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IS A PROMINENT STERNITE RELATED TO SEX RATIOS AND ABUNDANCE IN *CENTROBOLUS* COOK, 1897?

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Abstract- **Two species of *Centrobolus* were identified (*C. anulatus*, *C. inscriptus*) based on morphology and confirmed using Scanning Electron Microscopy (SEM) of the gonopod structure. Two sets of linear measurements were made from the SEM micrographs: (1) prominence of the stemite, and (2) colepood spine length; with (3) spine number. Sex ratios in two species were gaged. Sternite prominence and sex ratios were negatively related (r=-0.46, Z score=-1.80, n=16, p=0.04). Spine length and spine number and sex ratios were not related. *C. inscriptus* had the highest sternite prominence (0.5 or 50%) with low sex ratios, and *C. anulatus* had the lower sternite prominence (0.30 or 30%) with high sex ratios. Sterqnite prominence and spine length and absolute abundances were positively related (r=0.63, Z score=1.66, n=8, p<0.05). Spine number and absolute abundance were negatively related (r=-0.63, Z score=-1.66, n=8, p<0.05).**

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 [4-10]. *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 [34]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [33]. Spirobolida has two pairs of legs modified into gonopods on the eighth and ninth diplosegments [35]. In *Centrobolus* the coleopods are the anterior gonopods of leg-pair eight. They 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. In contrast, leg-pair nine is sperm-transferring [1]. The sternites (or stigma-carrying plates [36]) 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 endangered *Centrobolus* species [1, 2, 34]. These worm-like millipedes have female-biased SSD [4-24, 30]. From the results, correlations between coleopod sternite prominence, spine length, spine number, sex ratios, and abundance 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 the gonopod structure (*C. anulatus*, *C. inscriptus*). The gonopods were dissected from males of these two 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. Three sets of linear measurements were made from the SEM micrographs: (1) prominence of the stemite (%), (2) coleopod spine length (μM), and (3) coleopod spine number. Sternite prominence has been estimated before as a ratio of how far it extends from the basal region up to the top of the coleopod. 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, coleopod spine length, and coleopod spine number, abundance, and sex ratios were correlated here using Pearson's Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php). Sternite prominence, spine length, and spine number were correlated with sex ratios and abundance in two species (*C. anulatus*, *C. inscriptus*) using Pearson's Correlation Coefficient.

III. RESULTS

Sternite prominence and sex ratios were negatively related (Figure 1: r=-0.46245865, Z score=-1.80434156, n=16, p=0.03558883). Coleopod spine length and spine number were not related to sex ratios. Sternite prominence and absolute abundances were positively related (Figure 2: r=0.63046242, Z score=1.65957221, n=8, p=0.04850025). Spine length and absolute abundance were positively related (r=0.63046242, Z score=1.65957221, n=8, p=0.04850025). Spine number and absolute abundance were negatively related (r=-0.63046242, Z score=-1.65957221, n=8, p=0.04850025).

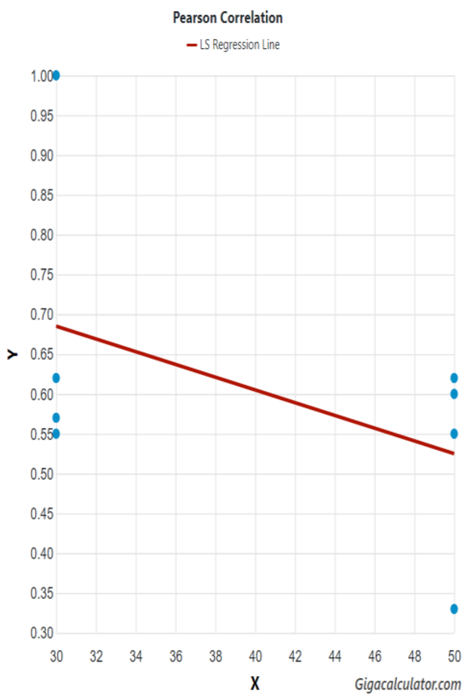

Figure 1. Relationship between sternite prominence (x) and sex ratios (y) for *C. anulatus* and *C. inscriptus*.

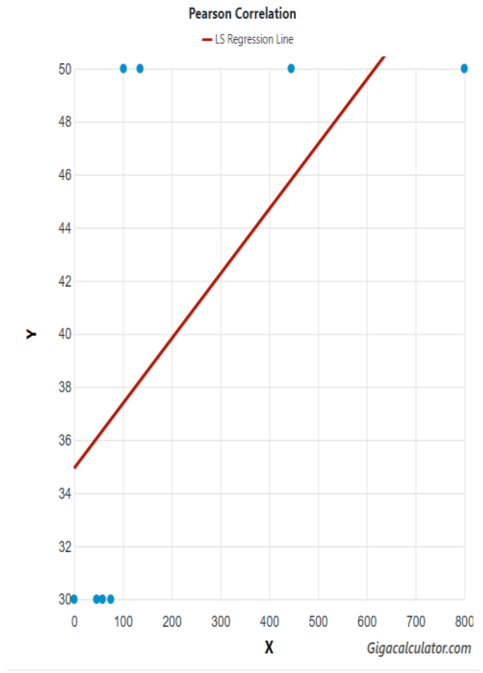

Figure 2. Relationship between sternite prominence (y) and absolute abundance (x) for *C. anulatus* and *C. inscriptus*.

