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Secondary zoospores in the algal endoparasite *Maullinia ectocarpii* (Plasmodiophoromycota)

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Key words: intracellular parasites, flagellar apparatus, Plasmodiophoromycota, brown algae, Ectocarpales

ABSTRACT: The present paper deals with the ultrastructure of zoospores produced by the plasmodiophorid *Maullinia ectocarpii*, living in the marine algal host *Ectocarpus siliculosus.* The zoospores described here are very similar to secondary zoospores of *Polymyxa graminis* and *Phagomyxa* sp. (the latter an algal endoparasite, also). Our results indicate that *M. ectocarpii* produces two types of plasmodia, and suggest that is a species with a complete life cycle, as it is known for all the Plasmodiophormycota that have been studied. Sporogenic and sporangial plasmodia produce, respectively, primary zoospores with parallel flagella within thick walled resting sporangia, and secondary zoospores with opposite flagella within thin walled sporangia.

Introduction

Maullinia ectocarpii I. Maier, E. R. Parodi, R. Westermeier et D. G. Müller, is a parasite of *Ectocarpus siliculosus* and other phaeophycean algae, and it is characterized by features specific for the plasmodiophorids and has been described as a new genus and species by Maier *et al*. (2000).

Plamodiophorids are obligate parasites of angiosperms (Kanyuka *et al*., 2003) and seaweeds (Schnepf, 1994). Considerations on the entire 18S rDNA of two genera of the group, *Spongospora* and *Plasmodiophora,* showed that they are not closely related to a range of protists and true fungi (Down *et al*., 2002). Many of them are involved in important diseases of brassicas, potato, cereal species and grass (Barr, 1979), and some of them are considered viral vectors, as for the rhizomania disease virus in sugar beet, and barley mild mosaic virus in cereals (Teakle, 1980; 1983).

Plasmodiophorid life cycles normally involve the production of two types of intracellular plasmodia: namely, sporogenic and sporangial. Resting spores and zoosporangia respectively produce two types of biflagellate zoospores, the primary and the secondary ones (Dylewski, 1990; Kanyuka *et al*., 2003).

In the original publication of *M. ectocarpii* (Maier *et al*., 2000) only one type of zoospores was described from thalli growing on both field and culture material of *Ectocarpus siliculosus* and other brown algae. In the present paper we describe a second type of zoospores

