IS MASS RELATED TO LATITUDE, LONGITUDE, AND WEATHER IN CENTROBOLUS COOK, 1897?

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***Abstract*-Four species of *Centrobolus* were identified (*C. digrammus*, *C. fulgidus*, *C. inscriptus*, *C. ruber*) based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopod structure. One set of measurements was made (1) body mass (g). Mass in three species was gaged. *C. inscriptus* had the heaviest mass (2.61 g) while *C. digrammus* males had the lightest mass (0.68 g). Mass was correlated with latitude (r=0.78, Z score=2.75, n=10, p<0.01). Female mass was correlated with latitude (r=0.91, Z score=2.12, n=5, p=0.02). Male mass was marginally correlated with latitude (r=0.77, Z score=1.44, n=5, p=0.08). Mass was correlated with longitude (r=0.75, Z score=2.59, n=10, p<0.01). Female mass was not related to longitude (r=0.60, Z score = 0.99, n=5, p=0.16). Male mass was marginally related to longitude (r=0.79, Z score=1.51, n=5, p=0.07). Mass was correlated with lowest relative humidity (r=-0.86, Z score=-3.42, n=10, p<0.01), month with the highest number of rainy days (r=0.80, Z score=2.87, n=10, p<0.01), month with the lowest number of rainy days (r=0.82, Z score=3.08, n=10, p<0.01), average temperature (r=0.77, Z score=2.67, n=10, p<0.01), rainfall (r=0.85, Z score=3.34, n=10, p<0.01), driest month (r=0.81, Z score=3.01, n=10, p<0.01), wettest month (r=0.70, Z score=2.30, n=10, p=0.01), and warmest month (r=0.78, Z score=2.75, n=10, p<0.01).**

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 a 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 [24]. It occurs in all the forests of the coastal belt from the Cape Peninsula to Beira in Mocambique [23]. The genital morphology and mechanics of copulation were figured in two *Centrobolus* species [1, 2]. These worm-like millipedes have female-biased SSD [3-8, 11-18, 21]. From the results, correlations between mass and latitude, longitude, and weather were checked.

II. MATERIALS AND METHODS

Four species of *Centrobolus* were identified based on morphology and confirmed using Scanning Electron Microscopy (SEM) of gonopod structure (*C. digrammus*, *C. fulgidus*, *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 measurements was made from live specimens. Mass was gaged using a Mettler Autobalance. Mass and latitude (Appendix I), longitude (Appendix II), and weather were correlated using a Pearson Correlation Coefficient (https://www.gigacalculator.com/calculators/correlation-coefficient-calculator.php).

III. RESULTS

There were correlations between mass and latitude (Figure 1: r=0.77699073, Z score=2.74557985, n=10, p=0.00302026). Female mass was corrrelated with latitude (Figure 2: r=0.90514094, Z score=2.12126308, n=5, p=0.01694977). Male mass was marginally correlated with latitude (r=0.76845044, Z score=1.43759410, n=5, p=0.07527467).

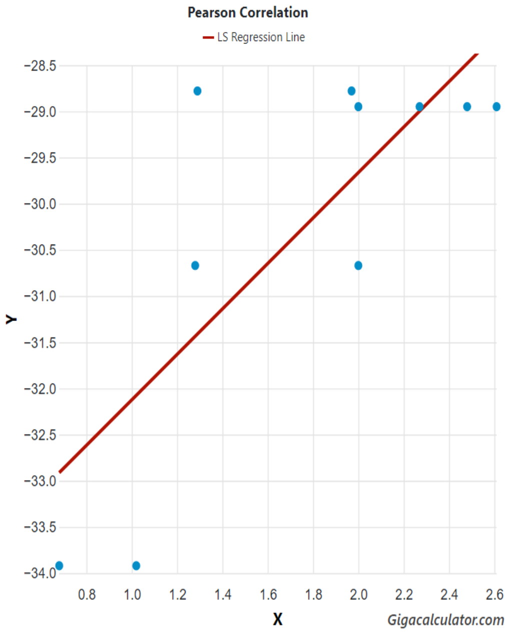

Figure 1. Correlation between body mass and latitude across four species of *Centrobolus* (*C. digrammus*, *C. fulgidus*, *C. inscriptus*, *C. ruber*).

