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Surface morphology of the marine parasite *Haplozoon axiothellae* Siebert (Dinoflagellata)

Brian S. Leander*, Juan F. Saldarriaga and Patrick J. Keeling

Canadian Institute for Advanced Research, Program in Evolutionary Biology, Department of Botany, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada; Fax: 604-822-6089, E-mail: bleander@interchange.ubc.ca

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The genus *Haplozoon* contains a group of intestinal parasites of marine worms with a bizarre cellular organization. Although the phylogenetic relationship of these parasites to other eukaryotes was once controversial, a few studies using light and transmission electron microscopy (TEM) and small subunit rDNA confirmed that they are aberrant dinoflagellates. Microscopical studies also suggested that haplozoans possessed a number of surface features that remained to be characterized. We have conducted a fine-scale examination of the surface of *Haplozoon axiothellae* Siebert, a parasite of the maldanid polychaete *Axiothella rubrocincta,* collected from two sites in the northeastern Pacific Ocean. Our findings indicated that the parasites are covered with a theca of barbs and small polygonal plates and possess a longitudinal row of ventral pores that resemble a kinety. Most surprisingly, we provide evidence that the entire organism is bounded by a single, continuous membrane, suggesting that haplozoans are not really multicellular but a syncytium compartmentalized by sheets of alveoli. We also demonstrated distinct clusters of unusual concave bodies over the surface of roughly half of the parasites observed. Septum-like junctions between individuals, distinctive threads reminiscent of pili, and their size ranges suggest that the concave bodies might be unusual episymbionts.

Key words: Alveolata; *Axiothella rubrocincta*; Blastodiniales; Episymbionts; Gymnodiniales; *Haplozoon axiothellae.*

Introduction

Marine parasitic dinoflagellates show extraordinary diversity in their morphology, range of hosts, and life history (Cachon and Cachon 1987; Coats 1999). Haplozoans are a group of intestinal parasites from marine worms with a highly unusual organization of differentiated cells, a body plan so bizarre that the early workers classified some of them as mesozoan animals and others as gregarine apicomplexans (Dogiel 1906; Calkins 1915; Shumway 1924). The light microscopical observations of Chatton (1919) led to predictions that haplozoans were dinoflagellates, and it was eventually shown that they release motile dinospores (Shumway 1924). Closer examination of one species with transmission electron microscopy (TEM) (Siebert 1973; Siebert and West 1974) and molecular phylogenies of small subunit rDNA (Saldarriaga et al. 2001) demonstrate that haplozoans are aberrant dinoflagellates that have adopted a "multicellular" or "colonial" trophont stage.

All but one species of *Haplozoon* Dogiel have been described with light microscopy from the Atlantic Ocean. These take on three basic forms: pyramidal (cells arranged in 3-dimensions as a

*corresponding author

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pyramid), pictinate (cells arranged in one plane as a triangular sheet), and linear (cells arranged as a single longitudinal row) (Chatton 1919; Shumway 1924). The remaining species, *Haplozoon axiothellae* Siebert, was collected from the eastern Pacific Ocean and described with light and transmission electron microscopy as a linear form that occasionally produced a double row of cells near the posterior end (Siebert 1973; Siebert and West 1974). These observations suggested that *H. axiothellae* had a number of surface features that were impossible to adequately characterize with TEM, such as patterns of thecal plates, the distribution of thecal barbs, surface details of the attachment apparatus, and patterns of thecal pores. Knowledge of these features could be important for inferring the phylogenetic relationship of *Haplozoon* to other dinoflagellates and for understanding the basic biology and structural organization of these enigmatic parasites.

Accordingly, we have examined the surface morphology of thirteen different individuals of *H. axiothellae* using scanning electron microscopy (SEM) to complement the two original papers describing *H. axiothellae* with TEM (Siebert 1973; Siebert and West 1974). We not only report new findings on the structures noted above, but we provide evidence for what we suspect are unusual episymbionts and evidence which indicates that each individual parasite is surrounded by a single membrane. It appears that haplozoans are not "multicellular" organisms but represent a novel organization of cell-like compartments that cannot be described as either a plasmodium or a multicellular colony.

Material and methods

Collection of organisms

Haplozoon axiothellae Siebert was isolated from the intestines of the marine maldanid polychaete *Axiothella rubrocincta* Johnston, the "bamboo worm". Hosts were collected at low tide (0.2–0.3 m above mean low tide) from two sites separated in time and space: the intertidal mudflats of Argyle Lagoon near Friday Harbor Marine Laboratories, San Juan Island, Washington, USA in March 2002 and the intertidal mudflats of Grappler Inlet near Bamfield Marine Station, Vancouver Island, Canada in April 2002. Parasites were found at both sites with similar frequency; roughly three out of five hosts were infected with *H. axiothellae*. Hosts were examined within two days after collection.

