Triatoma jatai sp. nov. in the state of Tocantins, Brazil (Hemiptera: Reduviidae: Triatominae)

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Triatoma jatai sp. nov. is the first new species of triatomine to be described in the state of Tocantins, in the northern region of Brazil. It was caught on rock outcrops in the wild environment and, more recently, invading homes. While T. jatai sp. nov. is morphologically similar to Triatoma costalimai, it is distinguished by its general colouring, differences in the blotches on the connexivum, wing size in females and external structures of the male genitalia. The type series has been deposited in the Entomological Collection and Herman Lent Collection, Oswaldo Cruz Institute-Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

Key words: Triatoma - taxonomy - Chagas disease vector - biodiversity - Brazil

The state of Tocantins (TO) is located in the northern region of Brazil; 91% of its territory is formed by the Cerrado (savanna-like) biome and the remaining 9% by the Amazonian biome (IBGE 2004). In 2006, the 139 municipalities that make up this state were classified regarding their degree of risk of vector transmission of Chagas disease, such that 24.47% presented a high risk, 52.51% medium risk and 23.02% low risk, with a triatomine fauna consisting of 12 species distributed across different ecotopes (Costa et al. 2003, Oliveira et al. 2008). The southern and southeastern regions of TO, which are considered to be endemic for Chagas disease, are within the Cerrado biome and are composed of a variety of vegetation types, such as cerrado (lightly wooded savanna-like terrain), cerradão (more heavily wooded savanna-like terrain), park-like terrain, campo limpo (savanna grassland) and campo rupestre (rocky open terrain). This last type consists of large numbers of calcareous rock outcrops scattered across the region that form the so-called "limestone scrub", which has the capacity to provide shelter for triatomine fauna and its food sources.

Specimens of *Triatoma costalimai* Verano & Galvão, 1959 have been caught in the municipalities of Aurora do Tocantins and Paranã, located in the southeast and south of the state. This species is now included in the species subcomplex *matogrossensis*, according to its molecular and biogeographical characteristics (Schofield & Galvão 2009).

Also in Paranã, triatomine specimens similar to *T. costalimai*, but unidentifiable from the classical morphological patterns have been caught.

According to Dujardin et al. (2010), the morphometric geometric of wings allows observing conformational changes undetectable by traditional morphological methods and has been used for taxonomic identification of cryptic species in Triatominae for the genus *Rhodnius* (Matias et al. 2001, Villegas et al. 2002, Rosa et al. 2012), *Triatoma* (Gumiel et al. 2003, Carbajal de la Fuente 2007, Carbajal de la Fuente et al. 2011) and *Mepraia* (Campos et al. 2011). The morphology and the morphometric geometric of wings are studied and described herein as bellowing to a new species of the genus *Triatoma* Laporte, 1832 and proposed a new taxonomic key for *Triatoma matogrossensis* subcomplex based on Obara et al. (2012).

MATERIALS AND METHODS

Specimens of *Triatoma jatai* sp. nov was captured on rock outcrops at four sites: on the ranch Fazenda Jatai (12°42'40"S 48°13'12"W), bordering the TO-387/296 highway on the stretch between Paranã and São Salvador (12°40'10"S 48°12'16"W and 12°40'40"S 48°11'36"W) on Fazenda Vereda, Bangue (12°26'05"S 48°03'59"W). All of these sites are within the municipality of Paranã (Figs 1, 2). T. costalimai from Aurora do Tocantins and Triatoma williami obtained from the National Laboratory and International Reference on Taxonomy of Triatominae (LNIRTT), Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro (RJ), Brazil, were used to comparison (license SISBIO 18014-1). The morphometric study of T. jatai sp. nov. and T. costalimai species was conducted on 30 males and 30 females on stereoscopic microscopy (Zeiss - SV6®). The male genital structures analyses were done on 10 specimens of *T. jatai* sp. nov. comparing with *T. costalimai* (Lent & Jurberg 1980).

