

3.2 ARTIGO 2:

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**A COMPARATIVE STUDY OF TESTIS FOLLICLES IN SPECIES OF *Triatoma*
(HEMIPTERA, TRIATOMINAE)**

SIMONE P. C. FREITAS^{1,2}, JACENIR R. SANTOS-MALLET², JANE COSTA³, ANA LÚCIA B. SOUZA⁴, JOSÉ E. SERRÃO⁵, TERESA C. M. GONÇALVES^{2*}

¹ Departamento de Biologia Animal, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brasil. ² Setor de Morfologia, Ultraestrutura e Bioquímica de Arthropódes e Parasitos, 21045-900, Instituto Oswaldo Cruz - FIOCRUZ, Av. Brasil, 4365, Rio de Janeiro, RJ, Brasil. ³ Laboratório de Biodiversidade Entomológica, 21045-900, Instituto Oswaldo Cruz - FIOCRUZ, Rio de Janeiro, RJ, Brasil. ⁴ Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia, 45.206-510, Jequié, BA. ⁵ Departamento de Biologia Geral, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brasil.

*Author for correspondence:

tcmonte@ioc.fiocruz.br

phone: ++21. 2598.4320

fax: ++21.2573.4468

ABSTRACT

In order to determine the existence of differentiation in the lengths of the testis follicles between three species and one subspecies of *Triatoma* a detailed morphometric analysis was carried out. Captures were performed at different sites where the species were described: Caicó, state of Rio Grande do Norte (*T. brasiliensis*), Petrolina, state of Pernambuco (*T. brasiliensis macromelasoma*), Juazeiro, state of Bahia (*T. juazeirensis*) and Espinosa, state of Minas Gerais (*T. melanica*). The records of the lengths of the testis follicles were submitted to statistic analysis. Results showed that all *Triatoma* studied have seven follicles in the testis, following the Triatominae pattern, which present different lengths. The morphometric data indicate the separation of the species in three groups: *T. brasiliensis*, *T. melanica*, and *T. juazeirensis*+*T. brasiliensis macromelasoma*. The obtained results are in agreement with other previous morphologic, ecologic, genetic, molecular, and phylogeographic studies, corroborating the importance of the morphometry on the testis follicles for taxonomic studies of triatomines.

Key words: Chagas' disease vector, reproductive tract male, morphology.

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INTRODUCTION

Morphology of male reproductive tract has been studied in different matter in insects (Woodward 1950; Forbes and Do-Van-Quy 1965; Louis and Kumar 1973; Bahadur 1975; Wheeler and Krutzsch 1992; Mikheyev 2004).

In Reduviidae (Heteroptera) the male reproductive tract is poorly developed in early nymphal instars, however the testis and the *vas deferens* change from the fourth instar completing its development in the fifth instar nymph (Carayon 1944). The male reproductive tract in Triatominae (Reduviidae) is formed by a pair of testes, two *vasa deferentia*, a pair of seminal vesicles, four pairs of accessory glands and an ejaculatory duct that open in the aedeagus (Barth 1958). In Triatominae the testis is an oval structure lined by a scrotal membrane that enclosed seven folded follicles, which may have variations among specimens, ageing, feeding condition and reproductive activity (Barth 1956).

The number, length and thickness of the testis follicles, in some species of Triatominae, can be used to characterize the genera status. *Panstrongylus* has seven narrow follicles with similar lengths, *Rhodnius* and *Psammolestes* have five short and fine follicles, and two medium and thick follicles, whereas *Triatoma* has three short and narrow follicles, two medium and thick, and two long and narrow ones, although in *Triatoma* variations in the lengths and thickness of the three follicle types may occur in different species (Schreiber et al. 1968; Silva and Schreiber 1971; Gonçalves et al. 1987).

Testes morphometry was used to revalidate the genus *Mepraia*, including *Triatoma spinolai* (Porter 1934) in this genus (Lent et al. 1994).

