The Journal of
Eukaryotic Microbiology

Published by the International Society of Protistologists

*J. Eukaryot. Microbiol.*, 59(3), 2012 pp. 232–245

DOI: 10.1111/j.1550-7408.2012.00616.x

# Molecular Phylogeny of Pacific Archigregarines (Apicomplexa), Including Descriptions of *Veloxidium leptosynaptae* n. gen., n. sp., from the Sea Cucumber *Leptosynapta clarki* (Echinodermata), and Two New Species of *Selenidium*

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ABSTRACT. Although archigregarines are poorly understood intestinal parasites of marine invertebrates, they are critical for understanding the earliest stages in the evolution of the Apicomplexa. Previous studies suggest that archigregarines are a paraphyletic stem group from which other lineages of gregarines, and possibly all other groups of apicomplexans, evolved. However, substantiating this inference is difficult because molecular phylogenetic data from archigregarines, in particular, and other gregarines, in general, are severely limited. In an attempt to help fill gaps in our knowledge of archigregarine diversity and phylogeny, we set out to discover and characterize novel lineages of archigregarines with high-resolution light and scanning electron microscopy and analyses of small subunit (SSU) rDNA sequences derived from single-cell (SC) PCR techniques. Here, we describe two novel species of *Selenidium*, namely *Selenidium idanthyrsae* n. sp. and *S. boccardiellae* n. sp., and demonstrate the surface morphology and molecular phylogenetic position of the previously reported species *S.* cf. *mesnili*. We also describe a novel genus of archigregarine, *Veloxidium leptosynaptae* n. gen., n. sp., which branches with an environmental sequence and, together, forms the nearest sister lineage to a diverse clade of marine eugregarines (i.e. lecudinids and urosporids). This molecular phylogenetic result is consistent with the hypothesis that archigregarines are deeply paraphyletic within apicomplexans, and suggests that convergent evolution played an important role in shaping the diversity of eugregarine trophozoites.

**Key Words.** Alveolata, marine gregarine, parasite, phylogeny.

ARCHIGREGARINES are single-celled, intestinal parasites of marine invertebrates and are inferred to have retained many characteristics found in the most recent common ancestor of all apicomplexans (Cavalier-Smith and Chao 2004; Cox 1994; Grassé 1953; Leander 2008; Leander and Keeling 2003). For instance, the extracellular trophozoites of archigregarines are similar in morphology and motility to their infective sporozoite stage and to the sporozoites of other apicomplexans (Cox 1994; Dyson et al. 1993, 1994; Kuvardina and Simdyanov 2002; Leander 2006; Ray 1930; Schrével 1968). Moreover, the oocysts of archigregarines contain just four infective sporozoites, and the life cycle of archigregarines is completed within a single host species (Grassé 1953; Schrével 1970; Théodoridès 1984). The nearest free-living sister lineages to the obligately parasitic Apicomplexa are photosynthetic chromerids and predatory, biflagellated colpodellids that, like some archigregarine trophozoites, possess an apical complex and utilize a vampire-like mode of feeding called "myzocytosis" (Barta and Thompson 2006; Cavalier-Smith and Chao 2004; Grassé 1953; Leander 2008; Leander and Keeling 2003; Schrével 1971a,b; Simdyanov and Kuvardina 2007). This insight along with molecular phylogenetic analyses of small subunit (SSU) rDNA has led to the hypothesis that archigregarines branched early within the Apicomplexa, and represent a paraphyletic stem group from which all other gregarines, and possibly the Apicomplexa as a whole, evolved (Leander 2008; Rueckert and Leander 2009).

There are several differences that distinguish archigregarine trophozoites from those of so-called "marine eugregarines" (e.g. lecudinids and urosporids). The trophozoites of archigregarines are generally spindle shaped and are capable of nematode-like bending and coiling motility (e.g. *Selenidium* spp.) (Rueckert and Leander 2009; Schrével 1970). The trophozoites of marine eugregarines, by contrast, are generally rigid and exhibit gliding motility (Leander 2008; Leander et al. 2003b; Rueckert et al. 2010). Moreover, trophozoites of marine eugregarines differ significantly from their infective sporozoite stages. The trophozoite surface of most eugregarines has hundreds of densely packed epicytic folds that run along the longitudinal axis of the cell (Leander et al. 2003b; Rueckert and Leander 2010; Rueckert et al. 2010). This stands in contrast to archigregarines that typically have many fewer longitudinal folds (i.e. 0–50) (Leander 2007, 2008; Leander et al. 2003b; Rueckert and Leander 2009; Schrével 1971a,b). The trophozoite surface of archigregarines is supported by microtubules, each surrounded by a transparent sheath, which are organized in one or more layers beneath the trilayered inner membrane complex (Leander 2007; Mellor and Stebbings 1980; Schrével 1971b; Stebbings et al. 1974; Vivier and Schrével 1964). This subcellular organization of microtubules is not present in the trophozoites of eugregarines (Leander 2008).

Of the approximate 65 species of archigregarines that have been described, roughly 56 belong to the genus *Selenidium* (Leander 2007; Levine 1971; Rueckert and Leander 2009). Levine (1971) proposed that several species of *Selenidium* should be considered eugregarines rather than archigregarines, if there is no report of merogony/schizogony (i.e. asexual reproduction of trophozoites) at any point during the life cycle. As a result, the genus *Selenidioides* was erected to accommodate the 11 members of *Selenidium* with reports of merogony, and the remaining members of *Selenidium* were moved into the order Eugregarinorida (Levine 1971). As pointed out previously, the presence or absence of merogony in the life cycle of any gregarine is difficult to substantiate, which makes this feature a poor indicator of phylogenetic relationships (Leander 2006, 2007; Rueckert and Leander 2009). Therefore, this study will consider *Selenidium* the main genus of archigregarines, which is consistent with most other contemporary work on this subject (Gunderson and Small 1986; Kuvardina and Simdyanov 2002; Leander 2006, 2007; Rueckert and Leander 2009; Schrével 1971a; Simdyanov and Kuvardina 2007; Théodoridès 1984).

Although our knowledge of archigregarines is based primarily on studies of species within the genus *Selenidium*, archigregarines are much more diverse. Close relatives of *Selenidium* show twisting and peristaltic movements (e.g. *Platyproteum vivax*) and highly modified morphology, such as matted hair-like projections on the trophozoite surface (e.g. *Filipodium*

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spp.) (Gunderson and Small 1986; Hoshide and Todd 1996; Leander 2006; Rueckert and Leander 2009). Ultrastructural information has been reported on fewer than 20 species of archigregarines, mostly *Selenidium* spp., and molecular phylogenetic data of SSU rDNA are available for a mere six species (e.g. *Selenidium terebellae*, *Selenidium pisinnus*, *Selenidium orientale*, *Selenidium serpulae*, *Filipodium phascolosomae*, and *Platyproteum vivax*); none of these DNA sequences has a well-supported phylogenetic position within the context of all major lineages of apicomplexans (Leander 2006, 2007; Leander et al. 2003b; Rueckert and Leander 2009; Rueckert et al. 2011). Improved understanding of archigregarine diversity, especially from a molecular phylogenetic perspective, is expected to lead to the discovery of novel gregarine lineages, and shed considerable light on the earliest stages in apicomplexan evolution.

Here, we characterize the surface morphology and molecular phylogeny of three species of *Selenidium* isolated from polychaete hosts, namely *Selenidium idanthyrsae* n. sp. and *Selenidium boccardiellae* n. sp. and the previously reported *Selenidium* cf. *mesnili*. We also describe and establish a novel genus of archigregarine, namely *Veloxidium leptosynaptae* n. gen., n. sp., from the intestines of a Pacific sea cucumber. Comparative morphology and molecular phylogenetic analyses of these new data helped evaluate whether archigregarines form a paraphyletic stem group from which several different eugregarine lineages evolved.

## MATERIALS AND METHODS

**Collection of organisms.** *Selenidium* cf. *mesnili* (Brasil 1909) and *S. boccardiellae* n. sp. were isolated from the Pacific polychaetes *Myxicola infundibulum* (Montagu, 1808) and *Boccardiella ligerica* (Ferronnière, 1898), respectively. Both host animals were collected by SCUBA diving in August 2010 at Ogden Point (48°25′43″N, 123°21′56″W), near Victoria, British Columbia, Canada. *Selenidium idanthyrsae* n. sp. was isolated from the polychaete *Idanthyrsus saxicavus* (Baird, 1863) collected in June 2010 in a dredge haul at 20 m depth near Wizard Islet, Bamfield, British Columbia, Canada (48°50′17″N, 125°08′02″W). *Veloxidium leptosynaptae* n. gen., n. sp. was isolated from the sea cucumber, *Leptosynapta clarki* (Heding, 1928), collected in May 2011 from intertidal mud at low tide, approximately 1.5 km inward from the mouth of Grappler Inlet, Bamfield, British Columbia, Canada (48°49′49″N, 125°8′15″W).

The intestines of all host animals were isolated using fine-tipped forceps and placed in a Petri dish filled with autoclaved filtered seawater. Intestinal contents were transferred to a well slide and viewed under a Leica (Wetzlar, Germany) DM IL inverted microscope. Individual trophozoites were manually isolated from the intestinal contents using a micropipette and washed at least three times in autoclaved filtered seawater, prior to examination with light microscopy and preparation for DNA extraction.

**Light and scanning electron microscopy.** Individual trophozoites were placed into a drop of seawater on a glass slide, secured with a coverslip and Vaseline, and viewed with differential interference contrast (DIC) using a Zeiss Axioplan 2 microscope (Carl-Zeiss, Göttingen, Germany) connected to a Leica DC500 color digital camera. Cell motility was recorded using an inverted Zeiss Axiovert 200 microscope connected to a PixeLink Megapixel color digital camera (PL-A662-KIT, Ottawa, Canada).

