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Is a prominent sternite related to spine length, spine number, copulation duration, and male width in *Centrobolus* Cook, 1897?

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Abstract

Four species of *Centrobolus* were identified (*C. fulgidus*, *C. anulatus*, *C. inscriptus*, *C. ruber*) based on morphology and confirmed using Scanning Electron Microscopy (SEM) of Gonopod structure. Two sets of linear measurements were made from the SEM micrographs: (1) prominence of the stemite, and (2) length of the spines present on the distal knob (which were counted). Copulation duration and male width were recorded. Sternite prominence and spine length were positively related (Kendall's τ=1, Z score=2.32, n=3, p=0.01). Sternite prominence and spine number were inversely related (Kendall's τ=1, Z score=-2.32, n=3, p=0.01). Spine length and spine number were inversely correlated (Kendall's τ=1, Z score=-2.32, n=3, p=0.01). There was a significant correlation between sternite prominence and copulation duration in the four species (Pearson's r=0.97, Z score=2.14, n=4, p=0.02). Sternite prominence was positively related to male width (r=0.96, Z score=1.90, n=4, p=0.03). This supports the function of the sternite as (hypoallometric) devices adapted toward sperm competition.

Keywords: Spine length, spine number, copulation duration, and male width, Scanning Electron Microscopy (SEM)

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 [3-8]. *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 [19]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [18]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [20]. 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 [22]) prevent lateral shifting (stabilizer) and stretch the vulva sac in a medial plane [2].

The genital morphology and mechanics of copulation were figured in four *Centrobolus* species ^[1]. These are worm-like millipedes that have female-biased SSD ^[3-8, 11-13]. From the results correlations between coleopod sternite prominence, spine length, and spine number were checked. Copulation duration and male width were also checked for correlations with sternite prominence.

Materials and Methods

Three species of *Centrobolus* were identified based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopod structure (*C. anulatus*, *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.

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SEM micrographs were examined and the individual components of the gonopods were identified according to the available species descriptions. Two sets of linear measurements were made from the SEM micrographs: (1) prominence of the stemite (%). This has been estimated before as a ratio of how far it extends from the basal region up to the top of the coleopod, and (2) the length of the spines present on the distal knob was estimated from measurements taken directly from SEM images. The collection of SEM micrographs for each species is particularly informative when comparisons are made between congruent views. These results have been published [1]. Sternite prominence, spine length, and spine number were correlated here using a Kendall Correlation Coefficient(https://www.gigacalculator.com/calculators/corr elation-coefficient-calculator.php). Sternite prominence was correlated with copulation duration in four species (C. fulgidus, C. anulatus, C. inscriptus, C. ruber) using a Pearson's Correlation Coefficient. Sternite prominence was correlated with male width in the same four species using a Pearson's Correlation Coefficient.

Results

Sternite prominence and spine length were positively related 1: Kendall's $\tau=1$, Z score=2.32379001. p=0.01006835). Sternite prominence and spine number were inversely related (Figure 2: Kendall's τ=-1, Z score=-2.32379001, p=0.01006835). Spine length and spine number were inversely correlated (Figure 3: Kendall's τ =-1, Z score=-2.32379001, p=0.01006835). Sternite prominence was marginally related to minimum temperature (r=-0.88803279, Z score=-1.41254232, n=4, p=0.07889522). There was a significant correlation between sternite prominence and copulation duration in the four species (Figure 4: Pearson's r=0.97257880, Z score=2.13789031, n=4, p=0.01626277). Sternite prominence was positively related to male width (r=0.95618289, Z score=1.89936295, n=4, p=0.02875832). Sternite prominence was also related to log male width (r=0.95525469, Z score=1.88864452, n=4, p=0.02946967) log sternite prominence was positively related to log male width (Figure 5: r=0.94192036, Z score=1.75480876, n=4, p=0.03964597); least-squares regression line was y = 0.22663911x + 0.83415338.

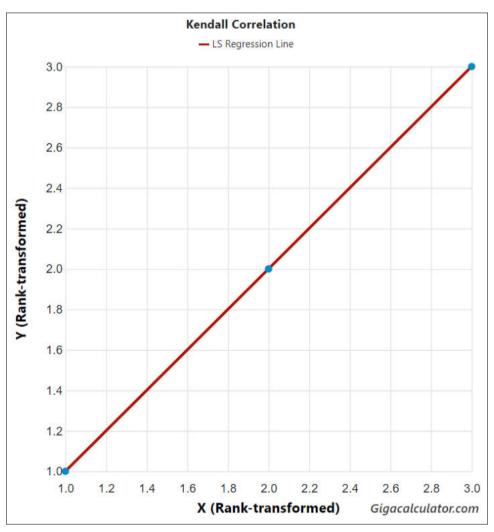


Fig 1: Positive correlation between sternite prominence (x) and spine length (y) for Centrobolus Gonopods

