ARE COLEOPOD SPINE LENGTH AND NUMBER RELATED TO MASS IN CENTROBOLUS COOK, 1897?

M. I. Cooper*
University of South Africa, South Africa.
*Correspondence email, cm.i@aol.com, +27714620070.

***Abstract*- Two species of *Centrobolus* were identified (*C. inscriptus*, *C. ruber*) based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopod structure. One set of linear measurements was made from the SEM micrographs: (1) coleopod spine length; and number. Mass in three species was gaged. Coleopod spine length (n=10, 23) and mass (n=273; 36) were positively related (r=0.76, Z score=1.73, n=6, p=0.04). Spines counted were inversely related to mass (r=-0.76, Z score=-1.73, n=6, p=0.04). *C. inscriptus* had the highest spine length (10µM) and the highest mass (2.48 g) while *C. ruber* males had the lowest spine length (2.5µM) and the lowest mass (1.28 g). This supports the function of the spine length as an allometric device in sperm competition or stimulation of cryptic female choice.**

I. INTRODUCTION

The red millipede genus *Centrobolus* is well known for studies on sexual size dimorphism (SSD) and displays prolonged copulation durations for pairs of individuals of the species [4-9]. *Centrobolus* is distributed in temperate southern Africa with northern limits on the east coast of southern Africa at -17° latitude South (S) and southern limits at -35° latitude S. It consists of taxonomically important species with 12 species considered threatened and includes nine vulnerable and three endangered species [25]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [24]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [26]. In *Centrobolus* the coleopods are the anterior gonopods of leg-pair eight and can be classed as paragonopods or peltogonopods because they are fused into a single plate-like structure and play a subsidiary role as inseminating devices while leg-pair nine are sperm-transferring [1]. The sternites (or stigma-carrying plates [27]) prevent lateral shifting (stabilizer) and stretch the vulva sac in a medial plane [3].

The genital morphology and mechanics of copulation were figured in two *Centrobolus* species [1, 2]. These worm-like millipedes have female-biased SSD [4-9, 12-19, 22]. From the results, correlations between coleopod spine length, number, and mass were checked for correlations.

• II. MATERIALS AND METHODS

Two species of *Centrobolus* were identified based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopod structure (*C. inscriptus*, *C. ruber*). The gonopods were dissected from males of these three species and prepared for SEM. Specimens were fixed, first in 2.5% glutaraldehyde (pH 7.4 phosphate-buffered saline) at 4 °C for 24 hours, then in osmium tetroxide (2%). Dehydration through a graded alcohol series (50%, 60%, 70%, 80%, 90% to 100% ethanol) and critical point drying followed. Specimens were mounted on stubs and sputter-coated with gold palladium. Gonopods were viewed under a Cambridge S200 SEM. SEM micrographs were examined and the individual components of the gonopods were identified according to the available species descriptions. One set of linear measurements was made from the SEM micrographs: (1) spine length (µM); and number. The collection of SEM micrographs for each species is particularly informative when comparisons are made between congruent views. These results have been published [1]. Mass was gaged using a Mettler Autobalance. Spine length, number, and mass were correlated using a Pearson Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php). Spine length and numbers were correlated with mass in two species

(*C. inscriptus*, *C. ruber*) using Pearson's Correlation Coefficient.

• III. RESULTS

Spine length (n=10, 23) and mass (n=88 & 88, 56 & 41; 18) were positively related (Figure 1: r=0.76188290, Z score=1.73324227, n=6, p=0.04152627). Least-Squares Regression Line y = 6.21927383 · x + -5.60193686.

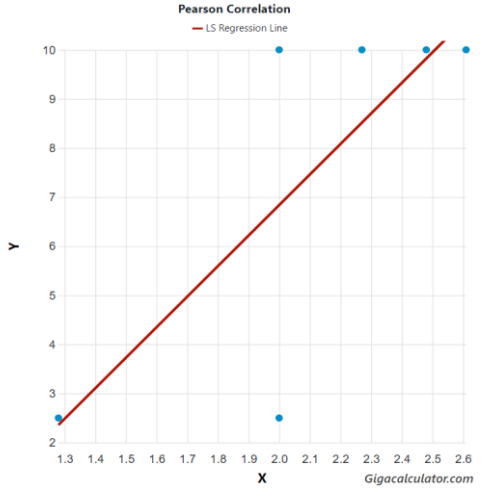

Figure 1. Relationship between the coleopod spine length (y) (µM) and mass (g) (x) across two species of *Centrobolus* (*C. inscriptus*, *C. ruber*).

Spines counted were inversely related to mass (Figure 2: r=-0.76188290, Z score=-1.73324227, n=6, p=0.04152627).

