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Article

Life cycles of chromerids resemble those of colpodellids and apicomplexan parasites

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With 5 figures

Abstract: Chromerids are alveolate algae with secondary plastids surrounded by four membranes, and with evolutionary positions close to the root of apicomplexan parasites. As both described chromerid species, *Chromera velia* and *Vitrella brassicaformis*, are, in spite of their phototrophy, distant relatives, there are some differences between their respective life cycles. Here, we summarize current knowledge of the life cycles of chromerid algae and the related colpodellids. We also describe zoosporangia formation and excystation in *C. velia*. We suggest that the formation of zoospores in *C. velia* is homologous to schizogony in apicomplexan parasites.

Keywords: *Chromera*, *Vitrella*, Chrompodelids, life cycle, schizogony, zoospore formation

Introduction

Alveolate algae with complex plastids, such as dinoflagellates, can form endosymbiotic relationships with corals (Harii et al. 2009; LaJeunesse 2001). In addition to the well-known symbiotic dinoflagellate genus *Symbiodinium*, members of a novel group of alveolate algae named chromerids have been isolated from Australian stony corals. The first species, *Chromera velia* (Fig. 1), was found in *Plesiastrea versipora* collected from Sydney Harbor and was formally described in 2008 (Moore et al. 2008). The second known chromerid species, *Vitrella brassicaformis* (Fig. 2), was isolated from *Leptastrea purpurea* from the Great Barrier Reef (Oborník et al. 2012). These algae are of particular interest due to their close phylogenetic relationship to apicomplexan parasites (Moore et al. 2008; Oborník et al. 2009; Janouškovec et al., 2010; Weatherby and Carter, 2013; Oborník & Lukeš 2013; Janouškovec et al. 2015). Apicomplexa belong to the SAR (Stramenopila + Aleveolata + Rhizaria) eukaryotic super group (Burki et al. 2007); they are known to cause many important human and veterinary diseases including malaria and toxoplasmosis. Apicomplexans typically possess an apical complex, a complex of membranous organelles used to penetrate host cells, and the apicoplast, a non-photosynthetic complex plastid (McFadden et al. 1996; Wilson et al. 1996; Köhler et al. 1997). This relic plastid contains a highly reduced genome consisting of a 35 kb circle and obviously originates from a secondary endosymbiotic event (Ralph et al. 2004; Oborník et al. 2009; Lim & McFadden 2010; McFadden 2011; Oborník & Lukeš 2013; Keeling 2014; Oborník & Lukeš 2015). The discovery of a plastid in the Apicomplexa suggested that the parasitic phylum was derived from a photosynthetic ancestor and instigated a search for photosynthetic relatives that could reveal the identity of this ancestor. The description of coral associated chromerid algae is therefore groundbreaking research: since they are related to the Apicomplexa and they contain regular photosynthetic plastids with genomes overlapping those of the apicoplast and dinoflagellate plastids, meaningful comparative studies can be carried out (Keeling 2008; Moore et al. 2008; Janouškovec et al. 2010). In addition to molecular phylogenies (Moore et al. 2008; Janouškovec et al. 2010; Janouškovec et al. 2015) the non-phylogenetic evidence for evolutionary relationship between chromerids and apicomplexans was also investigated. The presence of a non-canonical heme synthesis pathway in *C. velia*, which is partly homologous to that of apicomplexans (in the use of C4 pathway to synthesize aminolevulinate), provides a very strong argument for the common ancestry of *C. velia* and the Apicomplexa (Kořený et al. 2011). Furthermore, the structure of the plastid super operon suggests a common origin of apicomplexan, chromerid and heterokont plastids (Janouškovec et al. 2010). The use of non-canonical code for tryptophan (UGA) in some plastid encoded proteins has so

