# Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

**Mark Cooper**
School of Animal, Plant &
Environmental Sciences,
University of the Witwatersrand,
Johannesburg 2050, South Africa

# Julid millipede and spirobolid millipede gonopod functional equivalents

## Mark Cooper

### Abstract
Not all male genitalic structures function only in sperm transfer during the time of copulation (*in copula*), they perform copulatory courtship through movements and interactions with the female genitalia. These structures were considered 'functional luxuries' through natural selection, mate choice and conflict of interests. The genital biomechanics of *Centrobolus* millipedes have examined *ex copula* then *in copula* to make actual correlations between the male and female genitalia. Two methods were employed: (1) artificial simulation of gonopod movements and (2) freeze-fixation in order to understand their functional equivalents. After copulating for approximately 10 minutes pairs were freeze-fixed by pouring liquid nitrogen (-196 °C) upon them. After one month in 70% ethanol at -10 °C, the copulatory organs were dissected from the animals under the light microscope to remove the vulva sacs and associated tissue that concealed the regions of contact. Structure-function relationships of the male accessory genitalia (gonopods) were discussed in the spirobolidan millipede genus *Centrobolus* and compared to julid millipedes.

**Keywords:** Coleopod, gonopod, promerite, solenomerite, sternite, telopodite

## Introduction
The dual function of male genitalia in sperm displacement and transfer has been predicted from the combined examination of the ultra-structures of the male and female genitalia in the Odonata [9]. It is a widespread fact that not all male genitalic structures function only in sperm transfer during the time of copulation (*in copula*), but that they perform copulatory courtship through movements and interactions with the female genitalia in the Diplopoda [2-4]. Both these structures have evolved independently and may be considered 'functional luxuries' because they can induce cryptic female choice in stimulating structures on the female genitalia while facilitating rival-sperm displacement and sperm transfer [4]. In spirostreptidan millipedes, a single genitalic component (telopodite) may function in ejaculate placement, repositioning and displacement, and female stimulation [1]. This is truly a 'functional luxury'. Further functional equivalents are suggested in divergent taxa of julid and spirobolid millipedes.Genitalic complexity is probably underestimated in many species because they have only been studied in the retracted or relaxed state [3]. A 'functionless luxury', as opposed to a 'functional luxury' is defined as a genitalic structure that has no further function after male and female genitalia have coupled [6]. In some species it is possible to freeze-fix mating pairs *in copula* so as to establish how the genitalia of both sexes function so their sexual behaviour and genital mechanics can be well understood in copula, *e.g.* in acridids [10, 13], arachnids [5-7] and diplopods [1, 12]. The congruent fixation of the coupled genitalia is a way to test hypotheses centred around the co-adaptation of male-female genitalia [11].

The genital biomechanics of *Centrobolus* millipedes are examined *ex copula* then *in copula* to make actual correlations between the male and female genitalia. The aims here are to determine (1) contact zones within reach of the sperm stores; (2) what genitalic movements are performed syn-copulation; (3) the specific structure-function relationships of the male gonopods; (4) whether the male gonopods interact with the sensory regions of the bursa copulatrix and can function in cryptic female choice [4].

**Correspondence**
**Mark Cooper**
School of Animal, Plant &
Environmental Sciences,
University of the Witwatersrand,
Johannesburg 2050, South Africa

## 2. Materials and Methods

### 2.1 Observations

Gonopods of the four species, *C. inscriptus, C. anulatus, C. ruber* and *C. fulgidus*, were removed from sacrificed males and manipulated under the light microscope. The coleopods were pinched distally and pulled outwards so that the movement of the phallopods could be produced by slowly opening the gap between the anterior and posterior gonopods. The insertion of a pin behind the coleopods causing the phallopods to extend allowed them to become dehydrated in that position. One specimen from each species were chosen and prepared for SEM. The sequence of gonopod movements was reconstructed on the SEM micrographs using Adobe Photoshop 3.0 and Illustrator 7.0.

### 2.2 Experimentation

Five copulatory pairs of *C. inscriptus* were formed by introducing single males to females in plastic containers (13cm diameter). After copulating for approximately 10 minutes pairs were freeze-fixed by pouring liquid nitrogen (-196 °C) upon them. After one month in 70% ethanol at -10 °C, the copulatory organs were dissected from the animals under the light microscope to remove the vulva sacs and associated tissue that concealed the region of contact. The cleanest specimen was chosen and prepared for SEM.