Electron microscopy

About 15–20 parasites per individual host, one host from each of the two sites, were prepared for scanning electron microscopy (SEM). *Haplozoon* parasites were released into seawater by teasing apart the intestine of *A. rubrocincta* with fine-tipped forceps. The parasites were removed from the remaining gut material by micromanipulation and washed twice in seawater. Individuals were deposited directly into the threaded hole of a Swinnex filter holder, containing a 5 µm polycarbonate membrane filter (Coring Separations Div., Acton, MA), that was submerged in 10 ml of seawater within a small canister (2 cm dia. and 3.5 cm tall). A piece of Whatman filter paper was mounted on the inside base of a beaker (4 cm dia. and 5 cm tall) that was slightly larger than the canister. The Whatman filter paper was saturated with 4% OsO_4 and the beaker was turned over the canister. The parasites were fixed by OsO_4 vapors for 30 min. Six drops of both 8% gluataraldehyde and 4% OsO_4 were added directly to the seawater and the parasites were fixed for an additional 30 min. A 10 ml syringe filled with distilled water was screwed to the Swinnex filter holder and the entire apparatus was removed from the canister containing seawater and fixative. The parasites were washed then dehydrated with a graded series of ethyl alcohol and critical point dried with CO_2. Filters were mounted on stubs, sputter coated with gold, and viewed under a Hitachi S4700 Scanning Electron Microscope. Five parasites were recovered from stubs prepared from Argyle Lagoon and eight parasites were recovered from stubs prepared from Grappler Inlet. Some SEM data were presented on a black background using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA).

Results and discussion

General morphology

Individual parasites varied considerably in length (85–110 µm) and morphology (Figs 1, 2a–c). However, all individuals consisted of three fundamental units, which are termed according to Shumway (1924): an anterior "trophocyte", a midregion of "gonocytes", and posterior "sporocytes" (Figs 1, 2a). The ventral surface of each trophocyte contained an attachment apparatus used to hold the parasites to the endothelial lining of the host intestine (Figs 1, 2a). The posteriormost surface of each trophocyte was distinctly smooth and identified as the "neck", where the dorsal region of the neck was much broader (7–11 µm at maximum breadth) than the ventral region (Figs 1, 2a–c). We infer that the neck region contains the nucleus of the trophocyte as TEM data

demonstrated that the nucleus is located in the posterior third of each trophocyte (Siebert 1973; Siebert and West 1974).

A string of gonocytes at different stages of division sit just posterior to the trophocyte, and accordingly, the gonocytes may contain either one nucleus, two nuclei or some intermediate stage of nuclear division (Siebert 1973; Siebert and West 1974). This continuous process was manifested on the surface of the parasites as "mature" and "immature" junctions between gonocytes (Figs 2a–c). Mature junctions were identified as relatively deep indentations and immature junctions as shallow indentations (Fig. 2b). The posterior-most gonocytes often possess two nuclei, and according to Siebert and West (1974), these nuclei will divide without cell division to form tetranucleate sporocytes. Sporocytes, however, appear to have been defined differently by Shumway (1924) and Siebert (1973), and consequently there is ambiguity re-