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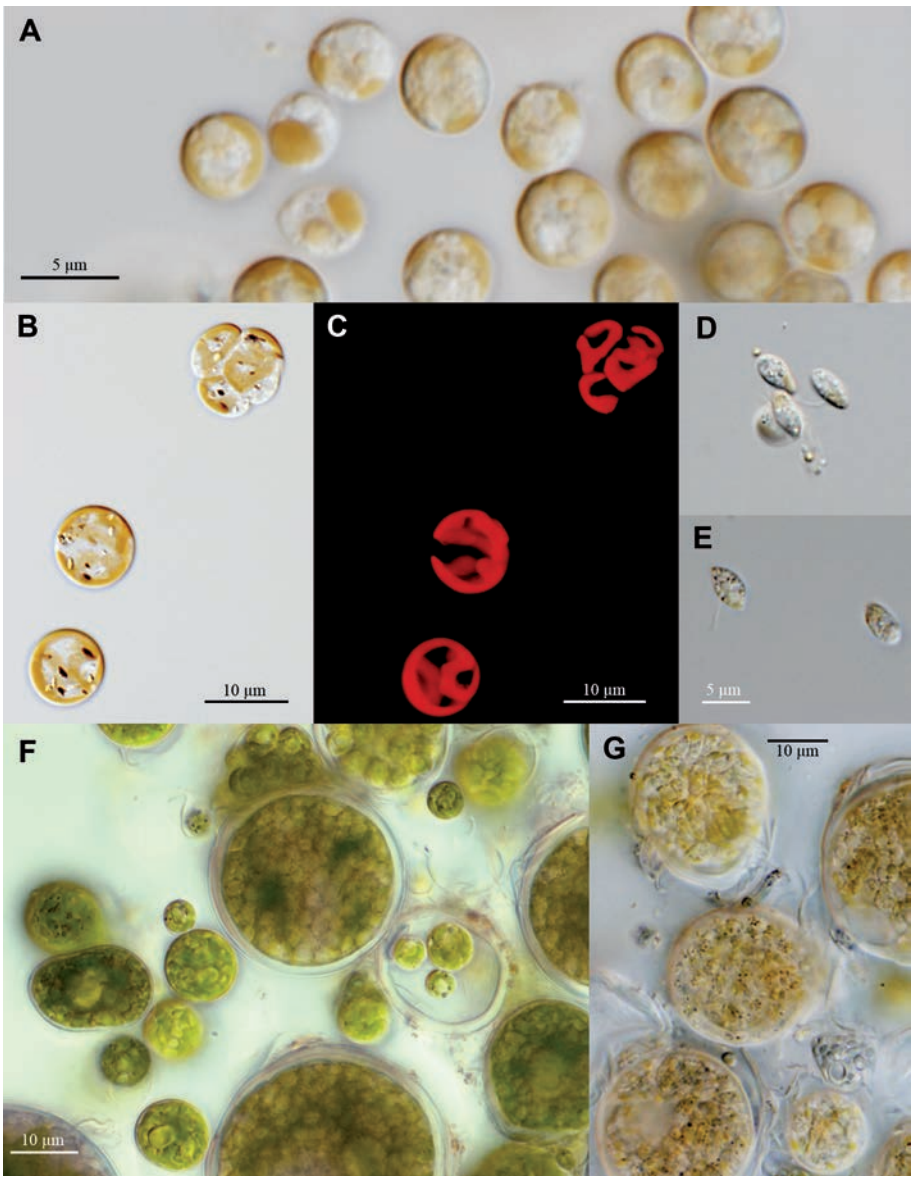

Fig. 1. Light microscopy of *Chromera velia* and *Vitrella brassicaformis*. (**A**) *C. velia* culture; (**B**) vegetative cell and autosporangium (top right) of *C. velia* with four autospores; (**C**) autofluorescence of plastid in *C. velia* vegetative cell and autosporangium; (**D**) zoospores of *C. velia*; (**E**) zoospores of *V. brassicaformis*; (**F**) vegetative cells and autosporangia of *V. brassicaformis*; (**G**) zoosporangia of *V. brassicaformis*.

far been reported exclusively in *C. velia* and coccidians – an advanced lineage of apicomplexan parasites. Analyses of transcriptomes from colpodellids (genera *Alphamonas*, *Colpodella* and *Voromonas*) and chromerids support the monophyly of "chrompodellids" (chromerids + colpodellids), with *V. brassicaformis* appearing at the root of the *Alphamonas* clade, while *C. velia* constitutes the earliest branch of the *Colpodella* + *Voromonas* clade (Janouškovec et al. 2015; Oborník & Lukeš 2015).