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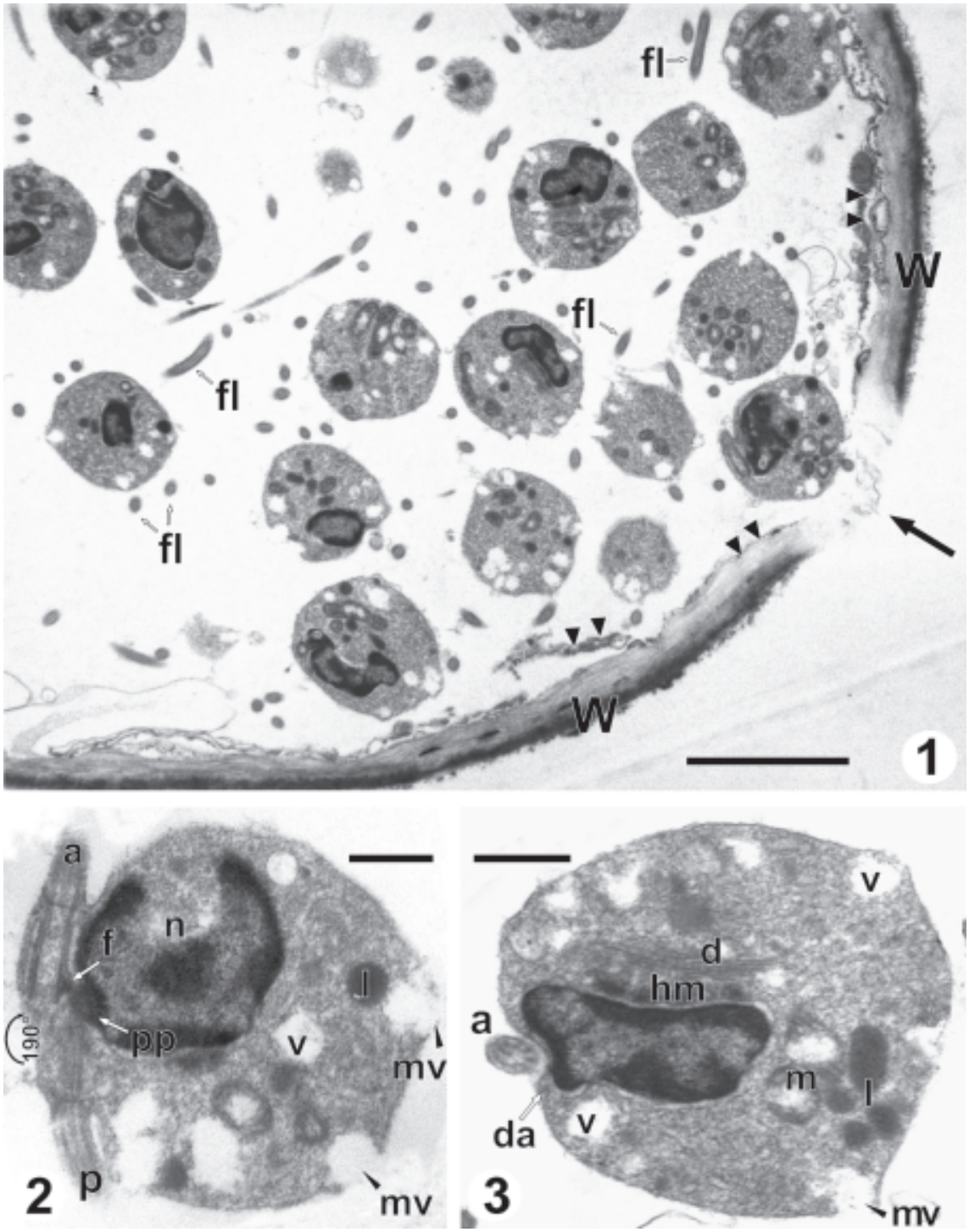
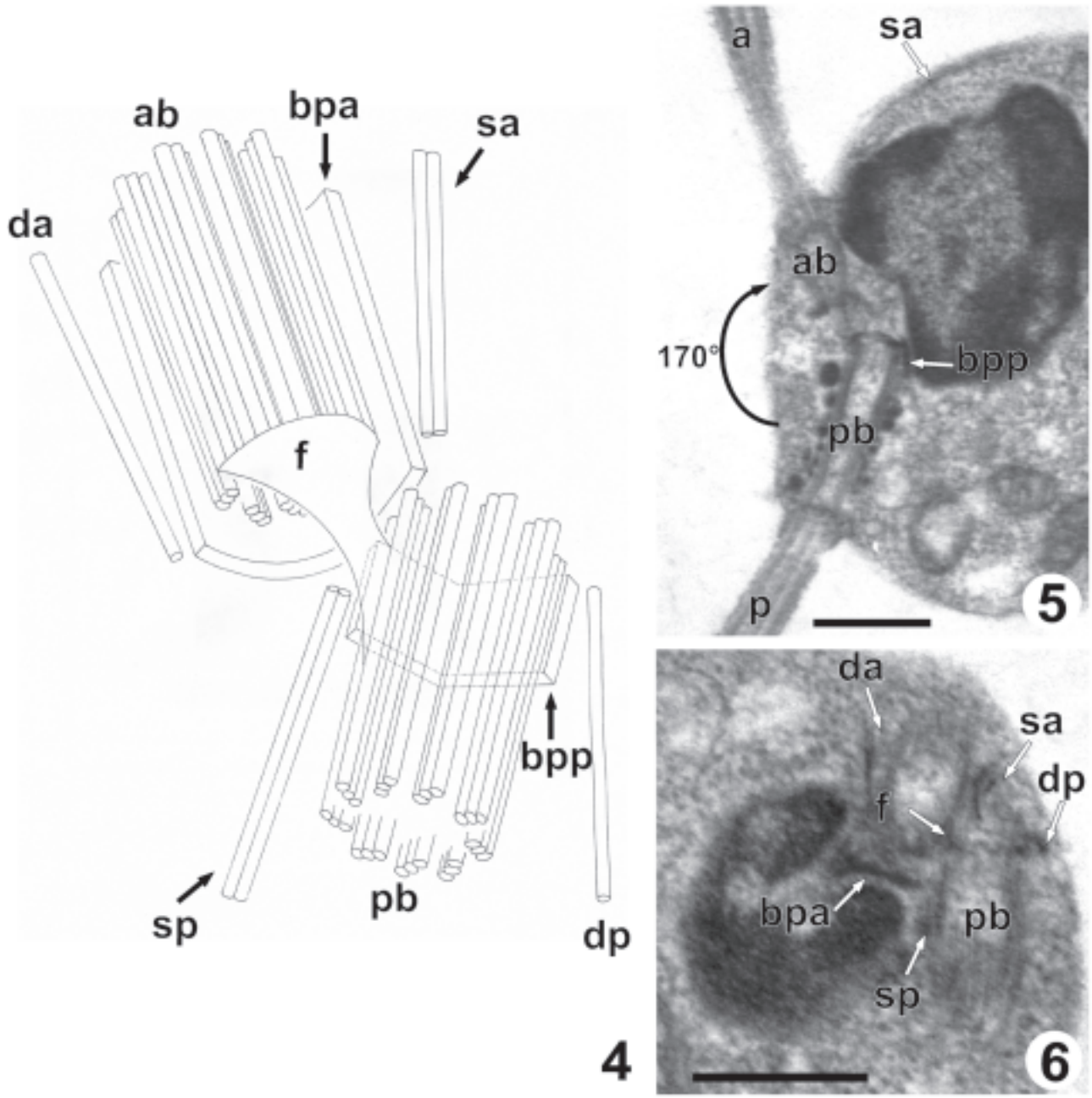


