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

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Abstract- Three 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. Weather gradients in three species were gaged. Coleopod spine length and number (n=10, 23) and weather were negatively (Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0) and positively (Kendall's τ=0.81649658, Z score=20000, n=3, p=0) related to four factos (highest and lowest relative humidity, months with the highest and lowest number of rainy days) and positively and negatively related to six factors (average annual temperature, precipitation, and hottest and coldest months), respectively. *C. inscriptus* had the highest spine length (10μM) and lowest spine number which was more negatively and positively related to lowest and highest relative humidities, more positively and negatively related to the months with the highest and lowest number of rainy days, and more positively and negatively related to average annual temperature, more positively and negatively related precipitation (highest and lowest), and more positively and negatively related to hottest and coldest months while *C. ruber* had the lowest spine length (2.5μM) and highest spine number which was less negatively and positively related to lowest and highest relative humidities, less positively and negatively related to the months with the highest and lowest number of rainy days, and less positively and negatively related to average annual temperature, less positively and negatively related precipitation (highest and lowest), and less positively and negatively related to hottest and coldest months. This supports the function of the spine length and number as environmentally adapted devices 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 three *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 and number with weather gradients were checked for correlations.

II. MATERIALS AND METHODS

Three 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), including 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]. Weather gradients were gaged using https://en.climate-data.org/africa/south-africa/kwazulu-natal. Spine length, number, and weather gradient factors were correlated using a Pearson Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php). Spine length and numbers were correlated with 13 weather factors in three species (*C. inscriptus*, *C. fulgidus*, *C. ruber*) using Pearson's Correlation Coefficient.

III. RESULTS

Spine length (n=10, 23) and number were related to:

<u>Highest relative humidity</u>

Spine length (Figure 1: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0) and number (Figure 2: Kendall's τ=0.81649658, Z score=20000, n=3, p=0).

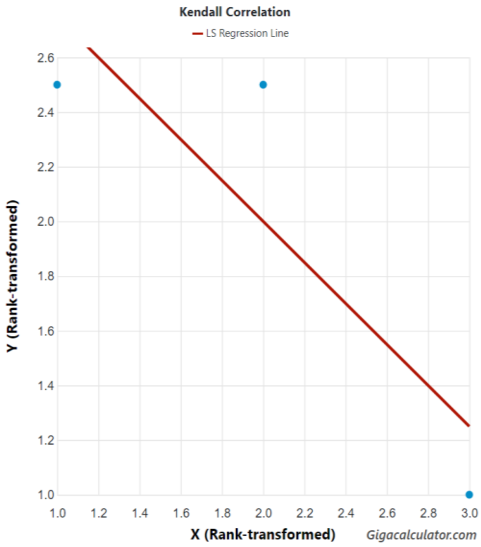

Figure1. Relationship between highest relative humidity and spine length in *Centrobolus* Cook, 1897.

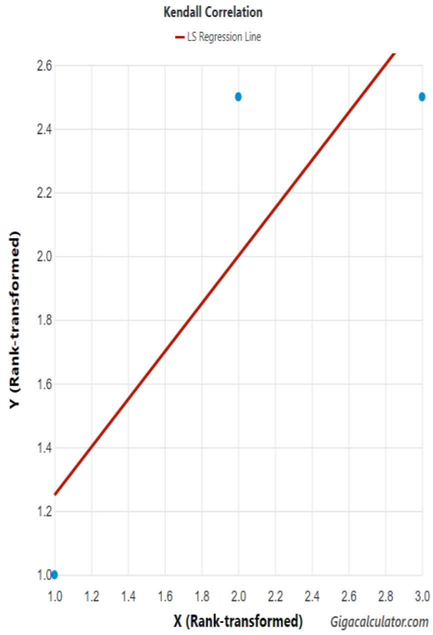

Figure 2. Relationship between highest relative humidity and spine number in *Centrobolus* Cook, 1897.

<u>Lowest relative humidity</u>

Spine length (Figure 3: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0) and spine number (Figure 4: Kendall's τ=0.81649658, Z score=20000, n=3, p=0).