Triatoma brasiliensis is now considered the most important Chagas' disease vector in the Northeast Brazil (Costa et al. 2003a). Recently, multidisciplinary studies (morphologic, biologic, ecologic, and molecular) on four distinct forms of this species clarified the subdivision of *T. brasiliensis* into three species *T. brasiliensis*, *T. melanica*, *T. juazeirensis*, and one subspecies *T. brasiliensis macromelasoma* (Costa et al. 2003b, Costa et al. 2006, Costa and Félix 2007).

Considering that morphometry may be a relevant tool in comparing species of Reduviidae, it was here used in order to determine the existence of differences in the testis follicles of distinct species of *Triatoma*. The following hypotheses were also tested: the existence of bilateral symmetry between testes; the division of the seven follicles into three groups (short, medium, and long) according to the length variation of the general pattern of the *Triatoma* genera.

MATERIALS AND METHODS

The insects were obtained from colonies initiated with specimens ($n > 30$ for each form) collected in the localities where the species of *Triatoma* were described (Lent and Wygodzinsky 1979; Costa et al. 1997a): Caicó, state of Rio Grande do Norte (*T. brasiliensis*); Petrolina, state of Pernambuco (*T. brasiliensis macomelasoma*), Juazeiro, state of Bahia (*T. juazeirensis*) and Espinosa, state of Minas Gerais (*T. melanica*). The colonies were maintained in the Section of Morphology, Ultrastructure and Biochemistry of Arthropods and Parasites, Oswaldo Cruz Institute, Rio de Janeiro, state of Rio de Janeiro, Brazil. Fifth instar male nymphs, sexed according to Lent and Jurberg (1969) were maintained in a dark glass flask (30x15 cm), covered with nylon screen at $29 \pm 1^\circ\text{C}$, $80 \pm 5\%$ RH and 12 hours photoperiod. Inside the flask a folded filter paper was placed to increase the contact surface and refuge, as well as removing the humidity excess. The insects were fed weekly with blood of Swiss mouse (Protocol CEUA - FIOCRUZ P0100-01).

After the imaginal moult the newly adults were starved during three days to avoid nutritional effect on the testes development. The male reproductive tract was dissected in a Petri dish containing saline solution for insect (0.1 M NaCl + 0.1 M KCl). The testes were isolated, identified as left and right and placed in plastic dishes (0.3 x 4.3 cm) filled with the same saline solution. Subsequently, the testis follicles were distended by the disruption of the scrotal membrane. Ten specimens of *T. brasiliensis* and *T. juazeirensis*, 13 of *T. brasiliensis macromelasoma* and 16 of *T. melanica* were studied.

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

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

RESULTS

The general aspect of the male reproductive tract of all species here studied follows the pattern already described for other Triatominae (Barth 1958, Freitas et al. 2007) (Figs 1).

The testes of species of *Triatoma* have seven testis follicles elongated and folded. Each follicle is narrowed in the proximal region forming a short canal, the *vas eferens* that converges to the *vas deferens*.

The *Triatoma* species studied have seven testis follicles (F1 - F7) of variable length (10.87 - 31.84 mm), which are similar between left and right testis (ANOVA, $F_{(1, 675)}=0.4227$; $P=0.5158$) (Fig. 2). The statistical analyses allowed the classification of the testis follicles in two long, represented for F1 and F2 (24.54 ± 6.20 mm), two medium, represented for F3 and F4 (20.02 ± 5.01 mm), and three short, represented for F5, F6 and F7 (13.09 ± 3.04 mm) (Table I).

These groups of follicles have different mean length among the species: long (*T. brasiliensis* - 30.93mm; *T. juazeirensis* - 22.04 mm; *T. brasiliensis macromelasoma* - 21.57 mm; *T. melanica* - 24.54 mm), medium (*T. brasiliensis* - 25.10 mm; *T. juazeirensis* - 16.30 mm; *T. brasiliensis macromelasoma* - 17.65 mm; *T. melanica* - 21.10 mm) and short follicles (*T. brasiliensis* - 15.88 mm; *T. juazeirensis* - 12.19 mm; *T. brasiliensis macromelasoma* - 13.31 mm; *T. melanica* - 11.73 mm) (Fig. 3).

