

3.1 ARTIGO1

Morphometry of the testis follicles in *Triatoma rubrofasciata* (De Geer, 1773)
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Morphometry of the testis follicles in *Triatoma rubrofasciata* (De Geer, 1773) (Hemiptera, Triatominae)

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Abstract—The male reproductive tract in Triatominae has a pair of testes, two *vasa deferentia*, a pair of seminal vesicles, four pairs of accessory glands, and an ejaculatory duct, which opens in the aedeagus. In species of the genus *Triatoma* each testis is formed by seven testicular follicles. Because *Triatoma rubrofasciata* has a common ancestor with species of *Triatoma* occurring in North America and because the length of testis follicles varies among different species of *Triatoma* a morphometrical analysis of the follicles was conducted. *Triatoma rubrofasciata* has seven testis follicles of variable length that are similar between left and right testes. The statistics allowed the classification in a long follicle, two medium follicles, two that are short, and two that are very short. This finding is compared with data available for other Triatominae and it is emphasized that the length of follicles testis should be included in future phylogenetic analysis of Triatominae.

Keywords: evolution; insecta; reproductive tract.

INTRODUCTION

The morphology of the internal male reproductive tract of insects is increasingly applied to systematic and phylogenetic studies (Woodward, 1950; Hodapp and Jones, 1961; Louis and Kumar, 1973; Wheeler and Krutzsch, 1992).

Early nymphal stages in the family Reduviidae have no significant development of the male reproductive tract; however, the testis and the *vas deferens* complete

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its development in the fifth stage (Carayon, 1944). The male reproductive tract in Triatominae comprises a pair of testes, two *vas deferentia*, a pair of seminal vesicles, four pairs of accessory glands, and an ejaculatory duct, which opens in the aedeagus (Barth, 1958). In these insects, the testis may show variation among specimens, depending on age, feeding status, and sexual activity. In species of the genus *Triatoma* each testis is formed by seven testicular follicles, which are enclosed by a peritoneal sheath. These follicles may be of different length, which can vary between individuals as well among the follicles of a testis (Barth, 1956).

The number, size, and thickness of the testis follicles of some Triatominae are consistent within certain genera: Species of *Panstrongylus* has seven thin follicles of similar length, species of *Rhodnius* and *Psammolestes* have five short and thin follicles and two medium and thick follicles, while *Triatoma* has three short and thin follicles, two medium and thick and two long and thin ones (Schreiber et al., 1968; Silva and Schreiber, 1971; Gonçalves et al., 1987). Moreover, morphometry of the testis follicles of *Triatoma spinolai* (Porter, 1934) was used as one of the arguments to include this species in the genus *Mepraia* (Lent et al., 1994).

Triatoma rubrofasciata (De Geer, 1773) is frequently found in coastal cities of Brazil, especially the city São Luís, Maranhão (Macario-Rebelo et al., 1999). Although *T. rubrofasciata* is occasionally found to be naturally infected with *Trypanosoma cruzi*, it is not considered an important vector of Chagas disease (Lucena and Magalhães Netto, 1939; Brazil et al., 1985). It is commonly infected with *Trypanosoma conorhini*, which infects rats (Sherlock and Serafim, 1972), and is considered the main vector of this parasite.

Triatoma rubrofasciata is widely distributed in the tropics of the Old and New World, but it is likely to be of a New World origin (Patterson et al., 2001). This species has furthermore been proposed to have a relatively basal phylogenetic position within Triatomini (Hypsa et al., 2002). Variation in length of the testis follicles is known to occur among Triatomini (Schreiber et al., 1968), and so we concluded that it would be an interesting project to inquire if *T. rubrofasciata* has different length of testis follicles in comparison to the species of Triatominae for which there are available data.

MATERIALS AND METHODS

From *T. rubrofasciata* of the urban area of São Luís, State of the Maranhão, Brazil, colonies were maintained in an insectary at the Department of Entomology, Oswaldo Cruz Institute, FIOCRUZ. Fifth instar male nymphs were sexed according to Lent and Jurberg (1969) and were kept in a dark glass flask (30 × 15 cm) closed with nylon screen at 29 ± 1°C and 80 ± 5% rh with 12 h photoperiod. Inside the flask a folded filter paper was placed to increase the contact surface and as a refuge, as well as to remove excess humidity. The insects were fed weekly with blood of Swiss mouse (Protocol CEUA – FIOCRUZ P0100-01).

Thirty specimens were used. After the imaginal moult the newly hatched adults were starved for three days to avoid nutritional effects on the testis development. The male reproductive tract was dissected in saline solution for insects (0.1M NaCl + 0.1M KCl). The testes were isolated, identified in left and right and placed in plastic plates (0.3 × 4.3 cm) filled with the same saline solution. Subsequently, the testis follicles were distended by disruption of the sheath lining the testis.