The geometric morphometric analysis of wings was performed with *T. jatai* sp. nov. (85 females and 67 males), *T. costalimai* (54 females and 67 males), *T. williami* (5 females and 10 males) and *Triatoma sordida* (14 females and 6 males), as the outgroup. The wings were extirpated

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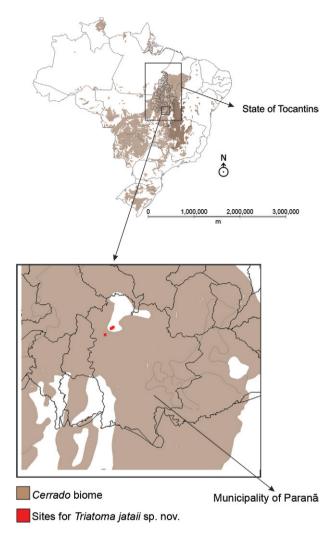


Fig. 1: map showing *Cerrado* biome and sites of capture of *Triatoma jatai* sp. nov, municipality of Paranã, state of Tocantins, Brazil.

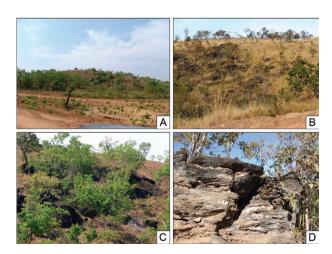


Fig. 2A: site of capture view; B-D: details of rock outcrops where *Triatoma jatai* sp. nov. was captured on the ranch Fazenda Jatai, state of Tocantins, Brazil.

with forceps and mounted among microscope slides and cover-slips with alcohol 70%. Photographs were taken using digital camera (Sony DSC - S70) and coupled manually on the ocular of a stereomicroscope Zeiss - SV6®. We used the 7 type-1 landmarks (vein intersections) according to Bookstein (1990). For comparison of wing size we used the isometric estimator known as centroid size (CS) derived from coordinates data. CS is defined as the square root of the sum of the squared distances between the centre of the configuration of landmarks and each individual landmark (Bookstein 1990). Shape variables (partial warps plus uniform components) were obtained using the Generalized Procrustes Analysis superimposition algorithm (Rohlf 1996). The method is based in the superimposition of each individual using least-square, eliminating effects of scale, orientation and position of the objects. The shape variables define the positional changes at each landmark in relation to a consensus shape.

Kruskal-Wallis tests corrected by Bonferroni method were used to analyse the CS between the analysed species. The Mahalanobis distances derived from shape variables were used to explore the shape proximity between species. Their statistical significance was computed by permutation tests (1,000 runs each) and corrected by Bonferroni method. These distances were used in unweighted pair-group method with arithmetic average (UPGMA) cluster analyses to produce dendrograms. Discriminant analyses were performed to evaluate the existence of significant differences to interspecific level and to reclassify the individuals using the discriminant functions and Mahalanobis distances.

For morphometric analysis we used CLIC v45 package developed by JP Dujardin (life.bio.sunysb/morpho). Statistics analysis was executed with JMP v4 (SAS Institute, Inc, Cary, NC, USA).

The material is deposited in the Entomological Collection, Oswaldo Cruz Institute (CEIOC), Fiocruz, and Herman Lent Collection, LNIRTT. The morphological and morphometrics descriptions are according to the taxonomic characteristics for the Triatominae proposed by Verano and Galvão (1959) and Lent and Wygodzinsky (1979).

RESULTS

T. jatai Gonçalves, Teves-Neves, Santos-Mallet, Carbajal-de- la-Fuente and Lopes, sp. nov. (Fig.3)

Diagnosis - T. jatai sp. nov. (Fig. 3) can be distinguished from the morphologically closer species T. costalimai (Fig. 4) mainly by the smaller size (Tables I, II) and general brown colour; by the pilosity of rostral segments, by the size and colour of the connexival spots, which are yellow, above an intersegmental suture, which is brown; by the shorter wing size of the females, finishing at the basal third of the seventh tergite and by the smaller size and shape of the structures of male external genitalia (Table III).