Chromerid ultrastructure, morphology and life cycles

Chromerids have been the subject of extensive ultrastructural studies, including descriptions of their vegetative life cycles (Wetherby et al. 2011; Oborník et al. 2011; Oborník et al. 2012; Portman et al. 2014). These studies revealed features typical of apicomplexan parasites – cortical alveoli subtended by a sheet of microtubules, micropores, a pseudoconoid and

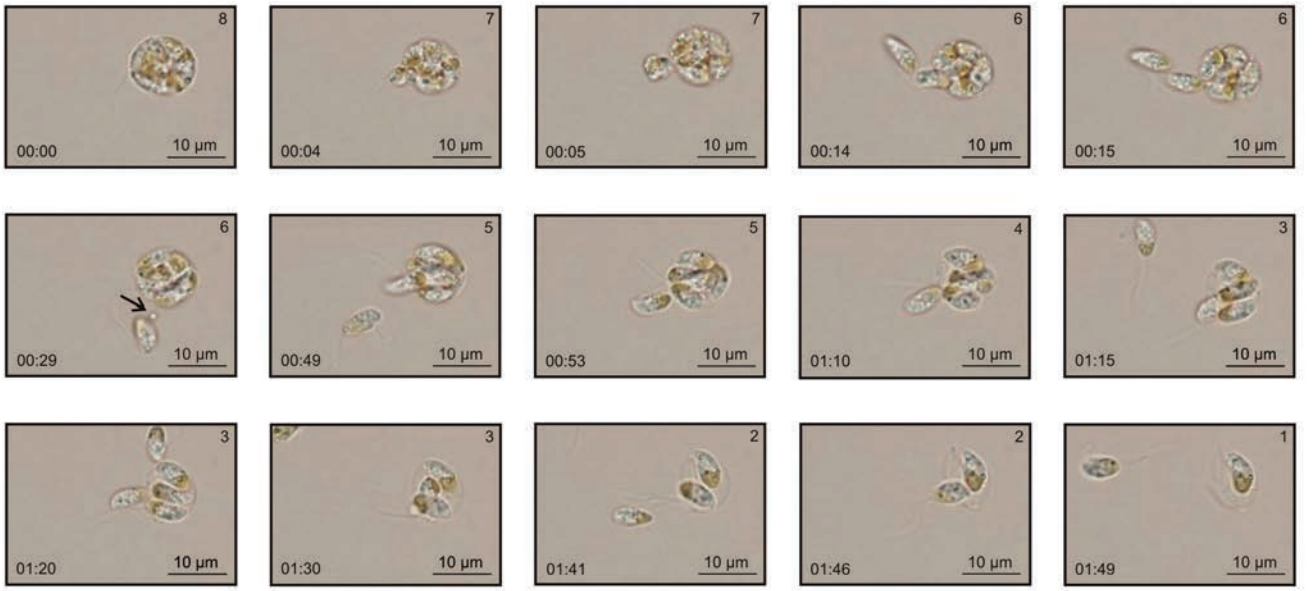

Fig. 2. Excystation of zoospores of *Chromera velia*. The entire process captured herein takes about two minutes. The zoospores starts to rotate within the zoosporangium that subsequently ruptures and spores are released one by one through a rupture in the wall. The release of the residual body is shown (6). Scale bar is 10 µm.