Twelve, eight, ten, and seven individual trophozoites of *S.* cf. *mesnili*, *S. boccardiellae* n. sp., *S. idanthyrsae* n. sp., and *V. leptosynaptae* n. gen., respectively, were prepared for scanning electron microscopy. Very few trophozoites are present in some hosts; for instance, only nine trophozoites of *V. leptosynaptae* were recovered from *L. clarki*. For each species, a 10-μm polycarbonate membrane filter was placed within a Swinnex filter holder (Millipore Corp., Billerica, MA, USA); the filter holder was then placed within a small beaker (4 cm diam. and 5 cm tall) that was filled with 2.5% (v/v) glutaraldehyde in seawater. The manually isolated trophozoites were placed directly into the Swinnex filter holder and fixed on ice for 15 min. Ten drops of 1% (w/v) $OsO_4$ were then added directly to the opening of the Swinnex filter holder, and the samples were post-fixed on ice for 30 min. A syringe was attached to the Swinnex filter holder, and distilled water was slowly run over the samples. A graded series of ethanol (30%, 50%, 75%, 85%, 95%, and 100%) was then used to dehydrate the fixed cells using the syringe system. Following dehydration, the polycarbonate membrane filters containing the trophozoites were transferred from the Swinnex filter holders into an aluminum basket submerged in 100% ethanol in preparation for critical point drying with $CO_2$. The dried polycarbonate membrane filters containing the trophozoites were mounted on aluminum stubs, sputter coated with 5 nm gold, and viewed under a Hitachi S4700 scanning electron microscope (Nissei Sangyo America Ltd., Pleasanton, CA, USA). Some SEM data were presented on a black background using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA, USA). The SEM stubs containing holotype and paratype material were deposited in the Beaty Biodiversity Research Centre Marine Invertebrate Collection, University of British Columbia, Vancouver, Canada.

**DNA isolation, gene amplification, cloning and sequencing of the *Selenidium* species.** Twelve to 20 individual trophozoites were isolated from host gut contents, washed until clean, at least three times, in autoclaved filtered seawater and transferred into 1.5 ml microfuge tubes. DNA was extracted using the MasterPure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, Madison, WI). A region of the small subunit (SSU) SSU rDNA (~ 1,800 bp) was targeted for DNA amplification and sequencing. Initial PCR was performed using a PCR bead (Illustra, PuReTaq Ready-To-Go PCR beads; GE Healthcare, Quebec, Canada), the extracted trophozoite DNA in 24 μl of $H_2O$, and 1 μl the following primer pair (total reaction volume of 25 μl): F1 5′-GCGCTACCTGGTTGATCCTGCC-3′ and R1 5′-GATCC TTCTGCAGGTTCACCTAC-3′ (Leander et al. 2003a). The thermocycler was set for the following conditions: initial denaturization of 96.0 °C for 5:00 min; 40 cycles of 96.0 °C for 30 s, 52.0 °C for 30 s (annealing temperature), and 72.0 °C for 2:00 min; and a final extension of 72.0 °C for 9:00 min. One microliter of the initial PCR product using primers F1–R1 was used as a template for two semi-nested PCR reactions using a higher annealing temperature of 58 °C in 25 cycles (all other PCR and thermocycler conditions as listed above) and the following primer pairs: F2 5′-GATCCCGGAGAGGGAG CTTGAG-3′ and R1 producing a 1,200-bp product; F1 and R2 5′-GCCTYGCGACCATACTTC-3′ producing a 1,000-bp product. PCR products corresponding to the expected sizes were gel isolated and purified using UltraClean 15 DNA Purification Kit (MO Bio, Carlsbad, CA, USA). Purified DNA was cloned into a pCR 2.1 vector using the TOPO TA cloning kit (Invitrogen, Frederick, MD, USA). Four clones from each species were PCR amplified and screened using vector primers in a 25-μl reaction with EconoTaq Master Mix (Lucigen Corp. Middleton, WI, USA) with the following thermocycler program: initial denaturization of 94.0 °C for 2:00 min; 30 cycles of 94.0 °C for 2:00 min., 50.0 °C for 30 s, and 72.0 °C for 1:30 min; and final extension of 72.0 °C for 9:00 min. Cloned

products corresponding to the expected sizes were sequenced using vector primers and ABI big dye reaction mix. Novel SSU rDNA gene sequences were initially identified using BLAST and confirmed with molecular phylogenetic analyses. New SSU rDNA sequences from *S.* cf. *mesnili*, *S. boccardiellae* n. sp., and *S. idanthyrsae* n. sp. were deposited into GenBank as JN857968, JN857969, and JN857967, respectively.

**PCR and sequencing of *Veloxidium leptosynaptae* n. gen., n. sp.** Three individual trophozoites of the nine recovered of *V. leptosynaptae* n. gen. et sp. were manually isolated, photographed, video recorded, and placed in two separate 0.2-ml PCR reaction tubes containing 10.0 μl FFPE DNA QuickExtract (DNA Extraction Kit, Epicentre Biotechnologies, Madison, WI). "*Veloxidium leptosynaptae* Isolate 1″ consisted of a single trophozoite, and "*V. leptosynaptae* Isolate 2″ consisted of two gamonts undergoing head-to-head syzygy (i.e. the onset of sexual reproduction). Both tubes were then placed in a thermocycler and incubated at 56.0 °C for 60:00 min and 98.0 °C for 2:00 min.

A PCR bead (illustra, PuReTaq Ready-To-Go PCR beads, GE Healthcare, Quebec, Canada), 1.0 μl of the F1-R1 primer pair, and 14.0 μl of autoclaved distilled water were added to each 0.2 ml tube containing 10.0 μl of the DNA extract to bring the total PCR reaction volume to 25.0 μl. The same primers, semi-nested PCR protocols, thermocycler programs, and sequencing protocols described above were used to sequence the SSU rDNA sequence from *V. leptosynaptae* n. gen., n. sp. The two new SSU rDNA gene sequences from the two isolates of this species were identical; the sequence was initially identified using BLAST, confirmed with phylogenetic analyses, and deposited in GenBank as JN857966.

**Molecular phylogenetic analyses.** The four new SSU rDNA sequences generated in this study were aligned with three sequences from dinoflagellates as the outgroup and 80 additional sequences representing the major subgroups of apicomplexans, forming an 87-taxon dataset. The alignment was then visually fine-tuned using MacClade 4 (Maddison and Maddison 2000); gaps and ambiguously aligned regions were excluded resulting in 1,008 unambiguously aligned sites. JModeltest (Guindon and Gascuel 2003; Posada and Crandall 1998) selected a GTR+I+Γ model of evolution under AIC and AICc (proportion of invariable sites = 0.1770, gamma shape = 0.6170). Garli-GUI (Zwickl 2006) was used to generate a maximum likelihood (ML) tree and ML bootstrap analysis (100 pseudoreplicates, one heuristic search per pseudoreplicate). A distance analysis was completed using PAUP 4.0 for the three *Selenidium* species isolated in this study (Swofford 1999).

Bayesian posterior probabilities were calculated using the program MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). We set our program for four Monte Carlo Markov chains starting from a random tree (MCMC; default temperature = 0.2), a gamma distribution, and a stop rule of 0.01 (i.e. when the average split deviation fell below 0.01, the program would terminate). A sum of 7,400,000 generations was calculated. Trees were sampled every 100 generations, with a prior burn-in of 500,000 generations. Burn-in was confirmed manually, and a majority-rule consensus tree was constructed. Posterior probabilities correspond to the frequency at which a given node is found in the post burn-in trees.

