

Proof Only

Intraspecific and Interspecific Phenotypic Differences Confirm the Absence of Cryptic Speciation in *Triatoma sordida* (Hemiptera, Triatominae)

Ariane Cristina Caris Garcia,^{1†} Jader de Oliveira,^{2,3†} Daniel Cesaretto Cristal,³ Luiza Maria Grzyb Delgado,¹ Isadora de Freitas Bittinelli,¹ Cleber Galvão,^{4*} Nilsa Elizabeth Gonzalez Britez,⁵ Hernán José Carrasco,⁶ João Aristeu da Rosa,³ and Kaio Cesar Chaboli Alevi^{1,3}

¹Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Instituto de Biociências de Botucatu, Distrito de Rubião Junior, Botucatu, São Paulo, Brazil; ²Laboratório de Entomologia em Saúde Pública, Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo (USP), São Paulo, Brazil; ³Laboratório de Parasitologia, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Araraquara, São Paulo, Brazil; ⁴Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz (FIOCRUZ), Pavilhão Rocha Lima, Rio de Janeiro, Brazil; ⁵Departamento de Medicina Tropical, Instituto de Investigaciones en Ciencias de la Salud (IICS), Universidad Nacional de Asunción, Campus Universitario, San Lorenzo, Paraguay; ⁶Laboratorio de Biología Molecular de Protozoarios, Facultad de Medicina, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela

Abstract. *Triatoma sordida* is an endemic Chagas disease vector in South America, distributed in Brazil, Bolivia, Paraguay, and Uruguay. Chromosomal, molecular, isoenzymatic, and cuticular hydrocarbon pattern studies indicate cryptic speciation in *T. sordida*. Recently, *T. rosai* was described from specimens from Argentina initially characterized as *T. sordida*. Although several authors assume that the speciation process that supports this differentiation in *T. sordida* is the result of cryptic speciation, further morphological and/or morphometric studies are necessary to prove the application of this evolutionary event, because the only morphological intraspecific comparison performed in *T. sordida* is based on geometric morphometry and the only interspecific comparison made is between *T. rosai* and *T. sordida* from Brazil that evaluated morphological and morphometric differences. Based on this, morphological analyses of thorax and abdomen using Scanning Electron Microscopy and morphometric analyses of the head, thorax, and abdomen among *T. sordida* from Brazil, Bolivia, and Paraguay, as well as *T. rosai*, were performed to assess whether the evolutionary process responsible for variations is the cryptic speciation phenomenon. Morphological differences in the thorax and female external genitalia, as well as morphometric differences in the head, thorax, abdomen, pronotum, and scutellum structures, were observed. Based on this, the evolutionary process that supports, so far, these divergences observed for *T. sordida* populations/*T. sordida* subcomplex is not cryptic speciation. Moreover, we draw attention to the necessity for morphological/morphometric studies to correctly apply the cryptic species/speciation terms in triatomines.

INTRODUCTION

Chagas disease is a neglected disease caused by the *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae), and transmitted mostly by the triatomines (Hemiptera, Triatominae)—hematophagous insects that defecate during the blood meal, which can lead to the release of the infective form of the parasite (trypomastigote) in case of the vector is infected by *T. cruzi*.¹ This disease can be divided into two main phases (acute and chronic) that can either be asymptomatic^{2,3} or cause cardiomyopathy, arrhythmias, megaesophagus, megacolon, cardiomegaly, and more rarely, polyneuropathy and cerebral vascular accident (in about 30–40% of chronic chagasic patients).⁴

Currently, there are 156 described species of triatomines.^{5–7} Although there are species with a greater or lesser degree of importance in the transmission of the Chagas disease (highlighting *Triatoma infestans*, *Panstrongylus megistus*, *T. brasiliensis*, *T. pseudomaculata*, and *T. sordida* that have greater vector skills in Brazil),⁸ all triatomines, of both sexes and at any stage of development after hatching, are considered as potential vectors of this disease described over 110 years ago.^{9,10}

Triatoma sordida is an endemic species in South America, distributed in Brazil, Bolivia, Paraguay, and Uruguay.^{6,8} Because of the high dissemination capacity of *T. sordida*,¹¹

which inhabits sylvatic, peridomestic, and domestic environments,^{10,12} and presents several food sources,^{13–18} this species has an appraisable ecological value.¹⁹ Broadly, infection rates by *T. cruzi* are relatively low, ranging from 0.5% to 16.2%.^{13–18} This information associated with the habit of nymphs and adults of *T. sordida* to defecate during the blood meal,²⁰ highlights the vectorial importance of this species in the transmission of the Chagas disease.¹⁸

Noireau et al.,²¹ based on isoenzymatic analyses, suggested that the cryptic speciation phenomenon was occurring among the *T. sordida* populations from Bolivia. However, Panzera et al.²² pointed out that the authors performed an interspecific comparison between *T. sordida* and *T. garciabesi*, which resulted in an elevated genetic distance. Taking into account this initial hypothesis associated with cryptic speciation in *T. sordida*, chromosomal, molecular, isoenzymatic, and cuticular hydrocarbon pattern studies were performed among the *T. sordida* populations from Brazil, Bolivia, and Argentina, and based on the observed divergences, the authors suggested that *T. sordida* may represent more than a taxon.^{22–24}

Recently, Alevi et al.⁶ described *T. rosai* from specimens from Argentina initially characterized as *T. sordida*. For the description of the new species, the authors used integrative taxonomy and compared several characteristics between *T. rosai* and *T. sordida* (morphological, morphometric, molecular, cytogenetics data, experimental crosses, electrophoresis pattern, molecular analyses, cuticular hydrocarbons pattern, geometric morphometry, cycle, and average time of life as well as geographic distribution). Among the characteristics that differentiate the species, it is worth mentioning that the average time of development and the life cycle of *T. rosai* is

*Address correspondence to Cleber Galvão, IOC/FIOCRUZ, Av. Brazil 4365, Pavilhão Rocha Lima, sala 505, 21040-360 RJ, Brazil. E-mail: clebergalvao@gmail.com

†These authors contributed equally to this work.

half of the observed for *T. sordida*.⁶ In addition, *T. rosai* has a higher rate of infection by *T. cruzi* (38.5%).⁶

Although several authors assume that the speciation process that supports this differentiation in *T. sordida* is the result of cryptic speciation,^{21–24} further morphological and/or morphometric studies are necessary to prove the application of this evolutionary event, because the only morphological intraspecific comparison performed in *T. sordida* was based on geometric morphometry of the head, wings, and pronotum,²⁵ and the only interspecific comparison made was between *T. rosai* and *T. sordida* from Brazil that evaluated morphological and morphometric differences.⁶ Both studies indicate that, possibly, cryptic speciation is not the best model to characterize the evolutionary process associated with *T. sordida*.

