

# Morphometric Analysis of a Population of Diplopods of the Genus *Rhinocricus* Karsch, 1881

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( diplopoda / *Rhinocricus* / morphometry / multivariate statistic / taxonomy )

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**Abstract.** Diplopods belonging to the subclass Helminthomorpha may present one or both leg pairs of the seventh diplosegment modified into structures that aid copulation, called gonopods. These structures are used as a taxonomic trait for the description of most species. In the genus *Rhinocricus* these structures are closely similar, so that it is difficult to distinguish species only on the basis of this trait. Two species, *R. padbergi* and *R. varians*, are found in the same habitat and present gonopods practically identical in shape; together they present a broad colour gradient, ranging from dark brown to light beige. Morphometric data for individuals of the experimental group were submitted to ANOVA and MANOVA, using Hotelling-Lawley Trace and generalized Mahalanobis distances ( $D^2$ ) tests. The results demonstrated a relationship between size and colour, with darker individuals being larger. On the basis of this preliminary analysis, we may suggest that the two species are distinct since dark individuals are distant from medium- and light-coloured individuals according to the  $D^2$  values. This seems to indicate a possible polymorphism of individuals belonging to *R. padbergi* which present close proximity in the values obtained. In all analyses, we observed that the main variables were diameter, length and telson size.

Diplopoda are popularly known in Brazil as piolhos de cobra, embuás, gongos, gôngolos or millipeds. They vary in length from 2 mm to 30 cm and range in colour from black to brown, with some species being red or orange.

According to Golovatch et al. (1995), the group is estimated to comprise 80 000 species, this being the third largest class of Arthropoda after Insecta and Arachnida. Only about 10 to 15% of these species have been described estimatively, including approximately 2500 genera and 16 families (Golovatch, 1997). The taxonomy of this group is confused in certain families

and genera. The most commonly used traits are colour, and configuration of segments and gonopods; the latter two are quite similar in some groups, being unfeasible to utilize them as specific traits. Within this context, we may cite the genus *Rhinocricus* Karsch, 1881.

Two species of this genus, *R. varians* Brölemann (1901) and *R. padbergi* Verhoeff (1938) present great similarity in most of the diagnostic traits and are frequently found in the same habitat. This similarity impairs the identification of these species since their gonopods are practically identical. One of the traits used to differentiate these two species is colour, with *R. varians* being darker than *R. padbergi* (Schubart, 1951); however, when several animals are compared, independently of the age or the size, a colour gradient ranging from dark brown to light beige is observed (Fig. 1).

*Rhinocricus varians* was considered by Brölemann (1901) as variation of *Rhinocricus nattereri* Humbert & Saussure, 1870 (Schubart, 1951). In 1951, Schubart considered *R. varians* as a species, characterized by a dark brown colour with shades of purple, metazonites lighter, antenna brown with last segments lighter, yellowish, legs chestnut-yellow, mean length of 73.5 mm, telson with a large and prominent pre-anal process. This trait was important to separate *R. varians* from *R. nattereri*, which does not present a prominent pre-anal process.

*R. padbergi* was also described initially as belonging to the *R. nattereri* group, but Schubart elevated it to a species level in 1944 (Schubart, 1944). For this author,



Fig. 1. Colour gradient in specimens of *Rhinocricus* sp.

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Abbreviation: ANOVA – analysis of variance

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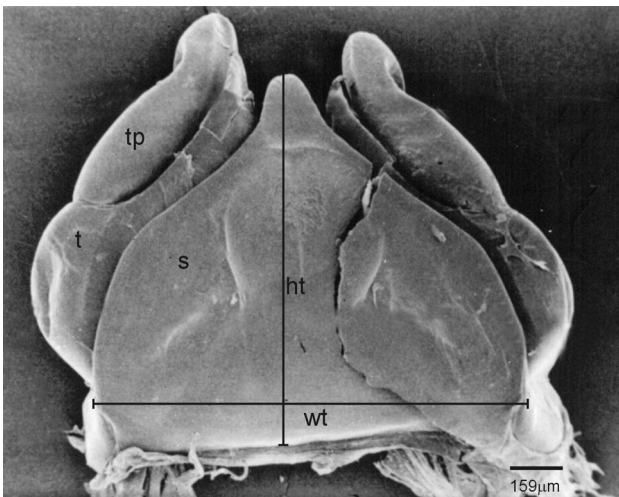


Fig. 2. Anterior gonopod of *Rhinocricus* sp.: ht, height, s, sternite, t, thigh, tp, telopodite, wt, width.

this species is easily recognized when alive by its chestnut-pink to acajou colour, telson darker and legs and antennae golden yellow-whitish. The mean length was 64.1 mm (Schubart, 1951).