Length: male 18.6 mm, female 20.15 mm; length and width of head, length of eye, synthlipsis, rostral segments, length and width of pronotum and width of abdomen are in the Tables I, II.

Head: brown, elongate, slightly rugose. Head four times longer than width (1:0.23) and longer than pronotum (1:0.7) or as long as the pronotum and scutellum together, without apical process of scutellum, covered with short setae; 1+1 glabrous area extending from the base to the antenniferous tubercles and to the ocelli. Anteocular region four times longer than postocular region (1:0.22). Clypeus narrow, extending gradually from the middle region. Genas sharp, projecting beyond apex of clypeus. Jugum angular apically. Eyes in lateral view attaining level of undersurface and remote from level of upper surface of head. Ratio of width of eye to synthlipsis 1:0.5. Antenniferous tubercles inserted into the middle of anteocular region of head. First antennal segment brown, reaching the middle of clypeus, with scattered setae in row. Second segment brown, hirsute, bristles longer than the diameter of the segment. Third segment with base dark and the remainder clear. Fourth segment clear. Both third and fourth segments covered with sparse short and long bristles, arranged perpendicularly to the segment. Ratio of antennal segments 1:4.5:2.7:2.4. Rostrum with the same colour as head, extending to prosternum, with sparse short hairs on first and second segments; third segment with numerous hairs. Ratio of rostral segments 1:3:1.

Pronotum: brown, rugose, length and width similar. Anterior lobe with 1+1 discal tubercles; lateral tubercles absent. Submedian carinae almost reaching the posterior margin of posterior lobe. Humeral angles rounded; may present 1+1 light spot.

Scutellum: brown, rugose, with a central V-shaped depression. Apical process cylindrical, as long as main body of scutellum; apex slightly elevated, rounded.

Hemelytra: males attaining or almost attaining apex of seventh urotergite. Females short, attaining basal third or middle of seventh urotergite. Corium and clavus brown; subcosta light. Corium with 2+2 ochre spots on basal and apical regions. Membrane gray with black veins.

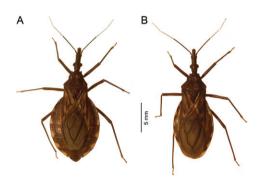


Fig. 3: Triatoma jatai sp. nov. A: female; B: male.

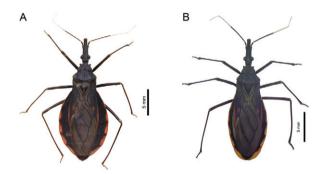


Fig. 4: Triatoma costalimai. A: female; B: male.

TABLE I

Measurements (in mm) of *Triatoma jatai* sp. nov. and *Triatoma costalimai* female captured in the municipality of Paranã and Aurora do Tocantins, state of Tocantins, Brazil

Gender	T. jatai sp. nov.					T. costalimai				
Variables	Min	Max	X	SD	S^2	Min	Max	X	SD	S^2
Total length	17.2	28.5	20.15	2.027	4.252	22.5	25.9	24.87	1.162	1.352
Width of abdomen ^a	6.3	9.4	8.15	0.698	0.504	8.4	10.7	9.55	0.879	0.772
Length of pronotum	2.3	3.2	2.79	0.208	0.044	3.1	3.5	3.27	0.160	0.025
Width of pronotum	2.3	3.1	2.62	0.260	0.069	2.6	3.2	2.95	0.207	0.042
Length of head	3.9	4.4	4.06	0.145	0.021	4.1	5.1	4.81	0.376	0.141
Width of head	0.8	1.0	0.90	0.047	0.002	1.0	1.1	1.08	0.037	0.001
Synthlipsis	0.8	1.0	0.85	0.056	0.003	0.9	1.1	0.97	0.075	0.005
Width of eyes	0.4	0.5	0.40	0.017	0.000	0.5	0.5	0.50	0.000	0.000
Length of 1st rostral segment	0.8	1.1	0.94	0.080	0.006	1	1.2	1.07	0.075	0.005
Length of 2nd rostral segment	2	3.0	2.54	0.223	0.051	3	3.3	3.14	0.127	0.016
Length of 3rd rostral segment	0.8	1.0	0.92	0.071	0.005	1	1.2	1.08	0.089	0.008

a: all the differences between species are significant, except for the female width abdomen (analysis of variance - p < 0.05); Max; maxima; Min: minimum; SD: standard deviation; S²: variance; X: average.

