



HAL
open science

Ultrastructure of *Selenidium pendula*, the Type Species of Archigregarines, and Phylogenetic Relations to Other Marine Apicomplexa

Joseph Schrével, Andrea Valigurová, Gérard Prensier, Aurélie Chambouvet, Isabelle Florent, Laure Guillou

► To cite this version:

Joseph Schrével, Andrea Valigurová, Gérard Prensier, Aurélie Chambouvet, Isabelle Florent, et al.. Ultrastructure of *Selenidium pendula*, the Type Species of Archigregarines, and Phylogenetic Relations to Other Marine Apicomplexa. *Protist*, 2016, 167 (4), pp.339-368. 10.1016/j.protis.2016.06.001 . hal-01338422

HAL Id: hal-01338422

<https://hal.sorbonne-universite.fr/hal-01338422>

Submitted on 28 Jun 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ORIGINAL PAPER

2
3 **Ultrastructure of *Selenidium pendula*, the Type Species of**
4 **Archigregarines, and Phylogenetic Relations to Other Marine**
5 **Apicomplexa**6
7 Joseph Schrével^{a,1}, Andrea Valigurová^b, Gérard Prensier^{c,2}, Aurélie
8 Chambouvet^d, Isabelle Florent^a, and Laure Guillou^e9
10 ^aUnité Molécules de Communication et Adaptation des Microorganismes, (MCAM, UMR
11 7245), Muséum National Histoire Naturelle, Sorbonne Universités, CNRS, CP 52, 57 Rue
12 Cuvier, 75005 Paris, France13 ^bDepartment of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2,
14 611 37 Brno, Czech Republic15 ^cCell Biology and Electron Microscopy Laboratory, François Rabelais University, 10
16 Boulevard Tonnellé, BP 3223, 37032 Tours Cedex, France17 ^dLaboratoire des Sciences de l'Environnement Marin (LEMAR), UMR6539
18 UBO/CNRS/IRD/IFREMER, Institut Universitaire Européen de la Mer (IUEM),
19 Technopole Brest Iroise, 29280 Plouzané, France20 ^eSorbonne Universités, Université Pierre et Marie Curie - Paris 6, CNRS, UMR 7144,
21 Station Biologique de Roscoff, Place Georges Teissier, CS90074, 29688 Roscoff cedex,
22 France23
24 Submitted February 3, 2016; Accepted June 12, 2016

25 Monitoring Editor : Frank Seeber

26
27 **Running title:** *Selenidium pendula*: Ultrastructure and Phylogeny28
29 ¹Corresponding author; fax +33 1 40 79 34 9930 e-mail schrevel@mnhn.fr (J. Schrével).31
32 ²This work is dedicated to the memory of Brigitte Arbeille-Brassart

34 Archigregarines, an early branching lineage within Apicomplexa, are a poorly-known
35 group of invertebrate parasites. By their phylogenetic position, archigregarines are an
36 important lineage to understand the functional transition that occurred between free-
37 living flagellated predators to obligatory parasites in Apicomplexa. In this study, we
38 provide new ultrastructural data and phylogenies based on SSU rDNA sequences
39 using the type species of archigregarines, the Selenidiidae *Selenidium pendula* Giard,
40 1884. We describe for the first time the syzygy and early gamogony at the
41 ultrastructural level, revealing a characteristic nuclear multiplication with
42 centrocones, cryptomitosis, filamentous network of chromatin, a cyst wall secretion
43 and a 9+0 flagellar axoneme of the male gamete. *S. pendula* belongs to a monophyletic
44 lineage that includes several other related species, all infecting Sedentaria Polychaeta
45 (Spionidae, Sabellaridae, Sabellidae and Cirratulidae). All of these *Selenidium* species
46 exhibit similar biological characters: a cell cortex with the plasma membrane - inner
47 membrane complex - subpellicular microtubule sets, an apical complex with the
48 conoid, numerous rhoptries and micronemes, a myzocytosis with large food vacuoles,
49 a nuclear multiplication during syzygy and young gamonts. Two other distantly
50 related *Selenidium*-like lineages infect Terebellidae and Sipunculida, underlying the
51 ability of archigregarines to parasite a wide range of marine hosts.

52

53

54 **Key words:** Archigregarines; Apicomplexa; *Selenidium pendula*; ultrastructure;
55 phylogeny; sporozoite.

56

57

58

59

60 **Introduction**

61

62 Apicomplexa, a large subgroup of the Alveolata, are unicellular parasites infecting a wide
63 range of invertebrate and vertebrate hosts. Most known Apicomplexa belong to Coccidia
64 and Haemosporidia and are involved in human and veterinary diseases (malaria,
65 toxoplasmosis, coccidiosis, babesiosis, piroplasmosis). However, a very large group of the
66 early branching Apicomplexa, the gregarines, is comparatively poorly known. Most of the
67 gregarines infect invertebrate hosts and usually do not have deleterious effects on their

68 hosts (Desportes and Schrével 2013). The number of Apicomplexa species is estimated to
69 be ~2,000-6,000, however the ability of gregarines to infect a wide range of insects could
70 significantly enhance this estimation to several thousand or more than one million (e.g., the
71 Coleoptera (beetles) class corresponds to about 40% of the insect biodiversity with an
72 expected species number around 1 to 2 million) (Schrével and Desportes 2013).

73 All apicomplexan species are characterized by an infective life stage, the so-called
74 zoite, a polarized cell with an original apical complex. This apical complex is an assembly
75 of specific organelles including club-shaped rhoptries, filament-like micronemes, dense
76 granules and apical polar rings. In Apicomplexa, the presence of a conoid in the apex of the
77 zoite, observed in coccidia and gregarines, defines the Conoidasida Levine, 1988. In
78 contrast, no conoid is observed in Haemosporidia and Piroplasmida designated
79 Aconoidasida by Mehlhorn et al. (1980). Except gregarines and some other taxa developing
80 in epicellular localization, such as cryptosporidia, most Apicomplexa have an intracellular
81 development in their host cells and there, the apical organelles as well as the conoid, play
82 an essential role in cell invasion processes through sophisticated cascades of molecular
83 interactions (Boothroyd and Dubremetz 2008; Bradley et al. 2005; Santos et al. 2009). In
84 Apicomplexa displaying an intracellular life style, the cycle usually occurs in two hosts, the
85 sexual phase being performed in the definitive host while asexual phases occur in one or
86 several intermediate hosts. Gametogenesis, as observed in Coccidia or Haemosporidia,
87 exhibits a clear anisogamy with production of small flagellated male gametes
88 (microgametes) and large non-flagellated female gametes (macrogametes). After
89 fertilization, the sporogony produces sporozoites in the definitive hosts while asexual
90 schizogony or merogony, producing merozoites, is realized in intermediate hosts. In contrast,
91 most gregarines exhibit an extracellular development and their entire life cycle usually
92 occurs within a single host. Their zoites transform into large vegetative cells, the
93 trophozoites, with an extraordinary diversity in their morphologies and behaviours. In
94 addition to this extracellular development, gregarines share a unique sexual phase. The
95 sexual association between two gamonts, named syzygy, produces a cyst where the
96 gametogenesis differentiates a large and equal number of male and female gametes; at this
97 stage, this cyst is called a gametocyst. Then, fertilization and sporogenesis take place
98 within the cyst yielding the final stages with the sporocysts usually containing each 8
99 sporozoites. These sporocysts can survive for a long period generally waiting for their
100 ingestion by their specific hosts. Gregarine biochemistry and physiology are still poorly
101 documented. Studies of their zoite apical apparatus as well as of the variation of their

102 cytoskeleton and microtubule organizing centers (MTOCs), with unique organization as the
103 6+0 or 3+0 flagellar axonemes described for some male gametes (Prensier et al. 1980;
104 Schrével and Besse 1975), contributed, however, to a more general understanding of many
105 biological aspects of Apicomplexa including pathogenic species.

106 Among Apicomplexa, there is a consensus on the stem group of archigregarines
107 commonly found in Polychaeta, Sipunculida and some Hemichordata. These marine
108 gregarines represent the earliest diverging lineage of Apicomplexa (Leander 2007a;
109 Schrével 1971b). The type species of archigregarines is *Selenidium pendula* Giard 1884 and
110 its life cycle was established during the second part of the 20th century (Schrével 1966,
111 1970). Beside this type species, a long series of contributions have been performed on other
112 *Selenidium* and related species at the cytological (Brasil 1907, Caullery and Mesnil 1899,
113 2000, Ray 1930, Reed 1933) and ultrastructural (Leander 2006, 2007b; Macgregor and
114 Thomasson 1965; Schrével 1968, 1970, 1971a; Simdyanov and Kuvardina 2007, Vivier
115 and Schrével 1964, 1966) levels and more recently also at the molecular level through the
116 analysis of SSU rDNA sequences (Leander et al. 2003; Leander 2006, 2007b; Rueckert and
117 Leander 2009; Wakeman and Leander 2012, 2013; Wakeman et al. 2014). Most of these
118 studies focused on the trophozoite stages with few descriptions on nutrition modalities
119 (Schrével 1968; Simdyanov and Kuvardina 2007). Additionally, these studies highlighted
120 several incongruities among Selenidiidae at the molecular level that could not be elucidated
121 in absence of the type species of the family. Here, we report on the cell organization of the
122 *Selenidium pendula* trophozoite with a special attention to the conoid, the abundance of
123 rhoptries and micronemes, and we provide the first ultrastructural description of the syzygy
124 (pairing stage), the early gamogony with the cryptomitosis and the secretion of the cyst
125 walls. We also provide the first phylogenetic analysis of the SSU rDNA gene sequences
126 encompassing the type species of archigregarines *S. pendula*. Molecular phylogenetic
127 analyses revealed three lineages within archigregarines, *S. pendula* belonging to the
128 Selenidiidae that includes parasites of Spionidae, Sabellidae, and Sabellariidae, all
129 polychaete annelids, as well as two Selenidiidae-like lineages, parasites of hosts belonging
130 to Terebellidae and Sipunculida, respectively.

131

132 **Results**

133

134 **The Trophozoite of *Selenidium pendula***

135 The mature *S. pendula* trophozoite is a crescent-shaped cell of about 150 μm in length with
136 a circular cross section of about 35 μm in diameter. The cell surface exhibits about 30
137 striations in phase contrast light microscopy as well as in scanning electron microscopy
138 (SEM), appearing as a series of longitudinal bulges of about 2.5-3 μm in width separated
139 by grooves (Fig. 1A). The trophozoite is inserted into the intestinal epithelium of the
140 *Scoelepis squamata* polychaete worm by a special apical apparatus called the mucron (Figs
141 1B, 2A-C). In transmission electron microscopy (TEM), a tropism for host cells rich in
142 granules can be observed (Fig. 1B). The mucron of *S. pendula* corresponds to the
143 attachment apparatus anchoring the parasite to the host epithelial cell. In SEM, the mucron
144 appears as a regular mammiliform area without bulges and grooves (Fig. 2A). After
145 detachment of the trophozoite, the trace of the mucron in the host cell is very regular with
146 sometimes a small hole in a subcentral position (Fig. 2B).

147 A series of short microvilli is seen at the periphery of the epithelial cells (Fig. 2B).
148 All around the trophozoite attachment, numerous long ciliary structures of the host
149 epithelium are observed (Figs 1A, 2B).

150 Asexual schizogony in *S. pendula* could be an explanation to the exceptional
151 clotting of trophozoites, with thousands and thousands of cells that obstruct the intestinal
152 lumen of some *Scoelepis squamata* hosts. In vivo, trophozoites are dispersed along the
153 host intestine, except for the first thirty segments. The distinction between these two
154 intestinal regions is facilitated by the yellow color of the first segments versus the green
155 color of the posterior region. Motility of the *S. pendula* trophozoites is clearly of pendular
156 type, as proposed by Giard (1884) for the species diagnosis, and the stroboscopic records
157 show regular pendular beats with a period of about 0.2 second (Golstein and Schr vel 1982
158).

159 In TEM cross sections, the bulges of *S. pendula* exhibit a characteristic
160 ultrastructure described for the first time in *Selenidium hollandei* (Vivier and Schr vel
161 1964). The plasma membrane is underlain by a regular flat vesicle designated as the inner
162 membrane complex (imc) while a very slight cell coat covers the cell surface. Under these
163 three cortical membranes, a regular set of longitudinal subpellicular microtubules and some
164 other dispersed microtubules within the cortical cytoplasm are seen below the bulges but
165 not in the area of the grooves (Fig. 3B-C). In TEM cross sections, each subpellicular
166 microtubule of *S. pendula* is surrounded by an electron-lucent sheath (Fig. 3C) as observed
167 in *S. hollandei* (Vivier and Schr vel 1964), *Platyproteum (Selenidium) vivax* (Leander

168 2006), *Selenidium serpulae* (Leander 2007) and *Selenidium terebellae* (Wakeman et al.
169 2013). Abundant mitochondria are present under the subpellicular network of the bulges.

170 Different ectoplasmic structures along the grooves are observed with lamellar
171 elements, dense material structures that crossed the imc and are in contact with the plasma
172 membrane (Fig. 3B, D-F). Under SEM, series of holes are observed in the grooves with an
173 irregular distribution and distances ranging from 0.3-0.4 μm to 0.8-0.9 μm (Fig. 3A). Such
174 a distribution seems to correspond to the opening sites of the above-mentioned ectoplasmic
175 structures and their density might indicate a role that was previously underestimated.

176 Interestingly, the longitudinal microtubular bundles, abundantly distributed beneath
177 the cortex in the trophozoite apical part corresponding to the mucron, could represent the
178 biogenesis site of the longitudinal networks of the subpellicular microtubules (Fig. 2D).

179

180 **Conoid and Myzocytosis**

181 The conoid of *S. pendula* is a truncated cone of about 225 nm height, with apical and distal
182 diameters about 260 nm and 1 μm respectively (Fig. 4A-C). In TEM cross sections, the
183 diameter of filaments is about 23-32 nm; 9 sections are well identified in one side of the
184 conoid, while only an opaque layer can be observed on the other side due to their spiral
185 organization (Fig. 4B-C). This structure is quite similar to the well-described conoid of
186 *Toxoplasma gondii* (Hu et al. 2002, 2006) but the apical polar ring is not present in the
187 distal part of *S. pendula* mucron and the preconoidal rings are not clearly identified in its
188 apical part but a dilatation of the imc and the ends of the subpellicular microtubules are
189 unambiguously demonstrated (Fig. 4A, C, white arrows). This imc dilatation could
190 correspond to a site of a Microtubule Organizing Center (MTOC) able to generate the
191 subpellicular microtubules since abundant bundles are found in the anterior area of the
192 trophozoite (Fig. 2D). In few TEM cross sections, dense structures corresponding to the
193 neck of the rhoptries are observed inside the conoid (Fig. 2C).

194 Myzocytosis, the predatory mode of nutrition characteristic of archigregarines, is
195 clearly illustrated in *S. pendula* with food vacuoles inserted inside the conoid (Figs. 2C,
196 4A-B). In the axis of the mucron, one or several clear food vacuoles, likely formed via the
197 conoid, are present (Fig. 2C). These food vacuoles are surrounded by many rhoptries and
198 micronemes, two apical organelles characteristic of zoites (Figs 1B, 2C). As shown by the
199 continuity of the food vacuole membrane up to its contact with the host epithelial cell, an
200 evagination process through the apex of the conoid has occurred, allowing the parasite to
201 suck out the nutriments from the host. This myzocytosis process starts at the top of the

202 conoid (Fig. 4A). The food vacuoles are large, reaching sometimes up to 7 μm , and several
203 additional food vacuoles of about 2 or 3 μm are observed in the axis of the trophozoite
204 (Figs 2C, 5A-B). The lumen of the food vacuoles has a low electron-dense aspect with
205 some vesicles and the membrane of the food vacuole exhibits a very irregular border with
206 numerous digitations.

207 Vital staining with low concentrations of neutral red (1 ‰) allowed to visualize
208 large vacuoles of about 4x2 μm located in the apex of the *S. pendula* trophozoite with
209 several small vesicles (data not shown). This observation is in agreement with a
210 fragmentation of the initial food vacuole into numerous vacuoles present in the anterior part
211 of the trophozoite (Fig. 5A).

212

213 **Rhoptries, Micronemes, and Intrareticular Granules in Trophozoites**

214 In addition to the conoid, the apical end of *S. pendula* trophozoites exhibits about 8-10
215 rhoptries corresponding to the long, electron-dense club-shaped, tubular or saccular
216 organelles. They appear in the trophozoite as cylindrical organelles reaching up to 6 μm in
217 length, with a diameter of 0.3-0.4 μm in the basal bulbous. At the apex, a rhoptry neck
218 could be observed. The rhoptry orientation usually follows the direction of the conoid. In
219 some cases, the rhoptry neck penetrates the conoid (Fig. 2C).

220 The rough endoplasmic reticulum (RER) and the Golgi apparatus of *S. pendula*
221 show an original association between the swollen cisternae containing numerous
222 intrareticular granules of about 0.5-1 μm and the first saccule of the cis-region of the Golgi
223 apparatus (Fig. 6D). Similar associations are observed in *S. hollandei* (Vivier and Schr vel
224 1966) but not in Selenidiidae species parasitizing Cirratulidae (Schr vel 1971), Serpulidae
225 (Leander 2006), Terebellidae (Wakeman et al. 2014) or Sipunculida (Simdyanov and
226 Kuvardina 2007, Leander 2007). Some micrographs show an accumulation of numerous
227 micronemes close to the nuclear envelope (Fig. 7D) or to the Golgi apparatus (Fig. 6C)
228 with annular sections likely corresponding to the neck of micronemes (Fig. 6B). The
229 relation of these RER-Golgi apparatus to the biogenesis of the rhoptries and/or the
230 micronemes is not clear, since numerous micronemes are mixed with large rhoptries (Fig.
231 6A).

232

233 **Nucleus and the Perinuclear Cytoplasm**

234 The ovoid nucleus of the *S. pendula* trophozoite is characterized by the presence of a large
235 spherical nucleolus of about 4-5 μm in diameter (Fig. 7A). No accumulation of chromatin
236 is observed in the nucleoplasm and the nuclear envelope lacks the nuclear lamina as
237 observed in *S. hollandei* (Schrével 1971a; Vivier 1967). The nuclear envelope, typically
238 comprising two membranes, is rich in nuclear pores (about 5 per μm) regularly distributed
239 all over the entire nuclear surface (Fig. 7C). In tangential sections, the pores appear as rings
240 of about 100-110 nm in their largest diameter with the presence of a central particle of
241 about 10 nm in diameter (Fig. 7C).

242 The periphery of the nucleus exhibits a special cytoplasmic area comprising a
243 regular, 0.5 μm thick fibrillar zone, lacking any organelle, and surrounding the nucleus in a
244 distance of 1.5-2 μm from the nuclear envelope (Fig. 7A-B, E). This fibrillar zone
245 corresponds to the axial ducts described in living cells (Schrével 1970).

246

247 **Apicoplast-like Organelles**

248 In the trophozoite of *S. pendula*, organelles with four membranes are frequently observed
249 (Fig. 5C) and they appear morphologically similar to the apicoplast of *Toxoplasma* and
250 *Plasmodium* (Lim and McFadden 2010) with some dense structures (Fig. 5D) or in contact
251 with multilamellar organelle (Fig. 5E).

