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Comparative Surface Morphology of Marine Coelomic Gregarines (Apicomplexa, Urosporidae): *Pterospora floridiensis* and *Pterospora schizosoma*

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ABSTRACT. Two species in the aseptate gregarine genus *Pterospora* from the Pacific and Gulf coasts were analyzed by scanning electron microscopy, which revealed characteristics not reported in other gregarines. The gamonts of these species had branching trunks that ended in terminal digits, and both species moved by cytoplasmic streaming and peristalsis. *Pterospora floridiensis* had surface pits and tracts of parallel ridges that bended and connected with one another. *Pterospora schizosoma* had irregular-shaped surface swellings that were usually arranged in rosette patterns. These unique surface features have not been reported for other gregarines, and are strikingly different from the surface features of many septate and aseptate gregarines that inhabit the intestinal lumena of their hosts and move by gliding. The correlation of *Pterospora*'s unique pellicular features to the habitat and cytoplasmic streaming characteristic of the genus may be significant, and may reflect an adaptation for development in coelomic environments.

Key Words. Acephaline, aseptate, *Axiothella mucosa*, *Axiothella rubrocincta*, gregarine, ultrastructure.

BAMBOO worms (Polychaeta: Maldanidae) are hosts to an unusual genus of aseptate gregarine, *Pterospora*. The genus was established by Labbé and Racovitza (1897) for gregarines collected on the coast of France, and since then six more species have been reported from maldanids in France, Russia, the United States, and Canada (for a review see, Landers 2001; Levine 1977). The gamonts of *Pterospora* have branching trunks of cytoplasm that bifurcate repeatedly, eventually forming terminal digits, the number of which varies for different species (Fig. 1, 2). The cells are found in syzygy in the coelom of their host and use peristalsis and cytoplasmic streaming to fill and empty the branching trunk system. Few ultrastructural studies of *Pterospora* exist. Landers (1991) published a scanning electron micrograph (SEM) of the *Pterospora demodendrion* gamont and an SEM of *Pterospora clymenellae* oocysts. Since that report, transmission electron microscopy (TEM) studies of *Pterospora floridiensis* have been published (Landers 1999, 2002). These three reports demonstrated that the gamont stage has an irregular surface of swellings and indentations and lacks the longitudinal epicytic folds characteristic of most eugregarines. Much has yet to be learned about the ultrastructure of these unusual gregarines, as a reconstruction of the complex surface based on TEM is very difficult. Thus, we undertook this comparative SEM study of *Pterospora* spp. from the Pacific and Gulf coasts to provide more information on the surface morphology of the gamont. It is anticipated that these *Pterospora* species will have a surface morphology distinct from previously studied intestinal gregarines, because of their coelomic habitat and non-gliding motility.

MATERIALS AND METHODS

Pterospora floridiensis was collected from the polychaete host *Axiothella mucosa*, obtained from St. Andrew Bay, FL, USA. Gamonts were fixed by two protocols: (1) OsO_4 vapor/ OsO_4+ glutaraldehyde protocol (Leander, Harper, and Keeling 2003) and (2) 4% (v/v) glutaraldehyde buffered in 0.05 M sodium cacodylate (pH 7.5) followed by post-fixation in 2% (w/v) OsO_4 in cacodylate buffer. After critical point drying in CO_2, the gamonts were sputter-coated with gold–palladium and viewed with an LEO 982 FE-SEM.

Pterospora schizosoma was collected from the host polychaete *Axiothella rubrocincta*, obtained from the mudflats at Argyle Lagoon on San Juan Island, Washington, USA (2004), and from Grappler Inlet, Vancouver Island, Canada (2002). The parasites were fixed using an OsO_4 vapor/OsO_4+glutaraldehyde protocol while in a Swinnex filter holder and then critical point-dried in CO_2 (Leander, Harper, and Keeling 2003). The cells were sputter-coated with gold and viewed with a Hitachi S4700 SEM.