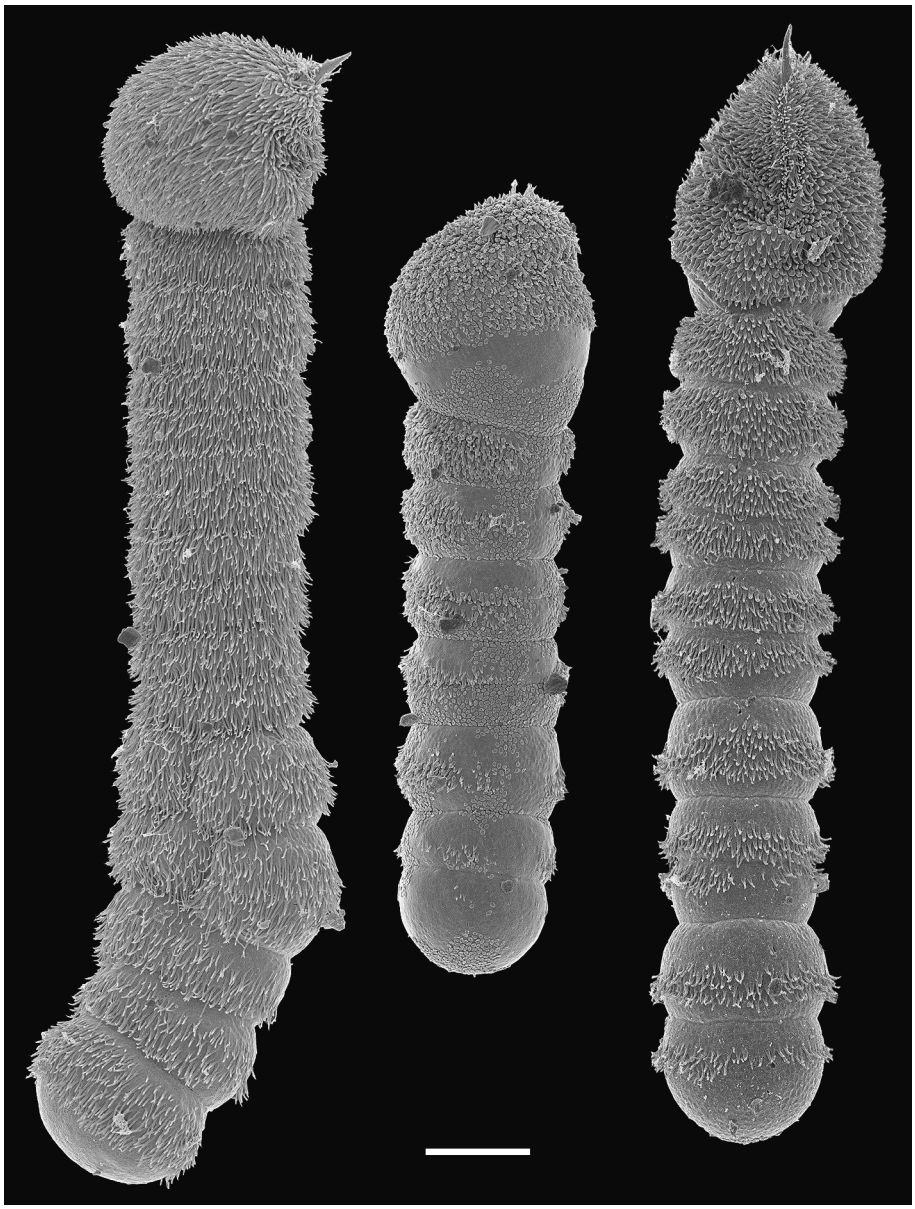
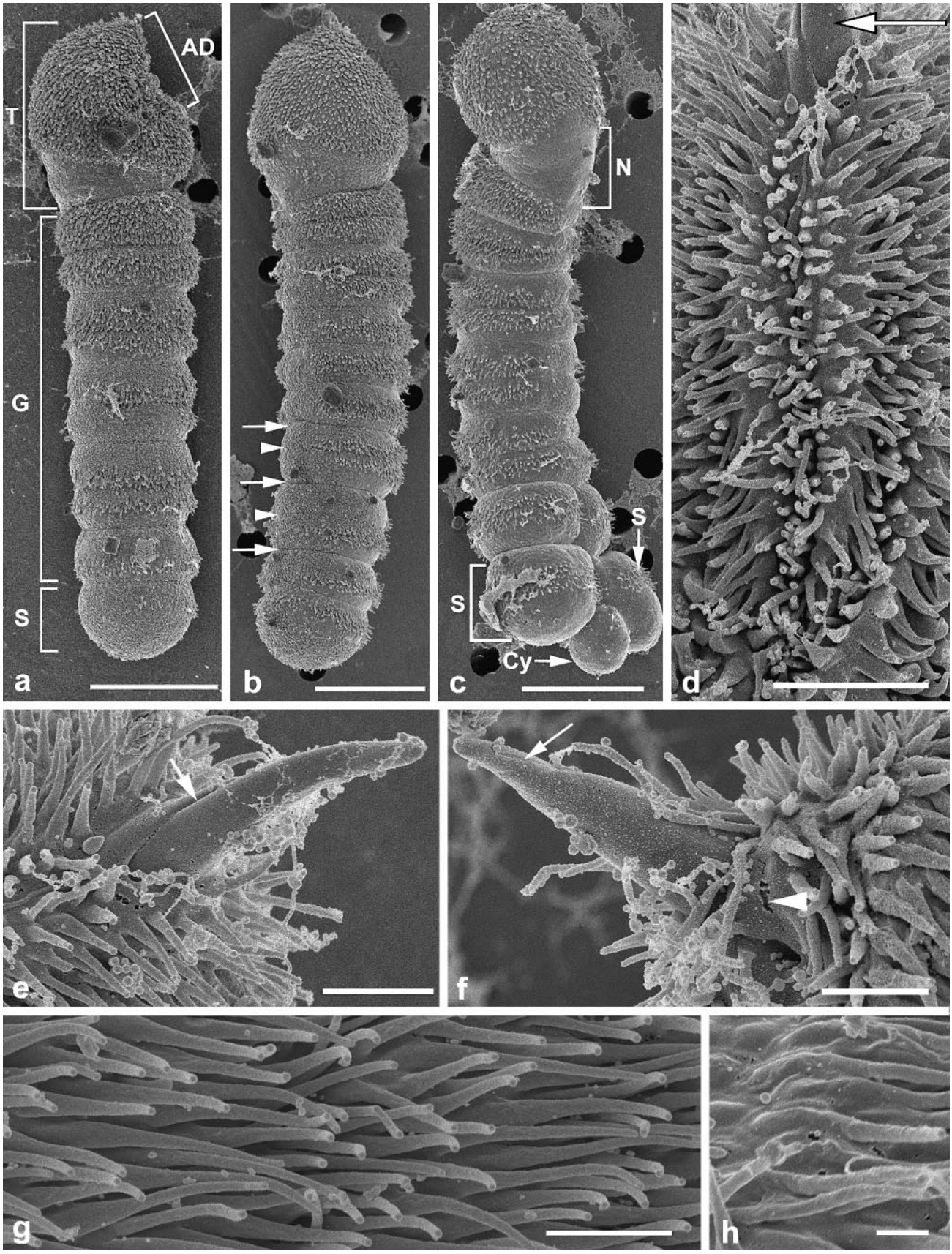


