Opinion

Marine gregarines: evolutionary prelude to the apicomplexan radiation?

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Gregarine apicomplexans inhabit the intestines, coeloms and reproductive vesicles of invertebrates. An emphasis on specific ancestral characteristics in marine gregarines has given the group a reputation of being 'primitive.' Although some lineages have retained characteristics inferred to be ancestral for the group, and perhaps apicomplexans as a whole, most gregarines represent highly derived parasites with novel ultrastructural and behavioral adaptations. Many marine gregarines have become giants among single-celled organisms and have evolved ornate surface structures. A comparison of gregarine morphology, placed in a modern phylogenetic context, helps clarify the earliest stages of apicomplexan evolution, the origin of *Cryptosporidium*, and specific cases of convergent evolution within the group and beyond.

What are marine gregarines?

Gregarine apicomplexans are large single-celled parasites that inhabit the intestines and other extracellular spaces of nearly every group of invertebrates, particularly annelids and insects (Figure 1). Because of their widespread presence in animals and little attention by the scientific community, most gregarines remain unknown [1–4]. Moreover, the high level of morphological variation within gregarine populations makes species delimitation within the group challenging, indicating that gregarine systematics will benefit from the development and implementation of DNA barcodes (see Glossary) [5]. The dearth

Glossary

Apical complex: A novel cell invasion apparatus positioned near the anterior end of apicomplexan cells comprising primarily a conoid, a polar ring and rhoptries. This apparatus has been used as the best defining features for the Apicomplexa, from which its name is derived, but it is also found in a variety of other myzozoans, such as colpodellids and perkinsids.
Apicomplexa: A clade of myzozoans, including the first ancestor to possess a closed conoid during the cell invasion stage in a parasitic lifecycle. This group does not include predatory flagellates with open-sided conoids (syn. pseudoconoids), such as colpodellids, or parasitic flagellates with open-sided conoids, such as perknisids, which are more closely related to dinoflagellates.
Colpodellids: Several genera of predatory biflagellates that have been shown to diverge near the phylogenetic origin of apicomplexan parasites, such as *Voromonas* and *Colpodella*. These flagellates share several homologous characteristics with apicomplexans, such as myzocytosis and an apical complex consisting of micronemes, rhoptries and a conoid.
Conoid: A cone-shaped cytoskeletal structure that functions as scaffolding for rhoptries of the apical complex. Conoids can be completely closed, as in many apicomplexans, or open-sided, as in colpodellids and perkinsids.
Cryptosporidiosis: An intestinal disease of vertebrates (especially mammals) caused by a *Cryptosporidium* infection. The main symptom is diarrhea, which can be chronic or fatal in immunocompromised patients.
Dinoflagellates: A diverse clade of biflagellates that share a novel flagellar apparatus and novel nuclear features including permanently condensed chromosomes that apparently lack canonical histones. Some dinoflagellates are predators, some are parasites, and roughly half are photosynthetic. Some of the photosynthetic species rank among the most important players in the primary fixation of carbon in the oceans, whereas others are responsible for toxic algal blooms.
DNA barcodes: A taxonomic method that uses a short genetic marker (e.g. the DNA sequence of the mitochondrial gene *cytochrome c oxidase I*) as a rapid means to identify that an organism belongs to a particular species. DNA barcodes rely on significant variation in DNA sequences between species and relatively uniform DNA sequences within species.
Epicytic folds: Longitudinally arranged folds on the surface of gregarine trophozoites formed by the plasma membrane and underlying inner membrane complex. The folds increase surface area for surface-mediated nutrition and facilitate actinomyosin-based gliding motility in eugregarines (e.g. *Lecudina* and *Gregarina*). The number and density of epicyctic folds around the cell periphery varies considerably in different species of gregarines (range = 4 to >300).
Monophyletic: A phylogenetic group of species that comprises a common ancestor and all of its descendants, as inferred from a specific cladogram.
Myzocytosis: A predatory mode whereby a cell is able to penetrate the cell surface and draw in the cytoplasmic contents of a prey or host cell.
Myzozoa: A clade of avleolates comprising the most recent common ancestor of apicomplexans and dinoflagellates and all of its descendents.
Oocysts: The resistant spore phase of apicomplexans that can remain viable outside a host for long periods of time. Oocysts are formed immediately after gamete fusion and eventually contain sporozoites.
Paraphyletic: A phylogenetic group of species that comprise a common ancestor and only some of its descendants, as inferred from a specific cladogram. A paraphyletic group is a partial subset of a more inclusive monophyletic group.
Perkinsids: Several genera of parasitic flagellates (e.g. *Perkinsus* and *Parvilucifera*) that diverge near the phylogenetic origin of dinoflagellates. These flagellates share several homologous characteristics with colpodellids and apicomplexans, such as myzocytosis and an apical complex comprising micronemes, rhoptries and an open conoid.
Plastids: A eukaryotic cell compartment in which photosynthesis occurs and any homologous compartment in which photosynthesis has been secondarily lost (e.g. the apicoplasts of apicomplexans). Plastids have their own genomes and can have different suites of light-harvesting pigments (e.g. those with chlorophyll a and b are most often referred to as 'chloroplasts').
Rhoptries: The primary extrusive organelle associated with the apical complex of apicomplexans, colpodellids and perkinsids. These organelles tend to be club-shaped where the narrower, distal ends are positioned just beneath the plasma membrane and are nested within the central opening of a conoid. Rhoptries release a variety of proteins and lipids associated with cell penetration and invasion.
Sporozoites: Spindle-shaped, haploid cells equipped with an apical complex that is used to infect the tissues of a new host; reside within oocysts.
Syndinians: Several genera of parasitic flagellates (e.g. *Amoebophrya*, *Hematodinium* and *Syndinium*) that diverge near the phylogenetic origin of dinoflagellates and perkinsids. The lifecycle of syndineans consists of zoospores that penetrate a host (e.g. copepods, polycistines and dinoflagellates) and develop into an undifferentiated sporangium. The growing sporangium eventually destroys the internal compartment of the host and differentiates into many minute zoospores (ranging from about 3 to 20 μm in length, depending on the species) that escape the host in order to infect a new one.
Trophozoites: Relatively large feeding cells that develop from sporozoites in apicomplexan life cycles.