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RESULTS

***Pterospora floridiensis*.** The gamonts consist of a large soma and two trunks that bifurcate to form a variable number of terminal digits, usually four to 18 per cell (Fig. 1, 3). Gamonts are always in syzygy unless separated during dissection of a host. Low-magnification SEM images revealed a uniform surface texture, which is porous and formed into circular elevations, giving a warty texture (Fig. 4, 5, 7, 8). Some specimens were covered with fine strands of adherent material, presumed to be mucus, which obscured the surface details to varying degrees (Fig. 4, 6). Higher magnification images (Fig. 6, 9–11) revealed many surface ridges that measure $\sim$ 75–90 nm in width and $\sim$ 200–400 nm in length. These ridges are arranged in parallel tracts that usually curve, divide, overlap one another, and surround distinctive surface pits (Fig. 10). The pits have an approximate diameter of 0.2 μm. The warty texture of the surface is related to the distribution of the tracts of ridges. The tracts of ridges are thrown up into elevated clusters with a diameter of $\sim$ 2.0 μm (Fig. 9, 11). Spaces in between these clusters reveal a relatively smooth pellicular surface containing fewer tracts of ridges. The ''warty'' surface created by the clusters of ridges is uniform over the entire cell, with the exception of the terminal digits and the interdigitating folds at the junctional site of the two gamonts. The terminal digits are bulbous and smooth when distended with cytoplasm, and crenulate when deflated (Fig. 5, 7, 8). Longitudinal folds (Fig. 4, 12) extend from the junctional site approximately 15 μm, and vary in their surface details as they approach the junction. Farthest from the junction, the folds have the clusters of pellicular ridges, forming the warty surface, although close to the junction the folds have fewer tracts of ridges and few if any elevated clusters (Fig. 12, 13).

Comparison of fixatives. *Pterospora floridiensis* was prepared using two fixation protocols, one in which osmium vapor was the initial step and one in which buffered glutaraldehyde was the initial step (see Materials and Methods). Both protocols resulted in very similar results. Figures 3–13 were chosen to provide a mixture of both protocols for comparative purposes. With both procedures, the cells are covered with clusters of ridges (cf. Fig. 5, 7, 9, 11), parallel tracts of ridges (cf. Fig. 6 and 10), and what is presumed to be mucus (cf. Fig. 4, 6). However, there was an apparent difference when comparing the area near the terminal digits and the digits themselves. Near the digits the pellicle is not as