a four-membrane plastid envelope (Janouškovec et al. 2010; Oborník et al. 2011; Oborník et al. 2012; Oborník & Lukeš 2013). Three distinctive life stages have been documented so far in *C. velia* (Oborník et al. 2011). Vegetative coccoid cells (Fig. 1A, B) are the predominant life cycle stage in culture. They divide by binary division and form autosporangia (cysts) covered by an additional membrane (Fig. 1B) with 2–4 coccoid cells. Both immotile stages are protected by a thick and resistant cell wall. In addition to non-motile stages, zoospores (Fig. 1D) with thin cell walls and two heterodynamic flagella have been observed. They exhibit a typical finger-like projection on the shorter flagellum and their cell shape resembles that of colpodellid trophozoites. The appearance of zoospores in culture is induced by light (Oborník et al. 2011) and is also inferred to be influenced by salinity (Guo et al. 2010). However, zoosporangia were not initially observed in *C. velia* and zoospores were thought to develop directly from coccoid cells (Oborník et al. 2011). Later, the formation of two zoospores from a single maternal cell (zoosporangium) was described by Portman et al. (2014). Here we show the presence of large zoosporangia (up to 15 µm in diameter) observed in culture, which contain even numbers (2–10) of zoospores. The process of excystation starts with the movement of zoospores inside the zoosporangium, which ultimately leads to rupture of the zoosporangium wall. The speed of excystation depends on the rupture – the larger the tear, the faster the release of the zoospores. The excystation shown in Figure 2 (for the movie see the link at the end of this article) took about 2 minutes and zoospores were released through a small rupture one by one. The release of a structure resembling the residual body in Apicomplexa was observed during the excystation of *C. velia* zoosporangia (Fig. 2 and the movie). However, we also observed much faster excystations where the zoosporangium wall ripped almost entirely around its circumference and zoospores were released simultaneously. Zoospores are highly abundant in culture between the 4th and 8th day after inoculation, when they can represent over 80% of the cells. Only asexual life stages have been observed in *C. velia*. Released autospores (immotile vegetative coccoid cells) divide forming autosporangia. In parallel, some vegetative cells develop to large zoosporangia with flagellated zoospores (Fig. 2). A zoospore can transform to a vegetative cell within 10 minutes and complete the life cycle (Oborník et al. 2011). The function of zoospores (Fig. 1D, E) is unknown; however, they may enable rapid dispersal and colonization of nearby areas. Furthermore, when the culture is exposed to concentrated high intensity light, all exposed cells disappear; it is possible that they form motile zoospores which subsequently escape from the exposed area. Since coral larvae infected by *C. velia* have been observed (Cumbo et al. 2013), it is tempting to speculate that zoospores function as an infective stage and invade corals (Fig. 3). At this point in time, however, nothing is known about *C. velia's* life cycle as a coral endosymbiont.

The ultrastructure of *Vitrella brassicaformis* is distinct from that of *Chromera velia*. The cell wall of vegetative cells and sporangia is laminated in *V. brassicaformis*, resulting in a cell shape resembling cabbage heads (Fig. 4A). We have