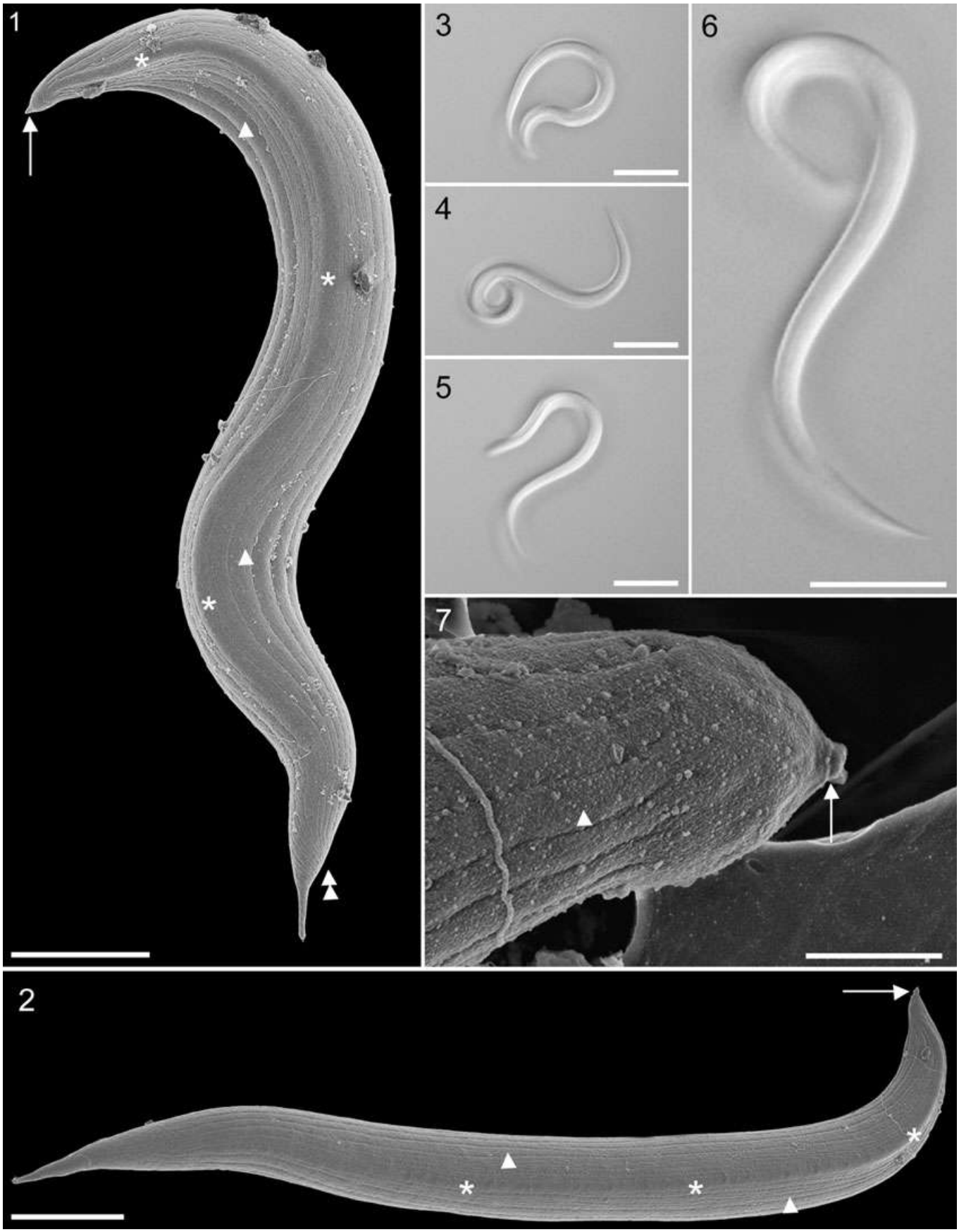
## RESULTS

***Selenidium boccardiellae* n. sp.** Trophozoites were vermiform, 158 μm long (range = 87–250 μm) and 10 μm wide (range = 10–12 μm; $N = 13$) (Fig. 1–7). An ellipsoidal nucleus was located in the middle anterior half of the cell and was 11 μm long (range = 10–12) and 5 μm wide (range = 4–6 μm) (Fig. 3–6). The trophozoites were compressed, especially near the anterior region. The mucron was dome-shaped and terminated with a conspicuous nipple (Fig. 1, 2, 7). Two pronounced ridges, one on each side of the compressed cell, formed near the base of the mucron and extended to about the middle of the cell (Fig. 12). Ten to twelve shallow longitudinal folds extended nearly the entire length of the trophozoites; the folds were absent on the extreme anterior, in the mucron region, and posterior ends (Fig. 1, 2, 7). The trophozoites tapered at each end, forming a narrow tip at the posterior end and the anterior end (Fig. 1, 2); the epicytic folds became helically arranged at the posterior end of the cell (Fig. 1). The trophozoites were capable of nematode-like coiling movements. Individual trophozoites were opaque-silver under light microscopy; there were no conspicuous accumulations of brown amylopectin granules within the cytoplasm (Fig. 3–6).

***Selenidium* cf. *mesnili*.** Trophozoites were conspicuously brown from an accumulation of amylopectin granules within the cytoplasm and were capable of a high degree of bending and coiling. The trophozoites were vermiform with tapered, but relatively rounded, anterior and posterior ends. The cells were 126.6 μm long (range = 85–157 μm; $N = 16$) and 20 μm wide (range = 18–24 μm) (Fig. 8–13). An ellipsoidal nucleus was 8 μm long (range = 7–9 μm) and 10 μm wide (range = 10–11 μm) (Fig. 10–12). The trophozoites underwent rapid changes in shape and were capable of shortening when agitated. The cell surface was covered with 22–24 deeply carved longitudinal folds that bifurcated near the anterior and posterior ends of the cell (Fig. 8, 9, 14, 15). The longitudinal folds were covered with densely packed transverse striations at ~ 3–4 striations/μm, except near the extreme anterior and posterior ends of the cell (Fig. 8, 9, 14, 15). The mucron was capable of being protracted (Fig. 8) and retracted (Fig. 9).

***Selenidium idanthyrsae* n. sp.** The trophozoites were relatively long, skinny, and flattened; the cells were 438 μm long (range = 450–543 μm; $N = 22$) and 15 μm (range = 14–16 μm) wide (Fig. 16, 18). The trophozoites were capable of rhythmic bending and coiling. A spherical nucleus was 14 μm in diam. (range = 13–15 μm) (Fig. 16). The posterior end was conspicuously compressed, forming a spade-like cell terminus, whereas the anterior end was more cylindrical, especially near the mucron (Fig. 18). The surface of the trophozoites was covered with 20–22 longitudinal folds that ran nearly the entire length of the cell; epicytic folds were absent at the extreme posterior end (Fig. 18). Transverse striations were absent on the entire cell, except near the base of the mucron; these transverse stria-

Fig. **1–7.** Differential interference contrast (DIC) micrographs and scanning electron micrographs (SEM) showing the general morphology and surface structure of trophozoites of *Selenidium boccardiellae* n. sp. isolated from the intestines of *Boccardiella ligerica*. **1–2.** SEMs showing elongated and slightly compressed trophozoites with 10–12 shallow epicytic folds (arrowhead). Distinct lateral ridges are visible near the anterior region of the cell (asterisk). The mucron is pointed (arrow), free of epicytic folds and narrows toward the extreme anterior end. The posterior end contains epicytic folds that twist at the terminal end (double arrowhead). **3–6.** DIC micrographs illustrating the bending, coiling, and thrashing movements of the trophozoites. **7.** SEM showing the mucron region (arrow) and longitudinal epicytic folds (arrowhead) at high magnification. Scale bars: Fig. 1–2 = 10 μm; Fig. 3–6 = 25 μm; Fig. 7 = 2 μm.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tions continued only 10–15 μm away from the mucron region and then disappeared (Fig. 17). A conspicuous collar that was 2 μm in diameter marked the base of the mucron (Fig. 17).

***Veloxidium leptosynaptae* n. gen., n. sp.** Trophozoites were vermiform, 74 μm long (range = 62–97 μm; $N = 9$) and 11 μm wide at the middle of the cell (range = 9–13 μm) (Fig. 19–23). The most distinctive feature of these trophozoites was their relatively rapid bending and thrashing motility. A spherical nucleus was located in the middle of the trophozoite and measured 10 μm in diameter (range = 9–11 μm) (Fig. 20). The cell surface consisted of raised transverse striations or rings at a density of 3–4 per micron; the transverse striations were most pronounced near the posterior end of the cell, and relatively faint toward the anterior end (Fig. 19). Longitudinal epicytic folds were absent. The mucron was inconspicuous and the anterior end was generally dome-shaped (Fig. 19, 20). The posterior end of the cell tapered to a point starting from the middle of the trophozoite. The posterior end of the trophozoites often formed a loop by curving back toward the middle of the cell (Fig. 21–23). Syzygy was head-to-head (Fig. 21–23). Conspicuous spherical granules or vacuoles, ~ 1–2 μm in diameter, were observed throughout the cytoplasm of the trophozoites (Fig. 20–23).

**Molecular phylogenetic positions of the new taxa.** Analyses of the 87-taxon data set resulted in a weakly supported backbone that gave rise to a dinoflagellate clade as outgroup and several different subclades of apicomplexans, including cryptosporidians, rhytidocystids, and a clade consisting of piroplasmids and coccidians (Fig. 24). Most of the gregarine sequences fell into three main clades: (i) a terrestrial gregarine clade consisting of monocystids, neogregarines, and eugregregarines from the intestines of insects; (ii) a clade of eugregarines from the intestines of crustaceans; and (iii) a clade of marine lecudinids and urosporids (excluding *Lecudina polymorpha* and related environmental sequences) (Fig. 24). The 11 sequences from archigregarines did not cluster together as a separate clade and, instead, branched in unresolved positions along the apicomplexan backbone. The three new *Selenidium* sequences reported here (i.e. *S.* cf. *mesnili*, *S. boccardiellae* n. sp., and *S. idanthyrsae* n. sp.) formed a robust clade with *Selenidium serpulae*. The distance analysis across 1,653 base pairs showed that the SSU rDNA of *S. serpulae* differed from those collected from *S.* c.f. *mesnili*, *S. idanthyrsae*, and *S. boccardiellae* by 8.6%, 9.6% and 9.1%, respectively. The SSU rDNA of *S.* c.f. *mesnili* differed from those of *S. idanthyrsae* and *S. boccardiellae* by 8.3% and 7.5%, respectively, and the SSU rDNA of *S. idanthyrsae* differed from that of *S. boccardiellae* by 8.1% (Table 1). The following sequences branched in unresolved positions from the apicomplexan backbone: (i) *Selenidium terebellae*, (ii) a clade consisting of *Filipodium phascolosomae* and *Platyproteum vivax*, (iii) a clade consisting of *S. orientale* and *S. pisinnus*, (iv) a clade consisting of *S. serpulae, S.* cf. *mesnili*, *S. boccardiellae* n. sp., and *S. idanthyrsae* n. sp, (v) a clade consisting of *Lecudina polymorpha* and two environmental sequences (AB252765 and AB275008), and (vi) a clade consisting of two environmental sequences of unknown cellular identity (AY179975 and AY179976).

