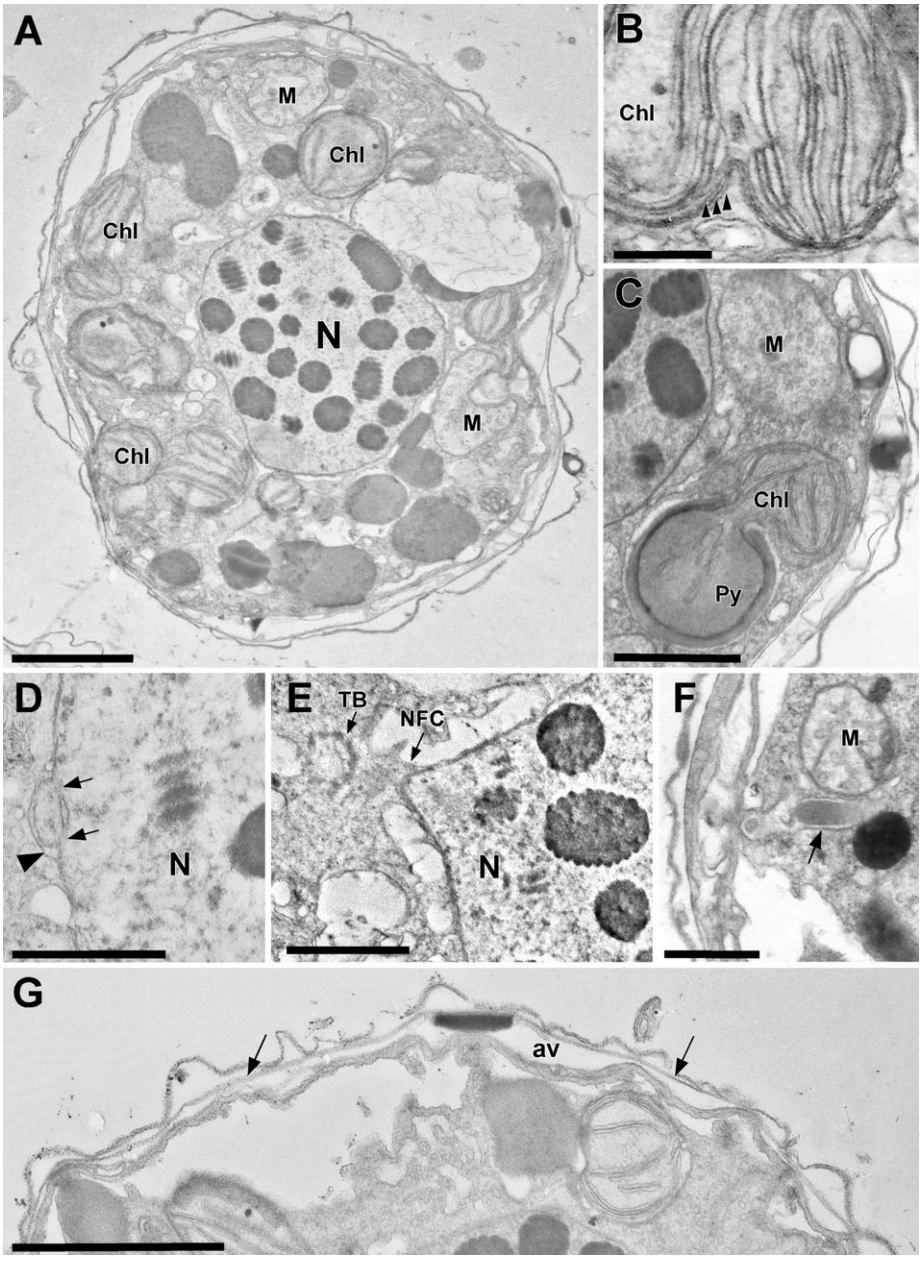

Fig. 7. Transmission electron micrographs of *Gymnoxanthella radiolariae* gen. et sp. nov. (A) Longitudinal section of the cell, scale bar = 2 μm. (B) Details of the chloroplast showing three membranes (arrowheads), scale bar = 0.5 μm. (C) Enlargement of a pyrenoid branching from a single chloroplast and enclosed in a starch sheath. The matrix is invaded by a few lamellae, scale bar = 1 μm. (D) Detail showing the nuclear envelope with a nuclear chamber (arrowhead) and nuclear pores (arrows), scale bar = 1 μm. (E) Longitudinal section of the flagellar apparatus, showing the attachment of the nuclear fibrous connective (NFC) to the nuclear extension and the proximal parts of the transverse basal body (TB), scale bar = 1 μm. (F) Trichocyst (arrow) and mitochondrion with tubular cristae, scale bar = 0.5 μm. (G) Detail of the amphiesmal vesicles containing plate-like structures (arrows), scale bar = 2 μm. av, amphiesmal vesicle; Chl, chloroplast; M, mitochondria; N, nucleus; Py, pyrenoid.

