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Life-cycle, ultrastructure, and phylogeny of *Parvilucifera corolla* sp. nov. (Alveolata, Perkinsozoa), a parasitoid of dinoflagellates

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Abstract

Recent studies of marine protists have revealed parasites to be key components of marine communities. Here we describe a new species of the parasitoid genus *Parvilucifera* that was observed infecting the dinoflagellate *Durinskia baltica* in salt marshes of the Catalan coast (NW Mediterranean). In parallel, the same species was detected after the incubation of seawater from the Canary Islands (Lanzarote, NE Atlantic). The successful isolation of strains from both localities allowed description of the life cycle, ultrastructure, and phylogeny of the species. Its infection mechanism consists of a free-living zoospore that penetrates a dinoflagellate cell. The resulting trophont gradually degrades the dinoflagellate cytoplasm while growing in size. Once the host is consumed, schizogony of the parasitoid yields a sporocyte. After cytokinesis is complete, the newly formed zoospores are released into the environment and are ready to infect new host cells. A distinguishing feature of the species is the radial arrangement of its zoospores around the central area of the sporocyte during their formation. The species shows a close morphological similarity with other species of the genus, including *P. infectans*, *P. sinerae*, and *P. rostrata*.

Keywords: Apical complex; HAB; Parasite; *Parvilucifera*; Phylogeny; Ultrastructure

Introduction

Marine parasitic protists are a key component of planktonic and benthic marine communities (Chambouvet et al. 2014; de Vargas et al. 2015) and there is considerable interest in their diversity, behavior, and ecology. Parasites may act as community regulators by causing changes in host population densities (Alacid et al. 2016), thereby indirectly affecting non-host species, which in turn alters community composition (Hatcher et al. 2012). The strong potential effects of parasitoids on the diversity of the marine community (Hudson et al. 2006) highlight the importance of parasitism on the structure of food webs (Lafferty et al. 2006).

In aquatic ecosystems, living organisms are almost constantly confronted with parasites (Jephcott et al. 2016). Many marine parasites belong to the eukaryotic protist lineage of Alveolates, which include ciliates, apicomplexans, dinoflagellates, marine alveolates (MALV), and the Perkinsozoa. All groups contain some parasitic representatives, and some are exclusively parasitic (Apicomplexa, Perkinsozoa,

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MALV). The presence of Perkinsozoa has been demonstrated in freshwater (Bråte et al. 2010), marine waters (de Vargas et al. 2015), and sediments (Chambouvet et al. 2014). These parasites infect shellfish (Andrews 1988), tadpoles (Chambouvet et al. 2015), and dinoflagellates (Park et al. 2004). Thus far, the diversity of Perkinsozoa is mainly represented by environmental sequences, with only two genera of Perkinsozoa described to date: *Perkinsus* (Mackin, Owen, Collier) Levine and *Parvilucifera* Norén et Moestrup. *Rastrimonas subtilis* (=*Cryptophagus subtilis*) Brugerolle is also included in Perkinsozoa (Brugerolle 2002, 2003), but molecular sequences are not available and its taxonomic affiliation remains to be confirmed.

The genus *Parvilucifera* was erected by Norén et al. (1999) based on the description of *P. infectans*, detected in waters of the North Sea. *Parvilucifera* infections were also reported in the Mediterranean Sea (Delgado 1999), on the French Atlantic coast (Erard-Le Denn et al. 2000), on the Baltic Sea (Johansson et al. 2006), and in waters as far ranging as the Indian Ocean, the North American Atlantic, and Tasmania (Norén et al. 2000). Later, the species *P. sinerae* (Figueroa et al. 2008), *P. prorocentri* (Leander and Hoppenrath 2008), and *P. rostrata* (Lepelletier et al. 2014) were additionally described. All of these organisms are parasitic for a wide range of dinoflagellate hosts (Garcés et al., 2013a; Lepelletier et al. 2014; Norén et al., 1999), with the exception of *P. prorocentri*, for which the host range could not be checked and its only known host is the benthic dinoflagellate *Prorocentrum fukuyoi* (Leander and Hoppenrath 2008). However, a partial specialization could exist in *Parvilucifera* parasitoids, as observed for *P. sinerae*, with its preference for certain dinoflagellate species (Alacid et al. 2016).