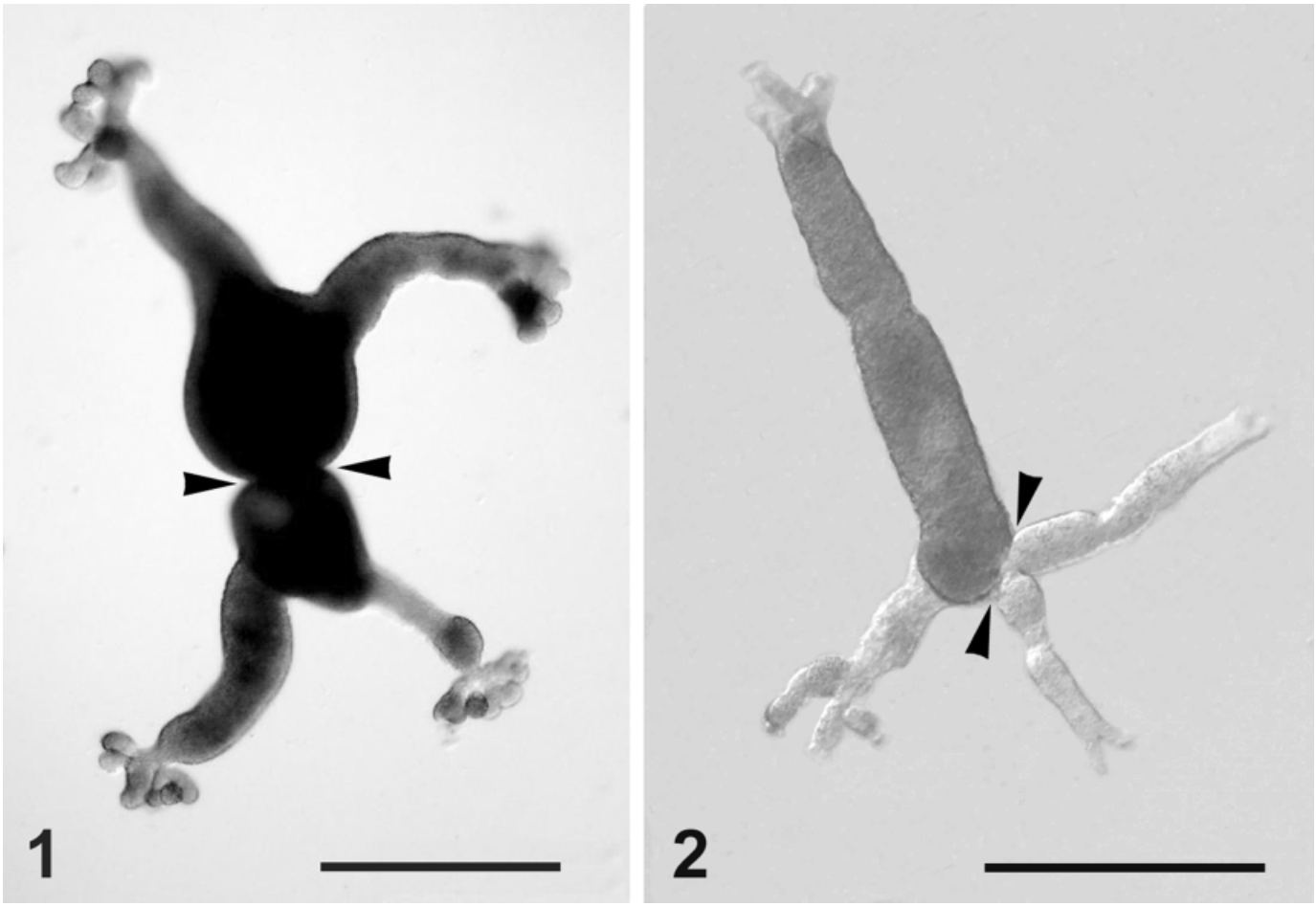

Fig. **1–2.** Light micrographs of live *Pterospora* gamont associations. Arrowheads demarcate the junctional site between gamonts in both figures. **1**. *Pterospora floridiensis* (scale bar = 200 μm). **2**. *Pterospora schizosoma* (scale bar = 100 μm).

contracted and therefore more smoothed out when using osmium vapor (Fig. 5, 8). This smoother surface makes a transition to the more contracted and crenulate surface proximal to the digits (Fig. 5). The terminal digits were fixed in both a bulbous, smooth condition as well as a contracted condition using osmium vapor (Fig. 5, 8), but were only fixed in contracted states using the buffered-glutaraldehyde protocol (Fig. 7, 8). In living gamonts, bulbous smooth terminal digits are observed during distention because of cytoplasmic streaming. This natural condition was best preserved with osmium vapor, which is the fixative we interpret to provide superior surface fixation in most specimens. It should be mentioned that the osmium vapor technique produced large blisters in some cells and did not always provide ideal fixation with our material (data not shown). This blistering was not observed with the buffered-glutaraldehyde protocol.

***Pterospora schizosoma*.** The majority of the cell consists of two elongated trunks that bifurcate to eventually form four to six terminal digits. There is a relatively short central soma from which the trunks project (Fig. 2). SEM revealed surface characteristics quite distinct from *P. floridiensis*. The osmium-vapor fixation protocol was used for all *P. schizosoma* images, as this protocol provided superior fixation of the *P. floridiensis* terminal digits. The surface morphology of the rest of the *P. floridiensis* gamont was nearly identical when organisms fixed by both methods were compared. Low-magnification images of *P. schizosoma* revealed a pellicle covered with distinctive swellings (Fig. 14, 17–19). The shape of the swellings is variable, although many are almost triangular, with a maximum side length of $\sim$ 1.8–3.6 μm. Some of the pellicular swellings are arranged in rosettes around shallow pits (Fig. 19), forming circular patterns over the trunk surface. The swellings have a uniform distribution on the trunk surface and extend toward the terminal digits. The uniform pattern of pellicular swellings does not continue to the terminal digits. The pellicle in the transition zone between the branches of the trunk and the digits becomes smoother, with fewer pronounced wrinkles or crenulations (Fig. 15). The terminal digits are elongated, unlike the bulbous spherical digits of *P. floridiensis*. Surface indentations that we interpret as pellicular pores were present on the smooth surface of the bulbous digits (Fig. 16), among the swellings of the trunk (Fig. 20), and in the transition zone at the base of the digits (Fig. 21).

DISCUSSION

Earlier TEM studies of *P. floridiensis* demonstrated a very irregular surface with numerous ridges and surface variations (Landers 1999, 2002). The ridges were arranged in parallel, elevated tracks, which bended and connected with one another on the surface. Longitudinal epicytic folds were not reported, with the exception of interdigitating folds in the immediate area of

