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Prevalence and seasonality of *Zoothamnium duplicatum* (Protozoa: Ciliophora) epibiont on an estuarine mysid (Crustacea: Mysida) in tropical mangrove brackish water

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Abstract: The estuarine mysid *Mesopodopsis tenuipes* (Crustacea: Mysida) was often found carrying the peritrich ciliate *Zoothamnium duplicatum* (Protozoa: Ciliophora) on its body. The prevalence and seasonality of this association were studied on the basis of a year-round survey conducted in the Merbok mangrove brackish water in north-western Peninsular Malaysia from March 2005 to February 2006. The frequency of mysids hosting ciliates varied from 0 to 57.3%, with an annual mean of 17.0%. Juveniles showed a significantly lower prevalence of ciliates than adult mysids. There was no difference in infestation prevalence between the sexes. An epibiotic renewal process could be assessed through observations in breeding females. The infestation prevalence and loads were appreciably lower in the females carrying earlier stages of embryos than in those with developed ones, indicating that zoothamnid ciliates may require two or more days to reach a saturation phase in terms of prevalence when ciliate reproduction is high. This, in turn, suggests that the high moulting frequency of the tropical mysid may inhibit high colonisation pressure by the epibiont. A periodic seasonal trend was not determined, although mysids in lower salinity conditions were more likely to carry epibionts than those at higher salinities.

Key words: epibiosis, Malaysia, mangrove estuary, *Mesopodopsis tenuipes*, *Zoothamnium duplicatum*

Epibiosis of ciliate protozoans on crustaceans is a frequent phenomenon in aquatic environments worldwide (Fernandez-Leborans & Tato-Porto 2000a, b, Fernandez-Leborans 2001, 2009). This association has traditionally been regarded as a commensal relationship. However, epibiotic ciliates sometimes affect host crustaceans adversely by increasing their susceptibility to predation, by hindering their movement, growth, reproduction, and ability to survive, or by reducing food availability through food competition with the epibiont (cf. Threlkeld et al. 1993, Utz & Coats 2005). Otherwise, epibionts could be direct food sources for the host crustacean (Barea-Arco et al. 2001). Over the last decades, the interaction between the parasite and host and its consequences within and/or between ecosystems have been gathering attention (Ohtsuka 2006, Wahl 2008).

Mysids are also subject to infestation by epibiotic ciliates (Fernandez-Leborans & Tato-Porto 2000a, b, Fernandez-Leborans 2001, 2009), nevertheless studies on the association between ciliates and mysids have seriously lagged behind those

of other crustaceans such as copepods, cladocerans, amphipods, euphausiids, and decapods. Most of the early literature simply recorded the presence/absence of the epibiont taxa, although several authors analysed the prevalence data in terms of seasonal variations (Evans et al. 1981, Hanamura 2000) and geographical gradients (Hanamura & Nagasaki 1996, Fernandez-Leborans 2003) in the temperate and boreal regions. In the tropics, Nair (1939) noted the incidence of zoothamnid ciliates on *Mesopodopsis orientalis* (Tattersall) in the brackish waters close to Madras (=Chennai), India. Recently, Fernandez-Leborans et al. (2009) reported the intersite characterisation of epibiotic ciliates on mangrove crustaceans in Malaysia and Thailand, in which *Zoothamnium duplicatum* Kahl was found to be the most common epibiont among the four ciliates that infested the *M. orientalis* complex. In this paper, the infestation prevalence of *Z. duplicatum* on *Mesopodopsis tenuipes* Hanamura et al. (formerly as *M. orientalis*) was examined throughout year according to the developmental stages of host mysids, host density, and environmental parameters in a tropical mangrove estuary in Merbok, Kedah State, on the north-western coast of Peninsular Malaysia.

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Mysids were sampled monthly at the morning low tide using a hand net (30 cm mouth width, 0.77 mm mesh width) in the intertidal zone at two sampling sites in the Merbok mangrove estuary (Stns. B and C: 5° 38'N, 100° 24'E), Peninsular Malaysia (Hanamura et al. 2008b), from March 2005 to February 2006. All samples were fixed immediately in approximately 5% formalin seawater and preserved prior to subsequent analyses. The sampling details, mysid density, and environmental conditions have been documented elsewhere (Hanamura et al. 2008b; as *M. orientalis*): for the taxonomic identity of the *M. orientalis* complex, see Hanamura et al. (2008a).

