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ORIGINAL PAPER

Morphology and molecular phylogeny of the marine gregarine parasite *Selenidium oshoroense* n. sp. (Gregarina, Apicomplexa) isolated from a Northwest Pacific *Hydroides ezoensis* Okuda 1934 (Serpulidae, Polychaeta)

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Abstract In this study, we describe a novel marine gregarine parasite, *Selenidium oshoroense* n. sp., isolated from *Hydroides ezoensis* Okuda 1934 (Serpulidae, Polychaeta). Trophozoites (feeding stages) of *S. oshoroense* n. sp. were isolated from the gut of *H. ezoensis* collected from the intertidal shore near Oshoro, Hokkaido, Japan, and prepared for analysis with scanning and transmission electron microscopy (SEM and TEM), and molecular phylogenetic analysis using 18S rDNA. Trophozoites of *S. oshoroense* n. sp. were, on average, 120 μm long and 13 μm wide. Observation of the cells under SEM and TEM revealed 23 longitudinally running epicytic folds on the surface of the cells. Peduncles, multimembrane-bound whorls, and inclusions were also observed and were indicative of surface-mediated nutrition. Myzocytotic feeding at the apical end of the cell was also indicated in sections of host gut tissue that were viewed under TEM, suggesting that myzocytosis could be a shared feature among this clade of *Selenidium* from tube-forming polychaetes. Molecular phylogenetic analysis of the 18S rDNA grouped *S. oshoroense* n. sp. within a clade of *Selenidium* from tube-forming polychaetes, sister to *S. serpulae*. The two 18S rDNA sequences generated from separate isolates of *S. oshoroense* n. sp. had a similarity of 0.997, but were 0.965 and 0.966 similar to *S. serpulae*. *S. oshoroense* n. sp. was differentiated from, *S. serpulae*, based on the absence of transverse striations on the surface of the epicytic folds and difference in ecological niche (host). Morphological differences were supported in phylogenetic analysis which grouped *S. oshoroense* n. sp. isolates, to the exclusion of *S. serpulae*.

Keywords Apicomplexa · Marine gregarine · Molecular phylogeny · Morphology · Parasite · *Selenidium* · Serpulidae

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Introduction

Selenidium Giard 1884 is a genus of marine gregarine apicomplexans that are commonly found infecting the intestines of polychaetes (Levine 1971; Ray 1930; Desportes and Schrével 2013a, b; Wakeman and Leander 2012, 2013b). Generally speaking, gregarines have been disproportionally studied, compared to their more infamous relatives such as *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*, which are notorious parasites of humans and other vertebrates. From an evolutionary perspective, marine gregarines are thought to have evolved early in the evolutionary history of apicomplexans, and some of these groups (e.g., *Selenidium*) represent what is possibly the earliest evolving of these lineages (Théodoridès 1984; Leander 2008).

Selenidium contains roughly 50 species (Levine 1971; Desportes and Schrével 2013a, b), and are found exclusively in the guts of the marine hosts, primarily annelids (polychaetes). Morphologically, this group is united by trophozoites (feeding stages) that have a general vermiform shape, relatively fewer (<50) longitudinally running epicytic folds

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on the surface of the cell, compared to lineages considered to be more derived (e.g., *Lecudina*, *Paralecudina*, or *Polyplicarium*) which contain hundreds of surface folds, and a bending/twisting movement which is posited to be driven by a network of microtubules below the trilayer membrane (Grassé 1953; Mellor and Stebbings 1980; Stebbings et al. 1974; Ray 1930); *Selenidium* also possesses an apical complex, and feeds via myzocytotis (Schrével 1968, 1971; Simdyanov and Kuvardina 2007), a (sucking) feeding strategy that has only been shown to exist in colpodellids (an early free-living group to apicomplexans; Brugerolle 2002; Kuvardina et al., 2002), perkinsids (early dinoflagellates) Perkins 1996; Saldarriaga et al. 2003), and gregarines (e.g., *Selenidium*) (Schrével 1971; Schrével et al. 2016).

Molecular data representing this group, and gregarines in general, is severely lacking. Any existing molecular data is primarily based on 18S rDNA, a marker that has shown utility in distinguishing and identifying closely related species (Rueckert et al. 2011; Wakeman and Leander 2013a; Wakeman et al. 2014), but is unable to delineate deeper relationships between gregarine groups, and to a larger extent, phylogeny in the context of Apicomplexa. Recent attempts have been made to reconcile relationships on a larger scale (Cavalier-Smith 2014), based on 18S rDNA molecular data, but these hypotheses are riddled with ambiguity. Larger datasets, comprised of multiple proteins, will be the most useful in settling any long-standing, chronic confusion at this level among gregarines and apicomplexans.

Still, understanding the diversity of *Selenidium* is of key importance because it remains one of the more poorly sampled gregarine lineages. Employing rDNA datasets can provide information on the coevolution between gregarines and their host group, host–parasite specificity, and the basic ecology and geographical distributions of these parasites.

To this end, this study describes *S. oshoroense* n. sp. from *Hydroides ezoensis*, a serpulid polychaete from the Northwestern Pacific near Oshoro, Hokkaido, Japan. Trophozoites of *S. oshoroense* n. sp. were isolated for molecular analyses using 18S rDNA, and morphological work using scanning and transmission electron microscopy (SEM and TEM).

Materials and methods

Collection of hosts and isolation of *Selenidium oshoroense* n. sp

Hydroides ezoensis (Serpulidae, Polychaeta) was collected at low tide from rocks near the Oshoro Marine Laboratory, Oshoro, Hokkaido, Japan (4312'33.26" N 14051'30.67" E) from April – May 2016. Worms were held in cool sea water, and transported to the laboratory, where their intestines were dissected using forceps and razors. A total of 10 hosts were collected and dissected; all 10 were heavily infected with *Selenidium oshoroense* n. sp. throughout the length of their digestive tract. Individual cells of *S. oshoroense* n. sp. were isolated using hand-drawn glass pipettes, and subsequently washed (until clean) in filtered, autoclaved seawater for further morphological and molecular analysis.

