

Life cycle and precopulatory mate guarding of *Goidelia japonica* (Copepoda: Poecilostomatoida: Echiurophilidae) associated with the echiuran *Urechis unicinctus*

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Abstract: The life cycle and precopulatory mate guarding behavior of the poecilostomatoid copepod *Goidelia japonica* associated with the spoon worm *Urechis unicinctus* (Echiura) are described based on specimens from 19 worms collected from western Japan. A total of 676 copepods were collected from the host's rectum (494 copepods) and body surface (182 copepods). Copepod numbers were significantly correlated with host weight. Copepods in the rectum consisted almost completely of copepodids (35%) and adult males (65%), with only one non-ovigerous adult female recorded. In contrast, those on the body surface were all adult males (24%) or females (76%), of which 58% were ovigerous. No nauplii or CI occurred. In the rectum, 36% of the adult males guarded 67% of the copepodids, consisting of CII–CV. From these results, their life cycle is considered as follows: nauplii and CI live as plankton; just before or after molting to CII, they enter the host's rectum and females are probably soon guarded by males; they grow up to CV there; CV females guarded by males move to the body surface just before the final molt; on the body surface females molt to adults and spawn eggs. Guarding the first symbiotic stage (CII) is a unique precopulatory behavior for symbiotic poecilostomatoid copepods. This can be explained from the viewpoint of evolutionary resolution of the intersexual conflict and advantage for early copepodids in precopula.

Key words: *Goidelia japonica*, life cycle, precopulatory mate guarding, symbiosis, *Urechis unicinctus*

Introduction

The crustacean subclass Copepoda contains about 13,000 species (Boxshall & Defaye 2008). According to Ho (2001), more than one-third of known copepods are symbiotic with other organisms, and their hosts range from sponges to marine mammals, including all major aquatic taxonomic groups. He noted that all aquatic animals are potential hosts for symbiotic copepods. Furthermore, symbiotic copepods can be very abundant on or in their hosts. Despite such an ubiquitous distribution, studies on the biology of symbiotic copepods are still very limited, compared to studies on their taxonomy.

The present study describes the life cycle during the

postnaupliar stages and mating behavior of the poecilostomatoid copepod *Goidelia japonica* Embleton, 1901 (Echiurophilidae) associated with the spoon (or innkeeper) worm *Urechis unicinctus* (von Drasche, 1881) (Annelida, Echiura). This echiuran lives in a U-shaped burrow in muddy and/or sandy sediments in lower inter- and subtidal zones of the Chinese continent and Japan, and is used as food in Japan, China and especially Korea (Abe et al. 2014). Echiuran burrows are known to serve as habitats for various symbionts, such as shrimps, crabs, bivalves and demersal fishes (Fisher & MacGinitie 1928, Anker et al. 2005, Itani et al. 2005, Goto 2017). In addition to the commercial and ecological importance of *U. unicinctus*, it has the potential to improve water quality by filtering suspended materials (Abe et al. 2014) and the quality of the organically contaminated coastal sediment by reducing acid volatile sulfide, COD, and total ignition loss (Kang et al. 2010). In Japan,

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U. uncinatus occurred previously as a common species in mud and sandy tidal flats, but its population has decreased dramatically due to reduction of their habitats during recent decades (Nishikawa 2012, Abe et al. 2014).

Goidelia japonica was first described from the rectum of *Urechis uncinatus* by Embleton (1901) based on Japanese specimens, and subsequently by Gooding (1963) based on specimens collected in Ehime, northwestern Shikoku Island, Japan, and Tokyo Bay, and by Kim (1998) on Korean specimens. The following other five poecilostomatoid species are known to be associates with echinurans: *Echiurophilus fizei* Delamare-Deboutteville & Nunes-Ruivo, 1955 with *Anelassorhynchus inansensis* (Ikeda, 1904) from Vietnam (Delamare-Deboutteville & Nunes-Ruivo 1955), *Hemicyclops mortoni* Boxshall & Humes, 1987 with *Ochetostoma erythrogrammon* Leuckart & Rüppell, 1828 from Hong Kong (Boxshall & Humes 1987), *Goidelia pelliviva* Kim I.H., 2000 with *Thalassema* sp. from Korea (Kim 2000), and the recently described *Echiuricopus aprilis* Kim I.H., 2016 and *Echiuricopus tenuipes* Kim I.H., 2016 with unidentified echinurans in Korea (Kim 2016). The number of known copepod species associated with Echiura is very few compared to those with other aquatic invertebrate groups such as Mollusca, Cnidaria, Echinodermata, Urochordata, Crustacea, and Polychaeta, with which more than a hundred copepod species have been reported as symbionts (Humes 1994).

