Precopulatory mate guarding and mating in *Tachidius discipes* (Copepoda: Harpacticoida)

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Abstract

Precopulatory mate guarding and mating behaviour of *Tachidius discipes* Giesbrecht, 1881, has been analysed using a video camera mounted on a microscope. Males preferably accompany fourth or fifth stage female copepodids during precopulatory mate guarding; only rarely are third copopodid stages guarded. Rare cases of males clasping juvenile males are also known. Males are active during mate guarding and constantly change the site of attachment of their antennules. This behaviour may ensure continued association of the adult male with the juvenile copepodid when the latter moults during the mate guarding phase.

The final moult of the female, once observed only, lasts only 2 minutes. Copulation immediately follows but is preceded by the male's control of the genital field of the female. This is done by the second, third and fourth legs of the male sliding over the female's genital field. Males copulate only with virgin females. A male will also copulate with a female that he has not guarded during her juvenile state. Previously inseminated adult females are rejected. Their condition is recognized even when the spermatophore has fallen off and when no egg sac is carried. Copulation takes only a few seconds. The spermatophore is transferred without the aid of any appendage and the male separates from the female shortly afterwards.

Males of *T. discipes* were found to have either one or two spermatophores in their gonoduct. Those with two were able to inseminate two females within about two to three hours. After the first or, in the case of a male with two spermatophores, after two copulations, 12.5 to 24 hours elapsed before he was able to inseminate another virgin female. Females are passive when investigated by a male and make no attempt to repel him. In *T. discipes*, a species exhibiting typical precopulatory mate guarding, male choice is a strongly expressed behaviour while female choice is not expressed.

Zusammenfassung

Das Paarungsverhalten von *Tachidius discipes* Giesbrecht, 1881, wurde mit Hilfe mikroskopischer Videoaufzeichnungen analysiert. Bei der Präkopula begleiten Männchen bevorzugt Weibchen des vierten oder fünften Copepodidstadiums und nur selten solche des dritten Copepodidstadiums. Gelegentlich wurden sogar juvenile Männchen von adulten Männchen gegriffen. Männchen sind während der Präkopulaphase sehr aktiv und verändern ständig die Griffposition der ersten Antennen. Dieses Verhalten mindert vermutlich die Gefahr, das jugendliche Weibchen zu verlieren, wenn es sich häutet.

Die Erwachsenenhäutung des Weibchen (nur einmal beobachtet) dauert nur 2 Minuten. Auf sie folgt die Kopulation, nachdem eine Genitalfeldkontrolle durch das Männchen stattgefunden hat. Diese wird mit den zweiten, dritten und vierten Beinpaaren des Männchens durchgeführt. Männchen kopulieren ausschließlich mit jungfräulichen Weibchen, sogar mit Weibchen die sie nicht in Präkopula begleitet haben. Bereits inseminierte Weibchen werden schnell erkannt, selbst wenn keine Spermatophore auf dem Geschlechtsfeld vorhanden ist und kein Eisack getragen wird, und wieder losgelassen. Die Kopulation dauert nur wenige Sekunden. Bei der Spermatophorenanheftung spielen die Extremitäten keine Rolle. Unmittelbar danach trennt sich das Männchen von dem Weibchen.

Es kommen Männchen sowohl mit einer als auch mit zwei Spermatophoren im Gonodukt vor. Solche mit zwei Spermatophoren können zwei Weibchen hintereinander innerhalb von zwei bis drei Stunden begatten. Nach der ersten bzw. im Falle von Männchen mit zwei Spermatophoren, nach der zweiten Kopulation, vergehen 12,5 bis 24 Stunden, bis diese Männchen erneut Weibchen inseminieren können. Weibchen verhalten sich während der Untersuchung durch das Männchen passiv und machen keinen Versuch, es abzuwehren. Es wird angenommen, daß bei *T. discipes*, als einem typischen Repräsentanten der Präkopulastrategie, männliches Wahlverhalten stark betont ist, während weibliches Wahlverhalten fehlt.

Introduction

Precopulatory mate guarding is of widespread occurrence within the Copepoda Harpacticoida. Adult males cling to female copepodids until the final moult after which copulation takes place (see Boxshall, 1990, for review). Such behaviour is interpreted as a strategy on the part of the male to monopolize a virgin female for insemination (Ridley, 1983; Burton, 1985). Published data on precopulatory mate guarding usually refer to the identity of copopodid stages found in association with males (see Kern et al., 1984) but very little is known about the behaviour of the pair during the long period of mate guarding. This has been called a "precopulatory passive phase" (Lazzaretto et al., 1993; 1994) suggesting that the partners are inactive during the association. However, males of *Tigriopus fulvus* Fischer, 1860, have been reported by these same authors to reject younger copepodids if more advanced female copepodids are encountered (Lazzaretto et al., 1993) indicating that males actively interrogate potential mates during this association. Burton (1985) observed similar behaviour of *Tigriopus californicus* Baker, 1912.

Only a few reports mention female or male behaviour when a female moults during precopulatory mate guarding, although this is one of the most critical moments during an association which may last for many days. A male's investment of time ranging from several days to over two weeks, as in *Tigriopus fulvus* (cf. Lazzaretto et al., 1994), can be wasted if the male looses the female during her moulting cycle. In *Tigriopus* spp., even first or second female copepodid stages (C I, C II) are clasped by the males (Ito, 1970; Koga, 1970; Takano, 1971; Burton, 1985; Lazzaretto et al., 1990) so there may be up to five moults of the female before copulation can take place.

Males of many species of harpacticoids clasp juvenile females with their antennules; however, the location on the female differs between different taxa. Males of the Laophontidae clasp the exopod of the fourth legs of the juvenile female (Lang, 1948), while males of *Coullana* sp. (Canuellidae) (T. Snell, pers. comm.), of *Mesochra*

lilljeborgi Boeck, 1864 (Canthocamptidae) (cf. Klie, 1950; Nilsson, 1987), and of *Huntemannia jadensis* Poppe, 1884 (Huntemanniidae) (cf. Feller, 1980) clasp the female's furca. Males of some Cletodidae, Harpacticidae, and Tachidiidae clasp the females at the posterior margin of the cephalothorax (Lang, 1948). This is the mode also displayed by males of *Tachidius discipes* Giesbrecht, 1881 (cf. Olofsson, 1917), but Willey (1929), who made live observations, and Dahms (1985), counting preserved material of pairs in precopulatory association, also found a few males embracing the female's third or fourth thoracic segments.