features that distinguish it from the others. It has a characteristically small body with a cell size 9.1–11.4 μm long, 5.7–9.4 μm wide, and a loop-shaped apical groove accompanied by a row of amphiesmal vesicles with the many small knobs. Other species with a cell size similar to that of *G. radiolariae* are rare in the genus *Gymnodinium*. They include *G. cnecoides* (9–14 μm long and 8–11 μm wide: Harris 1940), *G. japonicum* (8–12 μm long and 5–7 μm wide: Hada 1974), *G. nanum* (5 μm long: Wood 1963), *G. pumilum* (8–15 μm long and 6–12 μm wide: Larsen 1994), *G. punctatum* (10 μm long: Kofoid and Swezy 1921), *G. smaydae* (6–11 μm long and 5–10 μm wide: Kang et al. 2014), and *G. varians* (8–17 μm long and 6–12 μm wide: Kofoid and Swezy 1921). However, except for *G. smaydae*, these small gymnodinioid species have a much smaller degree of cingulum displacement than ours or do not have a displaced cingulum.

The species in the genus *Gymnodinium* that have the loop-shaped apical groove and amphiesmal vesicles with many small knobs are *G. aureolum*, *G. corollarium*, *G. impudicum*, *G. maguelonnense*, *G. smaydae*, and *G. trapeziforme* (see Kang et al. 2014). Most of them are much larger in size than *G. radiolariae*, but the only species possessing both the small body and the loop-shaped apical groove accompanied by amphiesmal vesicles with many small knobs is *G. smaydae*. *G. smaydae* is a marine mixotrophic dinoflagellate described by Kang et al. (2014). It