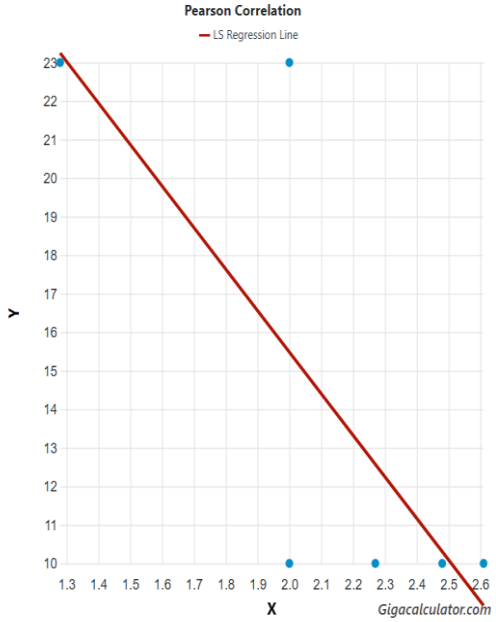

Figure 2. Relationship between the coleopod spine numbers (y) and mass (g) (x) across two species of *Centrobolus* (*C. inscriptus*, *C. ruber*).

• IV. DISCUSSION

The genital morphology and mechanics of copulation were figured in two *Centrobolus* species [1, 2]. A direct relationship between an ultrastructural feature (spine length and number) and the mass of the millipedes is compared which certainly supports the function of the spine as a device adapted for sperm competition [10, 30]. A relationship between this structural feature is present across two species suggesting adaptation to insemination. *C. inscriptus* had the most extended spine length (10µM) and the highest mass (2.48 g) while *C. ruber* males had the lowest spine length (2.5µM) and the lowest mass (1.28 g). Although there was neither a relationship between male nor female mass and spine length when mass is combined from both sexes a relationship exists. It can be challenging to understand the functionality and where there is no functional significance this could have been overlooked [25]. However, the spine lengths and their numbers in *Centrobolus* millipede coleopods predict a functional relevance in assuring paternity.

• V. CONCLUSION

New relationships between ultrastructural features of the morphology (spine length and number) and mass of the *Centrobolus* millipedes support the function of the spines as allometric devices adapted toward reducing sperm competition and assuring paternity. Spine length and number are related to body size across species.

• APPENDIX.

Male and female mass (g) and spine length (µM) in two species of *Centrobolus* with the first species (*C. inscriptus*) having two measurement sets.

C. inscriptus 2.48 (n=88), 10 (n=10) (male)
C. inscriptus 2.27 (n=88), 10 (n=10) (female)
C. inscriptus 2.00 (n=56), 10 (n=10) (male)
C. inscriptus 2.61 (n=41), 10 (n=10) (female)
C. ruber 1.28 (n=18), *2.5* (n=23) (male)
C. ruber 2.00 (n=18), *2.5* (n=23) (female)

• REFERENCES

1). M. I. Cooper, "Confirmation of four species of *Centrobolus* Cook (Spirobolida: Trigoniulidae) based on gonopod ultrastructure," Journal of Entomology and Zoology Studies, vol. 4, no. 4, pp. 389-391, 2016.