The final moult of the female is followed by copulation (Boxshall, 1990), but for this rather rapid process only a few observations have been reported for species exhibiting precopulatory mate guarding, as in *Harpacticus uniremis* Kröyer, 1842 (Harpacticidae) (cf. Williams, 1907), *Huntemannia jadensis* (cf. Feller, 1980), *Mesochra lilljeborgi* (cf. Nilsson, 1987), and *Harpacticella inopinata* Sars, 1908 (cf. Evstigneeva, 1993). In contrast, mating behaviour, including copulation, of harpacticoid species with postcopulatory mate guarding has been described in detail for four species of *Tisbe* Lilljeborg, 1853 (Tisbidae) and for *Paramphiascella fulvofasciata* Rosenfield & Coull, 1974 (Diosaccidae) (cf. Dürbaum, 1995). Males of these species engage in courtship behaviour after clasping exclusively adult females. In some species, females were able to rid themselves of the males already attached to them (Dürbaum, 1995).

In order to understand the reproductive biology of harpacticoid copepods, it is important to know if males can produce more than one spermatophore enabling them to inseminate more than one female (Boxshall, 1990). Johnson & Olson (1948) observed a male of *Tisbe furcata* (Baird, 1837) that mated with at least six females. Haq (1972) noted that males of *Euterpina acutifrons* Dana, 1848, are able to inseminate up to 11 females within 15 days. He observed the production of a second spermatophore in one male that took between 18 and 28 hours, yet it took another 20 hours before the second copulation was achieved. Burton (1985) found that males of *Tigriopus*

californicus were able to inseminate two to four females within three days, and Lazzaretto et al. (1994) also concluded from their observations on *Tigriopus fulvus* that males probably mate more often than females. Even more sparse is information on multiple mating of a single female with different males. This has been excluded for *Tigriopus californicus* (cf. Burton, 1985), but there are reports of females of different harpacticoids carrying more than one spermatophore (Krüger, 1911; Donner, 1928; Muus, 1967; Dürbaum, 1995).

In focus of the present study are behavioural observations of precopulatory mate guarding of *T. discipes* from field collected material and the analysis of mating behaviour with material originating from laboratory cultures by the aid of video equipment. Both of these issues are supported by morphological studies of fixed material, including precopulatory mate guarding pairs for SEM. Two experimental tests have been set up with the aim to find out, first, if males of *T. discipes* mate more than once and how often new spermatophores can be produced, and second, if males prefer certain types of females.

Materials and methods

Tachidius discipes was collected in the Wadden Sea south of the Island of Wangerooge in February 1993. The upper 5–10 mm of sediment was scraped off with a small plastic container and washed in a bucket of seawater. This water was filtered through a 50 μm gauze. The copepods were transported to the laboratory in containers with a little sediment. Observations made by placement of whole culture bowls under the stereo microscope revealed the manner of contacting juvenile females and the origin of a precopulatory mate guarding association. All couples of *T. discipes* in the phase of precopulatory mate guarding were sorted out under a stereo microscope and transferred to single petri dishes (volume 5 ml) filled with filtered seawater and a small amount of sediment. Determination of female copepodid stages was possible by checking the couple alive immediately after the transfer to the petri dish. All dishes were monitored under a stereo microscope for several hours a day to observe the moulting of the copepodids during mate guarding.

In order to observe copulatory behaviour, cultures of *T. discipes* were started from single females with egg sacs. These were isolated in small bowls containing about 200 ml of seawater. The bottom of the bowl was covered with heat-sterilized sediment from the original locality. The nauplii and

copepodids were fed with a mixture of dried and pulverized green algae (*Ulva* sp., *Enteromorpha* sp.) and red algae (*Ceramium* sp.). Copepodids that had reached the third or fourth stage were separated to prevent copulation. After they had reached the adult stage (= C VI) a female and a male were transferred to an observation chamber placed under a Dialux 20 EB microscope (Leitz) equipped with a CCD video camera. A professional SVHS video recorder was used to record and analyse the mating behaviour. This analysis was done by studying the recordings frame by frame on a high resolution monitor.

For SEM observation, animals were fixed in formaldehyde and dehydrated in alcohol. Several pairs in precopulatory mate guarding were anaesthetized with carbon dioxide or quickly frozen at –50 to –60°C. After thawing they were dehydrated in alcohol, dried with a critical point drying unit, mounted on SEM dishes and sputtered with gold.

Experiment 1: To test copulation frequency and spermatophore production of individual males, 10 males (4 with one spermatophore and 6 with two spermatophores visible in the gonoduct) were chosen that had copulated once. These were isolated from the females immediately after copulation and returned to their original petri dish. They were kept for different periods of time before being put into an observation or filming chamber containing a new adult virgin female in order to find out at which time they are able to copulate again. Males of *T. discipes* accept virgin adult females and readily mate with them if the opportunity to do so arises. Video recording was carried out from first contact of the male to the end of copulation.

Experiment 2: To test the ability of a male to distinguish a female at different stages of her reproductive cycle, a virgin male with a visible spermatophore in his gonoduct was placed with one of four kinds of adult females: (a) an inseminated female with a spermatophore on her genital field, (b) an inseminated female without an attached spermatophore, (c) an inseminated female carrying an egg sac (the spermatophore had already fallen off), and (d) a virgin female with an egg sac (if not inseminated, adult females produce a small egg sac after about one week in isolation). If no copulation was observed, the same male was brought together with an adult virgin female to test his ability to mate.

Results

Behaviour of single males

Males not involved in precopulatory mate guarding (*n* = min. 50) were observed in culture bowls to contact nearly every conspecific individual, juvenile or adult. Males use their clasping antennules for grasping the abdominal segments or cephalothorax of other individuals. Often only one antennule was used for grasping while the other was moved back and forth under the ventral
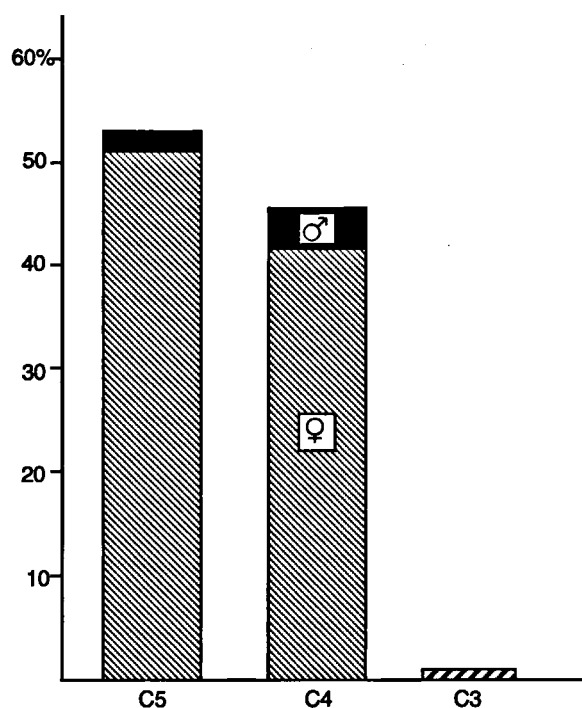
Fig. 1. Percentages of copepodid stages of *Tachidius discipes* found in association with males during precopulatory mate guarding (*n* = 357). The third copepodid stage (C III) could not be sexed.