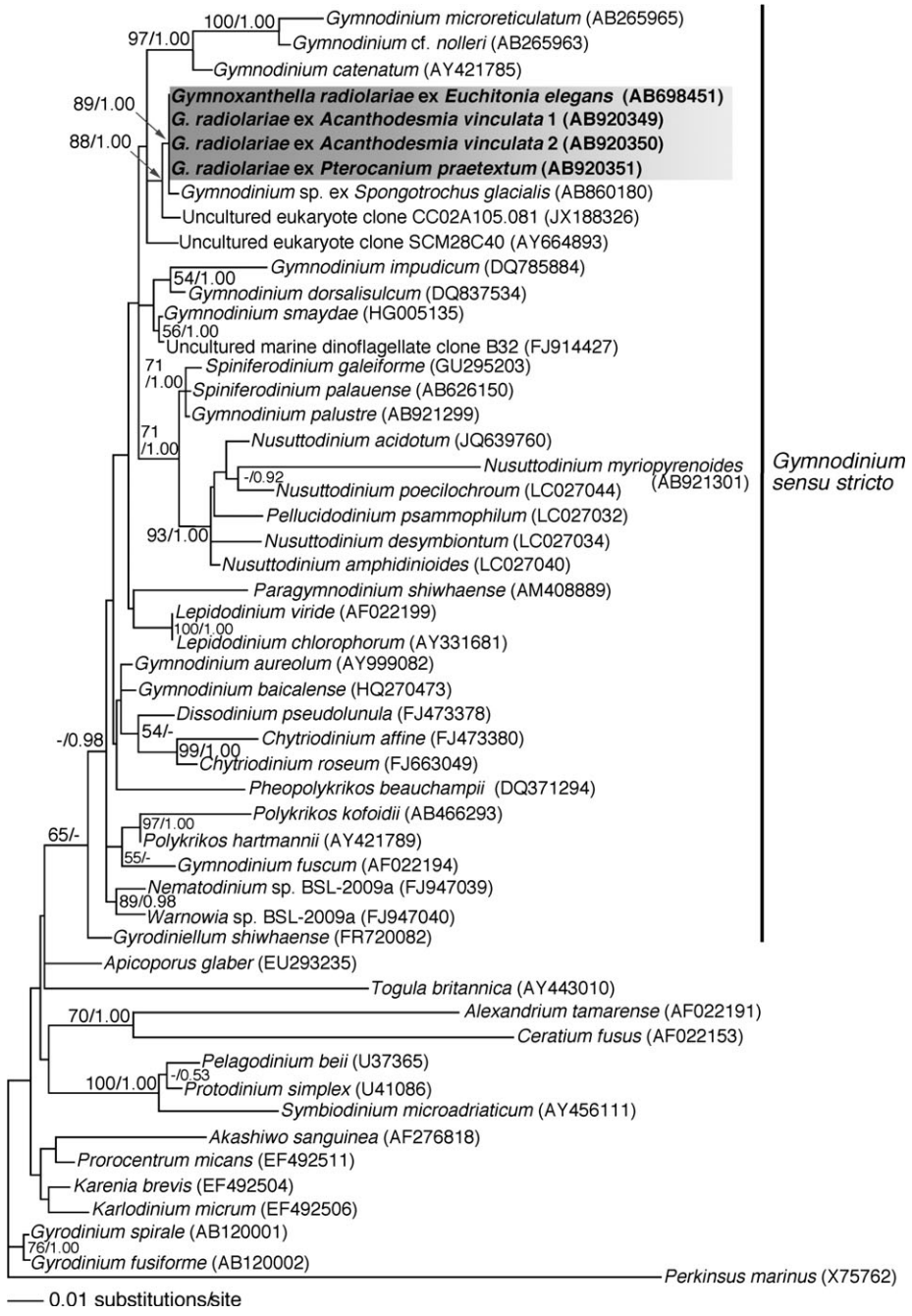

FIG. 8. SSU rDNA phylogenetic tree based on the maximum-likelihood method (52 taxa, 1,164 nucleotide sites) for our obtained and other dinoflagellate sequences already in the database. Bootstrap values (left number) above 50% and posterior probabilities (right number) over 0.50 are given at the respective nodes.

has the key characters of *Gymnodinium* s.s. and possesses a peduncle that is also found in *G. radiolariae*. In addition, *G. smaydae* phylogenetically forms a monophyletic clade with our species supported by moderate to high BV and PP in the LSU tree (Fig. 9).

However, in the SSU tree, *G. radiolariae* and *G. smaydae* do not form a monophyletic group (Fig. 8), and in the comparison of the SSU and LSU rDNA sequences of these two species, the differences between *G. radiolariae* and *G. smaydae* were 19 bases of 1,628 bp and 89 bases of 911 bp, respectively. Furthermore, close observation revealed morphological distinctions between these two species (Table 1). *G. radiolariae* has a rather large epicone in comparison to its hypocone, whereas *G. smaydae* has a smaller epicone than hypocone (Kang et al. 2014). The arrangement of the amphiesmal vesicles in the latitudinal series is also different: *G. radiolariae* has a latitudinal series of amphiesmal vesicles arranged in a total of 10 rows; four on the epicone, three in the cingular groove, and three on the hypocone, whereas *G. smaydae* has a total of 11 rows; 4 on the epicone, 3 in the cingulum, and 4 on the hypocone. The small knobs on the loop-shaped amphiesmal vesicles of the apical