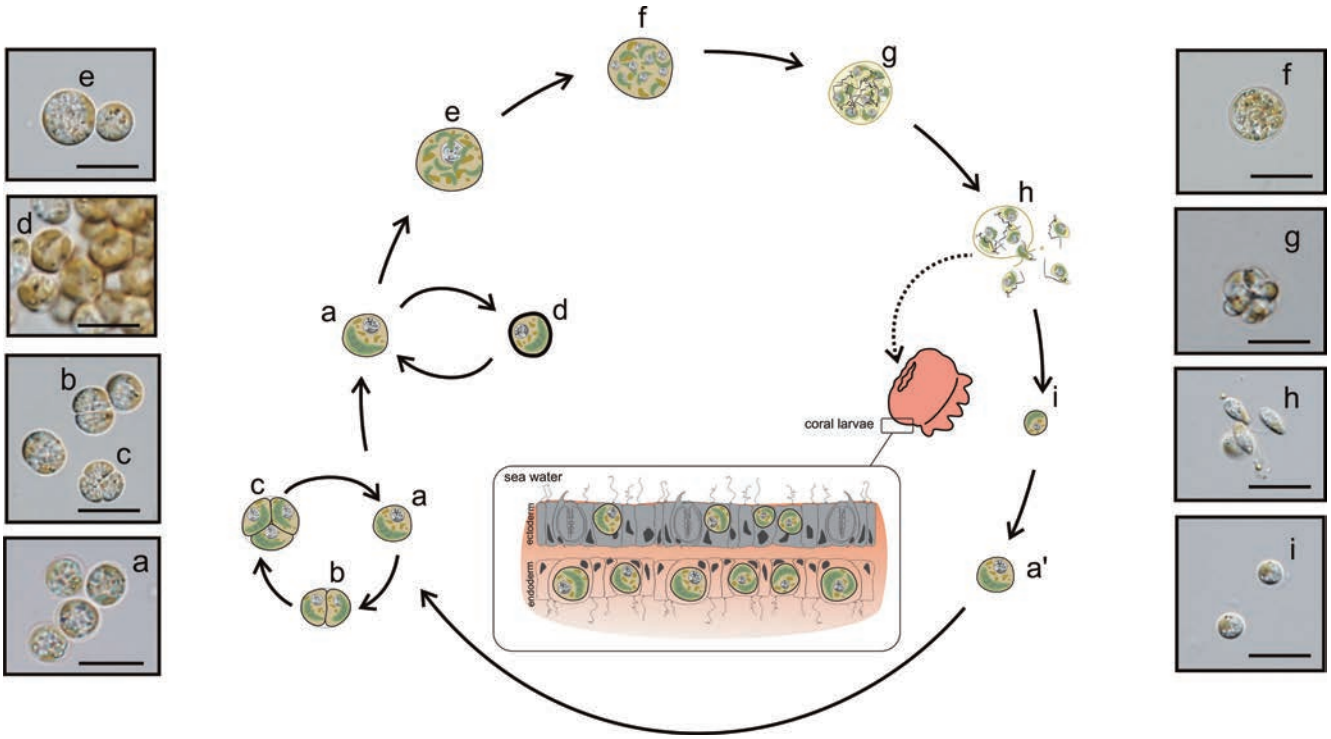

Fig. 3. Life cycle of *Chromera velia*: vegetative cell (**a, a′**) divides and form an autosporangium with dublets (**b**) to tetrades (**c**) of vegetative cells covered by additional membrane. The vegetative cell can develop to a zoosporangium (**g, h**) from which the zoospores are released through the rupture in the wall of sporangium. They are supposed to infect coral larvae or to encyst (**i**) and form a vegetative cell.

observed up to twelve cell wall layers. Although the cell wall is thick and multilayered, it is fully transparent, allowing for photosynthesis in all non-motile life stages. While vegetative cells of *C. velia* are all similar in size, ranging from 5–7 µm in diameter (Oborník et al. 2011), the size of vegetative cells in *V. brassicaformis* ranges from 3 µm (in released autospores) to 40 µm (in vegetative cells prior to sporangium formation) (Fig. 1F). Numbers of autospores and zoospores in sporangia are much higher in *V. brassicaformis* than in *C. velia*: they contain dozens of spores per sporangium (Oborník et al. 2012), in contrast to 4 autospores and 10 zoospores as maximum numbers for *C. velia* sporangia (Oborník et al. 2011; Oborník & Lukeš 2013). The zoosporangium of *V. brassicaformis* has a clearly visible operculum that readily serves as an exit point for the zoospores within (Oborník et al. 2012), in contrast to the unpredictable rupture of the zoosporangium wall in *C. velia*. Although *V. brassicaformis* seems like it should have, due to high numbers of spores in sporangia, higher reproductive rates, in culture it grows much more slowly than *C. velia* (Oborník et al. 2012; Flegontov et al. 2015). Zoospores of *V. brassicaformis* are also bi-flagellated, but the finger-like projection found in *C. velia* is not observed. Generally, the life cycle of *V. brassicaformis* is as follows: Autospores (immotile vegetative cells) are released and as vegetative cells grow from an initial size of 3 µm up to 30–40 µm. They subsequently form one of two types of sporangia: autosporangia (green) are full of small autospores (Fig. 1F) and zoosporangia (gray) contain dozens of zoospores (Fig. 1G). They are both released through an operculum in the wall of the sporangium (Oborník et al. 2012; Oborník & Lukeš 2013). We should note that zoospores are rare in cultures of *V. brassicaformis*, and the life cycle together with the fate of zoospores is not as well-described as it is in *C. velia*. The slow growth of *V. brassicaformis* in culture also makes it less amenable to use as a model organism.

