Journal of the Marine Biological Association of the United Kingdom

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Reproductive behaviour of the branchial ectoparasite *Bopyrus crangorum* (Isopoda: Bopyridae) following ecdysis of the host *Palaemon serrifer* (Caridea: Palaemonidae)

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Research Article

Cite this article: Burgos LA, Itani G (2023). Reproductive behaviour of the branchial ectoparasite *Bopyrus crangorum* (Isopoda: Bopyridae) following ecdysis of the host *Palaemon serrifer* (Caridea: Palaemonidae). *Journal of the Marine Biological Association of the United Kingdom* **103**, e67, 1–9. https://doi.org/10.1017/S0025315423000565

Received: 11 May 2023
Revised: 24 July 2023
Accepted: 31 July 2023

Keywords:
Biphasic moulting; bopyrid isopod; insemination; oviposition

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Abstract

The reproduction of bopyrid isopod parasites is thought to occur immediately following host ecdysis, but direct observations supporting this hypothesis are limited. The aim of this study was to describe the reproductive behaviour of the bopyrid isopod *Bopyrus crangorum* relative to host ecdysis based on video recordings. Several hours after host ecdysis, biphasic moulting of female parasites was observed. The cuticle of the posterior body was shed before that of the anterior body at an interval of 1 h. Two hours after female moulting, the male repeatedly moved from its initial position between the female pleopods and stopped at the anterior end of the fifth oostegite, immediately above the gonopore. To our knowledge, this repeated visiting behaviour by males has not been previously observed in bopyrid isopods. Oviposition through the female gonopore occurred 33 min later. The male-removal experiment showed that females with their males removed after visits to the gonopore oviposited eggs, whereas females with their males removed before visits did not. We propose that repeated visits by males to the gonopore are attempts to inseminate the female. We hypothesised that sperm are released onto the external surface of each gonopore and that the eggs are fertilised as they pass through the opening, which would explain the synchronous development of fertilised eggs inside the marsupium. The present study provides new information on the life history of bopyrid isopods, which allows for a better understanding of the host–parasite relationship.

Introduction

Bopyrid isopods are obligate ectoparasites that infest decapod crustaceans such as shrimp, crabs, and anomurans (Markham, 1986; Williams and Boyko, 2012). Infestation causes alterations in the morphology, behaviour, and reproductive capacity of their host (Yoshida, 1952; Beck, 1980a; Ito and Watanabe, 1992; Bass and Weis, 1999; Calado *et al.*, 2008; McGrew and Hultgren, 2011; Sherman and Curran, 2015; Corral *et al.*, 2019; Golin *et al.*, 2022). Bopyrids have a complex life cycle that requires two hosts: an intermediate host (calanoid copepod) and a definitive host (decapod crustacean). The embryos hatch as epicaridium, which attaches to an intermediate host and moults into a microniscus larva before further developing into a cryptoniscus larva (Lester, 2005; Williams and Boyko, 2012). After leaving the copepod, the cryptoniscus larva infects its final host (Anderson and Dale, 1981). The first larva to settle on the host becomes a female, whereas subsequent larvae develop into males (Reinhard, 1949).

In contrast to the abundance of studies on bopyrid taxonomy and its parasitic effects on its host, live observations of parasitic behaviour related to its life cycle have rarely been reported. Several studies have suggested that the reproduction of bopyrid isopods is completed between host moults (Beck, 1980b; Brinton and Curran, 2015), and fertilisation may be external within the female marsupium (Hiraiwa, 1934, 1936). However, direct observations supporting this hypothesis are limited. To our knowledge, the only study to document aspects of the reproduction and behaviour of the branchial bopyrid parasite in relation to host moulting was conducted by Cash and Bauer (1993). They noted that moulting of female *Probopyrus pandalicola* and spawning of eggs occur 1.5–6.5 and 6–24 h, respectively, after host ecdysis, and larvae were released 6–120 h prior. They also observed that the male moved from its position on the female pleopods to the fifth pereiopod and inside the marsupium before egg spawning, presumably to inseminate the female. However, owing to the limited number and resolution of video observations, their results remain inconclusive regarding whether insemination occurs through each gonopore or by the release of sperm inside the marsupium. Furthermore, these details are limited to a single species of bopyrid; therefore, the generality of the behaviour is yet to be clarified.

In the present study, the reproductive behaviour of the branchial parasite *Bopyrus crangorum* (Fabricius, 1798) was investigated. This bopyrid parasitises seven species of *Palaemon* shrimp from Europe, the Eastern Mediterranean, and the Indo-Pacific and one species of processid shrimp from Southwestern Europe (Markham, 1986). In Japan, *B. crangorum* infests two species of palaemonid shrimp, *Palaemon serrifer* and *P. pacificus* (Ito and Watanabe, 1992). Studies have been conducted on the parasitic effects of this bopyrid on its host (Yoshida, 1952; Ito and Watanabe, 1992), but to the best of our knowledge, no studies have been conducted on its

life history. We selected this species because its behaviour is easily observable through the transparent carapace of the host. The objectives of this study were to (1) confirm whether male reproductive behaviour follows that described by Cash and Bauer (1993), (2) record the details of female parasite oviposition, and (3) test whether oviposition occurs in the presence or absence of males using male-removal experiments. Our findings also provide details of female parasite moulting for the first time and data on the timing of larval release relative to host ecdysis.

Materials and methods

Sample collection and video observations

Infested host shrimp, *P. serrifer*, were collected from the tidal zone of Uranouchi Inlet, Tosa Bay, Kochi, Japan (33°26′0.46"N, 133°26′21.75"E) using a handheld dipnet with a 1 mm mesh net. Parasitised shrimp were identified based on the presence of distinct bulges in the branchial chamber and the presence of male and female *B. crangorum* (Figure 1). The shrimp were kept in individual jars covered with fine mesh nets and placed in an 18 L aquarium filled with filtered and aerated seawater. The water was maintained at a salinity of 28–30 and 24–26°C, and the shrimp were fed commercial flake (TetraFin®; Tetra, Blacksburg, VA, USA) daily. Prior to observation, the carapace length of the host and the body length of male and female parasites were measured by capturing digital images using a Canon EOS Kiss 4 Digital Camera (Canon, Tokyo, Japan) on a Nikon SMZ 1000 stereomicroscope (Nikon, Tokyo, Japan). The images were analysed using ImageJ software (Schindelin *et al.*, 2015).