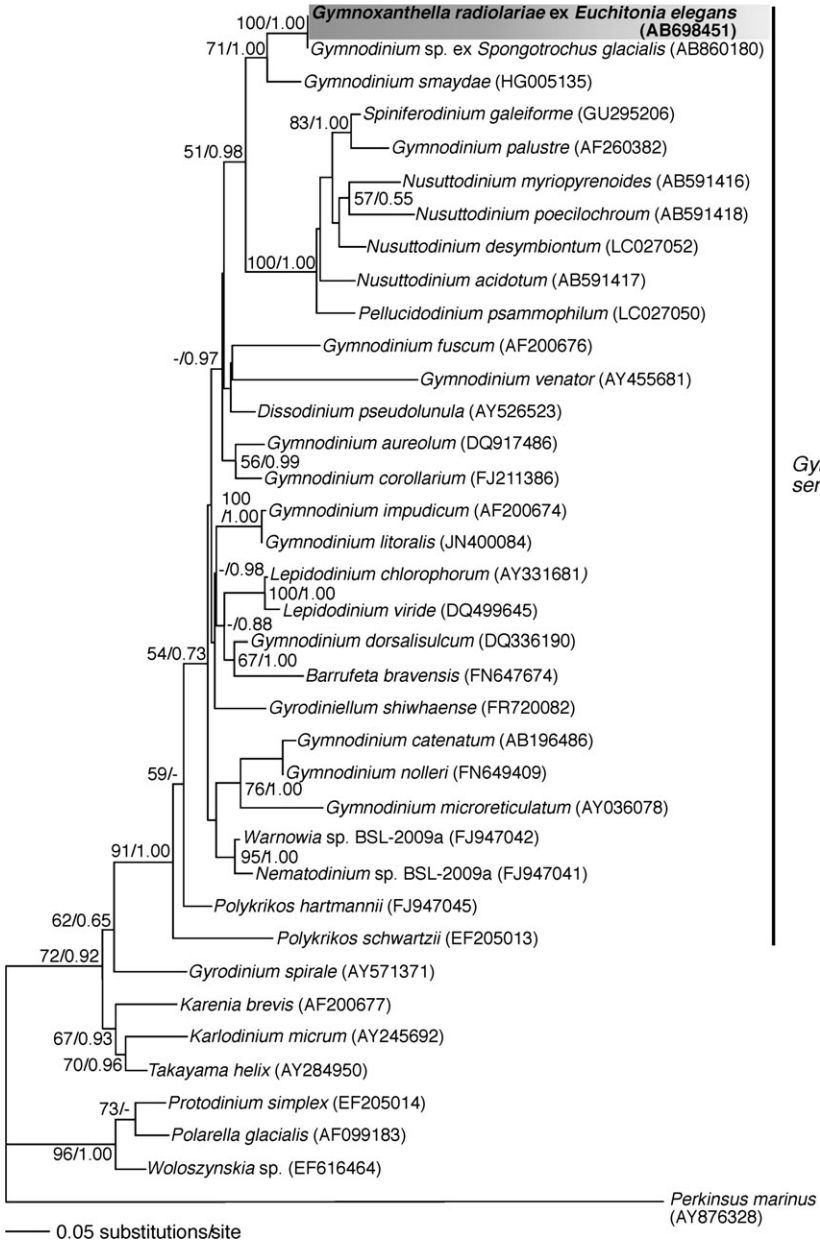

FIG. 9. LSU rDNA phylogenetic tree based on the maximum-likelihood method (37 taxa, 485 nucleotide sites) for our obtained and other dinoflagellate sequences already in the database. Bootstrap values (left number) above 50% and posterior probabilities (right number) over 0.50 are given at the respective nodes.

groove are a common feature of both species, however, our specimen had approx. Forty-four small knobs are present on the amphiesmal vesicles, whereas *G. smaydae* has approx. 74 (Kang et al. 2014). In addition, although both *G. radiolariae* and *G. smaydae* possess a peduncle, which is associated with feeding behavior, their feeding strategies seem to differ. Kang et al. (2014) described *G. smaydae* as having the phototrophic dinoflagellate *Heterocapsa rotundata* as prey, and they observed that the cells and chloroplasts of *H. rotundata* were digested by *G. smaydae*. They also reported that in spite of possessing permanent chloroplasts, *G. smaydae* died after about 40 d without prey (Kang et al. 2014). In contrast, the culture strain of our species is able to maintain for a long time after isolation without prey. In the present study, we confirmed a peduncle on the cultured cell of *G. radiolariae* However, it seems that when *G. radiolariae* is finally jettisoned into the environment prior to the reproduction or death of the host radiolarians, it transforms into motile cells and may take up nutrients from the marine environment and obtain energy by photosynthesis in order to survive; the peduncle may be a rudimentary organ. The morphological differences between *G. radiolariae* and *G. smaydae* combined with the results of our molecular phylogenetic analyses suggest that *G. radiolariae* is a new species.

Taxonomic affiliation of G. radiolariae. After Daugbjerg et al. (2000) defined *Gymnodinium* s.s., several

TABLE 1. Comparison of the measurements and cell morphology of *Gymnoxanthella radiolariae* gen. et sp. nov. and *Gymnodinium smaydae*.