Legs: brown with short hairs in row. Fore femora eight times as long as wide. Fore and mid femora with a couple of weak denticles subapically. Males with spongy fossulae on tibiae of foreleg; absent in female.

Abdomen: convex, polished, covered by short hairs, appearing glabrous. Spiracles clear adjoining connexival suture. Urosternites brown. Connexivum predominantly brown with basal clear triangular spots of variable width,

TABLE II

Measurements (in mm) of *Triatoma jatai* sp. nov. and *Triatoma costalimai* male captured in the municipality of Paranã and Aurora do Tocantins, state of Tocantins, Brazil

Gender	T. jatai sp. nov.					T. costalimai					
Variables	Min	Max	X	SD	S^2	Min	Max	X	SD	S^2	
Total length	16.6	21.9	18.64	1.249	1.561	19.9	22.8	21.47	0.784	0.615	
Width of abdomen ^a	6.0	7.9	6.94	0.486	0.236	5.6	8.4	6.99	0.834	0.696	
Length of pronotum	2.6	3.2	2.88	0.179	0.032	2.2	3.6	3.08	0.303	0.092	
Width of pronotum	2.2	3.0	2.55	0.188	0.035	2.5	3	2.77	0.176	0.031	
Length of head	3.6	4.2	3.91	0.131	0.017	4.1	4.8	4.51	0.152	0.023	
Width of head	0.7	0.9	0.84	0.056	0.003	0.9	1.0	0.96	0.047	0.002	
Synthlipsis	0.7	0.8	0.76	0.049	0.002	0.8	1.0	0.87	0.073	0.005	
Width of eyes	0.4	0.5	0.40	0.017	0.000	0.4	0.5	0.47	0.043	0.002	
Length of 1st rostral segment	0.5	1.0	0.90	0.116	0.013	0.8	1.2	1.00	0.090	0.008	
Length of 2nd rostral segment	2.0	2.9	2.45	0.214	0.046	2.7	3.2	2.94	0.124	0.016	
Length of 3rd rostral segment	0.7	1.0	0.88	0.077	0.005	0.6	1.0	0.98	0.099	0.010	

a: all the differences between species are significant, except for the male width abdomen (analysis of variance - p < 0.05); Max; maxima; Min: minimum; SD: standard deviation; S²: variance; X: average.

TABLE III

Morphological differences between *Triatoma jatai* sp. nov. and *Triatoma costalimai* Verano & Galvão, 1979