### 2.3 Explanation

Gonopod structures were studied and compared in julid and spirobolid taxa and functions and functional equivalents were tabulated.

## 3. Results

The male genital mechanics in three species of *Centrobolus* were successfully simulated, artificially manoeuvring the anterior and posterior gonopods [2]. In *Centrobolus* species the gonopod complexes could be positioned in a relaxed or retracted state and then brought to an everted state. These basic movements involve the basal region of the coleopod moving in an anti-medial fashion so that the phallopod extends aborally. The distal lamellae move in an arc so that it becomes the structure extended the furthest from the body. 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. After the pair were fixed, three significant contact zones between the male and female genitalia of *C. inscriptus* were recognized: (1) The distal telopodite of the coleopod, armed with spines, presses on the inside of the vulva; (2) The phallopod inserts the bursa copulatrix and pulls on the anterior junction between its two plates so that the insemination duct or furrow is exposed; (3) The sternite fits between the legs of the female so that the genitalia of the two sexes are symmetrical. The structure-function relationships of the male gonopods indicate that there are two processes involved in genitalic coupling. Stage I involves the coleopods and stage II the phallopods. The coleopods have a mechanical function that provides the phallopods with deeper access. Stage II involves the phallopods positioning within the bursa. Both pairs of gonopods were seated symmetrically within the female vulvae.

**Table 1.** Structure-function relationship of the male accessory genitalia (gonopods) in the spirobolidan millipede genus *Centrobolus*.

| Gonopod structure | Function | Functional equivalent |
|---|---|---|
| Coleopods | protective when retracted | julid promerite |
| 1.   distal telopodite | stretches the walls of the vulvae apart prevents slipping (i.e. mechanical) facilitates seating (i.e. sensory) places the gonopods (positioner) | clasping projection of the cheirite in *Craspedosoma transsilvanicam* (Tadler 1993) |
| 2.   stemite | prevents lateral shifting (stabiliser) stretches the vulva sac in a medial plane | |
| Phallopods | | julid solenomerite |
| 1.   sperm groove | directs sperm transfer (inseminator) | |
| 2.   distal lamella | enters the female bursa (see text) | |

## 4. Discussion

It is known that there has been a transition in the evolution of spirobolidan gonopods from proterospermous (sperm transfer by anterior gonopods) to opisthospermous (sperm transfer with posterior gonopods). In *Centrobolus* the coleopods can be classed as paragonopods or peltogonopods because they play a subsidiary role as inseminating devices [2]. As accessory plate-like structures their function is in protecting the gonopods proper. The phallopods are the gonopods proper that actually transfer the sperm. The distal lamellae of the phallopods do not enter the spermathecae as is also the case in spirostreptid and odontopygid millipedes, so direct ejaculate removal from the permanent sperm stores are mechanically impossible [1]. The end-plates and distal processes of the phallopods may function to remove rival male sperm from the temporary sperm stores and reposition self-sperm closer to the spermatheca and oviduct. Thus *Centrobolus* gonopods possess two of the five structures with putative functions in sperm displacement that are known in other millipedes [11]; (1) various projections on a moveable component (telopodite); and (2) tips on a distal process (opisthomerite) but not (3) flagellae (and pseudoflagellae); (4) brushes; and (5) end plates.

Three contact zones between male and female genitalia provide evidence that the male genitalia is more complex than simple casts and essentially sperm competition and cryptic female choice [2]. The female genitalia does not simply contain the male gonopods. The coupling of male-female genitalia in *C. inscriptus* suggests mutual mechanical co-adaptation between genitalia. The insertion of the phallopod into the bursa confirms that the males have access to the temporary sperm stores, that there is an exit, and that the gonopods have the opportunity to perform movements in copula. The orientation of the sperm groove into the bursa illustrates that sperm transfer and displacement may occur dualistically. Hence the phallopod is a functional luxury [4]. During stage I of *Centrobolus* gonopod, while the coleopods are stretching the vulvae, the phallopods evert. The process is best described as a hydropneumatic system, as discovered in the chordeumatid millipedes [11]. The phallopods become fully erectile when inflated by muscular action during stage II. Muscles that induce pressure changes are manifest by movements of specific structures or segments [9]. Thus there is movement of the phallopods within the female genital tract after coupling has been accomplished.

