Gonopod mechanics in *Centrobolus* Cook (Spirobolida: Trigoniulidae) II. Images

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Abstract
Gonopod mechanics were described for four species of millipedes in the genus *Centrobolus* and are now figured using scanning electron microscopy (SEM) with the aim to show the mechanism of sperm competition. Structures of sperm displacement include projections on a moveable telopodite and tips on a distal process (opisthomerite). Three significant contact zones between the male and female genitalia were recognized: (1) distal telopodite of the coleopod and the vulva, (2) phallopod and the bursa, (3) sternite and legs of the female.

Keywords: coleopods, diplopod, gonopods, phallopods

1. Introduction
The dual function of millipede male genitalia in sperm displacement and transfer were predicted from the combined examination of the ultrastructures of the male and female genitalia [1-3]. Genitalic structures function do not only in sperm transfer during the time of copulation, but that they perform copulatory courtship through movements and interactions with the female genitalia [4-5]. These 'functional luxuries' can induce cryptic female choice by stimulating structures on the female genitalia while facilitating rival-sperm displacement and sperm transfer. Genitalic complexity is probably underestimated in many species because they have only been studied in the retracted or relaxed state [4]. Historically, where animal genitalia have been examined in an everted state it has been exclusively for taxonomic purposes [6-14]. Here the mechanics of the gonopods of four *Centrobolus* spp. are figured.

2. Materials and Methods
Pairs of *Centrobolus* were isolated in plastic containers (13cm in diameter) and freeze-fixed by pouring liquid nitrogen (-196 °C) upon them. Gonopods of *C. inscriptus* (Attems, 1928), *C. annulatus* (Attems, 1934), *C. ruber* (Attems, 1928) and *C. fulgidus* (Lawrence, 1967) were removed and viewed under a light microscope (Wild). After one month in 70% ethanol at –10 °C the copulatory organs were dissected under a light microscope to remove the vulva sacs and all surrounding tissue. One specimen from each species was prepared for SEM and viewed/figured using Adobe Photoshop 3.0 and Adobe Illustrator 7.0.

3. Results and Discussion
The male genital mechanics in four species of *Centrobolus* were simulated by manipulating gonopods. The gonopod complexes were positioned in a relaxed retracted state and then everted. Basic movements involved the basal region of the coleopod moving anti-medially so that the phallopod extended aborally (Figure 1). The distal lamellae moved in an arc so that it became the structure extended furthest from the organism (Figure 1-3). Observations of *C. inscriptus* prior to fixation revealed that the female vulva was pulled out from the vulva sac by the grasping knob and bill of the coleopods. Once the gonopods were seated they became inflated and erectile (Figures 4-5). Three significant contact zones between the male and female genitalia were recognized: (1) distal telopodite of the coleopod and the vulva, (2) phallopod and the bursa, (3) sternite and legs of the female.
Fig 1: Scanning electron micrograph illustrating the male genital mechanics of the millipede *Centrobolus annulatus*. The phallopod (p) articulates from the base of the coleopod and extends in an aboral direction. The distal joint of the coleopod (d) opens sidewards in an anti-medial fashion, while the basal joint (b) remains static. In this specimen the phallopod has been fully extended to reveal the distal lamella (dl), the structure that interacts directly with the female genitalia during mating. The movement of this gonopod complex (arrows) is from a retracted towards an everted state. The forward motion is proposed to function in sperm competition through its displacement activity.

Fig 2: Scanning electron micrograph illustrating the male genital mechanics of the millipede *Centrobolus ruber*. The right phallopod (p) is fully everted with its distal lamella (dl) extended to extreme and the basal lobe (a) in an inflated state. In this species the coleopod, with its basal (b) and distal (d) telopodite joints, remains static rather than shifting laterally.

Fig 3: Scanning electron micrograph illustrating the male genital mechanics of the millipede *Centrobolus fulgidus*. The right gonopod (on the left) is in a retracted state while the left gonopod is everted. The phallopod (p) slides out from between the distal (d) and basal (b) telopodite joints of the coleopod so that the distal lamella (dl) remains furthest from the body. Simultaneously, the knob (k) and the bill (bi) of the coleopod shift laterally in an anti-medial fashion. The movement of this gonopod complex (arrows) is towards the everted state, revealing the gap between the anterior and posterior structures that is proposed to enhance sperm displacement.

Fig 4: Scanning electron micrograph of *Centrobolus inscriptus* male and female genitalia that were freeze-fixed in copula. Notice that the distal lamella (dl) of the posterior male intromittent organ, the phallopod (p), is seated near the operculum (op) of the female sperm storage organ, the bursa copulatrix (bc), which leads into the oviduct (o). The distal joint of the telopodite (d) of the anterior intromittent organ, the coleopod, is shifted anti-medially to distend the female vulval sac (v) while the basal joint of the telopodite (b) remains static.

Fig 5: An oral view of the freeze-fixed genitalia from a part of *Centrobolus inscriptus* in copula. The male gonopods remain firmly locked within the vulvae, the stemite (v) rests between the females second pair of legs (II), the basal joints (b) are inflated outwards, the phallos (p) are also inflated to give the gonopod complex a two-fold reach.

This evidence somewhat corroborates the lock-and-key hypothesis for the evolution of animal genitalia [15].

4. Conclusion
*Centrobolus* gonopods possess structures with functions in sperm displacement: projections on a moveable component (telopodite) and tips on a distal process (opisthomerite) [1]. Three significant contact zones between the male and female genitalia were recognized: (1) distal telopodite of the coleopod and the vulva, (2) phallopod and the bursa, (3) sternite and legs of the female.

5. References