252

253 **Nuclear Multiplication During the Syzygy and Young Gamonts**

254 The sexual phase of the *S. pendula* life cycle starts with the syzygy, characterized by the
255 pairing of two haploid trophozoites, now called gamonts: one male and one female. During
256 the young syzygy stage of *S. pendula*, the two gamonts are linked by their posterior parts,
257 while their pendular motility continues with waves starting from the apex to the posterior
258 end (Schrével, 1970).

259 In TEM, each gamont exhibits a similar intracellular organization with a nucleus of
260 about 20 μm in diameter containing a spherical nucleolus of about 5 μm in diameter
261 (Supplementary data 1). In each nucleolus, several clear areas are observed with sizes
262 varying from 0.3 to 1 μm in diameter (Supplementary data 1, arrows). The cell surface and
263 the cytoplasm of the two gamonts also exhibit a similar organization (Supplementary data
264 1).

265 A clear characteristic of archigregarines belonging to the family Selenidiidae is the
266 early nuclear multiplication within the two gamonts at the site corresponding to the initial
267 trophozoite nucleus that occurs before the encystment of the gamonts. The localization of

268 the nuclei at the initial site of the trophozoite nucleus is clearly shown by the DAPI staining
269 highlighting the DNA-containing structures (Fig. 8A-B). Bright spots are observed inside
270 spherical structures, each of them corresponding to a nucleus. In about two hours, the
271 pendular motility of each gamont is progressively reduced and cyst formation occurs with a
272 widening of the nuclear zone in the gamont's median plane (Fig. 8A-B). In TEM, the
273 concentration of the nuclei at this stage is not easy to observe due to the relatively high rate
274 of this process. In favourable cross sections, the nuclei are observed in the central area of
275 the gamont and before the secretion of the cyst wall. Each spherical nucleus is about 5 μm
276 in diameter (Fig. 8C). From this central site, the nuclei migrate to the periphery of each
277 gamont while the cyst wall is forming (Fig. 8D). In many nuclei of the gamonts,
278 centrocones and other stages of cryptomitosis were detected.

279

280 **Centrocones and Cryptomitosis in Gamonts**

281 The mitosis in *S. pendula* gamonts is a closed-mitosis, also called cryptomitosis, with the
282 persistence of the nuclear envelope as observed in all Apicomplexa (Francia and Striepen
283 2014). All the nuclei of the *S. pendula* gamonts are spherical with a diameter of about 5 μm
284 and many are associated to a cupule with microtubules radiating from the Microtubule
285 Organizing Center (MTOC) in order to form half-spindles (Fig. 9A-B). The chromatin is
286 localized all around the internal face of the nuclear envelope as shown in TEM images (Fig.
287 9A-B). This chromatin forms an electron dense filamentous network with spotty dark nodes
288 and in some cases, an important dense accumulation is observed inside the nucleoplasm
289 (Fig. 9A). This dense accumulation of at least 1 μm could correspond to the bright spots
290 visualized by the DAPI-staining (Fig. 8B). The distribution of chromatin in *S. pendula*
291 nucleus appears as a continuous filamentous network quite similar to the model of
292 Apicomplexa cryptomitosis proposed by Francia and Striepen (2014).

293 In *S. pendula* gamonts, many nuclei exhibit a centrocone resulting probably from
294 the high rate of nuclear divisions since the chronology from the syzygy to the encystment
295 of the gamonts represents only 2-3 hours (Schrével 1970). The centrocone depends upon
296 the MTOC that appears as an electron dense annular structure of about 200 nm in diameter.
297 From the MTOC, microtubules radiate and form a half-spindle that pushes the nuclear
298 envelope without penetration in the nucleus (Fig. 9A). The resulting cupule exhibits an
299 outer diameter of about 1.6-1.9 μm and the distance from the MTOC to the inner border of
300 the cupule is about 1.4-1.6 μm . This typical centrocone can duplicate and the second
301 centrocone migrates to the opposite direction of the initial cupule (Fig. 9B). Micrographs

302 with two centrocones are rather rare and an intranuclear spindle was not observed most
303 likely due to the high rate of the progamic nuclear division in *S. pendula*.

304 As the progamic nuclei migrate from the central part of the gamont to the periphery,
305 the cryptomitosis continues after this migration, since the duplication of the centrocones is
306 observed in the border of the cyst where the wall is secreted.

307

308 **Modifications of the Gamont Cell Surface and Secretion of the Gametocyst Wall**

309 When the gametocyst wall is forming, the cortical membranes of each gamont are strongly
310 modified (Fig. 10A-C). The plasma membrane is always present but the imc is disorganized
311 with a series of folds and clear dissociation from the plasma membrane (Fig. 10B-C). In
312 TEM, the gametocyst wall exhibits two major layers, a homogeneous internal layer of
313 about 500-700 nm in thickness and a fuzzy external layer with long filaments reaching
314 about 300 nm. The total thickness of the gametocyst wall at the beginning of gamogony is
315 about 1 μm . The secretion of this wall is the result of two types of vesicles, one with rather
316 electron dense components (vesicle 1) and the second with a network of very spotty
317 filaments (Fig. 10A). The mechanism of discharge of these two types of vesicles was not
318 clearly observed. As the gametocyst wall formation occurs only two hours after the early
319 syzygy step, the secretion is probably the result of accumulations of numerous intrareticular
320 granules in the cisterns of the rough reticulum endoplasm that represent storage material for
321 this process (Fig. 6D). However, a potential dual function of the RER-Golgi apparatus for
322 both the formation of rhoptries and micronemes and the storage of material for gametocyst
323 formation needs further investigations (Fig. 6A).

324

325 **The Gametocyst and the Sporocyst Walls**

326 The gametogenesis is a fast process in *S. pendula*, lasting about one hour (Schrével 1970).
327 After the series of progamic nuclear divisions yielding syncytium nuclei in the same
328 gametocyst, cellularization occurs, producing flagellated male gametes and female gametes
329 without flagellum. In TEM, the gametocyst wall is more compact with dense layers (Fig.
330 11A-B). The fuzzy coat observed at the beginning of the gamogony is now very irregular in
331 width and the internal homogenous layers are more electron dense (Fig. 11A). In some
332 cases the internal layers show a regular opaque layer of 0.3 μm and an irregular
333 homogenous layer with a lower electron density (Fig. 11A).

334 In cross sections, the flagellar axoneme of the male gamete of *S. pendula* exhibits a
335 9+0 pattern (Fig. 11B2). After fecundation, the life cycle moves into the sporogony phase
336 with the formation of sporocysts corresponding to the evolution of the zygotes toward the
337 sporozoite formation inside each sporocyst. A new secretion process occurs around this
338 sporocyst (Fig. 11C). The thickness of the sporocyst wall is about 0.1 μm with small thin
339 spine-like digitations of about 0.2 μm (Fig. 11C).

340

341 **Molecular Phylogenetic Analyses of the SSU rDNA Sequence**

342 Type species are important to build solid bridges between molecular phylogenies and
343 taxonomy. A phylogenetic tree was constructed using 115 sequences including nine novel
344 small subunit (SSU) rDNA sequences (two sequences from *S. pendula*, the type species for
345 Selenidiidae, one from *S. hollandei*, one from *Lecudina pellucida*, the type species for
346 Lecudinidae, and 5 from *L. tuzetae*, all specimen isolated from host organisms collected in
347 the Roscoff area, France) and 106 previously published ones available from public
348 databases, taking into account all available data for archigregarine species (Table 1).
349 Sequences known to produce extreme long branches in SSU rDNA-based phylogenies,
350 such as those of the gregarines *Trichotokara* spp. and *Pyxinia robusta*, were excluded from
351 this analysis. Globally, the Maximum Likelihood and the Bayesian tree topologies were
352 congruent (Fig. 12) and in good agreement with recently published phylogenies (Wakeman
353 and Leander 2013; Wakeman et al. 2014). The two early lineages emerging among
354 Apicomplexa were from marine gregarines with archigregarines and eugregarines.
355 Interestingly the phylogenetic position of the type species *Lecudina pellucida* (Fig. 12) fell
356 within the Lecudinidae, with a good support with lecudinids of tunicates represented by the
357 *Lankesteria* genus. In the terrestrial gregarines, the *Gregarina* lineage belongs to rather old
358 insects such as Coleoptera, Blattaria, and Orthoptera, in contrast to the *Ascogregarina*
359 lineage that infects more recent insects according to the most recent knowledge on insect
360 evolution (Misof et al. 2014).

361 An analysis of the SSU rDNA sequences clearly demonstrated the paraphyly of
362 Selenidiidae, which are split into three major groups (Fig. 13, Supplementary Material 2-4).
363 The type species *Selenidium pendula* is closely related to *Selenidium boccardiella*
364 (Wakeman and Leander 2013). These two gregarines infect members of the Spionidae
365 family of Polychaeta. Similarly, *S. hollandei* is closely related to *S. neosabellariae* and *S.*
366 *identhyrsae* (Wakeman and Leander 2013), these three species being parasites of hosts

367 belonging to Sabellariidae. Parasites infecting Spionidae and Sabellariidae diverged from
368 3.4 to 13.7 % from each other (sequence identity, Supplementary Material 2, 3).

369 *Selenidium* parasites of Terebellidae group form a second divergent lineage with a
370 wider global divergence with true Selenidiidae of 25.8-28.2 %, (Supplementary Material 2,
371 3). Finally, all Selenidiidae described in *Phascolosoma* formed a third group which is the
372 most divergent (26.3 - 28.8 % of divergence with the two precedent groups, Supplementary
373 Material 2, 3).

374

375 **Discussion**

376

377 ***Selenidium* spp. and archigregarines**

378 Since 2003, the morphology of some trophozoites of Selenidiidae and related
379 archigregarines was investigated using SEM and more than 25 SSU rDNA sequences were
380 deposited in the GenBank/EMBL/DDBJ databases (Leander 2003, 2007; Leander et al.
381 2003; Rueckert and Leander 2009; Wakeman and Leander 2012, 2013; Wakeman et al.
382 2014). However, data on sexual stages (gamogony and sporogony) were missing. By
383 combining electron microscopic descriptions with phylogenies using SSU rDNA sequence
384 data, new *Selenidium* species have been proposed such as *S. pisinnus* Rueckert and
385 Leander, 2009, *S. boccardiella* Wakeman and Leander, 2012, *S. idanthyrasae* Wakeman and
386 Leander, 2012, *S. neosabellariae* Wakeman and Leander, 2013, *S. sensimae* Wakeman and
387 Leander, 2013 and *S. melongena* Wakeman et al., 2014. A new genus *Platyproteum*
388 (Rueckert and Leander 2009) was erected to replace the former species *Selenidium vivax*
389 (Gunderson and Small 1986). A new enigmatic genus related to archigregarines was also
390 proposed as *Veloxidium* (Wakeman and Leander 2013). In their discussion, Leander and
391 co-workers produced a comparative table with morphological data of all Selenidiidae
392 described in the last century (Ray 1930; Schr vel 1970, 1971). Few mistakes within
393 Selenidiidae were reported in this table (table 2 in Wakeman and Leander 2012) as for
394 example the mention of *S. spionis*, presented as a parasite of *Polyrabdina spionis*.
395 *Polyrhabdina spionis* is in fact a lecudinid gregarine and the host of *S. spionis* is the
396 polychaete *Scolelepis fuliginosa* (Clapar de, 1870) now called *Malacoceros fuliginosus*
397 (Clapar de, 1870) (Schr vel and Desportes, 2013). However, the SSU rDNA sequences
398 analysis of *S. spionis* revealed lineages inside archigregarines and Leander and co-workers
399 underlined the importance of future work on additional *Selenidium*-like gregarines

400 especially the type species *S. pendula* (Wakeman et al 2014). This current work on the type
401 species *S. pendula* Giard, 1884 and on *S. hollandei* Vivier and Schrével, 1966 therefore
402 enlighten with less ambiguities parts of the evolutionary history of archigregarines.

403 The SSU rDNA sequence phylogeny trees with the different SSU rDNA sequences
404 of archigregarines (Table 1), show three clearly delimited lineages among Selenidiidae
405 (Figs 12-13, Supplementary Material 3, 4). A major group corresponds to the true-
406 *Selenidium* lineage, for which sexual stages (syzygy to sporocyst) have been described. Its
407 members are parasites of Sedentaria polychaetes, such as *S. pendula* that infects the
408 Spionidae family, *S. hollandei* infecting Sabellariidae and *Selenidium* cf. *meslini* infecting
409 Sabellidae. These true-*Selenidium* share common important features, such as a nuclear
410 multiplication during the syzygy, the gamogony and the sporocysts with usually four
411 sporozoites. Many archigregarines have developed atypical variations in their cell
412 morphology and their motility from pendular to rolling type, with subpellicular microtubule
413 sets under the inner membrane complex (imc), but without the gliding type observed in
414 eugregarines. Trophozoites of true-*Selenidium* exhibit a three-membrane cortex where the
415 imc forms a complete envelope underlying the plasma membrane, with sets of longitudinal
416 subpellicular microtubules running under the large folds designated as bulges (Schrével
417 1970a, 1971a; Schrével et al. 2013; Vivier and Schrével 1964). The grooves correspond to
418 the striations well described this last century by light microscopic (Brasil 1907; Ray 1930;
419 Schrével 1970). The cytoplasm beneath the grooves is devoid of microtubules but exhibits
420 micropores and residual membranous organelles in connection with the imc (Schrével et al.
421 2013; Vivier and Schrével 1964). These parasites feed by myzocytosis using the conoid
422 located at the apex of the trophozoite (Schrével 1968, Simdyanov and Kuvardina 2007, this
423 work).

424 In this true-*Selenidium* lineage, the sexual stage starts by the syzygy where the
425 formation of progametic nuclei is observed inside the gamont nucleus before encystment.
426 This observation in the type species *S. pendula* (Fig. 8) is the confirmation of histological
427 previous descriptions by Caullery and Mesnil (1900), Ray (1930), Reed (1933), Tuzet and
428 Ormières (1958) and in vivo observations by Schrével (1970). This gamogony is quite
429 different from all other eugregarines where the first gamogony division starts inside the
430 cyst and is followed by successive series of nuclear divisions called progamic mitoses
431 without cytokinesis such as in *Lecudina tuzetae* (Kuriyama et al. 2005). So, the *Lecudina*
432 type gamogony produces a syncytium until the cellularization process yielding the gametes.
433 Another clear difference concerns the degree of condensation of the chromatin with a

434 continuous filamentous network attached to the nuclear envelope all around the nucleus in
435 *S. pendula* cryptomitosis. Chromosome condensation does not seem to occur in *S. pendula*
436 in contrast to cryptomitosis of *L. tuzetae* (Kuriyama et al. 2005) and *Grebniackiella gracilis*
437 (Moblion-Noblot 1980). Sporogony then leads to spherical sporocysts that differentiate
438 usually into four sporozoites per sporocyst (Ray 1930; Schrével 1970).

439 Other *Selenidium*-like species infecting sipunculids and Terebellidae are only
440 known through their trophozoites and their localization within hosts (Leander 2006;
441 Wakeman et al. 2014). The intestinal trophozoite of *S. terebellae* Ray 1930 exhibits large
442 bulges but differences with the true-*Selenidium* have been observed. As an example, a
443 regular layer of about 30-33 nm in thickness (Supplementary Material 5 and Wakeman et
444 al. 2014) similar to the internal lamina of eugregarines (Schrével et al. 1983) or to some
445 euglenoid cortex (Mignot 1966) is attached to the imc. Numerous sets of longitudinal
446 subpellicular microtubules are immediately under this regular dense layer and many
447 residual membranous organelles are highly concentrated under the imc of the grooves
448 (Supplementary Material 5). *S. melongena* trophozoites were described in the same host as
449 *S. terebellae*, but inside the coelom, an unusual localization for archigregarines (Wakeman
450 et al. 2014). The cortex of *S. melongena* exhibits 30-40 epicytic folds helically arranged
451 along the axis of the cell. Surprisingly, although the subpellicular sets of microtubules were
452 not observed in TEM, a strong fluorescent labelling of alpha-tubulin was detected below
453 the helical folds. *S. melongena* are non-motile without pendular or rolling motility nor
454 gliding. According to Wakeman et al. (2014), such atypical cell organization of *S.*
455 *melongena* trophozoites seems to be closer to lecudinids than to Selenidiidae. These
456 original observations as well as the lack of description of syzygy and sporocysts require
457 future work, especially to explain the way by which *S. melongena* can infect its host.

458

459 The third *Selenidium*-like lineage described here corresponds to a group of intestinal
460 parasites of Sipunculida. These parasites are mainly known from SEM and TEM
461 observations on *Selenidium vivax* trophozoites (Leander 2006). Renaming of *S. vivax* as
462 *Platyproteum vivax* was supported by archigregarine flat shape when observed under TEM
463 with important sets of longitudinal subpellicular microtubules and numerous mitochondria
464 probably in relation to the very active plasticity of *S. vivax* (Rueckert and Leander 2009).
465 This cellular organization appears similar to that of *S. hollandei* (Schrével 1970). Here also,
466 descriptions of the syzygy with their characteristic progametic nuclei as well as the
467 sporocysts require clarifications. This point is also important for *Filipodium* trophozoites

468 where numerous microvilli from 1.6-10 μm long and about 0.15 μm in diameter were
469 clearly described in TEM (Hoshide and Todd 1996).

470 All archigregarines are intestinal parasites of Annelida belonging to the clade
471 Sedentaria except one *Selenidium metchnikovi* reported in Hemichordata (Léger and
472 Duboscq 1917). In contrast, many lecudinids are intestinal parasites of the clade Errantia
473 from Annelida (Schrével and Desportes 2013). This separation between archigregarines and
474 marine lecudinid eugregarines is probably related to the different modes of living of their
475 hosts. For instance, lecudinids are adapted to the errant and predatory life of Errantia while
476 archigregarines are adapted to sedentary life of Sedentaria with microphage species living
477 below stones, or as tube builders or ingesting sediment as the representatives of the family
478 Spionidae or surface deposit feeders with head appendages (Sabellidae, Sabellariidae).
479 Evolutionary history of Annelida is still poorly understood as the classic morphological
480 cladistic analysis with a monophyletic Polychaeta (Rouse and Fauchald 1997) was
481 challenged in the light of the recent molecular evidences. Today, Polychaeta are inferred to
482 be paraphyletic with the inclusion of the Clitella (earthworms) and the non-segmented taxa
483 Echiura and Sipunculida (Struck et al. 2011). Complexity of the phylogeny of *Selenidium*
484 species may reflect the one of their hosts. The true-*Selenidium* lineage within the
485 Selenidiidae family likely forms the core of archigregarines while the two other distant
486 lineages infecting respectively the Sipunculids and Terebellids orders, could be considered
487 as related *Selenidium*-like lineages. These results, deduced from molecular phylogeny
488 analyses need to be confirmed at the biological and cellular levels but are crucial since they
489 open new trends in evolutionary history among Apicomplexa.