Drawings of the male reproductive tract were made using a camera lucida and measurements were carried out with aid of a Japanese curvimeter CM 10 (Tokio Sakurai). As some testis follicles may be folded in 90° angle, measurements of the lengths were taken from both sides of each follicle, but only the larger value was considered (Gonçalves et al., 1987).

Morphometric data were submitted to variance analysis (ANOVA) at a significance of 5% using the software R (R, 2004).

RESULTS

The male reproductive tract of *T. rubrofasciata* comprises oval, whitish testes, which are placed between the 2nd and 5th abdominal segments. The *vas deferens* originates in the medium portion of the testis and runs into the posterior region of the insect body. In the medium region, the *vas deferens* dilates to form the seminal vesicle, and then acquires its normal diameter until it approaches the ejaculatory duct, which terminates in the aedeagus. Four accessory glands open in each *vas deferens*: the anterior, external, internal, and dorsal glands. The glandular duct starts at the point of junction of the four glands and terminates in the *vas deferens* of the ejaculatory duct.

Inside the testis there are seven elongated and folded testis follicles. Each follicle is narrow in its proximal region, which forms a short canal, the *vas eferens* which converge to the common *vas deferens* (fig. 1).

Triatoma rubrofasciata has seven testis follicles (F1-F7) of variable length (12.30-16.69 mm) (figs. 1 and 2), that are similar between left and right testes (ANOVA, $F_{(1,412)} = 0.4684$; $P = 0.4941$) (table 1). The analysis statistics allowed the classification of the follicles in a long one, represented by F1 (16.69 ± 2.65 mm), two medium follicles represented by F2 and F3 (15.76 ± 2.62 mm), two short follicles represented by F4 and F5 (14.59 ± 2.55 mm), and two very short follicles represented by F6 and F7 (12.67 ± 2.15 mm) (table 2, fig. 3).

DISCUSSION

The morphology of the male reproductive tract of the adult male of *T. rubrofasciata* is similar to the one observed in other species of *Triatoma* (Barth, 1956; Gonçalves, 1986).

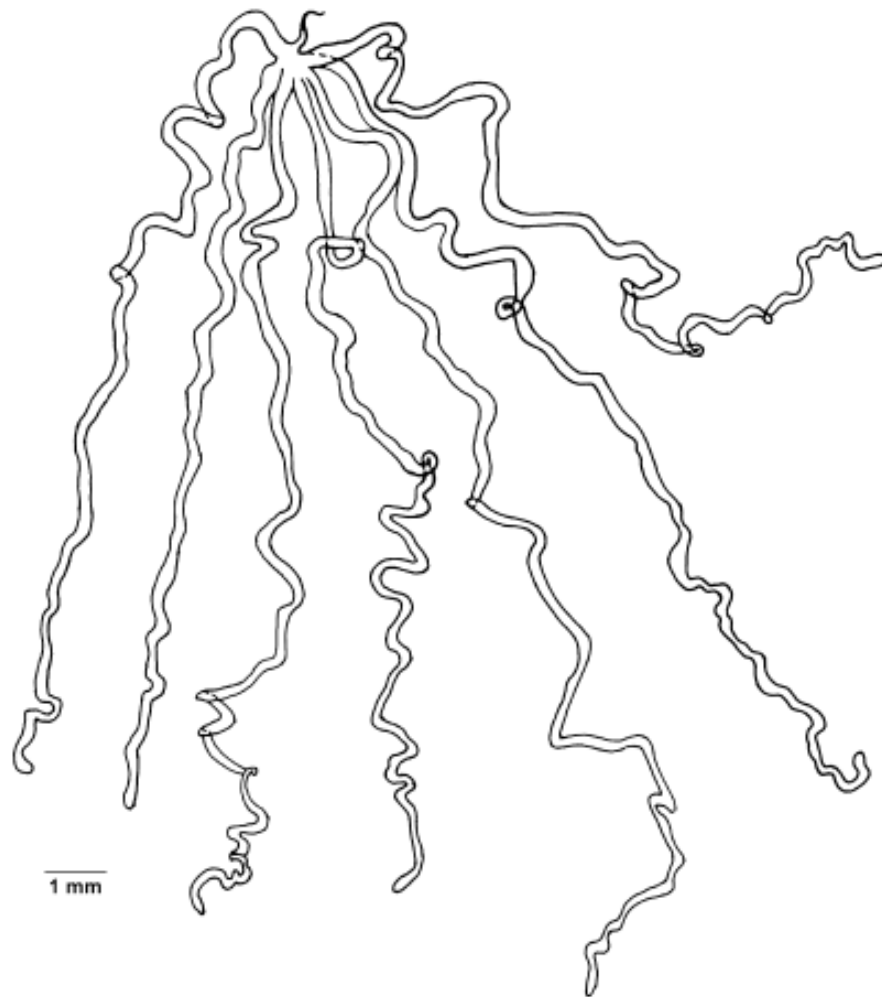


Figure 1. Schematic drawing of the testis follicles of *Triatoma rubrofasciata* (Hemiptera, Triatominae).