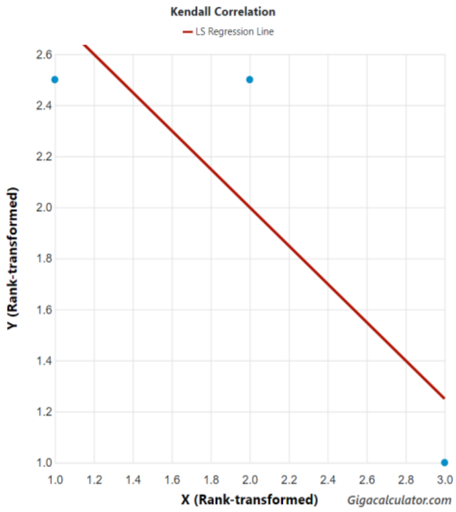

Figure 3. Relationship between lowest relative humidity and spine length in *Centrobolus* Cook, 1897.

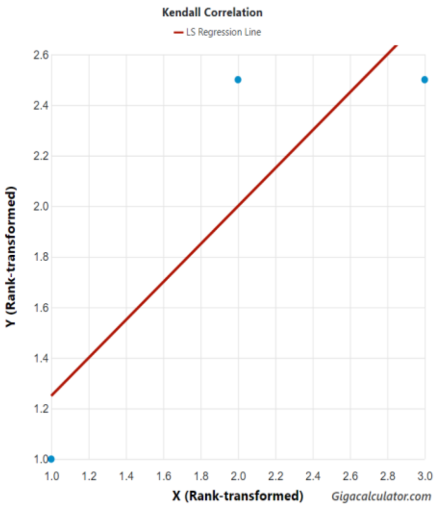

Figure 4. Relationship between lowest relative humidity and spine number in *Centrobolus* Cook, 1897.

The month with the highest number of rainy days

Spine length (Figure 5: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 6: Kendall's τ=0.81649658, Z score=-20000, n=3, p=0).

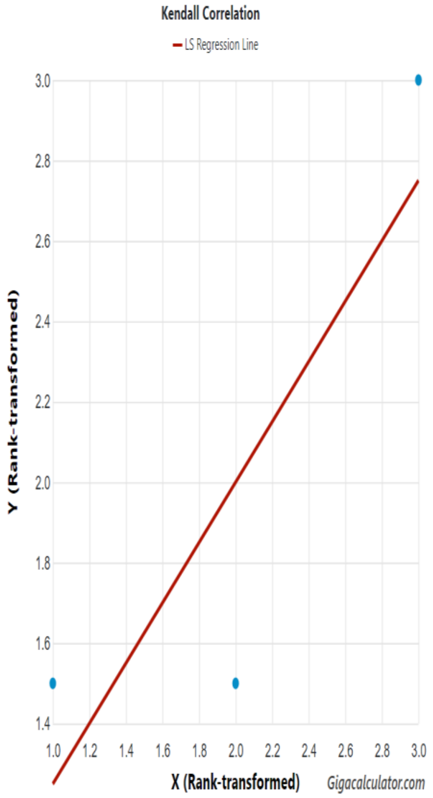

Figure 5. Relationship between a month with the highest number of rainy days and spine length in *Centrobolus* Cook, 1897.

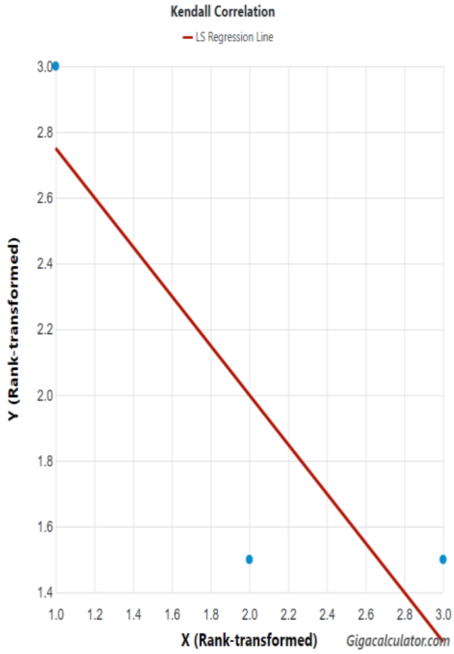

Figure 6. Relationship between a month with the highest number of rainy days and spine number in *Centrobolus* Cook, 1897.

