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Centrobolus anulatus (Attems, 1934) reversed sexual size dimorphism

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Abstract
The present research aimed to study relative sexual size dimorphism of *Centrobolus anulatus* compared to congenerics. Millipedes illustrated reversed sexual size dimorphism (SSD) as females were larger than males and broke the rule as this dimorphism increased with body size. SSD was calculated in 18 species of the genus *Centrobolus* and illustrated as a regression. The approximate relative position of *C. anulatus* was shown from measurements taken in South Africa (2018). The average size of *C. anulatus* was 74.23529412 X 5.685882 mm (n=17); males measured 69.2 X 5.264 mm (n = 5) and females were 76.3 X 5.861667 mm (n = 12). Log volume measurements were (females/x = 3.31359939 mm^3; males/y = 3.2377543 mm^3). The difference between the correlation coefficients for the species and the genus were not highly significant (r_a = 0.86207, r_b = 0.85; n_a = 17, n_b = 18; Z = 0.12; P (one-tailed) = 0.4522, P (two-tailed) = 0.9045). The mean volume ratio for *C. anulatus* was 1.02342521 which differed from 1 (t=3.59722; *p*-value = 000747; *p* <.01; n=17). Evidence suggests sexual bimaturism as proximate cause and competition as ultimate cause for SSD in *Centrobolus*.

Keywords: *Centrobolus anulatus*, dimorphism, millipede, SSD, size

1. Introduction
Sexual size dimorphism is prevalent in arthropods and females are usually larger than males. Behavioural patterns such as provisioning versus non- provisioning relate to SSD. Millipedes illustrate reversed sexual size dimorphism (SSD) and females are larger than males [23, 27, 29, 34]. Diplopoda are underrepresented in allometric analyses of sexual size dimorphism (SSD), although sexual size differences are known in body mass, length, width and leg dimensions of over half the taxa studied [5-9, 23, 26-27]. Size differences correlate with factors such as color, sexes, species, urbanisation and water relations [25-26, 31, 34]. Diplopoda resemble the majority of invertebrates where SSD is reversed [12-14, 20-22]. SSD has consequences for outcomes of sexual encounters in diplopod mating [1, 4-9, 11, 15, 25-27, 29, 34]. The allometry of SSD involves the detection of a relationship between body size and SSD and is known by Rensch's rule [32-33]. Rensch's rule may be explained by sexual selection and fecundity selection [24, 30]. The macro-evolutionary pattern is unresolved in Diplopoda. Here, Rensch's rule was tested in predicting SSD was not negatively correlated with diplopod body size in African forest and savanna taxa. SSD in the forest genus *Centrobolus* was investigated. SSD in forest millipedes have successfully been understood as volumetric measurements using *Centrobolus* to test Rensch's rule. The general trend of SSD has been calculated for *Centrobolus* and bimaturism shown [7]. The present study was aimed to illustrate the trend of SSD for the genus *Centrobolus* and estimate the position of *C. anulatus* relative to 18 congenerics in order to determine whether species follow the trend of Rensch's rule.

2. Materials and methods
Two factors were measured from *Centrobolus anulatus*: (1) body length (mm) placing a piece of string along the body length of individuals collected in South Africa (Table 1) and measuring the string alongside a rule (calibrated in mm), and (2) width (mm) with Vernier calipers. *C. anulatus* (Attems) were collected at the localities in Table 1 from KwaZulu-Natal, South Africa. Any measurement error associated with the transfer of measurement between string and rule would be the same for both sexes. Millipede SSD was also calculated in the genus *Centrobolus* [2-3, 5-9]. A regression of male volume on female volume was used to show the position of 18 species and the size of *C. anulatus* was taken as a volumetric measurement and inserted into a Microsoft (MS) Excel spreadsheet and converted using the log

(mathematical) equation. The chart for SSD in 18 species was captured, copied and exported using the snapshot function in the programme Soda Portable Document File (PDF) 8. It was pasted in a MS Word file.

2.1 Statistical Analysis

The basic descriptive figures were statistically compared using Statistica. Body length: width ratios were inputted into the formula for a cylinder. The mean values of length and width was obtained for 17 individuals of *C. anulatus*. Size was perceived as body volume and calculated based on the formula for a cylinder ($h.\pi.r^2$) where h is body length and r half of the width. SSD was estimated as the mean female volume divided by mean male volume and converted into a SSD index. Allometry for SSD was based on an allometric model where male size = α (female) $^{\beta}$. A Spearman's Rho calculation was made in order to test the correlation between the male and female data measurements at http://www.socscistatistics.com/tests/spearman/Default3.aspx. Correlation coefficients were compared at http://vassarstats.net/rdiff.html. SSD was compared against to 1 using a two-tailed t-test at http://www.socscistatistics.com/tests/studentttest/Default2.aspx.