Light microscopy, scanning electron microscopy and transmission electron microscopy

Differential interference contrast (DIC) images of the trophozoite stages of *Selenidium oshoroense* n. sp. were taken using a Zeiss Axioscop 2 *Plus* microscope connected to a Leica MC 120 HD digital camera. For SEM, cells were transferred to a glass coverslip submerged in 2.5% glutaraldehyde in seawater on ice for 15 min. After washing the cells 3 times for 5 min in seawater, the coverslip was place in 1% OsO_4 for 30 min, and subsequently washed with distilled water and dehydrated through a graded series of ethanol washes (30–100%) for 5 min at each step. Samples were critical point dried with CO_2, sputter coated with 5-nm gold and viewed using a Hitachi N-3000. For TEM, individual cells and small pieces of gut tissue were fixed in 2.5% glutaraldehyde in seawater on ice for 30 min, washed in seawater, and post fixed with 1% OsO_4 on ice for 1.5 h; both fixation steps were performed in the dark. Following the fixation with OsO_4, samples were washed in seawater, and dehydrated through a graded series of ethanol washes (30–100%), and acetone for 5 min at each step at room temperature. Samples were then placed in a 1:1 resin (Agar Low Viscosity Resin, Agar Sciences)/acetone mixture for 30 min, followed by a 100% resin mixture overnight at room temperature. Resin was exchanged the following day, and samples were polymerized at 68°C for 32 h. Samples were cut with a diamond knife, and viewed with a Hitachi-7400.

DNA extraction, PCR amplification, and sequencing of 18S rDNA

Two single-cell isolates of *Selenidium oshoroense* n. sp. were placed in 1.5-ml Eppendorf tubes. Total genomic DNA was extracted following the manufacturers protocol using an Epicentre MaterPure DNA and RNA purification kit. To amplify the 18S rDNA, an initial polymerase chain reaction (PCR) using Econotaq 2X Mastermix (Lucigen) was performed with the primers 5' - GCGCTACCTGGTTG ATCCTGCC - 3' and 5' - GATCCTTCTGCAGGTTCACC TAC - 3' using the following program on a thermocycler: initial denaturation 94°C for 2:00 min; 35 cycles of 94°C for 0:30 s, 52°C for 0:30 s, 72°C for 2:00 min; final extension 72°C for 7:00 min. Subsequently, 1 μl of that reaction was used as a template in a second round of amplification with the primer sets 5' - GATCCTTCTGCAGGTTCACCTAC - 3'

and 5' - GGTAGYGACAAGAAATAACAAC - 3', 5' - GCGCTACCTGGTTGATCCTGCC - 3' and 5' - GAYTACGACGGTATCTGATCGTC - 3', following the same thermocycler program described above (Wakeman and Leander 2013a). PCR products were purified using a Qiagen PCR purification kit; 1 μl of purified product was used in a sequencing reaction with ABI BigDye Terminator v1.1 (Applied Biosystems) and subsequently purified with ethanol, before being eluted in 18 μl of Hi-Di Formamide (Applied Biosystems) and sequenced on a 3130 genetic analyzer (Applied Biosystems). The two novel 18S rDNA sequences from *S. oshoroense* n. sp. were deposited in NCBI's GenBank (KX008972 and KX008973).

Phylogenetic analyses

The newly obtained 18S rDNA sequences from *Selenidium oshoroense* n. sp. were identified by BLAST. These sequences were then aligned with 78 additional sequences representing the diversity of gregarines, as well as other apicomplexan groups, and dinoflagellates (outgroup) using MUSCLE 3.8.31 (Edgar 2004). A second alignment containing 22 taxa, representing the diversity of *Selenidium* from tube-forming polychaetes including 1518 sites, was constructed in the same way described above. Both alignments were fine-tuned visually using Mesquite 3.04 (Maddison and Maddison 2015); gaps and unambiguous regions were omitted from the analysis.

Garli0.951-GUI (www.bio.utexas.edu/faculty/antisense/garli/Garli.html; Zwickl 2006) was used to run maximum-likelihood (ML) bootstrap analyses on both datasets. Jmodeltest 0.1.1 selected a general-time reversible (GTR+I+G) model of nucleotide substitutions under Akaike information criterion (AIC) and AIC with correction (AICc.; Posada and Crandall 1998) that incorporated invariable sites and a discrete gamma distribution (eight categories; GTR+Γ+I model: $\alpha = 0.6600$ and fraction of invariable sites = 0.1900, for the 18S alignment with 78 taxa, and $\alpha = 0.5890$ and fraction of invariable sites = 0.4370 for the 18 S alignment with 22 taxa). ML bootstrap analyses were performed on 500 pseudo-replicates, with one heuristic search per pseudo-replicate (Zwickl 2006), using the same program set to the GTR model+Γ+I. Bayesian analyses were performed using the program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The program was set to operate with GTR, a gamma-distribution, and four Monte Carlo Markov chains (MCMC; default temperature = 0.2). A total of 8,000,000 and 6,500,000 for the larger and smaller 18S alignments, respectively. Generations were calculated with trees sampled every 100 generations and with a prior burn-in of 1,000,000 generations (10,000 sampled trees were discarded; burn-in was checked manually). When the average split fell below 0.01, the program was set to terminate. All other parameters were left at the default setting. A majority rule consensus tree was constructed from 70 ,000 post-burn-in trees for the larger 18S rDNA dataset, while 55 ,000 trees were used for the 18S rDNA dataset with fewer taxa. Posterior probabilities correspond to the frequency at which a given node was found in the post-burn-in trees.

Results

General morphology of the trophozoite stage of *Selenidium oshoroense* n. sp

Trophozoites of *Selenidium oshoroense* n. sp. were found throughout the length of the intestinal tract (gut), and measured, on average, 120 μm long (80–175 μm, $n = 50$) and 13 μm wide (8–15 μm, $n = 50$; Figs. 1 and 2). The nucleus was ovoid and was positioned in the centre to posterior part of the trophozoite cells (Fig. 1). The surface of the cells was lined with 23 longitudinal epicytic folds (Fig. 3a and b). Over 50 trophozoite stages were carefully examined under SEM; transverse striations were not observed on any part of the cell (Fig. 2)

Ultrastructure of *Selenidium oshoroense* n. sp

Transmission electron micrographs of *Selenidium oshoroense* n. sp. revealed epicytic folds, subtended by microtubules running along the longitudinal axis of the cell (Fig. 3a–d). Mitochondria were observed throughout the cytoplasm of the cell, but were especially prevalent closer to the folds (edges) of the cells (Figs. 3c–e and 4a–c). Dense bodies (lipid droplets) and accumulated amylopectin were also seen throughout the cell, as were Golgi bodies (Fig. 3e), multimembrane-bound whorls, peduncles and inclusions (Figs. 3d, 4c and d). Sections through the host gut lumen contained trophozoites of *S. oshoroense* n. sp. In these sections, myzocytotic feeding by the parasite was indicated, as was a distinct conoid and rhoptries (Fig. 4e and f).