Fig. 1. Scanning electron micrographs showing the general morphology and diversity of *Haplozoon axiothellae*. Three individual organisms from the same host are presented in different orientations: right lateral view (left-hand organism), dorsal view (center organism), and ventral view (right-hand organism) (Bar = 10 µm).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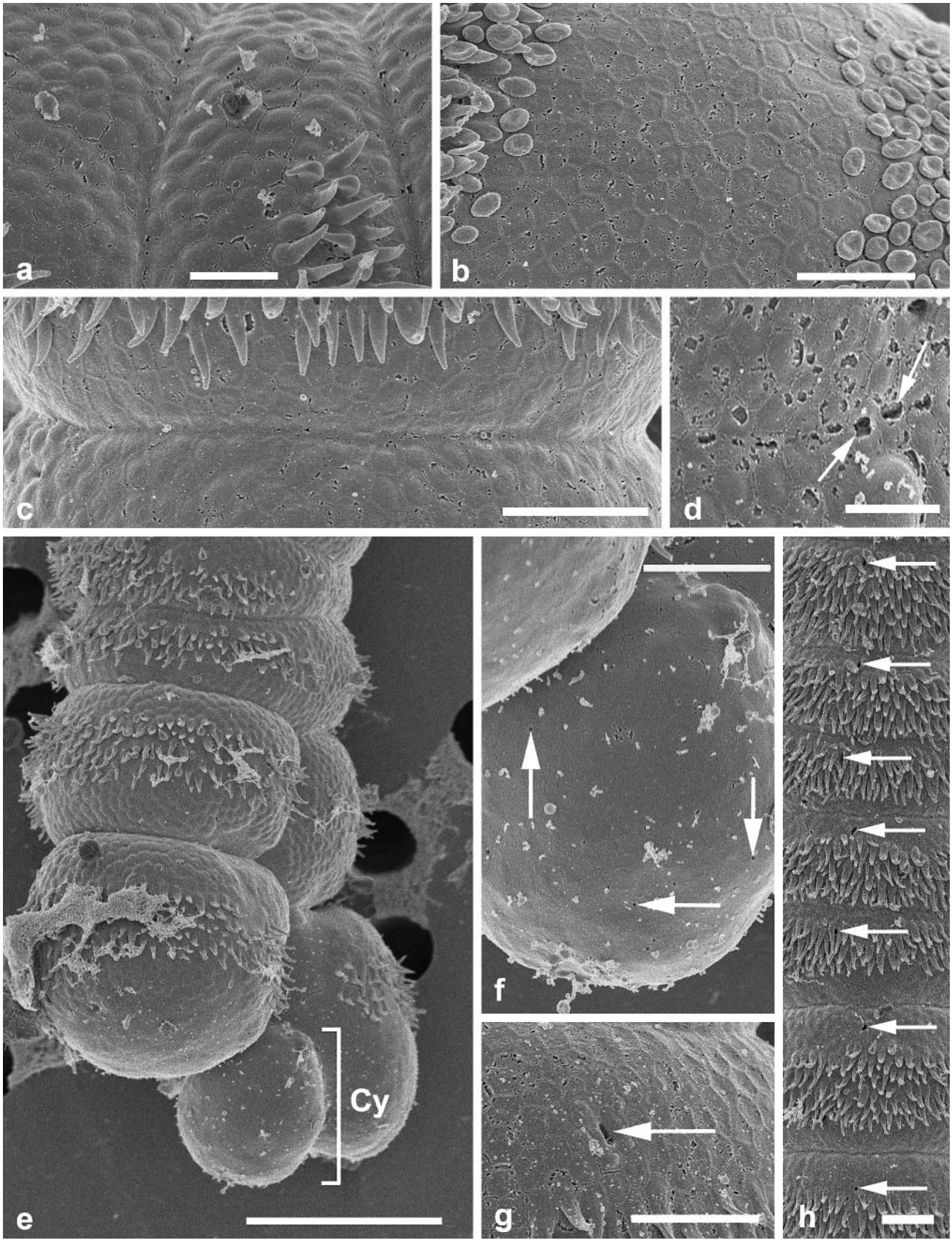
garding their identification. Siebert (Siebert 1973; Siebert and West 1974) distinguished sporocytes from gonocytes based on the degree of cytoplasm granularity and the number of nuclei present: one to two in gonocytes and four in sporocytes. By contrast, Shumway (1924) defined sporocytes as the posterior-most cell(s) of the parasite, which are distinctively bulbous in shape and tend to be more granular under the light microscope than gonocytes (Figs 1, 2a–c). We prefer the latter criteria, where the number of sporocytes per parasite depends only on the number of longitudinal rows present (Figs 2c and 3e). By this definition, the number of sporocytes in *H. axiothellae* is usually one (sometimes two, Fig. 1, 2a–c), and they are not necessarily tetranucleate; sporocytes may also have only one or two nuclei (Siebert 1973; Siebert and West 1974).

Eventually, the sporocytes (or "buds" from the sporocytes) fall off of the parasite, form a cyst, and leave the host with the faeces (Shumway 1924; Siebert 1973; Siebert and West 1974). In Atlantic haplozoans, the cyst contains motile dinospores that when released, infect a new host (Shumway 1924). However, neither cysts nor dinospores have been observed in *H. axiothellae*. Shumway (1924) stated from his observations of haplozoans that, "no evidence for the cell division of the sporocyte while attached to the colony has been found in the hundreds of specimens I have examined." By contrast, we have observed in *H. axiothellae* what appeared to be a dividing sporocyte and the pre-release of a putative cyst (Figs 2c and 3e). The surface of this putative cyst is quite different from typical gonocytes and sporocytes; it is described in more detail in the section dedicated to surface pores.