2). M. I. Cooper, "Elaborate gonopods in the myriapod genus *Chersastus* (Diplopoda: Trigoniulidae)," Journal of Entomology and Zoology Studies, vol. 3, no. 4, pp. 235-238, 2015.
3). M. Cooper, "Julid millipede and spirobolid millipede gonopod functional equivalents," Journal of Entomology and Zoology Studies, vol. 7, no. 4, pp. 333-335, 2019.
4). M. I. Cooper, "Sexual size dimorphism and corroboration of Rensch's rule in *Chersastus* millipedes," Journal of Entomology and Zoology Studies, vol. 2, no. 6, pp. 264-266, 2014.
5). M. I. Cooper, "Copulation and sexual size dimorphism in worm-like millipedes," Journal of Entomology and Zoology Studies, vol. 5, no. 3, pp. 1264-1266, 2017.
6). M. Cooper, "*Centrobolus anulatus* (Attems, 1934) reversed sexual size dimorphism," Journal of Entomology and Zoology Studies, vol. 6, no. 4, pp. 1569-1572, 2018.
7). M. I. Cooper, "The relative sexual size dimorphism of *Centrobolus inscriptus* compared to 18 congenerics," Journal of Entomology and Zoology Studies, vol. 4, no. 6, pp. 504-505, 2016.
8). M. I. Cooper, "Relative sexual size dimorphism in *Centrobolus fulgidus* (Lawrence) compared to 18 congenerics," Journal of Entomology and Zoology Studies, vol. 5, no. 3, pp. 77-79, 2017.
9). M. I. Cooper, "Relative sexual size dimorphism *Centrobolus ruber* (Attems) compared to 18 congenerics," Journal of Entomology and Zoology Studies, vol. 5 no. 3, pp. 180-182, 2017.
10). M. I. Cooper, "Competition affected by re-mating interval in a myriapod," Journal of Entomology and Zoology Studies, vol. 3, no. 4, pp. 77-78, 2015.
11). M. Cooper, "Re-assessment of rensch's rule in *Centrobolus*," Journal of Entomology and Zoology Studies, vol. 5, no. 6, pp. 2408-1410, 2017.
12). M. I. Cooper, "Size matters in myriapod copulation," Journal of Entomology and Zoology Studies, vol. 5, no. 2, pp. 207-208, 2017.
13). M. I. Cooper, "Sexual size dimorphism and the rejection of Rensch's rule in Diplopoda," Journal of Entomology and Zoology Studies, vol. 6, no. 1, pp. 1582-1587, 2018.
14). M. I. Cooper, "Allometry for sexual dimorphism in millipedes," Journal of Entomology and Zoology Studies, vol. 6, no. 1, pp. 91-96, 2018.
15). M. I. Cooper, "Trigoniulid size dimorphism breaks Rensch," Journal of Entomology and Zoology Studies, vol. 6, no. 3, pp. 1232-1234, 2018.
16). M. Cooper, "A review of studies on the fire millipede genus *centrobolus* (diplopoda: trigoniulidae)," Journal of Entomology and Zoology Studies, vol. 6, no. 4, pp. 126-129, 2018.
17). M. Cooper, "*Centrobolus sagatinus* sexual size dimorphism based on differences in horizontal tergite widths," Journal of Entomology and Zoology Studies, vol. 6, no. 6, pp. 275-277, 2018.
18). M. Cooper, "*Centrobolus silvanus* dimorphism based on tergite width," Global Journal of Zoology, vol. 3, no. 1, pp. 003-005, 2018.
19). M. Cooper, "Xylophagous millipede surface area to volume ratios are size dependent in forest," Arthropods, vol. 8, no. 4, pp. 127-136, 2019.
20). J. M. Dangerfield, S. R. Telford, "Seasonal activity patterns of julid millipedes in Zimbabwe," Journal of Tropical Ecology, vol. 7, pp. 281-285, 1991.
21). J. M. Dangerfield, A. E. Milner, R. Matthews, "Seasonal activity patterns and behaviour of juliform millipedes in south-eastern Botswana," Journal of Tropical Ecology, vol. 8, no. 4, pp. 451-464, 1992.
22). M. D. Greyling, R. J. Van Aarde, S. M. Ferreira, "Seasonal changes in habitat preferences of two closely related millipede species," African Journal of Ecology, vol. 39, no. 1, pp. 51-58, 2001.
23). G. I. Holwell, O. Kazakova, F. Evans, J. C. O'Hanlon, K. L. Barry, "The Functional Significance of Chiral Genitalia: Patterns of Asymmetry, Functional Morphology and Mating Success in the Praying Mantis *Ciulfina baldersoni*," PLoS ONE, vol. 10, no. 6, pp. e0128755, 2015.
24). R. F. Lawrence, "The Spiroboloidea (Diplopoda) of the eastern half of Southern Africa*," Annals of the Natal Museum, vol. 18, no. 3, pp. 607-646, 1967.
25). R. P. Mailula, "Taxonomic revision and Red List assessment of the 'red millipede' genus *Centrobolus* (Spirobolida: Pachybolidae) of South Africa," The University of Kwazulu natal, xxiii+289, 2021.
26). P. Sierwald , J. E. Bond, "Current Status of the Myriapod Class Diplopoda (Millipedes): Taxonomic Diversity and Phylogeny," Annual Review of Entomology, vol. 52, no. 1, pp. 401-420, 2007.
27). T. Wesener, P. Sierwald, J-F. Wägele, "Sternites and spiracles – The unclear homology of ventral sclerites in the basal millipede order Glomeridesmida (Myriapoda, Diplopoda)," Arthropod Structure & Development, vol. 43, no. 1, pp. 87-95, 2014.
28). X. J. Zahnle, P. Sierwald, S. Ware, J. E. Bond, "Genital morphology and the mechanics of copulation in the millipede genus *Pseudopolydesmus* (Diplopoda: Polydesmida: Polydesmidae)," Arthropod Structure & Development, vol. 54, pp. 100913, 2020.