In the first set of observations, the developmental stages of the isopod brood and the presence of host exuviae were monitored daily in individually isolated infested shrimp ($N = 25$). This allowed us to describe the order and timing of parasite reproductive stages relative to the host moult. The developmental stages of the isopod embryos were staged according to the embryonic colouration and morphology described by Beck (1980b) and Cash and Bauer (1993), as follows: egg; translucent or white; embryo I: yellowish, embryo reniform, and tissue around the yolk segmented; embryo II: tan with brown pigmentation, body segmentation well-developed but appendages not free, with little or no yolk; epicaridium larva: dark grey or black, embryo with free appendages, and well-developed eyes. The infested shrimp were observed until the parasites completed two to three full reproductive cycles or until the host died. The observations were performed between April and July 2022.

In the second set of infested shrimp ($N = 15$), female isopods that were brooding epicaridium larvae or in the intermoult phase were monitored every hour. Once the shrimp moulted, the time of moulting was recorded, and the infested shrimp were transferred to a dish with a sponge that had a rectangular cut in the middle. The cut was slightly larger than the shrimp's body and served as a substrate for the shrimp to cling to, which allowed the shrimp to remain on its side as the recording progressed. The dish was filled with seawater from the aquarium in which the shrimp were reared. Recordings were made using an Olympus Tough TG-6 (Olympus, Tokyo, Japan) mounted on a Nikon SMZ 1000 dissecting microscope. The observations were performed between May and October 2022. Parasite behaviour was recorded until complete oviposition of the eggs inside the marsupium was observed. The mean observation time was approximately 7 ± 2.7 h ($N = 15$).

Male-removal experiment

The male-removal experiment was conducted on two sets of post-moulting, infested shrimp. In the first set of shrimp ($N = 8$), the male was removed immediately after the female parasite had finished moulting, and for the second set ($N = 8$), the male was removed after its movement from the pleopod to the level of oostegite 5. The infested shrimp was held in a Petri dish, and then, using forceps, the shrimp's branchiostegite was gently lifted, and the male was removed. The shrimp were then placed in jars, returned to the aquarium, and monitored for oviposition. Once the parasite oviposited the eggs, the development of the embryo was monitored daily and staged according to embryonic colouration and morphology, as described above. Fisher's exact test was used to compare the percentage of oviposition, and

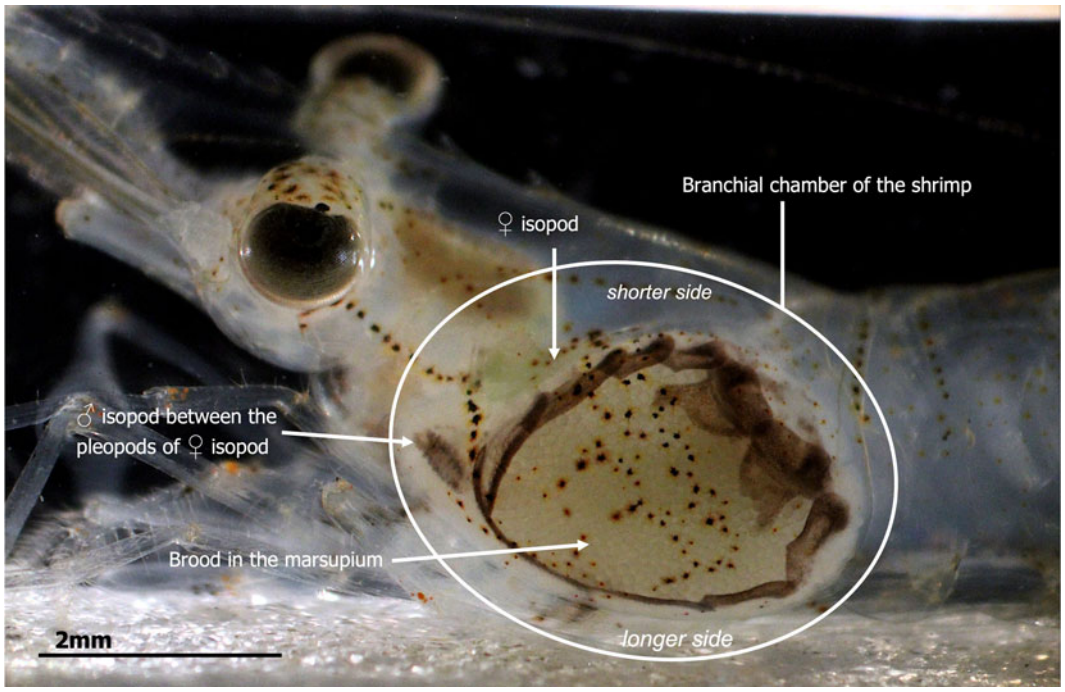

Figure 1. Positions of male and female *Bopyrus crangorum* inside the branchial chamber of *Palaemon serrifer*.

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Table 1. Duration of each developmental stage in the reproductive cycle of the bopyrid isopod *Bopyrus crangorum* on *Palaemon serrifer* expressed as median number of days

	Duration of isopod development		
Developmental stages	Median no. of days	Min	Max
Egg	2	2	4
Embryo I	3	2	3
Embryo II	3	2	3
Epicaridium	2	1	3
Full incubation	10	8	12

Only broods that have completed the entire cycle at 24–26°C ($N = 25$) were included in this analysis.

Mann–Whitney U test was used to compare the duration of host moult between treatments. All analyses were performed using JMP ver.14 (SAS Institute Inc. 2018).