In the literature several papers utilize morphometric analysis associated with multivariate statistical analysis to identify taxa, dimorphism between individuals, etc. (Chaud Netto, 1973; Fortuner and Maggenti, 1991; Mapatuna-Yasantha, 2002; Perequetti, 2002). In this paper we used a morphometric analysis, combined with multivariate statistical analysis, to verify whether there was one or more species in the test group.

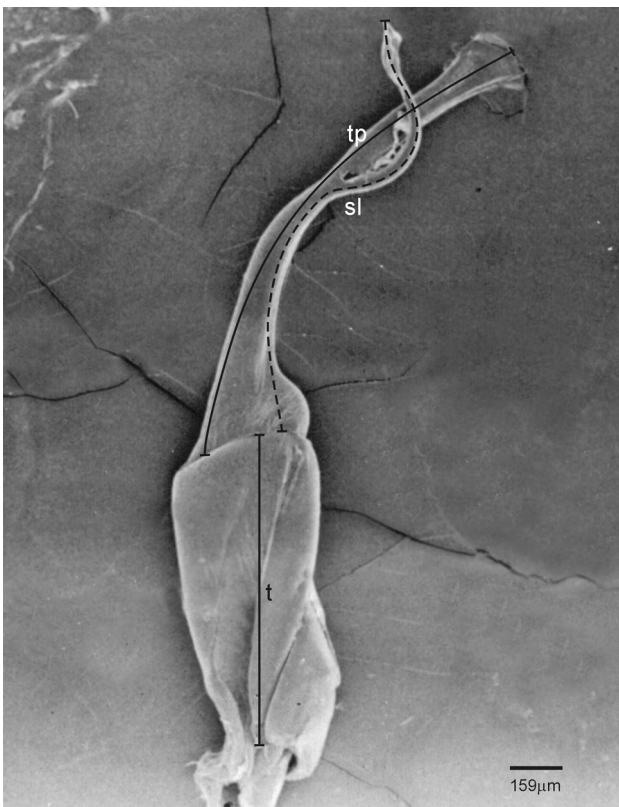


Fig. 3. Posterior gonopod of *Rhinocricus* sp.: sl, solenomerite, t, thigh, tp, telopodite.

In this context, among the tests used were: the Hotelling-Lawley Trace test, developed by Lawley (1938) and modified by Hotelling (1951), and the generalized Mahalanobis  $D^2$  distance. In the latter, the numeric value of the maximum possible separation between two groups is called generalized distance between two groups; it measures, on an independent scale from the one originally used for the many traits, the clarity of the disjunction between them (Pisani, 1966). The  $D^2$  value linking both groups is a pure number with properties of a common distance and it measures the extension of how they differ in shape and size (Barraclough and Blackith, 1962).

Table 1. Mean values of the variables studied in male and female individuals of the genus *Rhinocricus*

Variable	Colour pattern	n	Mean value
<b>Female</b>			
Body diameter	Light	12	5.8
	Medium	23	6.3
	Dark	22	7.3
	Total	57	6.6
Body length	Light	12	68.5
	Medium	23	69.2
	Dark	22	83.1
	Total	57	74.4
Length of the telson	Light	12	3.3
	Medium	21	3.1
	Dark	22	4.2
	Total	55	3.6
Number of segments	Light	12	55.2
	Medium	23	55.7
	Dark	22	56.5
	Total	57	55.9
<b>Male</b>			
Body diameter	Light	8	5.3
	Medium	23	5.9
	Dark	15	7.0
	Total	46	6.1
Body length	Light	8	59.0
	Medium	23	65.2
	Dark	15	81.7
	Total	46	69.5
Length of the telson	Light	8	3.1
	Medium	23	3.4
	Dark	15	4.1
	Total	46	3.5
Number of segments	Light	8	54.3
	Medium	22	53.5
	Dark	15	54.7
	Total	45	54.0

Note: the mean values are reported as mm, except for the variable “number of segments”.

