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Note

Mating plug in the planktonic copepod *Tortanus* (*Atortus*) *rubidus*

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The reproductive biology of planktonic copepods has been intensively studied to elucidate their population dynamics, life cycle, taxonomy, phylogeny and evolution (see review in Ohtsuka & Huys 2001). Their reproduction is composed of a sequence of pre-mating, mating, and post-mating: (1) mate recognition by the male; (2) capture of the female by the male; (3) transfer and attachment of spermatophore(s) by the male; (4) fertilization and release of eggs by the female; and (5) removal of discharged spermatophores by the female (Ohtsuka & Huys 2001).

After a spermatophore is delivered, sperm is stored in the seminal receptacles or the genital atrium to fertilize eggs (Barthélémy et al. 1998; Barthélémy 1999). In a part of the superfamily Diaptomoidea (=Centropagoidea), spermatophores with a species-specific coupling device play an important role in preventing heterospecific crossing (Blades-Eckelbarger 1991). Some species of the subgenus *Tortanus* (*Atortus*) belonging to the superfamily bear a highly complex coupling device on the spermatophore that almost entirely covers the female urosome (Fig. 1). Ohtsuka & Huys (2001) have suggested that since the coupling device is so tightly glued onto the female urosome, it may remain on it after all egg clutches are fertilized within the genital atrium. Here, we report the results of a study on the genitalia of *Tortanus* (*Atortus*) *rubidus* Tanaka, 1965, that strengthen the suppositions mentioned above.

Tortanus (*A.*) *rubidus* was collected from Tanabe Bay on the Pacific coast of the mainland Honshu, western Japan, at night on June 4, 1994, using a conical net (diameter 30 cm; mesh size 0.1 mm). Copepods were fixed with 10% neutralized formalin/sea-water immediately after collection. For the semithin sections the samples were dehydrated, embedded in Epon and then cut with an Ultracut Reichert-Jung.

The coupling device of the spermatophore entirely surrounds the female genital compound somite (Fig. 1). A part of the ventral face of the coupling device is completely perforated (Figs. 1a–e, 2b; arrow). The genital operculum is located beneath this large pore (ca. 60 µm in diameter) (Fig. 1c, d; go). Seminal products are stored in the genital atrium (Fig. 1e, f) as in other diaptmoideans without seminal receptacles (Barthélémy et al. 1998). Owing to the rudimentary female fifth leg, the coupling device tightly attached to the female urosome can not be removed after seminal products are completely discharged, and remains on her urosome. On the other hand, the spermatophore, which is easily visible in the testis (Fig. 2), has never been observed in inseminated females-probably because it drops immediately when empty.

All diaptomoideans, except the genus *Acartia* which has paired seminal receptacles, are considered to require multiple inseminations in order to fertilize all clutches of eggs (Barthélémy et al. 1998; Barthélémy 1999). In contrast, once a female of *T.* (*A.*) *rubidus* receives a spermatophore, no subsequent copulation seems to occur, and this is greatly beneficial for the male in propagating his own genes. Indeed this spermatophoral complex corresponds to the mating plug that is produced by the male of many insect species and injected into female gonopores to prevent subsequent copulations after they have transferred their sperm (Krebs & Davies 1978). Such a mating plug-like structure has never before been observed in copepods.

In the genus *Tortanus* there are two types of reproductive strategies (Ohtsuka et al. 2000): iteroparous and semelparous. Although the genus consists of five subgenera, the use of the latter strategy seems to be restricted to the seven species of the subgenus *Tortanus* (*Atortus*) where the fifth leg of females is rudimentary (functionless) and a coupling device on the spermatophore is complex: *T.* (*A.*) *rubidus*; *T.* (*A.*) *brevipes* Scott, 1909; *T.* (*A.*) *longipes* Brodsky, 1950; *T.* (*A.*) *bowmani* Othman, 1987; *T.* (*A.*) *digitalis* Ohtsuka & Kimoto, 1989; *T.* (*A.*) *ryukyuensis* Ohtsuka & Kimoto, 1989; *T.* (*A.*) *taiwanicus* Chen & Hwang, 1999. Interestingly, all these species are dis-

