Attachment of the peritrich epibiont *Zoothamnium intermedium* Precht, 1935 (Ciliophora, Peritrichia) to artificial substrates in a natural environment

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(With 1 figure)

Abstract

Peritrich ciliates are commonly found as epibionts, colonizing living organisms, or attached to non-living substrates in freshwater, estuarine and marine environments. Several species of peritrich epibionts are obligate, which means that they are able to only colonize other organisms, while others are facultative attaching to living or non-living substrates. The peritrich *Zoothamnium intermedium* is commonly found as epibiont on the copepod species *Acartia tonsa* and *Eurytemora affinis* in Chesapeake Bay, USA. Previous studies demonstrated that *Z. intermedium* is not able to attach to non-living substrates in the laboratory; with free-swimming stages (telotrochs) dying when living substrates are not available for colonization. The present study investigated the ability of *Z. intermdium* to colonize artificial substrates in the field. Observations were carried out while the peritrich ciliate was colonizing copepods in Rhode River, a tributary of Chesapeake Bay. Results demonstrated that four species of *Zoothamnium* were recovered from artificial substrates, but none of them was *Z. intermedium*. At the same time, *Z. intermedium* was colonizing adults and copepodites of *E. affinis* and *A. tonsa* during the whole study period. These results, in addition to laboratory observations, suggest that *Z. intermedium* is an obligate epibiont.

Keywords: ciliates, comensalism, *Acartia tonsa*, copepods, epibiosis

Colonização de substratos artificiais pelo peritríquio epibionte *Zoothamnium intermedium* Precht, 1935 (Ciliophora, Peritrichia), em um ambiente natural

Resumo

Ciliados peritríquios são normalmente encontrados como epibiontes, colonizando substratos vivos, ou em substratos inanimados em ambientes de água doce, estuarinos e marinhos. Muitas espécies de peritríquios epibiontes podem ser consideradas obrigatórias quando estão aptas a colonizar apenas substratos vivos, ou facultativos quando conseguem colonizar substratos vivos ou inanimados. A espécie de ciliado peritríquio *Zoothamnium intermedium* é encontrada colonizando os copépodos *Acartia tonsa* e *Eurytemora affinis* na Chesapeake Bay, EUA. Estudos preliminares demonstraram que *Z. intermedium* não consegue colonizar substratos inanimados em laboratório e que os estágios livre-natantes (telotróquios) morrem quando não estão expostos a algum substrato vivo. No presente estudo, foi investigada a habilidade de *Z. intermedium* colonizar substratos artificiais no campo. As observações foram realizadas no Rhode River, um afluente da Chesapeake Bay, enquanto *Z. intermedium* era encontrado colonizando copépodos. Os resultados demonstraram que quatro espécies de *Zoothamnium* colonizaram os substratos artificiais, mas nenhuma delas era *Z. intermedium*. No mesmo período, *Z. intermedium* foi encontrado colonizando copepoditos e adultos de *A. tonsa* e *E. affinis*. Estes resultados, juntamente com as observações de laboratório, sugerem que *Z. intermedium* é um epibionte obrigatório.

Palavras-chave: ciliados, comensalismo, *Acartia tonsa*, copépodos, epibiose.

1. Introduction

Epibiotic relationships involving sessile peritrichs and planktonic crustaceans are a widespread phenomenon in aquatic environments (e.g. Fenchel, 1965; Green, 1974; Hanamura, 2000; Song et al., 2002). Despite this wide distribution, epibiosis remains poorly understood especially with respect to the specificity of the relationship.

Studies emphasizing epibiont host specificity have pointed out that some peritrichs are able to attach to only

one or two host species, failing to colonize non-living substrates (Clamp, 1973; Henebry and Ridgeway, 1979; Gilbert and Schröder, 2003). By contrast, some authors demonstrated that other epibionts are opportunistic, being able to attach to living or non-living substrates (Mayén-Estrada and Aladro-Lubel, 2000). However, only a few studies have investigated the ability of a peritrich epibiont to attach to non-living substrates in the laboratory and/or in the field (Henebry and Ridgeway, 1979; Mayén-Estrada and Aladro-Lubel, 2000).

The peritrich ciliate *Zoothamnium intermedium* Precht, 1935 was found as epibiont on the calanoid copepods *Acartia tonsa* Dana, 1848 and *Eurytemora affinis* (Poppe, 1880) in Chesapeake Bay, USA. Previous studies (Utz and Coats, 2005) showed that *Z. intermedium* colonizes only these two species of copepods in the field, even when they are not the most abundant species in the zooplanktonic community. Laboratory studies also demonstrated that telotrochs of *Z. intermedium* failed to colonize non-living substrates, living only about 9 hours after being released from the colony without having a suitable host (Utz, 2003).

