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Linear morphometry of sexual and non-sexual traits of the rhinoceros beetle *Strategus aloeus* (L.) Coleoptera: Scarabaeoidea

Morfometría lineal de caracteres sexuales y no sexuales en el escarabajo rinoceronte *Strategus aloeus* (L.) Coleoptera: Scarabaeoidea

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RESUMEN

En este trabajo se analiza la morfometría lineal de caracteres sexuales y no sexuales del escarabajo rinoceronte *Strategus aloeus* (L.). Nuestros resultados sugieren que tanto los caracteres sexuales como los no sexuales son diferentes entre hembras y machos, así como la relación entre estas dentro de cada sexo. Los resultados dan evidencia de que los machos son más grandes que las hembras, pero las hembras muestran el mesonoto más grande. Aún más, es posible que en los machos exista una compensación entre la tibia y el mesonoto. Los resultados obtenidos en este estudio permiten la formulación de nuevas preguntas en el estudio morfométrico de esta especie.

Palabras clave: Morfometría lineal, *Strategus aloeus*, caracteres sexuales, Coleoptera.

ABSTRACT

The linear morphometry of sexual and non-sexual traits of the rhinoceros beetle *Strategus aloeus* (L.) is analysed. Our results suggest that these traits are different between males and females, and that are related differently among each sex. These results give evidence that males are bigger than females, but females show higher mesonotum. Furthermore, it is possible that there is a trade-off between tibia and mesonotum in a group of males. The results in this study raise new questions for future research upon the morphology of this species.

Key words: Linear morphometry, *Strategus aloeus*, sexual traits, Coleoptera.

The variation in size and shape of the horns in males of some species of Scarabaeoidea has been of great interest for researchers (Emlen *et al.* 2007, Moczek 2005, Tomkins & Moczek 2009). In Mexico one of the first studies in morphometry within the superfamily was published by the Mexican entomologist Miguel Ángel Morón (1995), who in his work "Review of the Mexican Species of *Golofa* Hope (Coleoptera: Melolonthidae: Dynastinae)" applied a morphometric analysis using the shape and size of the horns of the males to resolved systematics problems between the Mexican species of genus *Golofa*. Morón (1995) mentioned that these structures reflect part of evolutionary process within the group and provide valuable information.

Another genus in the superfamily of horned beetles (Dynastinae) that was studied by Morón is the genus *Strategus* Hope, 1837 (Morón 1976, Morón *et al.* 1997). This genus was established by William Kirby (1828), he listed four species to be included in the new genus (Ratcliffe 1976). One of these species was *Strategus aloeus* (L.), which is a species very widespread from North to South America. It is known that the life cycle of *S. aloeus* is about 11 months (Ahumada *et al.* 1995). Both adults and larvae feed on organic matter in the soil, roots, tubers or underground stems and sometimes they feed of plant xylem (Lugo- García 2011). This species, in some countries, has economic importance as a pest of some crops. In Colombia, the adult causes damage to palms less than four years of age, because it makes a hole near the base of the palm and penetrates until the meristem (Pallares *et al.* 2000). In Mexico *S. aloeus* has been reported as a major pest of agave due to damage caused by adults at the base of plants (Pérez-Domínguez 2006).

This species present moderate sexual dimorphism, males have three projections in the pronotum around a central cavity, one front horn-shape projection (horn) and two below hill-shape lateral prominences (adjacent horns). Females present bulges instead of horns (Morón *et al.* 1997, Ratcliffe 1976). These morphological characteristics are interesting for the study of the scaling relationships between horns and body size in Neotropical beetles.

Recently Álvarez *et al.* (2013) founded that the scaling relationships between horns and body size of a Mexican population of *S. aloeus* suggest horn positive allometry and differential development of principal horn and adjacent horns that might reflect strong sexual pressures. These authors explain this in an extreme reaction norm framework,

as suggested by the "positive allometry reaction norm model". However, the morphometric of sexual and non-sexual traits in this species is poorly understood, therefore, it is not known which traits in fact are sexual (besides horns) or non-sexual and how they are related.

The aim of our study was to investigate the linear morphometry of sexual and non-sexual traits of the rhinoceros beetle *S. aloeus*, to achieve a better understanding of the morphology of this species.