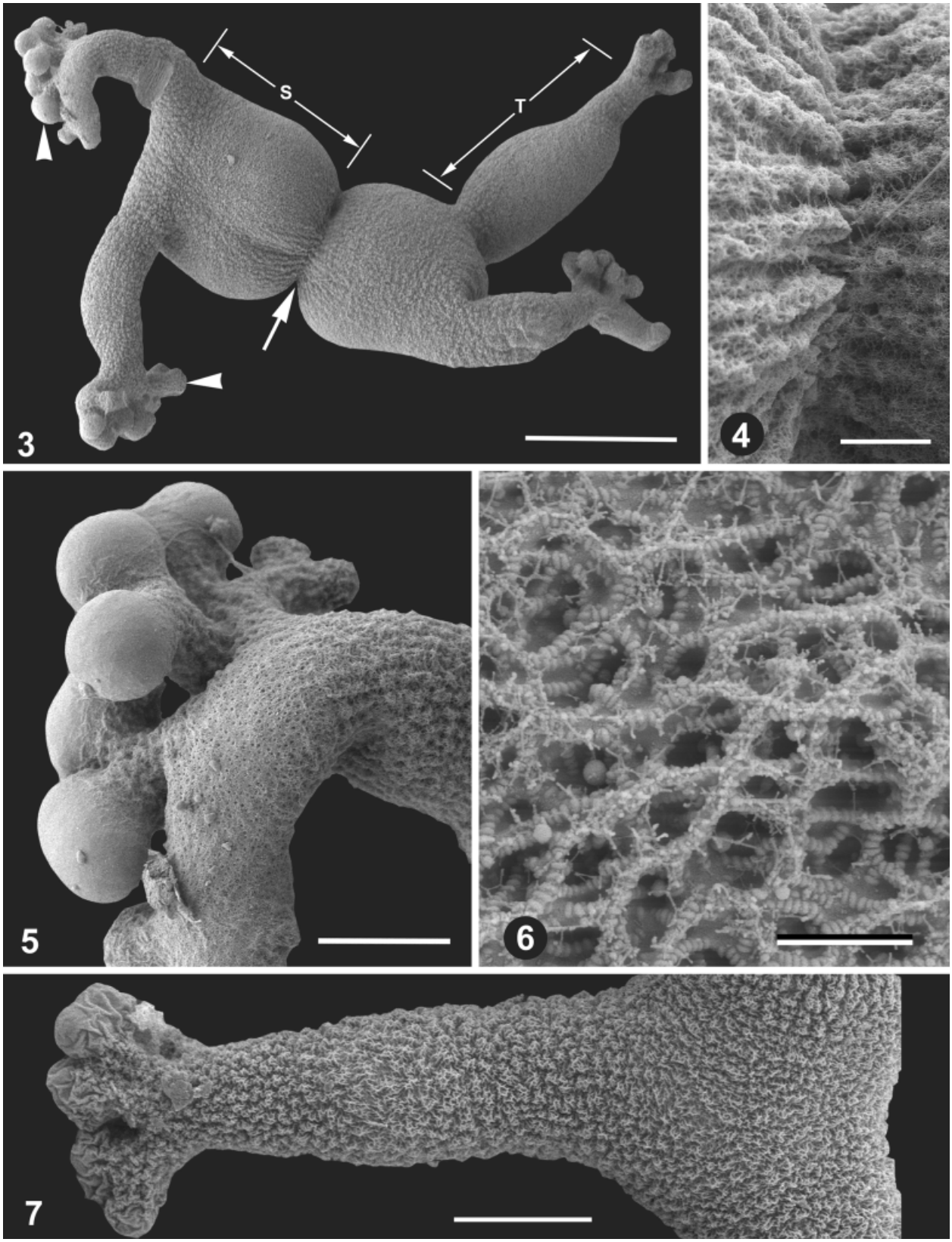

Fig. **3–7.** Scanning electron micrographs of the gamont surface of *Pterospora floridiensis*. Fig. 3, 5 and 6 are derived from an osmium-vapor fixation protocol, and Fig. 4 and 7 are derived from a buffered-glutaraldehyde fixation protocol (see Materials and Methods). **3**. Low-magnification image of a gamont pair (arrow, junctional site; arrowheads, terminal digits; S, soma; T, trunk) (scale bar = 100 μm). **4**. The junctional site between two gamonts, with fine strands, presumed to be mucus, over the surface. (scale bar = 7.5 μm). **5**. Enlargement of distended terminal digits present in the gamont pair in Fig. 3. (scale bar = 20 μm). **6**. High-magnification image of the pellicle, with tracts of parallel ridges, surface indentations, and fine ''mucus'' strands (scale bar = 2 μm). **7**. Trunk of a gamont showing the warty projections of the pellicle and deflated terminal digits. (scale bar = 20 μm).