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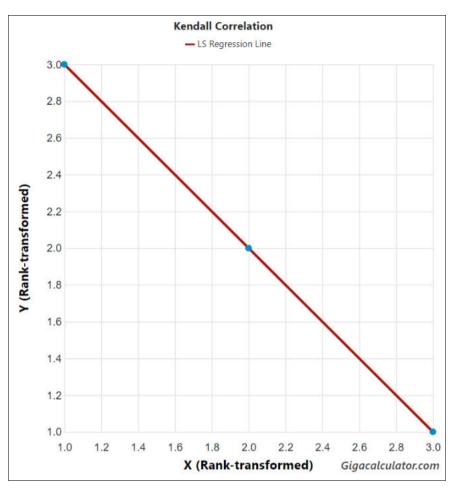


Fig 2: Negative correlation between sternite prominence (x) and spine number (y) for Centrobolus gonopods

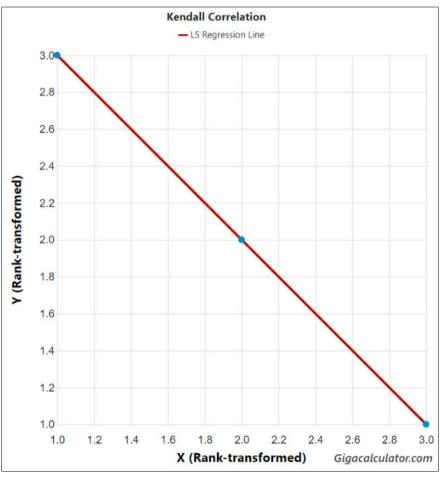


Fig 3: Negative correlation between spine length (x) and spine number (y) for Centrobolus gonopods

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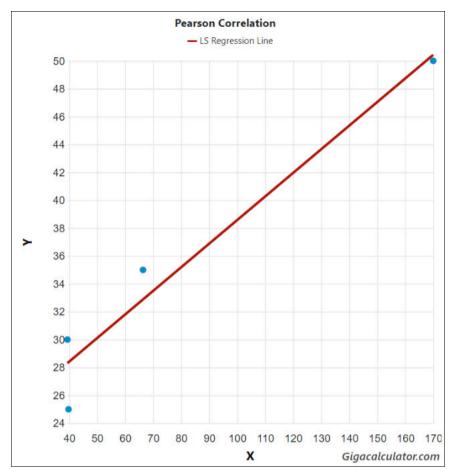


Fig 4: Positive correlation between sternite prominence in anterior gonopods (%) and copulation duration (X-axis) across four species of *Centrobolus* (*C. fulgidus*, *C. anulatus*, *C. inscriptus*, *C. ruber*)

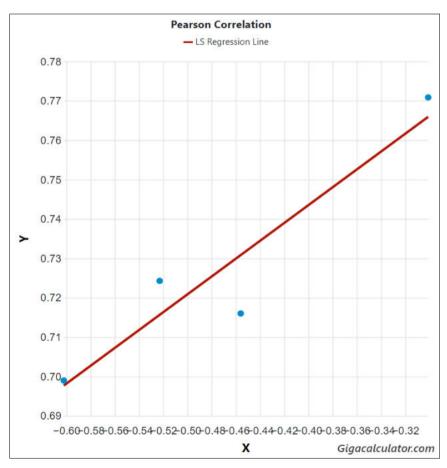


Fig 5: Positive correlation between *log* sternite prominence in anterior gonopods (%) and *log* male width in millimeters (Y-axis) across four species of *Centrobolus* (*C. fulgidus*, *C. anulatus*, *C. inscriptus*, *C. ruber*)

Discussion

The genital morphology and mechanics of copulation were figured in three Centrobolus species [1]. New relationships between the ultrastructural features (sternite prominence, spine length, spine number) of the millipedes' gonopods are compared which supports the function of the sternite and its accessory spines as devices adapted toward sperm competition [9, 23]. A relationship between one of the three structural features is present across species suggesting adaptation to insemination. It can be difficult to understand the functionality and where there is no functional significance this could have been overlooked [17]. However. the sternites in *Centrobolus* millipedes point toward a functional significance in assuring paternity. There was a hypoallometric allometric function relating prominence to male width which did not impact SSD. Hypoallometric relationships in genitalia have also been documented in the Lepidopteran Ostrinia latipennis [21], as was found in insects and maybe spiders [16].

Conclusion

New relationships between the ultrastructural features of the morphology (sternite prominence, spine length, spine number) of the *Centrobolus* millipedes' gonopods supports the function of the sternite and its accessory spines as hypoallometric devices adapted toward reducing sperm competition. A prominent sternite is related to spine length, spine number, copulation duration, and male width.

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