490 The enigmatic *Veloxidium leptosynaptae*, initially placed within archigregarines
491 after phylogenetic analyses (Wakeman and Leander 2012) was later included within
492 lecudinids and urosporids (Wakeman et al. 2014). In our phylogenetic studies, it also
493 groups with lecudinids and urosporids with strong supports (Fig. 12).

494

495 **Apicoplasts, Conoid, MTOC and Rhoptries are Major Cell Structures in the** 496 **Evolution of Apicomplexa**

497 Gregarines represent interesting models to investigate the evolution from free-living
498 flagellated alveolates status, likely photosynthetic, to obligatory parasites among
499 Apicomplexa.

500 In archigregarines, the presence of an apicoplast remains an open question. Presence
501 of a functional plastid is reported in *Chromera*, a free-living photosynthetic relative to

502 Apicomplexa (Lim and McFadden 2010). The apicoplast, a non-photosynthetic plastid of
503 red algae origin, is well documented in some Apicomplexa species such as *Plasmodium*,
504 *Toxoplasma*, *Eimeria*, *Babesia*, *Theileria*. This relict plastid is limited by four membranes
505 indicating its secondary endosymbiont origin. In the eugregarine *Gregarina niphandrodes*,
506 the apicoplast seems to be absent (Toso and Omoto 2007). Here in *S. pendula*, apicoplast-
507 like organelles are regularly observed in trophozoites at the ultrastructural level.
508 Interestingly Ray (1930) reported the visualization of a dark spot stained with Heidenhain's
509 haematoxylin, associated to each merozoite nucleus in *S. mesnili* parasitizing the
510 polychaete *Myxicola infundibulum*. Such an observation at the light microscopy level was
511 also observed by TEM, revealing the presence of an organelle with four membranes close
512 to the anterior part of each *S. hollandei* merozoite (Schrével 1971b).

513 The apical phagotrophy in the free-living predators of alveolates, with open conoid
514 and rhoptries, may be at the origin of the anchoring device of archigregarines like
515 *Selenidium*, characterized by their mucron and the myzocytosis function. The conoid of *S.*
516 *pendula*, similar to that of *S. hollandei* (Schrével 1968) and *S. orientale* (Simdyanov and
517 Kuvardina 2007), is conserved in large trophozoites and appears similar to the conoid of
518 sporozoites from eugregarines *Stylocephalus africanus* (Desportes 1969) and
519 *Ascogregarina (Lankesteria) culicis* (Sheffield et al. 1971). Among Conoidasida, the
520 conoid of *T. gondii* is the most investigated at the structural and molecular levels, with the
521 construction of unique coma-shaped tubulin sheets to form a spiral cone-shaped structure
522 (Hu and Murray 2002; Hu et al. 2006). As *S. pendula* is the archigregarine type species and
523 an early branching Apicomplexa, its conoid appears a good model to study the transition
524 between Apicomplexa with closed conoid and free-living alveolate ancestors with open
525 conoid, as found in the early branching dinoflagellates as *Colpodella* (Brugerolle 2002;
526 Leander et al. 2003) or *Psammosa pacifica* (Okamoto and Keeling 2014).

527 Recently, the hypothesis of molecular links between Apicomplexa and algal
528 ancestors was suggested with the demonstration of similar components in the apical
529 complex of Myzozoa and the flagellar apparatus of protists. This hypothesis was mainly
530 supported by the localization of striated fiber assemblies (Francia et al. 2012) and SAS-6
531 proteins (de Leon et al. 2013). *T. gondii* striated fiber assemblies (TgSFA2 and TgSFA3)
532 proteins whose orthologs are found in the rootlet associated with the basal bodies from
533 green algae, polymerize into a dynamic fiber that emerges from the centrosomes
534 immediately after their duplication (Francia et al. 2012). Genetic experiments showed that
535 the two proteins TgSFA2 and 3 play an essential role in the cell division of the *T. gondii*

536 since cytokinesis is blocked in their absence. This Tg SFA fiber thus provides a robust
537 spatial and temporal organizer for the parasite cell division. Also, Francia et al. (2012)
538 indicated that other comparable SFA fibers were observed in previous ultrastructural
539 studies on *Eimeria* (Dubremetz 1973, 1975) and *Plasmodium* (Schrével et al. 2008).

540 The SAS-6 protein is well known in the centriolar biogenesis of eukaryotes from
541 protists to vertebrates (Leidel et al. 2005; van Breugel et al. 2011). This protein was
542 described in the centrocone during *T. gondii* cryptomitosis (de Leon et al. 2013). In
543 addition a novel SAS-6 like (SAS-6L) protein family that shares an N-terminal domain
544 with SAS-6 but without the coiled-coil tails was localized above the *T. gondii* conoid (de
545 Leon et al. 2013). Genomic analyses showed that SAS-6L is an ancient protein found in
546 diverse eukaryotic lineages: *Trypanosoma*, *Leishmania*, ciliates and Apicomplexa (Hodges
547 et al. 2010; de Leon et al. 2013). In *Trypanosoma brucei* trypomastigotes, the Tb SAS-6L
548 was observed near the basis of the flagellum, consistent with the basal body location. In *T.*
549 *gondii*, the Tb SAS-6L antibody labelled the apex of tachyzoites, and after conoid extrusion
550 triggered by ionomycin treatment, it labelled the tip of the “true” conoid. The SAS-6L and
551 SAS-6 antibodies did not colocalize in *T. gondii*, the former one labelling the centriole and
552 the latter one labelling the conoid tip (de Leon et al. 2013).

553 Complex connections between the “pseudoconoid” or “incomplete conoid” and the
554 flagellar apparatus were also shown, by conventional TEM and 3D reconstruction, in the
555 apical complex of *Psammosa pacifica*, a predator relative of apicomplexans and early
556 dinoflagellates (Okamoto and Keeling 2014).

557 The MTOC of the centrocones of *S. pendula* appears as a disc similar to that
558 observed in other eugregarines such as *L. tuzetae* where 9 singlets could be detected in
559 favourable TEM sections (Kuriyama et al. 2005). From these MTOC discs, microtubules
560 radiated to form a cone involved in the cup-shaped invaginations of the nuclear envelope.
561 The continuity of these MTOC during the life cycle could be in agreement with a
562 centriolar-like structure since a 9+0 axonemal pattern is observed in *S. pendula* male
563 gamete (Fig.11B). The question of the subpellicular microtubule biogenesis is not clear.
564 The conoid is not, by itself, the MTOC since it is absent in the zoites of Hematosporida and
565 Piroplasmida. The two polar rings, observed at the apex of the *Eimeria* or *Plasmodium*
566 zoites were proposed as the MTOC sites generating the subpellicular microtubules (Russel
567 and Burns 1984), but these two polar rings were not observed in *S. pendula*. The imc
568 dilatation at the border of the proximal opening of the conoid could fulfil this function (Fig.
569 4). The exceptional accumulation of microtubule bundles in the anterior part of the mucron,

570 before the regular subpellicular microtubule sets of the epicytic bulges (Fig. 2D), is in
571 agreement with the strong labelling of *S. melongena* apex with fluorescent anti-alpha
572 tubulin (Wakeman et al. 2014). Biogenesis of these abundant microtubule bundles needs
573 further analysis.

574 Rhoptries are characteristic of the apicomplexan zoites and also of the Selenidiidae
575 trophozoites (Schrével et al. 2013 for a review). Interestingly, presence of numerous
576 intracytoplasmic thread-like bodies described by Ray (1930) in the apex of different
577 *Selenidium* trophozoites was visualized after iron haematoxylin staining (Heidenhain's
578 haematoxylin). By their sizes reaching 8-12 μm depending on the *Selenidium* species and
579 their localization, these thread-like structures could correspond to the rhoptries described
580 from TEM such as in *S. pendula* (Fig. 5A), *S. hollandei* (Schrével 1968) and *S. orientale*
581 (Symdyanov and Kurvidina 2007). Ray (1930) considered these thread-like structures as
582 one of the morphological characters of each *Selenidium* species, however the abundance of
583 rhoptries detected in TEM is in fact a general character for archigregarines (Schrével et al.
584 2013, for review). Biological functions of many apicomplexan rhoptry proteins remain
585 largely unknown. In *Plasmodium* and *Toxoplasma*, the most investigated apicomplexans at
586 the molecular level, there is growing evidence to suggest that the rhoptry neck proteins are
587 predominantly involved in host-cell adhesion with some sharing evolutionary origins
588 among apicomplexans. In contrast, the rhoptry bulb proteins appear mainly genus specific,
589 suggesting that they evolved secondarily to become highly specific to their host cells
590 (Counihan et al. 2013). In *S. pendula*, food vacuole membranes may have arisen from
591 numerous rhoptries localized within the apex. A strong membrane trafficking is expected to
592 produce the large and abundant food vacuoles observed during myzocytosis (Fig. 4A).
593 Therefore *Selenidium* rhoptry proteins could play a role in producing intracellular food
594 vacuole in contrast to Apicomplexa with an intracellular development, where the rhoptry
595 proteins seem involved in the parasitophorous vacuole elaboration such as in *Plasmodium*
596 and *Toxoplasma*.

597

598 **Archigregarines and Eugregarines: Two Early Branching Lineages Among** 599 **Apicomplexa**

600 The transition from the free-living alveolates to apicomplexan parasites was supported by
601 comparative ultrastructural studies and molecular phylogeny analyses of basal lineages,
602 such as dinoflagellates (together with perkinsids) and apicomplexans (including
603 colpodellids) (Leander and Keeling 2003). The myzocytosis is the most plesiomorphic

604 features of apicomplexans with archigregarines having a closed conoid (Schrével 1968,
605 1971b), and colpodellids the sister lineage of Apicomplexa with an open conoid (Kuvardina
606 et al. 2002). In perkinsids, representing the earliest diverging sister lineage of
607 dinoflagellates (Saldarriaga et al. 2003), an open conoid is also observed (Perkins 1996).
608 These three types of parasites also share rhoptry-like organelles and, together with their
609 phylogenetic positions, they confidently infer that a common ancestor of apicomplexans
610 and dinoflagellates had an apical complex involved in the acquisition of nutrients from the
611 cytoplasm of prey cells (Leander and Keeling 2003).

612 Among the high diversity of gregarines in invertebrates, Polychaeta, an animal class
613 known to be present at the Cambrian biodiversity explosion and to represent one of the
614 earliest Bilateria organisms (De Rosa et al. 2005; Schrével and Desportes 2013), is well
615 infected by gregarines. This situation supports the evolutionary prelude of marine
616 gregarines to the apicomplexan radiation (Leander 2007). The initial archigregarine
617 radiation is supported by the “hypersporozoite” cell organization of the trophozoite, the
618 myzocytosis and the pendular or rolling motility (Schrével 1971; Schrével and Desportes
619 2015). The subsequent eugregarine radiation, with an adaptation to the intestinal biome and
620 an extracellular development, could have emerged from intestinal lecudinid gregarines.
621 Here, their cell cortex is quite different from archigregarines by the presence of numerous
622 epicytic folds, without the regular sets of subpellicular microtubules but with a
623 sophisticated distribution of 12-nm filaments, apical rippled dense structures at the top of
624 the folds (Schrével et al. 1983; Vivier 1968). Their gliding motility depends upon an actin-
625 myosin system but the molecular mechanochemical properties are far from being
626 understood (Heintzelman 2004; Valigurová et al. 2013). The myzocytosis, similar to the
627 archigregarine model, is not observed in these marine eugregarines: their nutrition process
628 is realized through a bulbous attachment apparatus usually designated by mucron.

629 The gregarine colonization of the coelom in invertebrate hosts by transmigration of
630 the sporozoites through the intestinal epithelium and a coelomic development reveal
631 additional adaptations of eugregarines to their host environment. These adaptations are a
632 significant evolutionary step of marine gregarines as suggested by Leander (2007), and
633 represent an antithesis to any notion of “primitiveness”. One of the best evidence is the
634 unique adaptation of the coelomic eugregarine *Diplauxis hatti* to its host *Perinereis*
635 *cultrifera* where a strict synchronization is observed between the maturation of the
636 polychaete gametes and the sexual phases (gamogony and sporogony) of the parasite
637 (Prensier et al. 2008). This example illustrates how gregarines are well adapted to their host

638 environment. For instance, *D. hatti* is adapted to *P. cultrifera* but cannot invade other
639 Nereidae host as *Hedistes (Nereis) diversicolor* nor *Nereis pelagica*. The extreme
640 adaptation of some gregarines to their host environments could explain some unexpected
641 situations such as the reduction observed from the canonical 9+2 flagellar pattern, in the
642 male gametes, with a 9+0 pattern in *S. pendula* (this study), 6+0 in *L. tuzetae* (Schrével and
643 Besse 1975) and 3+0 in *D. hatti* (Prensier et al. 1980). The 9+0 pattern of *Selenidium*, close
644 to the 9+2 normality, may be correlated to a fertilization phase lasting about 1 hour in a 1-
645 day sexual phase (gamogony and sporogony), the 6+0 pattern of *L. tuzetae*, may result from
646 a fertilization realized in few hours within a cyst, during a 3 days sexual phase of the
647 *Lecudina* life cycle (Schrével 1969). More impressively, the 3+0 pattern in *D. hatti* could
648 have been selected over evolution because of the fertilization step lasting only few hours in
649 a highly extended complete life cycle, lasting 2.5 years (Prensier et al. 2008). Such
650 evolutionary proposal, suggesting that each gregarine develops its own programme
651 according to its environment is in agreement with the notion of regressive evolution in
652 microorganisms proposed by Lwoff (1944). This type of regressive evolution could
653 probably continue with other coelomic gregarines with the disappearance of the flagellum
654 in male gametes of *Gonospora* species as suggested from histology studies (Schrével 1963;
655 Trégouboff 1918). Expression of the own program of each coelomic eugregarines is also
656 observed with the variations in their epicytic cell surface transformations with digits,
657 surface swelling in *Pterospora*, microvillosities in *Diplauxis* or the development of
658 peristaltic motility instead of gliding, sometimes a pendular motility is observed in young
659 trophozoite and peristaltic motility during the fast growing period of the same trophozoite
660 as observed in *D. hatti* (see Schrével et al. 2013 for a review).

661

662

663 **Conclusion**

664

665 Molecular phylogenetic analyses of archigregarines demonstrate that *S. pendula*, the type
666 species of archigregarines, belongs to a lineage with a large number of *Selenidium* parasites
667 of Spionidae, Sabellaridae, Sabellidae, Cirratulidae families of the Sedentaria Polychaeta.
668 All these *Selenidium* exhibit similar biological characters such as the cell cortex with a
669 plasma membrane, imc (inner-membrane-complex) and subpellicular microtubules, the
670 apical complex with a conoid, the myzocytosis with large food vacuoles and abundance of

671 large rhoptry organelles, the nuclear multiplication during the syzygy and the early
672 gamonts. Two other *Selenidium*-like lineages are observed in the Terebellidae and
673 Sipunculida where the sexual characters are not available at this time. Such a status
674 underlines an adaptation of the family Selenidiidae to their host families and this first early
675 evolutive lineage could correspond to the transition step between the free-living flagellated
676 alveolates and the Apicomplexa, before the diversification of the marine eugregarines
677 without the typical myzocytosis realized through the conoid but with a gliding motility.

678
679

680 **Methods**

681

682 **Preparation of annelids and gregarines:** Isolates of the gregarine *Selenidium pendula*
683 Giard, 1884 type species, were collected from the intestine of the polychaete worm
684 *Scolecopsis squamata* (O. F. Müller, 1806) (previously named *Nerine cirratulus*, Delle
685 Chiaje, 1831) on the French coast of the English Channel at the "Station Biologique de
686 Roscoff", in 2007 then again in 2012. Isolates of the gregarines *Selenidium hollandei*
687 Vivier and Schrével, 1966, *Lecudina pellucida* (Mingazzini, 1891) type species and
688 different isolates of *L. tuzetae* Schrével, 1963 were also collected from the intestines of
689 polychaete worms from the same area, in 2007, 2012, 2013 and 2014 (Table 2).

690 After washing in seawater, each collected worm was kept, at the laboratory
691 temperature, in a separate Petri dish. The medium (seawater) was changed daily. For long-
692 term conservation, the collected worms were rinsed with 0.22 µm filtered seawater and
693 stored at 4 °C. In order to collect *Selenidium pendula* Giard, 1884, the anterior part of the
694 *Scolecopsis squamata* worms, with a yellow color, was discarded since the parasites were
695 always absent, then the worms were cut transversally in series of segments of about 1 to 1.5
696 cm of length. To collect *S. hollandei*, *L. pellucida* and *L. tuzetae*, a similar type of
697 microdissection was performed from their corresponding hosts, under a classic binocular
698 microscope, in order to expose the intestinal epithelial surface to the seawater. In addition,
699 and only in the case of *L. tuzetae*, cysts excreted with feces were collected from the Petri
700 dishes of individually kept *Neanthes (Nereis) diversicolor* (O. F. Müller, 1776).
701 Trophozoites of *S. pendula*, attached to the intestine, were easily detected, in spite of their
702 rather small sizes (usually 150 -180 µm x 30-35 µm), by their white color - contrasting to
703 the characteristic green color of the intestinal epithelium of the worm - and by their active

704 pendular movements. In highly infected *Scoelepis squamata*, trophozoites and sexual
705 stages of *S. pendula* (syzygies and young cysts) were also collected in Petri dishes, among
706 the gametes released from hosts during the dissection. *S. hollandei* trophozoites were easily
707 observed in host epithelium by their very active rolling movements, immediately after
708 sectioning the post abdominal segment of their hosts, *Sabellaria alveolata* Linnaeus, 1767.

709 **Electron microscopy:** For transmission electron microscopy (TEM), intestinal
710 epithelial tissues of *Scoelepis squamata* highly infected with trophozoites of *S. pendula*
711 were collected and fixed in 5 % (v/v) glutaraldehyde in 100-150 mM phosphate or 0.2 M
712 cacodylate buffer (pH 7.3), at 4 °C, for 6 to 12 hours. The syzygy and gametocytes of *S.*
713 *pendula*, not attached to the epithelium, were collected directly in the seawater from the
714 Petri dishes and fixed in the same conditions. After washing either in the same buffer or in
715 buffer containing 0.3 M sucrose, the samples were post-fixed with 1% (w/v) OsO₄ in the
716 same buffer for 1 hr, then processed through standard dehydration, infiltration, and
717 embedding procedures, in Epon or Araldite mixtures, with the corresponding solvents (i.e.
718 propylene oxide or acetone respectively), at room temperature. The blocks were thin
719 sectioned, collected on grids and stained with saturated uranyl acetate in 50% (v/v) ethanol
720 for 1-3 min then in lead citrate. Sections were observed with a Hitachi HU 11 E electron
721 microscopy (Hitachi Ltd, Japan) or a JEOL 1010 TEM.

722 For SEM, the intestines were open along the axis of the polychaete, and the body
723 parts highly infected by *S. pendula* were carefully washed in 0.22 µm-filtered seawater
724 before fixation in glutaraldehyde as done above for TEM. After the post fixation in 1%
725 OsO₄ in 0.2 M cacodylate buffer, specimens were dehydrated in a graded series of acetone,
726 critical point-dried in liquid CO₂ and coated with gold. The samples were examined in a
727 JEOL JSM-7401F FE SEM.