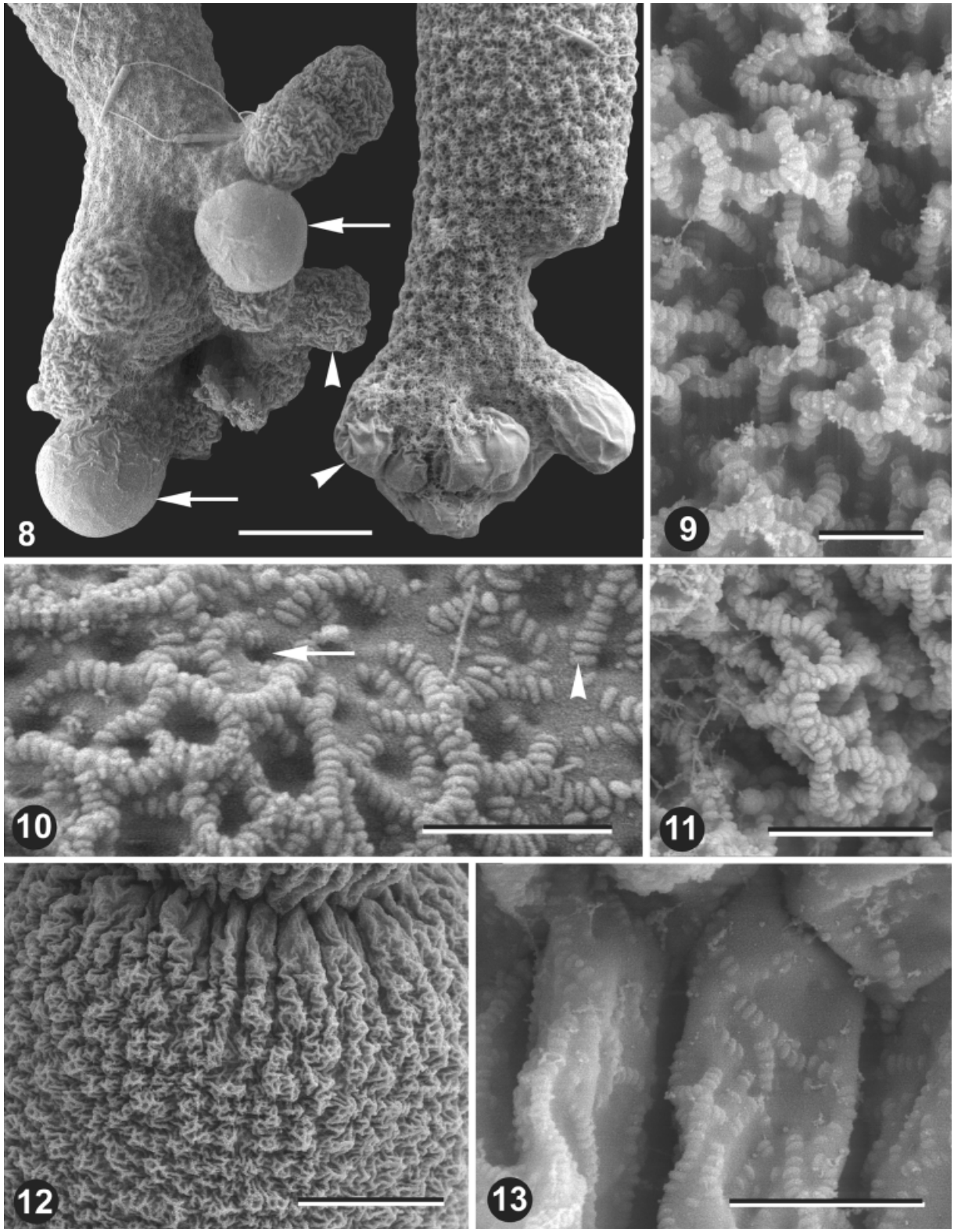

Fig. **8–13.** Scanning electron micrographs of the gamont surface of *Pterospora floridiensis*. Figure 8 (left image) and Fig. 11 are derived from an osmium-vapor fixation protocol; the remaining figures are derived from a buffered-glutaraldehyde fixation protocol (see Materials and Methods). **8**. Terminal digits in distended states (arrows) and deflated states (arrowheads) (scale bar = 20 μm). **9**. Higher magnification of the pellicle showing the pellicular ridges thrown up into elevated clusters (scale bar = 1 μm). **10**. Higher magnification view of the pellicle showing pellicular ridges (arrowhead) and pits (arrow) (scale bar = 2 μm). **11**. Pellicle fixed with osmium vapor demonstrating near identical ultrastructure present in the buffered-glutaraldehyde fixed cell in Fig. 9 (scale bar = 2 μm). **12**. Junctional site between two gamonts illustrating the transition from a ''warty'' condition (distal to the junction) to a smooth condition (proximal to the junction) (scale bar = 10 μm). **13**. Higher magnification view of the junctional folds in Fig. 12 showing relatively fewer tracts of ridges (compare with Fig. 10 from the same cell) (scale bar = 2 μm).