The month with the lowest number of rainy days

Spine length (Figure 7: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and number (Figure 8: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

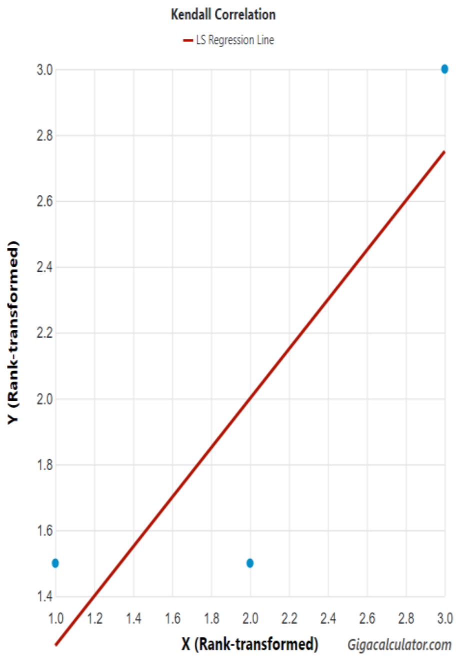

Figure7. Relationship between a month with the lowest number of rainy days and spine length in *Centrobolus* Cook, 1897.

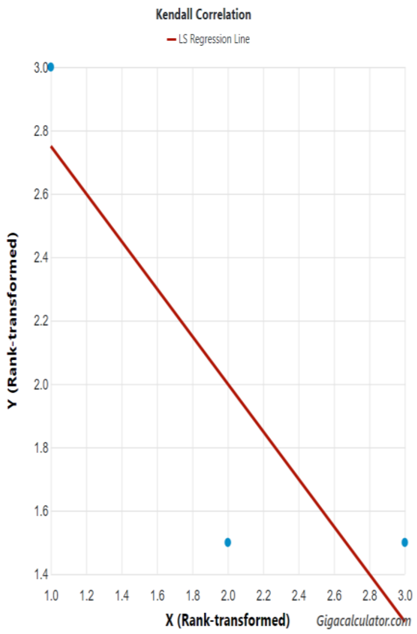

Figure 8. Relationship between a month with the lowest number of rainy days and spine number in *Centrobolus* Cook, 1897.

Average annual temperature

Spine length (Figure 9: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 10: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

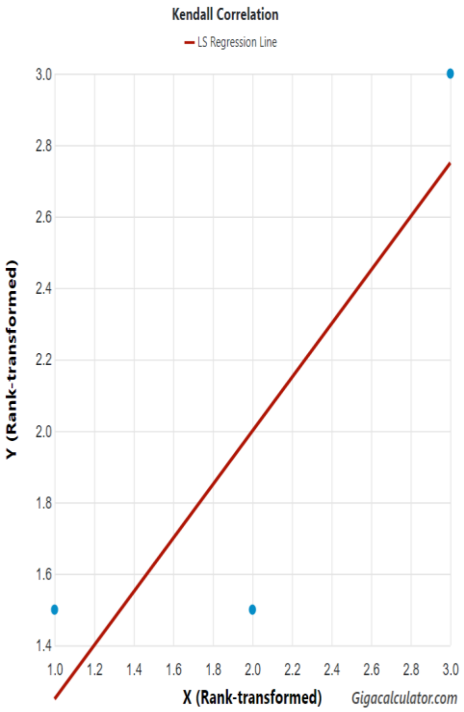

Figure 9. Relationship between average annual temperature and spine length in *Centrobolus* Cook, 1897.

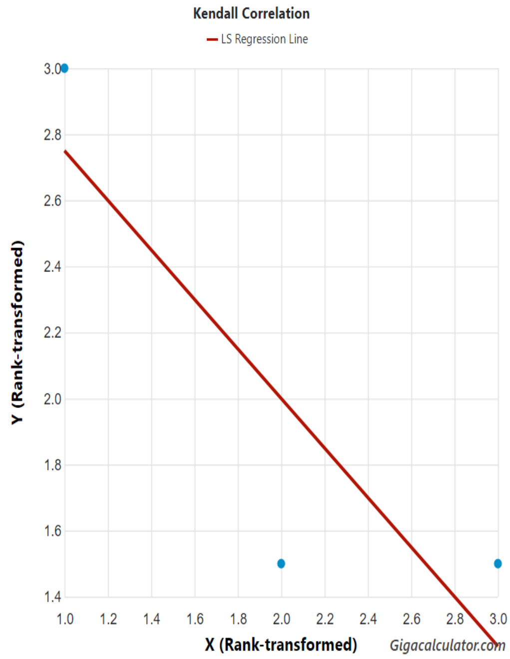

Figure 10. Relationship between average annual temperature and spine number in *Centrobolus* Cook, 1897.