side of the clasped individual. The position of the antennules is changed continuously. When gripping, the terminal segment of an antennule is pressed against the preceding bulbous segment thus enclosing the margin of any grasped body part. The decision whether to stay with the grasped individual for a longer time or to release it is a matter of seconds. If a male grasps another male with his antennules he usually releases within seconds (*n* = min. 25; for exceptions see under precopulatory mate guarding). When grasping adult females or copepodids (C I–C V) the male takes about 30 seconds to investigate the partner (*n* = min. 100). Very young copepodids (C I–C II) are usually released within the next 5 minutes.

Precopulatory mate guarding

When a male grasps a third to fifth copepodid stage female that is smaller than the male, they usually stay together until the final moult of the

female and subsequent copulation (this could be concluded from a spermatophore on the female's genital field). Precopulatory mate guarding was found to last about 30 to 48 hours when a copepodid IV was clasped (*n* = min. 30), and between 2 and 15 hours (*n* = min. 25) when a copepodid V was clasped. Fig. 1 shows the incidence of copepodid stages involved in precopulatory mate guarding. In 6% of all isolated pairs (*n* = 357), males attached themselves to juvenile males. Such pairs seemed to be as stable, i.e., they did not separate within a short range of time, as associated between males and female copepodids. In two cases, males stayed with their male partners from the fourth copepodid stage to the final moult of this partner, but a spermatophore was not transferred.

Males hold the margins of the copepodid's cephalothorax (Figs. 2 and 4A) or, more often, embrace the fourth and fifth thoracomere or the sixth thoracomere (Fig. 3). When grasping the sixth thoracomere, the distal segments of the male antennules are held ventrally in front of the female's fifth legs (Fig. 5). This grip is not permanent; position of the antennules frequently switches from the cephalothorax to the succeeding segments and vice versa. While antennules are used for clasping, the male's maxillipedes are held outstretched along the copepodid's abdomen or thorax (see Fig. 4A) and are also in continuous motion and maintain permanent contact with the female's body. The clasped copepodids are kept in check by constant adjusting movements of the antennules and maxillipedes. Third or fourth stage copepodids do not move much, whereas fifth female copepodids swim more actively than the attached males which are often dragged along. In culture bowls, males have never been seen feeding during precopulatory mate guarding, possibly because the dorsal surface of the copepodid lies close to the male's mouthparts.

The final moult of the female

This process was observed once. The male had fastened to the cephalothorax of a female copepo-
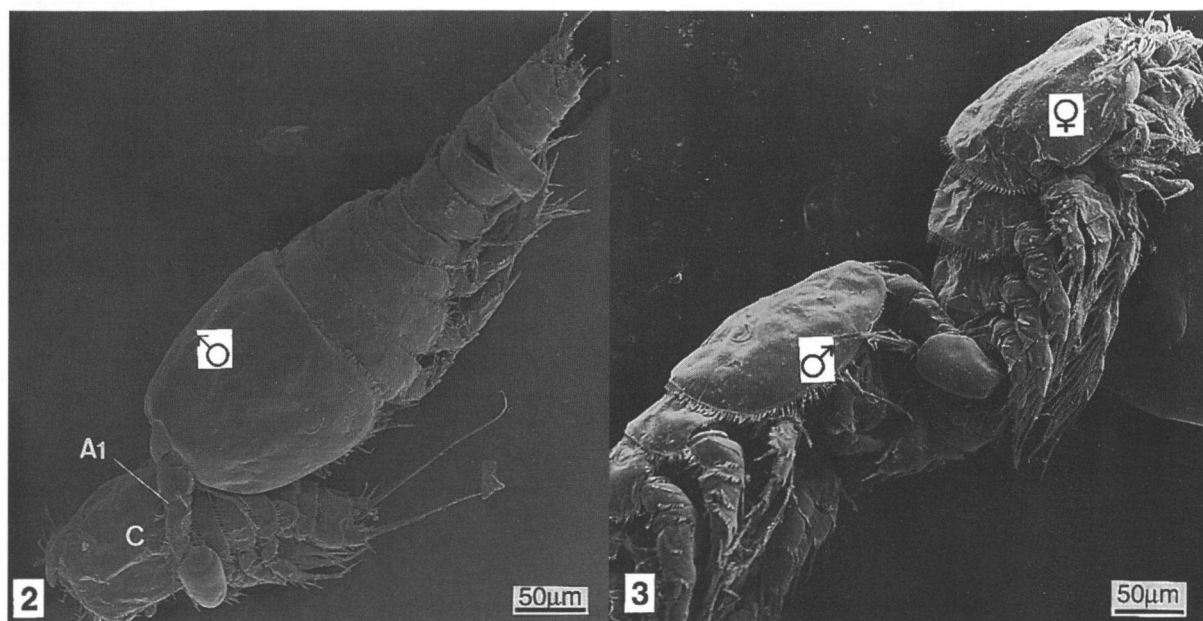
Fig. 2. Pair of *T. discipes* during precopulatory mate guarding: male clasps copepodid III (C) at margin of cephalothorax by the antennules (A1).
Fig. 3. Pair of *T. discipes* during precopulatory mate guarding: male clasps female (C V) around first abdominal segment.

did V, which remained on the bottom of the petri dish while the cuticle of the frontal part of the cephalothorax split open along the anterior-posterior body axis. The male reacted by changing his position of attachment to the cephalothorax every few seconds. Within the next two minutes the female emerged from the exuvium by extensions and contractions of the body. Strong peristaltic movements of her intestine were observed. As soon as the female left the old cuticle of the cephalothorax, the male switched his attachment to the new cuticle by alternating grips of the antennules (Fig. 4B). These movements continued until the female was completely free of the exuvium. Two minutes later the male climbed along the female's dorsal side down to the furca, assumed the copulation position, and, after checking the female's genital field, copulated (explained in detail below). Such mating behaviour appeared to be identical with that observed in experiments where males had been offered adult virgin females. Not all males may succeed in reclasping the female emerging from the old cuticle. Two of the 357 males found in precopu-

latory mate guarding were found swimming around with the exuvium (Fig. 6). In both cases the males were clasping the thoracic segments of the exuvium with their antennules.