Molecular Phylogeny of *Selenidium oshoroense* n. sp

Phylogenetic analysis of the 18S rDNA with 78 taxa resulted in well-supported clades comprised of dinoflagellates (outgroup), coccidians, pyroplasmids, cryptosporidians, two clades of terrestrial gregarines (terrestrial gregarines I and II), and eight clades of marine gregarines (Fig. 5). Clades were generally well-supported; however, the nodes between these groups had low to no support. The genus *Selenidium* comprised three lineages in the tree: one lineage from tube-forming polychaetes (containing the type species, *S. pendula*), another representing *Selenidium* from sipunculids, and *S. terebellae*. The two new sequences from the isolates of *S. oshoroense* n. sp. branched within the clade containing *Selenidium* from tube-forming

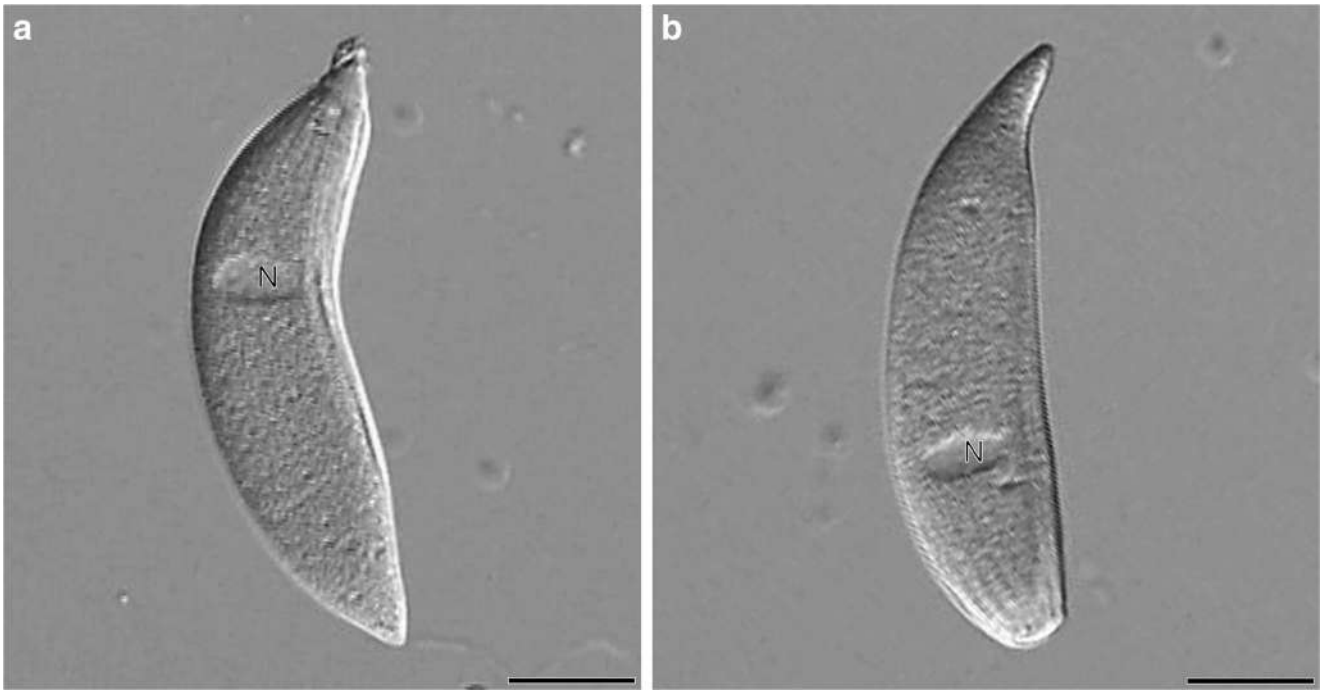


Fig. 1 Light micrographs of *Selenidium oshoroense* n. sp. **a**, **b**. Trophozoite stages of *S. oshoroense* n. sp. with a central-posterior nucleus (*N*). The anterior end of the cells are oriented towards the top of the figure. Scales = 10 μm

polychaetes. The sequences were nested within a clade containing *S. sensimae* and another species of *Selenidium* (KC110868). *S. oshoroense* n. sp. (Fig. 5).

In the 18S rDNA dataset containing 22 sequences of *Selenidium* from tube-forming polychaetes, nodes were generally well-supported throughout the tree, with only a few exceptions (Fig 6). *Selenidium oshoroense* n. sp. branched together, with high support, as a sister to *S. serpulae* (DQ683562). The pairwise distance between the 18S rDNA sequences of isolate 1 and isolate 2 of *S. oshoroense* n. sp. was 0.997 across 1,506 bp, while both those sequences had distances of 0.965 and 0.966 across 1521 bp and 1506 pb, when aligned with *S. serpulae* (DQ683562), respectively.