Cell shape	Epicone	Hypocone	Cell size	Cingulum	Apical groove	Apical row of amphiesmal vesicles	Sulcal extention	Number of longitudinal series	Chloroplast	Eyespot	Peduncle	Nucleus	Habitat	Source
Ovoidal	Conical	Hemispheroidal, slightly convex medially	L: 9.1–11.4 µm W: 5.7–9.4 µm	Wide, median, left-handed, displaced about 1/1 cingular width	Horseshoe-shape	Circular loop, accompanied by row of ~50 small knobs	Sulcal extention reaches apex	10	Several irregular, disc-shaped yellow-brown chloroplasts with one or two pyrenoids	No	Yes	Center or offset to one side of the cell	Symbiont of radioralia, marine	This study
Ovoidal	Slightly conical	Ellipsoidal	L: 6-11 µm W: 5-10 µm	Wide, median, left-handed, displaced about 0.4-0.6 of cell length	Horseshoe-shape	Circular loop, accompanied by row of ~70 small knobs	Sulcal extention reaches apex	11	Several, brown chloroplasts, arranged in bands, each with a pyrenoid	No	Yes	Anteior to center of the cell	Planktonic, marine	Kang et al. 2014

studies with increasing evidence obtained from molecular phylogenetic analyses showed that some species formed a distinct clade within *Gymnodinium* s.s., and that these species were distantly related to *Gymnodinium fuscum* (Ehrenberg) Stein, the type species of the genus *Gymnodinium*, and they possess unique morphological characteristics distinguishable from those of *G. fuscum*. Several new genera have been established in *Gymnodinium* s.s., namely *Barrufeta*, *Gyrodiniellum*, *Paragymnodinium*, *Pellucidodinium*, and *Nusuttodinium* (e.g., Kang et al. 2010, 2011, Sampedro et al. 2011, Takano et al. 2014, Onuma et al. 2015).

Our molecular identification of *G. radiolariae* as belonging to the *Gymnodinium* s.s. is in agreement with its morphology possessing four key characters of *Gymnodinium* s.s., however, *G. radiolariae* is also distantly related to *G. fuscum* in our molecular phylogenetic trees (Figs. 8 and 9). Both the SSU and LSU phylogenetic trees revealed close relationships among *G. radiolariae*, the photosynthetic dinoflagellates *G. palustre*, *Spiniferodinium galeiforme*, *S. palauense*, the heterotrophic dinoflagellate *N. desymbiontum*, *P. psammophilum*, and several species of the kleptochloroplastidic dinoflagellate *Nusuttodinium* (Figs. 8 and 9). *G. palustre* is a freshwater species with brown-colored, rod-shaped chloroplasts (Lewis and Dodge 2002). The genus *Spiniferodinium* was defined as possessing unique characteristics that produce a transparent, spiny, helmet-shaped cell covering in nonmotile vegetative cells (Horiguchi and Chihara 1987, Horiguchi et al. 2011). *P. psammophilum* and the species of the genus *Nusuttodinium* lack genuine chloroplasts (Takano et al. 2014, Onuma et al. 2015). Our species does not possess such a characteristic life stage or structures, and characteristically lives in symbiosis with radiolarians. As mentioned above, although our species morphologically resemble the asymbiotic species *G. smaydae*, they do not form a monophyly in our SSU phylogenetic tree. Thus, based on the consistent morphological, genetic, and ecological divergence of our species with *G. palustre*, *P. psammophilum*, and the genera *Nusuttodinium* and *Spiniferodinium*, we consider it justified to erect a new, separate genus and species *G. radiolariae* T. Yuasa et T. Horiguchi gen. et sp. nov. for the unarmored symbiotic dinoflagellates of radiolarians.

Comparison with other Gymnodinium*-like symbionts from radiolarians.* As mentioned in the *Introduction*, *Gymnodinium*-like symbionts in radiolarians have been reported by Hollande and Enjumet (1953), Hollande and Carré (1975), and Ishitani et al. (2014). In the report of Ishitani et al. (2014), a molecular phylogenetic analysis was used to identify *Gymnodinium* sp. obtained from radiolarian *S. glacialis*. The sequences of both *G. radiolariae* and *Gymnodinium* sp. from radiolarian *S. glacialis* were very similar: 2 bases of 1,792 bp for SSU rDNA and 1 base of 1,321 bp for LSU rDNA. Moreover, the

sequences of the ITS regions (ITS1, 5.8S rDNA, and ITS2) were identical. Although we have no morphological data on the symbiont from *S. glacialis*, these two species are considered conspecific based on their genetic similarity.