Table 2. Analysis of variance of the values obtained for the variables studied in males and females of the genus *Rhinocricus* of light, medium and dark colour

Variable	Scope	Sum of squares	df	Mean square	F	P
<b>Female</b>						
Body diameter	Between groups	21.577	2	10.789	13.587	0.000
	Within groups	42.878	54	0.794		
	Total	64.455	56			
Body length	Between groups	2697.164	2	1348.582	12.099	0.000
	Within groups	6018.731	54	111.458		
	Total	8715.895	56			
Length of the telson	Between groups	12.334	2	6.167	20.095	0.000
	Within groups	15.959	52	0.307		
	Total	28.293	54			
Number of segments	Between groups	17.03	2	8.515	1.562	0.221
	Within groups	296.339	54	5.488		
	Total	313.369	56			
<b>Male</b>						
Body diameter	Between groups	19.007	2	9.503	14.078	0.000
	Within groups	29.027	43	0.675		
	Total	48.034	45			
Body length	Between groups	3548.632	2	1774.316	19.243	0.000
	Within groups	3964.846	43	92.206		
	Total	7513.478	45			
Length of the telson	Between groups	6.548	2	3.274	10.140	0.000
	Within groups	13.883	43	0.323		
	Total	20.431	45			
Number of segments	Between groups	15.09	2	7.545	1.510	0.233
	Within groups	209.888	42	4.997		
	Total	224.978	44			

## Material and Methods

The specimens were collected in August 1998 at the Federal University of São Carlos (UFSCar), state of São Paulo, by Lilian Boccardo.

Due to the difficulty in identifying the species and the intermediated colour gradient observed between them, we collected and analysed diplopods, being careful not to identify them, treating them as one group.

Only adults, 57 females and 46 males, were used in the analysis. Body colour of specimens was defined as light, medium or dark.

Diameter, total body length and telson size were measured and the number of segments was recorded. The gonopods of all the males were removed and mounted on slides with synthetic resins, covered with coverslips and measured under an Axioskop microscope with the Axiohome system.

Regarding the anterior gonopod, we measured width, height and area (Fig. 2); posterior gonopods measurements were: length of the thigh, telopodite and solenomerite (Fig. 3).

The data obtained were submitted to analysis of variance (ANOVA) using the SPSS 11.0 statistical package.

Canonic analysis was also performed using the SAS package. The Hotelling-Lawley Trace test of this package was used to assess the differences in colour classes. The generalized Mahalanobis  $D^2$  distances were also estimated between diplopod groups of different colours.

## Results

To the (ANOVA), the comparison of the data obtained in this paper indicated significant differences in the variables studied with respect to colour, both for males and females. The variables that most affected the results were length and diameter of the body and telson length. The results obtained showed that darker individuals had larger measurements. The F values obtained for the variable "number of segments" were non significant (Tables 1 and 2).

We did not find any morphological differences among individuals based on the analysis of gonopod characters; we observed that all variables but thighs are correlated to the animal's colour. As a rule, the darker the animal, the larger its body measurements (Tables 3 and 4).

The ANOVA applied to the data for the traits analysed taking into consideration the sex of the

Table 3. Mean values of the variables concerning the gonopods of male individuals of the genus *Rhinocricus*