Precipitation

Spine length (Figure 11: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 12: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

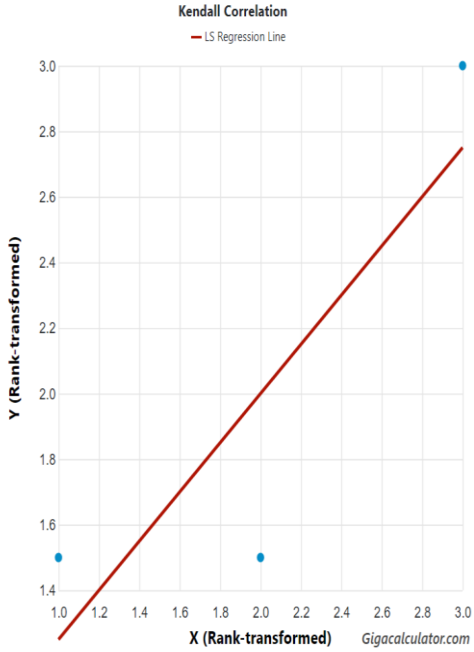

Figure 11. Relationship between precipitation and spine length in *Centrobolus* Cook, 1897.

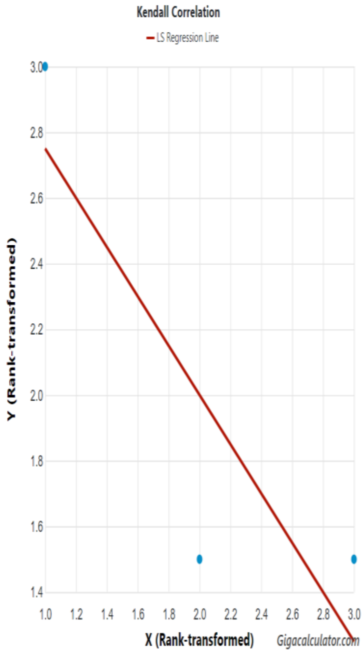

Figure 12. Relationship between precipitation and spine number in *Centrobolus* Cook, 1897.

<u>Lowest precipitation</u>

Spine length (Figure 13: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 14: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

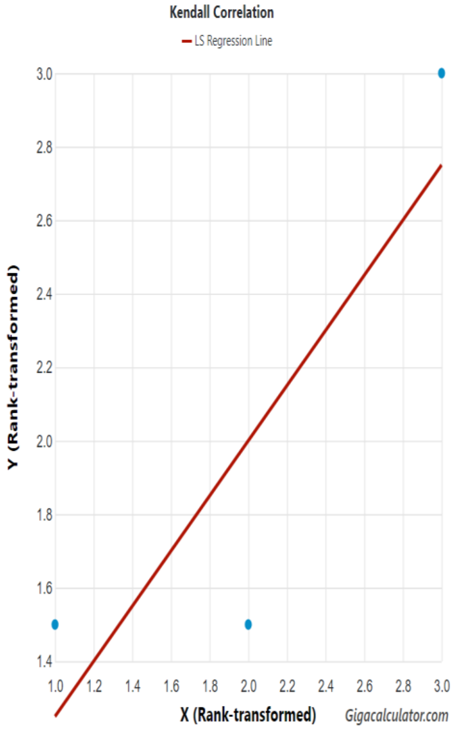

Figure 13. Relationship between lowest precipitation and spine length in *Centrobolus* Cook, 1897.

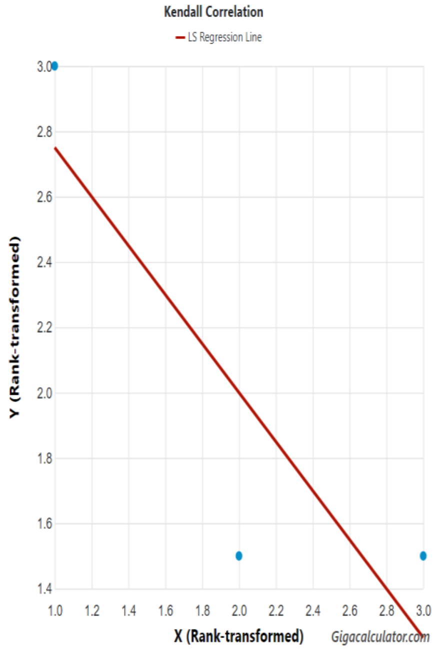

Figure 14. Relationship between lowest precipitation and spine number in *Centrobolus* Cook, 1897.