MATERIALS AND METHODS

We measured seven morphological variables in a sample of 92 males and 100 females of the rhinoceros beetle *S. aloeus*. These specimens are held in the Entomological Collection of the Instituto de Ecología AC, Xalapa, Mexico (IEXA) and belong to several localities from all over Mexico. We used a combined technique of measurement. First, each specimen was put on a grid plate, to make photographs with a digital camera (Kodak v550) and then make measurements of body length (BL), pronotum width (PW), humeral width (HW), mesonotum height (MH), back tibia length (TL) and adjacent horn length (AH) with the image program tpsDig (version 2.12). Second, horn length (HO) was measured with digital caliper because horn is projected, curved to the front over the head and we could not manipulate the specimens severely to make photographs. Body length was measured from the anterior tip of the pronotum to the base of the pygidium. Pronotum width was measured from the medial distal left edge to the medial distal right edge of the pronotum. Humeral width was measured from the medial distal left edge to the medial distal right edge of the elytra. Mesonotum height was measured from the upper edge to the lower edge of the mesonotum. Tibia length was measured from the tibia articulation with the femur to the tibia articulation with the tarsus. Adjacent horns length was measured from the base of the pronotum to the distal tip of the adjacent horns. As both adjacent horns were measured in all individuals (n= 100) and have no normal distribution (see results and discussion) and the same size (Wilcoxon-test: w = 4286, P = 0.7245) we only used data from left adjacent horn. Horn was measured from the point of the head articulation to the distal tip of the horn.

We searched for traits among our variables that allow an adequate estimation of body size. We analyse whether variables are more correlated using Pearson product-moment correlation among all pairs of traits in each sex, and we used highly correlated variables to analyse a general index of size and variance in shape among our sample, in a principal components analysis (PCA), which is a tool that can be used to summarize patterns of variability within sets of correlated variables (Manly 1986). We performed a PCA for each sex separately. We also search for sexual differences among morphological variables, we used a Student-*t* test for normal distributed variables and Wilcoxon test for no-normal distributed variables to compare each

variable shared between sexes. All analysis was performed in R Developmental Core Team (2011).

RESULTS

For females all morphometric measurements were normally distributed. For males BL, PW and HW were normally distributed, morphometric measurements of HO, AH, TL and MH were no normally distributed (Shapiro-Wilk test: HO, W = 0.9442, $P < 0.001$; TL, W = 0.8845, $P < 0.001$; AH, W = 0.9246, $P < 0.001$; MH, W = 0.844, $P < 0.001$). Morphometric measurements HO, AH, TL and MH have a bimodal tendency with a no normal distribution. However, log transformed data show a linear tendency for HO and AH, thus, residuals suggest linearity and normality.

We found statistically significant differences between males and females (Table 1). Morphometric measurements PW, HW and TL were bigger in males, conversely MH was bigger in females, BL was no statistically significant different between males and females (Table 1). We found strong correlation between all measured variables of females, in males MH was not correlated with other variables except for TL that is strong and negative correlated; TL was poor correlated with the rest of the variables (Table 2). Morphometric measurements BL, PW, HW, HO and AH were strong and positive correlated (Table 2).

Accordingly, we used only the non-sexual correlated variables in the PCA used to estimate patterns of variability within male and female variables. The PCA shows for males that PC1 explains 74% of the variances with an eigenvalue more than 5. The PC1 had strong negative loadings on BL, but low negative loadings on PW, HW and TL. The PC2 explains 22% of the variances with an eigenvalue more than 2. The PC2 had low positive loadings on BL, PW and HW, but had strong negative loadings on TL. In PCA the two traits that vary more for males were TL and BL, the trait that vary less was PW. The PCA shows for females that PC1 explains 95% of the variances with an eigenvalue more than 4. The PC1 had strong negative loadings on BL, but had low negative loadings on PW, HW, MH and TL. The PC2 explains 2% of the variance with an eigenvalue less than 1; therefore we do not take it into account. In PCA the trait that vary more for females was BL, the traits that vary less were TL, MH and PW (Table 3).