Based on this, morphological analyses of thorax and abdomen using Scanning Electron Microscopy (SEM) and morphometric analyses of the head, thorax, and abdomen among *T. sordida* from Brazil, Bolivia, and Paraguay, as well as *T. rosai*, were performed to assess whether the evolutionary process responsible for variations is the cryptic speciation phenomenon.

MATERIALS AND METHODS

Insects. The specimens used (*T. sordida* from Corumbá/MS/Brazil, Apolo/La Paz/Bolivia, and Paraguayan Chaco/Paraguay; *T. rosai* from Department San Miguel/Province of Corrientes/Argentina) were obtained from colonies maintained at the Triatomine Insectarium installed at the Faculty of Pharmaceutical Sciences (FCFAR/UNESP), Araraquara, São Paulo, Brazil, and from insectarium of the Department of Tropical Medicine at the National University of Asunción, Asunción, Paraguay.

Morphological studies in SEM. For morphological characterization of the triatomines in SEM (according to Rosa et al.²⁶), three individuals of each locality were used, emphasizing the study of the pronotum, scutellum, as well as female external genitalia. For this study, the insects were cleaned in ultrasound devices, dehydrated in alcoholic series, dried in an oven at 45° for 20 minutes, and then fixed in small aluminum cylinders with colorless enamel. Afterward, they were metallized by sputtering for 2 minutes with 10mA of power. After the metallization process, the samples were analyzed and photographed on the Topcon SM-300 SEM.

Morphometric studies. For measurement, adult specimens of *T. sordida* from Brazil (nine males and nine females), Bolivia (seven males and 10 females), and Paraguay (three males and three females), and *T. rosai* (seven males and seven females), were used. The measurements of the head, thorax, abdomen, pronotum, and scutellum, were performed using the Leica MZ APO Stereoscope Microscope and the image analysis system Motic Advanced 3.2 plus coupled. The data obtained were organized in the Excel 2007 spreadsheet program and presented as a table. The measurements of the thorax and abdomen parameters were statistically analyzed in the GraphPad Prisma program version 7 for Windows, using the unpaired *t*-test with Welch correction.

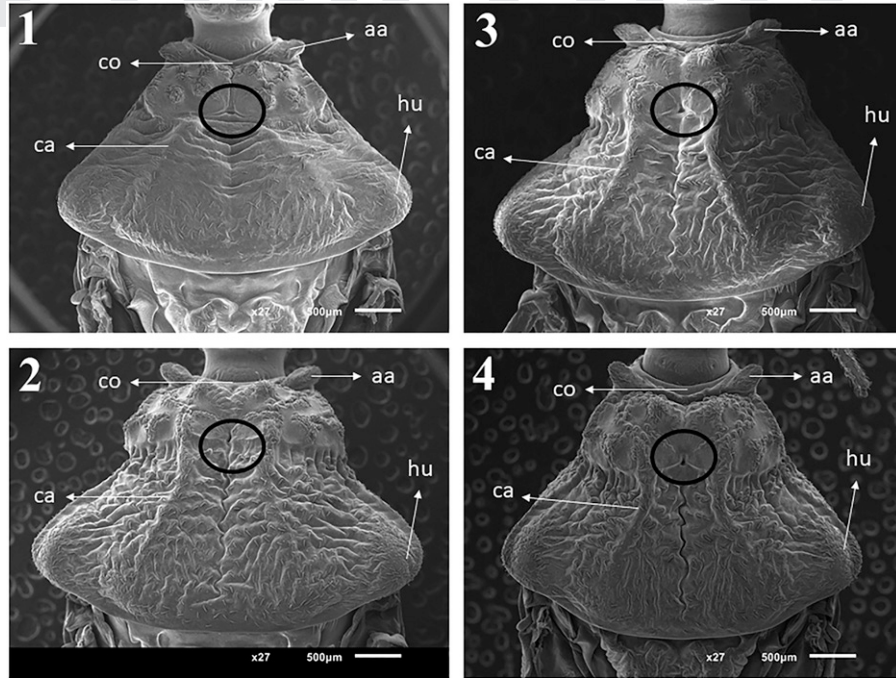
RESULTS

Morphology remarks. *Thorax.* Examining the images in the dorsal position of the thorax of *T. rosai* (Figures 1 and 5),

and *T. sordida* from Bolivia (Figures 2 and 6), Brazil (Figures 3 and 7), and Paraguay (Figures 4 and 8), it was possible to find some differences in the pronotum (Figures 1–4) and scutellum (Figures 5–8). In the pronotum, each specimen presented a format for the anterolateral angle (Figures 1–4, aa): in *T. rosai* (Figure 1, aa) is round and the tip is not tapered; in *T. sordida* from Bolivia and Paraguay (Figures 2 and 4, aa, respectively) is round and long, and the tip is not tapered; in *T. sordida* from Brazil (Figure 3, aa) is oval with tapered ends; the submedian carina is evident in *T. sordida* [Bolivia (Figure 2, ca), Brazil (Figure 3, ca) and Paraguay (Figure 4, ca)], whereas in *T. rosai* it is not very pronounced (Figure 1, ca). The specimens also presented different shapes for the humeral angles (Figures 1–4, hu): in *T. rosai* and *T. sordida* from Paraguay (Figures 1 and 4, hu, respectively) is narrow at the lateral line level of the pronotum; in *T. sordida* from Bolivia and Brazil (Figures 2 and 3, hu, respectively) is wide with a flap aspect. In addition, the specimens showed, by dorsal view of the pronotum, a clear differentiation in median pronotal depression (mpd): in *T. rosai* (Figure 1, circle) has an almost imperceptible or vestigial appearance; *T. sordida* Bolivia (Figure 2, circle) presents little noticeable mpd with a small depth aspect, while in *T. sordida* from Brazil (Figure 3, circle) and Paraguay (Figure 4, circle) it is clear and deeper. In the scutellum (Figures 5–8), the pattern of roughness and striations of its central portion (Figures 5–8, cps), and the length and shape of the scutellum apex (Figures 5–8, ap) is different among the specimens.