Since telotrochs of *Z. intermedium* were not able to colonize non-living substrates in the laboratory, and apparently display host-specificity, the main goal of the present study is to investigate if *Z. intermedium* is a common member of the benthic community in Chesapeake Bay, by testing its attachment to non-living substrates in the field.

2. Materials and Methods

To test if *Z. intermedium* is able to attach to non-living substrates in the field, 24 cover-slips placed in two small rectangular boxes (6 cm long x 3.5 cm wide) made with Plexiglas®, with open sides, were placed in the Rhode River (a tributary of the Chesapeake Bay). The traps were suspended from the dockside at the Smithsonian Environmental Research Center (38° 46' N and 76° 52' W), Edgewater, Maryland, for a period of seven weeks, between April 15th and June 3rd 2003. These dates were chosen because during this period *Z. intermedium* was present in the filed, colonizing *A. tonsa* and *E. affinis*. On the first three sampling dates, traps were left in the field for a period of seven days to allow enough time for colonization, which is dependent of water temperature. For all other sampling dates, traps were left in the field for a period of four days. At recovery time, traps were directly placed in a beaker filled with water from the site and taken to the laboratory.

Two cover slips from each set of traps were preserved with modified Bouin's fixative at a final concentration of 5% (Coats and Heinbokel, 1982), and four cover slips from each set were placed in small Petri dishes filled with water from the collection site for observation of live organisms with an inverted microscope (Invertscope, 25x magnification; Zeiss Corp.). Five fields of view in each cover slip were inspected for the presence of *Zoothamnium* Bory de St. Vincent, 1826 colonies. The total number of colonies and zooids in each field of view was recorded and the abundance of *Zoothamnium* per area was calculated.

The Protargol staining technique (Zagon and Small, 1970) was applied to the preserved material to confirm the identification of the *Zoothamnium* species that were observed alive attached to the cover slips. Filters were omitted from the preparation and cover slips were run throughout the procedure and mounted on glass slides. Protargol slides were observed using light microscopy, and morphological characteristics such as organization of the oral apparatus were observed for each *Zoothamnium* colony and compared to the described morphology of *Z. intermedium* attached to copepods (Utz and Coats, 2005).

To assess the occurrence of *Z. intermedium* on copepods, plankton samples were also collected from each site at the time of cover-slip recovery. Horizontal net tows were taken using a plankton net with 202 µm mesh size and 50 cm in diameter. A 125 mL sub-sample from the net tow was fixed with modified Bouin's fluid (Coats and Heinbokel, 1982) at a final concentration of 5%, and examined using a dissecting microscope (Bausch and Lomb; 30x magnification) to determine the presence of *Z. intermedium* on adults and copepodites of *Acartia tonsa* and *Eurytemora affinis*. Identification of *Z. ntermedium* was confirmed by staining infested copepods randomly selected from the samples (~5 copepods/sample). The Protargol staining technique (Zagon and Small, 1970) was run following the procedure described in Utz and Coats (2005), and diagnostic characteristics of *Z. intermedium* were observed using light microscopy. Infestation prevalence (percentage of copepods carrying epibionts) was calculated for adults and copepodites of both host species.

3. Results

Live observations and Protargol staining revealed that four species in the genus *Zoothamnium* (two of them tentatively identified as *Z. alternans* and *Z. arbuscula*) were present in the benthic community of the Rhode River over a period of seven weeks between April and June 2003, but none of them was identified as the same epibiont species (*Zoothamnium intermedium*) that colonizes populations of *A. tonsa* and *E. affinis* in the Chesapeake Bay and its tributaries. Assessment of the number of colonies and zooids of *Zoothamnium* per substrate area showed that this genus was the dominant peritrich from April 15th to April 21st, but was outnumbered by *Vorticella* spp. Linnaeus, 1767 on May 12th, showing an increase in subsequent samplings as shown in Figure 1a. During the same period, *Z. intermedium* was found attached to adult and juvenile stages of *A. tonsa* and *E. affinis* at an infestation rate that varied between 4.5 and 39% for adults and juveniles of *A. tonsa* and 6 and 25% for adults and copepodites of *E. affinis* (Figure 1b).