3. Results

The quantitative resolution of Rensch's rule for 18 species of *Centrobolus* together with the relative estimated position of *C. anulatus* is shown in Fig. 2. The average size of *C. anulatus* was 74.23529412 X 5.685882 mm (n=17); males measured 69.2 X 5.264 mm (n = 5) and females were 76.3 X 5.861667 mm (n = 12). Log volume measurements were (females/x = 3.31359939 mm^3; males/y = 3.2377543 mm^3) (Figure 3). The mean volume ratio for *C. anulatus* was 1.02342521. SSD was visible with the naked eye. There was a correlation between the log values for male and female volumes. The value of R was 0.86207 and the two-tailed value of P is 0.00134. By normal standards, the association between the two variables would be considered statistically significant. The difference between the correlation coefficients for the species and the genus was not highly significant (r_a = 0.86207, r_b = 0.85; n_a = 17, n_b = 18; Z = 0.12; P (one-tailed) = 0.4522, P = 0.9045) (Fig. 1). SSD was significantly different from 1 (t=3.59722; *p*-value = 000747; *p* <.01; n=17).

Table 1: *Centrobolus annulatus* morphological measurements.

KZN Museum Accession number	Sex (M/F)	Length (mm)_string	Width (mm)	Locality
NMSA25977	F	73	5,8	KZN, Mabengu forest, coast lowlands forest, on scrambler in forest
NMSA25994	F	65	5,6	KZN, Vernon Crookes Nature Reserve
NMSA25974	F	84	6,8	KZN, Mabengu forest, coastal lowlands forest, on forest floor with thick underground of ferns
NMSA25995	F	82	5,52	KZN, Vernon Crookes Nature Reserve, forest hillside, on pole at edge of camp
NMSA25910	F	71	6,22	KZN, Blythedale Beach, Farm Blyth Dale 1380
NMSA15551a	M	65	5,08	KZN, Hawaan forest
NMSA15551b	F	85	5,9	KZN, Hawaan forest
NMSA25930	F	74	5,7	KZN, Bluff Nature Reserve
NMSA16491	F	70	5,3	KZN, Kranzkloof Nature Reserve
NMSA25911a	M	78	5,8	KZN, Blythedale Beach, Farm Blyth Dale 1380
NMSA25911b	F	88	7,36	KZN, Blythedale Beach, Farm Blyth Dale 1381
NMSA25975	M	68	5,7	KZN, Mabengu forest, coastal lowlands forest, on felled coastal cabbage tree
NMSA15541a	F	68	5,12	KZN, Durban Bluff, off Berea rd.
NMSA15541b	M	68	4,82	KZN, Durban Bluff, off Berea rd.
NMSA15541c	M	67	4,92	KZN, Durban Bluff, off Berea rd.
NMSA15541d	F	72	5,00	KZN, Durban Bluff, off Berea rd.
NMSA15571	F	84	6,02	KZN, Harold Johnson Nature Reserve

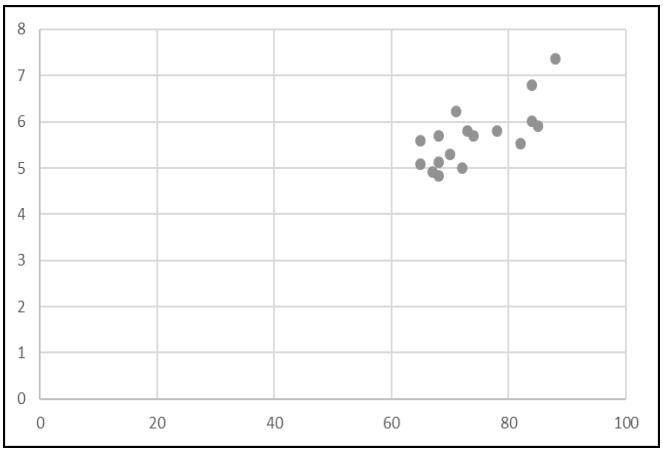

Fig 1: Quantitative resolution of sexual size dimorphism for 17 specimens of the millipede *Centrobolus anulatus*. Allometry for sexual size dimorphism (SSD) is based on the allometric model [28], male size = α (female size) $^{\beta}$; correlation coefficient, r = 0.86207 (P = 0.00134).