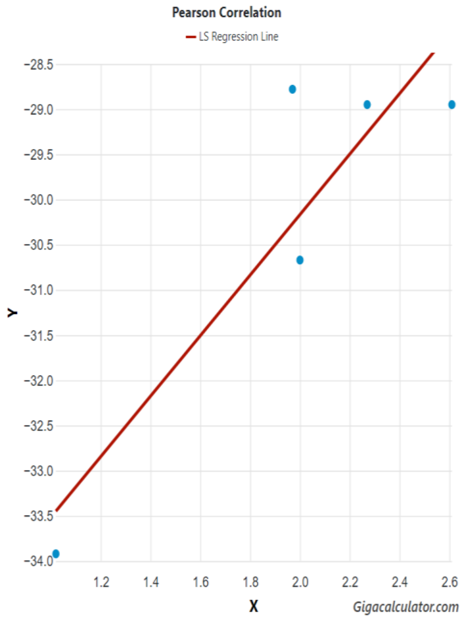

Figure 2. Correlation between female body mass and latitude across four species of *Centrobolus* (*C. digrammus*, *C. fulgidus*, *C. inscriptus*, *C. ruber*).

There were correlations between mass and longitude (Figure 3: r=0.75201970, Z score=2.58645369, n=10, p=0.00484849). Female mass was not related to longitude (r=0.60219853, Z score = 0.98512630, n=5, p=0.16228102). Male mass was marginally related to longitude (r=0.78866639, Z score=1.51022990, n=5, p=0.06549241).

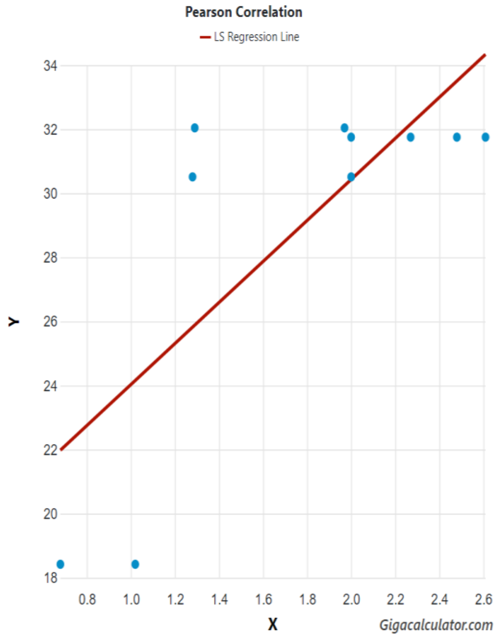

Figure 3. Correlation between body mass and longitude across four species of *Centrobolus* (*C. digrammus*, *C. fulgidus*, *C. inscriptus*, *C. ruber*).

There were correlations between mass and
<u>Lowest relative humidity</u>
r=-0.85996259, Z score=-3.42148828, n=10, p=0.00031145 (Figure 4).

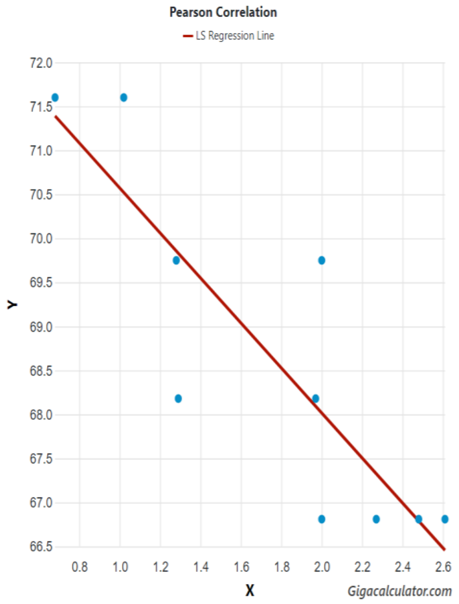

Figure 4. Relationship between lowest relative humidity and body mass across *Centrobolus*.

Month with the highest number of rainy days

r=0.79564981, Z score=2.87498861, n=10, p=0.00202028 (Figure 5).

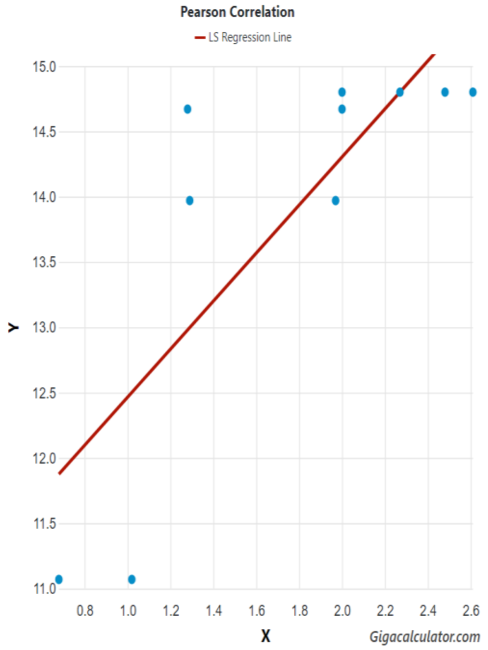

Figure 5. Relationship between month with the highest number of rainy days and body mass across *Centrobolus.*

Month with the lowest number of rainy days

r=0.82269987, Z score=3.08260474, n=10, p=0.00102606 (Figure 6).

