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Invited Review

The search for the missing link: A relic plastid in *Perkinsus*?

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ABSTRACT

Perkinsus marinus (Phylum Perkinsozoa) is a protozoan parasite that has devastated natural and farmed oyster populations in the USA, significantly affecting the shellfish industry and the estuarine environment. The other two genera in the phylum, *Parvilucifera* and *Rastrimonas*, are parasites of microeukaryotes. The Perkinsozoa occupies a key position at the base of the dinoflagellate branch, close to its divergence from the Apicomplexa, a clade that includes parasitic protista, many harbouring a relic plastid. Thus, as a taxon that has also evolved toward parasitism, the Perkinsozoa has attracted the attention of biologists interested in the evolution of this organelle, both in its ultrastructure and the conservation, loss or transfer of its genes. A review of the recent literature reveals mounting evidence in support of the presence of a relic plastid in *P. marinus*, including the presence of multimembrane structures, characteristic metabolic pathways and proteins with a bipartite N-terminal extension. Further, these findings raise intriguing questions regarding the potential functions and unique adaptation of the putative plastid and/or plastid genes in the Perkinsozoa. In this review we analyse the above-mentioned evidence and evaluate the potential future directions and expected benefits of addressing such questions. Given the rapidly expanding molecular/genetic resources and methodological toolbox for *Perkinsus* spp., these organisms should complement the currently established models for investigating plastid evolution within the Chromalveolata.

1. Introduction

1.1. Life cycle

Perkinsus spp. have a direct life cycle (Fig. 1): trophozoites proliferate intra- or extracellularly by palintomy (merogony or schizogony) giving rise to 4–32 (often 8–16) trophozoites, which are released upon rupture of the schizont cell wall (Perkins, 1996; Sunila et al., 2001). At the four-cell schizont stage, a functional diversification of the daughter cells has been proposed (Sunila et al., 2001). Whilst less frequent, binary fission has also been observed: trophozoite budding yields a mother cell surrounded by a thick wall and the daughter cell separated by a plasma membrane (Sunila et al., 2001). Sexual stages have been suggested (Perkins, 1996) and recent microsatellite analyses suggest that *Perkinsus marinus* utilises both sexual and asexual reproduction, and that over the short term selection acts upon independent parasite lineages rather than upon individual loci in a cohesive, interbreeding population (Thompson et al., 2011). Although the overall propagation process has been described in substantial detail, various aspects of cell division and maturation at the subcellular level remain poorly understood, especially regarding the segregation and function of some organelles and macrostructures (e.g. the large vacuole containing the vacuoplast and its precursors during trophozoite proliferation and maturation) and the zoosporulation process.

1.2. Natural history and phylogenetic position

In the early 1950s, the causative agent of widespread mass mortalities of eastern oysters (*Crassostrea virginica*) on the shores of Texas (USA) was identified and named *Dermocystidium marinum*. This microorganism was later renamed *Perkinsus marinus* and closely related *Perkinsus* spp. that affect various mollusc species worldwide were described during later years (Villalba et al., 2004). The gradual expansion of the geographic distribution of