Results

Timing of reproductive activities in relation to host moulting

Daily observations of female parasites on infested shrimp ($N = 25$) provided information on the timing and order of parasite reproductive stages, release of parasite larvae, host moulting, and oviposition into the female marsupium. The median interval between the release of the larva and the subsequent host moulting was 25 h (range: 15–92 h), while the median time interval between the host moulting and the oviposition was 6 h (range: 1–24 h). The broods were incubated for 8–12 days (median: 10 days), excluding the time when the female marsupium was empty before subsequent host moulting. The time interval of subsequent host moulting was 10–16 days (median: 11 days). The incubation duration by stage of development is shown in Table 1. The female parasites produced a maximum of seven successive broods (median: three broods) per female.

Moulting of females

Of the 15 post-moult, parasitised *P. serrifer*, 11 cases of complete biphasic moulting of female *B. crangorum* were recorded. In every case, the moulting process proceeded by first shedding the cuticle of the posterior body, including the fifth to seventh pereonites, pleons, pleotelson, and their respective appendages (pereopods, pleopods, and oostegite 5) (Figure 2A–C). Then, after 72.9 ± 27.9 min (range: 15.1–115.1 min), the female parasite shed the anterior half of the body cuticle, including the head, maxilliped, barbula, pereonites 1–4, and their respective appendages (Figure 2D–F). Prior to and during the posterior and anterior moults, vigorous contraction and folding of the female body

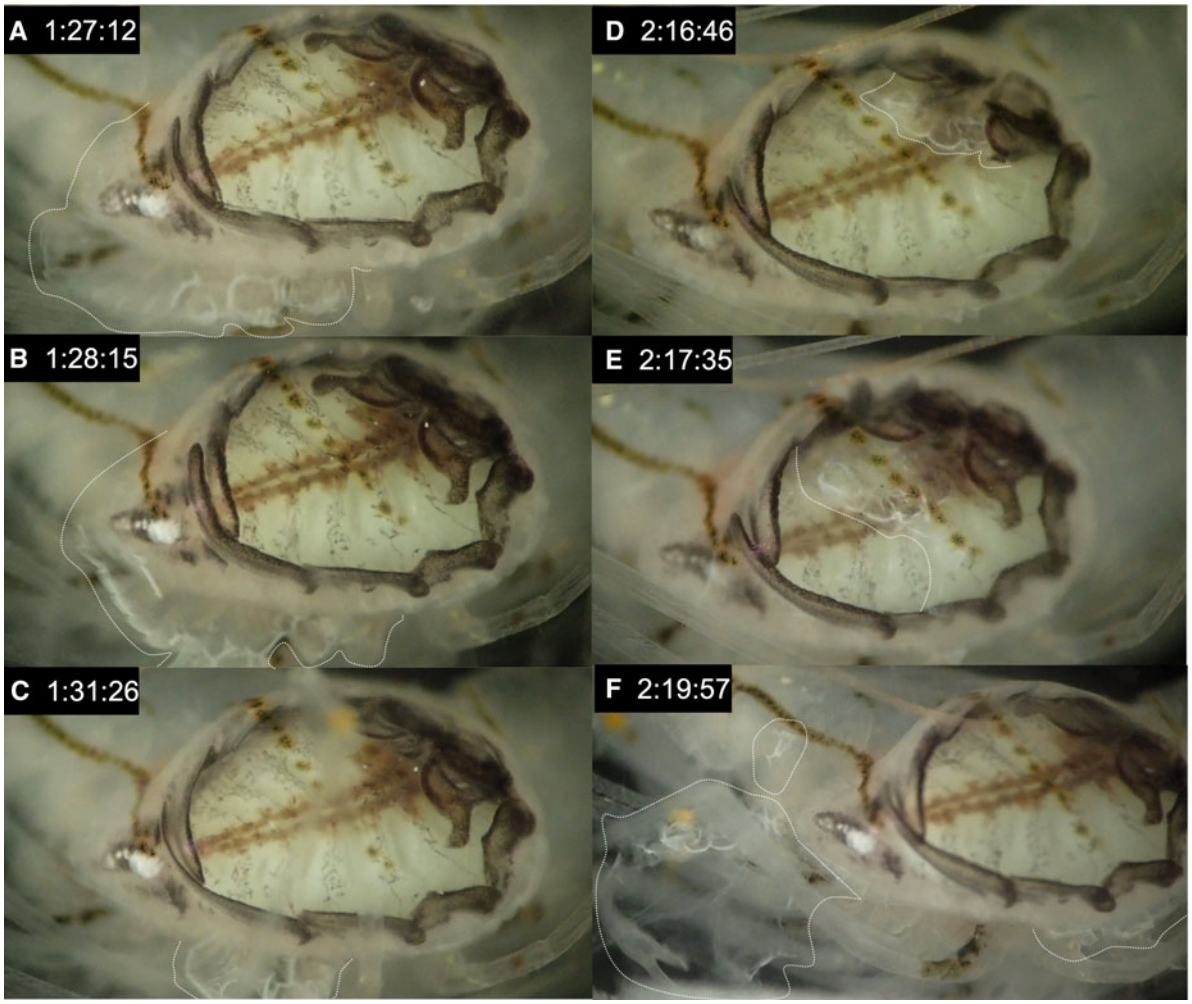

Figure 2. Moulting of female *B. crangorum*. Time elapsed after the host moulting is given. (A–C) Posterior moulting: (A, B) Cuticles from the 5th to the 7th pereon, pleon, and pleotelson loosen; (C) posterior cuticles were shed and exited the host's anterior branchial chamber. (D–F) Anterior moulting: (D) old cuticles of the head and maxillipeds start to moult; (E) anterior moult is almost complete from the head to the fourth pereon; (F) cuticles of the anterior body were shed and exited the anterior branchial chamber of the host. See also Supplementary Material 1: Video, 'Anterior moulting in female *B. crangorum*'.

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were observed until the cuticles loosened and were expelled through the anterior branchial chamber of the host (Supplementary material 1). In all cases, the posterior cuticle was shed in fragments (Figure 3A, B), whereas the cuticle of the anterior body was shed in one large piece (Figure 3C), except for pereiopods 1–4 and the lateral parts of the pereonites.