Mating behaviour

Mating was filmed in 32 cases in which a virgin male had been offered to an adult virgin female. Four distinctive processes can be recognized:
(1) Shifting onto the ventral side of the female: Only seconds after a male has clasped an adult female around the cephalothorax, he starts climbing down her body by alternate grips of the antennules until he reaches the last abdominal somite or the furca (Fig. 4C). Here he loosens one of the antennules, the attached antennule is twisted ventrally and he swings to the ventral side of the female's abdomen. This exercise is accompanied by strong paddling movements of the legs (Fig. 4D shows direction of paddling movements). What follows depends on the orientation of the female's legs. If the female's abdomen is
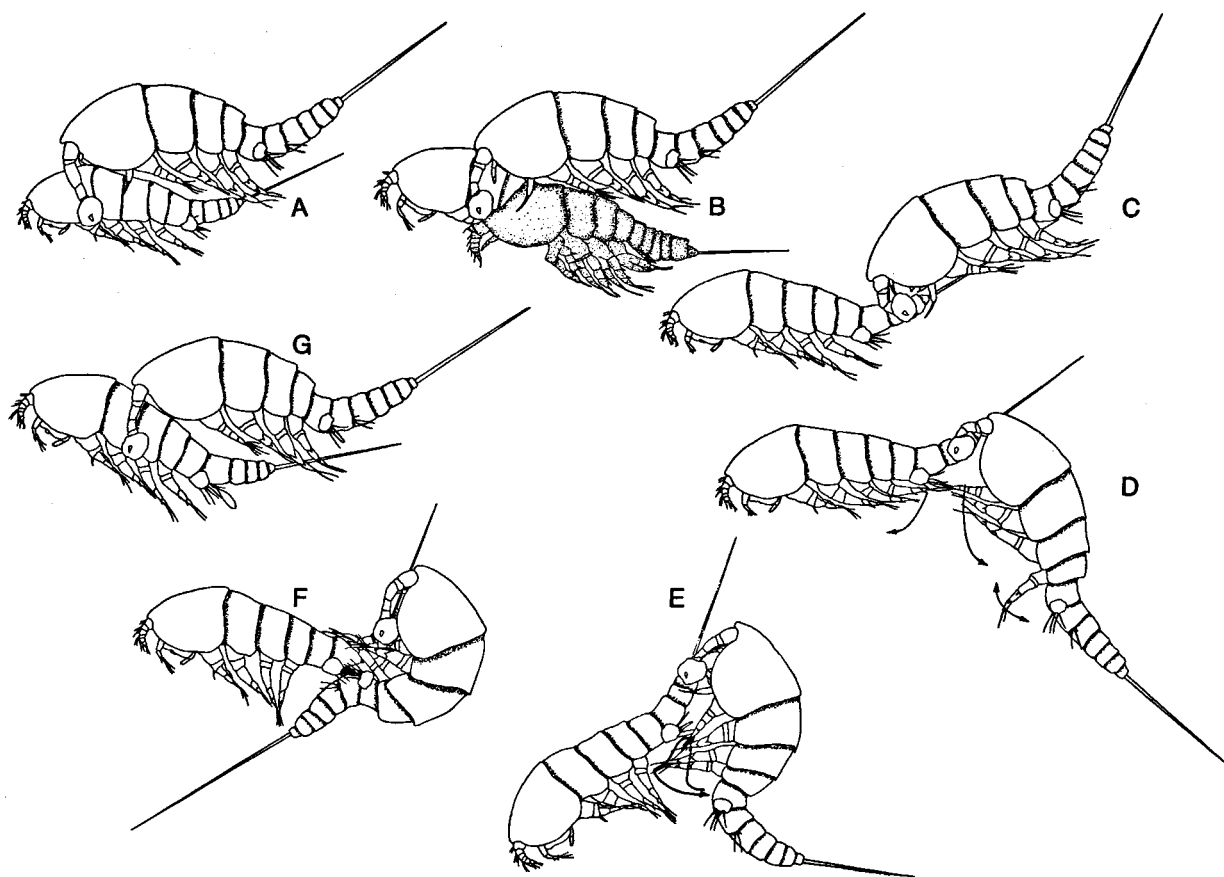
Fig. 4. Sequence of mating behaviour in *Tachidius discipes*: A, precopulatory mate guarding, male guards C V female; B, final moult of female, male reclasps female emerging from the exuvium (dotted); C, male climbs down the female's dorsal side; D, male swings to female's ventral side; E, pressing movements of the male, control of female's genital field; F, copulation; G, pair immediately after copulation.

not covered by her legs, the free antennule of the male is refastened, now reaching from the ventral to the dorsal side around the female's furca. Then the same procedure is repeated with the other antennule. The model of clasping in this position can be seen in Fig. 7.

If the female's legs cover the ventral side of her abdomen, the male first pushes her legs anteriorly with the help of his first, second and third legs. These are first stretched forward so that the first legs lie beneath the male's mouthparts and are then pushed backwards against the female's legs which are pushed anteriorly in the process (Fig. 4D). Paddling by the fourth legs keeps the male in close contact with the female. Immediately after this, the other antennule is loosened

and refastened in the same way as the first, grasping the dorsal side of the female's furca from beneath.