Approximately 100 mysids, whenever possible, were randomly extracted from each sample and categorised according to the sexual developmental stages (Hanamura et al. 2008b). A total of 2,240 individuals of mysids were examined in this study. Infestation prevalence, defined as the percentage of mysids with attached zoothamnid ciliates, was calculated using a stereomicroscope, and the samples were stained with methylene blue when necessary. The epibiont load was also calculated as the number of zooids on the host (no. of zooids host⁻¹). Data from the two study sites were combined.

In addition to seasonal analyses, 360 breeding females from selected collections (May, October, and November 2005), which were selected due to the highest zoothamnid ciliate incidence, were examined in order to evaluate the colonisation renewal process more precisely. The breeding females were categorised according to the degree of development of embryos carried in their brood pouches, and were denoted as F_I (eggs/embryos corresponding to Fig. 19 in Nair (1939), later on), F_{IIa} (Figs. 20–21) and F_{IIb} (Figs. 22–24), and F_{III} (Fig. 25). In statistical analyses, differences were considered significant at $p < 0.05$.

The colonial ciliate *Z. duplicatum* was frequently found infesting the posterior part of the mysid body (posteroventral part of the sixth abdominal somite and base of the uropod close to the anus) (Fig. 1), but also occasionally was attached to the pleopods, or rostral plate, or the eye stalks (see also Fernandez-Leborans et al. 2009).

This zoothamnid-mysid association was found almost as a year-round phenomenon at the study site, although the infestation prevalence of *Z. duplicatum* on *M. tenuipes* showed a wide range of variation from 0 to 57.3%, with an annual mean of 17.0%. The infestation prevalence reached its maximum in December 2005, followed by May and September–November 2005, while no colonisation of ciliates was observed in the June–July samples, and the colonisation remained at very low levels in March and August 2005 (Fig. 2a). The overall infestation prevalence of ciliates on mysids did not show any seasonal trend (run-test, $r=5$, $p > 0.05$; Tate & Clelland (1957)). Adult mysids exhibited two peaks of prevalence in May and December of 2005, and relatively higher incidences were observed from September 2005 to February 2006 (Fig. 2a). In immature mysids, the highest prevalence of zoothamnid ciliates was observed in the December 2005 sample, followed by those in October and September 2005. As compared with adults, juvenile mysids exhibited a smaller infestation peak in September

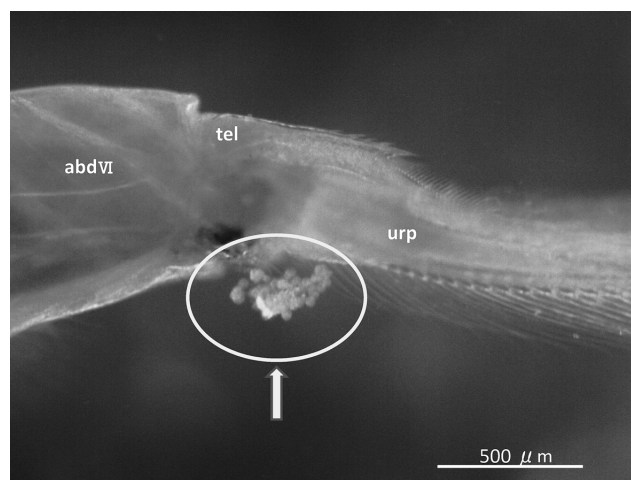


Fig. 1. Estuarine mysid *Mesopodopsis tenuipes* hosting a large colony of the peritrich ciliate *Zoothamnium duplicatum* (encircled): abdVI, sixth abdominal somite; tel, telson; urp, uropod.