The mean duration to complete the posterior moult was 14.5 ± 12.0 min (range: 3.1–42.3 min), while on average, the anterior moult lasted for 25.2 ± 20.0 min (range: 3.7–66.1 min). The total time of moulting for female *B. crangorum* was 109.5 ± 40.2 min (range: 61.8–181.9 min) (Table 2). The estimated mean time that elapsed from host moulting to female *B. crangorum* moulting was 104.2 ± 31.1 min (range: 66.0–148.0 min). During the process of female moulting, the male remained in its original position between the pleopods of the female with little movement, and moulting behaviour was not observed in the male. Furthermore, after host moulting and before the posterior and anterior moults, the eggs were already visible through the exoskeleton of the thoracic segments.

Male behaviour in relation to insemination

In 13 recordings of post-moulting shrimp, the male was observed to move from its original position between the pleopods of the female (Figure 4a1 and 4b1) and stop at the anterior end of oostegite 5. The movement was recorded at 286.4 ± 98.6 min after host ecdysis and 126.8 ± 50.5 min after the anterior moulting of the female parasite. The sequence of behaviour was as follows: (1) The male moved anteriorly along the lateral surface of oostegite 5 (Figure 4a2 & 4b2); (2) the male stopped on top of the fifth oostegite with its pleotelson aligned to the anterior end between the junction of oostegites 4 and 5 just above the female gonopore (Figure 4a3 & 4b3); (3) after a few minutes, the male moved again anteriorly and either turned to the inner lateral side of the marsupium (Figure 4a4–a6), sometimes on top of oostegites 1 and 2, or in the centre of the marsupium, turning upside down (Figure 4b4–b6); (4) then, the male moved posteriorly and back to the pleopod (Figure 4a7, a8; Figure 4b7, b8). In four recordings, the male stopped at the inner anterior margin of oostegite 5 with its pleotelson directed towards the gonopore of the female before moving back towards the pleopod of the female.

The male repeated the sequence of behaviours described above on both the longer and shorter sides of the female in variable order and frequency. Furthermore, before each movement, the male always moved back to its original position between the female pleopods. The mean time spent by the male on the shorter and longer sides was 1.2 ± 0.5 min and 1.3 ± 2.0 min, respectively, with an average of two or three visits per side. The mean time interval between one cycle of male movement was 9.5 ± 9.0 min. The total time of male movement was 65.5 ± 43.5 min (Table 2).

Oviposition to the marsupium

Egg oviposition was observed in 12 recordings of post-moulted shrimp. This occurred 33.4 ± 20.7 min after the last movement of a male. The eggs were released through the gonopore at the anterior inner base of oostegite 5 (Figures 5 & 6B, C). Eggs were released asynchronously either on the shorter ($N = 5$) or longer ($N = 6$) side first. Synchronous release of eggs was observed in only one case. The mean time to complete oviposition on both sides was 19.3 ± 12.9 min, and the time interval of release between sides was 2.1 ± 2.2 min (Table 2). During the process of oviposition from start to finish, the male did not move from its usual position between the female pleopods, except in one case where the male stopped at the distal end of oostegite 5 (longer side) inside the marsupium and then moved back to its original position after oviposition was completed. The mean time elapsed from host moulting to oviposition by the female parasite was 369.1 ± 113.6 min (range: 184.1–589.1 min).

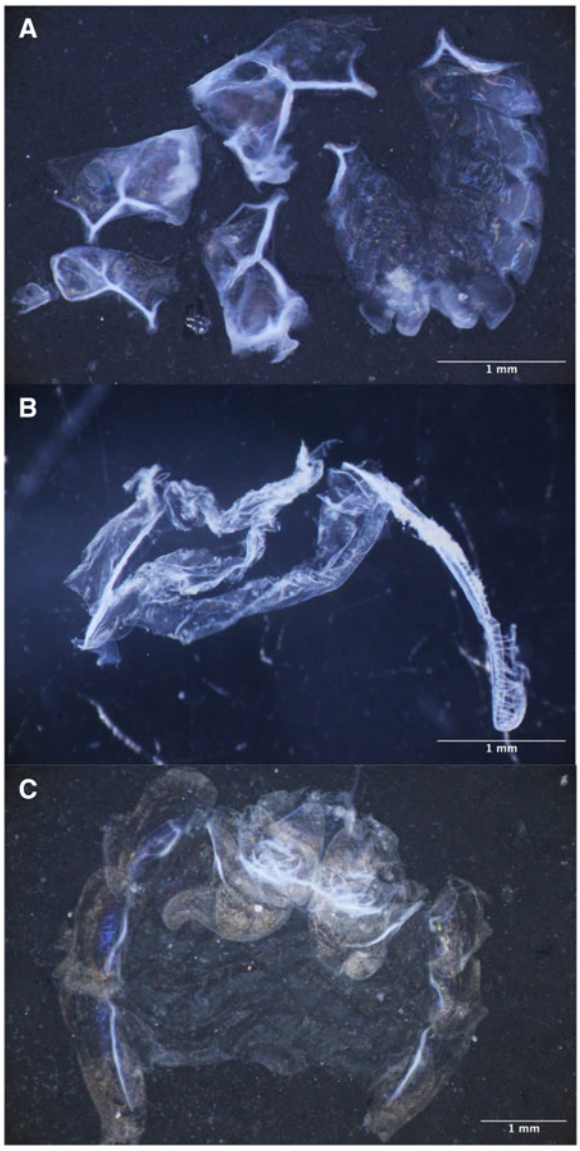

Figure 3. Exuviae of female *B. crangorum*. (A) Exuviae of pleon and part of dorsal pleopod; (B) exuviae of pereons 5–7 and oostegite 5; (C) exuviae of the anterior body.

Male-removal experiment

None of the eight female parasites with males removed after female ecdysis oviposited eggs until the next host moulting, which occurred 11 ± 1 days after male removal. In all cases, eggs were visible through the exoskeleton of the female thoracic segments. All eight female parasites with males removed after visits to the gonopore oviposited eggs. The percentage of oviposition significantly differed between treatments (Fisher's exact test; $P = 0.00016$) (Figure 7). Daily monitoring of the brood showed that all ovigerous female parasites completed embryonic development up to the epicardial stage and larval release, which occurred 10 ± 1 days after male removal. None of the females oviposited eggs again after subsequent moulting of the host, which occurred 12 ± 2 days after male removal. The duration of host moulting was not significantly different between treatments (U-test; $P > 0.05$) (Figure 7).