728 **DNA isolation and sequencing:** For the LG isolates (*S. pendula* LG, *S. hollandei*
729 LG, *L. pellucida* LG Table 1), groups of ~50-70 isolated trophozoites were washed at least
730 three times in 0.22 µm-filtered seawater and DNA was extracted from individual parasites
731 using a modified GITC (Guanidinium isothiocyanate) protocol (Chomczynski and Sacchi
732 2006). Individuals were placed in 50 µl of the GITC extraction buffer and crushed using an
733 adjusted micro-pilon (Kimble Chase®). Tubes were incubated at 72 °C for 20 min. Next,
734 one volume of cold isopropanol was added at -20 °C overnight for DNA precipitation. The
735 following day, samples were centrifuged (20,000 g, 15 min at 4 °C) and supernatants
736 removed. The DNA pellet was cleaned using 70% ethanol (100 µl), followed by a last
737 centrifugation (20,000 g, 10 min). Supernatant was removed and the DNA pellet was

738 hydrated into 20 μ l of sterile distilled water and stored at -20 $^{\circ}$ C. For *S. pendula* IF, a
739 group of \sim 50-70 isolated trophozoites were washed at least three times in 0.22 μ m filtered
740 seawater and genomic DNA was isolated by using a phenol-chloroform extraction
741 procedure as previously described for *Plasmodium falciparum* (Florent et al. 2000), and the
742 purified DNA pellet was rehydrated into 20 μ l of sterile distilled water and stored at -20 $^{\circ}$ C.

743 For *L. tuzetae* Roscoff 2012 IF462, DNA was isolated by using the phenol-
744 chloroform extraction procedure described above, from 2 cysts, collected from the feces of
745 a single *Neanthes (Nereis) diversicolor* (O. F. Müller, 1776) host individually kept in a
746 Petri dish. The purified DNA pellet was rehydrated into 20 μ l of sterile distilled water and
747 was stored at -20 $^{\circ}$ C. Finally, for the 4 remaining *L. tuzetae* Roscoff, DNA extractions were
748 performed using MasterPureTM Complete DNA and RNA Purification kit (Epicentre,
749 Illumina Inc. USA) following supplier's recommendations for Cell Samples manipulations,
750 with minor modifications, from respectively 7 cysts (IF131), 50 cysts (IF171 and IF172)
751 and 30 cysts (IF181). Briefly, each group of cysts was isolated from the feces of a single *N.*
752 *diversicolor* host individually kept in a Petri dish, from which each cyst was then
753 extensively washed, one by one, in three successive drops of 0.22 μ m filtered seawater
754 supplemented with antibiotics penicillin (100 U/mL), streptomycin (100 μ g/mL) and
755 gentamycin (50 μ g/mL) (Gibco, Life Technologies, USA) then pooled again. Then, isolated
756 and washed cysts were submerged in 300 μ L Tissue-and-Cell lysis solution, submitted to
757 five series of freezing (liquid nitrogen) and thawing (37 $^{\circ}$ C) before addition of Proteinase K
758 then RNase A and, after sample processing as recommended, isolated DNA pellets were
759 rehydrated in 35 μ l TE (10 mM Tris-pH 7.5 and 1mM EDTA) prior to subsequent storage
760 at -20 $^{\circ}$ C.

761 These DNA extraction products were then used as templates in various series of
762 PCR amplifications, in order to amplify the SSU rRNA gene of these gregarines, then
763 sequenced using the Sanger sequencing methodology.

764 **LG samples.** The PCR mix (15 μ l final volume) contained 1–6 μ l of the DNA extract,
765 330 μ M of each deoxynucleoside triphosphate (dNTP), 2.5 mM of MgCl₂, 1.25 U of
766 GoTaq® DNA polymerase (Promega Corporation), 0.17 μ M of both primers, 1 \times of buffer
767 (Promega Corporation). The PCR cycle, run in an automated thermocycler
768 (GeneAmp®PCR System 9700, Applied Biosystem, USA), was programmed to give an
769 initial denaturing step at 95 $^{\circ}$ C for 5 min, 35 cycles of denaturing at 95 $^{\circ}$ C for 1min,
770 annealing at 55 $^{\circ}$ C for 45 s and extension at 72 $^{\circ}$ C for 1 min 15 s, and a final extension step
771 at 72 $^{\circ}$ C for 7 min. PCR products were cloned into a TOPO TA cloning kit (Invitrogen®),

772 following manufacturer's recommendations. Inserts inside white colonies were screened by
773 PCR (same procedure as before). Positive PCR products were purified (ExoSAP-IT® For
774 PCR Product Clean-Up, USB®) and sequenced using the Big Dye Terminator Cycle
775 Sequencing Kit version 3.0 (PE Biosystems®) and an ABI PRISM model 377 (version 3.3)
776 automated sequencer with specific internal primers.

777 The list of primers used for both PCR amplifications and Sanger sequencing is provided in
778 the table of the Supplementary data 6.

779 **IF samples.** PCR amplifications were done using Hot firepol DNA polymerase as
780 recommended (Solis BioDyne, Estonia), in a 50 µl final volume supplemented with 2 mM
781 MgCl₂, 200 µM each dNTPs and 200 nM forward (P4+T or WL1) and reverse (EukP3)
782 primers (Supplementary Material 6) and 1µl of isolated gregarine DNAs. PCR cycles, run
783 in an automated thermocycler (GeneAmp®PCR System 9700, Applied Biosystem, USA),
784 were programmed to give an initial denaturation step at 95 °C for 4 min, 30 cycles of
785 denaturation at 95 °C for 30 s, annealing at 51°C for 30 s and extension at 72 °C for 2 min,
786 and a final extension step at 72 °C for 7 min. PCR products were purified using Illustra™
787 GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare, France) and were cloned
788 into pGEM®-T Easy vector (Promega, Madison WI, USA) using supplier's
789 recommendations. DNA sequences were obtained from positive clones selected by PCR
790 using T7 and Sp6 universal primers flanking the pGEM®-T Easy vector cloning site, using
791 T7, Sp6 and internal primers such as LWA1, LWA3, PIF3F and PIF3R (Table 6), by the
792 Sanger method (Beckman Coulter Genomics, Takeley, UK). Raw were edited using the
793 BioEdit 7.1.3.0 program (Hall 1999) and assembled by using MEGA6 (Tamura et al.
794 2013).

795 **Phylogenetic analyses:** SSU rDNA sequences from nine *Selenium* and *Lecudina* species
796 were aligned to 106 rDNA sequences from diverse eukaryotes, mostly corresponding to
797 representatives of Alveolata with one Rhizaria as outgroup. Sequences were aligned using the
798 online version of MAFFT version 7 (<http://mafft.cbrc.jp/alignment/server/> Katosh and Toh
799 2010), using the secondary structure of RNA (Q-INS-I option) and further refined manually
800 taking as a reference the secondary structure of *T. gondii* small subunit rRNA (Gagnon et al.
801 1996). Ambiguously aligned positions were manually removed which yielded a confident
802 alignment of 1350 positions. A GTR substitution model with gamma-distributed rate variation
803 across sites was suggested as the best-fit model by JModeltest V2.1.3 (Darriba et al. 2012).
804 Accordingly, a Bayesian phylogenetic tree was constructed with MrBayes v.3.2.3 (Ronquist et
805 al. 2012) using lset nst=6 rates=Invgamma Ngammacat=4 parameters. Four simultaneous Monte

806 Carlo Markov chains were run from random trees for a total of 13,000,000 generations in two
807 parallel runs. A tree was sampled every 1000 generation and 25% of the trees were discarded as
808 “burn-in”. A consensus tree was constructed from the post-burn-in trees and posterior
809 probabilities were calculated in MrBayes. Maximum Likelihood analyses were performed with
810 MEGA 6.06 (Tamura et al. 2013) using the GTR+G+I model. Bootstraps were estimated from
811 1,000 replicates.

812 The phylogenetic tree for the Selenidiidae lineage from polychate annelids (Fig. 13)
813 was constructed using the same alignment but for a subset of 20 sequences; all position
814 containing gaps and missing data were eliminated; there were a total of 1,416 positions in
815 the final dataset. Maximum Likelihood analyses were performed with MEGA 6.06 (Tamura
816 et al. 2013) using the GTR+G+I model. Bootstraps were estimated from 1,000 replicates.

817 **Estimate of evolutionary divergence between sequences:** Evolutionary
818 divergence between sequences was computed by using the MEGA 6.06 (Tamura et al.
819 2013) using a subset of sequences extracted from the main phylogenetic alignment. For the
820 analysis of the Selenidiidae lineage (Supplementary Material 4) the analysis involved 33
821 nucleotide sequences for 16 distinct species, there were a total of 2088 positions in the final
822 dataset and all positions containing gaps and missing data were eliminated.

823

824

825 **Acknowledgements**

826

827 Many thanks to Dr Isabelle Desportes (MNHN) for her fruitful comments, Dr Marc
828 Dellinger (MNHN) for help in primer design and Dr Marc Gèze (MNHN) for imaging on
829 the MNHN-CEMIM Platform. The careful illustrations of the figures were realized by
830 Doanh Baccam (MNHN). This study was supported by ECO-NET Project 2131QM (Égide,
831 France), PHC-Barrende projects 24663ND (2011-2012) and 31266SL (2014-2015),
832 Interdisciplinary Programs of the MNHN (ATM-Microorganismes, ATM-Emergence des
833 clades, des biotes et des cultures) and by the French governmental ANR under ANR-10-
834 LABX-0003 BCDiv, ANR-11-IDEX-0004-0 and the ANR-14-CE02-0007-01 (HAPAR).
835 AV was funded by the Czech Science Foundation project No. GBP505/12/G112 (ECIP),
836 and her travel expenses were supported by projects MEB021127 and 7AMB14FR013 from
837 the Ministry of Education, Youth and Sports of the Czech Republic.

838

839 **References**

- 840 **Boothroyd JC, Dubremetz JF** (2008) Kiss and split: the dual roles of *Toxoplasma*
841 rhoptries. *Nat Rev Microbiol* **6**:79-88
- 842 **Bradley PJ, Ward C, Cheng SJ, Alexander DL, Coller S, Coombs GH, Dunn JD,**
843 **Ferguson DJ, Sanderson SJ, Wastling JM, Boothroyd JC** (2005) Proteomic analysis of
844 rhoptry organelles reveals many novel constituents for host-parasite interactions in
845 *Toxoplasma gondii*. *J Biol Chem* **280**:34245-34258
- 846 **Brasil L** (1907) Recherches sur le cycle évolutif des *Selenidiidae*, Grégarines parasites
847 d'Annélides polychètes. I. La schizogonie et la croissance des gamétocytes chez *Selenidium*
848 *caulleryi* n. sp. *Arch Protistenkd* **16**:370-397
- 849 **Brugerolle G** (2002) *Colpodella vorax*: ultrastructure, predation, life-cycle, mitosis, and
850 phylogenetic relationships. *Eur J Protistol* **38**:113-125
- 851 **Caullery M, Mesnil F** (1899) Sur quelques parasites internes des Annélides. I. Grégarines
852 nématoides des annélides. *Trav Stat Zool Wimereux* **7**:80-99
- 853 **Caullery M, Mesnil F** (1900) Sur un mode particulier de division nucléaire chez les
854 Grégarines. *Arch Anat Microsc* **3**:146-167
- 855 **Chomczynski P. and Sacchi, N** (2006) The single-step method of RNA isolation by acid
856 guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nat*
857 *Protoc* **1**:581-585
- 858 **Counihan NA, Kalanon M, Coppel RL, de Koning-Ward TF** (2013) *Plasmodium*
859 rhoptry proteins: why order is important. *Trends Parasitol* **29**:228-236
- 860 **Darriba D, Taboada GL, Doallo R, Posada D** (2012) jModeltest 2: more models, new
861 heuristics and parallel computing. *Nat Methods* **9**:772-772
- 862 **de Leon JC, Scheumann N, Beatty W, Beck JR, Tran JQ, Yau C, Bradley PJ, Gull K,**
863 **Wickstead B, Morissette NS** (2013) A SAS-6-like protein suggests that the *Trypanosoma*
864 conoid complex evolved from flagellar components. *Eukaryot Cell* **12**:1009-1015
- 865 **De Rosa R, Prud'homme B, Balavoine G** (2005) Caudal and even-skipped in the annelid
866 *Platynereis dumerilii* and the ancestry of the posterior growth. *Evol Dev* **7**:574-587
- 867 **Desportes I, Schrével J** (2013, eds) *The Gregarines: The Early Branching Apicomplexa*.
868 Brill, Leiden, 781 p
- 869 **Dubremetz JF** (1973) Etude ultrastructurale de la mitose schizogonique chez la Coccidie
870 *Eimeria necatrix* (Johnson, 1930). *J Ultrastruct Res* **42**:354-376
- 871 **Dubremetz JF** (1975) Genesis of merozoites in the coccidia, *Eimeria necatrix*:
872 Ultrastructural study. *J Protozool* **22**:71-84

- 873 **Florent I, Mouray E, Dali Ali F, Drobecq H, Girault S, Schrével J, Sergheraert C,**
874 **Grellier P** (2000) Cloning of *Plasmodium falciparum* protein disulfide isomerase
875 homologue by affinity purification using the antiplasmodial inhibitor inhibitor 1,4-bis{3-
876 [N-(cyclohexyl methyl)amino]propyl}piperazine. FEBS Lett **484**:246-252
- 877 **Francia ME, Jordan CN, Patel JD, Sheiner L, Demerly JL, Fellows JD, de Leon JC,**
878 **Morrisette NS, Dubremetz JF, Striepen B** (2012) Cell division in apicomplexan
879 parasites is organized by a homolog of the striated rootlet fiber of algal flagella. PLoS Biol
880 10:e1001444
- 881 **Francia ME, Striepen B** (2014) Cell division in apicomplexan parasites. Nat Rev
882 Microbiol **12**:125-136
- 883 **Gagnon S, Bourbeau D, Levesque RC** (1996) Secondary structures and features of the
884 18S, 5.8S and 26S ribosomal RNAs from the apicomplexan parasite *Toxoplasma gondii*.
885 Gene **173**:129-35
- 886 **Goldstein SF, Schrével J** (1982) Microtubules and cell motility. SFRS, France,
887 CERIMES, www.cerimes.education.fr
- 888 **Gunderson J, Small EB** (1986) *Selenidium fallax* n.sp. (Protozoa, Apicomplexa) from the
889 sipunculid *Phascolosoma agassizii* Kerferstein 1867. J Parasitol **72**:107-110
- 890 **Hall TA** (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis
891 program for Windows 96/98NT. Nucleic Acids Symp Ser **41**:95-98
- 892 **Harper JD, Thuet J, Lehtreck KF, Hardham AR** (2009) Proteins related to green algal
893 striated fiber assemblin are present in stramenopiles and alveolates. Protoplasma **236**:97-
894 101
- 895 **Heintzelman MB** (2004) Actin and myosin in *Gregarina polymorpha*. Cell Motil
896 Cytoskeleton **58**:83-95
- 897 **Hodges ME, Scheumann N, Wickstead B, Langdale JA, Gull K** (2010) Reconstructing
898 the evolutionary history of the centriole from protein components. J Cell Biol **123**:1407-
899 1413
- 900 **Hoshida H, Todd KS** (1996) The fine structure of cell surface and hair-like projections of
901 *Filipodium ozakii* Hukui. Acta Protozool **35**:309-315
- 902 **Hu K, Roos DS, Murray JM** (2002) A novel polymer of tubulin forms the conoid of
903 *Toxoplasma gondii*. J Cell Biol **156**:1039-1050
- 904 **Hu K, Johnson J, Fraunholz M, Surajajjala S, DiLullo C, Yates J, Rossn DS, Murray**
905 **JM** (2006) Cytoskeletal Components of an Invasion Machine-The Apical Complex of
906 *Toxoplasma gondii*. PLoS Pathogens **2**(2):e13:121-138

- 907 **Katoh K, Toh H** (2010) Parallelization of the MAFFT multiple sequence alignment
908 program. *Bioinformatics* **26**:1899-1900
- 909 **Kuriyama R, Besse C, Geze M, Omoto CK, Schrével J** (2005) Dynamic organization of
910 microtubules and microtubule-organizing centers during the sexual phase of a parasitic
911 protozoan, *Lecudina tuzetae* (Gregarine, Apicomplexa). *Cell Motil Cytoskeleton* **62**:195-
912 209
- 913 **Kuwardina ON, Leander BS, Aleshin VV, Myl'nikov AP, Keeling PJ, Simdyanov TG**
914 (2002) The phylogeny of colpodellids (Alveolata) using small subunit rRNA genes
915 sequences suggests there are the free-living group to apicomplexans. *J Eukaryot Microbiol*
916 **49**:498-504
- 917 **Leander BS** (2006) Ultrastructure of the archigregarine *Selenidium vivax* (Apicomplexa)-A
918 dynamic parasite of sipunculid worms (host: *Phascolosoma agassizii*). *Mar Biol Res* **2**:178-
919 190
- 920 **Leander BS** (2007a) Marine gregarines: evolutionary prelude to the apicomplexan
921 radiation? *Trends Parasitol* **24**:60-7
- 922 **Leander BS** (2007b) Molecular phylogeny and ultrastructure of *Selenidium serpulae*
923 (Apicomplexa, Archigregarinia) from the calcareous tubeworm *Serpula vermicularis*
924 (Annelida, Polychaeta, Sabellida). *Zool Scripta* **36**:213-22
- 925 **Leander BS, Keeling PJ** (2003) Morphostasis in alveolate evolution. *Trends Ecol Evol*
926 **18**:395-404
- 927 **Leander BS, Harper JT, Keeling PJ** (2003) Molecular phylogeny and surface
928 morphology of marine aseptate gregarines (Apicomplexa): *Selenidium* spp. and *Lecudina*
929 spp. *J Parasitol* **89**:1191-205
- 930 **Leander BS, Kuwardina ON, Aleshin VV, Mylnikov AP, Keeling PJ** (2003) Molecular
931 phylogeny and surface morphology of *Colpodella edax* (Alveolata): insights into the
932 phagotrophic ancestry of apicomplexans. *J Eukaryot Microbiol* **50**:334-340
- 933 **Léger L, Duboscq O** (1917) Sporozoaires de *Glossobalanus minutus* Kow. *Eimeria*
934 *epidermica* n. sp.; *Eimeria beauchampi* n. sp.; *Selenidium metchnikowi* n. sp. *Ann Inst*
935 *Pasteur* **31**:60-74
- 936 **Leidel S, Delattre M, Cerutti L, Baumer K, Gonczi P** (2005) SAS-6 defines a protein
937 family required for centrosome duplication in *C. elegans* and human cells. *Nat Cell Biol*
938 **7**:115-125
- 939 **Lim L, McFadden GI** (2010) The evolution, metabolism and functions of the apicoplast.
940 *Philos Trans Royal Soc B* **365**:749-763