As previously mentioned, *C. velia* and *V. brassicaformis* are not closely related; they are recovered as isolated phototrophic lineages within the heterotrophic colpodellids (Gill & Slamovits 2014; Janouškovec et al. 2015; Oborník & Lukeš 2015). The two taxa combine to form a novel group, the "chrompodelids" (Janouškovec et al. 2015). In the life cycle of *Colpodella vorax* (Brugerolle 2002), trophozoites with two heterodynamic flagella encyst and divide into a four-celled cyst, similar to *C. velia*. New trophozoites are later released from the cyst. Trophozoites resemble chromerid zoospores and the cysts resemble the sporangia of *C. velia*, mainly due to the low and even number of trophozoites (spores) formed. In colpodellids conjugation between trophozoites has been reported (Simpson & Patterson 1996;

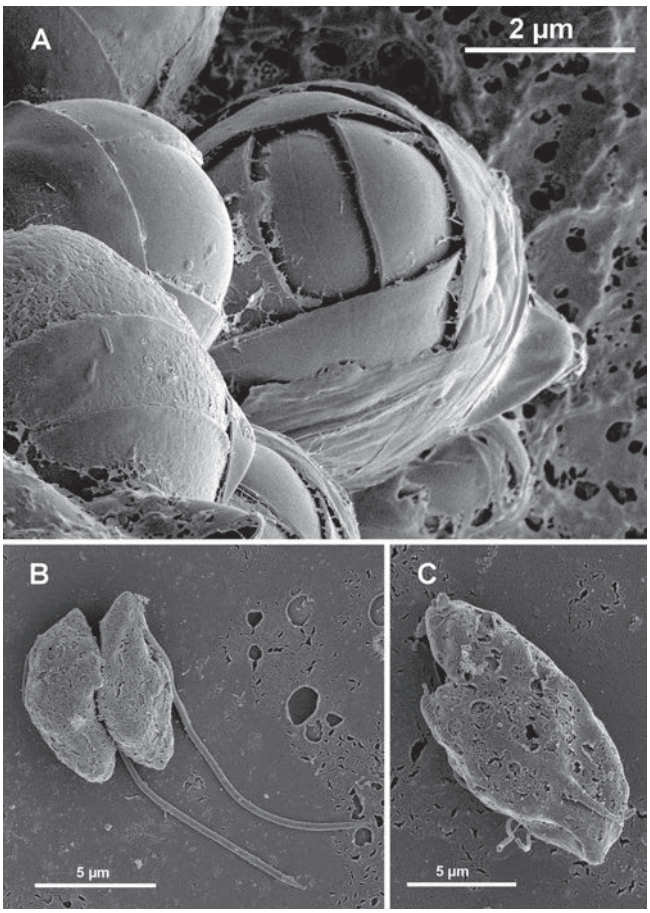

Fig. 4. (**A**) Scanning Electron Microscopy (SEM) picture of the vegetative cell of *Vitrella brassicaformis* resembles "cabbage head"; this shape gave together with full transparency of cell walls the alga name; (**B**) Possible fusion (or fission) of zoospores in chromerid alga *V. brassicaformis*. SEM picture was made using method described elsewhere (Moore et al. 2008).