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Table 2. The summary of duration and time intervals for each reproductive behaviour of *B. crangorum* (*n*, number of observations; min, minimum; max, maximum; SD, standard deviation)

Reproductive behaviours	Mean ± SD	Min	Max	*n*
1. Female moulting				
Posterior moult (min)	14.5 ± 12.0	3.1	42.3	11
Anterior moult (min)	25.2 ± 20.0	3.7	66.1	11
Interval between moult (min)	72.9 ± 27.9	15.1	115.1	11
Total moulting time (min)	109.5 ± 40.2	61.8	181.9	11
-Interval from moulting to male movement (min)	126.8 ± 50.5	66.0	235.0	
2. Movement of male				
Duration during each visit in the shorter side (min)	1.2 ± 0.5	0.3	2.2	26
Duration during each visit in the longer side (min)	1.3 ± 2.0	0.1	12.8	39
Time interval between moves (min)	9.5 ± 9.0	0.1	44.6	49
Number of visits along the shorter side	2.0 ± 1.0	1.0	3.0	13
Number of visits along the longer side	3.0 ± 1.0	1.0	7.0	13
Total time of male movement (min)	65.5 ± 43.5	20.8	153.2	
-Interval from male movement to oviposition (min)	33.4 ± 20.7	2.3	85.0	
3. Oviposition (min)	19.3 ± 12.9	5.5	39.0	12
Interval of oviposition between sides (min)	2.1 ± 2.2	0.0	7.4	12

Discussion

The reproductive behaviour of bopyrid isopods, particularly the insemination behaviour of dwarf males, has rarely been reported. In the present study, video observations of *B. crangorum* showed that the male repeatedly moved from its initial position between the female pleopods and stopped at the anterior end of oostegite 5 just above the gonopore. Movement was recorded after female ecdysis and immediately before oviposition. Cash and Bauer (1993) only observed male *P. pandalicola* within and on the lateral surface of the marsupium near pereiopod 5; however, recurrent

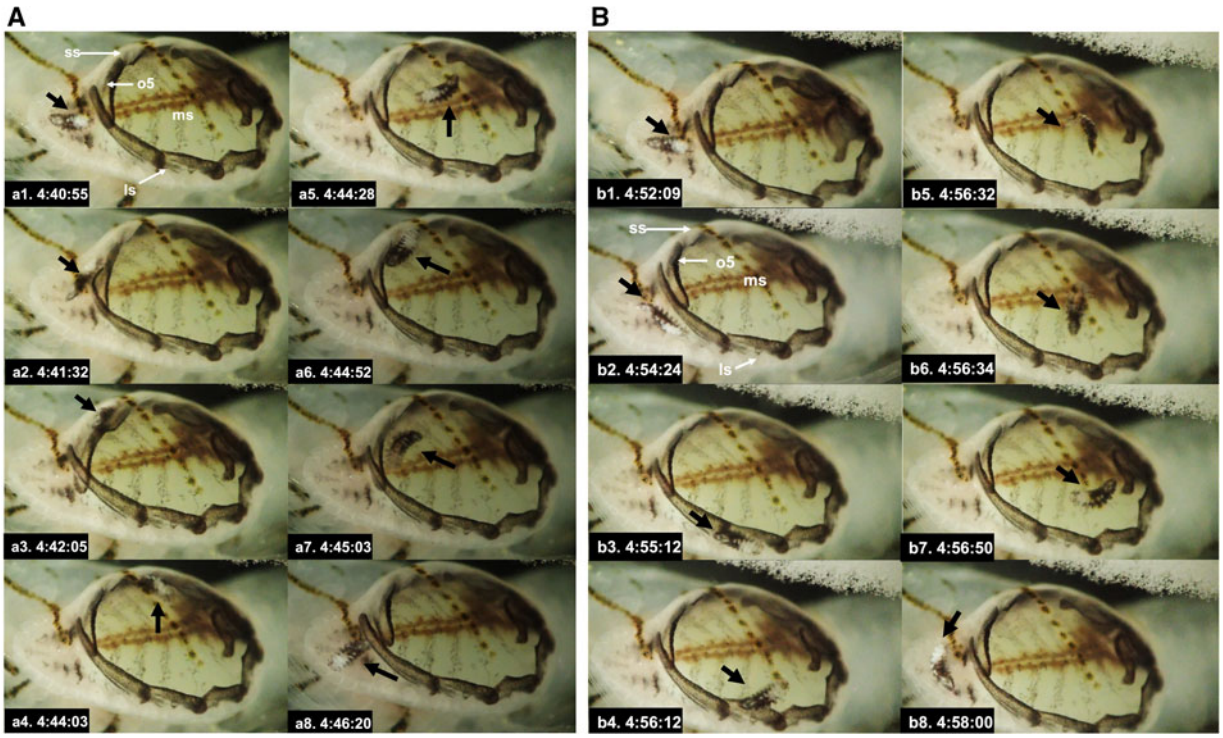

Figure 4. Sequence of male behaviour after the anterior moult and prior to oviposition in female *B. crangorum* (a. shorter side: b. longer side). The time elapsed after host moulting is provided. 1. Male in its original position between the pleopods of the female. 2. Male started to move anteriorly along oostegite 5 on the shorter side of female. 3. Male stopped on the anterior end of oostegite 5, pleotelson aligned in the junction of oostegites 5 and 4 above the gonopore. 4 and 5. Male started to move again and turned towards the marsupium of the female. 6. Male moved to the inner anterior base of oostegite 5. 7. Male began to move posteriorly. 8. Male back on pleopod of female. ms, marsupium; o5, oostegite 5; ss, shorter side; ls, longer side. See also Supplementary material 2: Video, 'Male behaviour in relation to insemination'.