- 941 **Lwoff A** (1944) L'évolution physiologique. Etude des pertes de fonction chez les
 942 microorganismes. Hermann Publ, Paris, 308 p
- 943 **Macgregor HC, Thomasson PA** (1965) The fine structure of two archigregarines,
 944 *Selenidium fallax* and *Ditrypanocystis cirratuli*. J Protozool **12**:438-443
- 945 **Mignot JP** (1966) Ultrastructure des Eugléniens. Etude de la cuticule chez différentes
 946 espèces. Protistologica **2**:51-117
- 947 **Misof B, Liu S, Meusemann K, et al.** (2014) Phylogenomics resolves the timing and
 948 pattern of insect evolution. Science **346**:763-767
- 949 **Molon-Noblot S, Desportes I** (1980) Etude ultrastructurale des mitoses gamogoniques de
 950 la Grégarine *Grebniackiella gracilis* Gr. parasite de la scolopendre *Scolopendra cingulata* L.
 951 Considérations sur les mitoses schizogoniques des Sporozoaires (Apicomplexa).
 952 Protistologica **16**:395-411
- 953 **Nei M, Kumar S** (2000) Molecular Evolution and Phylogenetics. Oxford University Press,
 954 New York, 333 p
- 955 **Okamoto N, Keeling PJ** (2014) The 3D structure of the apical complex and association
 956 with the flagellar apparatus revealed by serial TEM tomography in *Psammosa pacifica*, a
 957 distant relative of the Apicomplexa. PLoS ONE **9**(1):e84653
- 958 **Perkins FO** (1996) The structure of *Perkinsus marinus* (Mackin, Owen and Collier, 1950)
 959 Levine, 1978 with comments on the taxonomy and phylogeny of *Perkinsus* spp. J Shellfish
 960 Res **15**:67-87
- 961 **Prensier G, Vivier E, Goldstein S, Schrével J** (1980) Motile flagellum with a "3 + 0"
 962 ultrastructure. Science **207**:1493-4
- 963 **Prensier G, Dubremetz JF, Schrével J** (2008) The unique adaptation of the life cycle of
 964 the coelomic gregarine *Diplauxis hatti* to its host *Perinereis cultrifera* (Annelida,
 965 Polychaeta): an experimental and ultrastructural study. J Eukaryot Microbiol **55**:541-53
- 966 **Ray HN** (1930) Studies on some sporozoa in polychaete worms. I. Gregarines of the genus
 967 *Selenidium*. Parasitology **22**:370-398
- 968 **Reed N** (1933) Sporogony in *Selenidium mesnili* Brasil, a sporozoan parasite of *Myxicola*
 969 *infundibulum* Mont. Parasitology **25**:402-409
- 970 **Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B,**
 971 **Liu L, Suchard MA, Huelsenbeck JP** (2012) MrBayes 3.2: efficient Bayesian
 972 phylogenetic inference and model choice across a large model space. Syst Biol **61**:539-42
- 973 **Rouse GW, Fauchald K** (1997) Cladistics and Polychaetes. Zool Scripta **26**:139-204

- 974 **Rueckert S, Leander BS** (2009) Molecular phylogeny and surface morphology of marine
 975 archigregarines (Apicomplexa), *Selenidium* spp., *Filipodium phascolosomae* n. sp., and
 976 *Platyproteum* n. g. and comb. from North-Eastern pacific peanut worms (Sipuncula). J
 977 Eukaryot Microbiol **56**:428-439
- 978 **Russell DG, Burns RG** (1984) The polar ring of coccidian sporozoite: a unique
 979 microtubule-organizing centre. J Cell Sci **65**:197-207
- 980 **Saldarriaga JF, McEwan ML, Fast NM, Taylor FJ, Keeling PJ** (2003) Multiple protein
 981 phylogenies show that *Oxyrrhis marina* and *Perkinsus marinus* are early branches of
 982 dinoflagellate lineage. Int J Syst Evol Microbiol **53**:355-365
- 983 **Santos JM, Lebrun M, Daher W, Soldati D, Dubremetz JF** (2009) Apicomplexa
 984 cytoskeleton and motors: key regulators in morphogenesis, cell division, transport and
 985 motility. Int J Parasitol **39**:153-162
- 986 **Schrével J** (1963) Contribution à l'étude de trois Grégarines parasites d'Annélides
 987 Polychètes: *Lecudina elongata* Mingazzini 1891; *Lecudina tuzetae* Schrével 1963;
 988 *Gonospora varia* Léger 1892. Arch Zool Exp Gén **104**:125-142
- 989 **Schrével J** (1966) Cycle de *Selenidium pendula* Giard 1884, Grégarine parasite de *Nerine*
 990 *cirratus* Delle Chiaje (Annélide Polychète). Protistologica **2**:31-34
- 991 **Schrével J** (1968) L'ultrastructure de la région antérieure de la grégarine *Selenidium* et son
 992 intérêt pour l'étude de la nutrition chez les Sporozoaires. J Microsc Paris **7**:391-410
- 993 **Schrével J** (1969) Recherches sur le cycle des Lecudinidae grégarines parasites
 994 d'Annélides Polychètes. Protistologica **5**:561-588
- 995 **Schrével J** (1970) Contribution à l'étude à l'étude des Selenidiidae parasites d'Annélides
 996 Polychètes: I. Cycles biologiques. Protistologica **6**:389-426
- 997 **Schrével J** (1971a) Contribution à l'étude à l'étude des Selenidiidae parasites d'Annélides
 998 Polychètes: II. Ultrastructure de quelques trophozoites. Protistologica **7**:101-130
- 999 **Schrével J** (1971b) Observations biologiques et ultrastructurales sur les Selenidiidae et
 1000 leurs conséquences sur la systématique des Grégarinomorphes. J Protozool **18**:448-470
- 1001 **Schrével J, Besse C** (1975) Un type flagellaire fonctionnel de base 6+0. J Cell Biol **66**:492-
 1002 507
- 1003 **Schrével J, Desportes I** (2013) Introduction: Gregarines among Apicomplexa. Chapter 1, 7-
 1004 24 in Desportes I, Schrével J (eds) The Gregarines. The Early Branching Apicomplexa.
 1005 Brill, Leiden, pp 7-24
- 1006 **Schrével J, Desportes I** (2015) Gregarines. In Mehlhorn H (ed). Encyclopedia of
 1007 Parasitology, 4th edn, Springer, Berlin, Heidelberg, pp 1-47

- 1008 **Schrével J, Caigneaux E, Gros D, Philippe M** (1983) The three cortical membranes of
 1009 the gregarines. I. Ultrastructural organization of *Gregarina blaberae*. *J Cell Sci* **61**:151-74
- 1010 **Schrével J, Desportes I, Goldstein S, Kuriyama R, Prensier G, Vávra J** (2013) Biology
 1011 of Gregarines and their Host-parasite Interactions. Chapter 2, 25-195 in Desportes I,
 1012 Schrével J (eds) *The Gregarines. The Early Branching Apicomplexa*. Brill, Leiden, pp 25-
 1013 195
- 1014 **Schrével J, Asfaux-Foucher G, Hopkins JM, Vincent R, Bourgouin C, Prensier G,**
 1015 **Bannister LH** (2008) Vesicle trafficking during sporozoite development in *Plasmodium*
 1016 *berghei*: ultrastructural evidence for a novel trafficking mechanism. *Parasitology* **135**:1-12
- 1017 **Sheffield HG, Garnham PCC, Shiroishi T** (1971) The fine structure of the sporozoite of
 1018 *Lankesteria culicis*. *J. Protozool* **18**:98-105
- 1019 **Simdyanov TG, Kuvardina ON** (2007) Fine structure and putative feeding mechanism of
 1020 the archigregarine *Selenidium orientale* (Apicomplexa:Gregarinomorpha). *Eur J Protistol*
 1021 **43**:17-25
- 1022 **Struck TH, Paul C, Hill N, Hartman S, Hösel C, Kube M, Lieb B, Meyer A,**
 1023 **Tiedemann R, Purschke G, Bleidorn C** (2011) Phylogenomic analyses unravel annelid
 1024 evolution. *Nature* **471**:95-98
- 1025 **Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S** (2013) MEGA6: Molecular
 1026 Evolutionary Genetics Analysis Version 6.0. *Mol Biol Evol* **30**:2725-2729
- 1027 **Toso MA, Omoto CK** (2007) *Gregarina niphandrodes* may lack both a plastid genome
 1028 and organelle. *J Eukaryot Microbiol* **54**:66-72
- 1029 **Trégouboff G** (1918) Etude monographique de *Gonospora testiculi* Treg., grégarine
 1030 parasite du testicule de *Cerithium vulgatum* Brug. *Arch Zool Exp Gén* **57**:471-509
- 1031 **Tuzet O, Ormières R** (1958) *Selenidium flabelligerae* n. sp. parasite de *Flabelligera*
 1032 *diplochaitos* Otto (Annélide sédentaire). *Ann Sci Nat Zool* **13**:71-76
- 1033 **Valigurová A, Vaškovicová N, Musilová N, Schrével J** (2013) The enigma of
 1034 eugregarine epicytic folds: where the gliding motility originates? *Frontiers Zool* **10**:57
- 1035 **van Breugel M, Hirono M, Andreeva A, Yanagisawa HA, Yamaguchi S, Nakazawa Y,**
 1036 **Morgner N, Petrovitch M, Ebong IO, Robinson CV, Johnson M, Veprintsev D, Zuber**
 1037 **B** (2011) Structures of SAS-6 suggest its organization in centrioles *Science* **331**:1196-1199
- 1038 **Vivier E** (1967) Observations ultrastructurales sur l'enveloppe nucléaire et ses "pores" chez
 1039 les Sporozoaires. *J Microsc Paris* **6**:371-390
- 1040 **Vivier E** (1968) L'organisation ultrastructurale corticale de la grégarine *Lecudina*
 1041 *pellucida*; ses rapports avec l'alimentation et la locomotion. *J Protozool.* **15**:230-246

- 1042 **Vivier E, Schrével J** (1964) Etude au microscope électronique d'une Grégarine du genre
1043 *Selenidium* parasite de *Sabellaria alveolata* L. J Microsc Paris **3**:651-670
- 1044 **Vivier E, Schrével J** (1966) Les ultrastructures cytoplasmiques de *Selenidium hollandei*, n.
1045 sp., Grégarine parasite de *Sabellaria alveolata* L. J Microsc Paris **5**:213-228
- 1046 **Wakeman KC, Leander BS** (2012) Molecular phylogeny of Pacific Archigregarines
1047 (Apicomplexa), including descriptions of *Veloxidium leptosynaptae* n. gen., n. sp., from the
1048 sea cucumber *Leptosynapta clarki* (Echinodermata), and two new species of *Selenidium*. J
1049 Eukaryot Microbiol **59**:1-14
- 1050 **Wakeman KC, Leander BS** (2013) Molecular phylogeny of marine gregarine parasites
1051 (Apicomplexa) from tube-forming polychaetes (Sabellariidae, Cirratulidae and Serpulidae)
1052 including descriptions of two new species of *Selenidium*. J Eukaryot Microbiol **60**:514-525
- 1053 **Wakeman KC, Heintzelman MB, Leander BS** (2014) Comparative ultrastructure and
1054 molecular phylogeny of *Selenidium melongena* n. sp. and *S. terebellae* Ray 1930
1055 demonstrate niche partitioning in marine gregarine parasites (Apicomplexa). Protist
1056 **165**:493-511
- 1057

1057 **Figure legends**

1058

1059 **Figure 1.** Scanning and transmission electron microscopy of *Selenidium pendula*
 1060 trophozoites fixed to the intestine of the polychaete worm *Scoelelepis squamata* (**A-B**).
 1061 Abbreviations: bulge (B), dense granule (DG), food vacuole (FV), groove (G), intestinal
 1062 epithelium (IE), mucron (MU), rhoptry (R). **A.** SEM micrograph of trophozoites with their
 1063 apical region inserted into the intestinal epithelium, exhibiting on this face about 18
 1064 longitudinal bulges separated by grooves. The long filamentous structures covering the
 1065 intestinal epithelium correspond to ciliary structures (arrows). **B.** Longitudinal TEM section
 1066 of a trophozoite with the apical end designated as mucron containing a food vacuole and
 1067 numerous rhoptries. In the intestinal epithelium, the trophozoite preferentially anchors to
 1068 the host cells enriched in dense granules having mucous secretions.

1069

1070 **Figure 2.** Apex of the *Selenidium pendula* trophozoite (**A-D**). Abbreviations: bulge (B),
 1071 conoid (Co), food vacuole (FV), groove (G), intestinal epithelium (IE), microneme (mn),
 1072 microtubules (mt), microvilli (mv), mucron (MU), rhoptry (R). **A.** SEM micrograph of the
 1073 apex surface showing that bulges and grooves of the epicyte start from a regular
 1074 mammiliform area corresponding to the external surface of the mucron. Small folds
 1075 (arrows) are observed on the bulges located on the internal curvature of the cell **B.** SEM
 1076 micrograph of intestinal epithelium after the detachment of a mucron, with small microvilli
 1077 on the periphery, a small hole in the subcentral position (white arrow) and the long ciliary
 1078 structures (black arrow). **C.** TEM micrograph of a median longitudinal section of the apex
 1079 with several food vacuoles that enter via the conoid and are surrounded by an accumulation
 1080 of rhoptries and micronemes. **D.** TEM longitudinal section of the apical region (=
 1081 trophozoite apex with numerous micronemes and rhoptries) revealing that the subpellicular
 1082 microtubule bundles start before the differentiation of the epicytic bulges of the cell
 1083 surface.

1084

1085 **Figure 3.** Cell surface and cortex of *Selenidium pendula* trophozoite (**A-F**). Abbreviations:
 1086 bulge (B), groove (G), inner membrane complex (imc), microneme (mn), microtubules
 1087 (mt), mitochondrion (M), myelin-like structure (st myel), plasma membrane (pm), pore (p),
 1088 rhoptry (R), vesicle (ves). **A.** SEM view of the cell surface with the apertures of pores along
 1089 the grooves (arrows). **B-F.** TEM cross sections of the cortex with the plasma membrane,
 1090 the dilated inner membrane complex and the subpellicular microtubules under the epicytic

1091 bulges (C). In cross section, each microtubule is surrounded by a white hexagonal area.
 1092 Ectoplasmic organelles in the grooves, connected to the cortical membranes via the imc,
 1093 contain lamellar structures (arrow in B) or dense material (white arrow in D). These
 1094 organelles form an annular ring in cross section parallel to the cell surface (white arrow in
 1095 E) corresponding to the cross section of a micropore or myelin-like structures (F).

1096

1097 **Figure 4.** Conoid in the *Selenidium pendula* mucron (A-C). Abbreviations: dense granule
 1098 (DG), conoid (Co), food vacuole (FV), food vacuole membrane (fvm), inner membrane
 1099 complex (imc), host intestinal epithelium (IE), parasite plasma membrane (pm). **A-B.** Two
 1100 longitudinal sections of the *S. pendula* mucron, showing the conoid structure and the
 1101 opening, allowing a contact between the fvm and the host cell, visible in **A**. **C.** High
 1102 magnification showing the 9 cross sections of the microtubular network forming the
 1103 conoid.

1104

1105 **Figure 5.** Food vacuoles and rhoptries in the apex of the *S. pendula* trophozoite (A-B), and
 1106 apicoplast-like organelles (C, D, E). Abbreviations: conoid (Co), food vacuole (FV), host
 1107 intestinal epithelium (IE), microneme (mn), mitochondrion (M), fragmented food vacuoles
 1108 similar to pinocytotic vesicles (pv), rhoptry (R). **A.** TEM cross section with the initial food
 1109 vacuole passing through the conoid and the fragmented food vacuoles similar to the
 1110 pinocytotic vesicles (pv) observed in *S. hollandei* (Schrével 1968). Numerous rhoptries are
 1111 accumulated around these food vacuoles. **B.** Another cross section showing the irregular
 1112 shapes of the initial food vacuole and the intravacuolar vesicles. (C-E). Apicoplast-like
 1113 organelles, characterized by the presence of four membranes morphologically similar to the
 1114 apicoplast of *Toxoplasma* and *Plasmodium*.

1115

1116 **Figure 6.** Rhoptries, micronemes and Golgi apparatus (A-D). Abbreviations: amylopectin
 1117 granule (am), Golgi apparatus (Go), intrareticular granule (ig), microneme (mn),
 1118 mitochondrion (M), rhoptry (R). **A-B.** TEM cross sections of an accumulation of rhoptries
 1119 and micronemes within the cytoplasm. The micronemes appear as long-necked bottles, the
 1120 necks appear as dense rings in cross sections (white thick arrow in B). **C-D.** Golgi
 1121 apparatus and mitochondrion occur close to the micronemes; the *cis*-region of the Golgi
 1122 apparatus usually contains numerous intrareticular granules (D).

1123

1124 **Figure 7.** Nuclear area of *Selenidium pendula* trophozoite (A-E). Abbreviations:
 1125 amylopectin granule (am), intrareticular granule (ig), microneme (mn), nuclear pores (np),
 1126 nucleus (N), nucleolus (nu). **A-B.** TEM cross sections of the nucleus containing a spherical
 1127 nucleolus (A) and surrounded by the regular fibrillar zone without organelles (white arrows
 1128 in B). This fibrillar area is delimited by large vesicles of the rough endoplasmic reticular
 1129 containing numerous granules and amylopectin granules. **C.** Tangential section of the
 1130 nuclear envelope exhibits numerous pores. **D.** Occasional accumulation of micronemes can
 1131 be observed near the nucleus. **E.** Higher magnification of the micronemes and intrareticular
 1132 granules.

1133

1134 **Figure 8.** Nuclear development during the syzygy of *Selenidium pendula* (A-D).
 1135 Abbreviations: nucleus (N), cyst wall (CW). **A-B.** Fluorescence staining with DAPI
 1136 showing nuclei in the median plane of each gamont corresponding to the initial position of
 1137 the nucleus at the beginning of the syzygy stage. Their numbers are quite similar in the two
 1138 gamonts and some bright spots are observed in some nuclei (B). **C-D.** TEM cross sections
 1139 in an early cyst where the nuclei are accumulating in the central position of the gamont
 1140 while the cell wall is not secreted (C) and later after the secretion of the cyst wall where the
 1141 nuclei migrate to the gamont periphery (D).

1142

1143 **Figure 9.** Centrocones and mitosis stages during the gametogenesis of *Selenidium pendula*
 1144 (A-B). Abbreviations: centrocone (CC), chromatin (Ch), cyst wall (CW), dense layer (dl),
 1145 filamentous layer (fl), nuclear envelope (en), mitochondrion (M), microtubule-organizing
 1146 center (MTOC), microtubule (mt), nucleus (N), vesicle type 1 (V1) and type 2 (V2). **A.**
 1147 Early gametogenesis stage before the secretion of the gametocyst wall exhibiting
 1148 centrocone where the microtubules radiate from the MTOC to the cupule of the nuclear
 1149 envelope forming a truncated cone. The chromatin covers the inner face of the nuclear
 1150 envelope and a dense accumulation is observed in the nucleus. **B.** A second centrocone
 1151 migrating on the other side of the nucleus. No intranuclear spindle is observed, the
 1152 chromatin is attached to the persistent nuclear envelope. Two types of vesicles are observed
 1153 one with dense granules (V1) and a second one with filamentous material (V2). The
 1154 gametocyst wall exhibits an external filamentous layer and an internal dense layer.