Female external genitalia. Differences in the female external genitalia of *T. rosai* (Figures 9, 13, and 17), and *T. sordida* specimens from Bolivia (Figures 10, 14, and 18), Brazil (Figures 11, 15, and 19), and Paraguay (Figures 12, 16, and 20), were found. The analyses of the dorsal view showed that the shape of the eighth segment is trapezoidal in *T. sordida* Bolivia (Figure 10, VIII) and Brazil (Figure 11, VIII), and subtly oval in *T. rosai* (Figure 9, VIII) and *T. sordida* Paraguay (Figure 12, VIII). Besides that, the line that separates the seventh from the eighth segment in the central portion is straight in *T. sordida* from Bolivia (Figure 10) and Brazil (Figure 11), and slightly curved in *T. rosai* (Figure 9) and *T. sordida* Paraguay (Figure 12). Analyses of the posterior view demonstrated that the shape and length of the 10th segment brings an elongated and wide aspect in *T. rosai* (Figure 13, X) and *T. sordida* Bolivia (Figure 14, X) and a short and narrow in *T. sordida* from Brazil (Figure 15, X) and Paraguay (Figure 16, X). Yet, ventral view analyses showed that the limit of the seventh segment along with gonocoxites 8 and gonapophyses 8 is concave on the sides, and convex in the central portion in *T. rosai* (Figure 17, Gc8 and Gp8) and *T. sordida* Brazil (Figure 19, Gc8, and Gp8), while in *T. sordida* Bolivia (Figure 18, VII, Gc8, and Gp8, respectively) the sides are elevated, and the central portion is straight, and in *T. sordida* Paraguay (Figure 20, VII, Gc8, and Gp8, respectively) only the central portion is elevated.

Morphometry. The averages of the head length, inner distance between eyes, postocular head width, pronotum, scutellum, thorax and abdomen are shown in the Table 1. Except for the head length plus neck, all the structures measured (head, thorax, abdomen, pronotum, and scutellum) showed significant differences for at least one comparison between *T. rosai* and *T. sordida* (Brazil, Bolivia, or Paraguay), and among the populations of *T. sordida* (Table 2). In males, approximately 46% of the structures showed significant

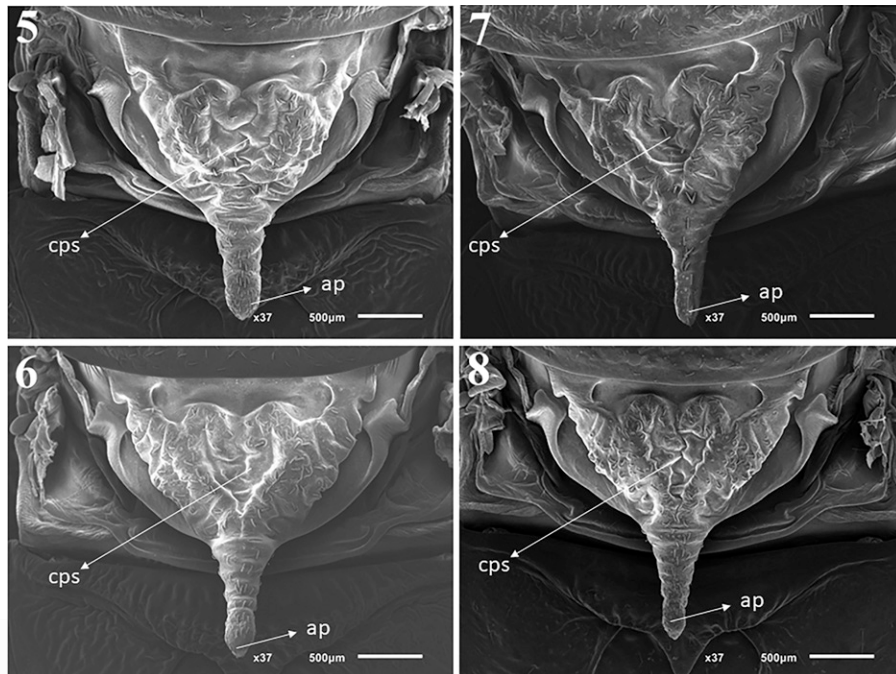


FIGURES 1–4. Details of pronotum: 1. *Triatoma rosai*; 2. *Triatoma sordida* Bolivia; 3. *Triatoma sordida* Brazil and 4. *Triatoma sordida* Paraguay. aa, anterolateral angle; ca, submedian carina; co, collar; hu, humeral angles; and circle, median pronotal depression.

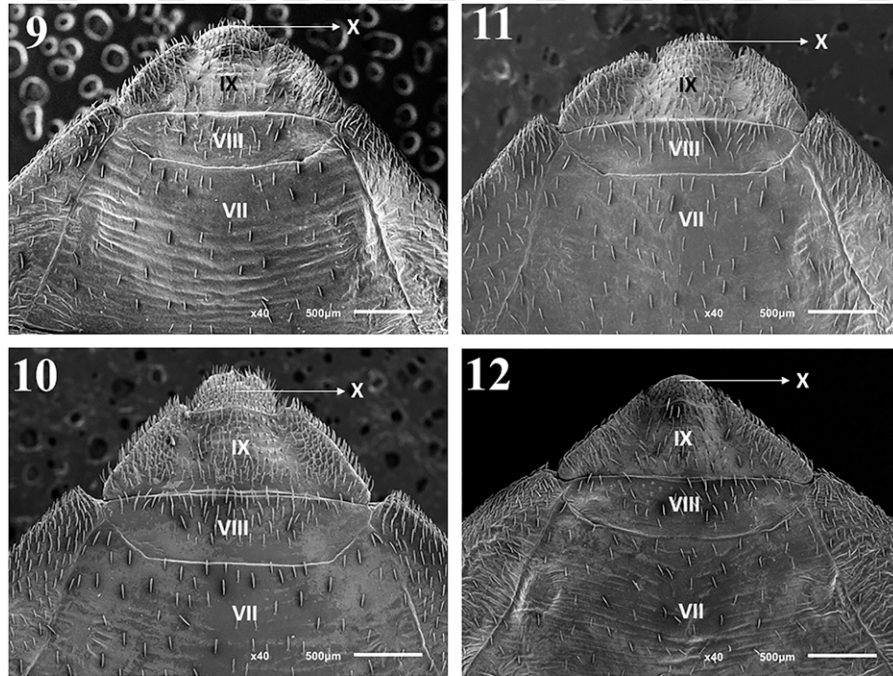
differences, whereas, in females, it was around 19% (Table 2). The decreasing sequence of allometric variation of the structures was: inner distance between eyes (75%) > abdomen (41.6%) > head length, pronotum, scutellum (33.3%) > thorax (25%) > postocular head width (16.6%) > head length plus neck (0%) (Table 2).