The current results closely parallel those of the study of the genital apparatus in julidan species of millipede, *Nemasoma vericorne* (Nemasomatidae), *Brachyiulus lusitanus*, *Unciger foetidus*, *Cylindroiulus bolete*, *Pachyiulus hungaricus* (Julidae) [8, 11]. *C. ruber* most closely resembles *N. varicorne* and *B. lasitanus* in that the vulvae remain within the vulva sac during copulation. The gonopods of *C. inscriptus* are functionally more similar to *U. foetidus* and *C. boleti* than the two aforementioned julidan species where parts of the gonopods form clasper-like structures that pull out the female vulvae from the vulva sacs [2, 12]. *Centrobolus* gonopods have functional equivalencies with the "lower" julids. The former possess coleopods and phallopods while the latter possess promerites and opisthomerites. The absence of an additional intermediate structure separates both of them from the "higher julids" that possess the mesomerite. The most obvious functional equivalent is between the julid solenomerite and the *Centrobolus* phallopod, both the sperm transferring structures which are introduced into the central funnel leading into the receptaculum seminis or bursa, respectively [2]. This is similar to the functional morphology of *B. lusitanus* and *C. boleti* where "a projection of the posterior gonopods (end-projection, brachite) fits into a slit anterior to the openings of the receptacula." [12]. A minor difference is that only the distal lamella of the *Centrobolus* phallopod enters the female bursa [2]. This may be realised through genitalic recognition or stimulation if a male-female contact zone of significantly smaller surface area were to be responsible for sperm destinies.

## 5. References

1. Barnett M. Sex in southern African Spirostreptida millipedes: mechanisms of sperm competition and cryptic female choice. Ph.D. thesis. University of Cape Town, South Africa, 1997, 1-179.
2. Cooper MI. Gonopod mechanics in *Centrobolus* Cook (Spirobolida: Trigoniulidae): II. Images. Journal of Entomology and Zoology Studies. 2016; 4(2):152-154.
3. Eberhard WG. Sexual selection and animal genitalia. Massachusetts, Harvard University Press, 1985, 1-244.
4. Eberhard WG. Female Control: Sexual Selection by Cryptic Female Choice. Princeton, Princeton University Press, 1996, 1-472.
5. Huber BA. Genital mechanics and sexual selection in the spider *Nesticus cellulanus* (Araneae: Nesticidae). Canadian Journal of Zoology. 1993; 71:2437-2447.
6. Huber BA. Copulatory mechanics in the funnel-web spiders *Histopona torpida* and *Tetrix denticulate* (Agelenidae, Araneae). Acta Zoologica. 1994; 75:379-384.
7. Huber BA. Genital morphology and copulatory mechanics in *Anyphaeana accentuata* (Anyphaenidae) and *Clubiona pallidula* (Ciubionidae: Araneae). Journal of Zoology, London. 1995; 235:689-702.
8. Jovanovic Z, Pavković-Lučić S, Ilić B, Vujić V, Dudić B, Makarov S *et al*. Mating behaviour and its relationship with morphological features in the millipede *Pachyiulus hungaricus* (Karsch, 1881) (Myriapoda, Diplopoda, Julida). Turkish Journal of Zoology. 2017; 41:1010-1023.
9. Córdoba-Aguilar A, Uhía E, Cordera Rivera A. Sperm competition in Odonata (Insecta): the evolution of female sperm storage and rivals' sperm displacement. Journal of Zoology. 2006; 261(4):381-398.
10. Song H, Marino-Perez R. Re-evaluation of taxonomic utility of male phallic complex in higher-level classification of Acridomorpha (Orthoptera: Caelifera). Insect Systematics and Evolution. 2013; 44:241-260.
11. Tadler A. Genitalia Fitting, Mating Behaviour and Possible Hybridization in Millipedes of the Genus *Craspedosoma* (Diplopoda, Chordeumatida, Craspedosomatidae). Acta Zoologica. 1993; 74:215-225.
12. Tadler A. Functional morphology of genitalia of four species of julidan millipedes (Diplopoda: Nemasomatidae; Julidae). Zoological Journal of the Linnean Society. 1996; 117:1-15.
13. Woller DA, Song H. Investigating the Functional Morphology of Genitalia during Copulation in the Grasshopper *Melanoplus rotundipennis* (Scudder, 1878) via Correlative Microscopy. Journal of Morphology. 2017; 278:334-359.