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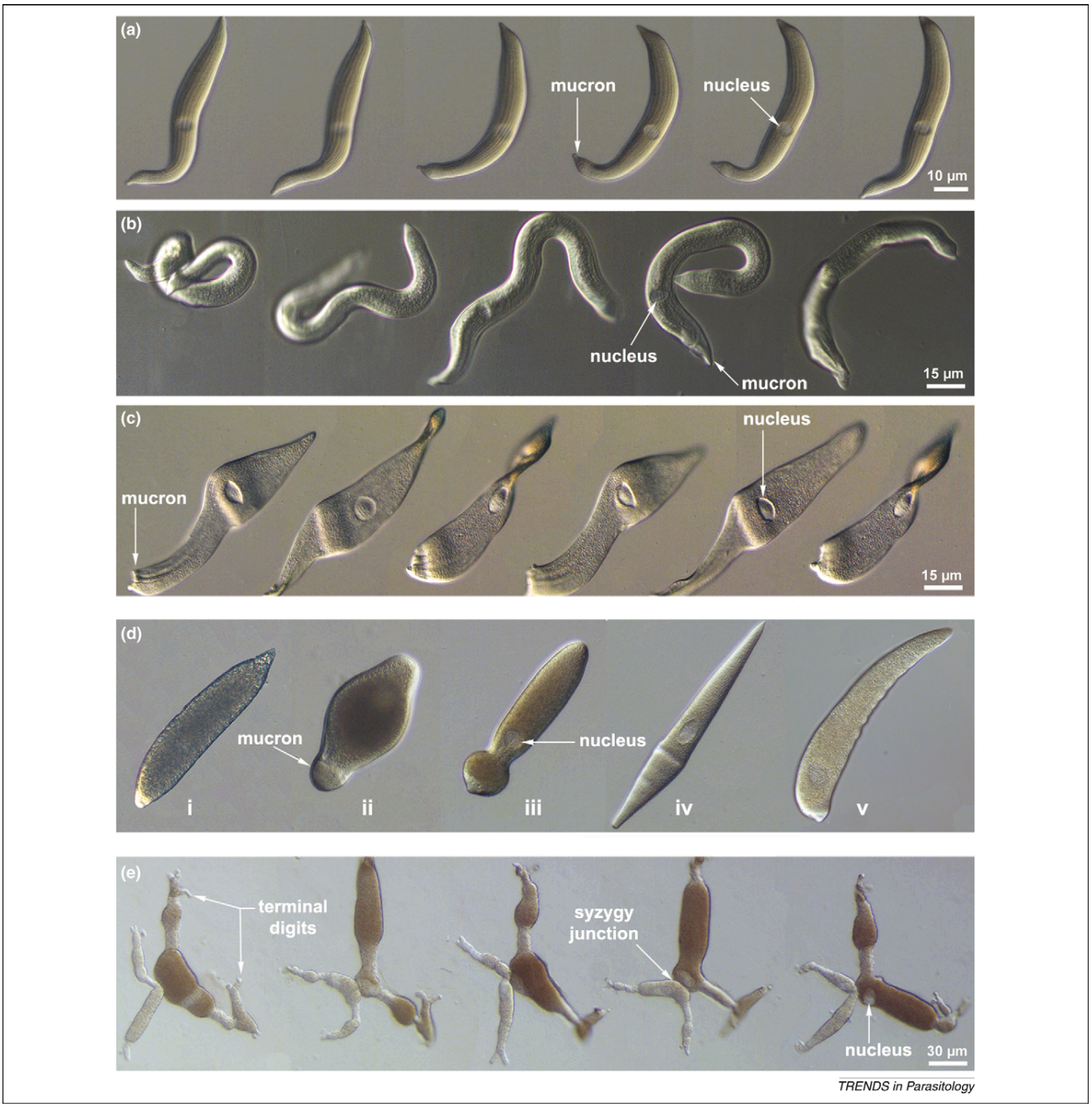