Organization of the amphiesma

The most striking feature on the surface of *H. axiothellae* was the presence of tapered "thecal barbs" that were about 0.5 µm thick at the base and ranged from 0.5–3.5 µm in length (Figs 1, 2d–h); we use this descriptor in place of the synonym "spines" (Siebert and West 1974). Aside from the neck, trophocytes were always completely covered with barbs, which, depending on the individual, could be either relatively narrow (Fig. 2g) or broad (Fig. 4d). In contrast with previous observations (Siebert and West 1974), gonocytes could also be completely covered with thecal barbs as in the left-hand parasite in Figure 1, but they most often possessed a distinct mid-ring of barbs that were most dense (approximately 3.5 barbs per square micron) on the ventral surface (Figs 1, 2a–c). Consequently, immature gonocyte junctions usually emerged within the mid-ring of the thecal barbs (Figs 1, 2a–c). The anterior-most gonocytes (the "primary gonocyte", Shumway 1924) had relatively more barbs than the rest of the gonocytes in the chain. Despite the report that the surface of sporocytes bears more thecal barbs than gonocytes prior to re-

Fig. 2. Scanning electron micrographs of *Haplozoon axiothellae* showing general anatomy and details of the adhesive apparatus (syn. suction disc) and thecal barbs (syn. spines). **a.** Right lateral view showing that, in most cases, the parasites are differentiated into three fundamental regions: an anterior trophocyte (T) containing an adhesive apparatus (AD), a trunk consisting of many linearly arranged gonocytes (G), and a terminal compartment called the "sporocyte" (S). Most of the trophocyte is covered with thecal barbs. Sporocytes are bulbous and free of thecal barbs on the posterior-most surface (Bar = 15 µm). **b.** Dorsolateral view showing two distinct types of junctions between adjacent gonocytes, defined by the degree of indentation: mature junctions (arrows) and immature junctions (arrowheads). Different junction types are inferred to indicate different stages of nuclear division, which is consistent with light and transmission electron microscopy data (Shumway 1924; Siebert 1973; Siebert and West 1974) (Bar = 15 µm). **c.** Left lateral view showing that in some cases the posterior region becomes subdivided into two rows of gonocytes; the sporocytes (S) give rise to a putative cyst (Cy-arrow) that will fall off and leave the host with the faeces. The anterior region of this organism shows the "neck" of the trophozoite (N), which is usually completely free of thecal barbs and significantly broader on the dorsal side (Bar = 15 µm). **d.** High magnification ventral view of the adhesive apparatus showing the base of a stylet (arrow) and two longitudinal ridges called "arms" by Siebert and West (1974). The arms are running vertically but are obscured by barbs (Bar = 2.5 µm). **e.** Ventral view of a stylet showing a "basal fold" (arrow) arranged spirally (anterior of organism is upper-right) (Bar = 1.5 µm). **f.** Right lateral view of a stylet showing a fold (arrow) near the apex. Distinct holes (arrowhead) near the stylet base suggest that the stylets are enveloped by an outer membrane that is continuous over the entire organism (Bar = 1.5 µm). **g.** High magnification view of thecal barbs (Bar = 1.5 µm). **h.** High magnification view of thecal barbs showing that they each stem from a single thecal plate (Bar = 0.5 µm).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lease (Siebert and West 1974), every sporocyte we observed tended to have either equal or fewer barbs than gonocytes. The bulbous posterior end of sporocytes was invariably completely void of thecal barbs, however, sporocytes possessed a mid-ring of barbs similar to those present on gonocytes. No barbs were present on the putative "cyst" described earlier (Fig. 3e, f).

Patterns of small alveoli were apparent wherever barbs were absent, except on the presumptive "cyst" (Fig. 3a, c). Alveoli were all of roughly the same size (0.8 µm across) and with either a pentagonal or hexagonal margin. The alveolar margins often had unequal side lengths. Each barb was subtended by a single alveolus (Figs 2h and 3a).