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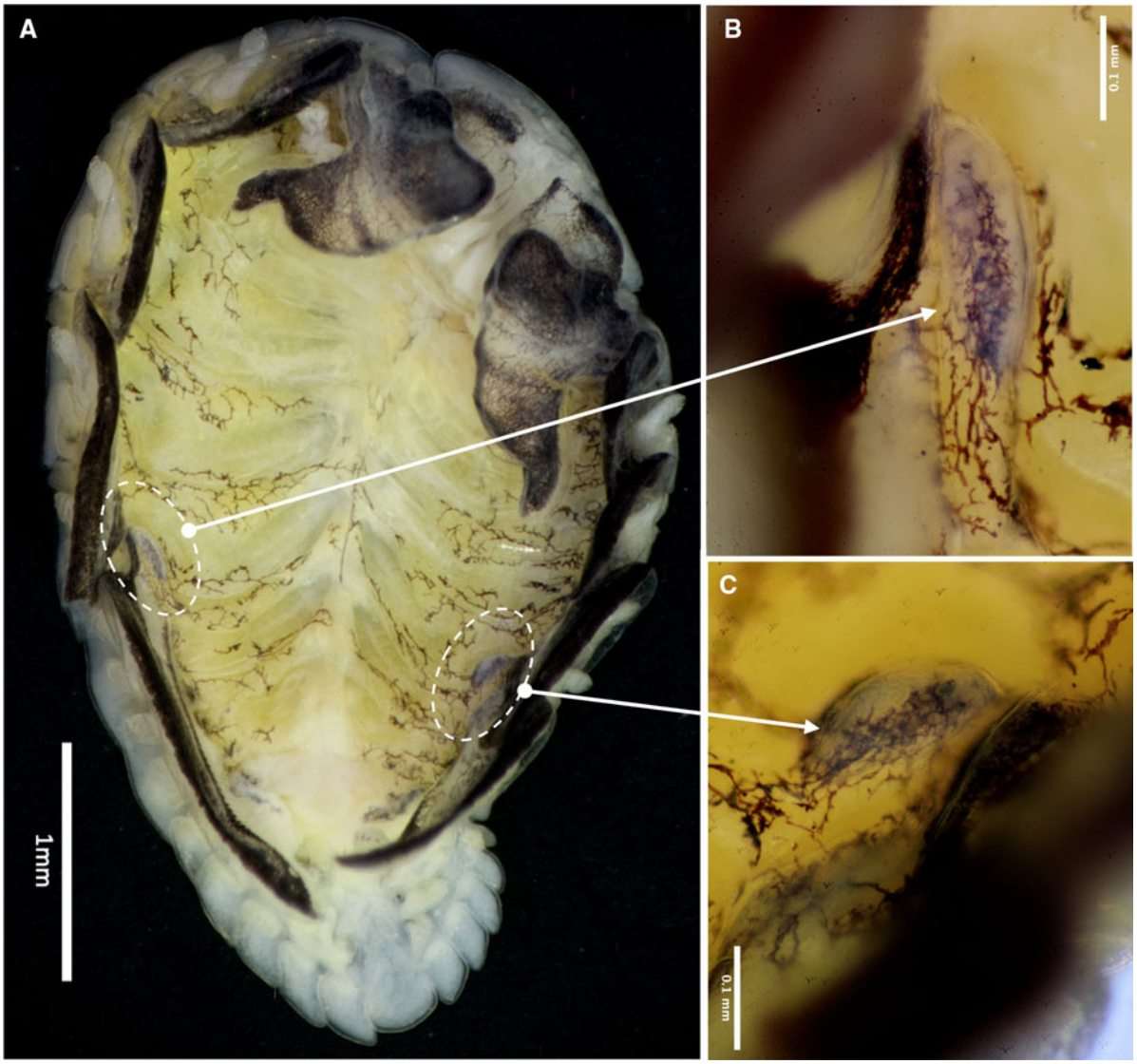

Figure 5. (A) Ventral surface of female *B. crangorum* showing the gonopores (genital aperture) in dashed circle on the fifth thoracic segment near the base of oostegite 5. (B, C) Closer view of the gonopores in the longer and shorter sides, respectively.

movement on both sides of the female was not documented. Males were previously considered to inseminate females either by (1) depositing sperm into the female gonopore (Cash and Bauer, 1993) or (2) releasing sperm inside the marsupium (Hiraiwa, 1936). The observations of the present study validate the first hypothesis. The repeated stops of the male *B. crangorum* on the anterior end of oostegite 5 were likely attempts to inseminate females through each gonopore. Conversely, the absence of male movement during and after oviposition contradicts the idea of simultaneous fertilisation. Although males were observed for a longer period inside the marsupium, this was always preceded by stops at the anterior end of oostegite 5. The reason the male always returned to its initial location before each movement is unknown but may be related to its proximity to the gonopore.

Oviposition by female *B. crangorum* into the marsupium was asynchronous. Although Hiraiwa (1936) suggested simultaneous oviposition, the 2 min delay in oviposition between the longer and shorter sides precludes simultaneous fertilisation. Observation of oviposition also confirmed the location of female gonopores, as noted in *Aparapenaeon japonica* (Hiraiwa, 1934) and *Bopyrina abbreviata* (Romero-Rodríguez *et al.*, 2016). The apertures are seen as two unequally sized clefts on the lateral and ventral sides of the fifth thoracic segment at the base of the oostegites. This feature appears to be common in bopyrids infesting the branchial chamber of hosts regardless of intraspecific differences. Unfortunately, the male genital aperture was not observed in *B. crangorum*, likely because of the minute size of its structure, as noted by Hiraiwa (1934). In most isopods, males have penes and an appendix masculina in the second pleopod, which are used for copulation and sperm transfer (Wilson, 1991). Females are inseminated internally through each gonopore via separate acts of copulation (Johnson, 1985). These structures are absent in male bopyrids, and females lack receptacles for sperm storage (Hiraiwa, 1934, 1936). Thus, based on the present observations, it can be inferred that the sperm is released directly onto the external surface of each gonopore, and that the eggs are fertilised as they pass through the opening. This may explain the synchronous development of embryos inside the marsupium (Beck, 1980b; Cash and Bauer, 1993; Brinton and Curran, 2015). However, further studies are needed to confirm this hypothesis. Histological examination of the female gonopore may confirm the presence of sperm prior to oviposition. The absence of the appendix masculina is uncommon in isopods and has only been reported in *Sphaeroma terebrans*, in which fertilisation occurs after the male releases sperm into a water current generated by the female pleopod (Messana, 2004).