FIGURES 1-3. Fig. 1. Mature secondary zoosporangium. The thin zoosporangium wall (arrowheads) remains very close to the cell wall of the host (W). The zoosporangium wall and the host cell wall have disrupted in the same region allowing zoospores release (arrow). Numerous profiles flagella (fl) are observed. Figs. 2-3. Longitudinal frontal (Fig. 2) and longitudinal lateral (Fig. 3) sections of zoospores showing a nucleus with abundant heterocromatin (n), profiles of circular mitochondria (m), lipid droplets (l) and globular vesicles (v). Adjacent to the nucleus and located in a large lateral depression, a single dictyosome appears (d) separated from the nuclear membrane by a homogeneus material with moderate electron density (hm). In Fig. 2, both flagella (*a* and *p*) were longitudinally sectioned. Flagella (*a* and *p*) emerged laterally in opposite directions one forwards (*a*) and the other backwards (*p*). In Fig. 3 the flagellum (*a*) was transversally sectioned and is seen from above in the section, running along an anterior invagination of the zoospore. Parts of the basal apparatus, i.e. the fibre (*f*), one of the roots (*da*) and the posterior plate (*pp*) have been sectioned. Scales bars = 5 μm (Fig. 1), 1 μm (Figs. 2 and 3).



FIGURES 4-6. *Maullinia ectocarpii* in *Ectocarpus siliculosus.* Fig. 4. Diagramatic depiction of the zoospores basal apparatus. (The microtubular roots are depicted sensu Barr and Allan, 1982). Two basal bodies, one anterior (*ab*) and another posterior (*pb*), form an external angle of 170-190º and they were slightly overlapped. Basal bodies were connected by a non-striated fiber (*f*). Underneath the anterior basal body (*ab*) a large dense basal plate was observed (*bpa*), straight in longitudinal view of the basal body and curved in cross sections of the basal body, whereas underneath the posterior basal body (*pb*) a smaller basal plate (*bpp*) appeared, straight in longitudinal view of the basal body and V-shaped in cross section sections of the basal body. Microtubular roots (*da*, *sa*) diverged from basal bodies ba and microtubular roots (*dp*, *sp*) diverged from basal bodies bp respectively, all four with similar angles of ~30º. The number of microtubules per root was inferred by the width of longitudinal sections of them. Fig. 5. Longitudinal section of the zoospore in which flagella and basal bodies are longitudinally sectioned and are viewed from the side. Flagella (*a* and *p*) emerged laterally in opposite directions one forwards (*a*) and the other backwards (*p*). Basal bodies form an external angle of 170º. The smaller basal plate (*bpp*) appeared, in longitudinal view. Fig. 6. Longitudinal section of the zoospore whose plane is perpendicular to the plane of Fig. 5 thus basal bodies are also longitudinally sectioned but frontally viewed. Note basal bodies are counterclockwise rotated and slightly overlapped. Microtubular roots (*da*, *sa*) and (*dp*, *sp*) diverged from basal bodies *ba* and *bp* respectively, with similar angles of ~30º. The large dense basal plate was observed (*bpa*). Scales bars = 1 μm.