DISCUSSION

The terms “cryptic species” and “cryptic speciation” have already been used several times in the Triatominae subfamily.^{21–24,27–34} Nevertheless, as proposed for *T. sordida*,²⁵ the use of these terms possibly occurred subjectively, since interspecific morphological/morphometric differences invalidate



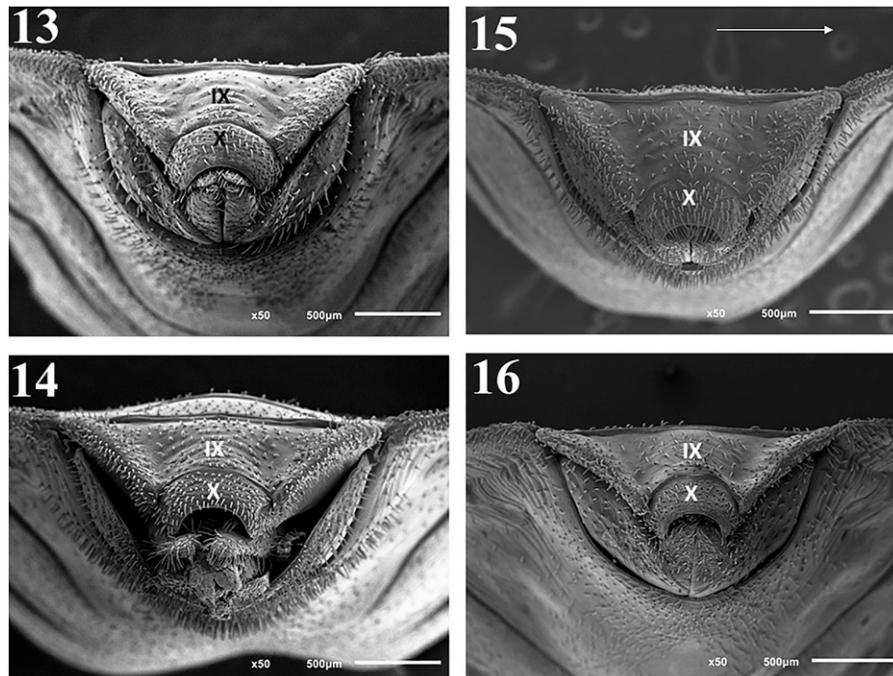
FIGURES 5–8. Details of scutellum: 5. *Triatoma rosai*; 6. *Triatoma sordida* Bolivia; 7. *Triatoma sordida* Brazil; and 8. *Triatoma sordida* Paraguay. ap, scutellum apex; cps, central portion of the scutellum.



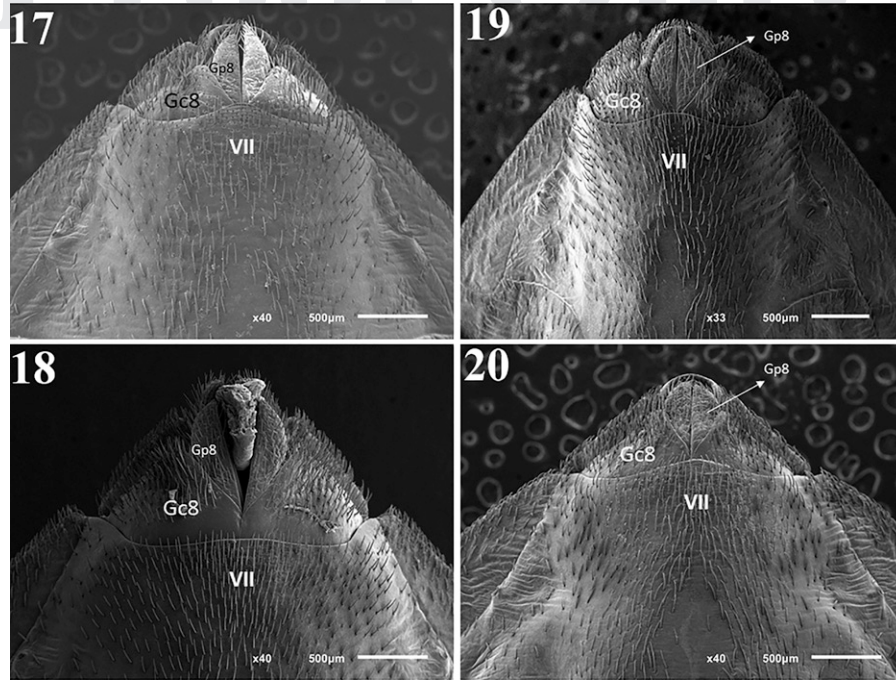
FIGURES 9–12. Details of female external genitalia by dorsal view: 9. *Triatoma rosai*; 10. *Triatoma sordida* Bolivia; 11. *Triatoma sordida* Brazil and 12. *Triatoma sordida* Paraguay. VII: seventh segment; VIII: eighth segment; IX: ninth segment, and X: 10th segment.

the application of these concepts.³⁵ Our results clearly show morphological/morphometric differences between *T. rosai* and *T. sordida* (Brazil, Bolivia, and Paraguay) and between *T. sordida* populations from Brazil, Bolivia, and Paraguay, which confirms that the event that underlies the divergences observed by chromosomal,^{22,23} molecular,²² isoenzymatic,²³ and cuticular hydrocarbon pattern²⁴ studies is not cryptic speciation.