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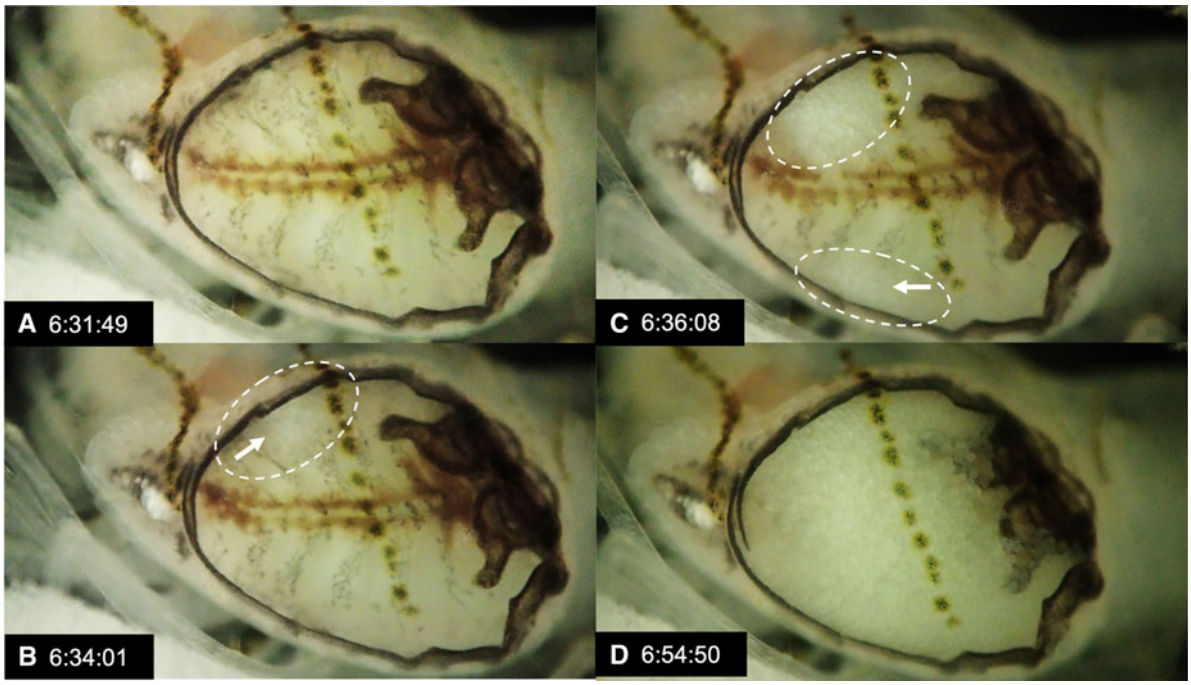

Figure 6. Video images of oviposition in the marsupium in female *B. crangorum* after male movement. Time elapsed after the host moulting is given. (A) Empty female marsupium prior to oviposition. (B) Mass of eggs released through the anterior inner base of oostegite 5 on the shorter side of the female. (C) Eggs were also released on the longer side of the female. (D) Oviposition completed. See also Supplementary material 3: Video, 'Oviposition in *B. crangorum*'.

The results of the male-removal experiment confirmed that eggs were fertilised following male movement. Despite the presence of oocytes, which were visible through the exoskeleton, females with males removed before male movement did not oviposit eggs. This indicates that the female bopyrid must be inseminated before oviposition and cannot store sperm (Hiraiwa, 1936; Cash and Bauer, 1993). Similar trends were observed in the free-living isopods *Asellus aquaticus* and *Tylos granuliferus* (Thompson and Manning, 1981; Suzuki *et al.*, 2013) and in the parasitic gnathiid *Elaphognathia cornigera* (Tanaka, 2019). Suzuki *et al.* (2013) suggested that male *T. granuliferus* secrete copulatory chemicals that stimulate oviposition in females, whereas in *A. vulgare*, physical stimulation of the female genital apparatus was proposed (Caubet *et al.*, 1998; Lefebvre and Caubet, 1999). In bopyrid isopods, the absence of a copulatory organ suggests that the stimulation for oviposition might involve hormonal release during insemination rather than physical, as hypothesised in *T. granuliferus* (Suzuki *et al.*, 2013). The presence of oviposition-stimulating factors in males has been reported in some species of insect (Lange and Loughton, 1985; Yi and Gillott, 1999). However, thus far, no such factors have been identified in crustaceans (Suzuki *et al.*, 2013). Further studies are needed on this aspect, particularly in parasitic isopods, which exhibit simplified reproductive structures compared to their free-living counterparts.

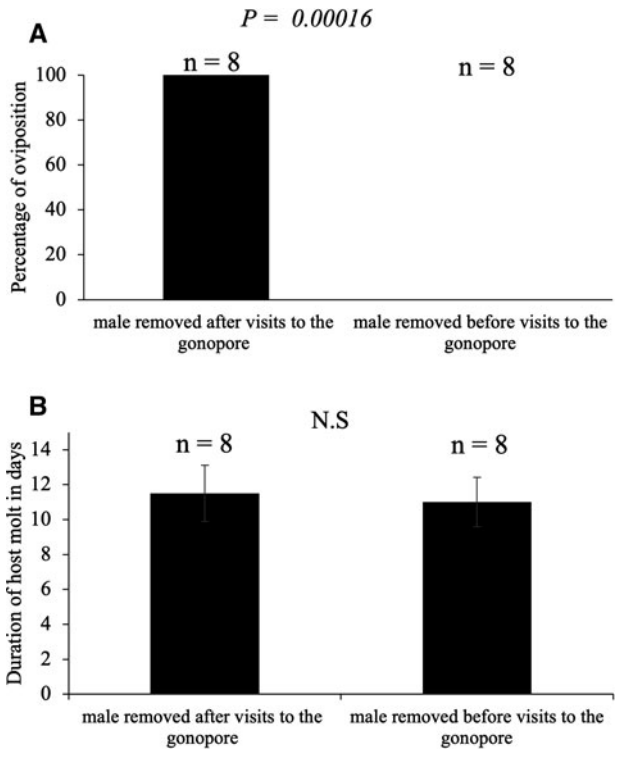

Figure 7. Results of male-removal experiment. (A) Percentage of oviposited female between the treatments. (B) Duration of host moulting between the treatments.