With regard to the testis follicles the number seven may be considered the plesiomorphic condition for Heteroptera, because this number was found in other species throughout the group (Woodward, 1950; Akingbohunge, 1983; Lemos et al., 2005). Triatominae have always seven follicles, however with different diameters and lengths in the genera *Panstrongylus*, *Rhodnius*, *Psammolestes* and *Triatoma* (Schreiber et al., 1968; Silva and Schreiber, 1971; Gonçalves et al., 1987). We found that *T. rubrofasciata* has one long, two medium, two short, and two very short testis follicles, and thus differs from the pattern (two long, two medium and three short) found in *Triatoma brasiliensis* Neiva, 1911, *Triatoma pseudomaculata* Corrêa and Espínola, 1964, *Triatoma sordida* (Stal, 1859) and *Triatoma vitticeps* (Stal, 1859) (Gonçalves et al., 1987). Among other Triatominae, species of *Rhodnius* (Rhodniini) have two short and five long testis follicles and *Panstrongylus herreri* Wygodzinsky, 1948 and *Panstrongylus megistus* Burmeister, 1835 (both Triatomini) have testis follicles of equal lengths (Gonçalves et al., 1987).

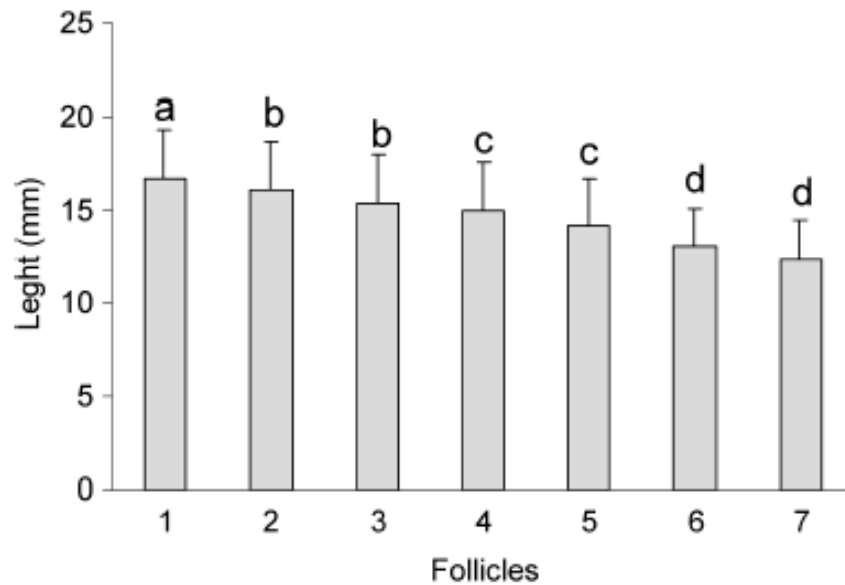


Figure 2. Length (mm) (mean \pm sd) of the seven testis follicles in *Triatoma rubrofasciata* (Hemiptera, Triatominae). Different letters above the bars in the same follicle indicates significant differences by the test F at 5%.

Table 1.

Length (mean \pm sd) of the seven testis follicles (F1-F7) of *Triatoma rubrofasciata* (Hemiptera, Triatominae).

Follicles	Left testis	Right testis
F1	17.16 \pm 2.55	16.87 \pm 2.78
F2	16.78 \pm 2.51	16.60 \pm 2.77
F3	15.90 \pm 2.56	16.51 \pm 2.67
F4	15.73 \pm 2.56	15.71 \pm 2.67
F5	14.82 \pm 2.23	14.37 \pm 2.73
F6	13.46 \pm 2.13	13.39 \pm 2.02
F7	13.56 \pm 2.40	13.12 \pm 1.98

Although the number of species of *Triatoma* so far studied for this feature is low, we suggest that the condition observed in *T. rubrofasciata*, i.e. the presence of four length categories of testis follicles, may be the plesiomorphic condition of this character among Triatomini, based on the assumption that *T. rubrofasciata* is the sister group of *Linshcosteus* and both of them are the sister taxon of the rest of the Triatomini (Hypsa et al., 2002). Thus categories of length of testis follicles other than four could be derived states for *T. brasiliensis*, *T. pseudomaculata*, *T. sordida*, *T. vitticeps*, *Rhodnius* spp. and *Panstrongylus* spp. Whether these are ordered or unordered states is not known, but it seems reasonable to believe that all testis follicles of the same size could be a derived state. *Rhodnius* and *Psammolestes* share a common type of testis follicles (Schreiber et al., 1968; Silva and Schreiber, 1971;

Table 2.