(2) The ventral sides of the partners are now opposed to each other at an angle of about 60 to 70°. It takes only 10 seconds from clasping the female to successful establishment on her ventral side. If a male fails to fix himself onto the female's ventral side, he climbs back to her dorsal side where he stays attached as in precopulatory mate guarding for a few seconds and then begins a new attempt to establish himself on her ventral side. Females are usually passive; only rarely do they disturb the males by performing swimming strokes. Female movements have never been observed to prevent a male from successfully at-
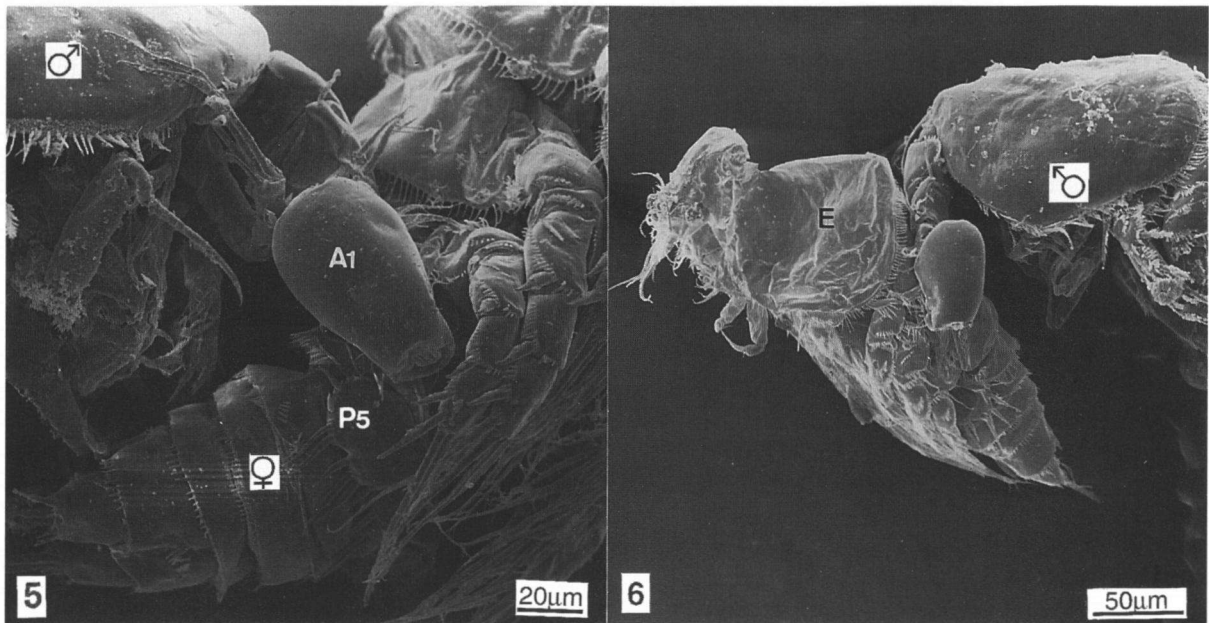
Fig. 5. Pair (female C V) in precopulatory mate guarding. Close-up of Fig. 3. P5 = fifth leg; A1 = antennule.
Fig. 6. Male clasping C V exuvium of female (E) around thoracic segments.

taching a spermatophore.

(3) Control of the female's genital field: As soon as the male has positioned himself on the female's ventral side, he starts pressing his abdomen rhythmically against that of the female. The cephalothorax of the male then is close to the female's furca, his thoracic somites are bent ventrally and his abdomen is curved dorsally. The male's legs are applied to the female's abdomen. He then starts to stretch the legs which slide along the female's ventral side and to push her fifth legs ventrally. This allows the second, third, and fourth legs of the male to contact the female's genital field, which normally is covered by her fifth legs. By flexing his body ventrally, the male's legs come to be pressed against those of the female, hers thus are pushed anteriorly. The pressing movements against the female's abdomen may be repeated three times per second and last for at most 15 seconds before the male pauses in this position (Fig. 4E) and then repeats the action.

Usually the female remains motionless. If a female becomes active, the male can be dislodged and may be pulled around for a few seconds; he then must regain his position at the female's ventral side. Elapsed time from clasping the female to copulation ranges from 25 to 360 seconds (mean = 132 seconds, SD = 91.2 seconds).

(4) Copulation: Shortly before extrusion of the spermatophore, the male's body is bent dorsad even more than during the preceding period of rhythmical pressing movements. His thoracic segments are flexed ventrally and his head lies under the female's furca, thereby pushing the furcal setae dorsally. Just after the spermatophore leaves the gonoduct, with its distal end first, the male's abdomen touches the genital field of the female. At the same time all the male's legs embrace the female's abdomen and press it against its own (Fig. 4F). This is repeated up to 15 times within 10 seconds. Copulation lasts only a few seconds (mean = 9.5 seconds, SD = 2 seconds) and in only one of the 32 cases took as long as 15 seconds.

Behaviour after copulation: As soon as the spermatophore is attached to the female, the male loosens one of the antennules, shifts to the dorsal side of the female, and refastens the antennule at the margin of the abdominal segments; the other antennule is repositioned similarly. The male then climbs by alternate gripping of his antennules to
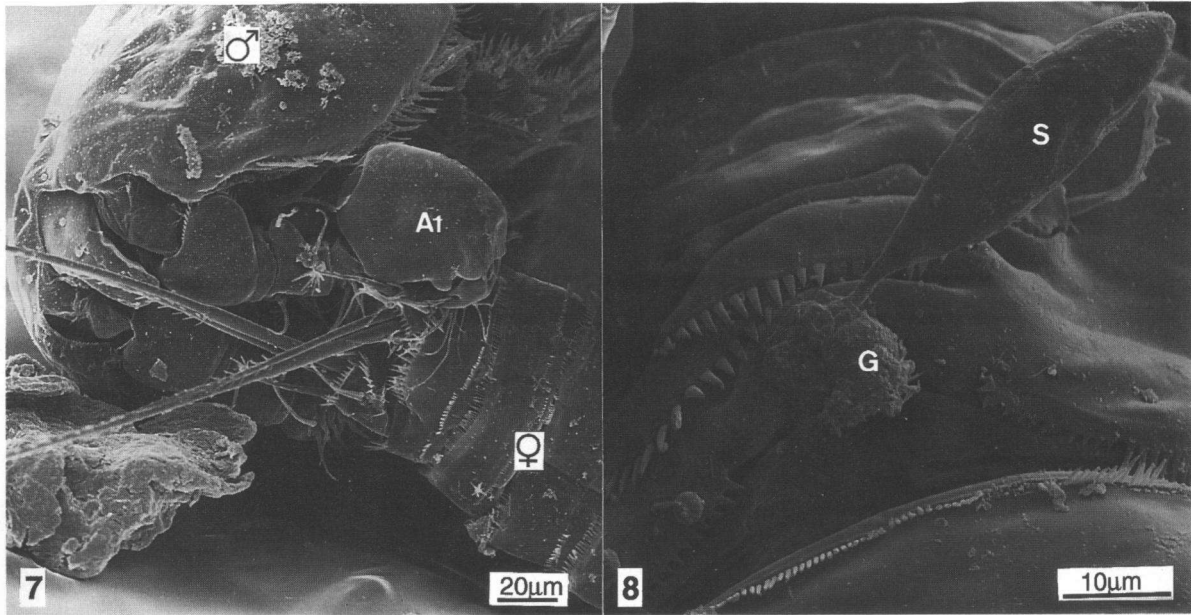
Fig. 7. Male of *T. discipes* attached to furca of adult female clasping it from ventral side. A1 = antennule.
Fig. 8. Spermatophore on genital field of female. G = glue; S = spermatophore.

the female's gonopore or thoracic segments and positions himself as during precopulatory mate guarding (Fig. 4G). The time from copulation to departure of the male does not exceed 5 minutes (mean = 94.2 seconds, SD = 75.6 seconds).