Variable	Colour pattern	n	Mean value (mm)
Height of the gonopod	Light	8	2.1
	Medium	23	2.2
	Dark	15	2.4
	Total	46	2.2
Width of the gonopod	Light	7	1.9
	Medium	23	2.0
	Dark	14	2.2
	Total	44	2.1
Area of the gonopod	Light	7	4.1
	Medium	23	4.5
	Dark	14	5.5
	Total	44	4.8
Length of the thigh1	Light	8	1.0
	Medium	23	1.1
	Dark	14	1.1
	Total	45	1.1
Length of the telopodite1	Light	8	1.9
	Medium	23	2.1
	Dark	14	2.2
	Total	45	2.1
Length of the solenomerite1	Light	8	1.8
	Medium	23	1.9
	Dark	14	2.0
	Total	45	1.9
Length of the thigh2	Light	6	1.0
	Medium	18	1.0
	Dark	13	1.1
	Total	37	1.1
Length of the telopodite2	Light	7	2.0
	Medium	19	2.0
	Dark	12	2.2
	Total	38	2.1
Length of the solenomerite2	Light	7	1.8
	Medium	19	1.8
	Dark	12	2.0
	Total	38	1.9

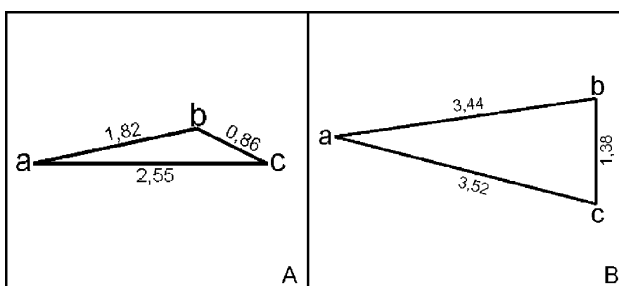


Fig. 4. Graphic presentation of the values of the generalized Mahalanobis distance: A: values reported as  $\sqrt{D^2}$  for males; B: values obtained for females. a, dark individuals; b, medium; c, light.

diplopods studied indicated that females had a larger body diameter than males and a larger number of segments. However, there was no difference between males and females regarding total body length or telson length (Tables 5 and 6).

Canonic analysis indicated that the first function was responsible for the variability detected among the three colour classes, with 96.13% and 81.60% for males and females, respectively. We may also conclude that telson length, diameter and size were the variables that most affected the results obtained for males and females.

The Hotellin-Lawley Trace test detected differences between colour classes, with a significant result for both males ( $P = 0.013$ ; d.f. = 66) and females ( $P = 0.0001$ ; d.f. = 96).

Mahalanobis analysis demonstrated proximity between the light and medium colour classes, with the dark class being more distant compared to the two previous ones. The values obtained can be visualized in Figure 4, which is an approximate graphic representation of the generalized Mahalanobis distances between the three colour classes studied. The diagram in Figure 4A was constructed with the D values, i.e., the square root of  $D^2$ , to establish a better proportionality between the points, facilitating the analysis and the understanding of the scheme.

## Discussion

Canonic analysis revealed a clear separation among the three colour classes studied in the space created by the first function of the canonic variable, which corresponded to a large proportion of significant variance (96.13% for males and 81.60% for females).

Analyses of variance combined with  $D^2$  values demonstrated that dark-coloured individuals are statistically more distant, from light- and medium-coloured individuals. The latter two are closer, and this might indicate a polymorphism regarding the coloration of individuals of *R. padbergi*.

The variance analysis taking into account gender indicated that females have a larger body diameter when compared to those of males, as well as a higher number of segments. This difference can be explained by the fact that females have ovaries containing oocytes in different developmental stages increasing body diameter (Hopkins and Read, 1992; Fontanetti and Saurengo da Cunha, 1993; Camargo-Mathias et al., 1998).

For both males and females, mean values of more than 7, 80, and 4 mm for diameter and length of the body and telson length seem to indicate a tendency to the darker colour and may lead to the identification of animals of the species *R. varians* in the sampling obtained in the present study.