The SSU rDNA sequences from *V. leptosynaptae* n. gen. n. sp. isolate 1 and *V. leptosynaptae* n. gen., n. sp. isolate 2 were identical; this sequence formed a robust clade with an environmental sequences (AB275006). This "*Veloxidium* clade" branched as the nearest sister lineage to the major clade of marine lecudinids and urosporids, excluding *L. polymorpha* and related environmental sequences, with robust statistical support.

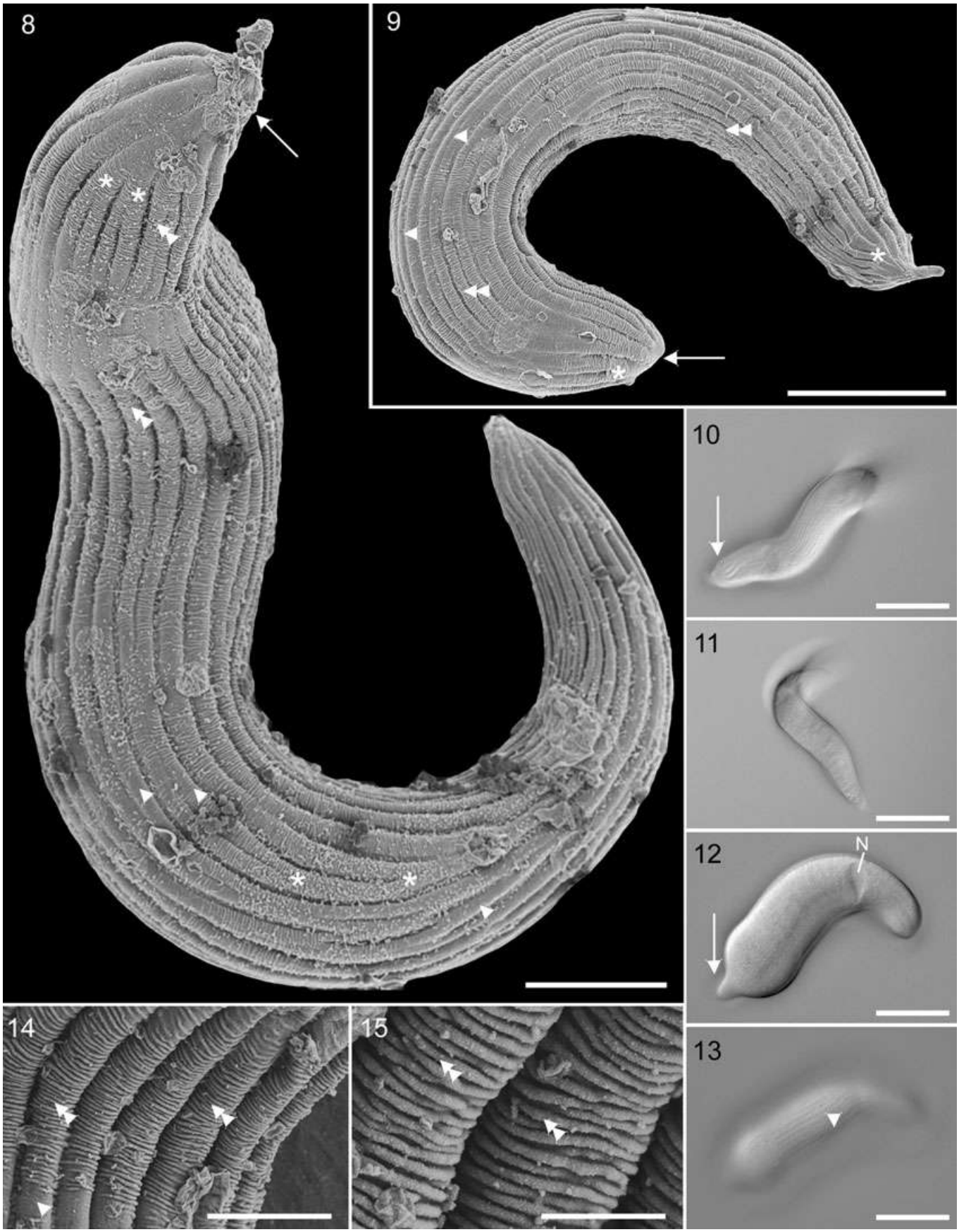
## DISCUSSION

Archigregarines are a poorly understood, but diverse assemblage of marine gregarines that are important for understanding early stages in the evolutionary history of gregarines and perhaps apicomplexans as a whole (Leander 2007, 2008). The main goal of this study was to discover and characterize additional species of archigregarines, to more comprehensively infer the phylogenetic relationships of these lineages within the Apicomplexa. Trophozoites of *Selenidium* cf. *mesnili* were isolated from the north Pacific *M. infundibulum* in this study. The host species, trophozoite motility and trophozoite morphology of *S.* cf. *mesnili* were indistinguishable from the original description of the type species (Brasil 1909; Ray 1930; Reed 1933; Schrével 1971a,b) (Table 2). However, because our isolate was collected from the Pacific Ocean and the type species of *S. mesnili* was collected from the Atlantic Ocean, we have chosen to designate our isolate as "*S.* cf. *mesnili*"; future work that determines an SSU rDNA sequence from the type host species, *Myxicola infundibulum*, collected in the east Atlantic, the type locality, will clarify whether or not this species has a broad geographical distribution. Nevertheless, this study has expanded our knowledge of this species or species complex with SEM data and a SSU rDNA sequence.

*Selenidium idanthyrsae* n. sp. was isolated from the intestines of *Idanthyrsae saxicavus*, which has not been reported as a host species of archigregarines until now. The trophozoites of *S. idanthyrsae* n. sp. were on average 438 μm long and 15 μm wide, which is longer than previously described species of *Selenidium*, except for *S. sabellaria*e, *S. fallax*, and *S. hollandei* (Levine 1971). However, the trophozoites in all three of these species differ from the isolate we describe here in several ways (Table 2): *S. fallax* has many more longitudinal folds (~ 90); *S. sabellariae* has transverse striations across the entire cell; and the anterior end of *S. hollandei* was reported to be "heart-shaped" (Table 2). Therefore, we established a new species for this isolate using host-specific, morphological, and molecular phylogenetic markers.

The trophozoites of *Selenidium boccardiellae* n. sp. were isolated from the intestines of *Boccardiellae ligerica* and were most similar in the number of longitudinal folds (10–12) and overall size with *S. spionis*, *S. cirratuli*, *S. polydorae,* and *S. rayi* (Levine 1971) (Table 2). However, all five species were isolated from a different host species and have trophozoites

Fig. **8–15.** Differential interference contrast (DIC) micrographs and scanning electron micrographs (SEM) showing the general morphology and surface structure of trophozoites of *Selenidium* cf. *mesnili* isolated from the intestines of *Myxicola infundibulum*. **8–9**. SEM showing the general elongate shape and surface morphology of the trophozoites. The surface of the trophozoites is adorned with 22–24 longitudinal epicytic folds (arrowhead) that disappear at the anterior (arrow) and posterior regions. The epicytic folds (arrowhead) bifurcate (asterisk) near the anterior and poster ends. Transverse striations (double arrowhead) on the epicytic folds are densely packed in some regions of the cell. The mucron region (arrow) and the posterior end are pointed. **10–13.** DIC micrographs illustrating the characteristic bending and thrashing motility of the trophozoites, including cell extension and contraction. Epicytic folds are visible in the DIC micrographs (arrowhead). The nucleus (N) is situated at the anterior or posterior parts of the cell. **14, 15.** High magnification SEM of the trophozoite surface showing epicytic folds (arrowhead) and transverse striations (double arrowhead). Scale bars: Fig. 8–9 = 10 μm; Fig. 10–13 = 25 μm; Fig. 14 = 3 μm; Fig. 15 = 1 μm.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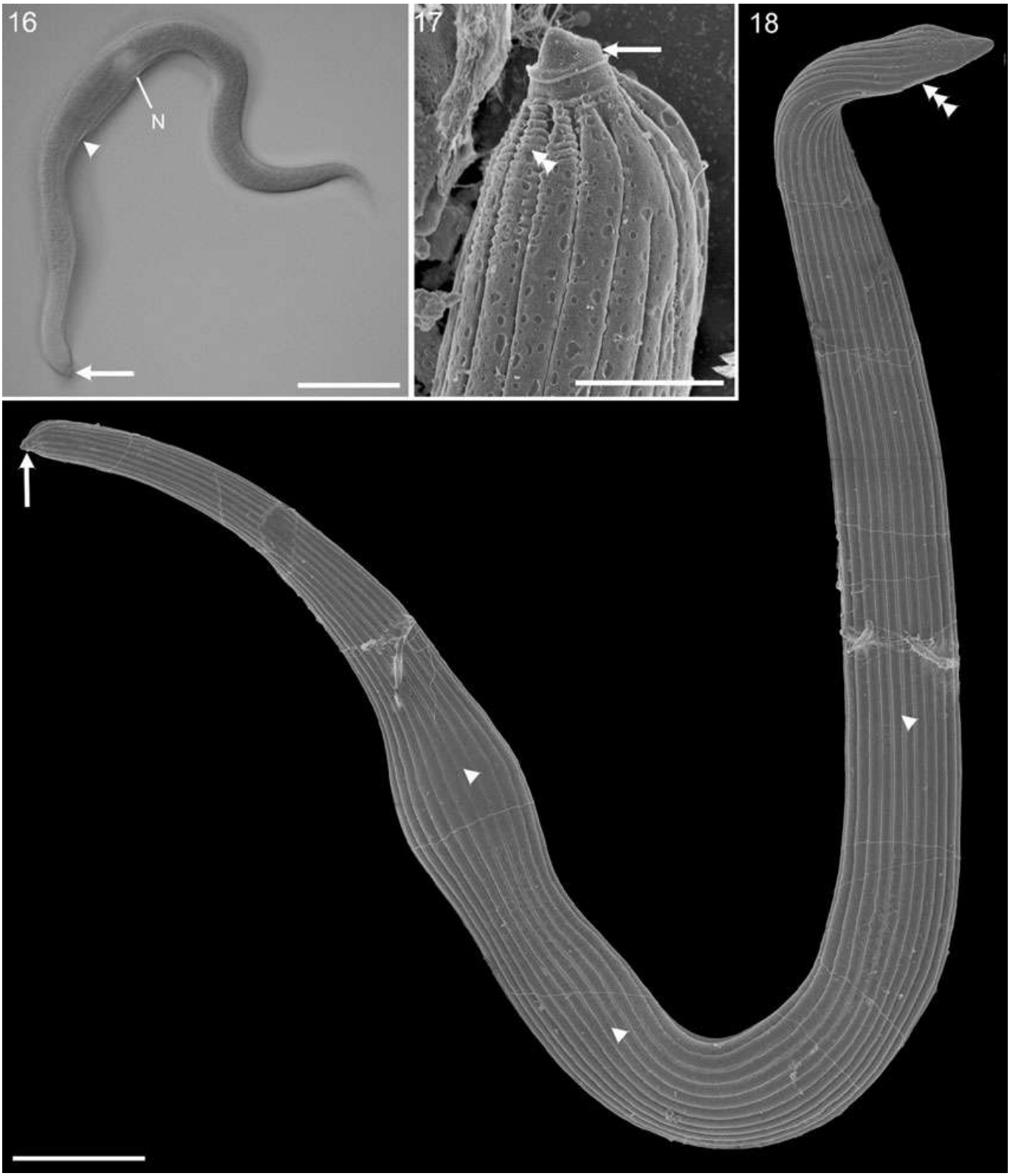