At present we have no way of positively determining whether our species and *Gymnodinium*-like symbionts reported by Hollande and Enjumet (1953) and Hollande and Carré (1975) are conspecific, because Hollande and Enjumet (1953) provided only one original illustration with regard to the motile stage of the symbiotic dinoflagellate (fig. 55d: Hollande and Enjumet 1953). This illustration shows only a smaller and more rounded epicone and smaller cingular displacement compared to *G. radiolariae*, and it has no characteristics that can be compared with some of the unique morphological features of our species (e.g., the arrangement of the amphiesmal vesicles or the small knobs on the loop-shaped amphiesmal vesicles of the apical groove). Moreover, Hollande and Enjumet (1953) clearly depicted in their figures 55b and 55c that the bi-nucleated and bi-flagellated stage, which is not *Gymnodinium*-like, came out of the theca. The caption also clearly mentions the theca in the phrase "sortant de leur coque," which translates to "coming out of their theca."

In light of the results reported by Hollande and Enjumet (1953), Hollande and Carré (1975) proposed *E. nutricola* (Brandt) sensu Hollande et Carré as a gymnodinioid symbiotic dinoflagellate from colonial and naked radiolarians. It is also difficult to compare the morphologies of what Hollande and Carré (1975) called *E. nutricola* with those of our species. Hollande and Carré (1975) showed several TEM images of the symbionts under a symbiotic state in radiolarians and they stressed that *E. nutricola* inside the host was surrounded by a cellulosic cell wall in contrast to *G. radiolariae*, which has no cellulosic cell wall in the symbiotic stage. In addition, the ultrastructural features of the nuclear chambers of the cells were not shown in the figures, and it was not possible to determine the other taxonomic features of the gymnodinioid dinoflagellate, while the pyrenoid was surrounded by a starch sheath with a typical peri-pyrenoid sac (Hollande and Carré 1975). These characteristics are incongruent with those of *G. radiolariae*.

Gast and Caron (1996) sequenced the symbiotic dinoflagellate from the naked radiolarian *Thalassicolla nucleata*, which corresponded to those identified in TEM studies as *E. nutricola* in Hollande and Carré (1975), and the culture strain isolated by Gast and Caron (1996) was reexamined by Gottschling and McLean (2013). The SSU rDNA sequence data of the symbiotic dinoflagellate from *T. nucleata* obtained by Gast and Caron (1996) and Gottschling and McLean (2013) were the same as the sequence data of the peridinioid dinoflagellate identified as *Brandtodinium* (=*Zooxanthella*) *nutricula* sensu Probert et Siano in Probert et al. (2014). In the present study, we analyzed the symbionts from two specimens of each of the radiolarian species of *A. vinculata* and *E. elegans*, and we detected that they all harbored *G. radiolariae* as a symbiont; moreover, the sequences of the dinoflagellate symbionts in the two specimens of radiolarian *S. glacialis* reported by Ishitani et al. (2014) from the Pacific Ocean were also the same. This suggests that the symbiotic interactions of polycystine radiolarians are probably not random, but rather selective, in contrast to the case of the acantharian radiolarians reported by Decelle et al. (2012), and suggests that the specimen which was examined and identified as the gymnodinioid *E. nutricola* sensu Hollande et Carré 1975 from the naked radiolarian *T. nucleata* may be the peridinioid dinoflagellate *Z. nutricula*.

Our present results clarified that the endosymbiont in radiolarians is the gymnodinioid dinoflagellate *G. radiolariae*. The results indicate that at least two dinoflagellate symbionts, peridinioid and gymnodinioid species, live in radiolarians. In addition, Taylor (1974) also observed *Amphidinium* sp. as a symbiotic dinoflagellate within radiolarians, suggesting the further biodiversity of symbionts of radiolarians. More detailed studies of the distribution of symbionts and their host radiolarians will provide better insight into the diversity and specificity of their relationships.

We are grateful to M. Elbrächter for his critical reading of the manuscript and many helpful suggestions, and for supervising "Appendix S2 in the Supporting Information" on the nomenclatural aspects of *Zooxanthella*. We would also like to express our appreciation to the editor and the anonymous reviewers for their suggestions on how best to improve our manuscript, and to one reviewer in particular, who provided much assistance on matters of nomenclature and etymology. We would also like to acknowledge A. Takemura, S. Harii, Y. Nakano, and S. Kadena of the University of the Ryukyus for their support and helpful discussions. This study was financially supported by funds for Collaborative Research of Tropical Biosphere Research Center, University of the Ryukyus to T. Yuasa; and by grants-in-aid from JSPS to O. Takahashi (grants no. 19540491 and 23540545).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Appendix S1. The ingredients for Daigo's IMK medium.

Appendix S2. Nomenclatural aspects of *Zooxanthella* and the related genera.