The influence of males on bopyrid ovarian maturation has been reported by Schuldt (1993) and Romero-Rodríguez *et al.* (2016), but each study showed contrasting results. Schuldt (1993) reported that the absence of male *Probopyrus ringueletti* prevented the completion of vitellogenesis, whereas in *B. abbreviata*, lone mature females showed oocytes with secondary vitellogenesis (Romero-Rodríguez *et al.*, 2016). Although the study did not test the influence of male on ovarian development, the results showed that females did not oviposit without insemination. This finding could explain the observed pattern in *B. abbreviata*, where mature oocytes were present in lone mature females. Additionally, Romero-Rodríguez *et al.* (2016) observed reabsorption of some oocytes in secondary vitellogenesis in the same female, which could explain the fate of undeposited eggs in bopyrid isopods. Oosorption due to the absence of males has been reported in females of the isopod *Thermosphaeroma thermophilum* (Jormalainen *et al.*, 1999).

The moulting process in parasitic isopods has only been described in the cymothoid *Mothocya renardi* in which all life

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stages undergo biphasic moulting, except for the free-living manca 1 (Panakkool-Thamban and Kappalli, 2020). Female *B. crangorum* exhibit the same biphasic moulting pattern as other isopods, where the posterior body cuticle moults first, followed by the anterior body cuticle (Carlisle, 1956; Vittori *et al.*, 2012). In other isopods, both the anterior and posterior cuticles of the body are shed in fragments (Tait, 1917; Panakkool-Thamban and Kappalli, 2020) or cast off in one piece (Carlisle, 1956). In the present study, the cuticles of the posterior body of *B. crangorum* were shed in fragments, whereas the anterior body was shed in one large piece. The interval between the posterior and anterior moults was comparable to that from a single observation reported by Cash and Bauer (1993) in *P. pandalicola,* which was 2.5 h. However, this period was much shorter than that of free-living isopods, which can last anywhere from 1 to 5 days (Tait, 1917; Johnson, 1985; Vittori *et al.*, 2012; Montesanto and Cividini, 2018), aquatic isopods, which can be 24 h (Marcus, 1990), and parasitic cymotoid isopods, which can be 2–3 days (Panakkool-Thamban and Kappalli, 2020). The shorter moulting cycle of bopyrid isopods is likely an adaptive strategy to deal with the shorter time required to complete the reproductive cycle before the next host ecdysis to avoid expulsion. Although it was previously noted that male parasites moult (Walker, 1984), moulting of male *B. crangorum* was not observed. The male did not move from its initial position between the female pleopods throughout female ecdysis, supporting the earlier hypothesis that migration to another host is unlikely, despite the mobility of the male (Itani *et al.*, 2002).

Based on the daily observations, the total incubation time of female *B. squillarum* at 24–26°C was slightly shorter than that observed in *P. pandalicola* infesting the grass shrimp *Palaemonetes paludosus* (11–14 days at 22–24°C) and *P. pugio* (11 days at 23°C) (Beck, 1980b; Brinton and Curran, 2015). This difference was probably due to the host species and environmental factors, particularly temperature, as the incubation period is shortened at higher temperatures (Brinton and Curran, 2015). The period between larval release and subsequent host moulting in the current study was within the range reported by Cash and Bauer (1993) (6–120 h) but longer than that observed by Brinton and Curran (2015) (0–2 days at 23°C). In contrast, the interval between host moulting and oviposition was shorter in *B. squillarum* (3–10 h) than in *P. pandalicola*, which reported a range of 6–24 h (Cash and Bauer, 1993).

In summary, although the animals were filmed while in a dish and may have deviated from the conditions in the rearing tank and their natural habitat (e.g. higher temperature or light exposure). All reproductive behaviours were consistently observed in all video recordings. The duration of each reproductive event varied but was comparable to the findings of Cash and Bauer (1993) and Brinton and Curran (2015), which indicated that these behaviours normally occur in bopyrid isopods. The present study confirmed that, similar to the closely related species *P. pandalicola*, the reproductive activities of *B. crangorum* (i.e. female moulting, male activity, and oviposition) were completed hours after host moulting. This is likely an adaptive strategy to survive the repeated moulting of the host and the typical pattern of bopyrid isopods infesting the host's branchial chamber. For a definitive conclusion, it is necessary to observe the reproductive behaviour of abdominal bopyrids because they have different body configurations and attachment sites. Based on the behaviour of males, insemination in *B. crangorum* likely occurs externally through female gonopores rather than by releasing sperm inside the marsupium. When male movement was not observed, the females did not oviposit their eggs, which suggest that females must be inseminated prior to oviposition. Although the mechanism of sperm transfer was not confirmed, these results provide new information regarding the insemination behaviour of male bopyrids parasitising the branchial chamber of their host. Further studies are needed to determine the mechanism of sperm transfer and how the parasite detects cues for host moulting.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315423000565.

Acknowledgements. We wish to thank the members of the Laboratory of Marine Symbiotic Biology, Kochi University, for providing assistance with the field sampling. We are also grateful to Dr Y. Henmi (Kyoto University) and Dr S. Kappalli (Central University of Kerala), whose feedback substantially improved the investigation. We acknowledge the insightful comments and suggestions of two anonymous reviewers.

Author's contributions. LAB performed the laboratory observations, data analysis, and writing the original draft. GI participated in the conceptualisation, the interpretation of the results, and revision of the manuscript. All authors read and approved the final manuscript.

Financial support. This work was supported by JSPS KAKENHI grant number JP21K05635 and JSPS Bilateral Program grant number JPJSBP120207715 to GI.

Competing interests. None.

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