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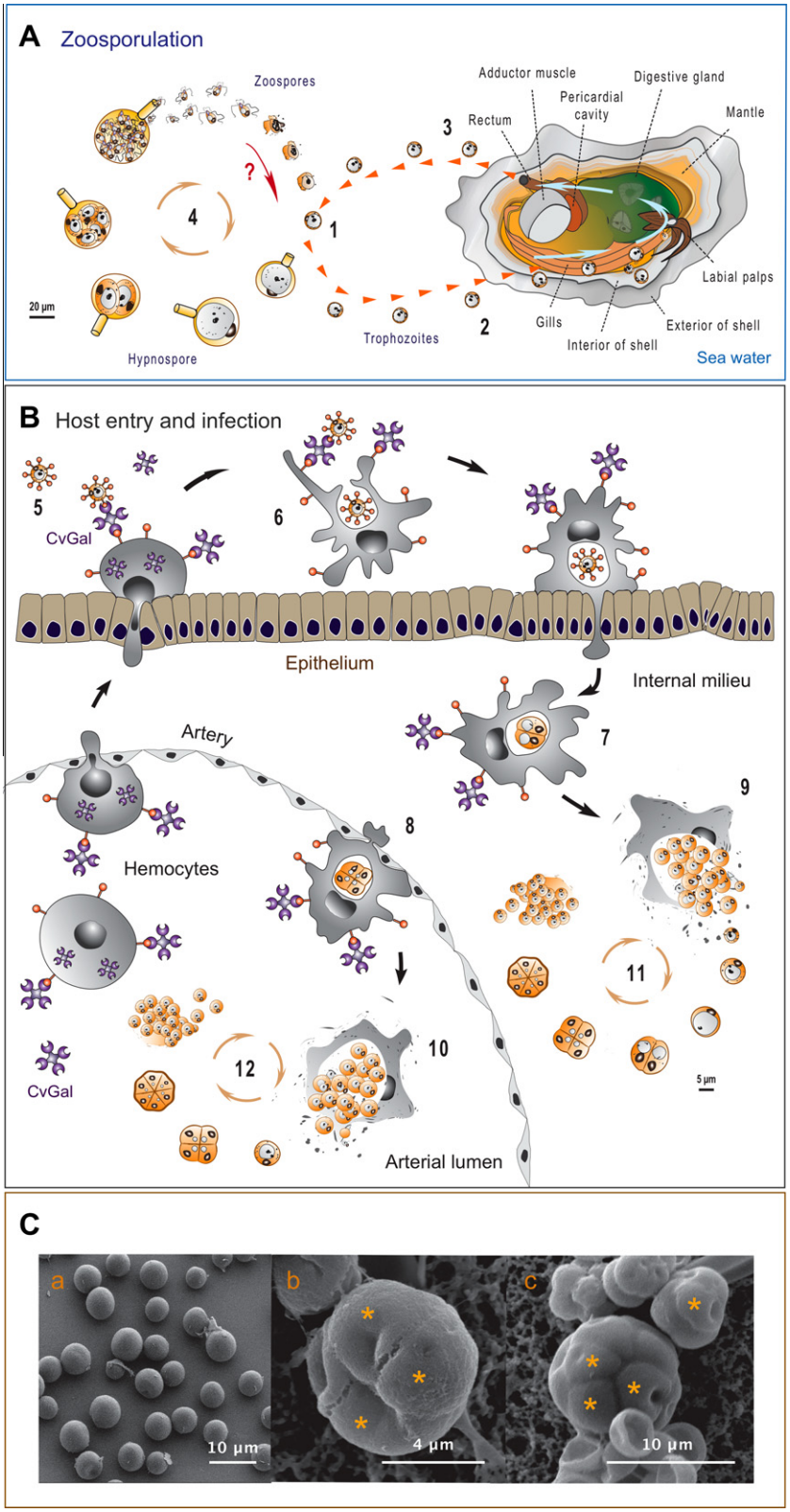

Fig. 1. *Perkinsus* life cycle. (A) Trophozoites in the water column (1) are taken by the oyster during filter-feeding (2), enter the paleal cavity and are directed through gills and palps towards the mouth. Trophozoites may be released into the water (3) from live oysters together with the pseudofeces and upon death of the oyster, from the decaying infected tissues (Bushek et al., 2002). Once released into the water column, trophozoites may sporulate (4): trophozoites enlarge, develop a discharge tube and after multiple rounds of division, release hundreds of zoospores into the water column. Whether zoospores develop into trophozoites remains an open question. (B) Once in the paleal cavity or the digestive tract (5), trophozoites displaying surface ligands for the oyster galectin CvGal (Tasumi and Vasta, 2007) are recognised and phagocytosed by the hemocytes (6) that can transmigrate to the internal milieu (7) and eventually into the vascular system (8). Parasites remain inside phagosome-like vesicles where they remain viable and multiply. When hemocytes disintegrate (9, 10), the released trophozoites can either be phagocytosed by neighbouring hemocytes or multiply extracellularly in both the internal milieu (11) and arterial lumen (12). The infected circulating hemocytes migrate throughout the host tissues where they may lyse and release trophozoites, leading to systemic infection and eventually host death. (C) In vitro culture of *Perkinsus marinus* trophozoites. Under scanning electron microscopy the cultured *P. marinus* trophozoite surface appears smooth (a, b). In trophozoites undergoing schizogony, the shapes of the daughter cells become apparent on the exterior surface (stars, b, c).