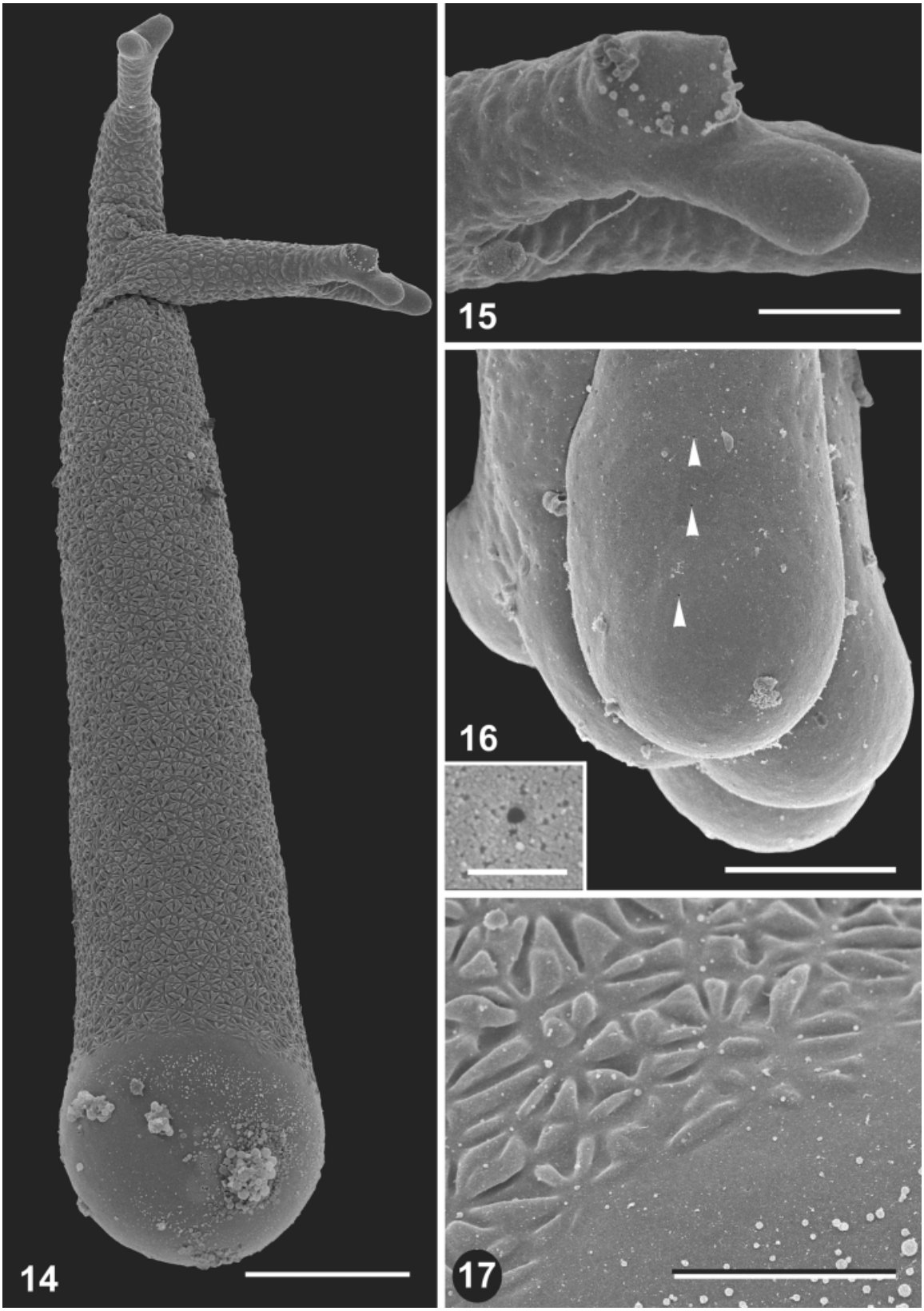

Fig. **14–17.** Scanning electron micrographs of the gamont surface of *Pterospora schizosoma*. All images derived from an osmium-vapor fixation protocol (see Materials and Methods). **14**. Low-magnification image of one trunk of a gamont (scale bar = 20 μm). **15**. Higher magnification view showing the terminal digits and the transition zone (left side of fig.) from trunk to digits of the gamont in Fig. 14 (scale bar = 10 μm). **16**. Enlargement of the terminal digits in a different gamont showing the presence and distribution of presumed pellicular pores (arrowheads) (Bar = 5 μm). Inset: High-magnification view of a presumed pellicular pore (scale bar = 0.25 μm). **17**. Pellicular surface enlargement from Fig. 14 near area where the cell has broken from the other trunk and reclosed (scale bar = 10 μm).

