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Especiação x Especiação críptica: o caso de *Triatoma sordida* (Stål, 1859) (Hemiptera, Triatominae)

Botucatu

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Zoologia) junto ao Programa de Pós-Graduação em Ciências Biológicas (Zoologia) do Instituto de Biociências de Botucatu, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu.

Orientador: Prof. Dr. Kaio Cesar Chaboli Alevi

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“Não basta ter belos sonhos para realizá-los. Mas ninguém realiza grandes obras se não for capaz de sonhar grande. Podemos mudar o nosso destino, se nos dedicarmos à luta para a realização de nosso ideal.”

Vladimir Ilyich Ulianov (Lenin)

Sumário

Resumo

Triatoma sordida é uma espécie endêmica da América do Sul, distribuída no Brasil, na Argentina, na Bolívia, no Paraguai e no Uruguai. Diversos estudos (cromossômicos, meióticos, isoenzimáticos e moleculares) sinalizaram polimorfismo e sugeriram que tenha ocorrido o fenômeno de especiação críptica em *T. sordida*, destacando que existem, pelo menos, duas possíveis espécies diferentes associadas à *T. sordida sensu stricto*, a saber, *T. sordida* Argentina (descrito recentemente como *T. rosai*) e *T. sordida* La Paz. Levando em consideração que o conceito de especiação críptica está pautado em espécies morfologicamente indistinguíveis que foram classificadas dentro de apenas um táxon nominal e, principalmente, que existem vários erros associados com a caracterização de espécies crípticas, a avaliação subjetiva das similaridades morfológicas e a falta de utilização de abordagens combinadas pode resultam na caracterização errônea desses táxons. Embora os estudos assumam que o processo de especiação que suporta a diferenciação de espécies dentro de *T. sordida* seja resultado da especiação críptica, estudos morfológicos são necessários para comprovar esse evento evolutivo, pois a única comparação morfológica entre *T. sordida* do Brasil, da Bolívia e da Argentina (*T. rosai*) foi realizada a partir da morfometria geométrica da asa. Diante disso, a presente dissertação teve como objetivo investigar a diversidade e descrever as características fenotípicas de *T. sordida* proveniente da Bolívia, Brasil, Paraguai e *T. rosai* (populações anteriormente consideradas como *T. sordida* da Argentina). Análises morfológicas e morfométricas foram realizadas e diferenças morfológicas no tórax e na genitália externa feminina, bem como diferenças morfométricas nas estruturas do tórax, abdômen, pronoto, escutelo e da cabeça foram observadas entre as populações alopátricas de *T. sordida* e *T. rosai*. Com base no exposto, fica evidente que o processo evolutivo que sustenta, até o momento, as divergências observadas para *T. sordida* não se fundamenta em especiação críptica.

Palavras-chave: Triatomíneos; Morfologia; Morfometria; Doença de Chagas

Abstract

Triatoma sordida is an endemic species in South America, distributed in Brazil, Argentina, Bolivia, Paraguay, and Uruguay. Chromosomal, molecular, isoenzimatic, and cuticular hydrocarbon pattern studies indicated polymorphisms and suggested cryptic speciation in *T. sordida,* highlighting that there are at least two possible different species associated with *T. sordida sensu stricto*, named *T. sordida* Argentina (recently described as *T. rosai*) and *T. sordida* La Paz. Considering the concept of cryptic species/speciation, based on morphologically indistinguishable species classified within only a nominal taxon, and the many mistakes associated with cryptic species characterization, the subjective evaluation of morphological similarities and lack of combined approaches may lead to the wrong characterization of these taxa. Although all authors assume that the speciation process supporting these differentiations 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, since the only comparison made among *T. sordida* from Brazil, Bolivia, and Argentina (*T. rosai*) analyzed the wing morphometric geometry. Based on this, the present dissertation aimed to describe phenotypical characteristics of *T. sordida* from Brazil, Bolivia, Paraguay and *T. rosai* (populations previously considered to be *T. sordida* from Argentina) to assess if the cryptic speciation is indeed the evolutionary event responsible for the possible new taxa differentiation associated with *T. sordida*. Morphological and morphometric analyses were performed and morphological differences in the thorax and female external, as well as morphometric differences in the head, thorax, abdomen, pronotum, and scutellum structures, were observed among the allopatric populations of *T. sordida* and *T. rosai*. Based on this, is evident that the evolutionary process that supports, so far, the divergences observed for *T. sordida* is not cryptic speciation.

