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GENERAL BIOLOGY

The Ultrastructure of the Zoospores of the Parasitic Dinoflagellate *Ichthyodinium chabelardi* Hollande et J. Cachon, 1952 (Alveolata: Dinoflagellata)

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Abstract—This is the first study on the ultrastructure of the zoospores of *Ichthyodinium chabelardi*, a parasitoid of the fish egg and early larval stages. The zoospores were characterized by the cell structure specific for dinoflagellates; particularly, cells contained large trichocysts and the "dinokaryon"-type nucleus. An unusual large electron-transparent zone was the only significant difference from the "classical" cell structure in Dinoflagellata. We did not find cell structures for the penetration to the host cell (microtubular basket, conoid, or secretory organelles such as rhoptries). The data on the fine structure of the zoospores of *I. chabelardi* agree with the results of molecular phylogeny; this allows us argue that excluding this species from Dinoflagellata and assigning it to Protalveolata was a mistake.

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Ichthyodinium chabelardi, an endoparasite of early larval stages of fish, was discovered in the Mediterranean Sea near the coast of Algeria [4]. Recently, it has been found widely in the Atlantic and Pacific oceans; it infects many fish species both in nature and in aquaculture, causing mass death at early developmental stages [1, 8, 9, 11, 12].

One or several individuals of *I. chabelardi* penetrate into the fish egg and pass there several endogenous stages of the life cycle, including the series of cell division divisions [3, 5, 8, 13]. As a result, the cell mass of the parasite replaces the contents of the yolk sac; its wall breaks, and the endogenous phase ends. This event takes place at the late stages of fish embryogenesis or, more usually, at early larval stages. The released cells of the parasite undergo a series of three monotomic cell divisions accompanied by the decrease in their size and change the cell shape and mode of the locomotion, which is ensured by a pair of flagella at these the stages [1]. After the third cell division, small and very motile zoospores appear; they are likely the invasive stages [1, 10].

Earlier, the ultrastructure of some stages of exogenous and endogenous phases of the *I. chabelardi* life cycle has been studied [3]; however, this publication suffered from serious contradictions and discrepancies with the data by other authors [1, 10].

The current study aimed at describing the fine structure of the zoospores of *I. chabelardi* using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

The zoospores obtained under laboratory conditions [1] were fixed in a 2.5% glutaraldehyde solution in the seawater (two series during 1 h each in the ice bath, in the darkness). Then, the material was washed three times for 20 min in filtered seawater and postfixed in a phosphate-buffered 2% solution of OsO_4 in (2 h in the ice bath, in the darkness). Then, the material was washed in distilled water for 20 min and dehydrated in a series of ethanol solutions of increasing concentrations (starting from 15% and ending at 96%, each stage took 20 min). For TEM, the material was dehydrated finally in the isopropanol and embedded into the Spurr medium (Ted Pella, United States). Prior to the fixation and each exchange of the fixing/dehydrating agent, the zoospores were concentrated in a centrifuge (350*g*, 10 min). In the embedding mixtures containing the resin, the centrifuging regime was set as 4000*g* for 20 min. Ultrathin sections were studied under JEM-100B and JEM-100C microscopes (JEOL, Japan).

For SEM study, the zoospores were placed into a polypropylene tube (containing 96% ethanol) and then processed in an Elmasonic D78224 ultrasonic bath (Elma, Germany) at 32 kHz for 30 min. The mixture was centrifuged (350*g*, 10 min), dehydrated in

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pure acetone, and critical point dried with CO_2. The samples were mounted on stubs and sputter-coated with gold–platinum. Then, they were studied using a JSM-6380LA scanning electron microscope (JEOL). The ultrastructure of the zoospores of *I. chabelardi* was described using analysis of the total series of sections of two zoospores and separate sections of several other specimens.