Parvilucifera species are endoparasitoids. Thus, once a host is infected by the biflagellated zoospore, the parasitoid degrades the cellular contents of the host and a sporangium develops inside the host cell. The mature sporangium gives rise to numerous zoospores that are eventually released into the environment in response to an external signal, e.g. dimethylsulfide, excreted by hosts (Garcés et al. 2013b). Zoospores contact their potential hosts, with subsequent penetration and the development of an infection strongly dependent on the degree of host susceptibility (Alacid et al. 2016).

Given their capability to kill their hosts, the wide range of dinoflagellates that are able to infect, and the high virulence observed in lab experiments, these parasites have generated interest as potential controllers of harmful algal blooms (Erard-Le Denn et al. 2000; Norén et al. 1999; Park et al. 2004). However, their partial specialization (Alacid et al. 2016) and their strain-specific infectivity (Råberg et al. 2014) hinder their implementation until the mechanisms underlying their specificity are better understood.

In this study, we used optical and electron microscopy to characterize the life-cycle stages and the ultrastructural features of a new species of *Parvilucifera* that infects several species of dinoflagellates. Its phylogenetic position is also presented, together with a morphological comparison with other species of *Parvilucifera* described to date.

Material and Methods

Detection, isolation, and culture of the parasitoid

The parasitoid was detected in two locations, in Catalan coastal waters (NW Mediterranean Sea) and along coast of the Canary Islands (North Atlantic). Samplings along the Catalan coast were carried out in May 2015, in the salt marshes of L'Estartit (42°01′44″N, 3°11′30″E). After several days of incubation, cells of the dinoflagellate *Durinskia baltica* present in the natural sample and found to be infected were transferred to well-plates. As cultures of the initial host were not available, different dinoflagellates were added to the wells. Those with *Alexandrium minutum* (strain AMP4) were infected and could be successfully maintained by the weekly transfer of aliquots (0.5 mL) of the infected culture into healthy, exponentially growing *A. minutum* cultures. The parasitoid was also isolated from Canary Island samples collected on September 2015 in tidal ponds of Lanzarote, in a rocky shore area (Charco del Palo, 29°04′50.7″N 13°27′07.2″W). In the original population, dinoflagellates were present at low density and there was no sign of infection. Thus, to reveal the possible presence of the parasite, 40-mL aliquots of the natural samples were inoculated with 1 mL of exponentially growing *A. minutum* strain AMP4; 3 days later the cultures were checked for infection and found to be positive. Parasitoid cultures were subsequently maintained following this same procedure. Based on previous experience with other species (Lepelletier et al. 2014; Norén et al. 1999; Turon et al. 2015), we attempted to maintain the parasitoids in the dormant sporangium stage or to slow down their life cycle by storing the cultures at 4 °C in the dark. However, both efforts were unsuccessful.

Optical microscopy

Infected cells were serially isolated, washed in filtered seawater, and transferred to well-plates with healthy *A. minutum* cells to obtain non-contaminated cultures. Parasitoid cultures were followed daily to identify the different life-cycle stages. Infected cells were observed directly either on the well-plates or in settling chambers under a phase-contrast Leica DM-IRB inverted microscope connected to a ProgRes C10 (JENOPTIK Laser, Optik, Systeme GmbH, Jena, Germany) digital camera. For the Canary Islands cultures, the procedure was similar but the cells were additionally washed in PBS, stained with DAPI (2 $\mu g\,mL^{-1}$), and observed at 1000 × magnification (Leica DMR, Germany) using a microscope camera (Axiocam HRC, Zeiss, Germany). The number of zoospores contained in each sporangium was counted from videos captured during zoospore release.