Figure 1. Light micrographs of marine gregarine trophozoites. Time series of differential interference contrast micrographs showing diverse cell shapes and movements in marine gregarine trophozoites. (a–c), archigregarines; (d), intestinal eugregarines; (e), a coelomic eugregarine (i.e. urosporidian). **(a)** *Selenidium serpulae* from a tubeworm; **(b)** *Selenidium* sp. from a tubeworm; **(c)** *Selenidium vivax* from a peanut worm; **(d)** Lecudinid eugregarines (not to same scale): (i) *Lecudina* sp. from a nemertean (scale bar = 70 μm), (ii) *Lecudina tuzetae* from an predatory polychaete (scale bar = 140 μm), (iii) *Lecudina* sp. from a deposit feeding polychaete (scale bar = 200 μm), (iv) *Lecudina* sp. from a deposit feeding polychaete (scale bar = 250 μm), (v) *Lankesteria abotti* from a tunicate (scale bar = 120 μm); **(e)** *Pterospora schizosoma* from a Pacific bamboo worm.

of expertise on gregarines must be addressed to (i) understand the breadth of evolutionary innovations and reoccurring properties in parasites, (ii) discover new systems for understanding mechanisms of symbiosis and infection and (iii) explore practical applications, such as new treatments for certain apicomplexan diseases (i.e. cryptosporidiosis) [6].

A revival in gregarine research will offer significant insights into apicomplexan biology and evolution. This is perhaps best exemplified by the unanticipated discovery of remnant plastids in gregarine relatives (e.g. *Plasmodium* and *Toxoplasma*) that provide novel targets for the treatment of malaria and toxoplasmosis [7,8]. Undoubtedly, improved knowledge of gregarines will help reconstruct one intriguing transformation: the evolution of obligate intracellular parasites from free-living photosynthetic ancestors [9,10].

Comparative morphology indicates that gregarines possess several characteristics retained from the most recent ancestor of all apicomplexans, such as extracellular

feeding stages, a life cycle involving one host, and a prevailing presence in marine environments. This suite of characteristics has resulted in gregarines being considered 'primitive' relative to apicomplexans with more complex life cycles involving vertebrates [11–13]. Molecular phylogenetic evidence is concordant with this interpretation and suggests that some gregarines diverge early within the Apicomplexa (Box 1) [14–16]. However, both molecular phylogenetic and ultrastructural data demonstrate that most gregarines are much more divergent than commonly assumed. For instance, the cytoskeleton of gregarine trophozoites is diverse and provides an excellent system for demonstrating evolutionary transformations in morphological characters. This diversity also provides compelling evidence for transitions between different modes of nutrition, modes of locomotion and host

Box 1. Deep phylogeny of the Apicomplexa

The Myzozoa comprise apicomplexans, dinoflagellates and several different lineages of predatory and parasitic flagellates that employ a myzocytosis-based mode of feeding (Figure I). Counterintuitively, parasitic flagellates called perkinsids and syndinians branch as the nearest sister groups to the primarily free-living (and often photosynthetic) dinoflagellates, and free-living predatory flagellates called colpodellids branch as the nearest sister groups to the parasitic apicomplexans [10,28,51,53]. The flagellated stages of perkinsids, syndinians and colpodellids share several morphological characteristics that have been retained from the most recent common ancestor of all myzozoans, such as an apical complex comprising an open conoid and rhoptries and two heterodynamic flagella [9,10].

Although the current status of apicomplexan molecular phylogeny provides some evidence of relationships that is congruent with morphological data, the overall backbone of apicomplexan phylogeny is poorly resolved. This lack of resolution severely limits our ability to infer early events in the evolution of the group and the characteristics of the most recent apicomplexan ancestor (Figure I, Node A). For instance, several enigmatic lineages of apicomplexans that infect intestinal systems of invertebrates, such as rhytidocystids, branch from the apicomplexan backbone without any clear sister groups [54,55]. The Coccidia sensu stricto and piroplasmids, which are intracellular parasites of both aquatic and terrestrial vertebrates, form a well-supported clade that also branches independently from the apicomplexan backbone (Figure I). Molecular phylogenetic data from gregarines have added additional complexity and uncertainty to the deepest relationships among apicomplexans, especially because most gregarine ribosomal genes are extremely fast evolving and prone to methodological artifacts [14–16,52].

Nonetheless, the emerging phylogeny for gregarines provides a framework for inferring character evolution within the group and challenges some traditional ideas about the systematics of *Cryptosporidium* [14,15,50,52]. If it is further demonstrated that *Cryptosporidium* descended from within gregarines, then at least two lineages of apicomplexans have independently co-evolved with vertebrate hosts and have converged on similar life history characteristics, namely *Cryptosporidium* and the clade comprising Coccidia sensu stricto and piroplasmids.