1155

1156 **Figure 10.** Reorganisation of the cortical membranes in *Selenidium pendula* gamonts and
 1157 ultrastructure of the gametocyst wall during the gametogenesis stage (A-D). Abbreviations:

1158 gametocyst wall (CW), dense layer (dl), epicytic folds (ef), filamentous layer (fl), inner
 1159 membrane complex (imc), plasma membrane (pm), vesicle type 1 (V1) and type 2 (V2). **A-**
 1160 **C.** TEM cross sections of the gamont's periphery where the epicytic folds are disorganized
 1161 with dissociation of the inner member complex under the plasma membrane. The wall of
 1162 the gametocyst exhibits a filamentous external layer and a more homogenous internal layer;
 1163 the vesicles of type 2 are probably involved in the cyst wall construction. **D.** Higher
 1164 magnification of the cyst wall with large amount of filaments attached to the surface of the
 1165 internal homogenous layer.

1166

1167 **Figure 11.** Gamonts with gametes and young sporocysts after the fertilization (**A-C**).
 1168 Abbreviations: amylopectin granule (am), axoneme (Ax), gametocyst wall (CW), dense
 1169 granule (DG), dense layer (dl), filamentous layer (fl), mitochondrion (M), nucleus (N),
 1170 sporocyst wall (SW). **A.** Gametocyst wall after the formation of the gametes exhibits a third
 1171 layer with more dense material under the two layers observed in more early gamont stages
 1172 (Figure 12). Residual amylopectin and dense granules are observed between the gametes
 1173 and the gametocyst wall. **B.** Cross sections of flagellar axonemes indicate a male gamete
 1174 (B) and two serial sections are of a 9+0 pattern. **C.** After the fertilization process the young
 1175 sporocysts are surrounded by a thin wall covered by very small spines.

1176

1177 **Figure 12.** Maximum Likelihood (ML) tree inferred on an alignment of 115 small subunit
 1178 (SSU) rDNA sequences corresponding to 9 *Selenidium* and *Lecudina* species from this
 1179 current study (highlighted in grey boxes) and 106 sequences from diverse eukaryotes
 1180 corresponding mostly to representatives of Alveolata with one Rhizaria as outgroup. The
 1181 Maximum Likelihood method is based on the General Time Reversible +G +I model (Nei
 1182 and Kumar 2000). The tree is drawn to scale, with branch lengths measured in the number
 1183 of substitutions per site. A branch was shortened by a multiple (3) of the length of
 1184 substitutions/site scale bar. There were a total of 1153 positions in the final dataset. ML
 1185 evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). Numbers at the
 1186 branches denote ML bootstrap percentage (first value). Bayesian posterior probabilities are
 1187 also indicated (second value). Black dots on branches denote bootstrap percentages above
 1188 99% and Bayesian posterior probabilities superior to 0.97.

1189

1190 **Figure 13.** Molecular phylogenetic analysis by Maximum Likelihood method of
 1191 Selenidiidae lineage retrieved from polychaete annelids (host families in bold black).

1192 The evolutionary history was inferred by using the Maximum Likelihood method based on
1193 the General Time Reversible model (Nei and Kumar 2000). The tree with the highest log
1194 likelihood (-5446.5092) is shown. A discrete Gamma distribution was used to model
1195 evolutionary rate differences among sites (5 categories with gamma parameter = 0.2711).
1196 The rate variation model allowed for some sites to be evolutionarily invariable (+I),
1197 39.8364% sites). The tree is drawn to scale, with branch lengths measured in the number of
1198 substitutions per site. Novel sequences are highlighted in grey boxes.
1199

Accepted Manuscript

1199 **Table 1.** List of the SSU rDNA sequence numbers of archigregarines and *Veloxidium*
 1200 initially included in this group and the references: 1. This work; 2. Leander et al. 2003; 3.
 1201 Leander et al. 2007; 4. Rueckert and Leander 2009; 5. Wakeman and Leander 2012; 6.
 1202 Wakeman and Leander 2013; 7. Wakeman et al. 2014.

Archigregarines	SSU rDNA sequences	Ref.	Host	Infraclass	Order	Family
<i>Selenidium pendula</i> LG	LN901443	1	<i>Scolecopsis squamata</i>	Canalipalpata	Spionida	Spionidae
<i>Selenidium pendula</i> IF	LN901444	1	<i>Scolecopsis squamata</i>	Canalipalpata	Spionida	Spionidae
<i>Selenidium boccardiella</i>	JN857969	5	<i>Boccardiella ligerica</i>	Canalipalpata	Spionida	Spionidae
<i>Selenidium mesnili</i>	JN857968	5	<i>Myxicola infundibulum</i>	Canalipalpata	Sabellida	Sabellidae
<i>Selenidium hollandei</i>	LN901445	1	<i>Sabellaria alveolata</i>	Canalipalpata	Sabellida	Sabellariidae
<i>Selenidium neosabellariae</i>	KC110871 KC110872 KC110873	6	<i>Neosabellaria cementarium</i>	Canalipalpata	Sabellida	Sabellariidae
<i>Selenidium identhysae</i>	JN857967	6	<i>Idanthysus saxicavus</i>	Canalipalpata	Sabellida	Sabellariidae
<i>Selenidium serpulae</i>	DQ683562	3	<i>Serpula vermicularis</i>	Canalipalpata	Sabellida	Serpulidae
<i>Selenidium sensimae</i>	KC110869 KC110870	6	<i>Spirobranchus giganteus</i>	Canalipalpata	Sabellida	Serpulidae
<i>Selenidium</i> Sp1	KC110863 KC110866 KC110867	6	<i>Spirobranchus giganteus</i>	Canalipalpata	Sabellida	Serpulidae
<i>Selenidium</i> Sp2	KC110864 KC110865 KC110868	6	<i>Spirobranchus giganteus</i>	Canalipalpata	Sabellida	Serpulidae
<i>Selenidium terebellae</i>	AY196709	2	<i>Thelepus</i> sp,	Canalipalpata	Terebellida	Theleponidae
<i>Selenidium terebellae</i>	KC890803 KC890804 KC890805 KC890806	7	<i>Thelepus japonica</i>	Canalipalpata	Terebellida	Theleponidae
<i>Selenidium melongena</i>	KC890799 KC890800 KC890801 KC890802	7	<i>Thelepus japonica</i>	Canalipalpata	Terebellida	Terebellinae
<i>Selenidium cf echinatum</i>	KC110874 KC110875	6	<i>Dodecaceria concarum</i>	Canalipalpata	Terebellida	Cirratulidae
<i>Selenidium vivax</i>	AF236097	2	<i>Phascolosoma agassizii</i>	Sipunculida	Phascolosimida	Phascolosomatidae
<i>Platyproteum vivax</i>	AY196708	4	<i>Phascolosoma agassizii</i>	Sipunculida	Phascolosimida	Phascolosomatidae
<i>Filipodium phascolosoma</i>	FJ832163	4	<i>Phascolosoma agassizii</i>	Sipunculida	Phascolosimida	Phascolosomatidae
<i>Selenidium pisinnus</i>	FJ832162	4	<i>Phascolosoma agassizii</i>	Sipunculida	Phascolosimida	Phascolosomatidae
<i>Selenidium orientale</i>	FJ832131	4	<i>Themiste pyroidea</i>	Sipunculida	Golfingiida	<i>Veloxidium leptosynaptae</i>
<i>Veloxidium leptosynaptae</i>	JN857966	5	<i>Leptosynapta clarki</i>	Echinodermata	Apodida	Synaptidae

1203

1204 **Table 2.** Summary of biological, geographical and molecular data, for original isolates in
 1205 this study. The number of corresponding stages used for DNA preparations is indicated; T,
 1206 trophozoite; C, cyst. Gene Accession numbers of the new sequences are available from the
 1207 EMBL database.
 1208

Gregarine	Host	Location	Isolate names	Stage	Gene Access number (18S)
<i>Selenidium pendula</i> Giard 1884	<i>Scoelepis squamata</i> (O. F. Müller 1806)	English Channel, Roscoff, Aber, Lat:48°43'35.25"N, Long:3°59'22.54"W.	<i>Selenidium pendula</i> LG	50-70 T	LN901443
<i>Selenidium pendula</i> Giard 1884	<i>Scoelepis squamata</i> (O. F. Müller 1806)	English Channel, Roscoff-Aber 2012, Lat:48°43'35.25"N, Long:3°59'22.54"W.	<i>Selenidium pendula</i> IF	50-70 T	LN901444
<i>Selenidium hollandei</i> Vivier & Schrével 1966	<i>Sabellaria alveolata</i> (Linnaeus 1767)	English Channel, Saint-Efflam-Ile Rouge Lat:48°40'57.96"N, Long:3°35'32.52"W.	<i>Selenidium hollandei</i> LG	50-70 T	LN901445
<i>Lecudina pellucida</i> (Mingazzini 1891)	<i>Perinereis cultrifera</i> (Grübe 1840)	English Channel, Roscoff-Ile de la Souris, Lat:48°43'41.73"N, Long:3°59'22.10"W.	<i>Lecudina pellucida</i> LG	50-70 T	LN901442
<i>Lecudina tuzetae</i> Schrével 1963	<i>Neanthes (Nereis) diversicolor</i> (O. F. Müller 1776)	English Channel, Roscoff-Penzé 2012, Lat:48°37'40.07"N, Long:3°57'13.40"W.	<i>Lecudina tuzetae</i> Roscoff 2012 IF132	7 C	LN901446
<i>Lecudina tuzetae</i> Schrével 1963	<i>Neanthes (Nereis) diversicolor</i> (O. F. Müller 1776)	English Channel, Roscoff-Penzé 2013, Lat:48°37'40,07"N, Long:3°57'13.40"W.	<i>Lecudina tuzetae</i> Roscoff 2013a IF181	30 C	LN901447
<i>Lecudina tuzetae</i> Schrével 1963	<i>Neanthes (Nereis) diversicolor</i> (O. F. Müller 1776)	English Channel, Roscoff-Penzé 2013, Lat:48°37'40.07"N, Long:3°57'13.40"W.	<i>Lecudina tuzetae</i> Roscoff 2013b IF462	2 C	LN901448
<i>Lecudina tuzetae</i> Schrével 1963	<i>Neanthes (Nereis) diversicolor</i> (O. F. Müller 1776)	English Channel, Roscoff-Penzé 2014, Lat:48°37'40,07"N, Long:3°57'13.40"W.	<i>Lecudina tuzetae</i> Roscoff 2014a IF171	50 C	LN901449
<i>Lecudina tuzetae</i> Schrével 1963	<i>Neanthes (Nereis) diversicolor</i> (O. F. Müller 1776)	English Channel, Roscoff-Penzé 2014, Lat:48°37'40,07"N, Long:3°57'13.40"W.	<i>Lecudina tuzetae</i> Roscoff 2014b IF172	50 C	LN901450

1209
 1210

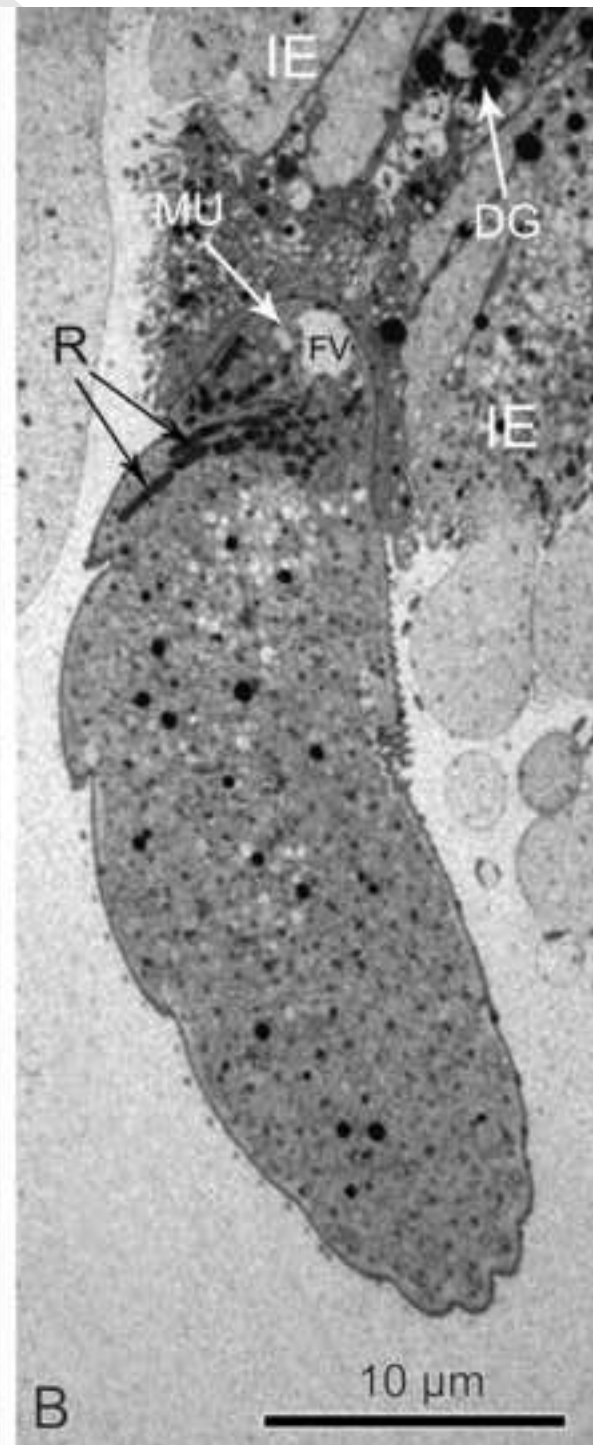
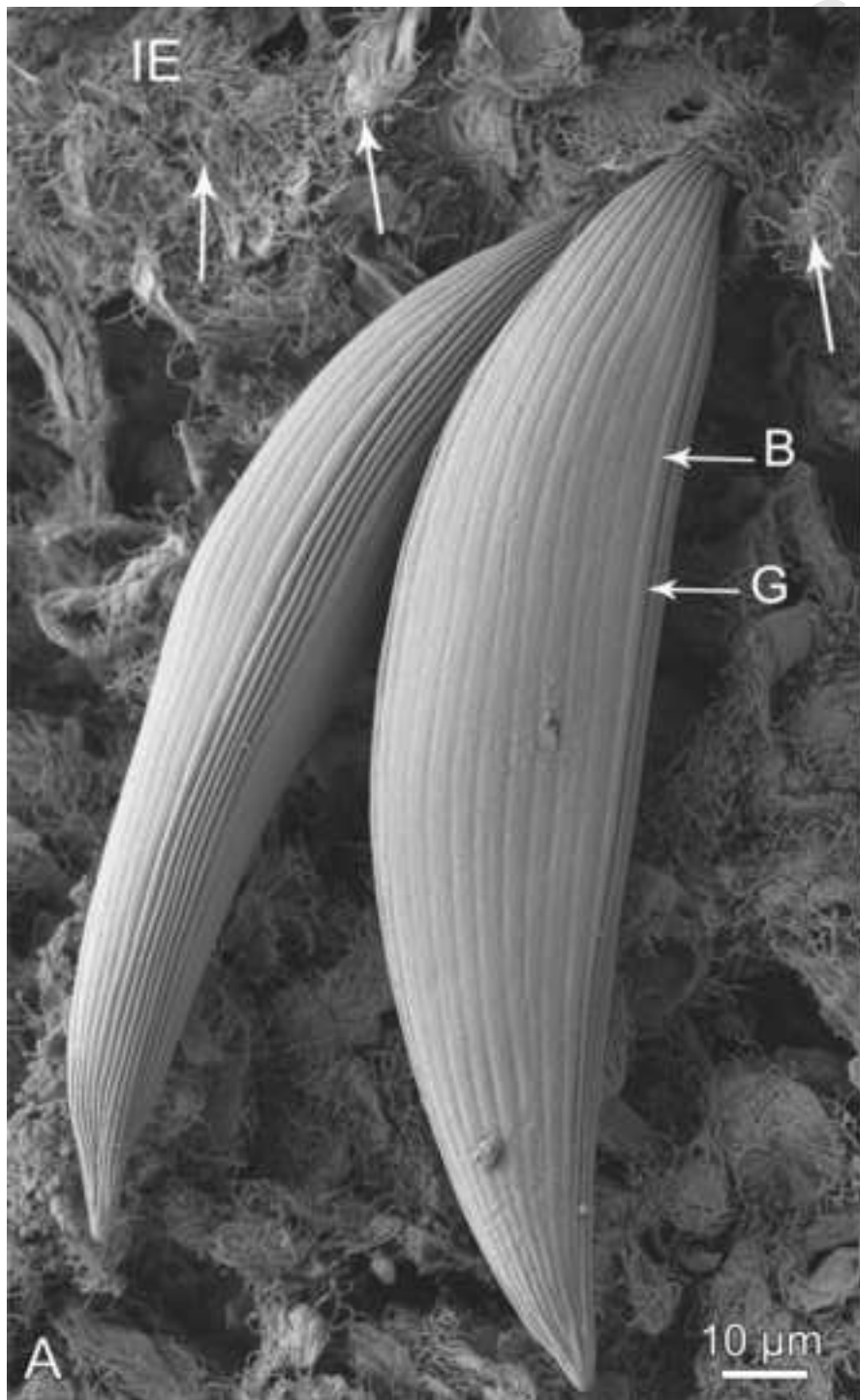