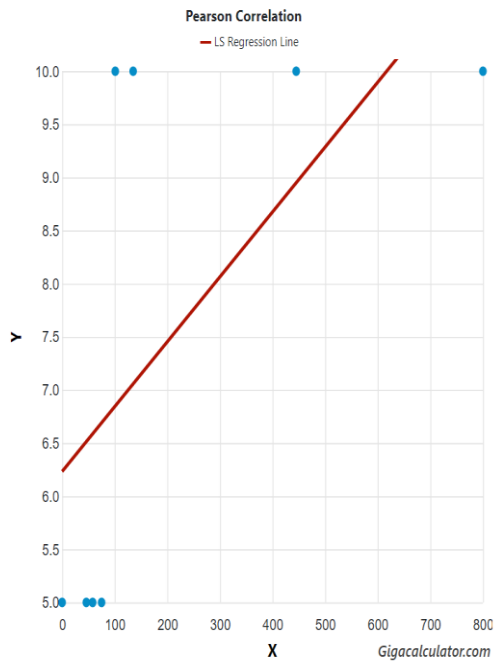

Figure 3. Relationship between spine length (y) and absolute abundance (x) for *C. anulatus* and *C. inscriptus*.

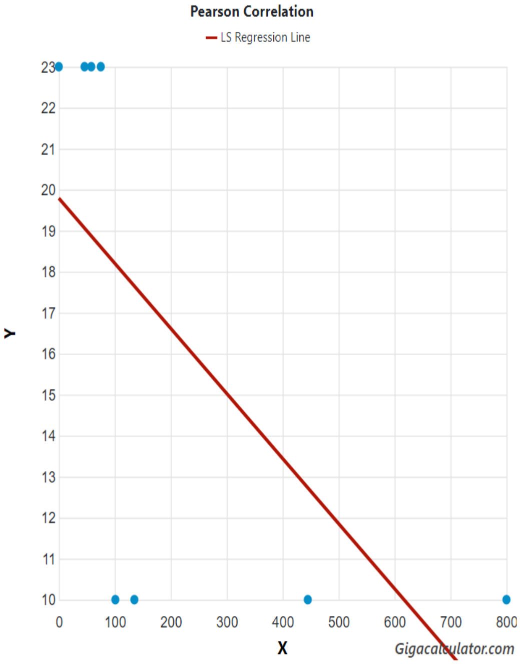

Figure 4. Relationship between spine number (y) and absolute abundance (x) for *C. anulatus* and *C. inscriptus*.

IV. DISCUSSION

The genital morphology and mechanics of copulation were figured in two *Centrobolus* species [1, 2]. A direct relationship between one ultrastructural feature (sternite prominence) and sex ratios (and abundance) in the millipedes is compared which certainly supports the function of the sternite as a device adapted to mating [11, 37]. A relationship between these structural features is present across two species suggesting adaptation to insemination. *C. inscriptus* had the highest sternite prominence (0.5 or 50%) and lowest sex ratios and highest abundance. *C. anulatus* had the lowest sternite prominence (0.30 or 30%) and higher sex ratios and lowest abundance. It can be challenging to understand the functionality [30] However, the sternites and accessory spines in *Centrobolus* millipedes predict a functional relevance in assuring paternity and mating or copulation. This was previously explained with a mechanical fit and stimulatory one-size-fits-all arguments [27, 28]. The results appear intuitive because the absence of *C. anulatus* on the ground substrate may statistically male-bias the adult sex ratio (ASR). Differences between the two species were expected and may be better explained concerning the correlation between sternite prominence and mating frequency or differentiating the ASR from the OSR [32]. The prominent sternite is evidence of sexual selection on males while the generation of a female-biased ASR in *C. inscriptus* is also evidence of conventional sex roles [32]. The costs of breeding, but not the operational sex ratio, may correctly predict the strength of sexual selection in this case [29].

V. CONCLUSION

New relationships between an ultrastructural feature of the morphology (sternite prominence) and sex ratios among the *Centrobolus* millipedes support the function of the sternite and its accessory structures as hypoallometric devices adapted toward negating sperm competition and assuring paternity among various sex ratios. A prominent sternite with longer fewer spines is adapted to function in female-biased sex ratios.

APPENDIX.

Male and female sex ratios (on the ground, in the trees, early, and late in a season) and sternite prominence (%), spine length (µM), and spine number in two species of *Centrobolus*.
1, 30 (*C. anulatus*).
0.55, 30 (*C. anulatus*).
0.62, 30 (*C. anulatus*).
0.57, 30 (*C. anulatus*).
0.55, 50 (*C. inscriptus*).
0.62, 50 (*C. inscriptus*).
0.60, 50 (*C. inscriptus*).
0.33, 50 (*C. inscriptus*).

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