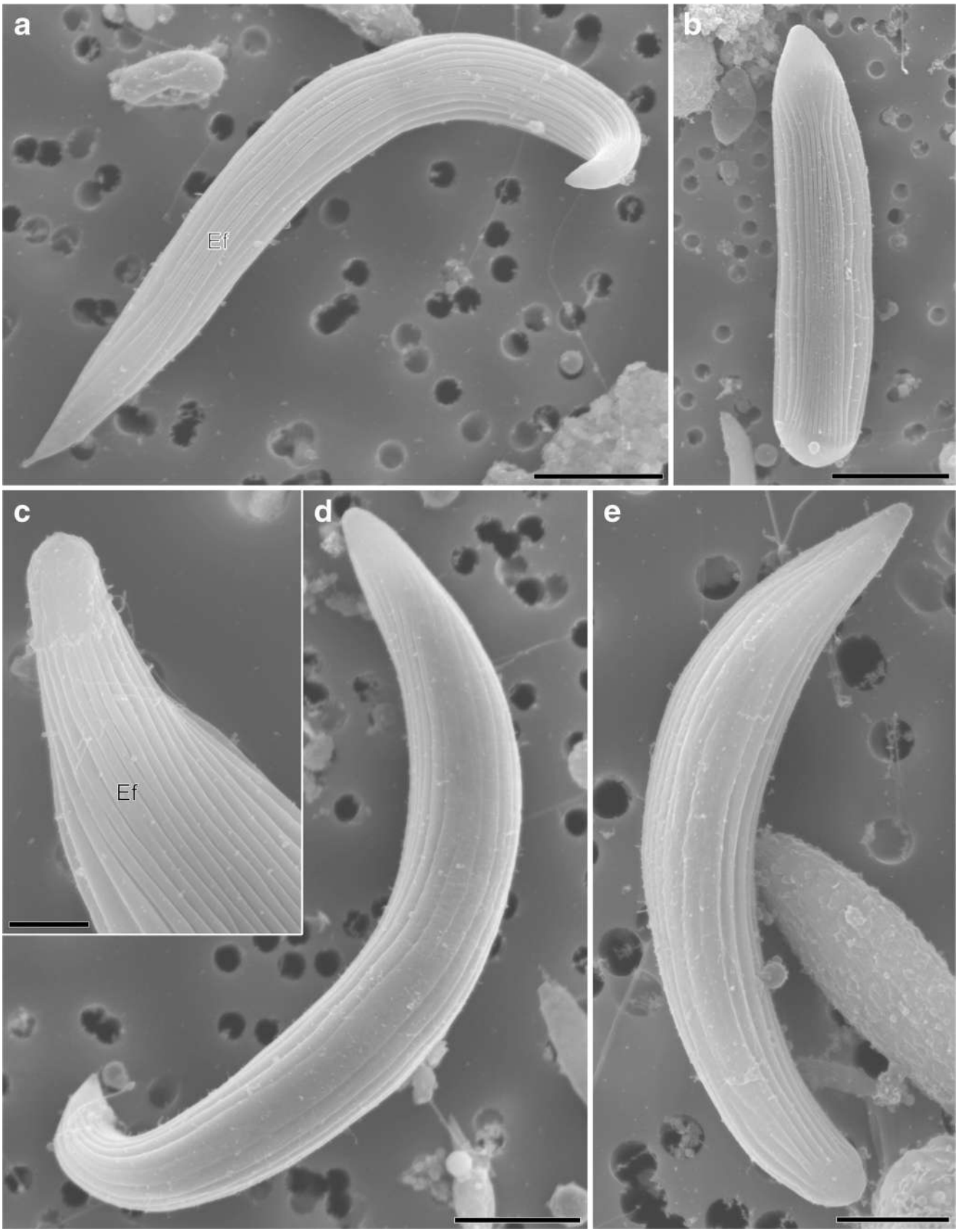
Discussion

This study describes a novel species of marine gregarine, *Selenidium oshoroense* n. sp., from the polychaete *Hydroides ezoensis* Okuda 1934 (Serpulidae, Polychaeta). *S. oshoroense* n. sp. was isolated from the intestines of *H. ezoensis* that where collected along the seashore near Oshoro, Hokkaido, Japan. Trophozoite stages of *S. oshoroense* n. sp. were collected for light microscopy, and phylogenetic analysis using 18S rDNA; other cells were fixed for more detailed investigations of the surface morphology and ultrastructure using SEM and TEM.

To date, five species have been described from serpulid polychaetes (*S. mercierellae*, *S. brasili*, *S. caulleryi*, *S. serpulae*, and *S. sensimae* (Brasil 1907; Caullery and Mesnil 1899; Levine 1971; Théodoridès and Laubier 1962; Wakeman and Leander 2012; 2013a). We determined that of the existing *Selenidium* species, *S. serpulae* bore the most resemblance to *S. oshoroense* n. sp. (Morphological traits of *S. oshoroense* n. sp. and other, closely related *Selenidium* are summarized in Supplementary Table 1). *S. serpulae* and *S. oshoroense* n. sp. share a similar number of surface folds, they parasitise closely related, serpulid hosts, and these gregarines have similar surface morphology. However, *S. serpulae* has transverse striations on the surface (Leander 2007) of its epicytic folds, while *S. oshoroense* n. sp. lacks these features.

S. serpulae and *S. oshoroense* n. sp. share general ultrastructural features, such as the organization of microtubules subtending the trilayer membrane, as well as peduncles and inclusions that apparently act in the process of acquiring nutrients through the surface of the cell membrane. Also, putative apicoplasts were not observed in either of these species, nor *S. hollandei* (Simdyanov and Kuvardina 2007), but have been reported in *S. pendula* (Schrével 1971; Schrével et al. 2016), the type species for *Selenidium*. Molecular data has recently shown that *S. pendula* is early-branching among *Selenidium* parasitizing tube-forming polychaetes (Schrével et al. 2016; also Fig. 6 in this study). Ultrastructural data for

Fig. 2 Scanning electron micrographs of *Selenidium oshoroense* n. sp. **a**, **b**. General view of trophozoite stages of *S. oshoroense* n. sp. with epicytic folds (Ef). **c**. High magnification of the anterior end of the cell showing epicytic folds (Ef). **d**, **e**. General view of trophozoite stages. Note that no transverse striations are visible. Scales: a, b, d, e = 15 μm; c = 2 μm