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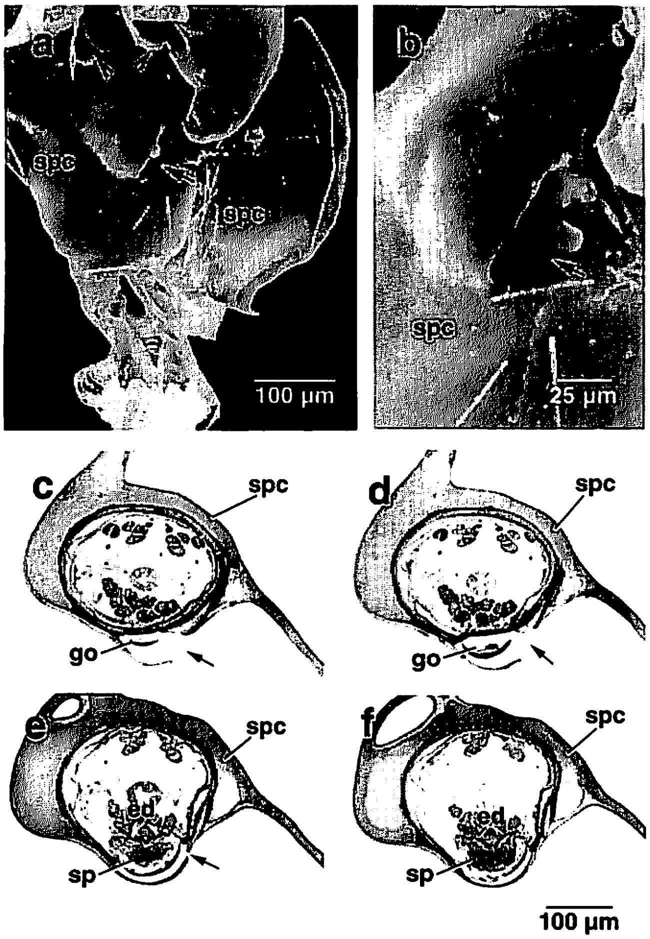

Fig. 1. Female genital compound somite of *Tortanus* (*Atortus*) *rubidus*. (a–b) SEM micrographs of the external ventral face and detail. (c–f) Photomicrographs of transverse sections (section (c) posteriormost, (f) anteriormost). Note the spermatophoral coupling device (spc) entirely surrounding the compound somite and exhibiting a ventral opening (arrow). Note also the rudimentary fifth legs (arrowheads). ed=egg-laying ducts, go=genital operculum, sp=seminal products.

tributed mainly in the tropical and subtropical waters of the West Pacific, although the subgenus has a broader distribution in the Indo-West Pacific extending to the Red Sea and South Africa (Ohtsuka & Kimoto 1989). Other congeners bear well-developed fifth female legs and a relatively simple coupling device on the spermatophore. Hence the female removes empty spermatophores with her fifth legs, and this likely requires multiple inseminations as in other diaptomoideans without seminal receptacles. Since the subgenus *Tortanus* (*Atortus*) is regarded as one of the most evolutionarily advanced groups in the genus (Ohtsuka & Reid 1998), the semelparous strategy of some members of the subgenus seems to be an evolutionary novelty.

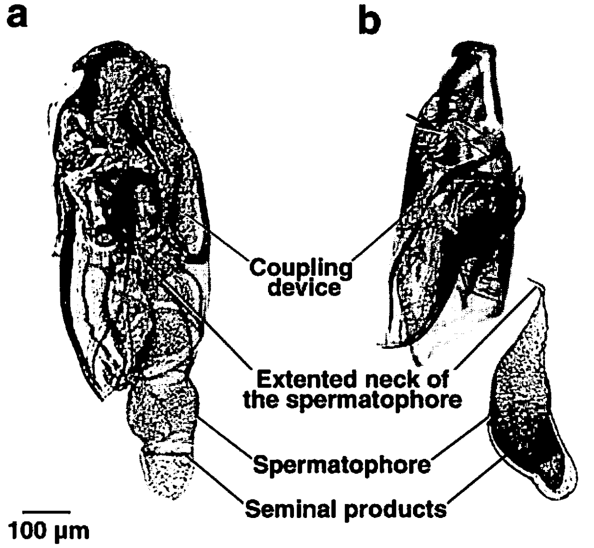

Fig. 2. Light microscopy of the spermatophoral complex of *Tortanus* (*Atortus*) *rubidus* as it appears in the testis (a) and after dissection (b). Note the opening of the coupling device (arrow) and the spermatophore proper filled with seminal products.

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