A zoospore cell (Fig. 1a) is of elongated shape and is 8–9 × 4–5 μm in size. The apical end of the cell is wide and rounded; it narrows gradually towards the caudal end. The cell carries a wide and shallow spiral groove with slightly sloping walls; the groove makes one turn counterclockwise when viewed from the apical end. This groove makes the cell asymmetric and of the spiral shape. The zoospore carries two smooth flagella; at least one of them lays in the groove partly. The cytoplasm of the cell carries well-developed mitochondria (Figs. 1d, 1g) and large inclusions that probably store reserve nutrients. These inclusions may resemble lipid globules (Figs. 1b, 1d–1f) or grains of storage carbohydrates (Fig. 1c). Interestingly, both types of the inclusions never occur at the sections of the same cell. In order to explain this phenomenon, additional cytochemical studies are required. Large (~3 × 0.3 μm) electron-dense structures that were identified as trichocysts are specific feature of the cells (Figs. 1b–1f). These structures are very elongated and look square or rhombic on cross-sections and diagonal sections, respectively. At the apical end of the cell, these organelles are distributed evenly and are oriented differently; their distal ends are located close to the plasmalemma (Figs. 1e, 1f). In the middle and the caudal end of the cell, where, probably, trichocysts are forming, they are oriented along the longitudinal axis of the zoospore; they closely neighbor the cell nucleus and even look embedded into it in some samples (Figs. 1b, 1c). The cell nucleus is located in the middle and in the posterior part of the cell; it is a dinokaryon. The condensation of chromatin varies greatly in the chromosomes of different cells studied, from homogenous compact electron-dense structures to loose structures with separate fibrils (Figs. 1g–1j). At some sections of the zoospores, there are paranuclear bodies (large globular structures with heterogeneous granular content) adjacent to the cell nucleus (Figs. 1c, 1h). A large electron-transparent structure of angulate shape is located in the caudal part of the cell (Fig. 1b). Its location corresponds to the location of the light-refracting body detected regularly in living zoospores [1]. The origin of this structure still remains unclear. Probably, this is a cavity remained after the mineral crystal has been dissolved during the fixation and dehydration, but this suggestion requires further study.

Our study of the ultrastructure of the zoospores of *I. chabelardi* revealed characteristics common for all dinoflagellates, including evolutionary advanced taxa [6]: their cells contained large trichocysts and a dinokaryon.

Despite thorough and detailed study of the series of the sections, we did not find structures resembling penetration organelles. Particularly, there were no components of the apical complex. If the zoospores studied are indeed the infecting stages, then the absence of specific penetration organelles may support the hypothesis of their penetration into the fish egg through the micropyle [9]. The large light-reflecting body in the caudal end of the cell (electron-transparent in TEM) is the only significant difference of the zoospores of *I. chabelardi* from those of other parasitic and free-living dinoflagellates.

The zoospores studied here are externally identical to those described by Skovgaard et al. [10] but differ significantly from the cells described by Gestal et al. [3] in both ultrastructure and external morphology. The latter article [3] have studied the ultrastructure of some stages of both endogenous and exogenous phases of the life cycle of *I. chabelardi*, namely the formation of zoosporangia via a namely environment and producing the zoospores by zoosporangia in the series of palintomic divisions of sporoblasts inside the common envelop has been described; these results contradict other studies on of this species [1, 5, 10]. The zoospores described in [3] were smaller (5 μm), of spherical shape, and they bore two flagella, one of them was not smooth (as in *I. chabelardi*) but was covered by mastigonemes arranged in a single longitudinal row. These results and their interpreting [3] suffer from some discrepancies and contradictions. Particularly, the cell nuclei of the studied sporangia and zoospores represent the standard eukaryotic nuclei whereas the typical dinokaryon is clearly seen on the microphotograph of the ultrathin section of the earlier exogenous stage (prior to the zoosporangia). The chromosomes of the dinokaryon were identified as rhoptries [3]. A wide microtubular band contacting the kinetosome of the flagellum (found at the same stage) was described as conoid, but the absence of these structures in the zoospores studied was not discussed at all. Nevertheless, the conclusion on the presence of rhoptries and conoid in the cells studied was the reason to exclude *I. chabelardi* from Dinoflagellata and to assign it to the paraphyletic group Protalveolata [3], which comprises organisms such as *Chromera, Colpodella*, and *Perkinsus* [2], and, finally, to rename it to *Perkinsoide chabelardi*.

Based on the results of this and our previous [1] studies as well as on the other publications cited above, the only possible explanation of all the discrepancies and contradictions in [3] is that only the earlier exogenous stage studied by Gestal et al. belonged to the