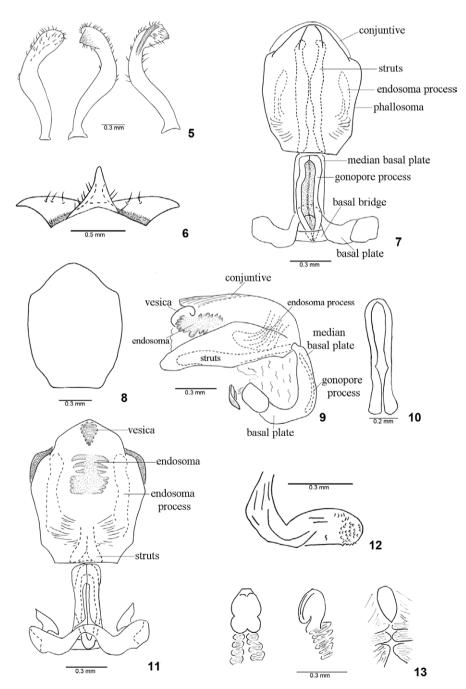
General aspects	<i>T. jatai</i> sp. n.	T. costalimai Dark brown or black				
General colour	Brown					
Rostrum	Second and third rostral segments with short hairs, numerous on third	Second and third rostral segments with numerous long hairs				
Hemelytra	Corium with 2+2 ochre spots on basal and apical regions. Males hemelytra attaining or almost attaining apex of seventh urotergite, females attaining basal third or middle of seventh urotergite	Corium with 2+2 red spots on basal and apica regions. Males and female hemelytra attaining apex of seventh urotegite				
Connexivum	Connexivum brown with apical clear triangular spot close to the basal intersegmental suture	Connexivum entirely dark brown or black, with orange-red marking of variable width extending along outer connexival border, interrupted by very narrow black bands on intersegmental suture				
Tibiae	Spongy fossulae on fore tibiae of male, absent in female	Spongy fossulae on fore and mid tibiae of male, absent in female				
Median process of pygophore	Triangular with arcuate base	Y shape with arcuate base				
Vesica	Vesica with recesses; 1+1 submedian and one in base; hooked in side view, elongated with flat base and pointed apex in dorsal view	Vesica without recesses, with flat base, curved in side view with rounded apex				
Phallosoma	Pentagonal	Ovoid				

near the intersegmental suture. Females with abdomen wider than in males (1:0.8).

Male genitalia: parameres cylindrical; upper third curved with laminar projections at 90°. Middle and inner surface hairy; outer side glabrous and hirsute on edge (Fig. 5). Median process of pygophore triangular with arcuate base (Fig. 6). Phallus ventral, showing pentagonal phallosoma (Figs 7, 8). Phallus lateral view showing elongated hollow struts; lateral arms attached apically and slightly separated in the base (Figs 9, 10). Phallus dorsal view with

endosomal aliform process, striated throughout its length; apical third with conspicuously sclerotised projections (Figs 11, 12). Vesica with recesses; 1+1 submedian and one in base; hooked in side view, elongated with flat base and pointed apex in dorsal view (Figs 9, 11, 13). Gonopore process cylindrical, hollow; outer edges removed almost reaching the median basal plate (Figs 7, 9). Median basal plate rectangular, welded to basal plate (Fig. 7).

Type-locality - Fazenda Jatai (12°42'40"S 48°13'12"W), municipality of Paranã.



Figs 5-13: *Triatoma jatai* sp. nov.; 5: parameres, side view, internal view and external view; 6: median process of pygophore; 7: phallus, ventral view; 8: phallosoma; 9: phallus, lateal view; 10: struts; 11: phallus, dorsal view; 12: endosoma process; 13: vesica, ventral, lateral and dorsal view.

Type data and depository - Holotype: female (27 IX 2007) and seven paratypes (4 males and 3 females) from Fazenda Jatai (12°42'40"S 48°13'12"W), municipality of Paranã; four paratypes (2 males and 2 females) from the same locality registrations 3401-3404 of Herman Lent Collection (LNIRTT/IOC).

Other material examined - One male for the description; four males for the genitalia study, 30 males and 30 females for the morphometric study.

Distribution - Municipality of Paranã (Fig. 1).

Etymology - The specific epithet is a noun in apposition referring to the capture site, Fazenda Jatai in the type locality, to where the local population was relocated during the construction of the Aproveitamento Hidrelétrico Peixe Angical, TO.

Key to the species of *Triatoma matogrossensis* subcomplex (based on Obara et al. 2012)

- Pronotum dark brown or reddish brown with 1+1 light spots on ad-humeral region; legs light yellowish brown with markings or annulus dark coloured; femora predominantly light coloured; connexivum yellow or yellowish brown with a straight dark strip

- Femora entirely dark, trochanters partially or entirely dark...... 6

Size variation - The Kruskal-Wallis test revealed significant differences (p < 0.001) between T. costalimai, T. williami, T. jatai sp. nov. and T. sordida being markedly lower wings of T. jatai sp. nov. (Fig. 14). The female wings of T. jatai sp. nov. are significantly smaller than the males (p < 0.001) (Fig. 14). No significant differences were found in the CS of males and females belonging to the species T. sordida.