a
Ef
b
c
Ef
d
e

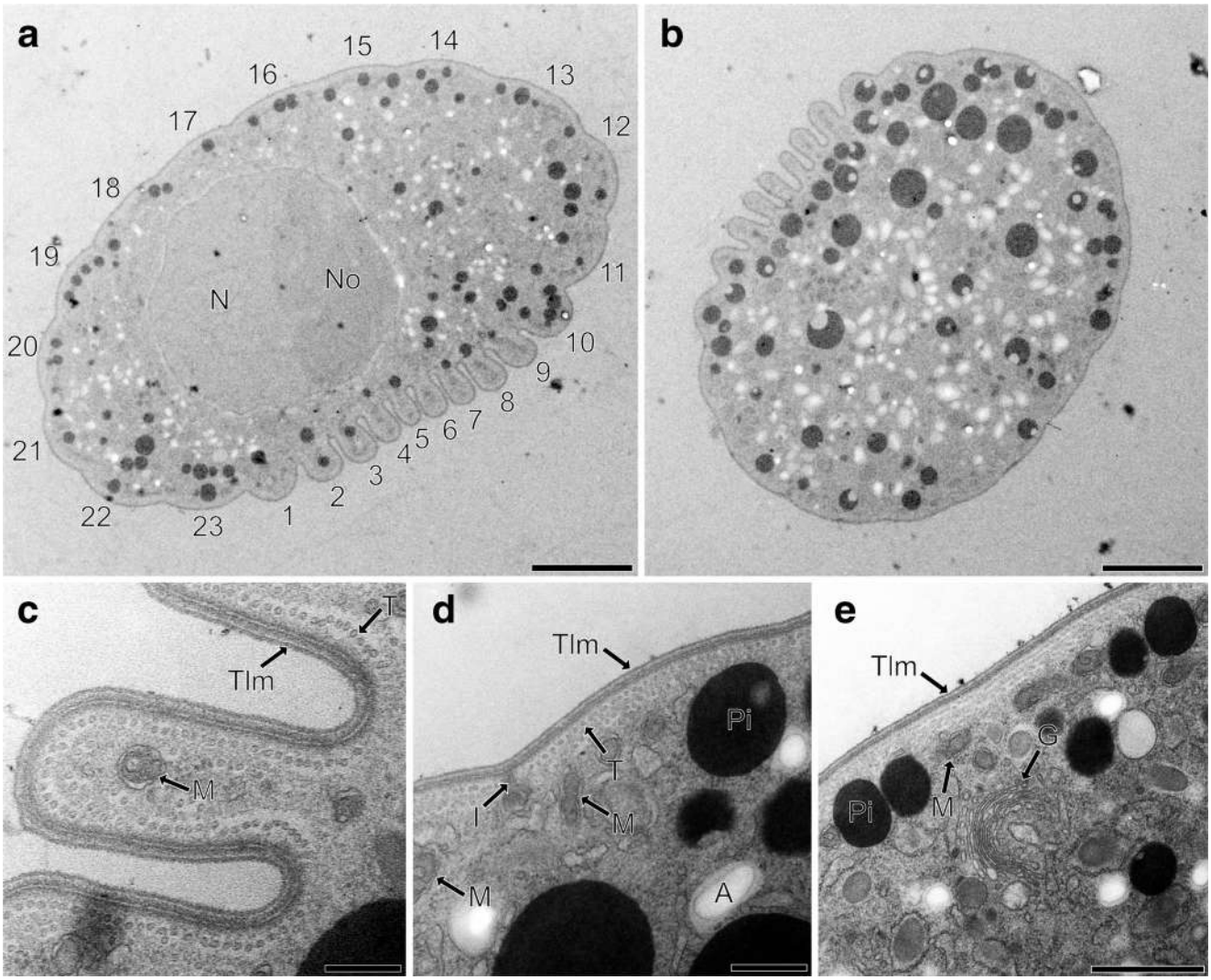


Fig. 3 Transmission electron micrographs of *Selenidium oshoroense* n. sp. **a**, **b**. Cross-sections of trophozoite stages showing 23 longitudinally running epicytic (surface) folds, nucleus (*N*), and nucleolus (*No*). **c**–**e**. High-magnification cross-sections showing the trilayer membrane (*Tlm*) of the epicytic folds, microtubules (*T*), mitochondria (*M*), electron-dense protein inclusions (*Pi*), accumulated amylopectin (*A*), cell inclusions (*I*), and a golgi body (*G*). Scales: a, b = 1 μm; c, d = 50 nm; e = 1 μm

this particular clade is scant, but this could suggest that larger differences such as the presence, absence, or reduction of apicoplasts within this *Selenidium* clade between species that are early-branching or recently derived.

One ultrastructural discrepancy was the presence of mitochondria. In this study, we were able to observe distinct mitochondria under TEM in *S. oshoroense* n. sp. The earlier work by Leander (2007) reported finding only putative mitochondria, suggesting that these organelles might have been highly reduced in that particular lineage. It is likely, however, that this ultrastructural difference between these two species is due to a fixation artefact during sample preparation.

Sections of the host gut that were prepared for viewing under TEM were able to show evidence of myzocytotic feeding in *S. oshoroense* n. sp. While this process was not readily observed in *S. serpulae* (Leander 2007), it has been reported extensively in *S. hollandei* (Simdyanov and Kuvardina 2007) and also in *S. pendula* (Schrével 1971; Schrével et al. 2016). Therefore, it is reasonable to think that myzocytosis is widely distributed among *Selenidium* in this clade. However, even when examining host tissue that is highly infected with trophozoites, getting a conclusive image that shows myzocytosis is challenging and rare, and therefore comprehensively mapping the distribution of this feeding characteristic among derived lineages of gregarines, or earlier-branching marine gregarines that have maintained ancestral characteristics, would be difficult.

In both the larger 18S rDNA alignment containing 78 taxa, and the smaller 18S rDNA alignment containing 22 taxa, the sequences from the two isolates of *S. oshoroense* branched together, to the exclusion of *S. serpulae*. The *S. oshoroense* n. sp. isolates had a 0.997 similarity, and were 0.965 and 0.966