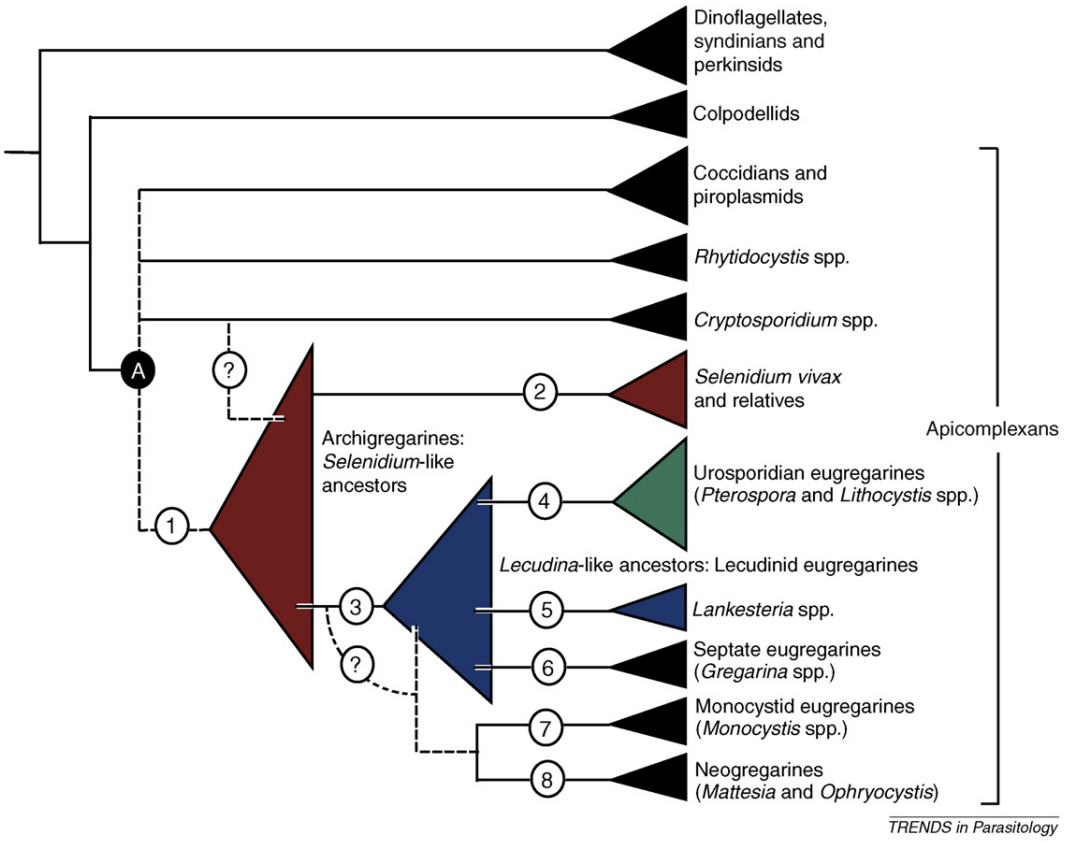

Figure I. Synthetic phylogenetic framework for the Myzozoa. An illustration of phylogenetic relationships based on morphological and molecular phylogenetic data, especially small subunit rDNA sequences. Triangles indicate species radiations and numbers indicate the inferred positions of character states as constrained by parsimony. Dashed lines and '?' highlight significant phylogenetic uncertainty. Red denotes archigregarines; blue denotes intestinal eugregarines; green denotes coelomic eugregarines (i.e. urosporidians); archigregarines and lecudinids are shown as paraphyletic stem groups. The 'A' indicates the most recent apicomplexan ancestor.

environments during the course of gregarine evolution. This ultrastructural diversity will be examined in a modern molecular phylogenetic context to highlight the significance of marine gregarines in reconstructing the early evolution and overall diversity of apicomplexans.

The gregarine life cycle

Gregarines are usually transmitted to new hosts by oral ingestion of oocysts in both aquatic and terrestrial environments. Some gregarine oocysts are transmitted with host gametes during copulation (e.g. *Monocystis*). Nonetheless, four or more sporozoites (depending on the species) equipped with an apical complex eventually escape from the oocysts, find their way to the appropriate body cavity and penetrate host cells in their immediate environment. The sporozoites emerge from a host cell, begin to feed and develop into larger trophozoites. In some gregarines, the sporozoites and trophozoites are capable of asexual replication, a process called schizogony (or merogony); most gregarines appear to lack schizogony in their lifecycles. Nonetheless, two mature trophozoites eventually pair up in a process called syzygy and develop into gamonts (Figure 1e). The orientation of gamonts during syzygy differs in different species (e.g. side-to-side and head-to-tail).

A gametocyst wall forms around each pair of gamonts, which then begin to divide into hundreds of gametes. Zygotes are produced from the fusion of two gametes and become surrounded by an oocyst wall, within which meiosis occurs to yield the sporozoites [17]. Hundreds of oocysts accumulate within each gametocyst, and are released via host feces or via host death and decay. Environmental DNA sequence surveys of organismal diversity indicate that gregarine oocysts are common constituents of sediments collected at varying depths of the ocean floor [18–20]. Interpretation of the cellular origins and biogeographies associated with these molecular data depends on an accurate and comprehensive phylogenetic context for gregarines.