Anker et al. (2005) reviewed symbiotic animals associated with echinurans. Most of these symbionts are considered as inhabitants in echinurans' burrows and commensals

with a tendency to kleptoparasitism. Copepoda is one of the few taxa of host-specific associates living on or in echinurans, and it appears that they are dominant endo- and ectosymbionts. However, there have, as yet, been no studies on the life cycle and behaviors of copepod symbionts associated with echinurans.

Materials and Methods

Nineteen individuals of *Urechis uncinatus* were collected from a tidal flat of the Kamogawa River (33.935°N, 133.155°E) on the northern coast of Ehime Prefecture, Shikoku Island, Japan, during a spring ebb tide on 4 June 2004. The sediment at the collection site was sandy mud. The worms were dug up from burrows using a shovel, and individually fixed and preserved in 5% formalin-seawater solution immediately after collection. In the laboratory, sample bottles were shaken to separate copepods off the body surface of the worm and all copepods in the sample waters were collected and examined under a stereoscopic microscope; these copepods are regarded as those associated with the host body surface. Then the rectums of the worms were dissected to collect copepods; prior to this operation, we dissected the whole digestive tracts of several worms and confirmed that copepods were found restrictedly in the rectums. We examined a dissected rectum twice for each worm under a stereoscopic microscope to collect copepods completely. No other copepods were found in the samples from the rectum and/or the body surface. Wet weights of the 19 worms, which were measured using an

Table 1. The numbers of *Goidelia japonica* collected from *Urechis uncinatus*. Values in brackets and parentheses indicate the numbers of individuals in precopula and of ovigerous females, respectively.

Worm ID	Wet weight (g)	Rectum			Body surface		Total copepods
		Copepodids II–V	Adult female	Adult male	Adult female	Adult male	
#1	14.40	15 [12]	0	29 [12]	6 (2)	2	52
#2	13.32	20 [20]	0	58 [20]	27 (16)	3	108
#3	10.00	16 [12]	0	36 [12]	11 (5)	2	65
#4	9.64	11 [10]	0	30 [10]	16 (11)	4	61
#5	9.25	11 [11]	0	32 [11]	7 (5)	1	51
#6	9.05	13 [13]	0	26 [13]	14 (4)	3	56
#7	7.69	14 [9]	0	27 [10]	7 (4)	2	50
#8	7.09	3 [3]	0	5 [3]	10 (8)	3	21
#9	6.35	1 [0]	1	8 [0]	4 (1)	1	15
#10	6.14	3 [1]	0	3 [1]	3 (2)	4	13
#11	5.95	6 [1]	0	5 [1]	2 (1)	2	15
#12	5.87	4 [0]	0	0 [0]	6 (4)	0	10
#13	5.75	23 [12]	0	29 [13]	6 (6)	2	60
#14	5.29	3 [0]	0	0 [0]	1 (1)	1	5
#15	5.29	9 [3]	0	5 [3]	2 (1)	1	17
#16	5.21	0 [0]	0	3 [0]	2 (1)	2	7
#17	5.01	0 [0]	0	1 [0]	3 (2)	4	8
#18	4.93	11 [3]	0	8 [3]	7 (4)	2	28
#19	4.92	8 [5]	0	17 [5]	5 (2)	4	34
Total		171 [115]	1	322 [117]	139 (80)	43	676

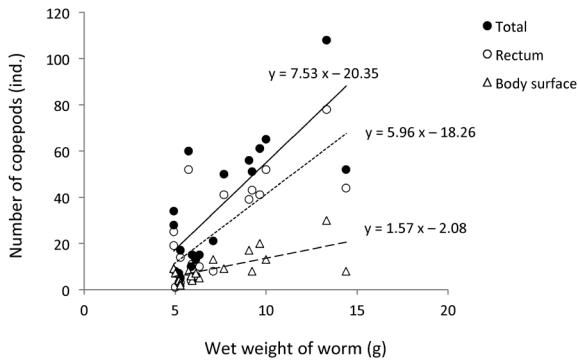


Fig. 1. The relationships between the number of *Goidelia japonica* and the wet weight of the host *Urechis unicinctus*. The solid, dotted, and broken regression lines represent regressions of the numbers of total copepods from the host, from the host rectum, and from the host body surface, respectively.