The morphometric multivariate techniques showed that some combination of morphological traits can help identify the species *R. padbergi* and *R. varians*. A sim-

Table 4. Analysis of variance of the data concerning the variables studied in male diplopods of the genus *Rhinocricus* considering three body colour classes

Variable	Scope	Sum of squares	df	Mean square	F	P
Height of the gonopod	Between groups	0.631	2	0.316	6.868	0.003
	Within groups	1.976	43	0.046		
	Total	2.607	45			
Width of the gonopod	Between groups	0.585	2	0.293	4.000	0.026
	Within groups	2.999	41	0.073		
	Total	3.584	43			
Area of the gonopod	Between groups	13.256	2	6.628	7.633	0.002
	Within groups	35.603	41	0.868		
	Total	48.859	43			
Length of the thigh1	Between groups	0.078	2	0.039	0.582	0.563
	Within groups	2.827	42	0.067		
	Total	2.905	44			
Length of the telopodite1	Between groups	0.493	2	0.247	7.634	0.001
	Within groups	1.357	42	0.032		
	Total	1.850	44			
Length of the solenomerite1	Between groups	0.241	2	0.120	3.955	0.027
	Within groups	1.277	42	0.030		
	Total	1.518	44			
Length of the thigh2	Between groups	0.157	2	0.079	1.954	0.157
	Within groups	1.369	34	0.040		
	Total	1.526	36			
Length of the telopodite2	Between groups	0.545	2	0.273	10.859	0.000
	Within groups	0.879	35	0.025		
	Total	1.424	37			
Length of the solenomerite2	Between groups	0.426	2	0.213	6.621	0.004
	Within groups	1.125	35	0.032		
	Total	1.551	37			

Table 5. Descriptive analysis of the data obtained for four variables studied in male and female diplopods of the genus *Rhinocricus*

Variable	Group	N	Mean	± SD	± SE	95% confidence interval for mean			
						lower bound	upper bound	min	max
Body diameter	F	57	6.569	1.073	0.142	6.284	6.854	4.3	10.3
	M	46	6.150	1.033	0.152	5.843	6.456	4.0	8.5
	Total	103	6.382	1.071	0.106	6.172	6.591	4.0	10.3
Body length	F	57	74.42	12.476	1.652	71.11	77.73	52	120
	M	46	69.52	12.922	1.905	65.68	73.36	45	100
	Total	103	72.23	12.849	1.266	69.72	74.74	45	120
Length of the telson	F	55	3.59	0.724	0.098	3.39	3.78	2	5
	M	46	3.54	0.674	0.099	3.34	3.74	3	5
	Total	101	3.57	0.698	0.069	3.43	3.71	2	5
No. of the segments	F	57	55.89	2.366	0.313	55.27	56.52	46	60
	M	45	54.02	2.261	0.337	53.34	54.70	49	58
	Total	102	55.07	2.491	0.247	54.58	55.56	46	60

F – female, M – male, SD – standard error, SE – standard deviation

Table 6. Analysis of variance of the values obtained for four variables studied in males and females of the genus *Rhinocricus* taking into consideration the sex of the diplopods

Variable	Scope	Sum of squares	df	Mean square	F	P
Body diameter	Between groups	4.477	1	4.477	4.020	0.048
	Within groups	112.489	101	1.114		
	Total	116.966	102			
Body length	Between groups	611.035	1	611.035	3.803	0.054
	Within groups	16229.373	101	160.687		
	Total	16840.408	102			
Length of the telson	Between groups	0.057	1	0.057	0.116	0.734
	Within groups	48.725	99	0.492		
	Total	48.782	100			
No. of the segments	Between groups	88.173	1	8.173	16.379	0.000
	Within groups	538.346	100	5.383		
	Total	626.519	101			

ilar research was developed by Calle et al., 2002, with *Anopheles* females. They studied five species of the subgenus *Nyssorhynchus* and their results with multivariate analysis permitted identification of the species with 90% certainty and without ambiguities.

The results obtained in morphometrical analysis used in this paper have shown that the size of the individual is connected with the body colour. These results confirm that dark animals have larger dimensions compared to medium and light animals.

Based on the morphometric data and statistical analyses conducted in this paper, we conclude that there is a separation between differently coloured individuals. As mentioned elsewhere, light- and medium-coloured individuals would belong to the species *R. padbergi*, presenting a colour polymorphism among individuals. Dark-coloured individuals would belong to the species *R. varians*, in agreement with the description by Schubart (1951), where the individuals of this species are larger in size (mean length 73.5 mm) when compared to those of *R. padbergi* (mean length 64.1 mm).

To the present, due to the few studies conducted with this group, it has not been possible to define what factors could generate the polymorphism observed in *R. padbergi*.

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