Diversity of marine gregarines

Gregarines have historically been separated into three categories based on habitat, host range and trophozoite morphology: archigregarines, eugregarines and neogregarines (Box 1). Some authors have also emphasized the apparent absence of schizogony [1]. Archigregarines are found exclusively in marine habitats, possess intestinal trophozoites that are similar in morphology to the infective sporozoites and are inferred to be the most representative of the ancestral gregarine [3,21,22].

Eugregarines are found in marine, freshwater and terrestrial habitats and possess large trophozoites that are significantly different in morphology and behavior from the sporozoites. Intestinal eugregarines are separated into septate and aspetate (mostly marine) gregarines depending on whether the trophozoite is superficially divided by a transverse septum (Box 1, Figure I, Step 6). Urosporidians are aseptate eugregarines that infect the coelomic spaces of marine hosts. Monocystids are aseptate eugregarines that infect the reproductive vesicles of terrestrial annelids (Box 1, Figure I, Step 7) and tend to branch closely with neogregarines.

Neogregarines are found in terrestrial hosts (e.g. insects), have reduced trophozoites and tend to infect tissues other than the intestines (Box 1, Figure I, Step 8). Molecular phylogenies coupled with comparative morphological evidence indicate that there are two nested radiations of marine gregarines: the initial archigregarine radiation and the subsequent eugregarine radiation.

Archigregarines and the early apicomplexan radiation

Most of the best-studied archigregarines fall within the genus *Selenidium*. The trophozoites of archigregarines tend to be vermiform and are capable of dynamic coiling movements like those found in apicomplexan sporozoites (Figure 1a,b). Moreover, the sporozoites and trophozoites in many archigegarines are similar in possessing an apical complex with a closed conoid used for intracelllular invasion (sporozoites) and myzocytosis (trophozoites) within intestinal environments [23–27]. Because free-living colpodellids possess an apical complex with an open conoid used in predatory modes of feeding, the origins of closed conoids and intestinal parasitism are inferred to be ancestral states for the Apicomplexa (Box 1, Figure I, node A) [9,10,28]. However, the morphology of gregarine trophozoites changed significantly following the exploitation of intestinal environments by archigregarine ancestors. For instance, ancestral flagella were lost, and increases in cell surface area occurred several times independently within gregarines, in association with the abandonment of a myzocytosis-based mode of feeding.

The surface of archigegarine trophozoites is usually inscribed with up to 50 longitudinal grooves that demarcate the intervening epicytic folds (Box 1, Figure I, Step 1; Figure 2a) [1,15,16,21,29–32]. Comparative morphology indicates that the number of epicytic folds increased steadily on the surface of trophozoites during the course of gregarine evolution, and epicytic folds are adaptations for increasing surface area within intestinal environments (Figure 2a–c). Transverse striations resulting from coiling movements are common on the surfaces of archigregarine trophozoites (Figure 1a–c; Figure 2d,e) [15,16,26,33–35]. One or more layers of microtubules subtend an elastic inner membrane complex and generate the forces responsible for these nematode-like movements (Figure 2f) [16,22,36,37]. The most dynamic archigregarines also possess dense, superficial accumulations of mitochondria that provide a consistent supply of ATP required to support the microtubule-associated motors underlying the cellular deformations [22,33]. Some archigregarines, such as *Selenidium vivax*, have abandoned the epicytic folds of their ancestors and instead have become extremely flattened [15,33,34]. These parasites resemble and behave much like the individual proglottids of some tapeworms (Figure 1c). Another lineage of archigregarines gave rise to species that further embellished the epicytic folds of trophozoites, a change that set the stage for the marine eugregarine radiation (Box 1, Figure I, Step 3). Archigregarines, therefore, represent a diverse and poorly circumscribed group that, on one hand, forms the paraphyletic stem from which all other gregarines evolved, and on the other hand, includes several divergent lineages that have become greatly specialized within the intestines of different marine invertebrates.