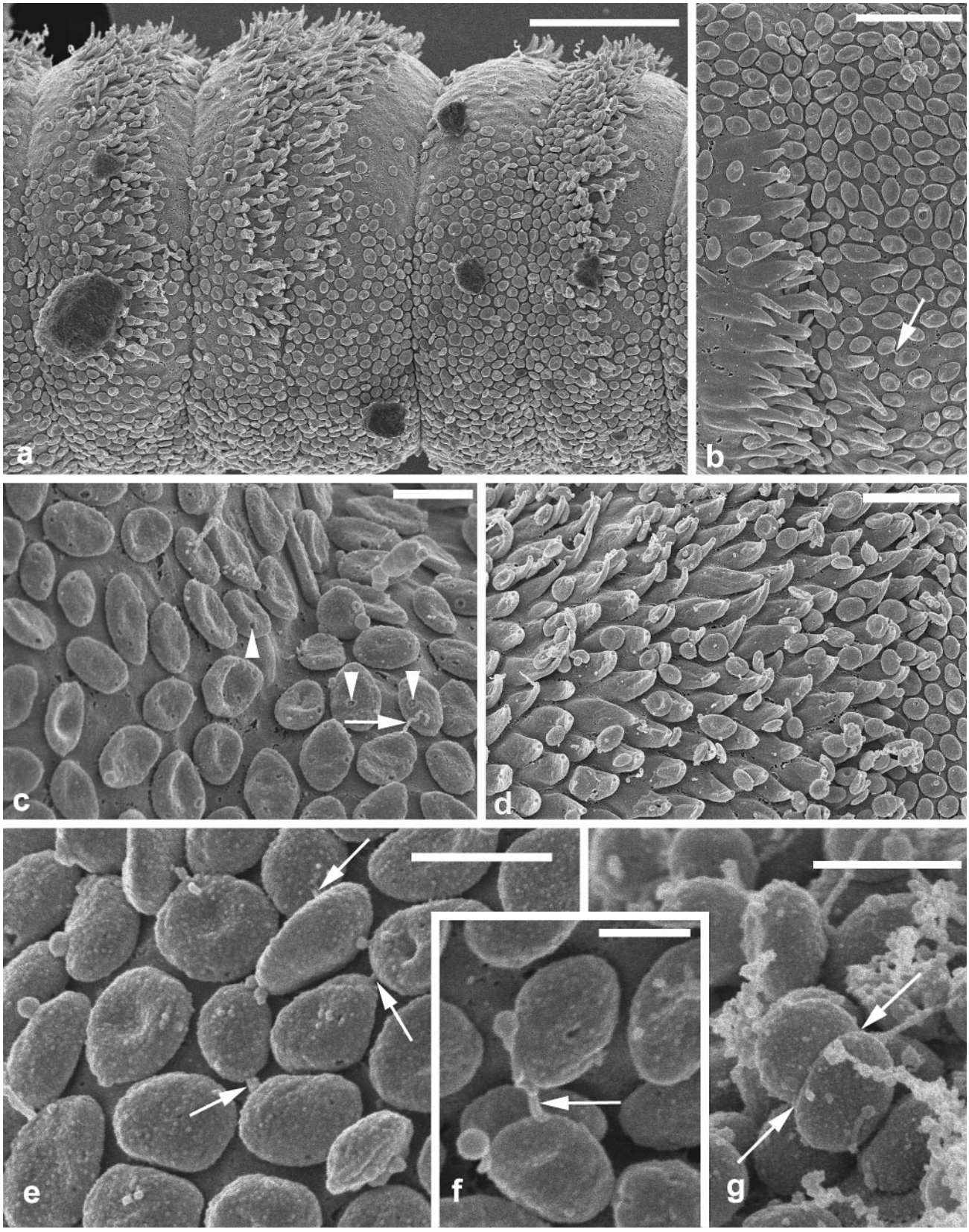
Most surprisingly, SEM data suggest very strongly that the entire organism is contained within a common outer membrane. So rather than being "multicellular" or "colonial" (Dogiel 1906; Calkins 1915; Shumway 1924; Siebert 1973; Siebert and West 1974), each parasite is perhaps more appropriately described as a "compartmentalized syncytium". This is best illustrated by a close examination of the junctions between gonocytes (Fig. 3a, 3c and 3d). A continuous membrane appeared to span the junctions regardless of whether the junction is "mature" or "immature"; alveolar boundaries never spanned gonocyte junctions.

Poorly fixed specimens possessed tears in the outer membrane; at the gonocyte junctions, the tears clearly showed the underlying alveolar membranes and the regions between tears accentuated the continuity of the outermost membrane (Fig. 3c, d). Membrane tears at gonocyte junctions sometimes showed the alveolar membranes descending to form the "septa" between gonocytes. Stylets are also covered by the same outer membrane as suggested by tears in the attachment apparatus (Fig. 2e, f). We also observed tears on and around the thecal barbs, which clearly showed a continuous, enveloping membrane. Re-examination of the original TEM data was consistent with this interpretation, but the membranes between gonocytes were so densely stacked that their number could not be determined. Moreover, the outermost membrane showed no signs of a "furrow" at gonocyte junctions (Fig. 3c), which would be the expected morphology if two separate membranes (one from each neighbouring gonocyte) were actually pressed together at these locations.

Attachment apparatus

Haplozoon axiothellae attaches to the host with the ventral surface of the trophocyte, which was marked by a distinct concavity in lateral view (Fig. 2a). The apical margin of this concavity often supported a "stylet" (syn. proboscis, Shumway 1924) that probes the host tissue by protraction and retraction (Figs 1, 2e, and 2f). The stylet had a uniform surface but was marked by distinct folds that were arranged spirally (Fig. 2e, f). A ventral view of the attachment apparatus demonstrated two longitudinal ridges (syn. arms, Siebert and West 1974) about 8.2 µm in length that were separated by a groove roughly 0.5 µm in width and positioned immediately posterior to the stylet (Fig. 2d). SEM data demonstrate that the attachment apparatus or "suction disc" is much longer (8.2 µm) than the 2.5 µm length previously reported (Siebert and West 1974). The longitudinal ridges appeared to be functionally associated with the stylet and may be involved in the adhesive function of the apparatus and absorption of nutrients from the host (Shumway 1924; Siebert and West 1974). Thecal barbs covered the entire surface of the attachment apparatus without any obvious pattern (Fig. 2d–f).