The spermatophore and its discharge

The spermatophore of *Tachidius discipes* is flask-shaped. Its length is about 60 μm, the maximum breadth 15 μm. It is deposited directly on the female's gonopore and fixed there by a glue-like substance (Fig. 8). With light microscopy the contents of the spermatophore appear as two different substances. The distal one-fourth to one-fifth of the total volume is granular and is separated from the rest of the contents by a smooth membrane. The proximal substance is pressed out by swelling of the distal substance in the 15 minutes following attachment. The distal substance retains its granular quality for another 15 minutes (Fig. 9). In two cases, spermatophores were not properly placed and fell from the female's genital field. These unattached spermatophores discharges within six to seven minutes. As observed

in 6 instances, discharged spermatophores drop off two to three hours after copulation.

Experiment 1: Copulation frequency and spermatophore production of the males

Ten males of *T. discipes* with one or two spermatophores in their gonoduct were tested for their
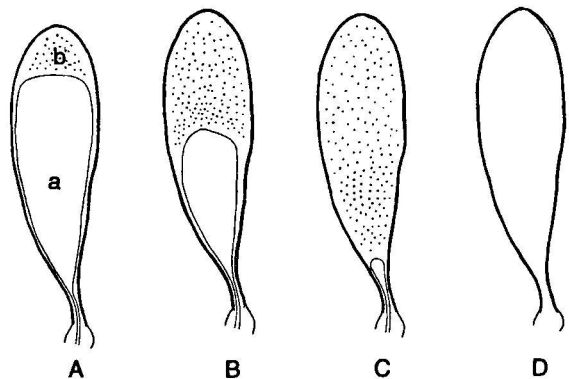

Fig. 9. Discharge of spermatophore of *T. discipes*: A, condition immediately after copulation; B, 5 minutes later; C, 15 minutes later; D, 30 minutes later; a = proximal substance; b = distal substance. Spermatophore drawn without fertilization tube.

copulation frequency and spermatophore production. Of 30 checked adult males, 6 had two spermatophores in their gonoduct. The second lies behind or slightly underneath the first in the gonoduct, is smaller, and seems to be fully developed (Fig. 10).

The results shown in Fig. 11 give the differences between the two groups in copulation frequency and spermatophore production. The exact time needed for the production of a new spermatophore could not be determined. Five different reactions were observed when males were offered a second, third or later virgin mate:

(1) Copulation: Behaviour as explained above, spermatophore transfer.

(2) Clasping: The female was clasped by the male, but no attempt was made either to shift to the female's ventral side or to copulate. Males departed about one to five minutes after first contact.
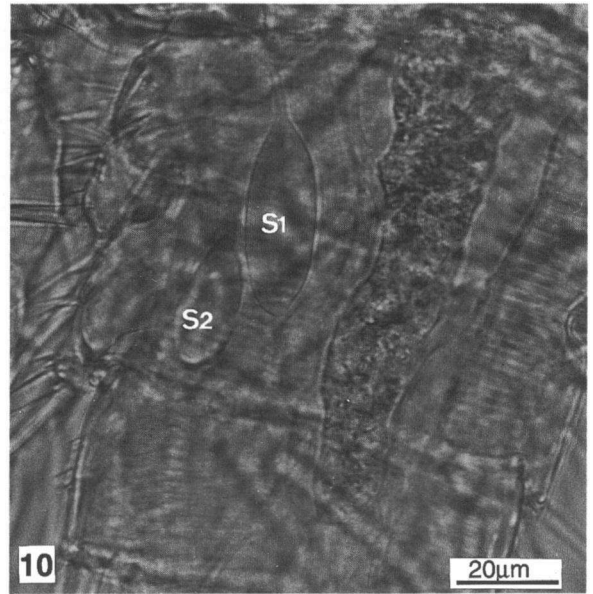
(3) Accompanying: The male clasped the female

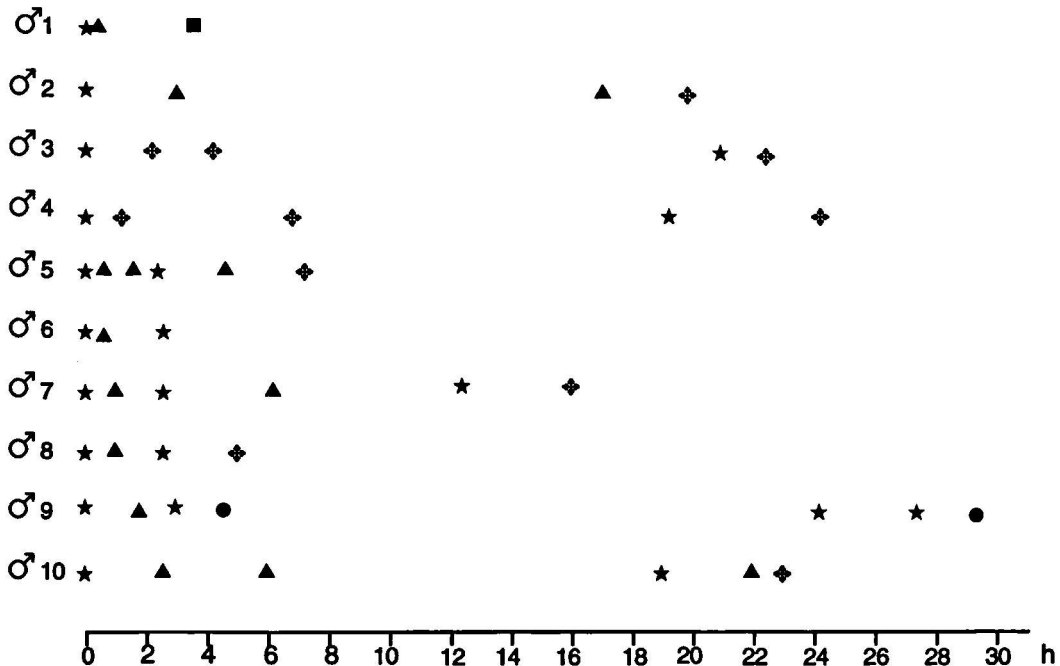*Fig. 10.* Abdomen of a male of *T. discipes* with two spermatophores (S1, S2) in gonoduct.