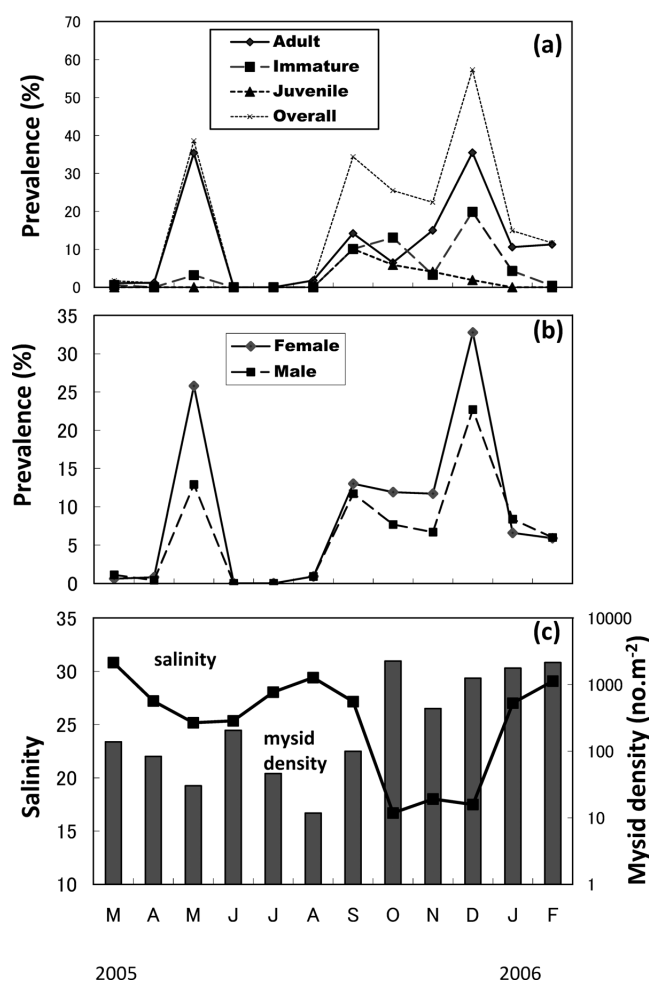


Fig. 2. Seasonal changes in infestation prevalence (%) for *Zoothamnium duplicatum* epibiosis on mysids in the Merbok mangrove estuary according to developmental stages (a) and sexes (b) of *Mesopodopsis tenuipes*, and changes in salinity and host mysid's abundance (c).

2005. The infestation prevalence was significantly different between the developmental stages of mysids (Kruskal-Wallis test, 7.21, $p < 0.05$), and juveniles were the least vulnerable group when compared to adults (Scheffé test, 7.21, $p < 0.05$). There was no difference in infestation prevalence between the sexes (Fig. 2b) (Kruskal-Wallis test, 0.05, $p > 0.05$). Epibiont loads on mysids were relatively low, and heavily infested mysids (>20 zooids host^{-1}) accounted for only 3.4% of the total mysids observed in the seasonal analyses. The infestation prevalence of zoothamnid ciliates in *M. tenuipes* in the Merbok mangrove estuary (17.0% in annual mean) was much lower when compared to $>90\%$ prevalence of peritrich ciliates attached to *Archaeomysis articulata* Hanamura in Ishikari Bay, northern Japan, in which the epibiont loads were also higher (often >50 zooids host^{-1}) (Hanamura 2000). In Lake Michigan, more than half of the population of *Mysis diluviana* Audzijonte & Väinölä (as *M. relicta*) were heavily infested (>20 zooids) by a suctorian ciliate, *Tokophrya* sp. (Evans et al. 1981). Furthermore, Fernandez-Leborans (2003) found that the number of peritrich ciliates *Vorticella* sp. attached to the Lake Lüsiai mysids was 390 (mean).

Amongst the heavily infested mysids, larger females carried more zoothamnid ciliates and decreasing frequency was as follows (total $N=76$): breeding females (52.6% in prevalence), adult males (21.1%), adult females (13.2%), immature females (6.6%), juveniles (3.9%), and immature males (2.6%). In the breeding females caught during the periods when ciliate incidence was high, heavily infested females were found only among those with developed embryos ($N=29$); F_{III} (16.5%), F_{IIb} (12.8%), F_{IIa} (0%), and F_I (0%). Similar results have often been reported for other ciliate-crustacean associations (Xu 1992, Hanamura 2000, Fernandez-Leborans 2003), while some other studies have indicated host-size independence (Evans et al. 1981). Also, an inverse relationship between infestation intensity and size/age of host has even been reported (Utz & Coats 2005). Warm water species/population of mysids have shorter inter-moult periods than those in colder water habitats (Mauchline 1980), and this could be a factor hampering more intensive colonisation pressure from epibionts (Threlkeld et al. 1993). Indeed, the infestation prevalence could exceed 80% in breeding females (see discussion below), which may have the longest instar duration of the mysid population.