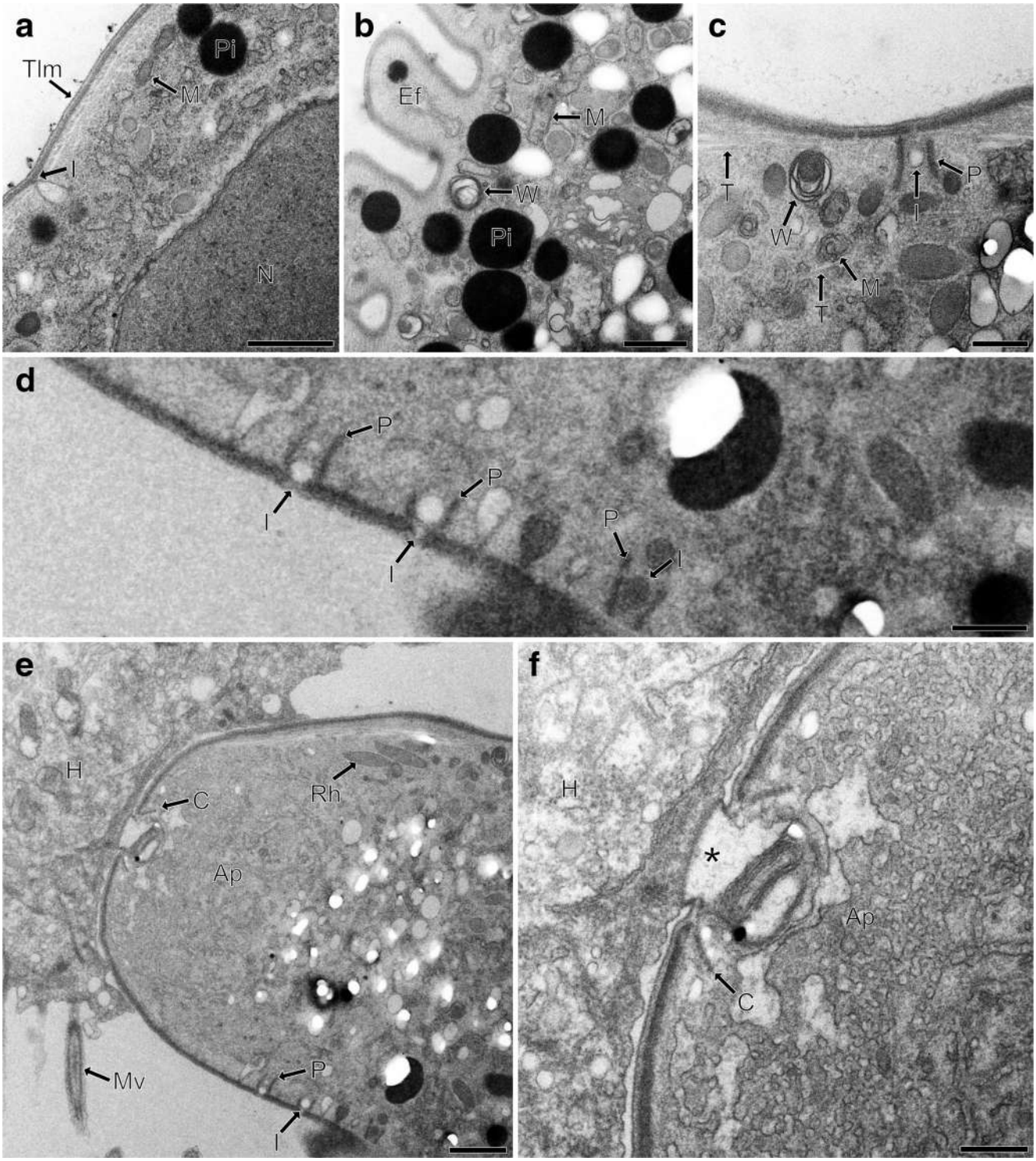
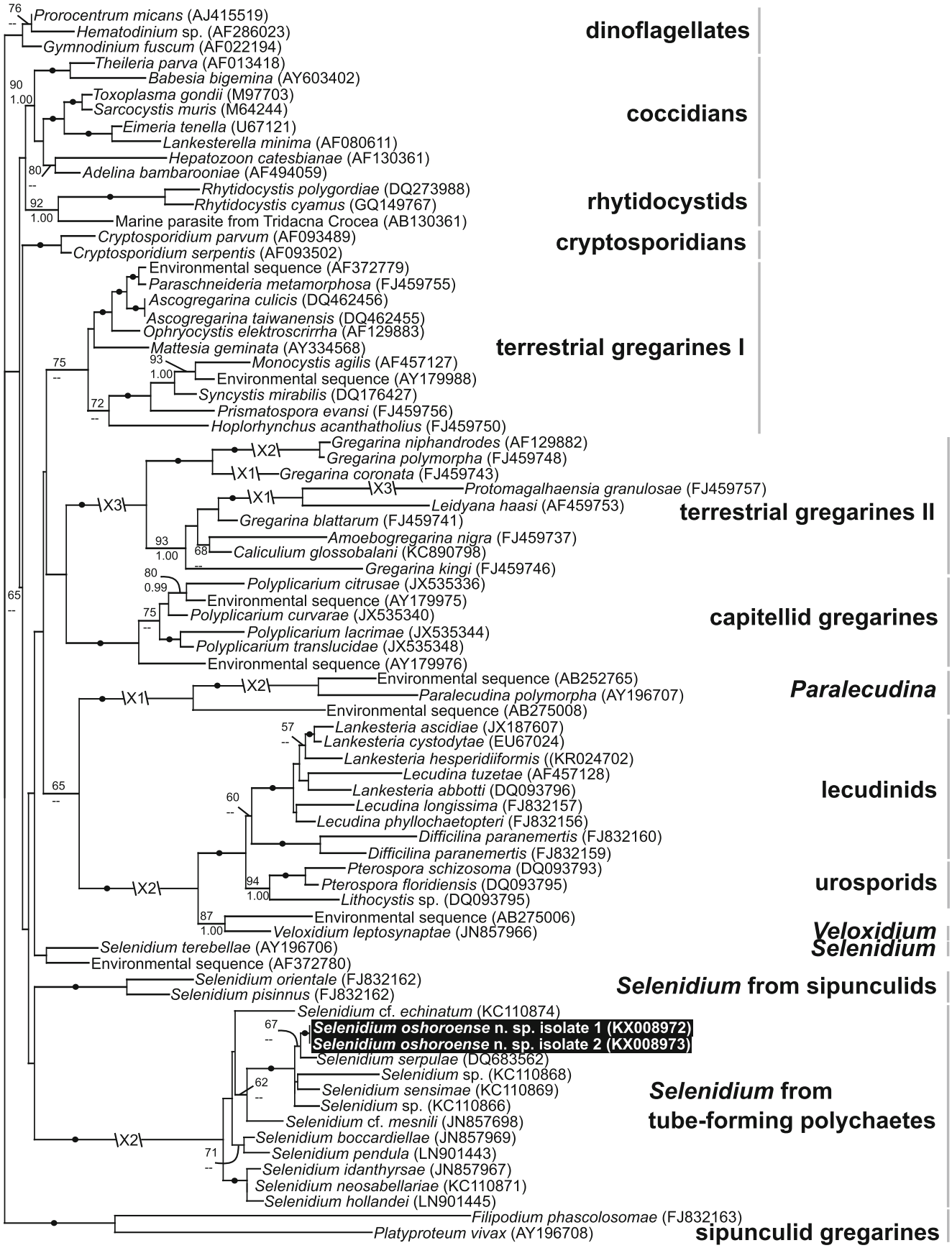