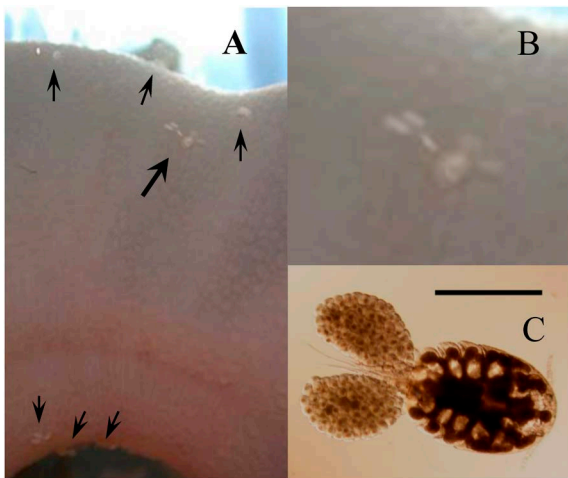


Fig. 2. *Goidelia japonica*. A: living individuals (indicated by arrows) on the body surface of *Urechis unicinctus*; B: enlarged photo of an ovigerous female (indicated with a big arrow in A); C: ovigerous female on glass slide (scale bar 0.50 mm).

electronic balance, ranged from 4.9–14.4 g (average 7.4 g).

Developmental stages of copepods were distinguished under a stereoscopic microscope. Adult copepods were identified by the genital compound somite or presence of egg clutches for females and six urosomites for males. Copepodid stages were distinguished by the number of leg pairs on the prosome (two for CI, three for CII, and four for the later stages) and the number of urosomites (three for CI–CIII, four for CIV, and five for CV) following the description of copepodid stages of the poecilostomatoid copepod *Hemicyclops japonicus* Itoh & Nishida, 1993 (Itoh & Nishida 1995).

Results

Abundance of copepods

Goidelia japonica was collected from every spoon



Fig. 3. Precopulatory adult male of *Goidelia japonica* guarding a CII individual.

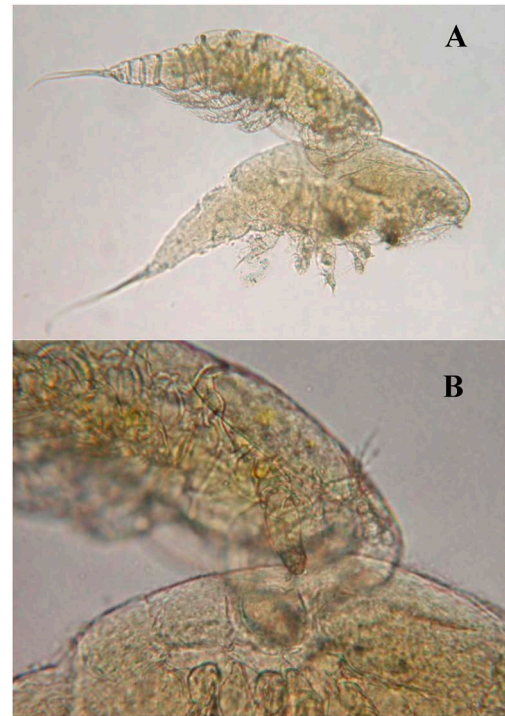


Fig. 4. *Goidelia japonica*. A: a male clasping a CV female; B: enlarged photo of A, showing a male clasping with maxillipeds.

worm and the total number of copepods from the 19 worms was 676, of which 494 (73%) were from the rectum and 182 (27%) were from the body surface (Table 1). Copepod nauplii and CI were not found at all. The stage-specific individual numbers of CII–CV and adults, excluding two copepodids for which the stages could not be distinguished, were 37, 74, 18, 40, and 505, respectively, of which the ratios to the total numbers of copepods were 5.5, 11.0, 2.7, 6.0, and 75.1%, respectively. Adult males (365 individuals) were 2.6 times more numerous than adult females (140 individuals).