According to the statistic analysis were detected: *T. brasiliensis* (ANOVA, $F_{(3, 680)}=45.011$; $P<0.001$), *T. melanica* (ANOVA, $F=21.579$; $P<0.001$) the most divergent for the seven follicles studied, and the *T. juazeirensis*+*T. brasiliensis macromelasoma* had similar follicles length (ANOVA, $F_{(7, 677)}=1.8392$, $P=0.07714$) (Figs 2 and 3).

DISCUSSION

The number of seven testis follicles may be considered the plesiomorphic condition for Heteroptera (Akingbohunge 1983). In Triatominae, studies on testis morphometry showed that this subfamily has always seven follicles, with different width and length variation among the genera *Panstrongylus*, *Rhodnius*, *Psammolestes* and *Triatoma* (Schreiber et al. 1968; Silva and Schreiber 1971; Gonçalves et al. 1987). In the present study, the follicle length of the *Triatoma* species showed a pattern that agrees with the *Triatoma*: long, medium and short follicles.

None significant difference in follicles lengths between the left and right testes was observed, supporting the hypothesis of bilateral symmetry like on other studied species, as well as their classification in three categories: two long, two medium and three short (Gonçalves et al. 1987).

Significant differences in the length of the testis follicle were observed between *T. brasiliensis* and *T. melanica*, allowing their differentiation and corroborating previous studies that showed the higher morphologic variation in the egg exochorion (Costa et al. 1997a) and the genetic distance (Costa et al. 1997b; Monteiro et al. 2004) between this two species.

We suggest that the morphometric differences in the testis follicle length of the *T. brasiliensis* and *T. melanica* may be due to reproductive barriers, because they are geographically more isolated than *T. juazeirensis* and *T. brasiliensis macromelasoma* (Costa et al. 1998)

The hypothesis of similarity in the follicle length can be rejected for *T. brasiliensis* and *T. melanica*, whereas for *T. juazeirensis* and *T. brasiliensis macromelasoma* no significant differences are evident, corroborating this hypothesis.

The data here presented emphasize the importance that morphometric studies of testis follicles showed be associated with others combinations of characters to address important issues on *Triatoma* taxonomy.

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Table I: Analyses of variance (ANOVA) of the testis follicles in species of *Triatoma* (Hemiptera, Triatominae). * Follicles with significant difference in the length by the test F at 5%. N=sample size.

Follicle groups	N	Df	F	P
F7F6	662	4	0,949	=0,434
F7F6F5 (short)	666	4	2,287	=0,059
F7F6F5F4*	670	4	55,673	<0,001
F4F3 (medium)	670	4	1,769	=0,133
F4F3F2*	674	4	16,954	<0,001
F1F2 (long)	674	4	1,726	=0,142

LEGENDS SECTION

Fig. 1: Schematic drawn of the male reproductive tract in species of *Triatoma* (Hemiptera, Triatominae). T - testes, vd - vas deferens, sv - seminal vesicle, g1 - accessory gland I, g2 - accessory gland II, g3 - accessory gland III, g4 - accessory gland IV, gd - gland duct.