Fig. 4 Transmission electron micrographs of *Selenidium oshoroense* n. sp. **a**, **b**. High-magnification cross-sections near the epicytic folds (*Ef*) showing cell inclusions (*I*), mitochondria (*M*), electron-dense protein inclusions (*Pi*), and the double membrane-bound nucleus (*N*), multimembrane-bound whorls (*W*). **c**. High-magnification tangential-section showing microtubules (*T*), multimembrane-bound whorls (*W*), peduncles (*P*) and cell inclusions (*I*). **d**. High-magnification tangential-section showing peduncles (*P*) and cell inclusions (*I*). *E*. Section through host gut tissue (*H*) with microvillae (*Mv*), showing a trophozoite stage of *S. oshoroense* n. sp. (*Ap*)., a distinct conoid (*C*) at the anterior end, rhopteries (*Rh*), peduncles (*P*), and cell inclusions (*I*). **f**. High-magnification image showing the interface of *S. oshoroense* n. sp. (*Ap*) and the host gut tissue (*H*), showing the stacked conoid (*C*), and the interface between the parasite and host (*) during myzocytotic feeding. Scales: a, b = 1 μm; c = 200 μm; d = 200 nm; e = 500 nm; f = 100 nm


Prorocentrum micans (AJ415519)
Hematodinium sp. (AF286023)
Gymnodinium fuscum (AF022194)
dinoflagellates
Theileria parva (AF013418)
Babesia bigemina (AY603402)
Toxoplasma gondii (M97703)
Sarcocystis muris (M64244)
Eimeria tenella (U67121)
Lankesterella minima (AF080611)
Hepatozoon catesbianae (AF130361)
Adelina bambarooniae (AF494059)
coccidians
Rhytidocystis polygordiae (DQ273988)
Rhytidocystis cyamus (GQ149767)
Marine parasite from Tridacna Crocea (AB130361)
rhytidocystids
Cryptosporidium parvum (AF093489)
Cryptosporidium serpentis (AF093502)
cryptosporidians
Environmental sequence (AF372779)
Paraschneideria metamorphosa (FJ459755)
Ascogregarina culicis (DQ462456)
Ascogregarina taiwanensis (DQ462455)
Ophryocystis elektroscirrha (AF129883)
Mattesia geminata (AY334568)
Monocystis agilis (AF457127)
Environmental sequence (AY179988)
Syncystis mirabilis (DQ176427)
Prismatospora evansi (FJ459756)
Hoplorhynchus acanthatholius (FJ459750)
terrestrial gregarines I
Gregarina niphandrodes (AF129882)
Gregarina polymorpha (FJ459748)
Gregarina coronata (FJ459743)
Protomagalhaensia granulosae (FJ459757)
Leidyana haasi (AF459753)
Gregarina blattarum (FJ459741)
Amoebogregarina nigra (FJ459737)
Caliculum glossobalani (KC890798)
Gregarina kingi (FJ459746)
terrestrial gregarines II
Polyplicarium citrusae (JX535336)
Environmental sequence (AY179975)
Polyplicarium curvarae (JX535340)
Polyplicarium lacrimae (JX535344)
Polyplicarium translucidae (JX535348)
Environmental sequence (AY179976)
capitellid gregarines
Environmental sequence (AB252765)
Paralecudina polymorpha (AY196707)
Environmental sequence (AB275008)
Paralecudina
Lankesteria ascidiae (JX187607)
Lankesteria cystodytae (EU67024)
Lankesteria hesperidiiformis ((KR024702)
Lecudina tuzetae (AF457128)
Lankesteria abbotti (DQ093796)
Lecudina longissima (FJ832157)
Lecudina phyllochaetopteri (FJ832156)
Difficilina paranemertis (FJ832160)
Difficilina paranemertis (FJ832159)
lecudinids
Pterospora schizosoma (DQ093793)
Pterospora floridiensis (DQ093795)
Lithocystis sp. (DQ093795)
urosporids
Environmental sequence (AB275006)
Veloxidium leptosynaptae (JN857966)
Veloxidium
Selenidium terebellae (AY196706)
Environmental sequence (AF372780)
Selenidium
Selenidium orientale (FJ832162)
Selenidium pisinnus (FJ832162)
Selenidium from sipunculids
Selenidium cf. echinatum (KC110874)
Selenidium oshoroense n. sp. isolate 1 (KX008972)
Selenidium oshoroense n. sp. isolate 2 (KX008973)
Selenidium serpulae (DQ683562)
Selenidium sp. (KC110868)
Selenidium sensimae (KC110869)
Selenidium sp. (KC110866)
Selenidium cf. mesnili (JN857698)
Selenidium boccardiellae (JN857969)
Selenidium pendula (LN901443)
Selenidium idanthyrsae (JN857967)
Selenidium neosabellariae (KC110871)
Selenidium hollandei (LN901445)
Selenidium from tube-forming polychaetes
Filipodium phascolosomae (FJ832163)
Platyproteum vivax (AY196708)
sipunculid gregarines

◀ **Fig. 5** Maximum likelihood (ML) and Bayesian inference derived from the phylogenetic analyses of 18S rDNA from *Selenidium oshoroense* n. sp. and 76 other taxa representing the diversity of gregarine apicomplexans, core apicomplexans, and dinoflagellates (outgroup) over 1207 unambiguously aligned sites. This tree was inferred using the GTR+Γ+I substitution model (−ln L = 21,864.8449, gamma shape = 0.6600, proportion of invariable sites = 0.1900). Numbers at the nodes denote the ML bootstrap percentage and Bayesian posterior probabilities, respectively. *Black dots* on branches denote instances were bootstrap support values and Bayesian posterior probabilities of 95/0.95 or higher were recorded. Bootstrap and Bayesian values less than 55 and 0.95 were not added to this tree. The novel sequence from *S. oshoroense* n. sp. generated in this study is highlighted in a *black box*. Some branches were shortened by multiples of the length of the substitutions/site scale bar (e.g., 1X)

similar to that of *S. serpulae*. As a generality, gregarines infecting closely related hosts tend to also be closely related (with some exceptions). More recently, this idea has been supported/reflected in molecular studies (Rueckert et al. 2011, 2015; Wakeman and Leander 2013a). To this extent, our study corresponds with recent work that has accumulated rDNA from *Selenidium* and suggests a high degree of host specificity and specialization between *Selenidium* and their polychaete host groups (Schrével et al. 2016). For instance, *Selenidium* clades tend to be exclusive to polychaete families ranging from Serpulidae, Sabellidae, Sabellaridae, Cirratulidae, and Spionidae. Future work combing molecular phylogenetic data from the hosts and their respective gregarine parasites could answer fundamental questions related to coevolution and host specificity in these groups.