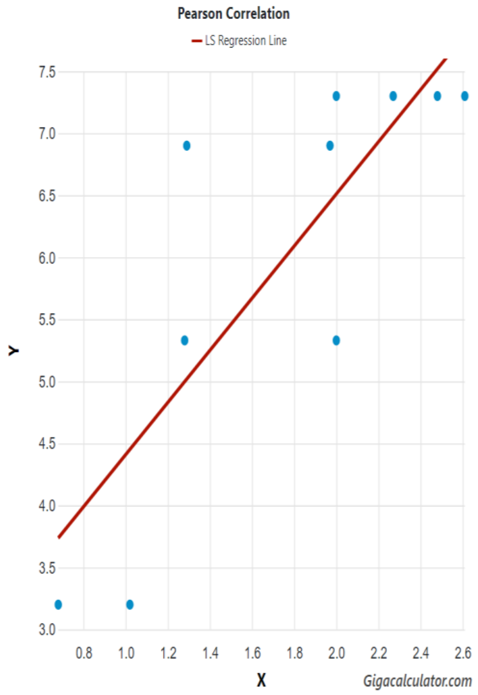

Figure 6. Relationship between month with the lowest number of rainy days and and body mass across *Centrobolus*.

Average temperature

r=0.76544968, Z score=2.67021205, n=10, p=0.00379021 (Figure 7).

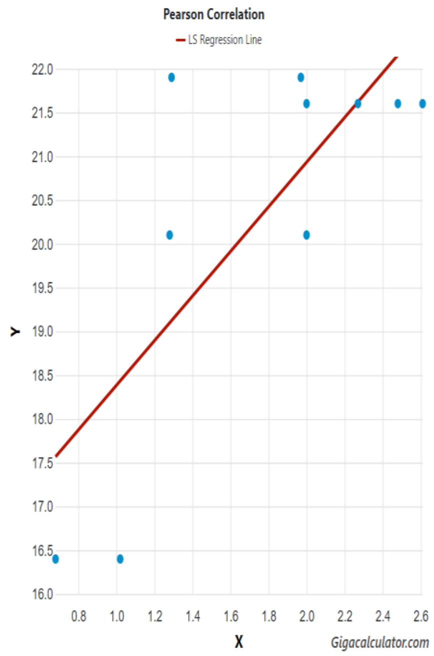

Figure 7. Relationship between average temperature and body mass across *Centrobolus*.

Rainfall

r=0.85125369, Z score=3.33546710, n=10, p=0.00042584 (Figure 8).

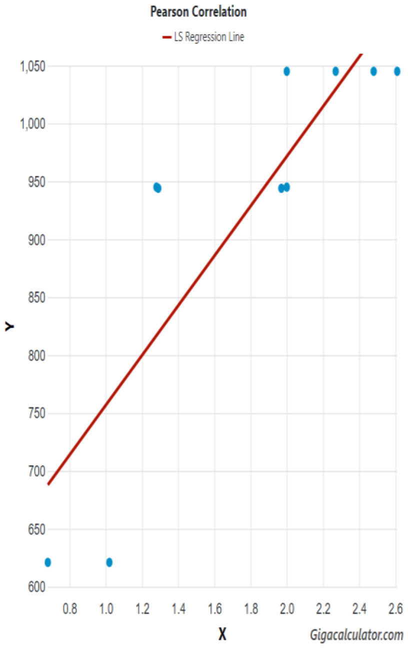

Figure 8. Relationship between rainfall and body mass across *Centrobolus*.

Driest month

r=0.81365072, Z score=3.01016956, n=10, p=0.00130558 (Figure 9).

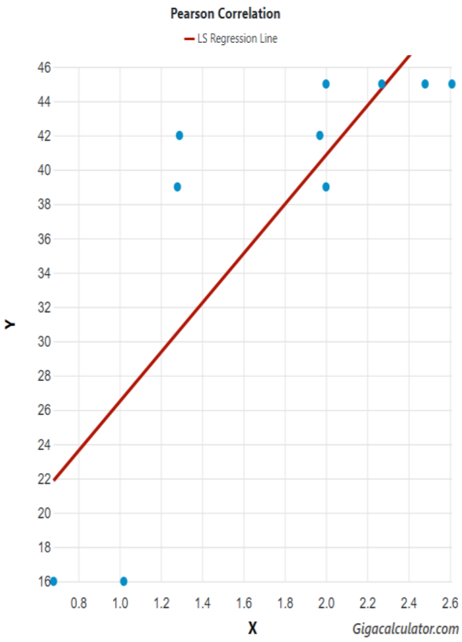

Figure 9. Relationship between driest month and body mass across *Centrobolus*.