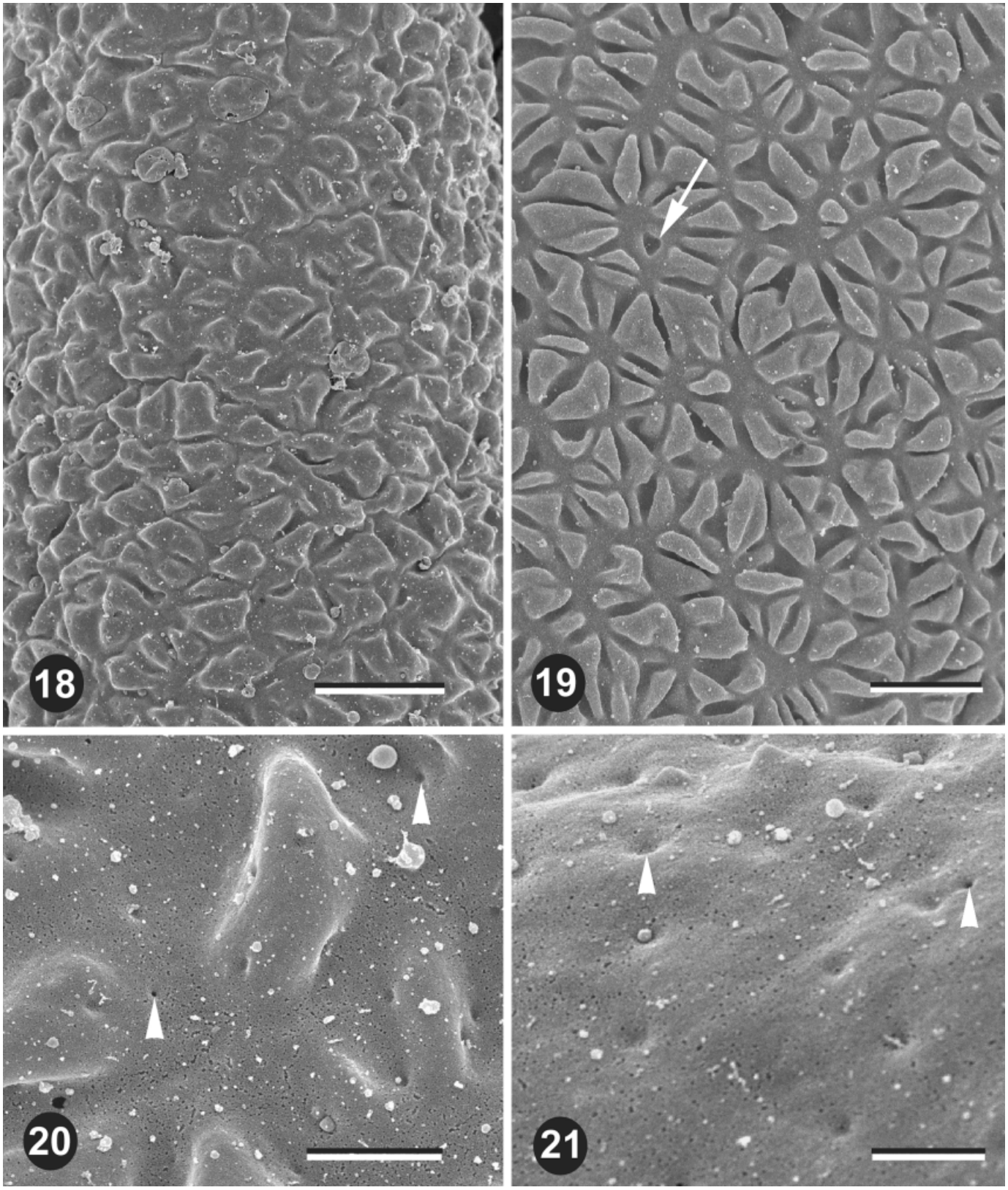

Fig. **18–21.** Scanning electron micrographs of the gamont surface of *Pterospora schizosoma*. All images derived from an osmium-vapor fixation protocol (see Materials and Methods). **18**. Pellicle of a gamont collected from Argyle Lagoon, San Juan Island, WA, USA (2004) showing irregular surface elevations (scale bar = 5 μm). **19**. Pellicle of a gamont collected from Grappler Inlet, Bamfield, BC, Canada (2002) showing irregular surface elevations similar to those present in Fig. 18 (scale bar = 5 μm). Arrow: shallow surface pit. **20**. High-magnification view of a trunk showing the presence and distribution of presumed pellicle pores among the irregular surface elevations (arrowheads) (scale bar = 1 μm). **21**. High-magnification view of the base of a terminal digit showing the presence and distribution of presumed pellicle pores (arrowheads) (scale bar = 0.25 μm).