Fig. 3. Scanning electron micrographs of *Haplozoon axiothellae* showing details of the theca. **a.** The junctions between gonocytes (left hand junction) and between the trophocyte and the anterior-most gonocyte (right-hand junction) are enveloped by a continuous membrane (Bar = 1.5 µm). **b.** The theca is comprised of numerous hexagonal and pentagonal alveoli that are roughly 0.70 µm across (Bar = 1.5 µm). **c.** High magnification view showing the continuous membrane across a mature gonocyte junction (Bar = 2.5 µm). **d.** Poorly fixed specimens produced membrane tears (arrows) that help illustrate the continuity of the outer membrane across the gonocyte junctions (Bar = 0.5 µm). **e.** Details of a specimen that has generated two bulbous sporocytes and a putative cyst (Cy) at the posterior end (Bar = 10 µm). **f.** High magnification view of the putative cyst showing the absence of thecal plates and the presence of minute pores (arrows) distributed over the surface (Bar = 2.5 µm). **g.** High magnification view of a distinct pore (arrow) positioned on the ventral surface of a gonocyte near the anterior junction (Bar = 1.5 µm). **h.** These pores (arrows) are arranged in a longitudinal row along the ventral surface of the organism (Bar = 2.5 µm).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Pores

Two distinct types of surface pores were observed on *H. axiothellae*. One type was associated with the putative cyst emerging from the sporocytes shown in Figures 2c and 3e. Close examination of this "cyst" revealed minute (about 60 nm in diameter) but distinct pores scattered across its surface; we were unable to find similar pores on any other specimen. It is possible that the pores indicate the presence of trichocysts, however, the pores were much smaller than the usual docking sites associated with the trichocysts of dinoflagellates. Moreover, there was no evidence of ejected trichocysts anywhere in the preparation, which is usually the case for dinoflagellates fixed for electron microscopy. More studies are necessary to understand the significance of these pores on the surface of this specialized compartment.

The second type of pore that ranged from 0.20–0.25 µm in diameter was found exclusively on the ventral surface of gonocytes (Fig. 3g, h). A single pore was present near the anterior junction of each gonocyte, and collectively, the pores were arranged in a single longitudinal row along the ventral side of the parasite (Figs 1 and 3h). Although no associated cinguli and sulci were present, this row of pores was reminiscent of the monokinety found in some multinucleate dinoflagellates such as *Polykrikos*. It is possible that each ventral gonocyte pore represents a vestige of the flagellar pore present in most dinoflagellates. However, no evidence of flagellar systems was found in the TEM studies (Siebert 1973; Siebert and West 1974). One study presented evidence of thecal openings (Siebert and West 1974), but it was unclear whether these openings were simply artefactual breaks in the theca like those found with the SEM (Fig. 2f and 3d). Moreover, unusual trichocyst-like bodies were found in the TEM study, but they were never associated with an opening. Although the linear pattern of ventral gonocyte pores is intriguing, the significance and specific functions of these pores are unclear.

Episymbionts?

Seven of the thirteen individuals observed with SEM possessed numerous concave bodies that were distributed as distinct clusters over much of the parasite's surface (Figs 1, 2a, 2b, 3b and 4); four of eight parasites from Grappler Inlet and three of five parasites from Argyle Lagoon possessed the concave bodies. These structures were not observed in previous studies using TEM (Siebert 1973; Siebert and West 1974). The bodies were flat, circular to oval in shape, and usually 0.5 µm in length (the mean, n = 30) but ranged in size from 0.4 µm to 1.5 µm. The concave shape of the bodies was unlikely to be due to dessication as there was no evidence of "shrinkage" on any other specimen including unambiguous rod-shaped bacteria found in the same preparation. The distribution of the bodies was most dense on regions devoid of thecal barbs; however, they were also distributed haphazardly over areas covered with barbs (Fig. 4d). Dense clusters of concave bodies were present at the posterior-most end of the sporocytes and on the necks of trophocytes. The concave bodies were almost never distributed as more than a single layer, but when this did occur, many of the bodies were noticeably more spherical and septum-like junctions between bodies were evident (Fig. 4g). In most cases, the concave bodies were evenly spaced and did not touch neighbouring bodies (Fig. 4). On occasion, however, distinctive threads connected adjacent bodies (Figs 4b, c, e and f), which were most conspicuous in areas free of debris (Fig.

Fig. 4. Scanning electron micrographs of *Haplozoon axiothellae* showing putative episymbionts (concave bodies). **a.** Low magnification view showing the distribution of episymbionts on adjacent gonocytes (Bar = 5 µm). **b.** Slightly higher magnification view showing the diversity of epibiotic shapes and a putative pilus (arrow) between adjacent episymbionts (Bar = 2 µm). **c.** High magnification view showing the concave shape of the putative episymbionts, pore-like openings (arrowheads), and what may be an extended pilus (arrow) (Bar = 0.5 µm). **d.** The putative episymbionts are not only found on the smooth regions of the host but are also distributed haphazardly over areas dense with thecal barbs (Bar = 1.5 µm). **e.** and **f.** High magnification views showing structures resembling pili (arrows) (Bar = 0.5 µm and 0.25 µm, respectively). **g.** High magnification view showing a presumptive septum (arrow) between episymbionts (Bar = 0.5 µm).