Conceptual problems associated with the terms cryptic species/speciation may lead to a misconceived characterization of new species.³⁶ Struck et al.³⁶ highlighted that the subjective evaluation of morphological similarities and the lack of use of combined approaches also result in problems in the application of these terms. In Triatominae, it is usual to characterize morphologically similar species and/or sister species as cryptic species.^{22,33} It is acceptable that polymorphisms



FIGURES 13–16. Details of female external genitalia by posterior view: 13. *Triatoma rosai*; 14. *Triatoma sordida* Bolivia; 15. *Triatoma sordida* Brazil and 16. *Triatoma sordida* Paraguay. IX: ninth segment, and X: 10th segment.



FIGURES 17–20. Details of female external genitalia by ventral view: 17. *Triatoma rosai*; 18. *Triatoma sordida* Bolivia; 19. *Triatoma sordida* Brazil and 20. *Triatoma sordida* Paraguay. VII: seventh segment; Gc8: gonocoxite; and Gp8: gonapophyse.

(mainly genetic) that support significant differences from the taxonomic point of view lead to the use of the term cryptic species/speciation (as occurred to *T. dimidiata*).^{37,38} However, after further studies and morphological and/or morphometric differences are observed, these terms should not be used to characterize evolutionary processes.

Rhodnius robustus was considered a complex of cryptic species, as genetic differences phylogenetically supported distinguished four possible lineages within *R. robustus* (characterized as lineages I, II, III, and IV).²⁸ Currently, through analyses based on integrative taxonomy (including among them morphological and morphometric analyses), it is known that two of these lineages represent two valid taxa, namely, *R. montenegrensis* (lineage II) and *R. marabaensis* (lineage III),^{26,39–44} not characterizing cryptic speciation in the *R. robustus* complex.

Triatoma pintodiasi is a related species of *T. circummaculata*.³³ Based on the great morphological similarity of these taxa, *T. pintodiasi* was presented as a *T. circummaculata* cryptic species.³³ Yet, when describing *T. pintodiasi*, the authors brought morphometric and morphological differences that

make the application of this term unviable.³³ Likewise, Alevi et al.⁴⁵ analyzed *T. vitticeps* from different Brazilian states and, based on high genetic distance, suggested that *T. vitticeps* is a complex of cryptic species or subspecies. Hence, an application of that term will only be valid in case the new taxon/taxa do not present morphological/morphometric divergence(s).

Morphological,^{31,32} morphometry,²⁹ cytogenetics,²⁷ cuticular hydrocarbons,³⁰ and reproductive isolation³⁴ analyses suggested the existence of *T. dimidiata* cryptic species.^{42,43} However, morphological^{31,32} and morphometric²⁹ divergences observed since 2004 for different *T. dimidiata* populations demonstrated that the term cryptic species/speciation has always been misused.^{27,29,31,32}

Recently, based on integrative taxonomy, two new species closely related to *T. dimidiata* were characterized: *T. mopan* and *T. huehuetanguensis*.^{46,47} Even though the term cryptic species/speciation had already been used several times for *T. dimidiata*,^{27,29,31,32} Dorn et al.⁴⁶ and Lima-Cordón et al.⁴⁷ do not, at any time, use these terms in the description of the new taxa, because morphological and/or morphometric

TABLE 1
Measurement averages of *Triatoma rosai* and *Triatoma sordida* from Bolivia, Brazil, and Paraguay

Specimens	Structures							
	HL	IE	PHW	PRO	SCU	THO	ABD	
Male	<i>T. rosai</i>	5,174	0,951	1,490	4,717	3,077	6,704	15,117
	<i>T. sordida</i> Bolivia	5,254	1,058	1,485	4,457	3,077	6,498	15,202
	<i>T. sordida</i> Brazil	4,857	0,966	1,381	4,107	2,816	6,093	14,084
	<i>T. sordida</i> Paraguay	5,273	1,062	1,463	4,564	3,243	6,622	14,070
Female	<i>T. rosai</i>	5,055	1,003	1,486	4,429	3,215	6,531	16,418
	<i>T. sordida</i> Bolivia	5,417	1,132	1,570	4,603	3,158	6,632	17,118
	<i>T. sordida</i> Brazil	5,208	1,066	1,510	4,221	3,214	6,459	16,167
	<i>T. sordida</i> Paraguay	5,528	1,135	1,610	4,744	3,375	6,842	17,442

HL = head length; IE = inner distance between eyes; PHW = postocular head width; PRO = pronotum; SCU = scutellum; THO = thorax; ABD = abdomen.

Proof Only

TABLE 2

Meaningfulness of the comparative parameters of *Triatoma rosai* and *Triatoma sordida* from Bolivia, Brazil, and Paraguay

Comparison		Structures							
		ABD	HLN	HL	IE	PHW	PRO	SCU	THO
Male	<i>T. s. BRA</i> x <i>T. r.</i>	0.01	0.18	0.01	0.34	0.01	0.0002	0.02	0.003
	<i>T. s. BRA</i> x <i>T. s. PAR</i>	0.49	0.20	0.02	0.05	0.07	0.08	0.0001	0.04
	<i>T. s. BRA</i> x <i>T. s. BOL</i>	0.01	0.11	0.01	0.01	0.01	0.03	0.01	0.05
	<i>T. r. x T. s. PAR</i>	0.004	0.09	0.22	0.04	0.26	0.28	0.05	0.35
	<i>T. r. x T. s. BOL</i>	0.40	0.25	0.21	0.001	0.44	0.06	0.50	0.12
Female	<i>T. s. PAR</i> x <i>T. s. BOL</i>	0.006	0.19	0.45	0.47	0.30	0.35	0.02	0.31
	<i>T. s. BRA</i> x <i>T. r.</i>	0.32	0.21	0.18	0.02	0.3	0.18	0.5	0.39
	<i>T. s. BRA</i> x <i>T. s. PAR</i>	0.18	0.4	0.10	0.05	0.11	0.02	0.22	0.12
	<i>T. s. BRA</i> x <i>T. s. BOL</i>	0.04	0.18	0.10	0.03	0.10	0.05	0.32	0.25
	<i>T. r. x T. s. PAR</i>	0.23	0.23	0.05	0.01	0.07	0.06	0.21	0.15
	<i>T. r. x T. s. BOL</i>	0.40	0.25	0.21	0.001	0.44	0.06	0.50	0.12
	<i>T. s. PAR</i> x <i>T. s. BOL</i>	0.40	0.38	0.30	0.47	0.29	0.22	0.15	0.23

T. s. = *Triatoma sordida*; *T. r.* = *Triatoma rosai*; BRA = Brazil; PAR = Paraguay; BOL = Bolivia; ABD = abdomen; HLN = head length plus neck; HL = head length; IE = inner distance between eyes; PHW = postocular head width; PRO = pronotum; SCU = scutellum; THO = thorax. Bold values indicate statistically significant.

differences were observed among species related to *T. dimidiata* (which, have already been considered as *T. dimidiata*),^{37,38} emphasizing that the authors were based correctly on the application of evolutionary terms.