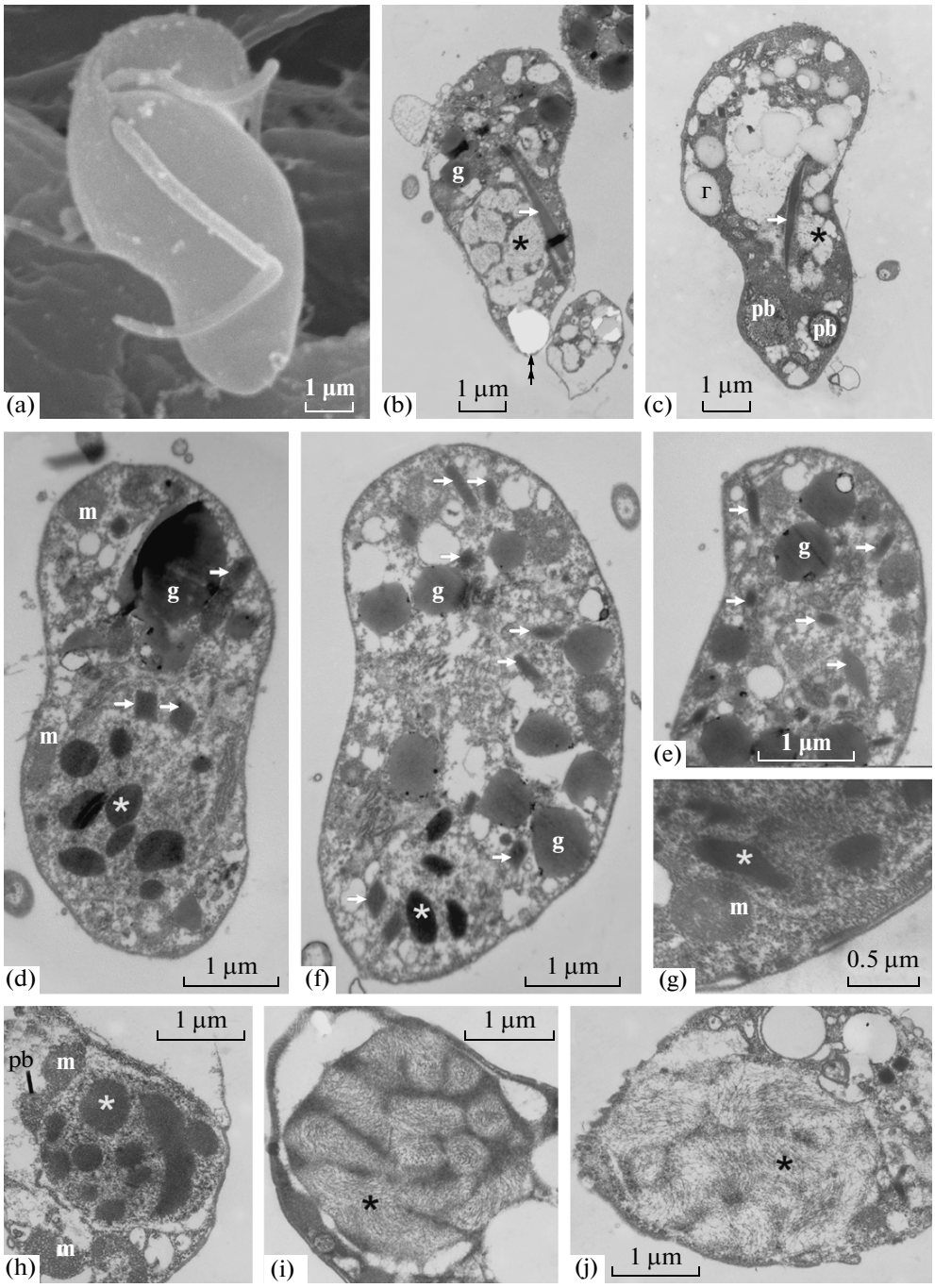

Fig. 1. The ultrastructure of zoospores of *I. chabelardi*. (a) General morphology of the zoospore (SEM); (a–c) longitudinal sections; (d–f) diagonal sections at various levels; (g) the zone of cytoplasm carrying a mitochondrion (m); (h– j) variants of chromosome decondensation within the nucleus. Abbreviations: (g) globules (likely, carrying the storage molecules); (m) mitochondria; (pb) paranuclear bodies; asterisks mark chromatin, arrows mark trichocysts, and double arrow marks à caudal electron-transparent structure.

genuine *I. chabelardi*, whereas all the subsequent stages (zoosporangia and zoospores) clearly belonged to some other parasitic protist probably, to all appearance not even a dinoflagellate. Therefore, the conclusions on the taxonomical position and following changes in the taxonomy of this species are groundless, which also agrees with results of molecular phylogenetic analyses [7, 10].

In addition, our studies on the fine structure of the zoospores of *I. chabelardi* show that this species undoubtedly belongs to Dinoflagellata, just as other

representatives of Syndinea likely do; and and recent molecular phylogenetic data [7, 10] do not conflict this statement.

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