Shape variables - The individuals projected onto the two first canonical factors show a separation between *T. jatai* sp. nov. and *T. costalimai* species. However, exist an overlap between the polygons from *T. williami* and *T. sordida* species (Fig. 15). The permutation test corrected by Bonferroni showed that *T. jatai* sp. nov. is significantly different from other species (p < 0.001). The derived UPGMA dendogram showed a clear separation between species (Fig. 16). Rates of reclassification of individuals to *T. jatai* sp. nov. and *T. costalimai* were considered satisfactory (48-64%) and for *T. willami* were acceptable (70% and 80%) and for the females of *T. sordida* lower (35%) and higher for males (82%). The contribution of canonical factors resulted in 59%, 30% and 6% for the first three factors.

DISCUSSION

A study on the triatomine fauna of the southern and southeastern regions of TO was conducted between 2004-2008, covering a total of 11 municipalities: Peixe, São Salvador, Paranã, São Valério, Ponte Alta do Bom Jesus, Taguatinga, Aurora do Tocantins, Lavandeira, Combinado, Novo Alegre and Arraias (Gonçalves et al. 2006).

In the southeastern region, in the municipalities of Ponte Alta do Bom Jesus, Taguatinga, Aurora do Tocantins and Paranã, specimens of *T. costalimai* infected with *Trypanosoma cruzi* (Chagas 1909) were collected

from wild environments among volcanic rock outcrops. Aurora do Tocantins was the only municipality in which specimens of this species positive for *T. cruzi* were also caught in peridomestic areas.

The type locality for *T. costalimai* is the district of Manhã, municipality of Taguatinga, state of Goiás, and specimens were collected from limestone outcrops (Verano & Galvão 1959).

The specimens of *T. jatai* sp. nov., which were restricted to the municipality of Paranã, were also caught in the wild environment but, unlike *T. costalimai* in this region, among limestone outcrops and without the presence of infection by *T. cruzi*. To investigate infection due to *T. cruzi*, faeces and/or urine of both species were obtained by means of abdominal compression and were seeded into Novy-MacNeal-Nicolle culture medium.

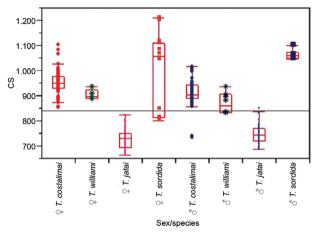


Fig. 14: variation of size [centroid size (CS)] among *Triatoma jatai* sp. nov., *Triatoma costalimai*, *Triatoma williami* and *Triatoma sordida*. Box plot indicate median of group as a line in the middle of quartiles (25 and 75 closed the box and 10 and 90 as lines in the extremes of each box). Line dot denotes the grand mean. The point at centre of the boxes shows the distribution of specimens.

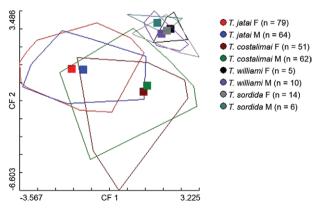


Fig. 15: canonical variate analysis of *Triatoma jatai* sp. nov., *Triatoma costalimai*, *Triatoma williami* and *Triatoma sordida*. Centroid distribution (multivariate mean) on canonicals factors (CF) 1 and 2. F: female; M: male.

Recently, three adult specimens (1 male and 2 females) were also collected from inside a home [A Feitosa, unpublished observations (Health Department of the State of Tocantins)].