Panzeria et al.,²² considering the species *T. sordida*, *T. patagonica*, *T. guasayana*, and *T. garciabesi*, suggested cryptic speciation for the *T. sordida* subcomplex proposed by Schofield and Galvão⁴⁸ (currently the *T. sordida* subcomplex is a monophyletic group formed by the species *T. sordida*, *T. rosai*, *T. garciabesi*, *T. jurbergi*, *T. matogrossensis*, and *T. vandae*).^{6,49} However, even if subtle, there are morphological and/or morphometric differences between all studied species.^{24,25,50–53} Besides, our results point out significant morphological/morphometric differences between *T. rosai* and *T. sordida* (Brazil, Bolivia and Paraguay) and between the different *T. sordida* populations from Brazil, Bolivia, and Paraguay, confirming the morphology/morphometry data presented by Alevi et al.⁶ and geometric morphometry data presented by Naterro et al.²⁵ Therefore, once more, the term cryptic speciation does not apply.

Based on this, the evolutionary process that supports, so far, these divergences observed for *T. sordida* populations/*T. sordida* subcomplex is not cryptic speciation. Moreover, we draw attention to the necessity for morphological/morphometric studies to correctly apply the cryptic species/speciation terms in triatomines.

Received March 19, 2021. Accepted for publication May 27, 2021.

Published online September 7, 2021.

Financial support: We appreciate the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process number 2017/05015-7, 2018/12039-2, 2018/24116-1, 2019/17581-2), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), PAHO/HDP/HDR/RG/VEN3 223 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brazil (CAPES)—Finance Code 001 for financial support.

Authors' addresses: Ariane Cristina Caris Garcia, Luiza Maria Grzyb Delgado, and Isadora de Freitas Bittinelli, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Instituto de Biociências de Botucatu, Botucatu, São Paulo, Brazil, E-mails: ariane.garcia@outlook.com, lu.grzyb@gmail.com, and isadora_bittinelli@hotmail.com. Jader de Oliveira, Laboratório de Entomologia em Saúde Pública, Departamento de Epidemiologia, Faculdade de Saúde Pública, Universidade de São Paulo (USP), São Paulo, Brazil, and Laboratório de Parasitologia, Universidade Estadual Paulista "Júlio

de Mesquita Filho" (UNESP), Faculdade de Ciências Farmacêuticas, Araraquara, São Paulo, Brazil, E-mail: jdr.oliveira@hotmail.com. Daniel Cesaretto Cristal and João Aristeu da Rosa, Laboratório de Parasitologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Faculdade de Ciências Farmacêuticas, Araraquara, São Paulo, Brazil, E-mails: daniel.cristal@hotmail.com and joaoaristeu@gmail.com. Cleber Galvão, Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil, E-mail: clebergalvao@gmail.com. Nilsa Elizabeth Gonzalez Britez, Departamento de Medicina Tropical, Instituto de Investigaciones en Ciencias de la Salud (IICS), Universidad Nacional de Asunción, Campus Universitario, San Lorenzo, Paraguay, E-mail: gbritez.nilsa@gmail.com. Hernán José Carrasco, Laboratorio de Biología Molecular de Protozoarios, Facultad de Medicina, Instituto de Medicina Tropical, Universidad Central de Venezuela, Los Chaguaramos, Caracas, Venezuela, E-mail: hjcarrasco@yahoo.com. Kaio Cesar Chaboli Alevi, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Instituto de Biociências de Botucatu, Botucatu, São Paulo, Brazil, and Laboratório de Parasitologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Faculdade de Ciências Farmacêuticas, Araraquara, São Paulo, Brazil, E-mail: kaiochaboli@hotmail.com.

REFERENCES

- World Health Organization, 2020. *Chagas Disease (American trypanosomiasis)*. Geneva, Switzerland: WHO. Available at: <http://www.who.int/chagas/en/>. Accessed March 10, 2021.
- Bern C, 2015. Chagas' disease. *N Engl J Med* 373: 456–466.
- Stanaway JD, Roth G, 2015. The burden of Chagas disease estimates and challenges. *Glob Heart* 10: 139–144.
- Perez-Molina JA, Molina I, 2018. Chagas disease. *Lancet* 391: 82–94.
- Galvão C, 2020. Taxonomia dos Vetores da Doença de Chagas da Forma à Molécula, quase três séculos de história. Oliveira J, Alevi KCC, Camargo LMA, Meneguetti DUO, eds. *Atualidades em Medicina Tropical no Brasil: Vetores*. Acre: editora Athenas, 9–37. Available at: <https://doi.org/10.35170/ss.ed.9786586283129.01>.
- Alevi KCC et al., 2020. *Triatoma rosai* sp. nov. (Hemiptera, Triatominae): a new species of Argentinian Chagas disease vector described based on integrative taxonomy. *Insects* 11: 830.
- Zhao Y, Galvão C, Cai W, 2021. *Rhodnius micki*, a new species of Triatominae (Hemiptera, Reduviidae) from Bolivia. *ZooKeys* 1012: 71–93.
- Galvão C, 2014. Vetores da doença de Chagas no Brasil. *Sociedade Brasileira de Zoologia*. Available at: <https://doi.org/10.7476/9788598203096>.
- Chagas C, 1909. Nova tripanozomíaze humana: estudos sobre a morfologia e o ciclo evolutivo do *Schizotrypanum cruzi* n. gen., n.