4b, c). One plausable explanation for all of these observations is that these unusual concave bodies may be episymbionts. Their size range is extremely small but is consistent with some bacteria, picoeukaryotes, and symbiotic archaea (Preston et al. 1996; Vainshtein and Kudryashova 2000; Schulz and Jorgensen 2001; Huber et al. 2002). It is crucial to observe these unusual bodies with transmission electron microscopy before drawing any confident conclusions about any possible symbiotic relationship. Nonetheless, if these are episymbionts, then they are among the smallest cells known and have a novel morphology suggesting further examination of these bodies could be very informative.

Systematic remarks

The genus *Haplozoon* is clearly a member of the dinoflagellates; the nuclei of the parasitic life stage appear to be dinokaryotic, and the organism is surrounded by alveoli that, at least in *H. axiothellae*, contain thecal plates (Siebert 1973; Siebert and West 1974). The free-living, unicellular stage is even more dinoflagellate-like in possessing longitudinal and transverse flagella oriented in the usual dinoflagellate fashion (Chatton 1919; Shumway 1924). Molecular data also supports this view as SSU rDNA-based phylogenetic trees of dinoflagellates consistently put *H. axiothellae* within the dinoflagellate clade (Saldarriaga et al. 2001).

The exact position of *Haplozoon* within the dinoflagellates is much more controversial. Traditionally, the genus has been considered a member of the order Blastodiniales, a group of parasitic dinoflagellates that is defined by the presence of non-dinokaryotic nuclei in certain stages of their life cycle (Fensome et al. 1993). However, *Haplozoon* may well be completely dinokaryotic. The parasitic life stage is certainly dinokaryotic, and although the nucleus of the motile stages has never been investigated, they also probably contain a dinokaryon; in organisms with both dinokaryotic and non-dinokaryotic phases, the motile phases are always dinokaryotic (Cachon and Cachon 1987). No true blastodinialean SSU sequences have ever been obtained (*Amyloodinium ocellatum*, formerly considered to be a blastodinialean, has been shown to have a peridinialean thecal plate organization, Landsberg et al. 1994), and so the question of the relatedness of *Haplozoon* to true Blastodiniales cannot yet be addressed using molecular methods.

The pattern of small, polygonal alveoli across the surface of *H. axiothellae* is similar to that present on members of the Gymnodiniales (e.g. *Amphidinium carterae*, *A. herdmanii*, and *Gymnodinium fuscum*; Dodge and Crawford 1970; Hansen et al. 2000). This is consistent with morphological observations (and predictions) indicating that the dinospores of an Atlantic species of *Haplozoon* are most similar to *Gymnodinium*-like dinoflagellates (Chatton 1919; Shumway 1924). We suspect that the closest living relatives of *Haplozoon* might be members of the Gymnodiniales, even though the group traditionally contains only athecate species. It should be mentioned, however, that thin thecal plates (or plate-like material) have been observed in the alveoli of several gymnodinialeans, including for example several species of *Woloszynskia* and the type species of *Gymnodinium, G. fuscum* (e.g. Dodge and Crawford 1970; Hansen et al. 2000). Unfortunately, SSU sequences do not help in answering this question: no particular position of *Haplozoon* within dinoflagellates is strongly supported by SSU phylogenies (Saldarriaga et al. 2001).

Concluding remarks

The structure of *H. axiothellae* is significant in many respects, not the least of which is the extreme sophistication of its many adaptations to its parasitic mode of life. The infection mechanism and life history of this organism are highly adapted, and it is interesting to consider the origin and evolution of these features in the light of the evolutionary position of *Haplozoon*. It is clear from comparative morphology and rRNA phylogenies that *Haplozoon* is not only related to dinoflagellates, but actually evolved from within dinoflagellates (Chatton 1919; Shumway 1924; Siebert 1973; Siebert and West 1974; Saldarriaga et al. 2001). The transition from a free-living phototroph to this parasite is remarkable. On one hand, some structural features that characterize *Haplozoon* can be traced to homologous features in dinoflagellates. The barbs, which may be an adaptation to increase surface area, are clearly derived from alterations in the shape of thecal plates. Similar alterations are made by other dinoflagellates to form other surface projections. Likewise, the ventral pores may be derived from flagellar pores which, in many dinoflagellates, also serve as openings for pusules. Therefore, the ventral pores in *Haplozoon* may

persist in the absence of flagella, basal bodies and microtubular roots (Siebert 1973; Siebert and West 1974), because of the (excretory) functions of underlying pusules. One the other hand, the attachment apparatus of *Haplozoon*, composed of the adhesive ridges and the probing stylet, is an extraordinarily suite of structures that have no identifiable homologues in dinoflagellates.

Perhaps the most interesting feature of *Haplozoon*, however, is its overall body plan. *Haplozoon* is not exactly multicellular because all evidence suggests that the entire organism is enclosed by a single plasma membrane. At the same time, the order and complexity of *Haplozoon* cells is beyond a syncytium or plasmodium, because the organism is compartmentalized in a specific and orderly fashion using the alveolar membranes. This compartmentalization achieves some of the advantages accrued by muticellularity, such as the ability to differentiate regions for specific functions. No other organism shares this peculiar level of organisation, and perhaps it is unlikely that such a body plan would evolve outside the Alveolata, as members of this group are equipped with the internal membrane system that made this "compartmentalized syncytium" possible.

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