Fig. 11. Copulation frequency of males of *T. discipes* with one spermatophore (♂ ♂ 1–4) and with two spermatophores in the gonoduct (♂ ♂ 5–10). Each symbol indicates a virgin female which was introduced to a male, the time which elapsed after the first copulation of the male, and the reaction of the male: ★ = copulation, ● = pseudocopulation, ▲ = clasping, ■ = accompanying, ✦ = no reaction. For explanations of the behaviour, see text.

as in precopulatory mate guarding and stayed with her possibly until copulation, which was not observed but took place at least 3 hours after first contact. The possibility that the male separated from the female and later reclasped her again for copulation could not be excluded. The female was isolated and produced viable nauplii.

(4) Pseudocopulation: After checking the female, the male performed normal behaviour before copulation (shifting onto the female's ventral side, pressing movements, positioning for spermatophore transfer), but no spermatophore was expelled. Males departed within one minute after pseudocopulation.

(5) No reaction: Although a male came very close to a female, he did not make contact with her in the next 20 minutes, neither by attaching himself with the antennules to her body nor to her genital field.

A male with two spermatophores was able to copulate with a second virgin female within 2 to 3 hours. Males with one spermatophore in the gonoduct were unable to copulate again in the same short interval. Of the four males with one spermatophore, male 1 clasped a female and later copulated with her, male 2 clasped a newly offered virgin female 20 minutes to 3.5 hours after first copulation without being able to copulate with her. Males 3 and 4 initially showed no response to the newly offered females but were able to copulate again 19.5 to 21 hours after first copulation.

Males with two spermatophores, when offered new females some hours later after the second copulation, behaved as did males with one spermatophore after their only copulation. Male 5 clasped the new female for a short period and male 8 showed no reaction. Male 9 tried to copulate without being able to expel a spermatophore (pseudocopulation). Subsequently, male 9 produced two new spermatophores and, after copulating with two more virgin females, showed the same behaviour when offered another female. Yet another male with two spermatophores (male 7) copulated for a third time 8 hours after the second copulation but did not copulate a fourth

time, although presumably he still carried a spermatophore in his gonoduct.

Experiment 2: Male choice behaviour

Isolated virgin males of *T. discipes* with one spermatophore in their gonoduct were offered one of four kinds of conspecific adult females which differed in the following ways:

(a) Inseminated female with a spermatophore attached to the genital field ($n = 3$): A male clasped the female in the usual manner and immediately climbed to her ventral side. He started pressing her abdomen, as described above, against the ventral side. His third and fourth legs came into contact with the genital field of the female and the adhering spermatophore. In two cases after repeated pressing movements, the male stopped this behaviour after a few seconds and left the female. The third male stayed attached to the ventral side for a further 20 minutes and clasped the attached spermatophore with one antennule without dislodging it.

(b) Inseminated females without adhering spermatophore ($n = 3$): A male controlled the female's genital field as explained under (a) but withdrew within a minute without copulation.

(c) Inseminated females with egg sac ($n = 4$): A female was clasped from the dorsal and lateral sides; the male thus came into contact with the egg sac with one of his antennules. Between 10 to 20 seconds after shifting to the ventral side of the female, the male left her without having pressed his abdomen against that of the female.

(d) Virgin female with small egg sac (females of *T. discipes* extrude a small egg sac even though not inseminated) ($n = 1$): Male behaviour before and after copulation was the same as observed for a male with a virgin adult female without an egg sac. During control of the genital field and copulation the small egg sac was pressed ventrally by the third and fourth legs of the male.

All males used for the experiments (a), (b), and (c) copulated successfully with virgin females offered to them directly after they had refused the first females in the experiment.

Discussion

Precopulatory mate guarding and mating of *Tachidius discipes* is a complex behaviour; many aspects were not reported previously.

Clasping other individuals

When a male makes contact with conspecific individuals, the antennules are not only used for clasping but also for checking mainly the ventral side of the other individual by moving back and forth. Probably via such antennule movements males can recognize the sex of the clasped individual. Contact pheromones have been found in the harpacticoid *Coullana* sp. in exactly the areas of the female's body that are clasped by the males, e.g., the furca (Snell & Carmona, 1994). Pheromonal signals may also serve as markers during copulation. *T. discipes* has not yet been examined for such contact pheromones.

Precopulatory mate guarding and moulting of the copepodid

Precopulatory mate guarding in *T. discipes* includes active behaviour of the male. During precopulatory mate guarding the attachment position of the female is often changed by alternate fastening of the antennules to the cephalothorax or the third or fourth thoracic segments, which explains why Dahms (1985), using preserved material, found males clasping females in different positions. While Dahms found most males attached to the cephalothorax of the female, in the present study most males attached to the free thoracic segments. This difference may be explained if males embrace the thoracic segments less firm than the cephalothorax. The latter may also be more powerful during fixation. Dahms (1988) noted that increasing body size of female harpacticoids may influence the clasping mode in favour of the more effective clasping of the cephalothorax. The reason for the different clasping positions could be that males recognize the time of the next moult more easily when attached

near possible sutures through which pheromones or other substances are released. Two clasping modes have also been found in *Nannopus palustris* Brady, 1880 (Huntemanniidae). While Willey (1929) reported that males clasped females around the urosome, Lang (1948) only saw them clasping females around the margin of the cephalothorax. Lang suggested that Willey (1929) may have observed only a transient position.

As observed for *Tisbe* spp. (Dahms & Schminke, 1993; Dürbaum, 1995) the male maxillipedes of *T. discipes* have no clasping function but the male may reduce the copepodid's freedom of movement by holding her abdomen with the open claw.