- sp., agente etiológico de nova entidade morbida do homem. *Mem Inst Oswaldo Cruz* 1: 159–218.
10. Lent H, Wygodzinsky P, 1979. Revision of the Triatominae (Hemiptera—Reduviidae) and their significance as vectors of Chagas' disease. *Bull Am Mus Nat Hist* 163: 123–520.
 11. Forattini OP, Ferreira OA, Rocha e Silva EOR, Rabello EX, 1975. Aspectos ecológicos da tripanossomíase americana. VII—Permanência e mobilidade do *Triatoma sordida* em relação aos ecótopos artificiais. *Rev Saude Publica* 9: 467–476.
 12. Noireau F, Gutierrez T, Flores R, Breniere F, Bosseno MF, Wisnivesky-Colli C, 1999. Ecogenetics of *Triatoma sordida* and *Triatoma guasayana* (Hemiptera: Reduviidae) in the Bolivian Chaco. *Mem Inst Oswaldo Cruz* 94: 451–457.
 13. Castro GB, Machado EMM, Borges EC, Lorosa ES, Andrade RE, Diotaiuti L, Azeredo BVM, 1997. *Trypanosoma cruzi* peridomiciliar transmission by *Triatoma sordida* in the municipality of Patis, Gerais State, Brazil. *Mem Inst Oswaldo Cruz* 92: 434.
 14. Diotaiuti L, Azeredo BVM, Busek SCU, Fernandes AJ, 1998. Controle do *Triatoma sordida* no peridomicílio rural do município de Porteirinha, Minas Gerais, Brasil. *Pan American Journal of Public Health* 3: 21–25.
 15. Lorosa ES, Andrade RE, Santos SM, Pereira CA, 1998. Estudo da infecção natural e da fonte alimentar do *Triatoma sordida* (Stål, 1859), (Hemiptera - Reduviidae) na região norte de Minas Gerais, Brasil, através da reação de precipitina. *Entomol Vectores* 5: 13–22.
 16. Breniere SF, Waleckx E, Barnabé C, 2016. Over six thousand *Trypanosoma cruzi* strains classified into discrete typing units (DTUs): attempt at an inventory. *PLoS Negl Trop Dis* 10: e0004792.
 17. Rossi JCN, Duarte EC, Gurgel-Gonçalves R, 2015. Factors associated with the occurrence of *Triatoma sordida* (Hemiptera: Reduviidae) in rural localities of central-west Brazil. *Mem Inst Oswaldo Cruz* 110: 192–200.
 18. Alevi KCC, Garcia ACC, Oliveira J, Rosa JA, 2020. Resgatando dados ecológicos, biológicos, epidemiológicos, taxonômicos e sistemáticos de *Triatoma sordida* (Stål, 1859) (Hemiptera, Triatominae). *Atualidades em Medicina Tropical no Brasil. Vetores* 1: 122–136.
 19. Gurgel-Gonçalves R, Ferreira JBC, Rosa AF, Bar ME, Galvão C, 2011. Geometric morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species. *Med Vet Entomol* 25: 84–93.
 20. Crocco LB, Catalá SS, 1996. Feeding and defaecation patterns in *Triatoma sordida*. *Mem Inst Oswaldo Cruz* 91: 409–413.
 21. Noireau F, Gutierrez T, Zegarra M, Flores R, Breniere F, Cardozo L, 1998. Cryptic speciation in *Triatoma sordida* (Hemiptera: Reduviidae) from the Bolivian Chaco. *Trop Med Int Health* 3: 364–372.
 22. Panzera F, Pita S, Nattero J, Panzera Y, Galvão C, Chavez T, 2015. Cryptic speciation in the *Triatoma sordida* subcomplex (Hemiptera, Reduviidae) revealed by chromosomal markers. *Parasit Vectors* 8: 495–504.
 23. Panzera F, Hornos S, Pereira J, Cestau R, Canale D, Diotaiuti L, Dujardin JP, Perez R, 1997. Genetic variability and geographic differentiation among three species of triatomine bugs (Hemiptera-Reduviidae). *Am J Trop Med Hyg* 57: 732–739.
 24. Calderón-Fernández GM, Juárez MP, 2013. The cuticular hydrocarbons of the *Triatoma sordida* species subcomplex (Hemiptera: Reduviidae). *Mem Inst Oswaldo Cruz* 108: 778–784.
 25. Nattero J, Piccinalli RM, Lopes CM, Hernandez ML, Abraham L, Lobbia PA, Rodríguez CS, Carbajal-de-la-Fuente NA, 2017. Morphometric variability among the species of the Sordida subcomplex (Hemiptera: Reduviidae: Triatominae): evidence for differentiation across the distribution range of *Triatoma sordida*. *Parasit Vectors* 10: 412.
 26. Rosa JA et al., 2012. Description of *Rhodnius montenegrensis* n. sp. (Hemiptera, Reduviidae: Triatominae) from the state of Rondônia, Brazil. *Zootaxa* 3478: 62–76.
 27. Panzera F et al., 2006. Chromosomal variation and genome size support existence of cryptic species of *Triatoma dimidiata* with different epidemiological importance as Chagas disease vectors. *Trop Med Int Health* 11: 1092–1103.
 28. Monteiro FA, Wesson DM, Dotson EM, Schofield CJ, Beard CB, 2000. Phylogeny and molecular taxonomy of the *Rhodniini* derived from mitochondrial and nuclear DNA sequences. *Am J Trop Med Hyg* 62: 460–465.
 29. Bustamante DM, Monroy C, Menes M, Rodas A, Salazar-Schettino PM, Rojas G, Pinto N, Guhl F, Dujardin JP, 2004. Metric variation among geographic populations of the Chagas vector *Triatoma dimidiata* (Hemiptera: Reduviidae: Triatominae) and related species. *J Med Entomol* 41: 296–301.
 30. Calderón-Fernández G, Juárez MP, Ramsey J, Salazar Schettino PM, Monroy MC, Ordóñez R, Cabrera M, 2005. Cuticular hydrocarbon variability among *Triatoma dimidiata* (Hemiptera: Reduviidae) populations from Mexico and Guatemala. *J Med Entomol* 42: 780–788.
 31. Catalá S, Sachetto C, Moreno M, Rosales R, Salazar-Schettino PM, Gorla D, 2005. Antennal phenotype of *Triatoma dimidiata* populations and its relationship with species of phyllosoma and protracta complexes. *J Med Entomol* 42: 719–725.
 32. Jurberg J, Noireau F, Galvão C, Carcavallo RU, Rocha DS, Lent H, 2005. Uma iconografia dos Triatomíneos. *Entomol Vectores* 11: 457–494.
 33. Jurberg J, Cunha V, Cailleaux S, Raigorodski R, Lima MS, Rocha DS, Moreira FFF, 2013. *Triatoma pintodiasi* sp. nov. do sub-complexo *T. rubrovaria* (Hemiptera, Reduviidae, Triatominae). *Rev Panamazonica Saude* 4: 43–56.
 34. García M, Menes M, Dorn PL, Monroy C, Richards B, Panzera F, Bustamante DM, 2013. Reproductive isolation revealed in preliminary crossbreeding experiments using field collected *Triatoma dimidiata* (Hemiptera: Reduviidae) from three ITS-2 defined groups. *Acta Trop* 128: 714–718.
 35. Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I, 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol Evol* 22: 148–155.
 36. Struck TH et al., 2018. Fiding evolutionary processes hidden un cryptic species. *Trends Ecol Evol* 33: 153–163.
 37. Dorn PL, Calderon C, Melgar S, Moguel B, Solorzano E, Dumonteil E, Rodas A, de la Rúa N, Garnica R, Monroy C, 2009. Two distinct *Triatoma dimidiata* (Latreille, 1811) taxa are found in sympatry in Guatemala and Mexico. *PLoS Negl Trop Dis* 3: e393.
 38. Dorn PL et al., 2016. Hypothesis testing clarifies the systematics of the main central American Chagas disease vector, *Triatoma dimidiata* (Latreille, 1811), across its geographic range. *Infect Genet Evol* 44: 431–443.
 39. Souza ES, Atzinger NCBV, Furtado MB, Oliveira J, Dameli JN, Vendramini DP, Gardim S, Rosa JA, 2016. Description of *Rhodnius marabaensis* sp. N. (Hemiptera: Reduviidae: Triatominae) from Pará State, Brazil. *ZooKeys* 621: 45–62.
 40. Carvalho DB, Congrains C, Chahad-Ehlers S, Pinotti H, Brito RA, Rosa JA, 2017. Differential transcriptome analysis supports *Rhodnius montenegrensis* and *Rhodnius robustus* (Hemiptera, Triatominae) as distinct species. *PLOS ONE* 12: e0174997.
 41. Monteiro FA, Weirauch C, Felix M, Lazoski C, Abad-Franch F, 2018. Evolution, systematics, and biogeography of the Triatominae, vectors of Chagas disease. *Adv Parasitol* 99: 265–344.
 42. Brito RN, Geraldo JA, Monteiro FA, Lazaoski C, Souza RCM, Abad-Franch F, 2019. Transcriptome-based molecular systematics: *Rhodnius montenegrensis* (Triatominae) and its position within the *Rhodnius prolixus-Rhodnius robustus* cryptics-species complex. *Parasit Vectors* 12: 305.
 43. Castro MRJ, Goubert C, Carareto CMA, Monteiro FA, Vieira C, 2020. Homology-free detection of transposable elements unveils their dynamics in three ecologically distinct *Rhodnius* species. *Genes (Basel)* 11: 170.
 44. Ravazi A, Oliveira J, Alevi KCC, 2020. Taxonomia e sistemática da tribo Rhodniini (Hemiptera, Triatominae): uma mini-revisão. *Atualidades em Medicina Tropical no Brasil. Vetores* 1: 38–48.
 45. Alevi KCC, Garcia ACC, Guerra AL, Moreira FFF, Oliveira J, Rosa JA, Azeredo-Oliveira MTV, 2018. *Triatoma vitticeps* (Stål, 1859) (Hemiptera, Triatominae): a Chagas disease vector or a complex of vectors? *Am J Trop Med Hyg* 99: 954–956.
 46. Dorn PL, Just AS, Dale C, Stevens L, Galvão C, Cordon RL, Monroy C, 2018. Description of *Triatoma mopan* sp. n. (Hemiptera, Reduviidae, Triatominae) from a cave in Belize. *ZooKeys* 775: 69–95.