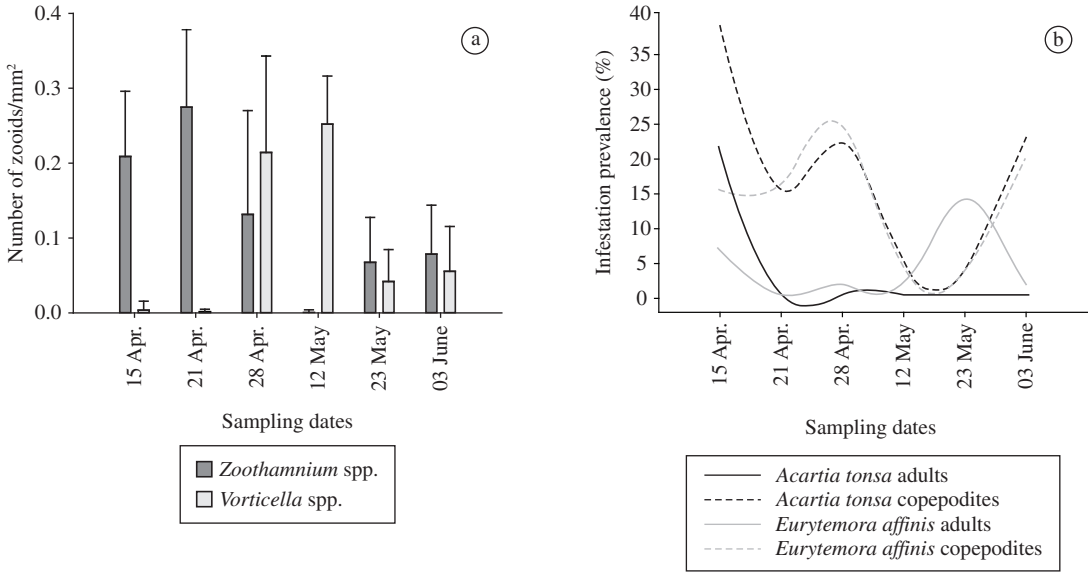

Figure 1. Attachment of peritrich ciliates to artificial substrates and copepods in the field. a) Number of peritrich zooids/mm² of cover-slip recovered on six sampling dates (between April 15th and June 3rd, 2003) from the Rhode River, a tributary of the Chesapeake Bay. Bars are mean numbers of zooids calculated from eight replicates and error bars are the Standard Error of the Mean. and b) Infestation Prevalence (%) of *Z. intermedium* on adults and copepodites of *A. tonsa* and *E. affinis* collected from the Rhode River on six sampling dates between April 15th and June 3rd, 2003.

No infestation was detected for adults of *A. tonsa* on some sampling dates in April, May and June (see Figure 1b). Infestation prevalence was very low for adults and copepodites of *E. affinis* in samples collected on May 12th as shown in Figure 1b.

4. Discussion

The present study investigated the attachment to non-living substrates of the peritrich epibiont *Zoothamnium intermedium*. According to Utz (2003), free-swimming stages of *Z. intermedium* are not able to colonize non-living substrates in the laboratory. The same pattern was observed in the field, when the peritrich was colonizing its primary hosts, but was not found colonizing cover-slips in the field, as other species of *Zoothamnium*.

Selection of suitable substrates for settlement has been the subject of several studies including invertebrate larvae, especially barnacle species. In general, invertebrate larvae show a pattern of extensive initial exploration of the substrate, finally settling where more individuals of their own species are found (Knight-Jones and Crisp, 1953). This searching and settlement behavior would help avoid unfavorable environments where the adult will have a lower chance of survival, since early post-settlement mortality can sometimes be extremely high (Gosselin and Qian, 1996). This similar pattern of settling next to conspecifics displayed by different species of invertebrate larvae was suggested to be convergent (Knight-Jones and Crisp, 1953), and might be the same utilized by telotrochs of peritrich ciliates (Langlois, 1975).

Peritrich epibionts can choose their host based on the availability of space and the access to resources (facultative), or based on chemical attraction to a specific host or group of hosts, not being able to attach to non-living substrates (obligate). *Zoothamnium intermedium* found as epibiont on Chesapeake Bay copepods is an example of obligate epibiont, since it failed to attach to glass surfaces in the laboratory (Utz, 2003) and to glass cover-slips in the field, as was demonstrated by live and Protargol staining observations of peritrichs attached to cover-slips sampled from nature. At the same time, *Z. intermedium* was colonizing copepods, demonstrating that free-swimming stages were available for potential colonization of non-living substrates if possible.

Although *Z. intermedium* was not found attached to cover slips, four other species in the genus *Zoothamnium* (including *Z. arbuscula* Ehrenberg, 1839 and *Z. alternans* Claparède et Lachmann, 1859) were found to be very abundant from April to the beginning of May, when they started to be outnumbered by *Vorticella* spp. Gross (1986), in a study characterizing species of *Zoothamnium* from the Patuxent River, a subestuary of the Chesapeake Bay using the morphological characters revealed by the Protargol staining technique, also found four species of this genus colonizing slides, but none of them with *Z. intermedium* found attached to copepods.

The results presented here suggest that *Zoothamnium intermedium* is not a common member of the benthic community of the Chesapeake Bay, and probably is an obligate epibiont colonizing preferentially *Acartia tonsa* and *Eurytemora affinis*.

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