The number of total copepods from a worm showed a significantly positive correlation with the wet weight of the worm ($r=0.77$, $p<0.001$) (Fig. 1). Significant positive

relationships with the wet weight of the worm were also observed for copepods from the rectum ($r=0.76$, $p<0.001$) and from the body surface ($r=0.66$, $p<0.01$).

In the rectum, copepods consisted of 171 copepodids (35%) and 323 adult males (65%), along with only one non-ovigerous adult female. In contrast to the number of copepodids in the rectum, no copepodids were found on the body surface. About one-fourth (24%) of the adult copepods on the body surface were male and three-fourths (76%) were female, of which more than a half (58%) were ovigerous (Fig. 2).

Precopulatory mate guarding

A total of 115 pairs of copepods in precopula were observed in 14 rectums among the 15 worms, which had both adult males and copepodids in their rectums (Table 1). No precopula pairs were found in the surface copepods. Copepods guarding other copepods were all adult males and guarded copepods were various copepodid stages from CII to CV. The precopulatory guarding of CII (Fig. 3) was not rare; the stages of guarded copepodids were not completely recorded in this study, but five of the six CII individuals recorded were guarded. Precopula of one copepodid guarded by two males were observed in worms #7 and #13. In the rectum, 36% of adult males guarded copepodids, and these comprised 67% of total copepodids. The number of precopula pairs per host showed a significantly positive correlation with the wet weight of the worm ($r=0.803$, $p<0.001$).

Precopulatory guarding was performed by claspings the dorsal joint between the first and second prosomal somites using maxillipeds (Fig. 4).

Discussion

The present study revealed that *Goidelia japonica* lives not only in the rectum but also on the body surface of *Urechis unicinctus*. In previous studies, poecilostomatoid copepods associated with echiurans were collected either from hosts' digestive tubes (Embleton 1901, Delamare-Deboutteville & Nunes-Ruivo 1955, Gooding 1963) or body surfaces, the latter specimens being collected from burrow waters (Boxshall & Humes 1987), washings of echiurans (Kim 2016), or directly from the hosts' skin (Kim 2000). The present result that they inhabit both the internal and external surfaces of echiurans is the first finding for poecilostomatoid copepod symbionts with echiurans.

No nauplii or CI stages were collected from the worms, indicating that only the stages from CII to adult are symbiotic. However, the developmental stage and adult sex composition were quite different between those in the rectum and on the body surface. Copepods in the rectum consisted completely of adult males and copepodids with the exception of a single non-ovigerous female, whereas on the body surface they were mostly adult females and much smaller numbers of adult males. No copepodids were found on the body surface. Noteworthy findings in the present study are

that more than a half of the females on the body surface were ovigerous and that most copepodids, including CII stage, in the rectum were guarded by males in precopula. These results lead us to propose the following scenario for the life cycle of *Goidelia japonica*. They hatch out from females on the body surface of *Urechis unicinctus* and live apart from the host as plankton until CI. Just before or after molting to CII, they enter the rectum of a host through the anus, and female copepodids are probably soon guarded by adult males. They grow up to CV in the rectum. CV females in precopula with adult males move to the body surface before the final molt. On the body surface, they quickly molt to adult females, copulate with males, and spawn eggs.

In her original description of *Goidelia japonica* from the rectum of *Urechis unicinctus*, Embleton (1901) described nothing about copepodids or precopulatory behavior, but stated that no adult females have egg-sacs. Gooding (1963) observed only two females of *G. japonica*, in contrast to many males (107) and copepodids (159), collected from the rectum of a single specimen of *U. unicinctus*. Since their collections were from the rectums, their observations are consistent with the present results.

The characteristic life cycle of *Goidelia japonica*, i.e. planktonic from nauplius to CI and symbiotic from CII to the adult stage, is common to the poecilostomatoid *Hemicyclops* spp., of which planktonic CI has a characteristic body shape different from the later stages and had therefore previously been identified as a different genus "*Saphirella*" (Itoh & Nishida 1995, 2007). Do et al. (1984) described the complete life cycle of *Pseudomyicola spinosus* (Raffaele & Monticelli, 1885), one of the most common poecilostomatoid copepods associated with bivalve mollusks. This copepod is planktonic during all naupliar stages and symbiotic during the copepodid and adult stages. The body shape of CI of this species is not characteristic but is similar to those of the later stages. Compared with this, the characteristic shape of CI in *Hemicyclops* is considered to be due to a planktonic life different from the symbiotic life led by the later stages. This suggests that CI of *G. japonica* also has a different body shape from the later stages, and thereby that it is impossible to find CI in field samples and, instead, culture from eggs in the laboratory or molecular biological analyses are necessary for identification.