produced by *M.ectocarpii,* hosted by *E.siliculosus.*

The present paper deals with the ultrastructure of these zoospores, with emphasis in the configuration of the flagellar apparatus. These apparatuses are a stable attribute of mature fungal flagellate cells, and may serve to characterize cell types as wells as to taxonomically identify the organisms (Lange and Olson, 1976; Hardham, 1987; Dick, 1997). Its potential has been clearly perceptible also in high level fungal taxonomy (Dylewski, 1990; Cavalier-Smith, 2002; Prillinger *et al*., 2002), although it may be relevant at the generic level too (Barr and Allan, 1982).

Although the genus *Maullinia* clearly seems to have affinities with all plasmodiophorid genera, its precise taxonomical affinities within this group are not clear in the present state of knowledge. Motile cells features have prove to be the most important criterion to infer phylogenetic relationships among the plasmodiophorids (Talley *et al*., 1978; Clay and Walsh, 1997), even though the origin of the simple cruciate root pattern in plasmodiophorids is not obvious owing to the absence of detailed root structures (Cavalier-Smith, 2002).

Materials and Methods

Infected filaments of *Ectocarpus siliculosus* were collected in the coast of Maullín city (X Región, Chile) and were fixed for 2 h in 1% glutaraldehyde in 0.05 M Na-cacodylate buffer at 5ºC, postfixed for 2 h in 1% OsO_4, dehydrated through a graded acetone series, embedded with Spurr's low viscosity resin (Spurr, 1969) and included using flat embedding (Reymond and Pickett-Heaps, 1983). Forty nine zoospores were serially sectioned and analyzed. Thin sections were obtained with a diamond knife (Diatome Ltd., Bienne, Switzerland) in a Reichert-Jung Ultracut ultramicrotome (C. Reichert Optische Werke, Wien, Austria), mounted on Formvar coated grids and stained with uranyl acetate and lead citrate. Sections were observed with a Jeol 100 CX-II electron microscope (Jeol Ltd., Akishima, Tokio, Japan) at the Centro Científico Tecnológico Bahía Blanca (CONICET-CCT- BB).