Water salinity had a broad range from 16.70 to 30.83 (annual mean 25.13 ± 4.93) depending on the survey (Fig. 2c) (see also Hanamura et al. 2008b). Despite the fact that a Kendall rank correlation test failed to find a significant relationship (Fig. 3a), there was a weak correlation between the incidence of zoothamnid ciliates and salinity, as infestation levels become higher with decreasing salinity ($r = -0.62$, $p < 0.05$), and salinity is likely to be correlated to the population dynamics of *Z. duplicatum*. López et al. (1998) found that, in a freshwater reservoir in western Venezuela, the incidence of epibionts dominated by *Epystylis* species increased in close correspondence to the rainy season. However, it remains unclear whether the increase in prevalence was the result of low salinity or is due to other factors. Salinity changes in the studied estuary

occur basically as a consequence of rainfall and could contribute to possible increasing the inflow of organic matter from the surrounding land, which is essential for the ciliates. Eutrophication in the aquatic environment is undoubtedly one of the major factors in enhancing the colonisation of epibiotic ciliates (Henebry & Ridgeway 1979, Xu 1992). The water temperature remained constant at around 30°C (annual mean $30.1^\circ\text{C} \pm 1.12$) throughout the year except for December 2006, when an unusually low value of 27°C was recorded; hence, water temperature is not a strong factor contributing to the epibiotic fluctuations observed in this study. The epibiotic system, however, may be regulated by complex interactions between the epibiont and host or between biological and environmental parameters or by a combination of both bio- and abiotic factors (Utz & Coats 2005).

Higher infestation prevalence and also load have occasionally been observed when the host species were more abundant, but also many neutral or negative observations have been recorded (cf. Threlkeld et al. 1993, Utz & Coats 2005). The density of mysids at the study site showed a broad range of fluctuations from 12 to $2,273 \text{ indiv. m}^{-2}$ (annual mean $709 \text{ indiv. m}^{-2}$) (Fig. 2c). The infestation prevalence was irrelevant to the catch abundance of host mysids ($r = 0.26$, $p > 0.05$) (Fig. 3b). Epibiotic ciliates including *Z. duplicatum* at the studied site were able to utilise a variety of crustacean substrates (Fernandez-Leborans et al. 2009) included *M. tenuipes*, which was not the sole host for the ciliates.

The females in the early breeding phases (F_I and F_{IIa}) showed a significantly lower incidence of ciliates than those in the later ones (F_{IIb} and F_{III}) (χ^2 test, 78.34, $p < 0.05$); the proportion of mysids with ciliates attached (and also epibiont loads) intensified in the late phases of breeding females, reaching well beyond 80% (Fig. 4). The life cycle of sessile peritrich ciliates is generally comprised of a stalked, sessile feeding stage (trophont) and a free-swimming dispersal stage (telotroch), although the mechanism inducing these phases is not well-known. *Zoothamnium duplicatum* settled less readily on the new exoskeleton of mysids, and its attachment appeared to be intensified after the F_{IIa} phase of breeding mysids. A similar pattern, slow at first and exponential later, was observed by Xu (1992) in a ciliate-cladoceran association in nature.

The development of mysid embryos progresses in a maternal brood pouch, while females do not moult during the breeding process (Mauchline 1980). This makes the embryos, such as the parthenogenic eggs of cladocerans (Threlkeld et al. 1993), useful as a chronological indicator. An examination of epibiosis in egg-bearing females, therefore, can be advantageous to assess how the colonisation of ciliates proceeds from the time of their last ecdysis, which takes place shortly before egg production. Nair (1939) found that egg extrusion of *M. orientalis*, the closest relative of *M. tenuipes*, takes place at night, and these eggs (or embryonic larvae) subsequently spend about 96 h (4 days) in the brood pouch at a water temperature of $24\text{--}29^\circ\text{C}$, where the first moult (Egg stage $I \rightarrow IIa$) occurs after about 24 h. If this time duration is applicable to *M. tenuipes*, the *Z. duplicatum* population would require at least