DISCUSSION

Our results suggest that traits are different between males and females. These traits are related differently among each sex. Traits of females had less amounts of variation they were more stable than those of males. Also there is sexual size dimorphism in *S. aloeus*. Males in general were bigger than females. However, females show higher mesonotum. Interestingly, there was no difference in BL. Thus, *S. aloeus* females could develop higher mesonotum because it is possible that they need room for fat reserves, which will be used to develop eggs (see Fairbairn *et al*. 2007). Also, in some insects species such as water striders, when females

Table 1. Summary statistics of untransformed morphological variables and Student-*t* test and Wilcoxon test (w) comparison of males (*n* = 92) and females (*n* = 100).

| | Mean | | Comparison test | |
	Male	Female	statistic	*P* value
Body length	47.40±3.52	48.08±3.35	*t* = -1.372	0.171
Pronotum width	25.28±2.40	21.47±1.64	*t* = 12.804	0.001
Humeral width	27.77±2.44	26.60±2.02	*t* = 3.614	0.001
Mesonotum height	11.41±3.60	15.77±1.19	w = 1729	0.001
Back tibia length	14.91±3.47	9.57±0.74	w = 8310	0.001
Horn length	6.31±2.28	Na	Na	Na
Adjacent horn length	12.80± 2.58	Na	Na	Na

Table 2. Pairwise correlation among males and females morphological variables. Above the diagonal: *P*-values of Pearson's product-moment correlation. Bellow the diagonal: standard Pearson's product-moment correlation coefficients.

| | | Males | | | | | | | Females | | | | |
		1 BL	2 PW	3 HW	4 MH	5 TL	6 HO	7 HA	1 BL	2 PW	3 HW	4 MH	5 TL
1	**Body lenght**		0.001	0.001	0.77	0.001	0.001	0.001		0.001	0.001	0.001	0.001
2	**Pronotum width**	0.92		0.001	0.86	0.001	0.001	0.001	0.9		0.001	0.001	0.001
3	**Humeral width**	0.92	0.94		0.88	0.001	0.001	0.001	0.97	0.91		0.001	0.001
4	**Mesonotum height**	0.031	-0.018	0.015		0.001	1	0.6	0.93	0.82	0.91		0.001
5	**Back tibia length**	0.46	0.5	0.48	-0.75		0.001	0.001	0.87	0.81	0.86	0.83	
6	**Horn length**	0.83	0.88	0.85	-0.001	0.47		0.001	Na	Na	Na	Na	Na
7	**Adjacent horn length**	0.77	0.85	0.83	-0.054	0.48	0.92		Na	Na	Na	Na	Na

Table 3. Principal component analysis of correlated morphological variables. General PCA explaining the variance in males (96.9%) and females (97.7%).

| | Males | | Females | |
	PC1	PC2	PC1	PC2
Body length	-0.637	0.365	-0.763	-0.333
Pronotum width	-0.431	0.190	-0.349	0.873
Humeral width	-0.438	0.217	-0.454	0.089
Back tibia length	-0.464	-0.884	-0.149	-0.021
Mesonotum height	Na	Na	-0.256	-0.341
Eigenvalues:	5.21	2.82	4.37	0.67
% Variance explained	74.9	22	95.5	2.2

are bigger than males is, in general, because some part of the abdomen is expanded to allow eggs (see Fairbairn *et al*. 2007).

By other side, our results suggest that HO and AH show linear tendencies despite apparently bimodality, which agree with recent research (Álvarez *et al*. 2013). Our analysis show that BL was not a good body size index, instead PW appears to be a better one. The rationale for this is that, sexual traits show larger variance than non-sexual traits (Cotton *et al* 2004 a, b), for example secondary sexual traits tend to be condition dependent and highly variable, instead, traits that are kept throughout life and are of high functional importance, such as skeletal characters, tend to be developmentally very stable (see Møller 1993; Cotton *et al*. 2004b).

It is interesting that TL and MH of males shown bimodal tendencies, and both were strong negative correlated. There is a group of males (*n* = 27) with very short back tibias but with very high mesonotum, however, this group is not related by geographical distribution. These traits were

different between males and females (see results). We looked for TL and MH mean in these individuals (Mean: TL = 9.78 ± 0.78 mm; MH = 16.52 ± 1.28 mm); apparently these traits resemble more to female traits than those of common males. It is rare that this does not affect the size of horn and adjacent horns (secondary sexual traits). Hence, it appears that there is a trade-off between TL and MH in those males. MH could be a secondary sexual trait and its development could be affecting the development of TL (see Nijhout & Emlen 1998; Simmons & Emlen 2006).

The results in this study raise new questions for future research upon the morphology of this species. For example, apparently the mesonotum is a sexual trait that develops differently in males and females. Is for males advantageous to have a high mesonotum? Is the size of mesonotum related to sexual interactions or mating behaviour for males? How *S. aloeus* use the mesonotum? Thus, the development and function of sexual and non-sexual traits should be investigated more thoroughly.

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