In the above scenario, we consider that most copepodids in the rectum are guarded by adult males, sometimes from CII. The basis for this is the high ratio of copepodids in precopula to all copepodids. Given that the sex ratio of copepodids is not so different from that of the adult stages (male/female=2.6) and that most copepodids in precopula are female, at least from CIV and later stages, which are sexually dimorphic stages (Do et al. 1984, Itoh & Nishida 1995), the high ratio (67%) of copepodids in precopula implies that almost all female copepodids, including CII, are guarded by males.

In this scenario, precopula males and female CV stages move to the body surface just before the final molt, where she copulates with the precopula males on the body surface. This supposition is based on the result that one-fourth of the copepods on the body surface were male. If females molt and copulate in the rectum, males would not need to go out from the rectum and as a result males on the body surface would be much less numerous. If this were the case then the question arises as to the reason for the lower number of males than females on the body surface, because if females move from the rectum to the body surface together with males then the sex ratio should be even on the body surface. Two possible reasons for this situation present themselves. One is that some males could return to the rectum after copulation. The much higher number of adult males than copepodids in the rectum seems to corroborate this answer. The other is that females could live on the body surface longer than males. This answer is also highly plausible, especially if females could spawn repeatedly using sperm in the seminal receptacle.

Precopula is widespread in podoplean copepods, especially Harpacticoida and Siphonostomatoida (Boxshall 1990). For example, Kern et al. (1984) listed reported clasplings by males for 17 harpacticoid species, of which 13 included precopulatory mate guarding. As for Siphonostomatoida, precopulatory mate guarding is a common behavior, and their life cycles with precopulatory guarding have been studied for species, such as *Cardiodectes* sp., *Lernaeocera branchialis* (Linnaeus, 1767), and *Lepophtheirus pectoralis* (Müller O.F., 1776) (Boxshall 1990). In Poecilostomatoida, only four species have hitherto been known to have precopulatory guarding behavior, i.e. *Pseudomyicola spinosus*, *Hemicyclops spinulosus* Itoh & Nishida, 1998, *Pseudanthessius tortuosus* Stock, Humes & Gooding, 1964 and *Pennatulicola pterophilus* (Stock, 1962) (Itoh & Nishida 2008). These species are all symbionts with benthic invertebrates. A remarkable difference of *G. japonica* from the four other species is that *G. japonica* males guard copepodids from the first symbiotic stage II onwards. The youngest stages of the four other species guarded by adult males are CIII (Stock et al. 1962, Gotto 1979, Do et al. 1984), except for *H. spinulosus*, of which the youngest guarded stage was unknown because observation of precopulatory guarding behavior was made only on a CV female and two adult males collected from the field (Itoh & Nishida 2008). *Pseudomyicola spinosus* and *H. spinulosus* start symbiotic life at CI (Do et al. 1984, Itoh & Nishida 2008), indicating that adult males do not guard CI or CII, even if they encounter them. Precopulatory mate guarding from mid- or late copepodid stages seems common also in harpacticoid copepods (Kern et al. 1984).

The timing to start precopulatory guarding is interpreted as an evolutionary resolution of the intersexual conflict that males favor a longer duration to assure paternity in case of potential sperm competition while females favor a shorter duration because of the potential costs of guarding

(Jormalainen 1998). Based on this theory, the reason why *G. japonica* males guard mates from the youngest symbiotic stage could be explained as follows. Adult males are much more numerous than copepodids in the rectum and therefore competition between adult males for precopulatory mates is severe. On the other hand, the costs of guarding females are low because there is no predatory risk in the echiuran rectum; no predatory animals were found in our observation. Guarding by adult males may give advantages rather than risks, especially to early copepodid stages, because guarded early copepodids could retain themselves more easily against excretory action in the rectum than non-guarded copepodids. Embleton (1901) noted that *G. japonica* specimens adhered very firmly to the epithelial lining of the host's rectum. This suggests that retention in the rectum is not easy for early copepodids.

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