Figure 2.tif

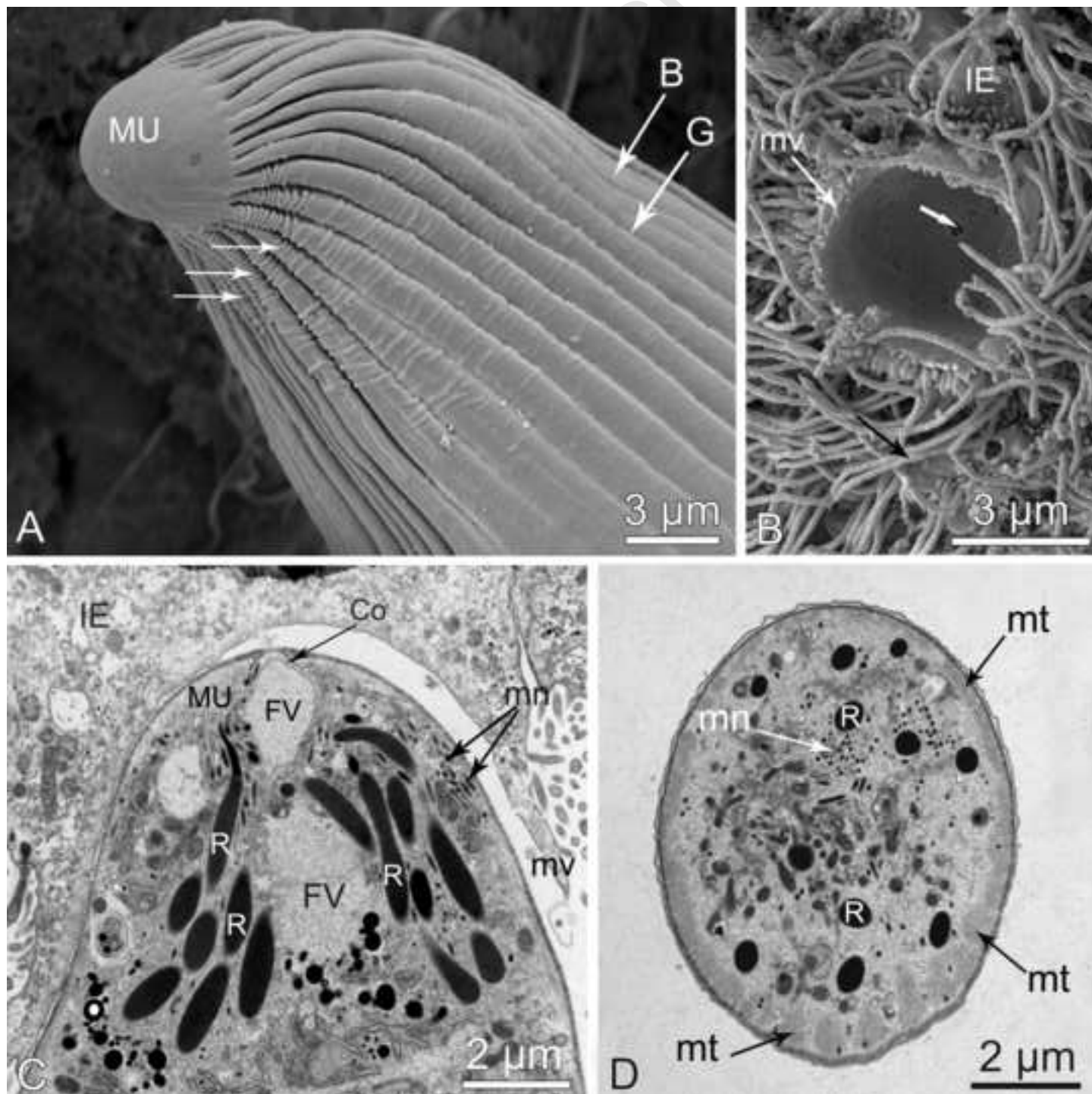
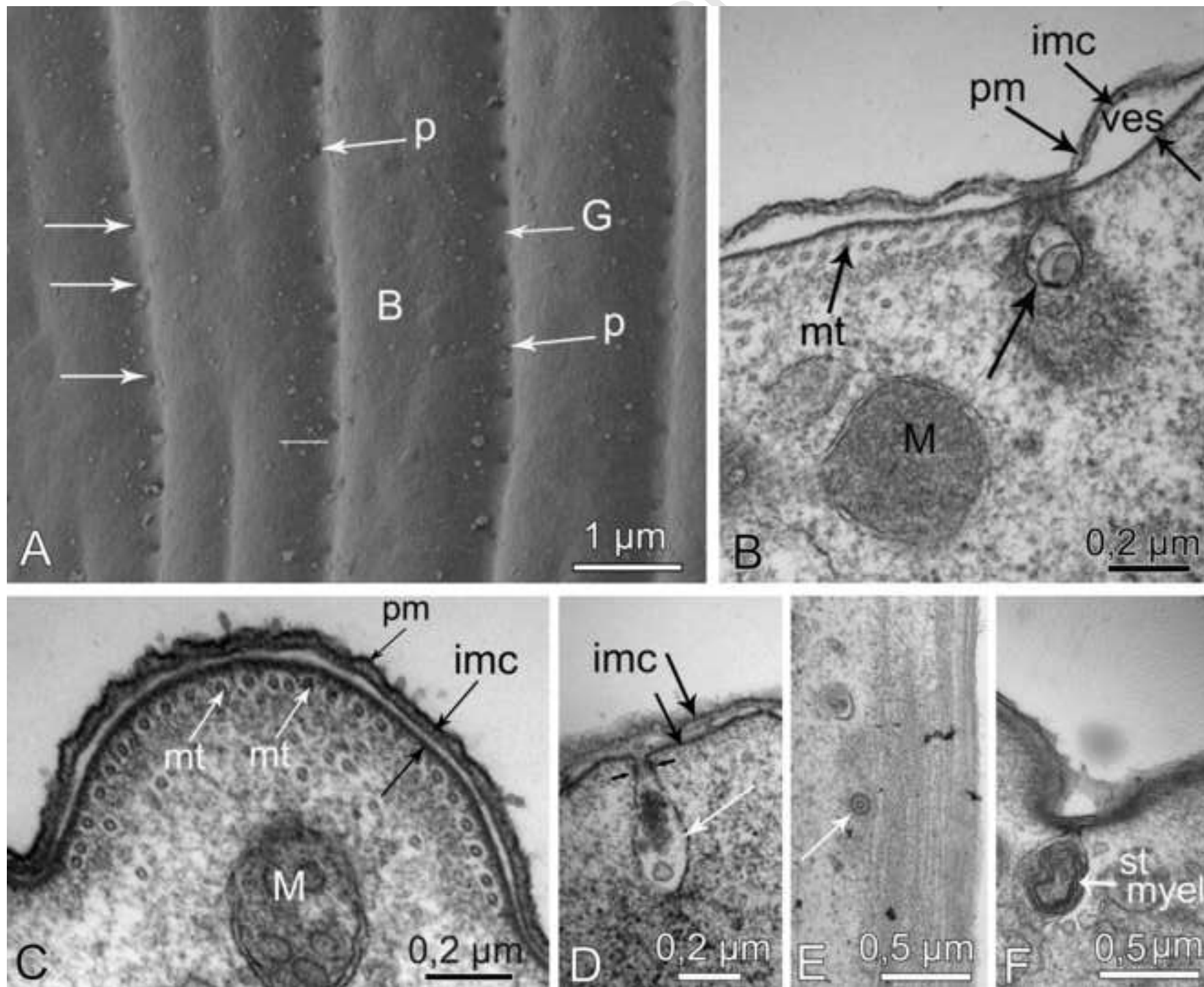
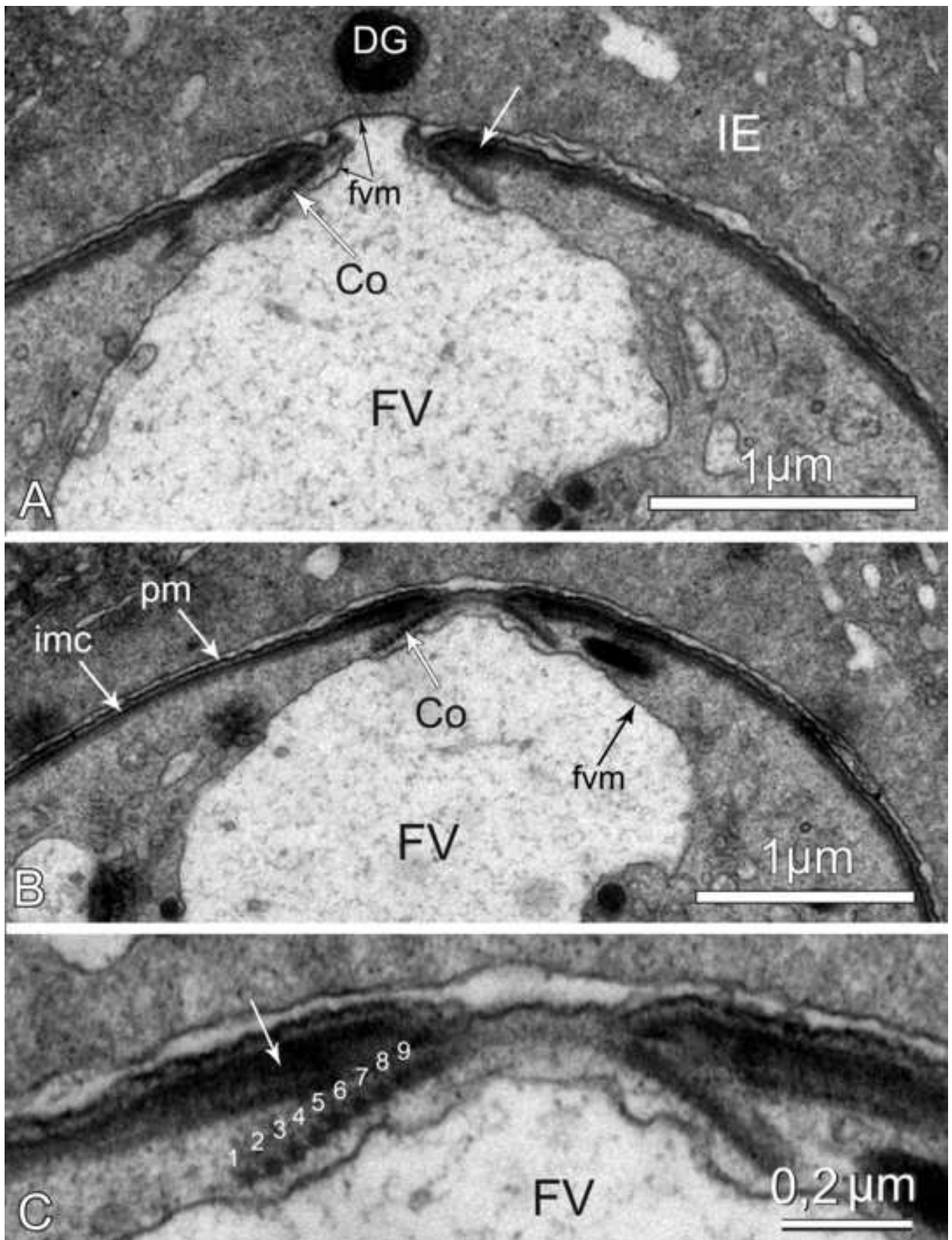
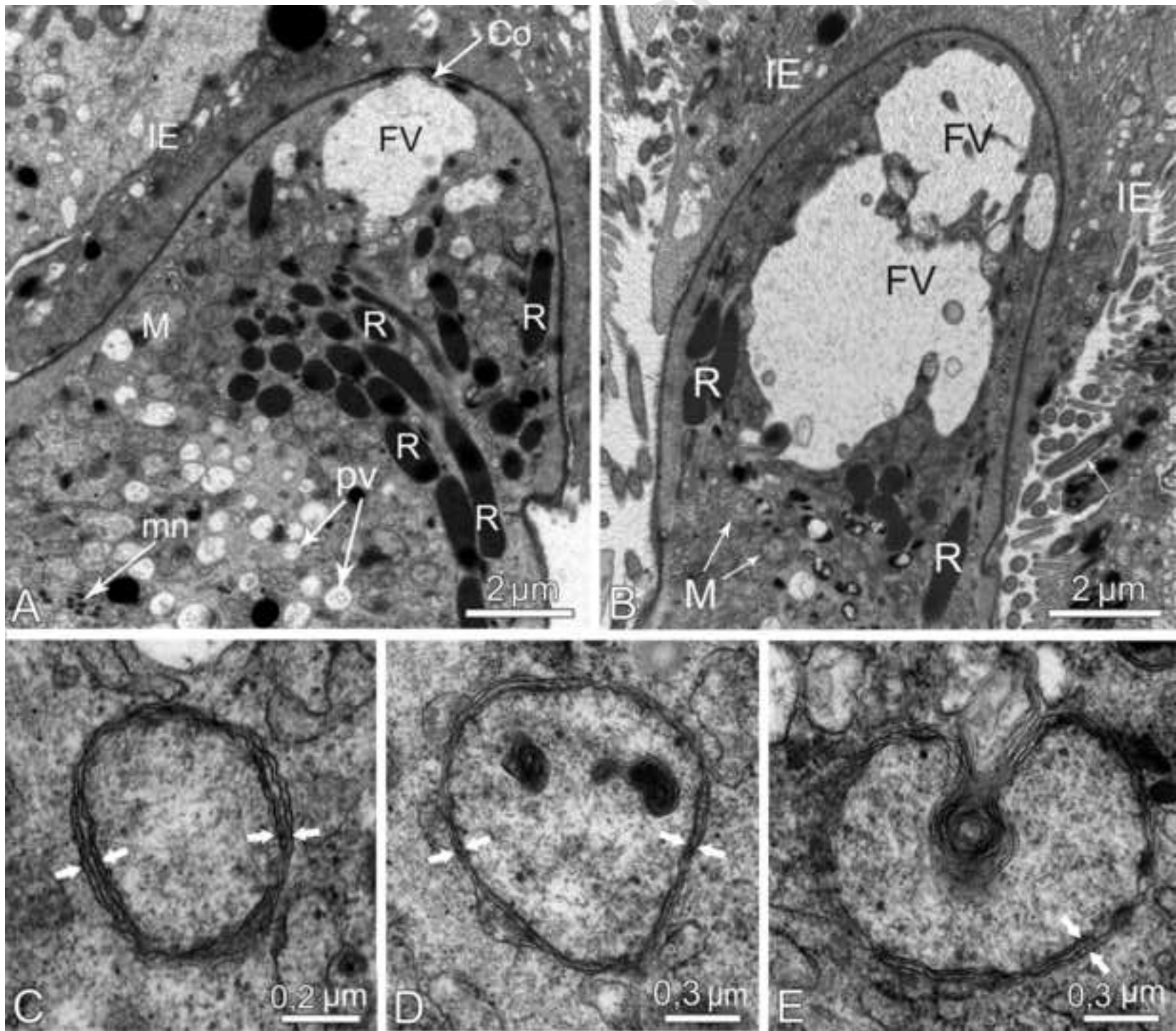
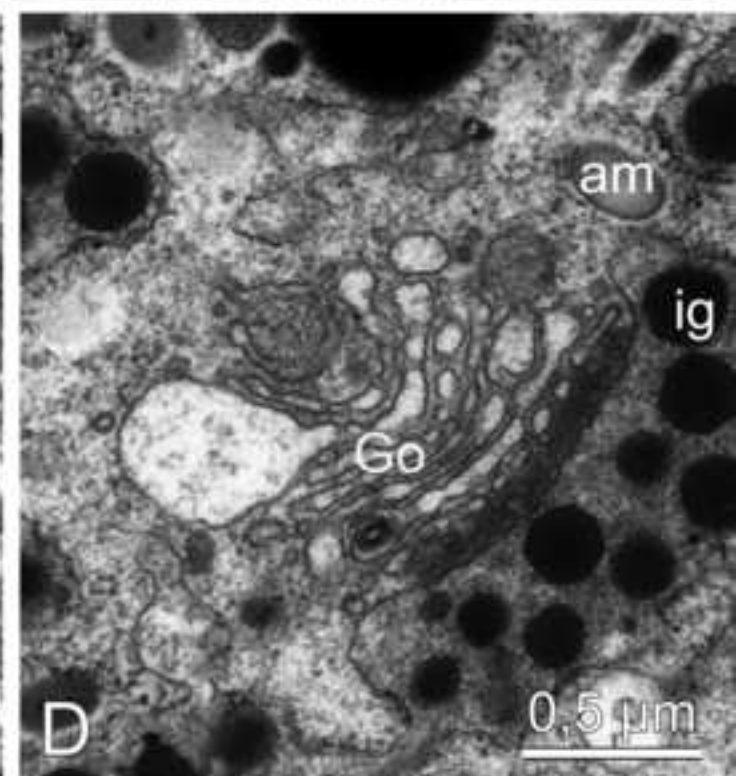
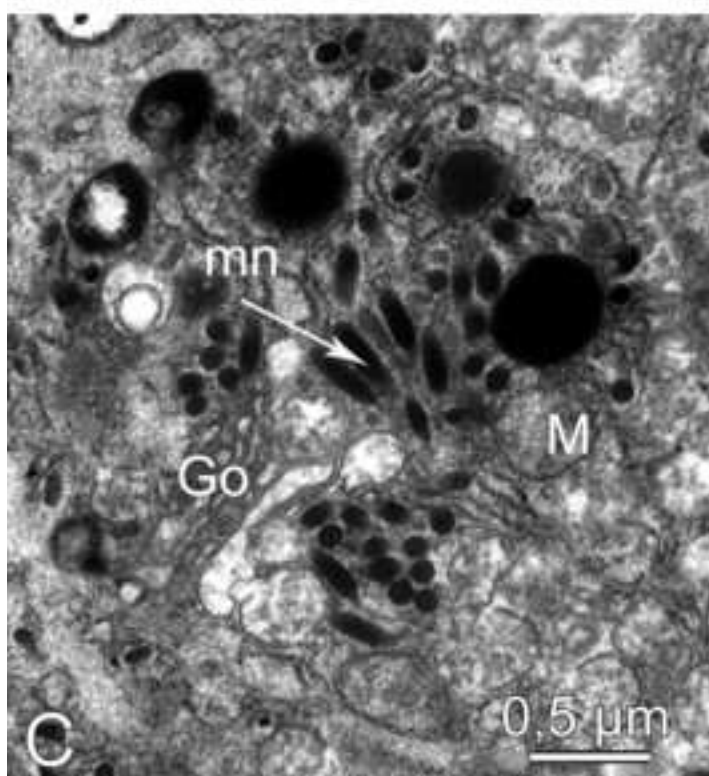
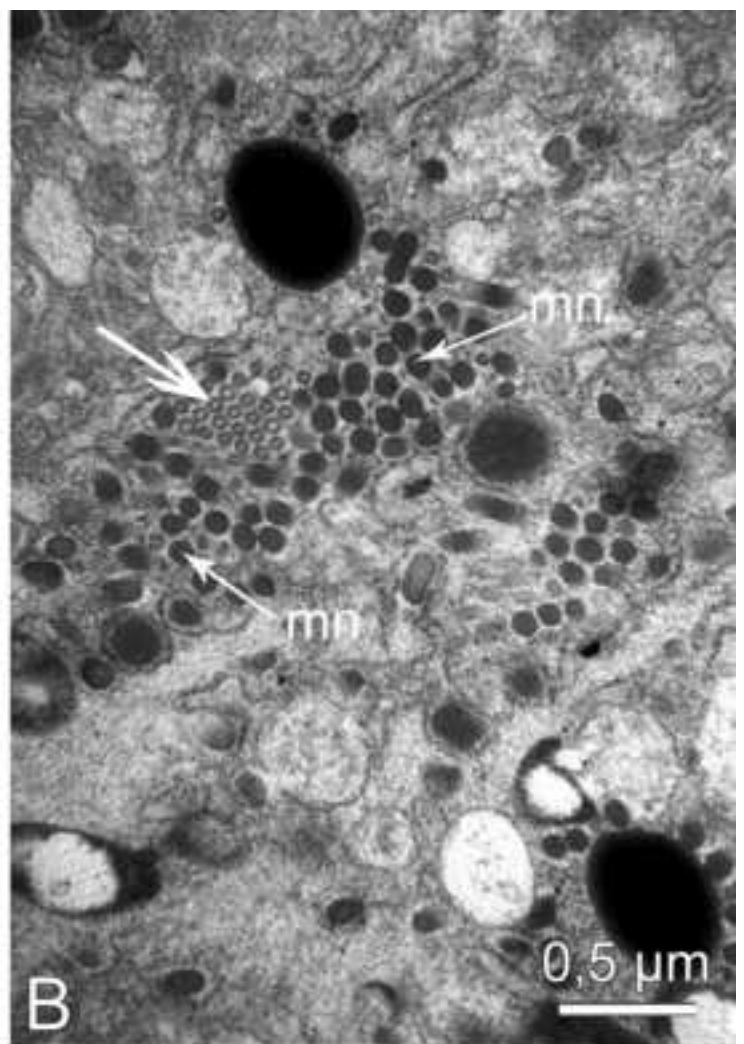
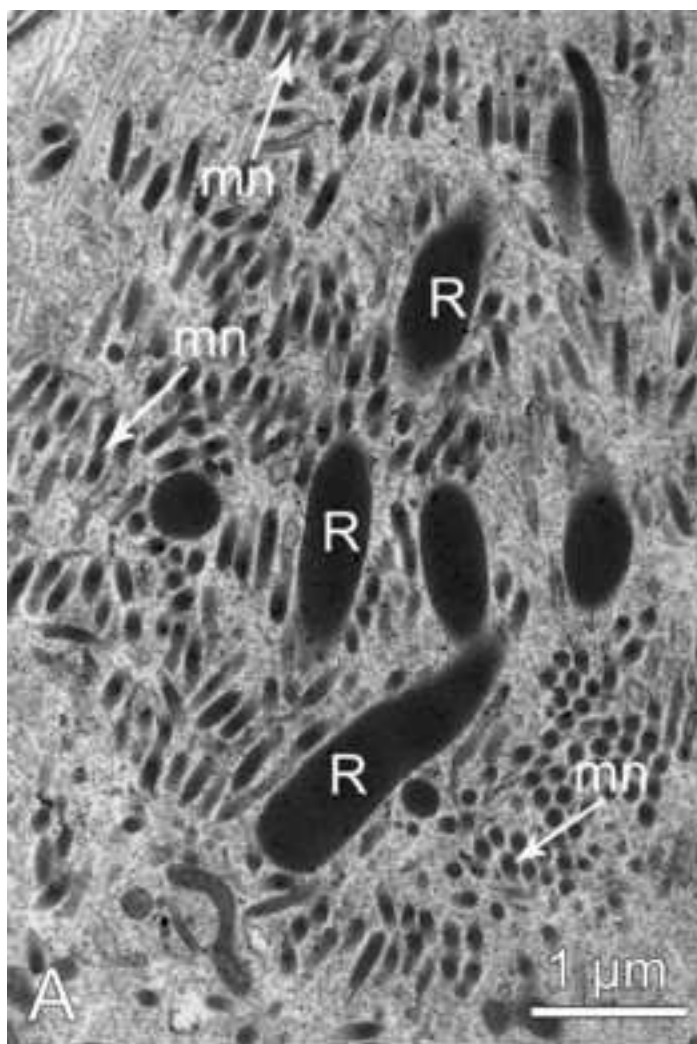