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

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

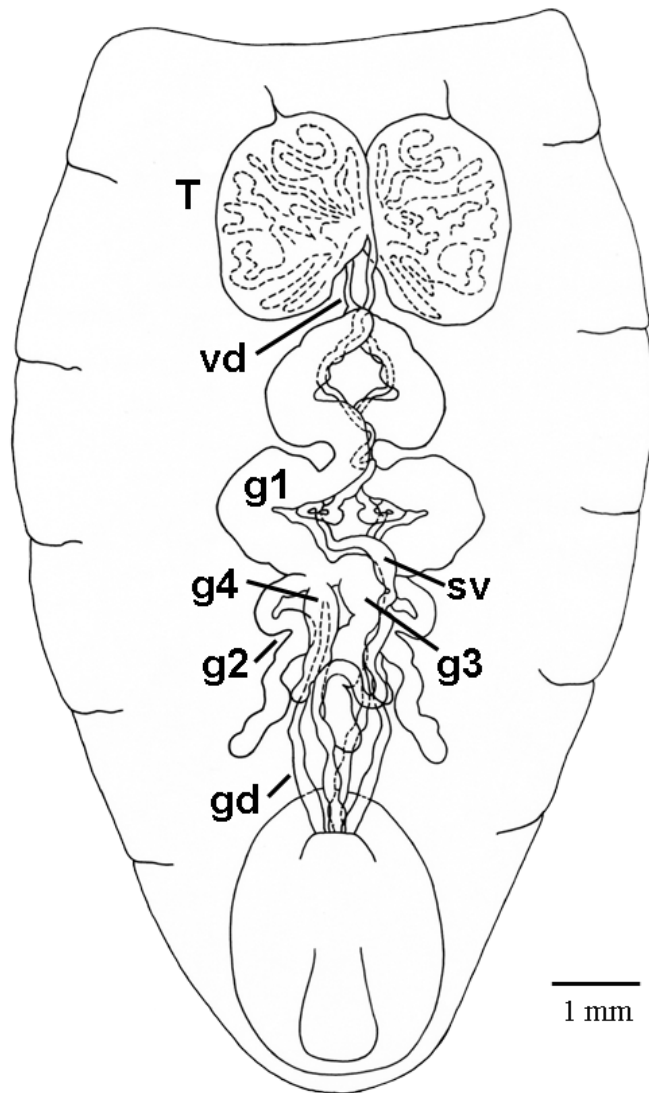


