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Structures related to attachment and motility in the marine eugregarine *Cephaloidophora* cf. *communis* (Apicomplexa)

Magdaléna Kováčiková[a,*], Timur G. Simdyanov[b], Andrei Diakin[a], Andrea Valigurová[a]

[a] *Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic*
[b] *Department of Invertebrate Zoology, Faculty of Biology, Lomonosov Moscow State University, Leninskiye Gory 1-12, Moscow 119234, Russian Federation*

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Abstract

Gregarines represent a highly diversified group of ancestral apicomplexans, with various modes of locomotion and host-parasite interactions. The eugregarine parasite of the barnacle *Balanus balanus, Cephaloidophora* cf. *communis,* exhibits interesting organisation of its attachment apparatus along with unique motility modes. The pellicle covered gregarine is arranged into longitudinal epicytic folds. The epimerite is separated from the protomerite by a septum consisting of tubulin-rich filamentous structures and both are packed with microneme-like structures suggestive of their function in the production of adhesives important for attachment and secreted through the abundant epimerite pores. Detached trophozoites and gamonts are capable of gliding motility, enriched by jumping and rotational movements with rapid changes in gliding direction and cell flexions. Actin in its polymerised form (F-actin) is distributed throughout the entire gregarine, while myosin, detected in the cortical region of the cell, follows the pattern of the epicytic folds. Various motility modes exhibited by individuals of *C.* cf. *communis*, together with significant changes in their cell shape during locomotion, are not concordant with the gliding mechanisms generally described in apicomplexan zoites and indicate that additional structures must be involved (e.g. two 12-nm filaments; the specific dentate appearance of internal lamina inside the epicytic folds).

Keywords: Actin; α-Tubulin; Apicomplexa; Cell motility; Eugregarine; Myosin

Introduction

Apicomplexans (Apicomplexa Levine 1980, emend. Adl et al. 2012) are one of the most investigated group of protists, comprising exclusively parasites of vertebrates as well as invertebrates. Besides important pathogens of human and agricultural animals (*Toxoplasma gondii*, *Plasmodium* spp., *Cryptosporidium* spp., and *Eimeria* spp.), this group comprises highly diversified basal lineages, including gregarines. Gregarines are obligate parasites that inhabit a wide range of

Abbreviations: AB, alcian blue; CLSM, confocal laser scanning microscopy; FITC, fluorescein isothiocyanate; IFA, indirect immunofluorescent assay; IMC, inner membrane complex; LM, light microscopy; PBS, phosphate buffered saline; PFA, paraformaldehyde; RR, ruthenium red; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TRITC, tetramethylrhodamine isothiocyanate.

*Corresponding author. Fax: +420 549 49 8331.
E-mail address: kovacikova@sci.muni.cz (M. Kováčiková).

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terrestrial, freshwater, and marine invertebrates and urochordates (Desportes and Schrével 2013). In particular, gregarines occurring in marine environments have retained specific characteristics inferred to be ancestral and are considered to be deep-branching apicomplexans. Molecular phylogenetic evidence is concordant with this interpretation (Leander 2008). In general, the development of gregarines takes places in the digestive tract, reproductive organs and body cavities of their hosts, hereby explaining their varied shapes and motility modes. Gregarine trophozoites are attached to host tissue by a specialised apical part forming a mucron or an epimerite (Schrével and Desportes 2015). After the vegetative phase of their life cycle, trophozoites detach and transform into sexual stages, called gamonts, which are usually motile (Schrével and Desportes 2015).