Analysis of variance (ANOVA) of the testis follicles groups in *Triatoma rubrofasciata* (Hemiptera, Triatominae).

Follicles	N	df	F	P
F7F6 (very short)	414	1	2.6677	=0.1032
F7F6F5*	415	1	15.394	<0.001
F5F4 (medium)	415	1	2.99	=0.08406
F5F4F3*	416	2	3.701	<0.05
F3F2 (short)	416	1	2.3789	=0.1237
F3F2F1*	417	1	5.7026	<0.05
F1 (long)	416	1	52.177	<0.001

*Follicles with significant difference in the length by the test F at 5%. N = sample size.

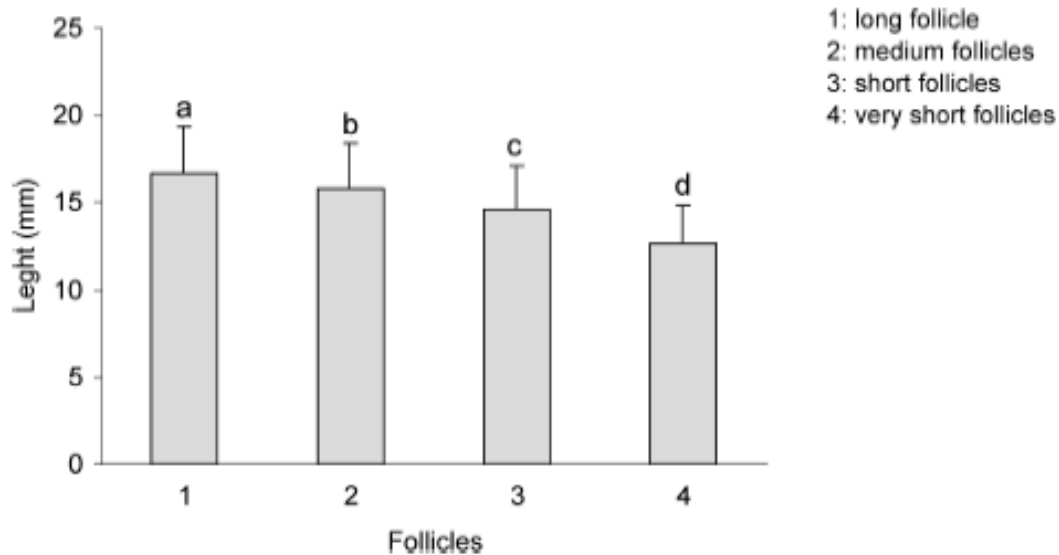


Figure 3. Length (mm) of the four groups of testis follicles in *Triatoma rubrofasciata* (Hemiptera, Triatominae). Different letters above the bars indicates significant differences by the test F at 5%.

Gonçalves et al., 1987), and mapped on the hypothesis by Hypsa et al. (2002), the presence of only two types of follicles could provide further evidence that both genera are derived from a common ancestor. *Panstrongylus* spp. have a derived condition of length of testis follicles, because in all studied species, they have testis follicles of the same size (Gonçalves et al., 1987). This assertion is supported by Hypsa et al. (2002) who have pointed out that *Panstrongylus* has a short time between its origin and subsequent radiation. In the phylogenetic tree proposed by Hypsa et al. (2002), the South American species of *Triatoma* form a clade, although six species of *Triatoma* display a position branching outside of South American clade. Gonçalves et al. (1987) analyzed the length of testis follicles for only four South American *Triatoma* species, which had three categories of follicles testis

length, suggesting that this character state could be an apomorphy for the South American species of *Triatoma*, although analyses of this character in other *Triatoma* species should be necessary.

In their molecular study, Hypsa et al. (2002) have pointed out that *T. rubrofasciata* is the sister group of *Linshcosteus* and both of them are the sister taxon of the rest of the Triatomini, but using solely the length of testis follicles in the transformation series herein presented, *T. rubrofasciata* is more similar to the common ancestor for both Rhodniini and Triatomini. This assertion is highly hypothetical and an analyze of follicle testis in more species of North and South American *Triatoma* as well as in *Meccus*, *Mepraia*, *Linshcosteus* and *Nesotriatoma* (*sensu* Hypsa et al., 2002) could corroborate the above proposed relationships.

Indeed we propose that morphometry data of testis follicles are useful to be included in future phylogenetical analyses of Triatominae.

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