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New Insights into the Parasitoid *Parvilucifera sinerae* Life Cycle: The Development and Kinetics of Infection of a Bloom-forming Dinoflagellate Host

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Parvilucifera sinerae **is a parasitoid of dinoflagellates, the major phytoplankton group responsible for harmful algal bloom events. Here we provide a detailed description of both the life cycle of *P. sinerae*, based on optical, confocal, and transmission electron microscopy observations, and its infection kinetics and dynamics. *P. sinerae* completes its life cycle in 3–4 days. The zoospore encounters and penetrates the host cell within 24 h after its addition to the host culture. Inside the host, the parasitoid develops a trophocyte, which constitutes the longest stage of its life cycle. The trophocyte replicates and divides by schizogony to form hundreds of new zoospores contained within a sporangium. Under laboratory conditions, *P. sinerae* has a short generation time, a high rate of asexual reproduction, and is highly prevalent (up to 80%) in the *Alexandrium minutum* population. Prevalence was shown to depend on both the parasitoid inoculum size and host density, which increase the encounter probability rate. The parasitoid infection parameters described in this study are the first reported for the genus *Parvilucifera*. They show that *P. sinerae* is well-adapted to its dinoflagellate hosts and may be an important factor in the termination of *A. minutum* blooms in the natural environment.**

Key words: *Parvilucifera*; parasitoid; dinoflagellate; prevalence; alveolate.

Introduction

Marine dinoflagellates are an abundant group of microplankton, some of which produce potent toxins and cause harmful algal blooms (HABs). HAB events have a negative impact on the exploitation of seafood resources, pose a threat to human health, and alter marine trophic structure (Van Dolah 2000; Zingone and Enevoldsen 2000). However, the microalgae that cause HABs serve as hosts for several eukaryotic parasites (Park et al. 2004 and references therein). Recent interest in parasites derives from the impact that they may exert in the control of dinoflagellate populations, especially HAB species. This has led to an increase in both experimental studies (Kim et al. 2004; Maranda 2001; Park et al. 2002a, b; Park et al. 2004) and modelling-based analyses (Llaveria et al. 2010; Montagnes et al. 2008; Salomon and Stolte 2010). Under specific conditions, some parasites cause high mortality of their hosts and thus facilitate

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the decline of their blooms (Coats et al. 1996; Mazzillo et al. 2011). Accordingly, the use of these parasites as control agents in bloom mitigation has been proposed (Anderson 1997; Chambouvet et al. 2008; Erard-Le Denn et al. 2000; Norén et al. 1999). However, the infection prevalence in natural populations of dinoflagellates is typically low to intermediate (Coats and Bockstahler 1994; Chambouvet et al. 2008; Velo-Suárez et al. 2013). In addition to causing mortality, eukaryotic parasites can induce a shift in the physiology and life-history stages of their hosts (Chambouvet et al. 2011; Toth et al. 2004) and they may also stimulate their sexual reproduction and therefore increase the rate of genetic recombination (Figueroa et al. 2010).

The greatest diversity of eukaryotic parasites belongs to Alveolates (including Dinoflagellates, Apicomplexa, and Perkinsozoa among others), which are characterized by the presence of cortical vesicles (alveoli) that subtend the plasma membrane (Cavalier-Smith 1993; Leander and Keeling 2003; Zhang et al. 2011). This group is well represented in aquatic and terrestrial ecosystems (Díez et al. 2001; Guillou et al. 2008; Massana et al. 2004; Richards et al. 2005) and includes pathogenic species that cause economic losses (Mackin 1951) and important human diseases, such as malaria parasites of the genus *Plasmodium* (Kaplan et al. 2000; Nahlen et al. 2005). In the marine ecosystem, the genera *Amoebophrya* (Syndiniales, Dinophyceae) and *Parvilucifera* (Perkinsozoa) are widespread and composed exclusively of parasites that infect dinoflagellates, including HAB species (Garcés et al. 2013a; Guillou et al. 2008; Park et al. 2004). Studies of *Amoebophrya* account for most of the knowledge of parasitism in dinoflagellates whereas little is known about the diversity, ecology, and host effects of *Parvilucifera* species.

The genus *Parvilucifera* belongs to the Perkinsozoa together with the two parasitic genera *Perkinsus* and *Rastrimonas* (originally described as *Cryptophagus*) (Brugerolle 2003; Norén et al. 1999). As an early branch in alveolate evolution, *Parvilucifera* species possess many of the features of dinoflagellates and apicomplexans, making their study interesting from an evolutionary point of view (Leander and Keeling 2003). Thus far, four *Parvilucifera* species have been described, with *P. infectans* and *P. sinerae* as the most closely related species with respect to their morphology, host range, and molecular phylogenetics (Figueroa et al. 2008; Garcés et al. 2013a; Garcés and Hoppenrath 2010; Norén et al. 1999). The morphologies of the sporozoite and the sporangium distinguish *P. rostrata* from *P. infectans* and *P. sinerae*, but the host range of all three species is very similar (Lepelletier et al. 2014). By contrast, the morphological features of *P. prorocentri* are a combination of those of the perkinsids and syndineans (Leander and Hoppenrath 2008). These morphological differences and the phylogenetic distance with the other *Parvilucifera* species suggest the need for its reclassification. (Hoppenrath and Leander 2009; Leander and Hoppenrath 2008; Lepelletier et al. 2014). All four *Parvilucifera* species complete their life cycle within a single host organism that is then consumed and killed. Therefore they are referred to as parasitoids (Lafferty and Kuris 2002). Their life-cycle can be summarized as follows: A small biflagellate zoospore penetrates the host cell and then develops into the trophocyte. This trophont gradually becomes a sporocyte (referred to as the sporangium), which is the replicative stage resulting in many zoospores that are released into the marine environment to infect new hosts (Garcés et al. 2013a; Lepelletier et al. 2014; Norén et al. 1999). Several stages of the *P. sinerae* life cycle have been observed and both the time needed by sporangia to germinate and the infection rates in different host species have been established (Figueroa et al. 2008; Garcés et al. 2013a). The ultrastructure of the sporangium and of the zoospores of *P. sinerae* was described by Garcés and Hoppenrath (2010) and it supported the classification of these organisms in the genus *Parvilucifera*. However, the wide range of morphological and structural changes induced in infected host cells by *Parvilucifera* are poorly characterized with respect to parasitoid development and survival. Specifically, the life-cycle stages of the parasitoid during infection and the kinetics of infection, including parasitoid generation time, prevalence in susceptible hosts, and host mortality rate, have yet to be determined. Here we provide the first detailed characterization of the *P. sinerae* life cycle during its infection of the bloom-forming toxic dinoflagellate *A. minutum*. By using several different microscopy techniques we were able to follow the kinetics of infection by this parasitoid, including determination of the different stages of infection, parasitoid development time, the duration of each stage of infection, parasitoid mortality and prevalence, and the host mortality rate, and to quantify the effect of the inoculum size on parasitoid prevalence. These data will contribute to an understanding of the potential effect of this parasitoid on its host population.