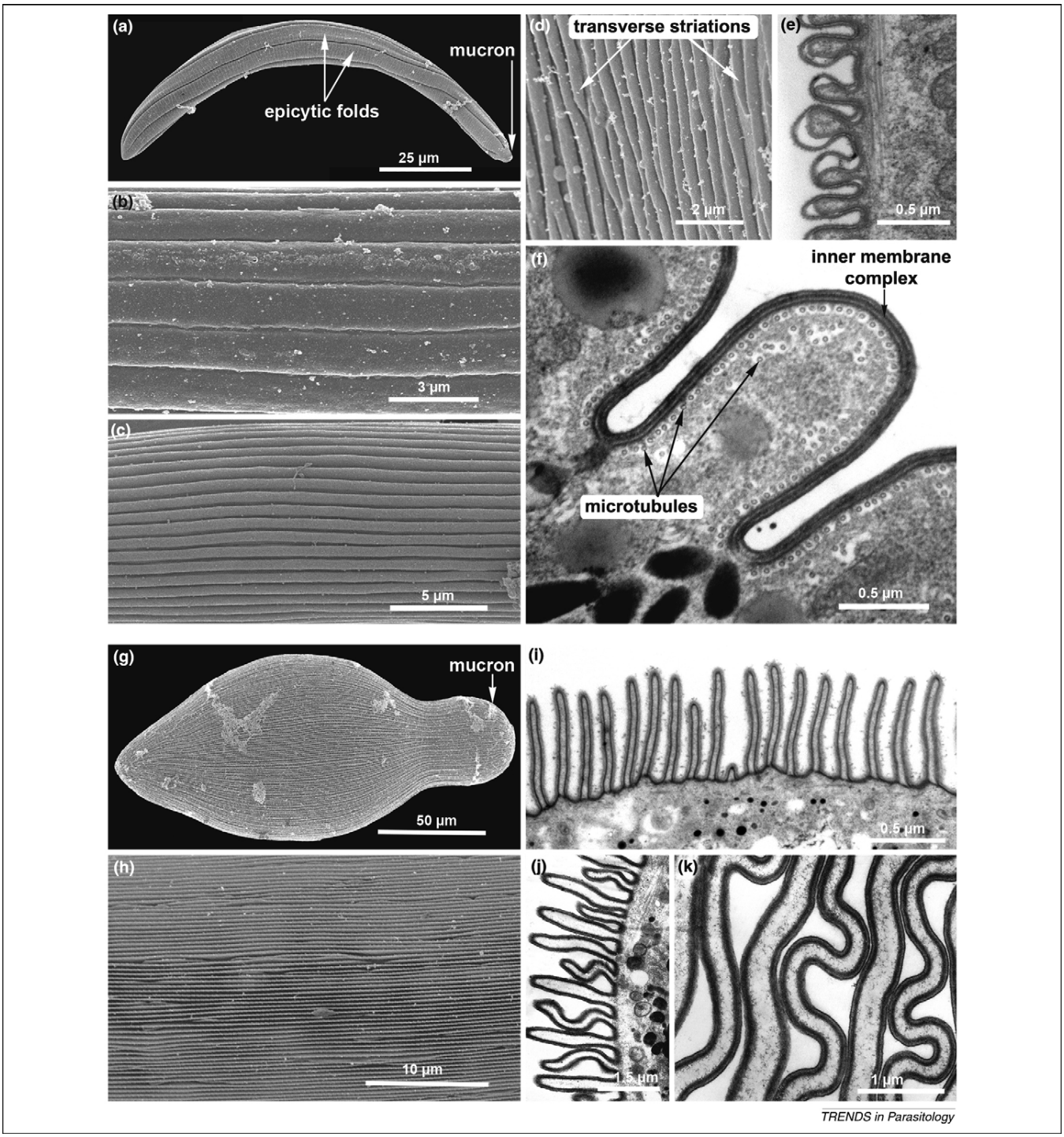

Figure 2. Electron micrographs of cytoskeletal diversity in intestinal gregarine trophozoites. (a–f), archigregarines; (g–k), intestinal eugregarines. **(a–c)** Scanning electron micrographs showing the number and density of epicytic folds in (a) *Selenidium terebellae* from a spaghetti worm [15], (b) *Selenidium serpulae* from a calcareous tube worm and (c) *Selenidium* sp. from a peanut worm. **(d)** Scanning electron micrograph showing the transverse striations of *S. vivax* [33]. **(e)** Transmission electron micrograph showing the transverse striations of *S. vivax* [33]. **(f)** High-magnification transmission electron micrograph through the epicytic folds of *S. serpulae* [16]. **(g,h)** Scanning electron micrographs showing the number and density of epicytic folds in (g) *Lecudina tuzetae* and (h) *Lecudina polymorpha.* **(i–k)** Transmission electron micrographs of (i) *L. tuzetae* and (j,k) the undulating epicytic folds of *L. pellucida* [40]. The incremental evolution of more densely packed longitudinal epicytic folds correlates with the loss of the conoid and myzocytosis, and the gain of surface mediated nutrition and gliding motility. (a) Reproduced, with permission, from Ref. [15]; (f) reproduced with permission, from Ref. [16]; (j-k) reproduced, with permission, from Ref. [40].

The marine eugregarine radiation: adaptation within the intestinal microcosm

Eugregarines comprise the majority of known gregarines, owing to the fact that many species infect insects [4]. Most of the eugregarines that inhabit marine invertebrates have been classified within the poorly circumscribed family Lecudinidae and genus *Lecudina* [2,38], and possess several features retained from the most recent eugregarine ancestor (Box 1, Figure I, Step 3) [3,13–15,21,22]. Other genera of marine eugregarines are usually separated on

the basis of host range; for instance, the *Lecudina*-like parasites of urochordates are classified within *Lankesteria* (Figure 1d[v]; Box 1, Figure I, Step 5) [14,39]. Nonetheless, the trophozoites of marine eugregarines are considerably diverse in shape, and possess dense arrays of longitudinal epicytic folds (Figure 1d; Figure 2g–k). This surface area optimizes surface-mediated nutrition in intestinal environments, and explains the loss of an apical complex (and myzocytosis) in eugregarine trophozoites and the development of a more bulbous attachment apparatus or 'mucron' (Figure 1d; Figure 2g; Box 1, Figure I, Step 3). Unlike marine eugregarines, the mucron has become considerably pronounced and diverse in the septate eugregarines of insects and is usually referred to as an 'epimerite' in these species.