<u>**Most precipitation**</u>

Spine length (Figure 15: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 16: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

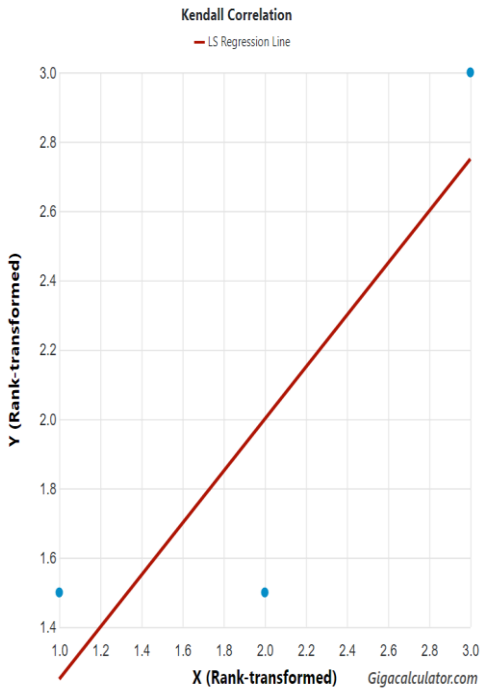

Figure 15. Relationship between highest precipitation and spine length in *Centrobolus* Cook, 1897.

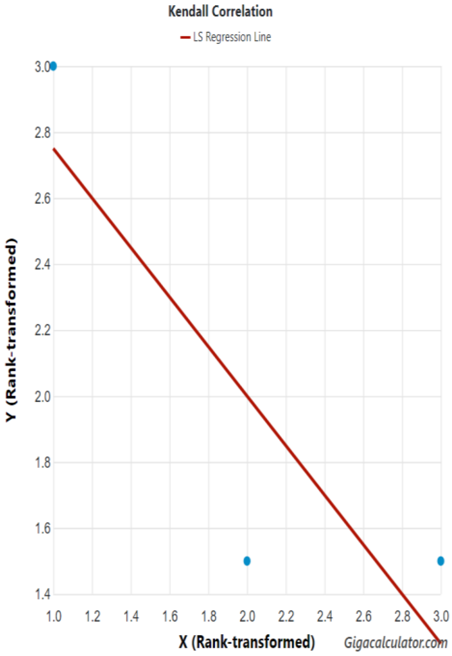

Figure 16. Relationship between highest precipitation and spine number in *Centrobolus* Cook, 1897.

Hottest month

Spine length (Figure 17: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 18: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

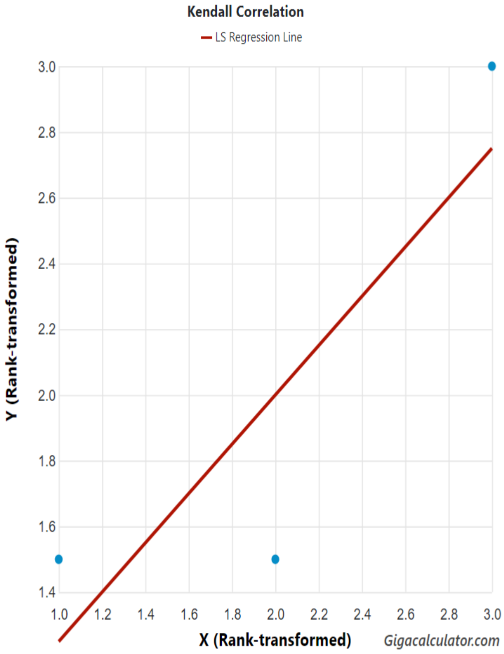

Figure 17. Relationship between hottest month and spine length in *Centrobolus* Cook, 1897.

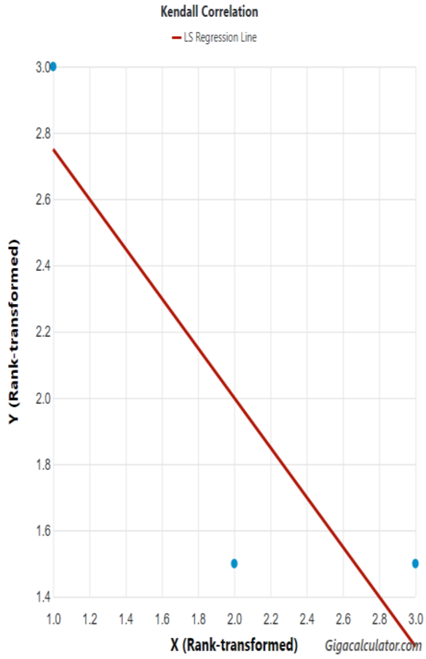

Figure 18. Relationship between hottest month and spine number in *Centrobolus* Cook, 1897.