Results

Zoospore ultrastructure

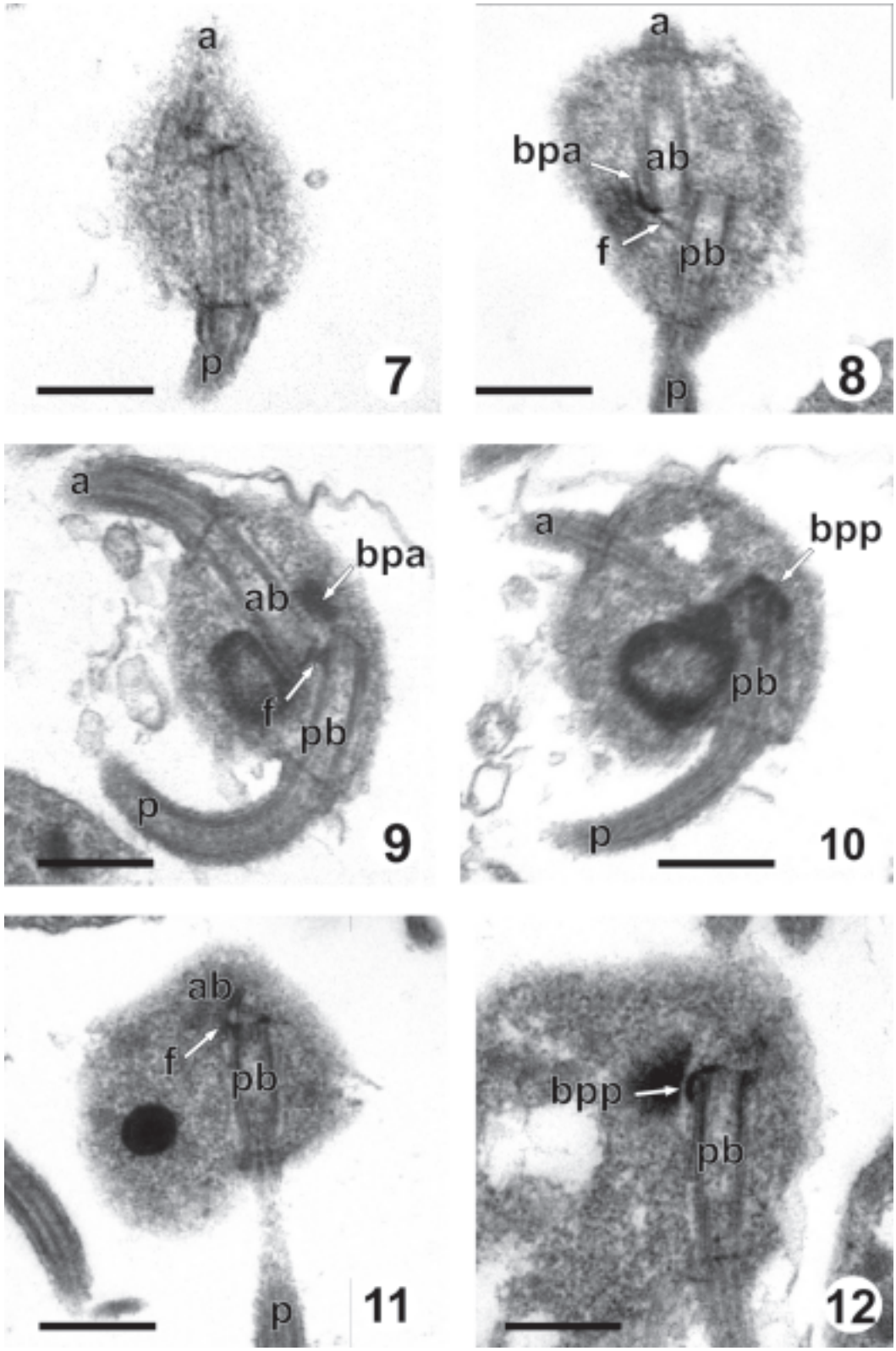
Biflagellate zoospores are formed in thin-walled zoosporangia (Fig. 1). The moderately electron dense wall of the zoosporangium (Fig. 1, arrowheads) remained close to the host cell wall. When mature, both the zoosporangium wall and the host's cell wall disrupted in the same region and simultaneously, allowing zoospore release (Fig. 1, arrow). Mature zoospores showed a nucleus with abundant heterochromatin (Fig. 2, *n*). Circular mitochondrial profiles (*m*), lipid droplets (*l*) and both central (*v*) and marginal (*mv*) globular vesicles were observed (Figs. 2 and 3). Marginal vesicles opened to the exterior of the zoospore body (arrowheads). Adjacent to the nucleus and located in a concavity of it, a single dictyosome (Fig. 3, *d*) appeared separated from the nuclear membrane by a homogeneous material of moderate electron density (*hm*).