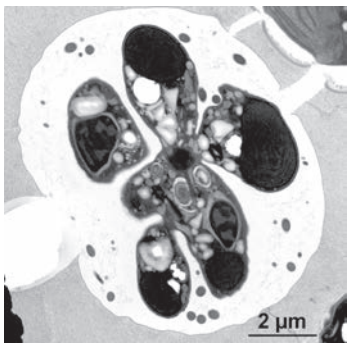

Fig. 5. Transmission Electron Microscopy (TEM) picture shows formation of zoospores in the zoosporangium of *Chromera velia* that highly resembles schizogony in apicomplexan parasites. TEM picture was made using method described elsewhere (Moore et al. 2008).

Brugerolle 2002), suggesting a sexual phase in their life cycle; sexual stages have not been observed in *C. velia* (Oborník et al. 2011, 2012). Some electron micrographs of *V. brassicaformis* flagellates appear to depict cell fusion (Fig. 4); since we have only observed this behavior a few times out of hundreds of images, however, we believe that conjugation occurs rarely, if at all. Nevertheless, due to a possible homology with *Alphamonas edax*, we speculate that zoospores can fuse together; this suggests the occurrence of sexual reproduction in the *V. brassicaformis* clade (Fig. 4B, C).

The almost complete absence of synapomorphies in the life cycles of chromerids and apicomplexan parasites demonstrates the degree to which apicomplexan parasites are derived, because it is rather difficult to find any obvious developmental similarities between the two groups. *Chromera velia* contains a pseudoconoid, a primitive apical complex similar to that used for feeding by colpodellids and apicomplexans, which has not yet been found in *V. brassicaformis* (Oborník et al. 2011; Portman et al. 2014). When the zoosporangium of *C. velia* was investigated by transmission electron microscopy (TEM), zoospores appeared to be highly organized in the sporangium, forming a star-like structure similar to that observed during apicomplexan schizogony (Fig. 5), a stage in the asexual reproduction of apicomplexans involving multiple fission of the parasite nucleus followed by fragmentation of the cytoplasm. Similar arrangements of flagellated cells in a cyst were shown in *Colpodella tetrahymenae* intracyst division (Cavalier-Smith & Chao 2004), which is not, however, proposed to form multinuclear cells (Brugerolle 2002) as it is in the Apicomplexa. It is very likely that life cycles vary substantially between colpodellids, because they differ between chromerids and even in the genus *Colpodella*: resting cysts of *C. vorax* develop through two consequential mitotic divisions to the four-celled cyst, followed with formation and release of trophozoites (Brugerrole 2002), while *C. unguis* was documented to form trophozoites directly from the resting cyst, followed by feeding and fission of the trophozoites (Mylnikov 2009). The observation of dividing trophozoites in colpodellids opens the possibility that the inferred fusion of *V. brassicaformis* zoospores discussed above (Fig. 4B, C) could instead be fission.

Conclusions

Due to their high trophic diversity, we cannot at this time propose a general life cycle for chrompodellids; however, it almost certainly includes motile zoospores (trophozoites) as a predominant stage in the colpodellid cycles, and vegetative cells likely dominate chromerid life cycles. As colpodellids lost photosynthesis at some point in their evolutionary history; the predominance of trophozoites with an apical

complex-like feeding apparatus could reflect a reliance on predation for energy acquisition. Since photosynthesis seems to be optimized in chromerid vegetative cells, this may have resulted in their predominance within chromerid life cycles (Oborník et al. 2011). Zoospores of *C. velia* and trophozoites of colpodellids are formed in a process resembling schizogony in apicomplexan parasites; this represents the only known developmental synapomorphy between the Apicomplexa and their close relatives the chrompodelids.

This paper is accompanied by a youtube video showing excystation of the zoosporangium of *C. velia*: https://www.youtube.com/watch?v=o1NqX9BjCJY

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