Fig. **16–18.** Differential interference contrast (DIC) micrographs and scanning electron micrographs (SEM) showing the general morphology and surface structure of trophozoites of *Selenidium idanthyrsae* n. sp. isolated from the intestines of *Idanthyrsae saxicavus*. **16.** DIC micrograph showing the pointed anterior (arrow), bending movement and longitudinal epicytic folds (arrowhead). The nucleus (N) was in the middle to posterior region of the trophozoites. **17.** High magnification SEM of the pointed mucron (arrow); transverse striations (double arrowhead) are only visible at the base of the mucron. **18.** SEM showing the vermiform shape of the trophozoites and 20–22 epicytic folds (arrowhead), which are absent from the extreme anterior (arrow) and compressed posterior (triple arrowhead) region of the cell. Scale bars: Fig. 16 = 50 μm; Fig. 17 = 3 μm; Fig. 18 = 10 μm.

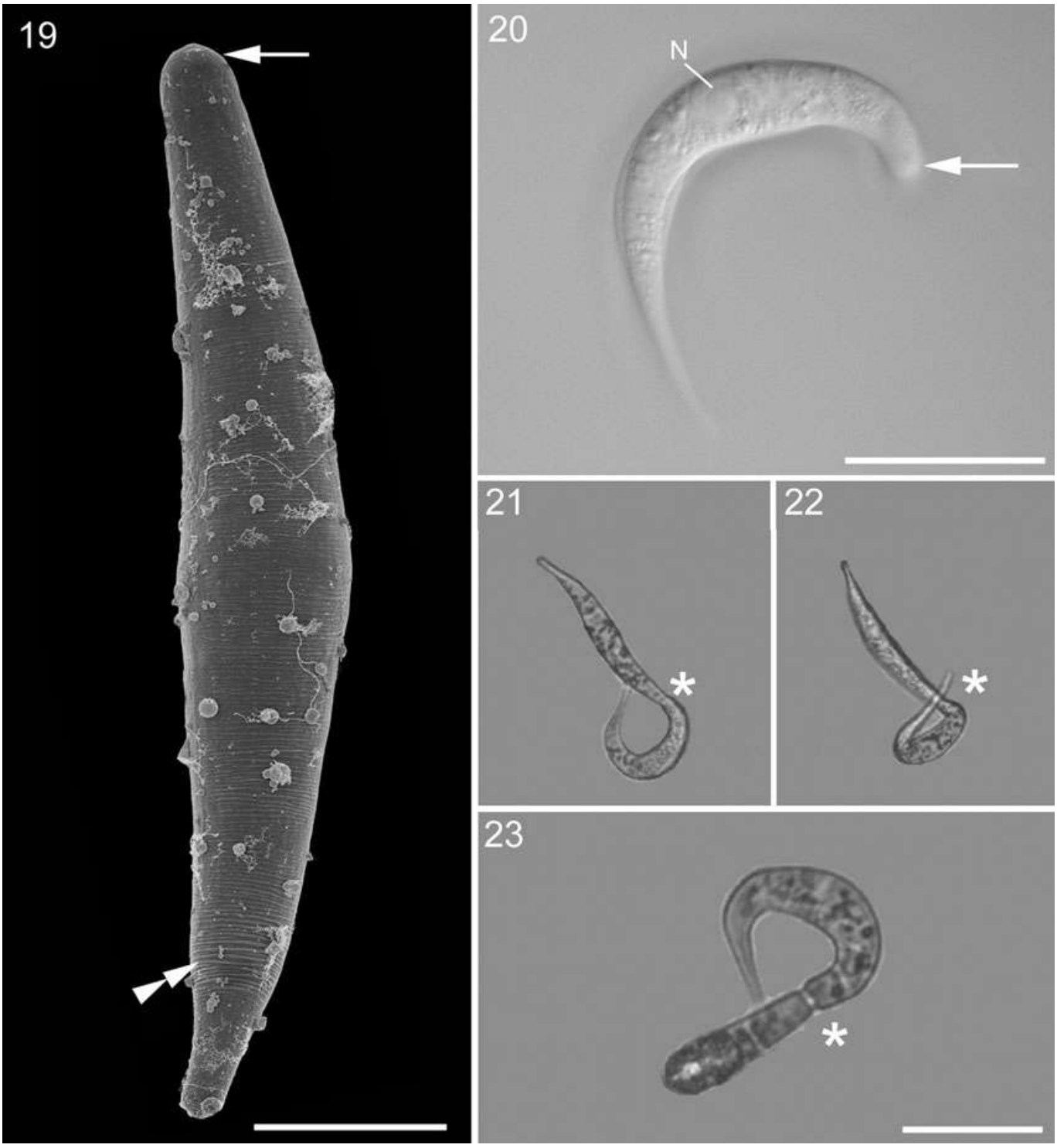


Fig. **19–23.** Differential interference contrast (DIC) micrographs and scanning electron micrographs (SEM) showing the general morphology and surface structure of trophozoites of *Veloxidium leptosynaptae* n. gen., n. sp. isolated from the intestines of *Leptosynaptae clarki*. **19.** SEM showing general morphology and spindle-shape of the trophozoites. The mucron (arrow) is dome-like and blunt. Epicytic folds are not present. A dense array of transverse striations (double arrowhead) is present over most of the cell surface. **20.** DIC image of "isolate 1" collected for single-cell PCR and subsequently molecular phylogenetic analysis. The nucleus (N) was located in the middle of the cell. **21–23.** DIC image of "isolate 2" collected for PCR and subsequently molecular phylogenetic analysis. The micrographs showing head-to-head syzygy. The junction (asterisk) between the two gamonts is the same width as the mucron of an individual trophozoite; the terminal ends of each gamont are tapered, curved, and pointed in a way that is identical to the posterior end of an individual trophozoite. Prominent spherical granules, roughly 1 μm in diam., were observed within the cytoplasm of the cell. Scale bars: Fig. 19 = 10 μm; Fig. 20 = 50 μm; Fig. 21–23 = 50 μm.

that differ in the shape of the mucron: *Selenidium boccardiellae* n. sp. has a pointed mucron, *S. spionis* has a depressed sucker-like mucron, *S. cirratuli* has a cone-shaped mucron, and both *S. polydorae* and *S. rayi* have mucrons described as "nipple-like" or "knob-like" (Levine 1971). Moreover, the flattened cell shape of the trophozoites in *S. boccardiellae* n. sp. distinguishes this species from these other four species, which are not flattened. Therefore, we established a new species for this isolate using host-specific, morphological, and molecular phylogenetic markers.

Our molecular phylogenetic analyses resulted in three different lineages of *Selenidium* species: an *S. terebellae* lineage, a clade consisting of *S. orientale* and *S. pisinnus* from the intestines of sipunculids, and a clade consisting of *S. boccardiellae*, *S.* cf. *mesnili*, *S. idanthyrsae*, and *S. serpulae*. Whether or not these three lineages form a more inclusive "*Selenidium* clade" or occupy different positions along the apicomplexan backbone was equivocal in our analyses. The trophozoites of the *Selenidium* species share several morphological features: a vermiform cell, a pointed mucron and posterior tip, nematode-like bending motility, and relatively few (i.e. between 4 and 44) longitudinal epicytic folds (Rueckert and Leander 2009; Simdyanov and Kuvardina 2007) (Table 2). Within the context of available molecular phylogenetic data, the fewest numbers of longitudinal epicytic folds are found in *S. terebellae* (4–6) and *S. boccardiellae* (10–12); the largest number is found in *S. pisinnus* (40–44). The trophozoites of *S.* cf. *mesnili*, *S. idanthyrsae*, *S. orientale*, and *S. serpulae* have an intermediate number of longitudinal epicytic folds (i.e. 18–30), suggesting that a gradual increase in the number of longitudinal folds occurred in the evolutionary history of archigregarines, presumably as a way to increasing surface area for acquiring nutrients within the intestines of their hosts. Moreover, like *P. vivax*, *S. boccardiellae* and *S. orientale* have trophozoites that are considerably flattened, which also increases surface area. The different molecular phylogenetic positions of these three species suggest that flattened trophozoites have evolved several times independently.