The incremental increase in the number of epicytic folds in eugregarines is correlated with the evolution of stiff cells and a distinctive mode of gliding motility (Box 1, Figure I, Step 3). In some eugregarines, the epicytic folds are capable of undulations that push intestinal fluids over the trophozoite surface (Figures 2j,k). Initially, this behavior was interpreted to be the mechanism underlying gliding motility across a secreted layer of mucilage [40,41]. However, current evidence demonstrates that gliding motility is instead facilitated by an actinomyosin system organized beneath the longitudinal edge of each epicytic fold (Figure 2h,i) [42]. Unlike archigregarines, eugregarine trophozoites do not possess layers of microtubules beneath the inner membrane complex and are not capable of bending, except near the mucron in some species (Figure 2i–k). Accordingly, the actinomyosin-based gliding in eugregarines is a key innovation that distinguishes members of this group from archigregarine species (Box 1, Figure I, Step 3).

Most apicomplexans are adapted to intracellular environments and have become smaller and more streamlined over time (e.g. *Plasmodium* and coccidians). By contrast, the trophozoites of eugregarines have adapted to spacious extracellular environments by becoming giants among single-celled organisms and by increasing cytoskeletal complexity. Although intestinal eugregarines can be impressively large, the grandest and perhaps most bizarre apicomplexans known are specialists of the coeloms of marine polychaetes.

Colonization of the coelomic microcosm

Traversal of sporozoites across the boundary formed by the closely associated intestinal wall and visceral peritoneum gave rise to a lineage of eugregarines that inhabit coelomic spaces, namely urosporidians (Box 1, Figure I, Step 4) [14,43,44]. The trophozoites of many urosporidians possess surface crenulations, peristaltic motility and a bifurcating shape with terminal digits (Figure 1e; Figure 3a–f)