Figure 1

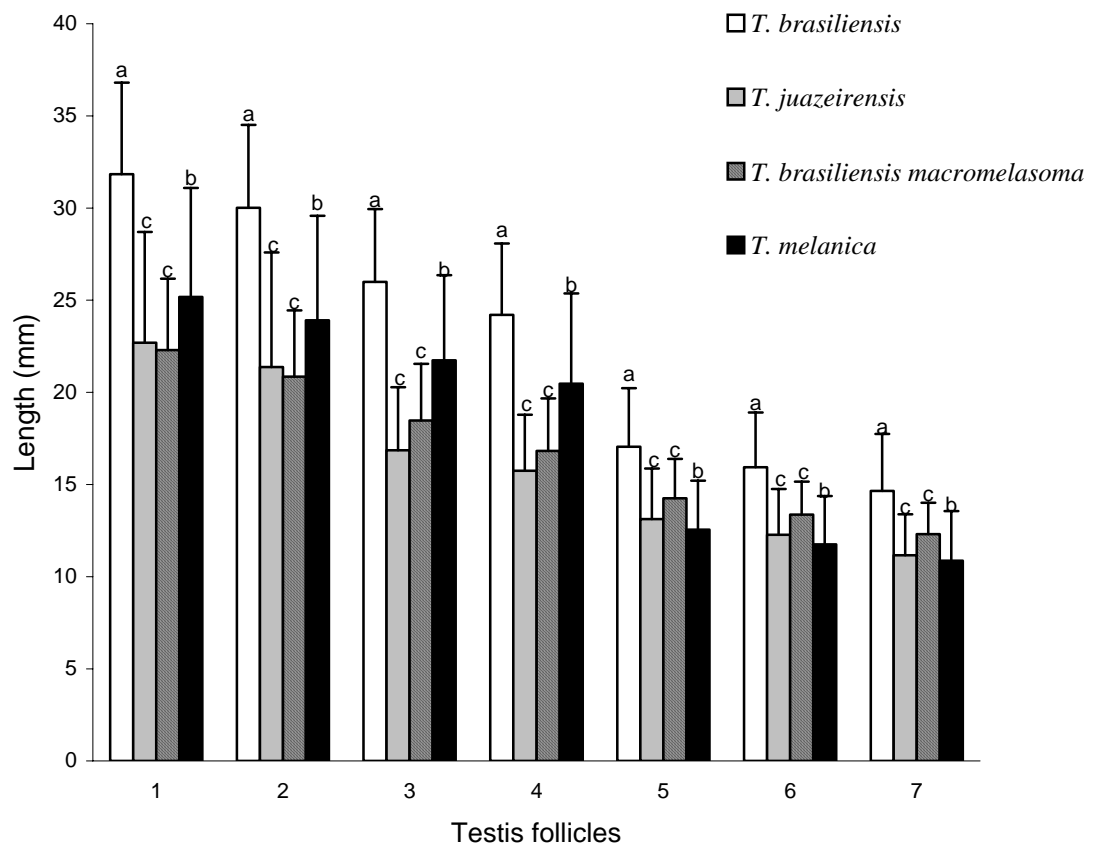


Figure 2

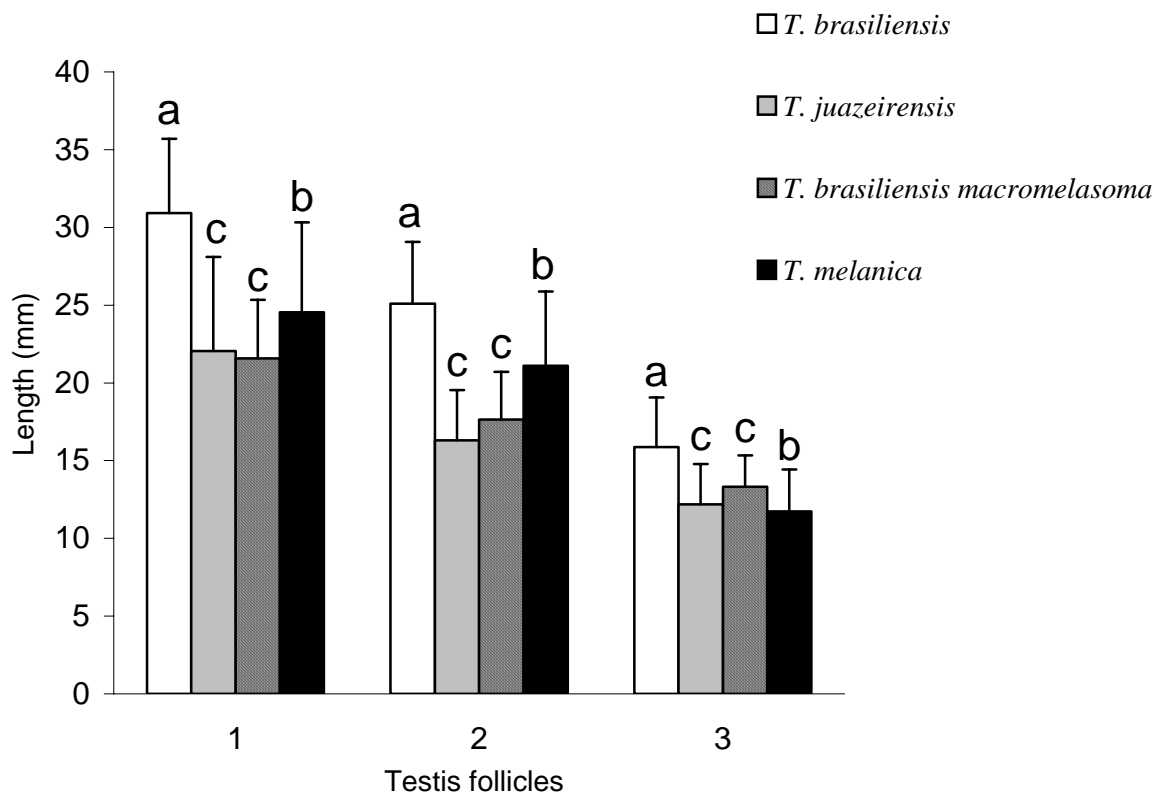


Figure 3