Males of *T. discipes* prefer to clasp fourth and fifth copepodids. Clasping of advanced copepodid stages has been observed in several other species (Ito, 1971; Jewett & Feder, 1977; Castel, 1979; Walker, 1981; Burton, 1985; Evstigneeva, 1993). This practice has the advantage of reducing the male's time investment, as discussed extensively by several authors (Ridley, 1983; Kern et al., 1984; Burton, 1985), but also reduces the risk of losing the copepodid when it moults. Such mishaps have been observed in the present study, and Nilsson (1987) likewise described males of *Mesochra lilljeborgi* holding exuvia of female copepodids and swimming around with them. Possibly contact pheromones (cf. Snell & Carmona, 1994) on the exuvium are the reason why the male remains attached to the exuvium.

Clasping of certain copepodid stages may also give hints as to the population structure as discussed by Kern et al. (1984) and Burton (1985). The lower the percentage of receptive females (e.g., in *Tigriopus californicus* only virgin females are attractive), the higher the pressure on the males to find a suitable mate (Burton, 1985). The same applies to *T. discipes*. As found in experiment 1, males are able to mate more than once, while females are not. Muus (1967) found the sex ratio of *T. discipes* to be strongly female biased (76% female). Thus males have a good chance finding a female copepodid which they can accompany until her final moult and to copulate with her thereafter. They may even succeed in finding an adult virgin female to mate with at

once. As observed in the present study, males of *T. discipes* have a flexible mating system insofar as they are able to copulate with an adult virgin female without previous mate guarding.

The final moult of female harpacticoids of species exhibiting precopulatory mate guarding has not been observed in detail. Williams (1907) described that of *Harpacticus uniremis*. While the female emerged from the exuvium, the male clasped her between the thoracic and abdominal segments; the whole procedure lasted 5 minutes. In *Huntemannia jadensis* it took about 2 minutes (Feller, 1980) but nothing is said about the mode of reclasping of the female. For *Mesochra lilljeborgi* and *Amonardia normani* Brady, 1872, it is only known that moulting takes about 15 minutes and follows a phase of low activity (Nilsson, 1987). Evstigneeva (1993) reported that a male of *Harpacticella inopinata* (Harpacticidae) loosened his grip while the female was moulting and waited nearby, presumably, as she states, eating the exuvium before reclasping the emerged female. From what has been observed in *T. discipes*, it appears unlikely that males should separate from the females before copulation after having invested so much time accompanying them. This investment would be lost if another male detected the unguarded female and copulated with her.

Control of the female's genital field

This male behaviour can be observed after the final moult of the female or after a male clasps an adult virgin or an adult female already inseminated. It was not observed when a male investigated a female with a developing egg sac. While controlling the female, the male's legs slide over her genital field and touch her fifth legs. It was not possible to follow these movements in detail.

During experiment 2 there were no signs of repelling behaviour by the female during either the control or the first clasping phase. This suggests that female choice is absent. Decision whether to mate or not is often made in less than

a minute and appears to be a decision made by the male. Behaviour prior to copulation is a much more complex interaction between the sexes in four species of *Tisbe* and of *Paramphiascella fulvofasciata*. Courtship in these species lasts from a few to 15 minutes in *T. holothuriae* to 135 minutes in *Paramphiascella fulvofasciata* (cf. Dürbaum, 1995) and is very different from the behaviour of *T. discipes*. In at least some of these species, females are able to physically reject males and all have special mechanisms for responding to male courtship by increasing passivity or by exposing their genital field. Only when this happens, the male is able to attach a spermatophore (Dürbaum, 1995).

Copulation

Copulation of *T. discipes* takes only a few seconds, similar in time to few other species of harpacticoids in which it has been studied (Fahrenbach, 1962; Haq, 1972; Dürbaum, 1995). The spermatophore is extruded distal end first, as in *Paramphiascella fulvofasciata* and *Tisbe* spp., and attached to the genital field without the aid of any appendages, as in *P. fulvofasciata*, while in *Tisbe* spp. the legs transmit the spermatophore (Dürbaum, 1995). Unlike those of the latter, males of *T. discipes* embrace the female's abdomen with their first to fourth legs during transfer of the spermatophore, which is attached with a glue-like substance. The spermatophore's position is secured by repeated pressing of the male's abdomen against it and the female's genital field. This copulatory behaviour is most similar to *P. fulvofasciata* (Dürbaum, 1995).

Discharge of the spermatophore and number of matings per female

Discharge of a spermatophore takes from 15 to 30 minutes. This is considerably longer than the time the female is accompanied by the male after copulation, and postcopulatory mate guarding apparently does not occur in *T. discipes*. Postcopu-

latory mate guarding should be applied when the inseminating male accompanies the female for at least the time necessary for discharge of the spermatophore (Dürbaum, 1995). Generally, it appears that harpacticoids exhibiting precopulatory mate guarding lack a postcopulatory mate guarding phase (Dürbaum, 1994), perhaps because the male's investment would be too high if he guarded a female not only prior to, but also after copulation (see discussion in Parker, 1984). In *T. discipes*, there is probably no need to protect the discharge of the spermatophore by postcopulatory mate guarding because males always rejected females that were already inseminated and no females were found carrying more than one spermatophore. Burton (1985) did not observe mating behaviour in *Tigriopus californicus*, a precopulatory mate guarding species, but was able to prove by electrophoresis of enzymes of their progeny that females never mated twice.

Spermatophore production and male's mating sequence

As shown here, males of *Tachidius discipes* are able to fertilize up to four females within 30 hours and some carry two spermatophores in the gonoduct that can be extruded separately and can be used for two copulations within 2 hours. Both one and two spermatophore-bearing males can produce new spermatophores within 12 to 24 hours. The question arises why those with two spermatophores to not outcompete those with one and are in fact rarer than the latter. A possible explanation is that having two spermatophores is advantageous only when the male shortly after his first copulation can find another adult virgin female, or one on the brink of her final moult. This may happen rarely and the usual situation will be that of precopulatory mate guarding starting with a copepodid IV, or even III, female. In this case, a male with two spermatophores cannot use the second one at once but has to wait for the new female's final moult, just as does a male with only one spermatophore.

The presence of two spermatophores in one

gonoduct has not previously been described for harpacticoid copepods. Reports that males transfer two spermatophores at the same time refer to cases where these develop in two gonoducts. Females of the Canuellidae usually carry two spermatophores (Huys & Boxshall, 1991). A male *Canuella perplexa* T. & A. Scott, 1893, with two expelled spermatophores is illustrated by Glatzel (1988: fig. 17), suggesting that two spermatophores are transferred simultaneously. Willey (1929) noticed that all males of *Nannopus palustris* had a pair of spermatophores in their frontal urosome. However, mating behaviour and spermatophore transfer in these species are still unknown.

Acknowledgements

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