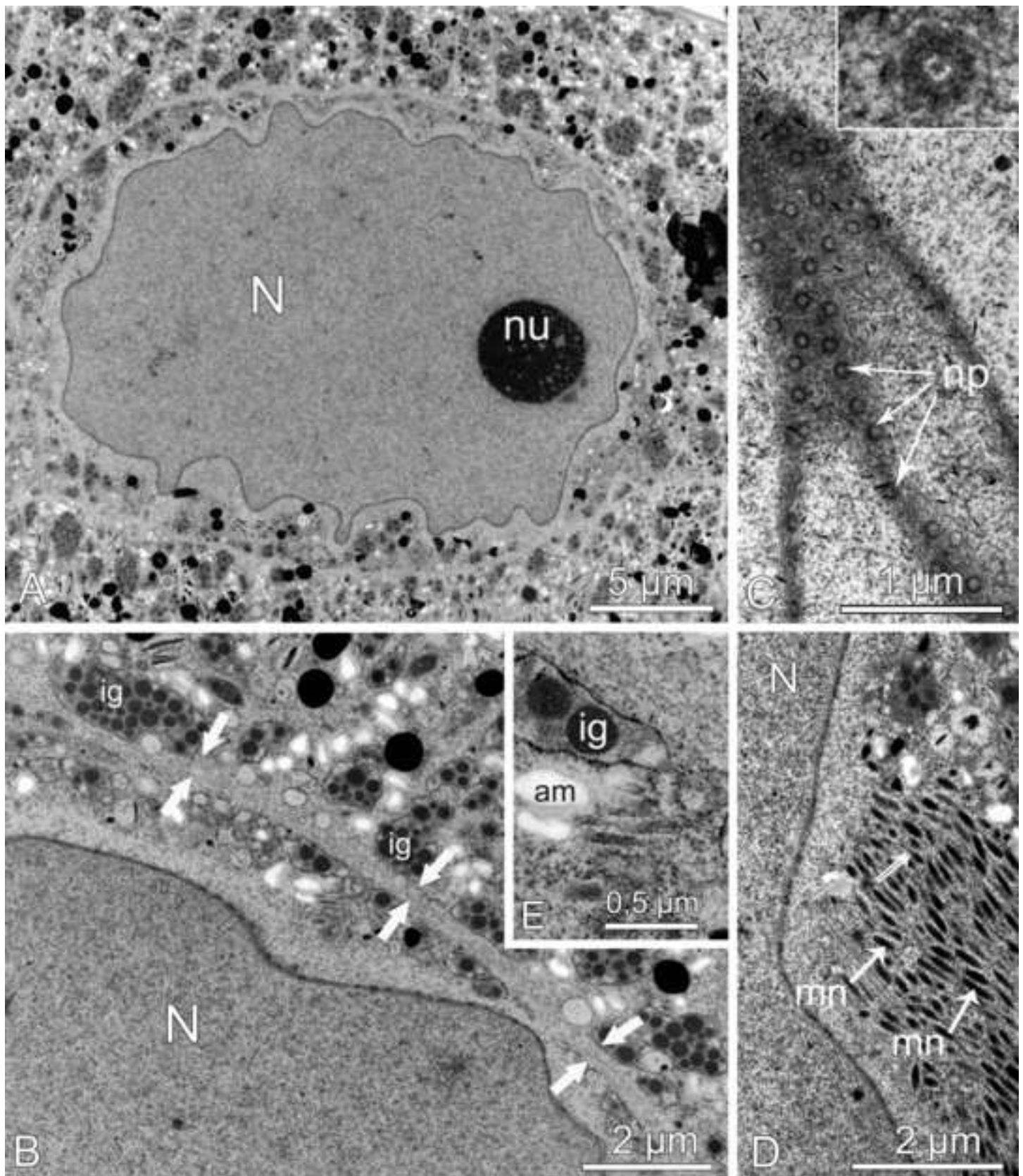
Figure 3.tif

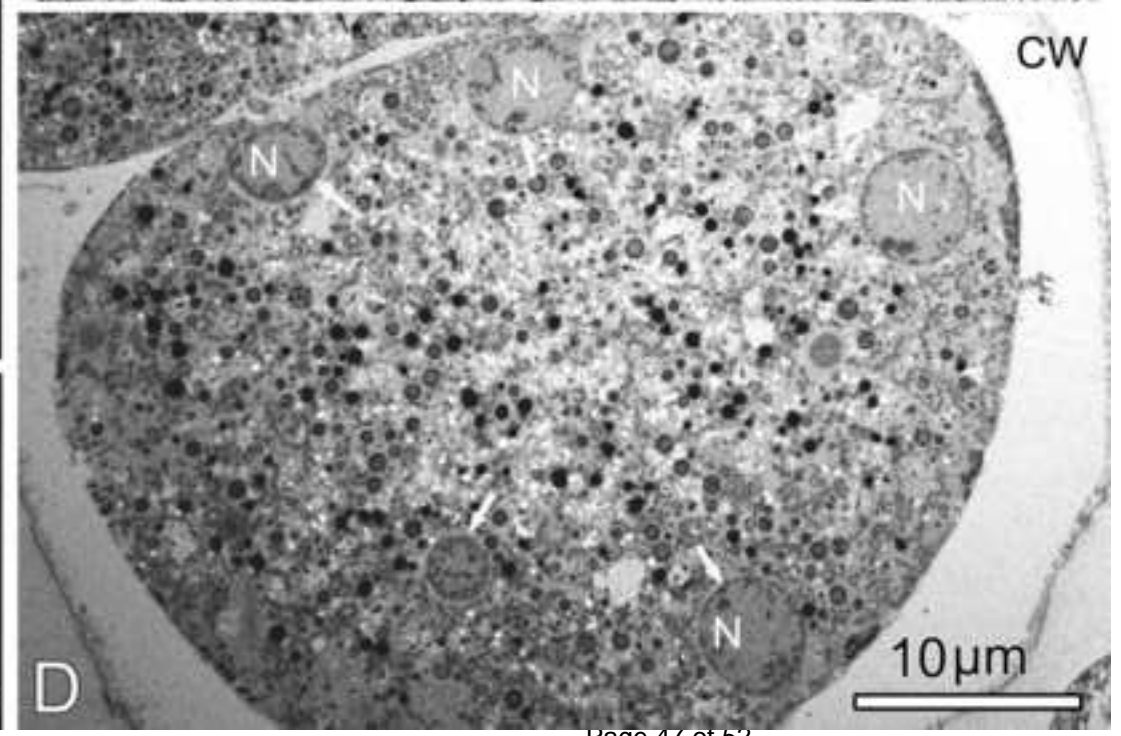
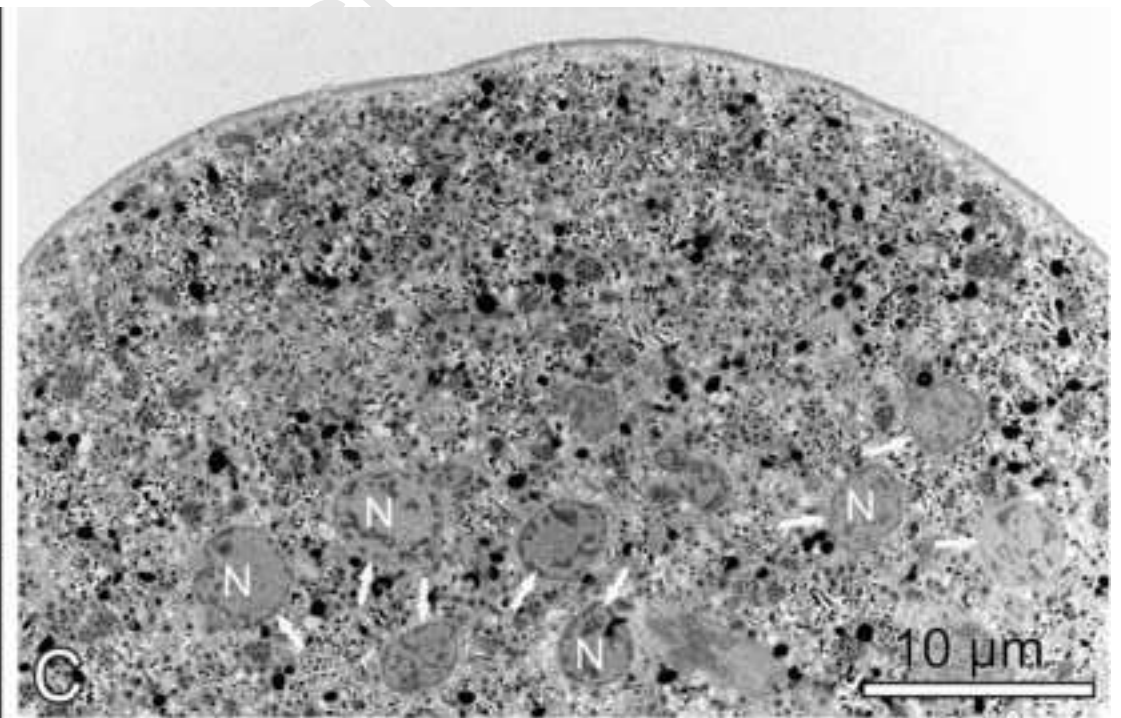
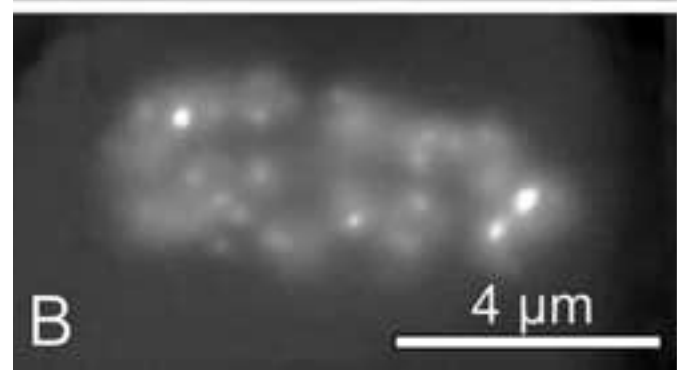
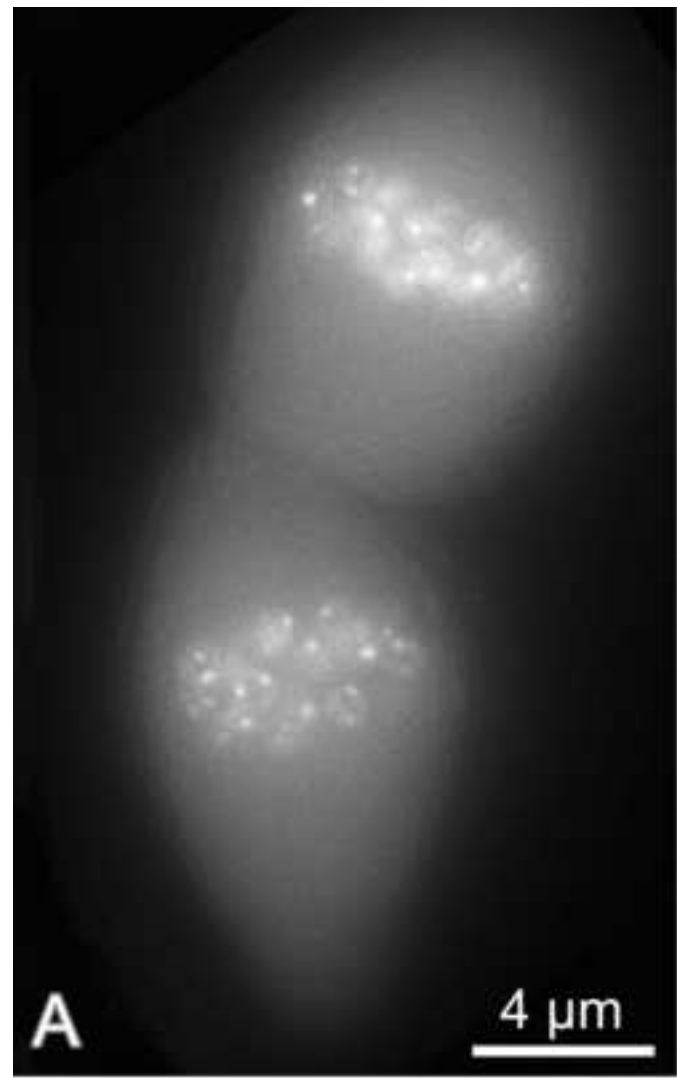












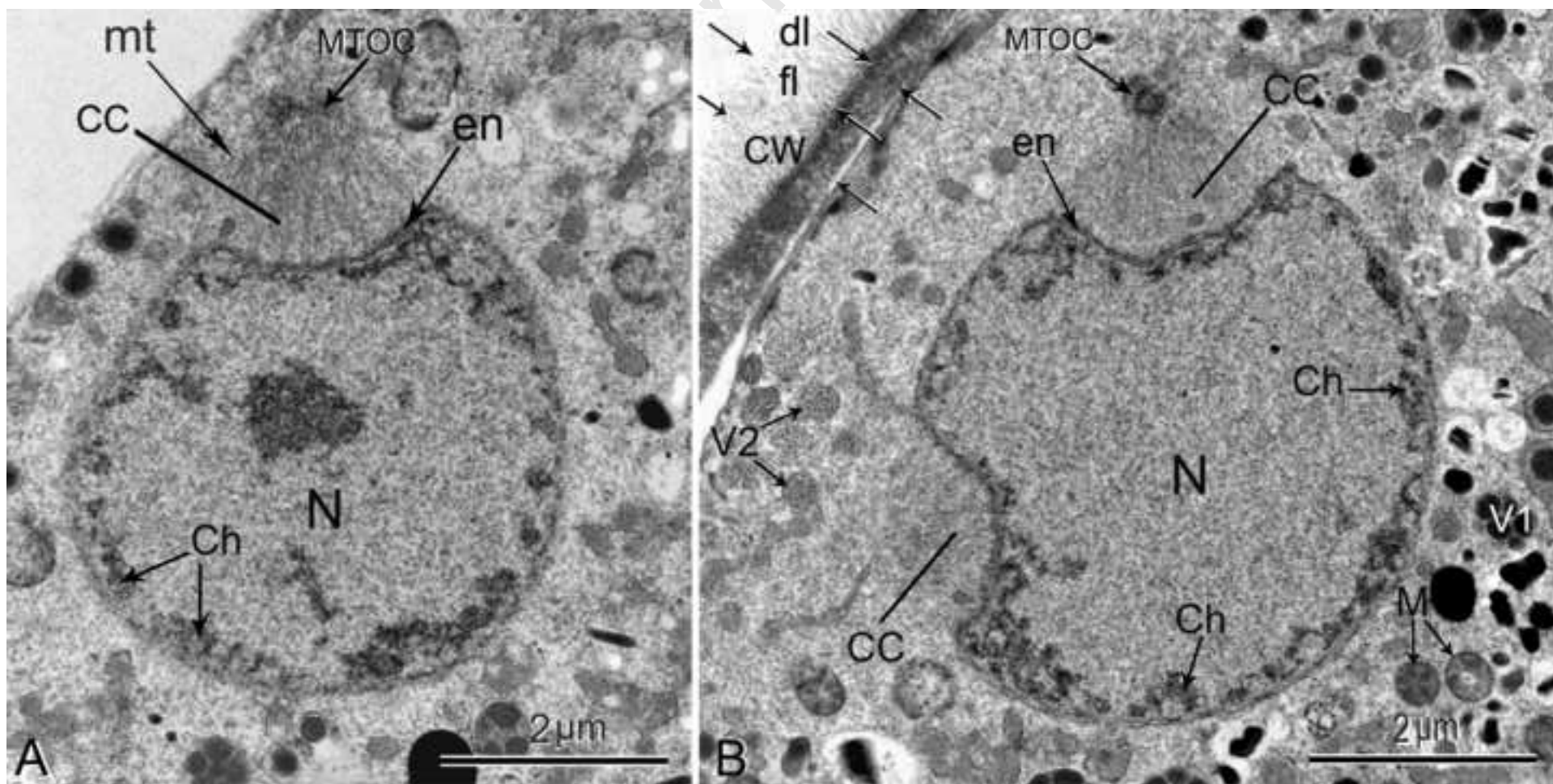


Figure 10.tif