Coldest month

Spine length (Figure 19: Kendall's τ=0.81649658, Z score=20000, n=3, p=0) and spine number (Figure 20: Kendall's τ=-0.81649658, Z score=-20000, n=3, p=0).

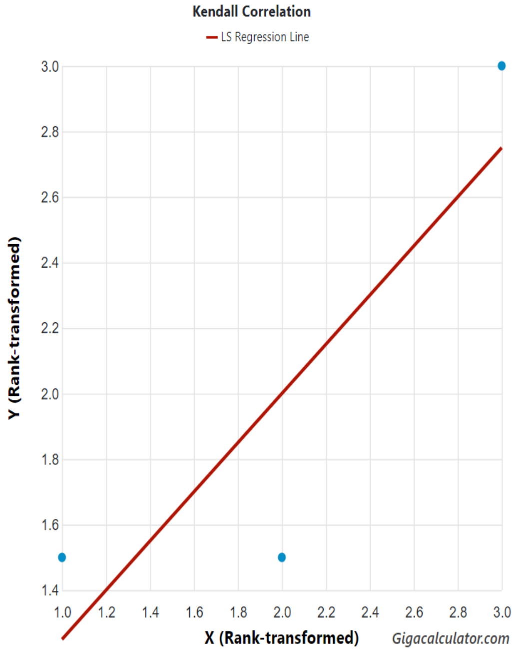

Figure 19. Relationship between the coldest month and spine length in *Centrobolus* Cook, 1897.

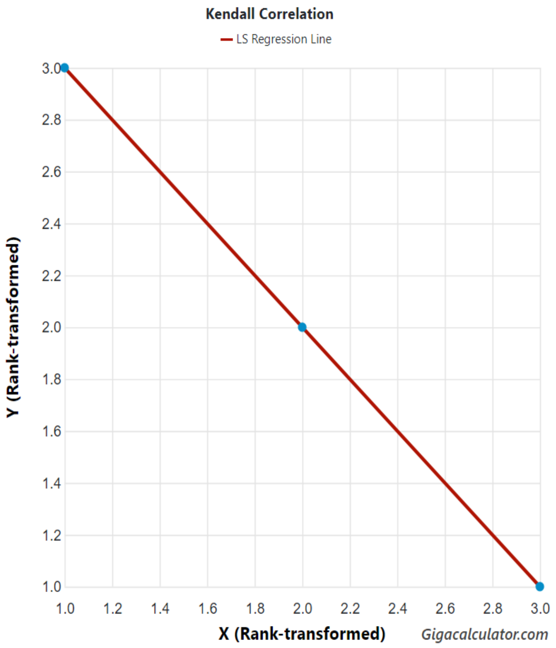

Figure 20. Relationship between the coldest month and spine number in *Centrobolus* Cook, 1897.

• **IV.** DISCUSSION

The genital morphology and mechanics of copulation were figured in three *Centrobolus* species [1, 2]. A direct relationship between an ultrastructural feature (spine length and number) and the weather gradients are compared which certainly supports the function of the coleopod spine as a device adapted for sperm competition under different environmental conditions [10, 30]. A relationship between this structural feature is present across two species suggesting adaptation to insemination in different environmental conditions. *C. inscriptus* had the most extended spine length (10µM) and lowest spine number which were more negatively and positively related to lowest and highest relative humidities, more positively and negatively related to the months with the highest and lowest number of rainy days, and more positively and negatively related to average annual temperature, more positively and negatively related precipitation (highest and lowest), and more positively and negatively related to hottest and coldest months while *C. ruber* had the lowest spine length (2.5µM) and highest spine number which was less negatively and positively related to lowest and highest relative humidities, less positively and negatively related to the months with the highest and lowest number of rainy days, and less positively and negatively related to average annual temperature, less positively and negatively related precipitation (highest and lowest), and less positively and negatively related to hottest and coldest months. It can be challenging to understand the functionality and where there is no functional significance this could have been misjudged without weather gradients [25]. However, the spine lengths in *Centrobolus* millipede coleopods predict a functional relevance in assuring paternity evolved under weather gradients.

• **V.** CONCLUSION

New relationships between ultrastructural features of the morphology (spine length) and weather gradients in the *Centrobolus* millipedes support the function of the spines as allometric devices adapted toward reducing sperm competition and assuring paternity.

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