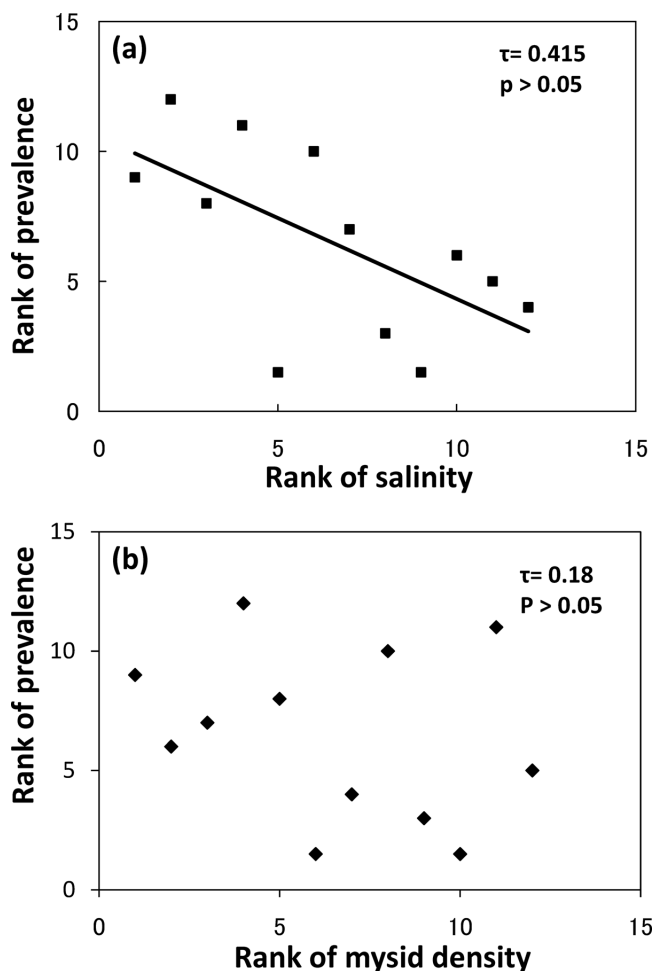


Fig. 3. Relationships between the infestation prevalence and salinity (a) and between the infestation prevalence and mysid abundance (b).

two days to achieve a stationary phase of settlement, although the epibiont loads may continue to increase gradually with the age of the mysid (the duration of the instars). The egg size of *M. tenuipes* is slightly larger than that of *M. orientalis* (0.42 mm in mean vs. 0.37 mm) (Hanamura et al. 2008b), although the incubation time for the embryos of *M. tenuipes* in the studied estuary is assumed to be not very different from that of *M. orientalis* in terms of egg size and water temperature, which significantly influence the incubation time of embryos (Wittmann 1984).

There was an absence of zoothamnid ciliates on the sergestid shrimp *Acetes japonicus* Kishinouye sampled in a marine habitat (Fernandez-Leborans et al. 2009, Hanamura pers. obser.). Similarly, we did not detect any incidence of *M. orientalis* carrying *Z. duplicatum* despite conducting a year-round survey off the southern coast of Penang Island, Malaysia (Hanamura pers. obser.). In contrast, another species of sergestid shrimp *Acetes sibogae* Hansen and/or juvenile of the banana shrimp *Fenneropenaeus merguensis* (De Man), both of which were typical residents of the inner mangrove swamp, were regarded as preferential substrates for the ciliates (Fer-

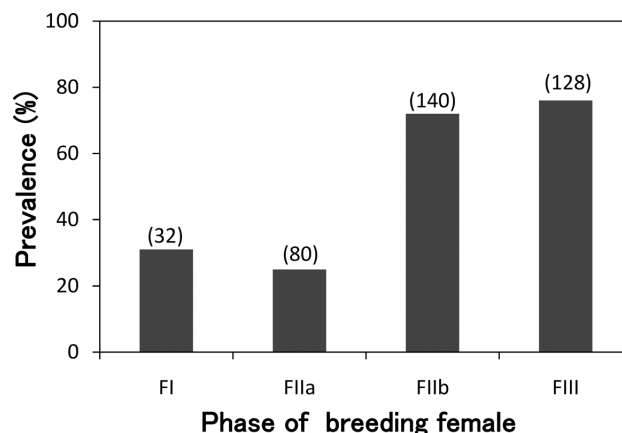


Fig. 4. Infestation prevalence in female *Mesopodopsis tenuipes* carrying embryos of each developmental stage. Numbers in parentheses indicate individual numbers of mysids examined.

nandez-Leborans et al. 2009). These findings suggest that this type of interaction, between *Z. duplicatum* and planktonic crustaceans, could be uncommon in marine coastal habitats. The presence of zoothamnid ciliates may be a useful bio-indicator for discriminating a crustacean population with a closer affinity to a mangrove estuary than a coastal habitat.

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