47. Lima-Cordón RA, Monroy MC, Stevens L, Rodas A, Rodas GA, Dorn PL, Justi AS, 2019. Description of *Triatoma huehuetenanguensis* sp. n., a potential Chagas disease vector (Hemiptera, Reduviidae, Triatominae). *ZooKeys* 820: 51–70.
48. Schofield CJ, Galvão C, 2009. Classification, evolution, and species groups within the Triatominae. *Acta Trop* 110: 88–100.
49. Belintani T, Oliveira J, Pinotti H, Silva LA, Alevi KCC, Galvão C, Rosa JA, 2020. Phylogenetic and phenotypic relationships of the *Triatoma sordida* subcomplex (Hemiptera: Reduviidae: Triatominae). *Acta Trop* 2012: 105679.
50. Carcavallo RU, Cichero JA, Martínez A, Prosen AF, Ronderos R, 1967. Una nueva especie del género *Triatoma* Laporte (Hemiptera, Reduviidae, Triatominae). *Jornadas Entomoepidemiológicas Argentinas* 2: 43–48.
51. Jurberg J, Galvão C, Lent H, Monteiro F, Lopes CM, Panzera F, Perez R, 1998. Revalidação de *Triatoma garciabesi* Carcavallo, Cichero, Martínez, Prosen & Ronderos, 1967 (Hemiptera: Reduviidae). *Entomol Vectores* 5: 107–122.
52. Gurgel-Gonçalves R, Ferreira JBC, Rosa AF, Bar ME, Galvão C, 2011. Geometric morphometrics and ecological niche modelling for delimitation of near-sibling triatomine species. *Med Vet Entomol* 25: 84–93.
53. González-Britez NE, Carrasco HJ, Martínez Purroy CE, Feliciangeli MD, Maldonado M, López E, Segovia M, Rojas de Arias A, 2014. Genetic and morphometric structures of *Triatoma sordida* (Hemiptera: Reduviidae) from the eastern and western regions of Paraguay. *Front Public Health* 2: 149.