The invasive stages (zoites) of apicomplexans are characterised by a unique set of organelles called the apical complex. Their pellicle consists of a plasma membrane which is underlain by a closely apposed inner membrane complex (IMC). The pellicle is associated with numerous cytoskeletal elements such as microtubules, a network of intermediate filament-like proteins, actin, and myosin (Morrissette and Sibley 2002). While motile apicomplexan zoites are reported to employ a unique mechanism of substrate-dependent gliding facilitated by a conserved form of the actomyosin motor (the so called glideosome concept) first proposed for *Toxoplasma gondii* and *Plasmodium* spp. (Kappe et al. 2004; Keeley and Soldati 2004; Opitz and Soldati 2002), the precise machinery involved in the motility of apicomplexan ancestral groups remains unclear. Indeed, in gregarine trophozoites and gamonts, several types of motility have been described. For example, the pendular or rolling movement in archigregarines of the family Selenidiidae Brasil, 1907 seems to be facilitated by regular sets of subpellicular microtubules (Schrével et al. 1974; Stebbings et al. 1974). Meanwhile, the motility mode mostly displayed by intestinal eugregarines is a form of progressive linear gliding usually without obvious changes in cell shape (King 1981, 1988) and accompanied by the secretion of mucus leaving a trail behind (Mackenzie and Walker 1983; Valigurová et al. 2013; Walker et al. 1979). The pellicle of most eugregarines forms numerous longitudinal epicytic folds. Electron microscopic analyses showed these folds to form lateral undulations, which were suggested to provide the force behind the gregarine gliding (Schrével and Philippe 1993; Vávra and Small 1969; Vivier 1968). Actin and myosin restricted to the cell cortex were identified in eugregarines of the genus *Gregarina* Dufour, 1828, and their involvement in gliding motility was proposed (Ghazali et al. 1989; Heintzelman 2004; Valigurová et al. 2013). As subpellicular microtubules were not observed in the investigated species of eugregarines, the mechanism of their gliding motility must differ from that described by the glideosome concept (Valigurová et al. 2013). Another type of motility, exhibited by coelomic (Urosporidae Léger, 1892 and Monocystidae Bütschli, 1882) and some intestinal eugregarines (e.g. *Didymophyes gigantea*), is the so-called peristaltic or metabolic movement (Desportes and Schrével 2013; Diakin et al. 2016; Hildebrand and Vinckier 1975; Landers and Leander 2005; Leander et al. 2006; MacMillan 1973). These diverse modes of gregarine motility represent specific adaptations to parasitism in different environments (Valigurová et al. 2013).

The present study focuses on the eugregarine *Cephaloidophora* cf. *communis* (Cephaloidophoridae Kamm, 1922) parasitising the barnacle *Balanus balanus* Linnaeus, 1758 (Crustacea: Cirripedia). We performed ultrastructural and immunological analyses of structures, which are expected to be related to the attachment to host tissue and to the unique motility mode displayed by the trophozoites and gamonts of this eugregarine.

Material and Methods

The gregarine *Cephaloidophora* cf. *communis* was isolated from the intestine of its marine crustacean host *Balanus balanus*. Hosts were collected between 2013 and 2015 from the White Sea environment close to the White Sea Biological Station of Lomonosov Moscow State University (66°33.190′N, 33°06.550′E). Parasites were separated from host's intestine in filtered seawater using entomological needles and then transferred to embryo dishes with a 30 mm cavity for careful washing in filtered seawater and subsequent fixation procedures. The dissection and manipulation of parasites were performed using a stereomicroscope MBS-1 (LOMO, Russia). For native preparations, individual gregarines were put on microscope glass slides with filtered seawater and their motility was monitored using a Leica DM 2000 light microscope connected to a DFC 420 digital camera (Leica Microsystems, Germany).

For transmission electron microscopy (TEM), specimens were fixed in an ice bath in 2.5–3% (v/v) glutaraldehyde in filtered seawater or in 0.15 M cacodylate buffer (pH 7.4). Some specimens were fixed with 3% glutaraldehyde–ruthenium red [0.15% (w/v) stock solution in Milli-Q water] in cacodylate buffer or with 2.5% glutaraldehyde–alcian blue [1% (w/v) stock solution in Milli-Q water] in filtered seawater. Fixed samples were rinsed 3× for 20 min and post-fixed in 2% (w/v) OsO_4 for 2 h in the same buffer as used for fixation. After rinsing 3× for 20 min in the same fixation buffer and after dehydration in an acetone series, specimens were embedded in Epon (Polybed 812). Ultrathin sections were obtained with diamond knives using a Leica EM UC6 ultramicrotome (Leica Microsystems, Germany) and stained with uranyl acetate and lead citrate. Sections were examined under a JEM-1010 (Jeol, Japan).

For scanning electron microscopy (SEM), specimens were fixed in 2.5–5% (v/v) glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4), washed 3× for 15 min in cacodylate buffer, post-fixed in 2% (w/v) OsO_4 for 2 h in the same buffer, and finally washed 3× for 15 min in cacodylate buffer. After dehydration in an acetone series, parasites were critical