The presence of transverse striations on the surface of trophozoites is a distinctive feature of many archigregarines; in the context of our phylogenetic analysis, transverse striations are present in *S.* cf. *mesnili*, *S. serpulae*, *S. terebellae*, *P. vivax*, the anterior region of *S. idanthyrsae*, and *V. leptosynaptae* n. gen., n. sp. (Leander 2006, 2007; Leander et al. 2003b; Rueckert and Leander 2009). Transverse striations have not been observed on the trophozoites of *S. boccardiellae*, *S. orientale*, and *S. pisinnus* (Rueckert and Leander 2009). The phylogenetic distribution of archigregarines without transverse striations suggests that this feature has been lost at least three times independently: once in *S. boccardiellae*, once in the most recent ancestor of *S. orientale* and *S. pisinnus*, and once in the most recent ancestor of marine lecudinids and urosporids (excluding *L. polymorpha* and close relatives).

***Veloxidium leptosynaptae* n. gen., n. sp.** Twenty-five species of apicomplexans have been described from echinoderms so far, 23 eugregarines, one *Selenidium* species, and one coccidian (Barel and Kramers 1977; Jangoux 1987). The eugregarine species constitute the Urosporidae and fall within three genera: *Cystobia*, *Lithocystis*, and *Urospora* (Coulon and Jangoux 1987; Jangoux 1987). Similar to so many other lineages of gregarines, molecular information available for gregarines from echinoderms is sparse. Only a single DNA sequence collected from a putative *Lithocystis* sp. isolated from an echinoid is available; this sequence was not definitively linked to any morphological features of the trophozoites or other life history stages (Leander et al. 2006). The description and phylogenetic analysis of *V. leptosynaptae* n. gen., n. sp., in this study, represents the first morphological description of a gregarine isolated from an echinoderm in tandem with molecular phylogenetic data.

The motility and morphological characteristics of *V. leptosynaptae* are reminiscent of other archigregarines, particularly behavioral traits exhibited by members of *Selenidium*. Syzygy in this species was head-to-head. Light micrographs and videos show trophozoites of *V. leptosynaptae* bending, twisting, and thrashing in an almost identical manner to that of many *Selenidium* spp. (e.g. *S. boccardiellae*, *S. idanthyrsae*, and *S.* cf. *mesnili*, in this study). However, the motility of these trophozoites was also distinctive in the relatively rapid manner in which it occurred. The trophozoite surface of *V. leptosynaptae* was not adorned with longitudinal epicytic folds and instead was covered with a dense array of transverse striations, most similar to those observed in the archigregarines *P. vivax* and *Digyalum oweni* (Dyson et al. 1993, 1994; Gunderson and Small 1986; Leander 2006; Rueckert and Leander 2009).

Mingazzini (1893) originally described the only other *Selenidium* archigregarine from a holothurian, namely *Selenidium synaptae* from the intestines of *Synapta* sp., which was subsequently accepted by Levine (1971). The original description of *S. synaptae* documented four longitudinal epicytic folds on the surface of the trophozoites, a feature that is not present in *V. leptosynaptae* n. gen., n. sp. Therefore, the host species and the morphology of our isolates of *V. leptosynaptae* do not conform to the description of *S. synaptae* (Table 2). With the exception of *Platyproteum*, none of the other eight previously described archigregarine genera has a trophozoite stage that has transverse surface striations and lacks longitudinal epicytic folds (Rueckert and Leander 2009). The trophozoites of *Platyproteum*, however, differ markedly from those in *V. leptosynaptae* because they are greatly flattened, have a distinctive wave-like pattern of motility, and are found in sipunculids. Moreover, the SSU rDNA sequence generated from *V. leptosynaptae* is very distantly related to the sequence from *Platyproteum* (Fig. 24). Therefore, it was necessary to establish a new genus for this unusual isolate.

**Character evolution and the "archigregarine" morphotype.** There are two very important realizations in any interpretation of character evolution within the context of available molecular phylogenetic data from gregarines: (i) The archigregarine concept has never been adequately tested with molecular phylogenetic data and is, at most, a convenient category of trophozoite morphotypes with little or no taxonomic value; (ii) the deepest relationships within the Apicomplexa are almost completely unresolved with available molecular phylogenetic data. Analyses of SSU rDNA sequences do not pro-

Fig. **24.** Maximum likelihood (ML) tree based on 87 small subunit (SSU) rDNA sequences and 1,008 unambiguously aligned sites using the GTR + I + Γ substitution model (- ln L = 18528.74970, gamma shape = 0.6170, proportion of invariable sites = 0.1770). Bootstrap support values are given at the top of branches, and Bayesian posterior probabilities are given at the bottom. Thick branches represent bootstrap support values and Bayesian posterior probabilities 95/0.95 or greater. Bootstrap values less than 55, and Bayesian posterior probabilities less than 0.90 were not added to this tree. The four sequences generated in this study are highlighted in black boxes.

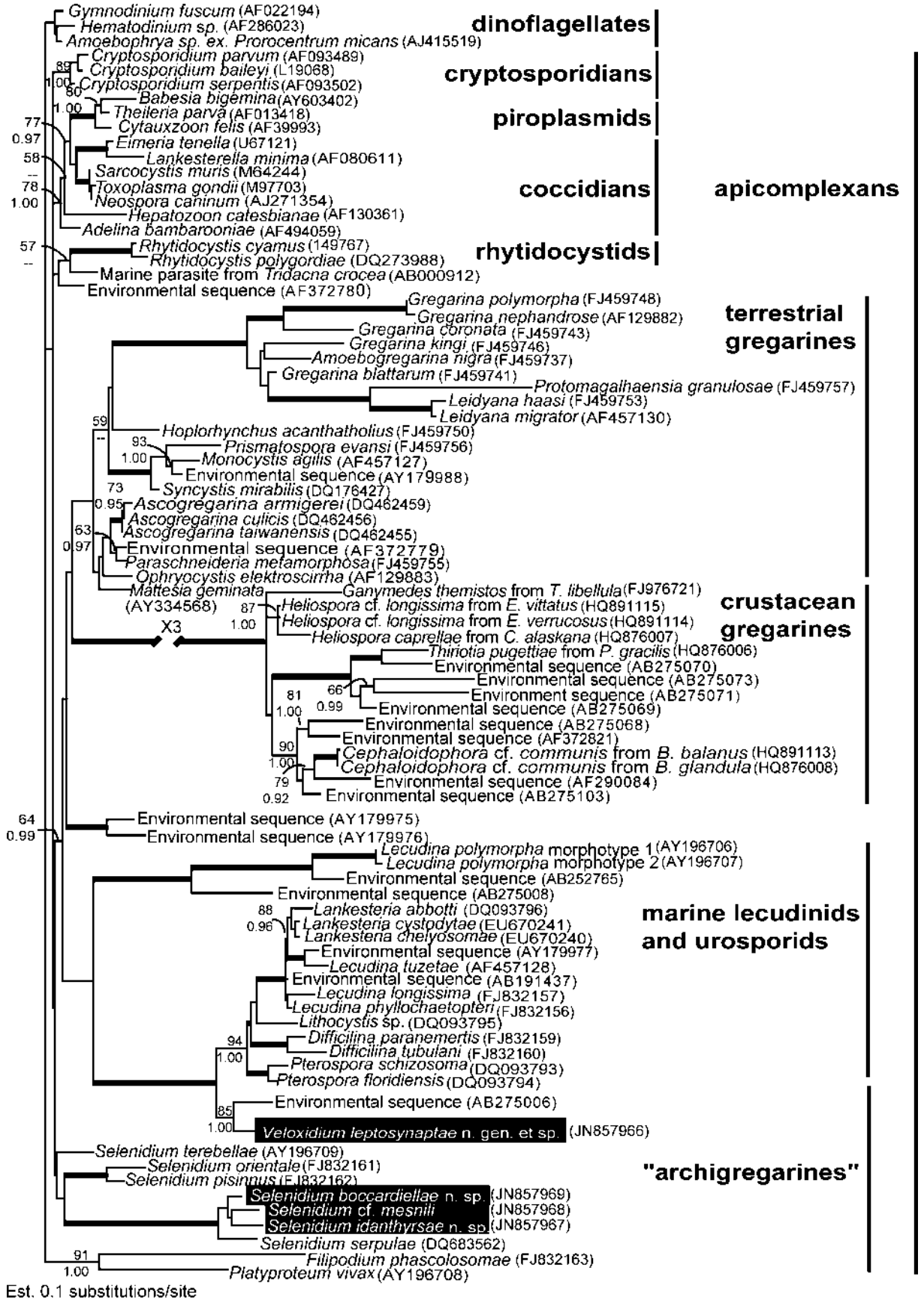
Gymnodinium fuscum (AF022194)
Hematodinium sp. (AF286023)
Amoebophrya sp. ex. Prorocentrum micans (AJ415519)
dinoflagellates
Cryptosporidium parvum (AF093489)
Cryptosporidium baileyi (L19068)
Cryptosporidium serpentis (AF093502)
cryptosporidians
Babesia bigemina (AY603402)
Theileria parva (AF013418)
piroplasmids
Cytauxzoon felis (AF39993)
Eimeria tenella (U67121)
Lankesterella minima (AF080611)
Sarcocystis muris (M64244)
Toxoplasma gondii (M97703)
Neospora caninum (AJ271354)
Hepatozoon catesbianae (AF130361)
coccidians
apicomplexans
Adelina bambarooniae (AF494059)
Rhytidocystis cyamus (149767)
Rhytidocystis polygordiae (DQ273988)
rhytidocystids
Marine parasite from Tridacna crocea (AB000912)
Environmental sequence (AF372780)
Gregarina polymorpha (FJ459748)
Gregarina nephandrose (AF129882)
Gregarina coronata (FJ459743)
Gregarina kingi (FJ459746)
Amoebogregarina nigra (FJ459737)
Gregarina blattarum (FJ459741)
Protomagalhaensia granulosae (FJ459757)
Leidyana haasi (FJ459753)
Leidyana migrator (AF457130)
terrestrial gregarines
Hoplorhynchus acanthatholius (FJ459750)
Prismatospora evansi (FJ459756)
Monocystis agilis (AF457127)
Environmental sequence (AY179988)
Syncystis mirabilis (DQ176427)
Ascogregarina armigerei (DQ462459)
Ascogregarina culicis (DQ462456)
Ascogregarina taiwanensis (DQ462455)
Environmental sequence (AF372779)
Paraschneideria metamorphosa (FJ459755)
Ophryocystis elektroscirrha (AF129883)
Mattesia geminata (AY334568)
Ganymedes themistos from T. libellula (FJ976721)
Heliospora cf. longissima from E. vittatus (HQ891115)
Heliospora cf. longissima from E. verrucosus (HQ891114)
X3
Heliospora caprellae from C. alaskana (HQ876007)
crustacean gregarines
Thiriotia pugettiae from P. gracilis (HQ876006)
Environmental sequence (AB275070)
Environmental sequence (AB275073)
Environment sequence (AB275071)
Environmental sequence (AB275069)
Environmental sequence (AB275068)
Environmental sequence (AF372821)
Cephaloidophora cf. communis from B. balanus (HQ891113)
Cephaloidophora cf. communis from B. glandula (HQ876008)
Environmental sequence (AF290084)
Environmental sequence (AB275103)
Environmental sequence (AY179975)
Environmental sequence (AY179976)
Lecudina polymorpha morphotype 1 (AY196706)
Lecudina polymorpha morphotype 2 (AY196707)
Environmental sequence (AB252765)
Environmental sequence (AB275008)
Lankesteria abbotti (DQ093796)
Lankesteria cystodytae (EU670241)
Lankesteria chelyosomae (EU670240)
Environmental sequence (AY179977)
Lecudina tuzetae (AF457128)
Environmental sequence (AB191437)
Lecudina longissima (FJ832157)
Lecudina phyllochaetopteri (FJ832156)
Lithocystis sp. (DQ093795)
Difficilina paranemertis (FJ832159)
Difficilina tubulani (FJ832160)
Pterospora schizosoma (DQ093793)
Pterospora floridiensis (DQ093794)
marine lecudinids and urosporids
Environmental sequence (AB275006)
Veloxidium leptosynaptae n. gen. et sp. (JN857966)
Selenidium terebellae (AY196709)
Selenidium orientale (FJ832161)
Selenidium pisinnus (FJ832162)
Selenidium boccardiellae n. sp. (JN857969)
Selenidium cf. mesnili (JN857968)
Selenidium idanthyrsae n. sp. (JN857967)
Selenidium serpulae (DQ683562)
"archigregarines"
Filipodium phascolosomae (FJ832163)
Platyproteum vivax (AY196708)
Est. 0.1 substitutions/site