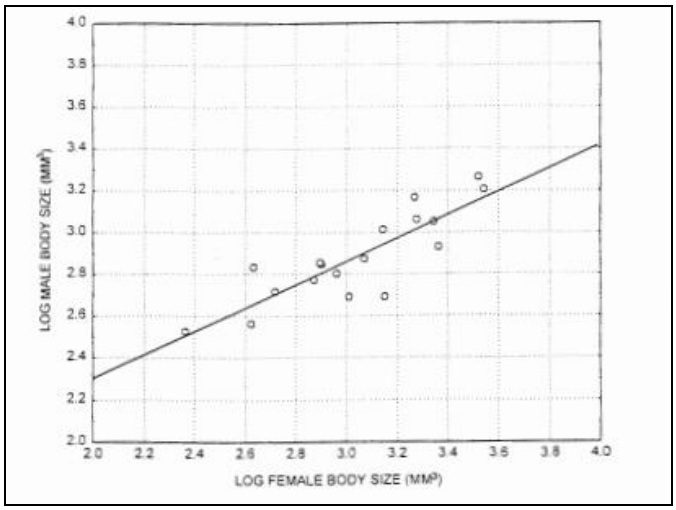

Fig 2: Quantitative resolution of sexual size dimorphism for 18 species of millipedes of the genus *Centrobolus*. Allometry for sexual size dimorphism (SSD) is based on the allometric model [28], male size = α (female size) $^{\beta}$; correlation coefficient, r = 0.85.

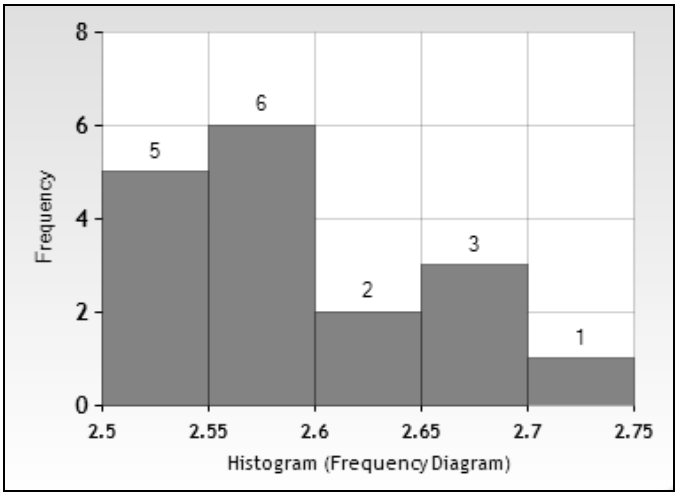

Fig 3: Distribution frequency histogram for male and female volumes of *Centrobolus anulatus*.

4. Discussion

Previous studies on SSD in invertebrates and these results consistently give a positive correlation and break the rule [5, 10, 12-19, 36]. Figure 1 shows the finding for *Centrobolus anulatus* where the regression of log male volume on log female volume was highly significant with a positive slope of 0.86207; showing females get larger than males with an increase in body size [2-3, 7-8]. SSD was significantly different from 1 in this species. Mean volume ratio of 1.02342521 for *C. anulatus* was a trend for the genus in Fig. 2. As a proximate cause for SSD in millipedes the evidence suggests the Sexual Bimaturism hypothesis [7]. As ultimate cause for SSD this together with ecological evidence suggests intersexual competition [2]. Evidence for sexual selection on dimorphism based on the relative size dimorphism in *C. anulatus* implies size would be important in determining the outcome of mating [11, 15, 34-35]. The mechanism based on a conflict of interests is known in *C. inscriptus* [8]. In the millipede *Doratogonus uncinatus* female choice for mating partners is "size selective" [35]. The cross-mating experiments in *Centrobolus* suggest a combination of size assortative mating without a size based preference operates [2]. Studies of diplopod sexual dimorphism may include more taxa and make use of the length and width measurements to calculate volumes using the geometric morphometric approach shown here for finding causal relationships of dimorphism.

5. Conclusion

Precise measurements for *C. anulatus* show sexual size dimorphism with small males and larger females.

6. Acknowledgement

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