the junction with another gamont. Our observations here have provided a surface view of these same features, revealing a much more extensive pattern of pellicular ridges (arranged in tracts) as well as pellicular pits, and a distinctive pattern of wart-like elevations on the surface. The function of the tracks of ridges is unknown, although their presence has been demonstrated in *P. floridiensis* using multiple fixation protocols in this and previous studies (Landers 1999, 2002). The wart-like elevations evident at low magnification result from a clustering of the tracts of ridges, an arrangement that supports the assumption that the tracts are contractile. An additional argument supporting a contractile function for the tracts rests in their distribution, as they diminish in frequency near the gamont junctional site where the cell does not move as actively (Landers 2002).

The osmium-vapor fixation protocol and buffered-glutaraldehyde protocol gave similar results. The basic structure of the pellicle was similar using each fixative, although there were some differences evident in the digits and their connection to the trunks. Many digits were fixed in a smooth condition using osmium vapor, indicating that they were filled with cytoplasm and turgid when fixed, and remained in that condition during the fixation process. Buffered glutaraldehyde did not give this result, suggesting that turgid digits, if present at the moment of fixation, deflated after contact with the fixative. In the area connecting the digits to the trunks, the pellicle of the osmium-fixed cells was less crenulate than the rest of the cell, suggesting that either cytoplasmic movement or pellicular contraction may have been more pronounced when fixed with the buffered glutaraldehyde or the digit pellicle could have shrunk because of osmotic differences between the cell and the glutaraldehyde fixative.

The ultrastructure of *P. schizosoma* was markedly different from that of *P. floridiensis*. The distinctive pellicular tracts and ridges were not present, but instead, a pattern of surface elevations covered the cell. Future studies of *P. schizosoma* using TEM are needed to determine whether they share characteristics with the decorations on the *P. floridiensis* pellicle. The *P. schizosoma* elevations are uniform in general size and shape, and are arranged in circular patterns on the surface. The pellicular surface makes a transition to a smooth surface at the digits, which was also observed in *P. floridiensis*. Pellicular pores, present in all areas of the *P. schizosoma* pellicle, were not observed in *P. floridiensis* but are likely present, although difficult to observe on such an intricate surface.

Overall, this study has demonstrated marked surface variability in two species of *Pterospora*. Each species moves by peristaltic waves of cytoplasm and resides in the coelom of their host (Landers 2001; Landers and Gunderson 1986). Each species also lacks the longitudinal epicytic folds and gliding motility found in many sepatate and aseptate eugregarines that reside in the intestinal lumena of invertebrate hosts (e.g. Leander, Harper, and Keeling 2003; Mackenzie and Walker 1983; Sanders and Poinar 1973; Schrével et al. 1983; Vávra and Small 1969; Vivier 1968; Walker et al. 1979). The correlation between the irregular surface features of *Pterospora* and their habitat and type of movement may be significant, and may reflect an adaptation for development in coelomic environments.

In general, aseptate gregarines exhibit a wide range of surface features, including longitudinal epicytic folds (Desportes 1974; Vivier 1968), alternating primary and secondary ridges (Warner 1968), cytoplasmic hairs (cytopilia), cytoplasmic papillae (Sathananthan 1977, Warner 1968), undulating membranes, broad folds, deep grooves, transverse striations (Leander, Harper, and Keeling 2003; Schrével 1971a, b), regularly arranged epicytic knobs (Ciancio, Scippa, and Cammarano 2001), and the patterns of ridges and swellings described here. Coelomic gregarines are known to move by strong waves of contraction and cytoplasmic streaming, as has been described in *Pterospora* (see for example: Landers 1991, 2001; Landers and Gunderson 1986) and *Urospora* (Watters 1962), but little was known of the surface features of these gregarines. Even although the tracts of ridges found on the surface of *P. floridiensis* and the swellings of *P. schizosoma* are clearly different in detail, these surface structures have only been described in gregarines that inhabit the coelomic compartments of their hosts. As further studies of coelomic gregarines are reported, a pattern of surface features and their relationship with habitat and movement may emerge. Our data suggest that the novel surface features of *Pterospora* correlate with growth and development in a coelomic cavity. This hypothesis may be tested by future ultrastructural studies of other genera that also reside in coelomic habitats.

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