Table **1.** Distance matrix comparing 1,653 bp of the small subunit (SSU) rDNA sequences from *Selenidium serpulae*, *S.* cf. *mesnili*, and the two new species of *Selenidium* reported here.

| | *Selenidium serpulae* | *S.* cf. *mesnili* | *S. idanthyrsae* n. sp. | *S. boccardiellae* n. sp. |
|---|---|---|---|---|
| Selenidium serpulae | — | | | |
| *S.* cf. *mesnili* | 0.08557 | — | | |
| *S. idanthyrsae* n. sp. | 0.09581 | 0.08336 | — | |
| *S. boccardiellae* n. sp. | 0.09121 | 0.0752 | 0.08066 | — |

vide (statistical) support for or against any of the relationships between the major clades of gregarines (especially genera referred to as "archigregarines") and other groups of apicomplexans (rhytidicystids, coccidians + prioplasmids, and cryptospordidians).

The current molecular phylogenetic data contain several different species of gregarines with trophozoites that conform to the "archigregarine" morphotype (e.g. spindle-shaped or vermiform cells with transverse striations, bending motility, and relatively few or no longitudinal epicytic folds). These species fall within a minimum of five different clades/lineages, four of which do not have any sister group and therefore have unresolved phylogenetic positions along the apicomplexan backbone. The clustering of these lineages at the bottom of Fig. 24 reflects convenience of illustration (i.e. it provides a way to bracket species with the archigregarine morphotype) and does not reflect any reliable evidence for phylogenetic relationships.

Nevertheless, the strongly supported phylogenetic position of the *Veloxidium* clade was the most exciting discovery of this study because the new species reported here conforms to the archigregarine morphotype yet branches as the nearest sister group to a large and diverse clade of marine eugregarines (i.e. urosporids and most lecudinids). Two reasonable interpretations exist for this situation: (i) *Veloxidium* is a descendant of eugregarines, and the archigregarine morphotype in this species reflects convergent evolution with (distantly related) *Selenidium* species; or (ii) the archigregarine morphotype in *Veloxidium* reflects morphostasis of traits that are shared with *Selenidium* species and their most recent common (archigregarine) ancestor.

As articulated in Leander (2008), we favor the second interpretation, specifically that available data is consistent with the hypothesis that species with the archigregarine morphotype represent a large paraphyletic (stem) group from which many other clades of gregarines evolved (Cox 1994; Grassé 1953; Leander 2008; Leander and Keeling 2003; Leander et al. 2003a; Rueckert and Leander 2009; Théodoridès 1984; Vivier and Desportes 1990). An expectation of this hypothesis is that different species with the archigregarine morphotype will form the nearest sister lineage to several different major groups of eugregarines that currently branch from the apicomplexan backbone without resolution. The phylogenetic position of *Veloxidium* conforms to this expectation. This hypothesis would be unsupported/falsified if all or most gregarine species with the archigregarine morphotype clustered exclusively within one clade (i.e. monophyletic) and/or were phylogenetically scattered *within* several different major groups of eugregarines (i.e. polyphyletic). This distribution of archigregarine species is not supported by current molecular phylogenetic data.

Three relatively large clades of eugregarines are usually recovered in molecular phylogenetic analyses of SSU rDNA sequences: (i) terrestrial eugregarines plus neogregarines, (ii) eugregarines form crustacean hosts, and (iii) marine lecudinid and urosporid eugregarines, excluding *L. polymorpha* and close relatives (Rueckert et al. 2011) (Fig. 24). Most members of these clades have trophozoites with a cortex consisting of a dense array of longitudinal epicytic folds and with gliding motility that is distinct from the bending and twisting movements of archigregarines. The most parsimonious explanation from previous work was that this combination of features evolved in the most recent common ancestor of eugregarines (Leander et al. 2003a, 2006). However, the robust molecular phylogenetic position of *V. leptosynaptae* as the nearest sister group to a diverse clade of marine lecudinids and urosporids provides support not only to the hypothesis that archigregarines form a paraphyletic stem group from which other gregarines evolved, but also that trophozoites with gliding motility and dense arrays of longitudinal epicytic folds (e.g. most eugregarines) evolved several times independently. Continued exploration, discovery, and molecular characterization of new archigregarine species will test these inferences and provide some of the greatest insights into the earliest stage of apicomplexan evolution.

## TAXONOMIC SUMMARY

Phylum Apicomplexa Levine, 1970
Class Conoidasida Levine, 1988
Subclass Gregarinasina Dufour, 1828
Order Archigregarinorida Grassé, 1953
Family Selenidiidae Brasil, 1907
Genus *Selenidium* Giard, 1884
*Selenidium boccardiellae* n. sp. Wakeman and Leander

**Diagnosis.** Trophozoites are vermiform and partially flattened, mean length = 158 μm (range = 87–250), mean width = 10 μm (range = 10–12). Cells are slightly compressed forming two lateral ridges, especially in the anterior region. The mucron tapers and is pointed. The surface is inscribed with 10–12 shallow epicytic folds. The epicytic folds swirl near the tapered posterior end. The nucleus is ellipsoidal (11 × 5 μm) and located in the middle upper half of the cell. Trophozoites are capable of bending, coiling and trashing movements.

**Gene sequence.** A sequence of the SSU rDNA is deposited as GenBank accession No. JN857969.

**Type locality.** Odgen Point, Victoria, British Columbia (48° 25′43″N, 123°21′56″W), Canada. Subtidal 7m.

**Type habitat.** Marine

**Type host.** *Boccardiella ligerica* (Ferronnière, 1898) (Metazoa, Annelida, Polychaeta, Spionida, Spionidae)

**Location in host.** Intestinal lumen

**Holotype.** Fig. 1. Image taken from the holotype fixed on a gold sputter-coated SEM stub. The stub has been deposited in the Beaty Biodiversity Research Centre (Marine Invertebrate Collection; voucher: MI-PR114) at the University of British Columbia, Vancouver, Canada.