Flagellar apparatus

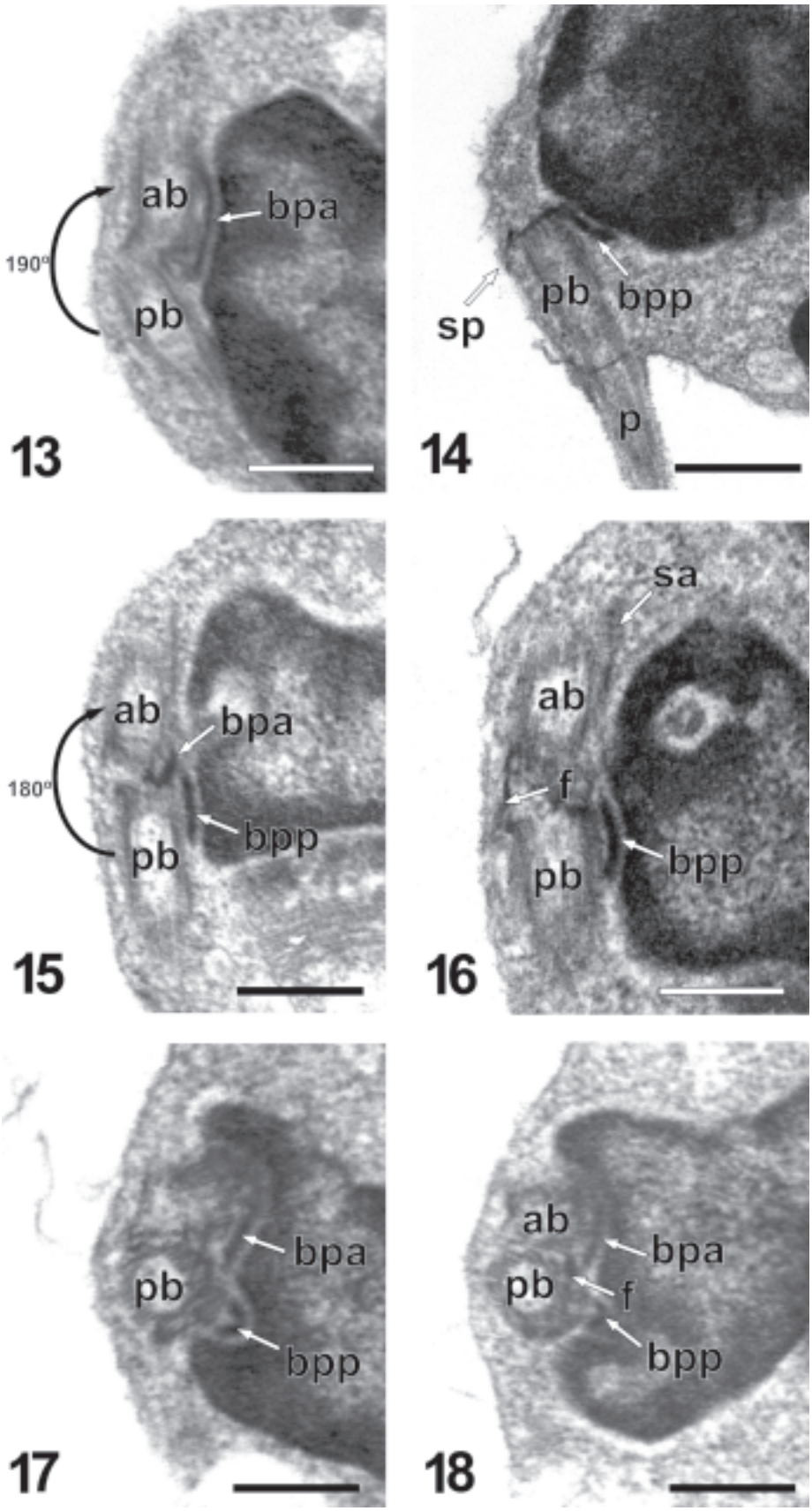
Two flagella emerged laterally though in opposite directions (Figs. 2 and 5) one forward (*a*) and the other backward (*p*). Their basal portions run along invaginations of the zoospore (Fig. 3). The configuration of the basal apparatus is diagrammatically depicted in Figure 4. It presented two basal bodies, one anterior (*ab*) and another posterior (*pb*), both located in a lateral concavity of the nucleus (Figs. 5 and 6). Both basal bodies formed an exterior angle of 170-190º to one another in lateral views, whereas in frontal views they were slightly overlapped, in a counterclockwise direction (Figs. 7-11). Basal bodies were connected by a non-striated fiber (Figs. 6 and 9, *f*). Underneath the anterior basal body (*ab*) a large dense basal plate (*bpa*) appeared as straight or curved, in either longitudinal or cross sections of the basal body, respectively (Figs. 6, 13, 15, 16, 17, 18) whereas underneath the posterior basal body (*pb*) a smaller basal plate (*bpp*) appeared as straight or V-shaped, in either longitudinal or cross sections of the basal body, respectively (Figs. 5, 10, 12, 14, 15, 17 and 18). Microtubular roots (*da*, *sa*) diverged from both basal bodies, with similar angles of ~30º, and they run close to the plasma membrane (Figs. 3, 5 and 6). The number of microtubules of each root could not be determined on the basis of cross sections but could be inferred (as depicted in Fig. 4) from the width of the longitudinal profiles of the roots.

Discussion

We have confirmed the existence of zoospores similar to those reported in the original description of *Maullinia ectocarpii*, (Maier *et al*., 2000) which bore



FIGURES 7-12. *Maullinia ectocarpii* in *Ectocarpus siliculosus.* Longitudinal sections of zoospores in which basal bodies are longitudinally sectioned and frontally viewed. Figs. 7-8 and 9-10. Two pairs of contiguous sections of a series. Fig. 7. Flagella (a and p) emerged in opposite directions one forwards (a) and the other backwards (p). Basal bodies (ab and pb) are counterclockwise rotated and slightly overlapped. The large dense basal plate (*bpa*), the smaller basal plate (*bpp*) and the fiber (*f*) are observed.