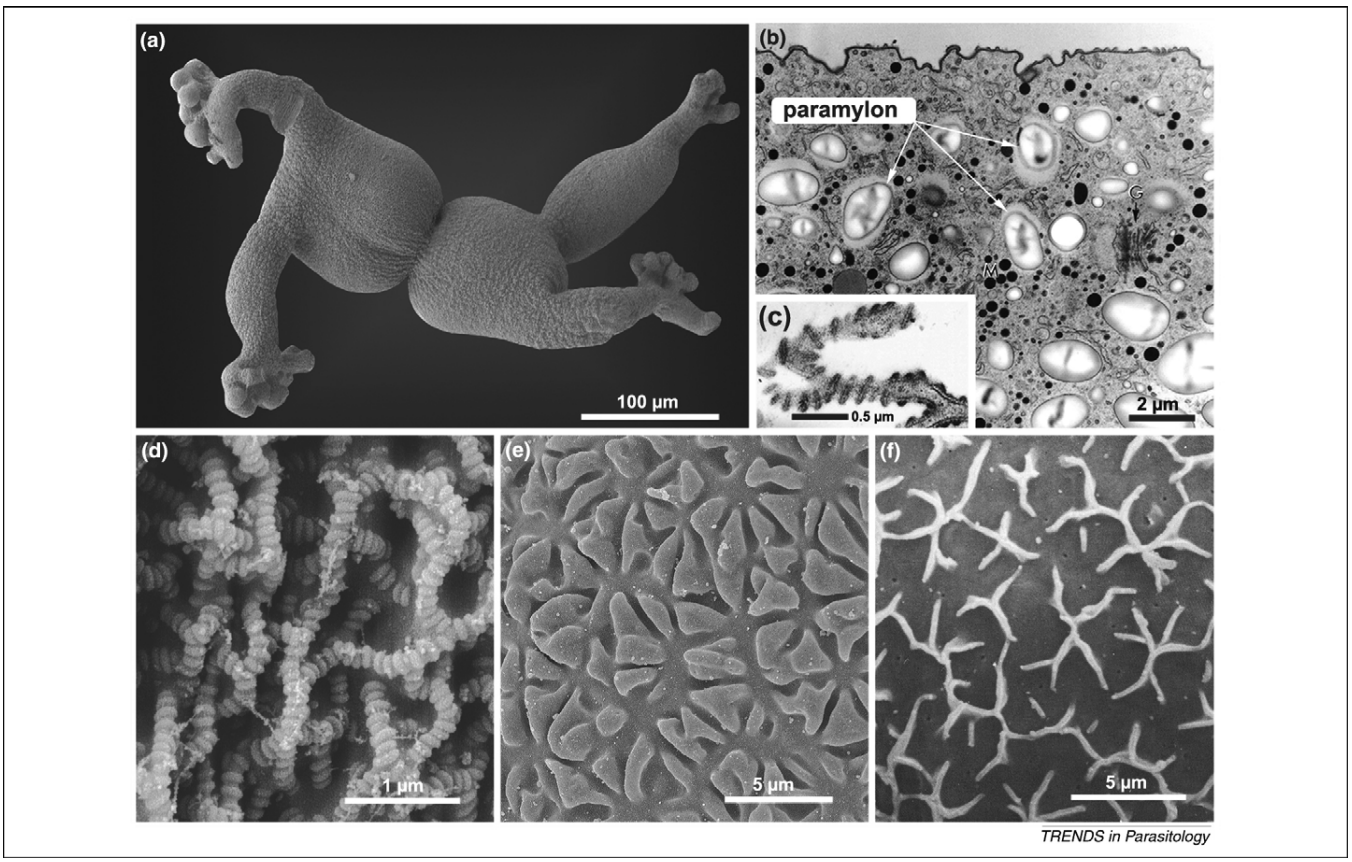

Figure 3. Electron micrographs of the cytoskeletal diversity in coelomic eugregarines (i.e. urospordians). **(a)** Scanning electron micrograph of a gamont pair of *Pterospora floridiensis* in syzygy (color distinguishes individual cells) [43]. **(b,c)** Transmission electron micrographs of *Pterospora floridiensis* [48]. **(d)** High magnification scanning electron micrograph of *Pterospora floridiensis* from a Gulf Atlantic bamboo worm [43]. **(e)** High magnification scanning electron micrograph of *Pterospora schizosoma* from a Pacific bamboo worm [43]. **(f)** High magnification scanning electron micrograph of *Lithocystis foliacea* from an Atlantic heart urchin [47]. The longitudinal epicytic folds and gliding motility of intestinal eugregarines have been replaced by convoluted cell surfaces with diverse morphologies and peristaltic and pulsating cell movements. (a–e) reproduced, with permission, from Ref. [43]; (f) reproduced, with permission, from Ref. [47].

[43,45–47]. Urosporidians also tend to lack attachment structures and instead form gamont pairs that pulsate freely within coelomic fluid (Figure 1e). Individual gamonts in *Pterospora*, for instance, can be V-shaped or Y-shaped, and accordingly, gamont pairs appear X-shaped (Figure 1e; Figure 3a). The trunk of each gamont bifurcates repeatedly into terminal digits, where the number of digits differs in different species [43]. The terminal digits are sequentially inflated and deflated as the cytoplasm is pushed into and out of each trunk (Figures 1e, 3a). The function(s) of this dynamic pattern of cellular deformation, the exceedingly large cell sizes (e.g. 300 μm in length) and the associated diversity of surface texture in urosporidian trophozoites is unclear (Figure 3d–f). However, these features correlate with coelomic habitats and other non-intestinal environments, and presumably facilitate a poorly understood mechanism for nutrient acquisition.

The diversity of urosporidians demonstrates intermediate character states associated with the evolutionary transformation of trophozoites following the colonization of coelomic environments. Some urosporidians have a dense packing of sinuous epicytic folds and glide along the inner coelomic wall (e.g. *Urospora*) [47]. The vermiform trophozoites of some *Lithocystis* species have retained widely spaced epicytic folds, but have replaced gliding for peristaltic motility [47]. Other urosporidians (e.g. *Pterospora*) combine peristalsis with complex patterns of surface ridges and pits (Figure 3b–f) [43,47,48]. Molecular phylogenies have confirmed that urosporidians are nested within marine intestinal eugregarines and have transformed ancestral epicytic folds and gliding into surface crenulations and peristalsis (Box 1, Figure I, Step 4) [14]. As such, urosporidians represent some of the largest and most bizarre apicomplexans known and, by all standards, are the antithesis to any notion of 'primitiveness'.

Concluding remarks

Literature on gregarines reflects a great deal of taxonomic work based on only a few characters, and it is widely scattered in different languages and obscure journals, making familiarity with gregarine research challenging [1,2,38,44]. Moreover, few gregarines have been examined using electron microscopy, and even fewer have been studied at the molecular level. Improved understanding of gregarine diversity will provide significant insights into the molecular, cellular and life history properties of apicomplexans and provide the comparative data necessary for understanding the early evolution of intracellular parasitism in the group. Molecular phylogenetic studies on gregarine diversity, particularly archigregarines, are also expected to help pinpoint the closest living relatives of *Cryptosporidium* and elucidate the ancestral condition from which these important parasites evolved [9,15,49–52]. Current molecular evidence is consistent with the hypothesis that archigregarines and *Cryptosporidium* are among the earliest diverging lineages within the Apicomplexa (Box 1).

The perception that gregarine apicomplexans as a whole are 'primitive' is grossly misleading, however. Gregarine diversification produced some of the most evolutionarily specialized apicomplexans known, and tracing behavioral and ultrastructural characters onto a current phylogenetic framework illustrates compelling cases of character evolution within the group. For instance, gregarine variation reflects the properties of specific host compartments (e.g. intestines, coeloms and reproductive vesicles) and demonstrates novel, albeit poorly understood, ways in which these parasites have solved basic biological problems, such as locomotion and nutrition. Hopefully, the comparative approach outlined here will stimulate new awareness and research on the phylogeny, cell biology and evolution of this important and captivating group of parasites.

Acknowledgements

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