**Paratypes.** Fig. 3–6

Table **2.** Morphological comparisons of *Veloxidium* n. gen., *Selenidium pendula* (type species) and other *Selenidium* species.

| | *S. pendula* (type species) | *S. terebellae* | *S. orientale* | *S. pisinnus* | *S. serpulae* | ***S.* cf. *mesnili*** | ***S. boccardiellae* n. sp** | ***S. spionis*** |
|---|---|---|---|---|---|---|---|---|
| Host | *Nerine cirratulus* | *Terebella lapidaris* | *Themiste pyroides* | *Phascolosoma agassizii* | *Serpula vermicularis* (*contortuplicata*) | ***Myxicola infundibulum*** | ***Boccardiella ligerica*** | *Polyrabdina spionis* |
| Host tissue | Intestines | Intestines | Intestines | Intestines | Intestines | **Intestines** | **Intestines** | Intestines |
| Locality | E. Atlantic | E. Pacific and E. Atlantic | E. and W. Pacific | E. Pacific | W. pacific | **W. Pacific** | **W. Pacific** | W. Pacific |
| Trophozoite Shape | Spindle-shaped | spindle-shaped | Spindle-shaped, flattened | Oblong to elliptoid | Spindle-shaped | **Spindle-shaped** | **Spindle-shaped, partially flattened** | Spindle-shaped |
| Tophozoite Size (L x W, μm) | 180 × 30–40 | 30–330 × 12–40 | 120–300 × 15–40 | 64–100 × 9–25 | 100–150 × 7–25 | **85–157 × 18–24** | **87–250 × 10–12** | 60–80 × 10–12 |
| Nucleus Shape | Rounds to oval | Oval (ellipsoidal?) | Ellipsoidal | Ellipsoidal | Ellipsoidal | **Ellipsoidal** | **Ellipsoidal** | Unknown |
| Nucleus Size (L × W, μm) | 18–33 × 13–32 | 35 × 15 | 8–15 × 10–25 | 5 × 11 | 7–10 × 5–11 | **7–9 × 10–11** | **10–12 × 4–6** | Unknown |
| Position of nucleus | Middle | Posterior half | Middle | Anterior half | Anterior half | **Anterior to Posterior** | **Anterior half** | Unknown |
| Motility behavior | Bending, twisting, pendulum-like | Bending, twisting | Bending, twisting | Pending, twisting | Beding, twisting, contract/stretch | **Beding, twisting, contract/stretch** | **Bending, twisting** | Bending, twisting |
| Number of long. epicytic folds | 20–30 | 4–6 | 18–20 | 40–44 | 14–23 | **22–24** | **10–12** | 20–30 |
| Transverse surface folds | Unknown | Yes | No | No | Yes | **Yes** | **No** | Unknown |
| Shape of mucron | Pointed | Pointed to slightly round | Pointed | Pointed | Pointed to round | **Pointed to round** | **Pointed** | Sucker-like, depressed |
| Literature | Levine (1971); Schrével (1970) | Leander et al. (2003a,b) | Rueckert and Leander (2009); Simdyanov and Kuvardina (2007) | Rueckert and Leander (2009) | Levine (1971); Leander 2006; | **Brasil (1909); Schrével (1970); This study** | **This study** | Levine (1971) |

| *S. cirratuli* | *S. polydorae* | *S. rayi* | ***S. idanthyrsae* n. sp** | *S. sabellariae* | *S. fallax* | *S. hollandei* | ***V. leptosynaptae* n. gen., n. sp.** | *S. synapta* |
|---|---|---|---|---|---|---|---|---|
| *Cirratulus cirratus* | *Polydora ciliata* | *Cirratulus filiformis* | ***Idanthyrsus saxicavus*** | *Sabellaria alveolata* | *Cirriformia tentaculata* | *Sabellaria alveolata* | ***Leptosynaptae clarkii*** | *Synapta* sp. |
| Intestines | Intestines | Intestines | **Intestines** | Intestines | Intestines | Intestines | **Intestines** | Intestines |
| W. Atlantic | W. Pacific | W. Pacific | **W. Pacific** | E. Atlantic | E. Atlantic | E. Atlantic | **W. Pacific** | E. Atlantic |
| Spindle-shaped, flattened | Spindle-shaped | Spindle-shaped | **Spindle-shaped, partially flattened** | Spindle-shaped | Spindle-shaped | Spindle-shaped, elongate, flattened | **Spindle-shaped** | Spindle-shaped |
| Unknown | 100–150 × 10–12 | 80–100 × 15–20 | **450–543 × 9–11** | 220 × 16 | 300–500 × 10–30 | Up to 500 × 20–30 | **62–97 × 9–13** | Unknown |
| Unknown | Unknown | Unknown | **Spherical** | Ovoid | Unknown | Spherical or ovoid | **Spherical** | Spherical |
| Unknown | Unknown | Unknown | **13–16 × 9–10** | 16 × 13 | Unknown | 16 × 6–8 | **9–10 × 9–11** | Unknown |
| Unknown | Unknown | Unknown | **Middle to posterior half** | Unknown | Unknown | Unknown | **Middle to posterior half** | Middle |
| Bending, twisting | Bending, twisting | Bending, twisting | **Bending, twisting** | Bending, twisting | Bending, twisting | Bending, twisting | **Bending, twisting (rapid)** | Bending, twisting |
| About 12 | 10–12 | 10–12 | **20–22** | Unknown | About 90 | About 16 | **None** | 4–8 |
| Unknown | Unknown | Unknown | **Yes** | Yes | Unknown | Unknown | **Yes** | Unknown |
| Cone-shaped | Nipple-like | Knob-like | **Pointed** | Pointed, spine present | Knob-like | Heart-shaped | **Round** | Pointed |
| Levine (1971) | Levine (1971) | Levine (1971) | **This study** | Levine (1971) | Levine (1971) | Levine (1971) | **This study** | Mingazzini (1893) |

Archigregarines described in this study are highlighted in bold.

**Etymology.** The specific epithet is named for the genus of the polychaete host in which this species was found.

*Selenidium idanthyrsae* n. sp. Wakeman and Leander

**Diagnosis.** Trophozoites are elongated with a compressed and spade-like posterior end. Trophozoites have an average length of 438 μm (range = 450–543 μm) and an average width of 15 μm (range = 14–16). The spherical nucleus (14 × 14 μm) was positioned in the middle to posterior part of the cell. The cell surface was lined with 20–22 epicytic folds. Transverse striations are present on parts of the anterior near the anterior part of the cell near the mucron. The mucron is pointed, protruding 2–3 μm. This species is capable of bending and coiling mements.

**Gene sequence.** A sequence of the SSU rDNA is deposited as GenBank accession No. JN857967.

**Type locality.** Wizard Islet (48°50′17″N, 125°08′02″W). Subtidal 20m. Bamfield, British Columbia, Canada.

**Type habitat.** Marine

**Type host.** *Idanthyrsae saxicavus* (Baird, 1863) (Metazoa, Annelida, Polychaeta, Sabellariidae)

**Location in host.** Intestinal lumen

**Holotype.** Fig. 18. Image taken from the holotype fixed on a gold sputter-coated SEM stub. The stub has been deposited in the Beaty Biodiversity Research Centre (Marine Invertebrate Collection; voucher: MI-PR115) at the University of British Columbia, Vancouver, Canada.

**Paratype.** Fig. 16

**Etymology.** The specific epithet is named for the genus of the polychaete host in which this species was found.

*Veloxidium* n. gen. Wakeman and Leander

**Diagnosis.** Trophozoites are vermiform with a spherical nucleus located in the middle of the cell. The trophozoite surface lacks longitudinal epicytic folds and has transverse striations. Syzygy is head-to-head. Trophozoites are capable of dynamic and distinctively rapid bending movements.

**Type species.** *Veloxidium leptosynaptae*

**Etymology.** The genus name is taken from "*Velox*" (Latin) = "rapid" and refers to the fast bending and thrashing motility of the trophozoites.

*Veloxidium leptosynaptae* n. sp. Wakeman and Leander

**Diagnosis.** Trophozoites are vermiform, mean length = 74 μm (range = 62–97 μm) and mean width = 11 μm (range = 9–13 μm). A spherical nucleus (10 × 10 μm) is located in the middle of the cell. The surface of the trophozoites lacks longitudinal epicytic folds and is covered with a dense array of transverse striations. The anterior mucron region is dome-like. The posterior end tapers to a point and in some individuals bends characteristically at an angle. Syzygy is head-to-head. The trophozoites are capable of dynamic bending and twitching movements.

**Gene sequence.** A sequence of the SSU rDNA is deposited as GenBank accession No. JN857966.

**Type locality.** Grappler Inlet. Muddy intertidal. Bamfield, British Columbia (48°49′49″N, 125°8′15″W), Canada.

**Type habitat.** Marine

**Type host.** *Leptosynapta clarki* (Heding, 1928) (Metazoa, Echinodermata, Holothuroidea, Synaptidae, Leptosynapta)

**Location in host.** Intestinal lumen.

**Holotype.** Fig. 19. Image taken from the holotype fixed on a gold sputter-coated SEM stub. The stub has been deposited in the Beaty Biodiversity Research Centre (Marine Invertebrate Collection; voucher: MI-PR116) at the University of British Columbia, Vancouver, Canada.

**Paratypes.** Fig. 20–23

**Etymology.** The specific epithet is named for the genus of the polychaete host in which this species was found.

## ACKNOWLEDGMENTS

This research was supported by grants from the National Science and Engineering Research Council of Canada (NSERC 283091-09) and the Canadian Institute for Advanced Research, Program in Integrated Microbial Biodiversity.

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*Received: 10/19/11, 01/23/12; accepted: 01/23/12*