FIGURES 13-18. *Maullinia ectocarpii* in *Ectocarpus siliculosus.* Figs. 13-16 and Figs. 15-16. Two pairs of contiguous longitudinal sections of zoospores in which basal bodies are obliquely sectioned and are viewed from the side. Basal bodies form an exterior angle of 180º-190º. The large dense basal plate (bpa), the smaller basal plate (*bpp*) and the fiber (*f*) are observed. Figs. 17-18. Transversal sections of zoospores in which basal bodies are transversally sectioned. The large dense basal plate (*bpa*), the smaller basal plate (*bpp*) and the fiber (*f*) are observed. Scales bars = 1 μm.

flagella emerging in the same direction and almost parallel to each other (their basal bodies showed an external angle of 15-45º), and which were produced within thick-walled sporangia. We have described here the existence of another zoospore type, which showed flagella emerging in opposite directions, and which was produced within thin-walled sporangia.

Maier *et al*. (2000) interpreted that the single type observed by them were secondary zoospores, thus assuming that the *M. ectocarpii* life cycle involved only the sporangial plasmodia. Nevertheless, the authors did not disregard the possibility that only part of the parasite´s life cycle was observed in that study, and that the sporogenic phase might occur indeed in different culture conditions or in different hosts. In the zoospores described here, which were produced in thin walled zoosporangia, the basal bodies consistently displayed an external angle of 170-190º, thus the flagella were emerging in opposite directions.

In most plasmodiophorid species in which secondary zoospores have been studied with transmission electron microscopy, the configuration of their basal apparatuses (in particular, the angle that basal bodies form to one another), is similar (see Miller *et al*., 1985; Braselton, 1995; Dylewski, 1990) to the angle found between basal bodies of the previously described zoospores of *M. ectocarpii* (Maier *et al*., 2000). For that reason it was sensible that the authors interpreted them as secondary zoospores. However, it is relevant for the present investigation, (1) that the zoospores described here are almost identical to the plasmodiophorid zoospores found by Schnepf (1994) in an algal endoparasite of the centric marine diatom *Bellerochea mallus* (Bacillariophyceae, Heterocontophyta), which were considered as related to those of the genus *Phagomyxa*, and (2) that the zoospores ultrastructure and the configuration of their flagellar apparatus of the zoospores described here are similar to those of the bona fide secondary zoospores of *Polymyxa graminis* (Barr and Allan, 1982). These similarities suggest a close affinity of these two genera with *Maullinia* and also introduce a key to interpret the real nature of both types of zoospores. If we consider the wall of the sporangia described in the present paper it is evident that it was extremely thin and clearly different to the thick wall of the zoosporangia described in the original paper, which recalled the cysts' walls that develops into primary zoospores in some other plasmodiophorids. Consequently, there are reasons to plausibly conclude that the previously described zoospores of *Maullinia ectocarpii* are in fact primary zoospores produced in a thick walled resting sporangium, homologous to the clusters of single celled resting spores or sporocysts of other plasmodiophorids (Alexopoulos *et al*., 1996).

The production of two types of zoospores by *M. ectocarpii* suggest that two types of plasmodia are produced, which are known to infect different host tissues in other genera (Dylewski, 1990; Alexopoulos *et al*., 1996; Kanyuka *et al*., 2003). However, owing to the cellular homogeneity of *E. siliculosus*, both types of plasmodia develop within any cell of the algal thallus.

Further experimental studies are needed to accurately understand the life cycle of *M. ectocarpii* and the precise nature of the two types of zoospores. Based on our ultrastructural analysis we assume that the life cycle of *M. ectocarpii* involves two plasmodia, (1) a sporogenic plasmodium, which develops thick walled, resting sporangia, and which produces primary zoospores (with flagella emerging in the same direction and basal bodies forming an angle of 15º-45º), and (2) a sporangial plasmodium, which develops thin walled sporangia, that produces secondary zoospores with opposite flagella and basal bodies forming an angle of 170º-190º. Thus the life cycle of this species would most probably be diplo-haplont, as it is known for all the studied plasmodiophorid species. However, sexual reproduction of *M. ectocarpii* is currently not known, but we may predict that secondary zoospores might be able to act as isogametes.

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