Both species are too close morphologically, but may be differentiated mainly by the size, general colour, size and shape of wings, connexivum and intersegmental sutures. *T. jatai* sp. nov. is brown and significantly smaller than *T. costalimai*, which present the general colour black. The wings of female are visibly shorter, ending at the level of the seventh urotergite, while in *T. costalimai* the wings cover the eighth urotergite. *T. jatai* sp. nov. show a connexivum with subtriangular yellow connexival blotches of varying width, located in the apical region proximal to the chestnut brown intersegmental sutures, different of *T. costalimai*, where the connexivum have a reddish outline, weakly interrupted at the level of the intersegmental sutures by a black band and completely black at the internal limit with the abdomen.

Structures of the male external genitalia of *T. jatai* specimens did not show intraspecific variation, but they are smaller than *T. costalimai* described by Lent and Jurberg (1980). *T. jatai* shows the median process of pygophore is triangular; the vesica show recesses, hooked in side view, the phallosoma has a trapezoidal shape, while *T. costalimai* the median process of pygophore has any shape, the vesica show no recesses, less hooked in side view, and the phallosoma is ovoid.

Pires et al. (1995) identified differences of genital structures for two populations of *Triatoma infestans* as well Costa et al. (1997) to *Triatoma brasiliensis* population. However, the last author state that a large and representative samples should be examinated before using for diagnosis. This corroborates the results of the present study where a multidisciplinary analysis was important to a taxonomic identification.

Regarding the CS, females of *T. jatai* sp. nov. have smaller wings than *T. costalimai* and *T. williami*. Sexual dimorphism in body size may be observed in both *T. jatai* sp. nov. and *T. costalimai* species, as observed for other triatomine species where females are larger than males (Márquez et al. 2011, Gaspe et al. 2012). Significant differences were observed comparing the sexes between the species where *T. jatai* sp. nov. was significantly lower than *T. costalimai*. Only the abdomen width was bigger in *T. jatai* sp. nov. males, which may be associated to nutritional status, which does not influence the other morphological characteristics.

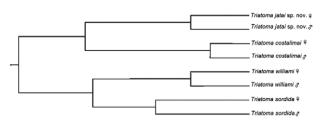


Fig. 16: unweighted pair-group method with arithmetic average dendogram derived from Mahalanobis distances based on wings shape.

The geometric morphometrics wings allowed to differentiate *T. jatai* sp. nov. from the species *T. costalimai* and *T. williami*, which are close morphologically (Lent & Wygodzinsky 1979).

The wings shape showed the separation between *T. jatai* sp. nov. and the remaining species. Thus, the UPG-MA Mahalanobis distances tree evidenced that *T. jatai* sp. nov. and *T. costalimai* clustered in different clades, corroborating the differences found by other tools presented in this paper, showing once again the contribution of geometric morphometrics to clarify taxonomic differences.

The main vector causing endemicity of Chagas disease in the southeastern region of TO is the species T. infestans (Klug 1834), which was eliminated from domestic environments in 2002 (Oliveira et al. 2008). In turn, the native species started to occupy the vacant ecotopes and participate in transmission of T. cruzi as commented by Costa and Lorenzo (2009). T. costalimai is now taking on epidemiological importance because of the numbers of notifications of colonisation by this species in peridomestic and domestic areas, with the presence of infection by T. cruzi, thus showing the importance of maintaining epidemiological surveillance in relation to these species. Although T. jatai sp. nov. was caught in the wild environment, on rock outcrops located close to a settled area and although it was negative for T. cruzi, it was also recently found in a home on a ranch close to the type locality. This occurrence in an area with human activity shows that the importance of entomological surveillance focusing on T. costalimai should be extended to T. jatai sp. nov.

The subfamily Triatominae, for which up to the year 2012, 144 species had been recorded, with 62 occurring within Brazilian territory (Poinar 2005, Schofield & Galvão 2009, Frias-Lasserre 2010, Gurgel-Gonçalves et al. 2012, Rosa et al. 2012), now with *T. jatai* sp. nov. numbers 145 species and 64 in Brazil.

Bionomic studies aiming to elucidate the vector potential of *T. jatai* sp. nov. for transmission of the parasite *T. cruzi* are being conducted.

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