In conclusion, this study chose to establish a new species to describe the diversity exhibited by *Selenidium oshoroense* n. sp. This new species distinguished itself from other closely related species (i.e., *S. serpulae*) based on the lack of transverse striations on the epicytic folds of the trophozoite stages, the corresponding molecular difference in the 18S rDNA that was supported in phylogenetic analysis that grouped two isolates of *S. oshorosensis* n. sp., to the exclusion of *S. serpulae*, and the difference in ecological niche (the host, *Hydroides ezoensis*) from which *S. oshoroense* n. sp. was isolated.

Selenidium oshoroense n. sp. Wakeman and Horiguchi

Description. Trophozoites vermiform with an average length and width, at the widest part, of 120 and 13 μm, respectively. Cells light-brown. The posterior end tapers to a fine point, or rounded; anterior end tapers or is rounded. A spherical nucleus (12 μm) is positioned in the middle or posterior of the cell. Trophozoites move by undulating, bending and twisting. The cell surface is lined by 23 longitudinally running epicytic folds; transverse striations not present.

DNA sequence. SSU rDNA sequences (GenBank KX008972-KX008973).

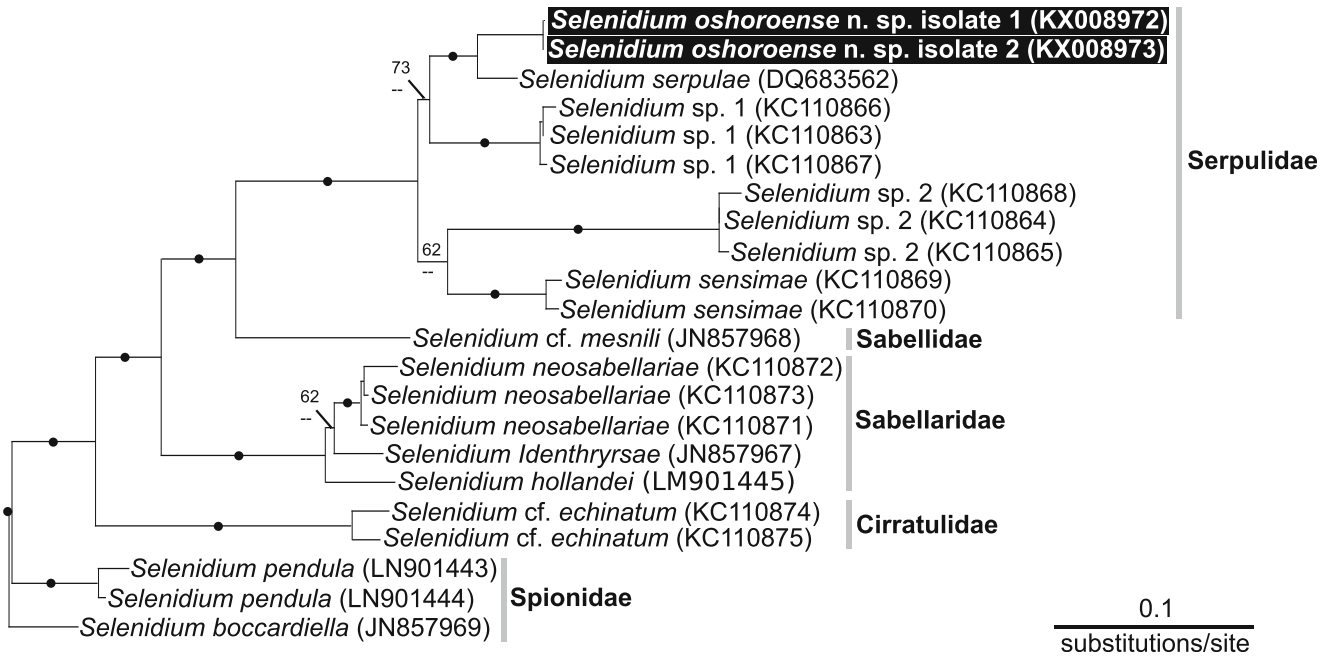


Fig. 6 Maximum likelihood (ML) and Bayesian inference derived from the phylogenetic analyses of 18S rDNA from *Selenidium oshoroense* n. sp. and 20 other taxa representing the diversity of *Selenidium* from tube-forming polychaetes over 1518 unambiguously aligned sites. This tree was inferred using the GTR+Γ+I substitution model (−ln L = 6,078.3768, gamma shape = 0.5890, proportion of invariable sites = 0.4370). Numbers at the nodes denote the ML bootstrap percentage and Bayesian posterior probabilities, respectively. *Black dots* on branches denote instances were bootstrap support values and Bayesian posterior probabilities of 95/0.95 or higher were recorded. Bootstrap and Bayesian values less than 55 and 0.95 were not added to this tree. The novel sequence from *S. oshoroense* n. sp. generated in this study is highlighted in a *black* box. Some branches were shortened by multiples of the length of the substitutions/site scale bar (e.g., 1X)

Type locality. Oshoro Marine Station, Oshoro, Hokkaido, Japan (4312'33.26" N 14051'30.67" E). Host in tubes on rocks; Intertidal 0.2 m below mean sea level.

Type habitat. Marine.

Type host. *Hydroides ezoensis* Okuda 1934 (Annelida, Polychaeta, Serpulidae).

Location in host. Intestinal lumen.

Type material: Parasites on gold sputter-coated SEM stubs have been deposited in the Biodiversity Collection at Hokkaido University (KCW_S_Osh1)

Etymology. The species name, *oshoroense*, refers to locality (Oshoro, Hokkaido) from where the host and parasite were collected.

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