Wettest month

r=0.70193382, Z score=2.30472045, n=10, p=0.01059109 (Figure 10).

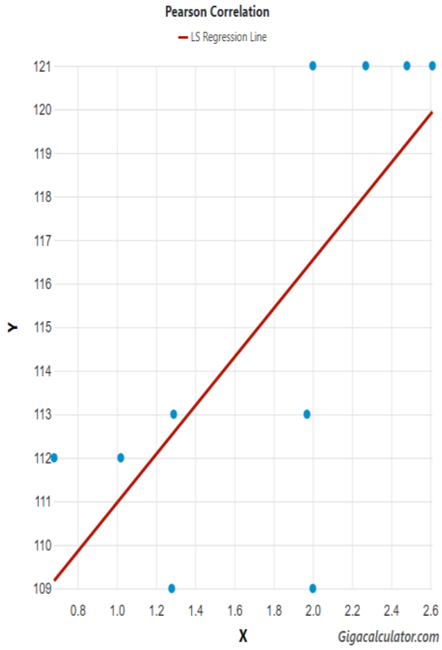

Figure 10. Relationship between wettest month and body mass across *Centrobolus*.

Warmest month

r=0.77698928, Z score=2.74557018, n=10, p=0.00302035 (Figure 11).

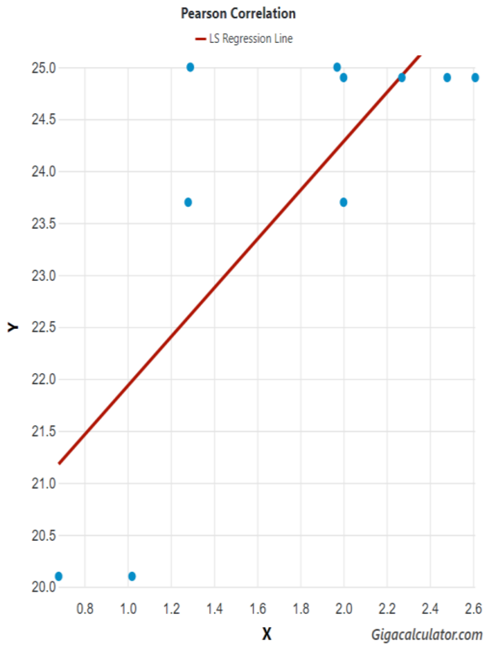

Figure 11. Relationship between warmest month and body mass across *Centrobolus*.

IV. DISCUSSION

The genital morphology and mechanics of copulation were figured in four *Centrobolus* species [1, 2]. A direct relationship between mass and latitude, longitude, and weather in the red millipedes is compared which certainly supports size-based gradients [9]. A relationship between mass and latitude and mass and longitude is present across four species suggesting adaptation across geographical gradients. *C. inscriptus* has the heaviest mass (2.61 g) and was found further northeast while *C. ruber* males had the lightest mass (1.28 g) and were found further southwest. Female mass was correlated with latitude, and male mass was marginally correlated with latitude. Female mass was not related to longitude and male mass was marginally related to longitude.

V. CONCLUSION

Several new relationships between the body mass of the *Centrobolus* millipedes and latitude, longitude, and weather support a geographical gradient driving body size evolution across species.

APPENDIX I.

Male and female mass (g) and latitude (degrees South) in four species of *Centrobolus* with the first species (*C. inscriptus*) having two measurement sets.

C. digrammus 0.68, -33.92 (n=6) (male)
C. digrammus 1.02, -33.92 (n=6) (female)
C. fulgidus 1.29, -28.78 (n=11) (male)
C. fulgidus 1.97, -28.78 (n=11) (female)
C. insciptus 2.48, -28.95 (n=88) (male)
C. insciptus 2.27, -28.95 (n=88) (female)
C. insciptus 2.00, -28.95 (n=56) (male)
C. insciptus 2.61, -28.95 (n=41) (female)
C. ruber 1.28, -30.67 (n=18) (male)
C. ruber 2.00, -30.67 (n=18) (female)

APPENDIX II.

Male and female mass (g) and longitude (degrees East) in four species of *Centrobolus* with the first species (*C. inscriptus*) having two measurement sets.

C. digrammus 0.68, 18.42 (n=6) (male)
C. digrammus 1.02, 18.42 (n=6) (female)
C. fulgidus 1.29, 32.04 (n=11) (male)
C. fulgidus 1.97, 32.04 (n=11) (female)
C. insciptus 2.48, 31.75 (n=88) (male)
C. insciptus 2.27, 31.75 (n=88) (female)
C. insciptus 2.00, 31.75 (n=56) (male)
C. insciptus 2.61, 31.75 (n=41) (female)
C. ruber 1.28, 30.51 (n=18) (male)
C. ruber 2.00, 30.51 (n=18) (female)

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