Keywords: Chagas disease vector, triatomines, morphology, morphometry, evolution

1. INTRODUÇÃO

Os triatomíneos (Hemiptera, Triatominae) são insetos hematófagos de grande importância para a saúde pública, pois são considerados como a principal forma de transmissão do protozoário *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), agente etiológico da doença de Chagas (WHO, 2019), a qual atinge cerca de oito milhões de pessoas e coloca em risco de infecção, aproximadamente, 25 milhões de pessoas no mundo, sendo o tratamento com os anti-tripanosomatídeos (Benznidazol e Nifurtimox) efetivos apenas na fase aguda da doença (WHO, 2019).

Atualmente, existem 156 espécies descritas na subfamília Triatominae (sendo 153 espécies vivas e três espécies fósseis), agrupadas em 18 gêneros e cinco tribos (ALEVI et al., 2020; GALVÃO, 2020; ZHAO et al., 2021). Embora existam espécies com maior ou menor grau de importância na transmissão da doença de Chagas [com destaque para *Triatoma infestans* (Klug, 1834), *Panstrongylus megistus* (Burmeister, 1835), *T. brasiliensis* (Neiva, 1911), *T. pseudomaculata* (Corrêa & Espínola, 1964) e *T. sordida* (Stål, 1859), que apresentam maiores competências vetoriais no Brasil (GALVÃO, 2014)], todos os triatomíneos, de ambos os sexos e em qualquer fase do desenvolvimento após a eclosão (N1, N2, N3, N4, N5 e adultos), são considerados como potenciais vetores dessa doença.

Triatoma sordida é uma espécie endêmica da América do Sul, distribuída no Brasil, na Argentina, na Bolívia, no Paraguai e no Uruguai (GALVÃO, 2014). Embora seja considerada predominantemente peridomiciliar, apresenta capacidade de colonizar domicílios e formar colônias intradomiciliares, o que pode ser potencializado pela capacidade de dispersão (DANTAS et al., 2018), destacando-a entre as prioridades para campanhas de controle de vetores no Brasil, principalmente em áreas de ocupação agrícola nos estados de São Paulo, Minas Gerais e Goiás em que os índices de captura de *T. sordida*

têm preocupado as autoridades de saúde (DIOAIUTI et al. 1995; SILVA et al. 2005; OLIVEIRA e SILVA 2007).

Essa espécie, em conjunto com *T. patagonica* (Del Ponte, 1929), *T. guasayana* (Wygodzinsky & Abalos, 1949) e *T. garciabesi* (Carcavallo et al. 1967), foi agrupada no subcomplexo *T. sordida* (SCHOFIELD e GALVÃO, 2009). Contudo, diversos estudos sinalizam polimorfismo em *T. sordida*: Panzera et al. (1997) observaram variação cromossômica para o padrão de heterocromatina constitutiva e no comportamento meiótico para *T. sordida* proveniente do Brasil e da Argentina; Noireau et al. (1998), com base em análises isoenzimáticas relataram alta distancia genética e sugeriram especiação críptica para *T. sordida* de diferentes localidades da Bolívia; Panzera et al. (2015), com base em dados cromossômicos (padrão de heterocromatina constitutiva e marcação por FISH com sondas de DNAr 45S) e moleculares (gene mitocondrial COI) também apontam para o fenômeno de especiação críptica em *T. sordida*, destacando que existem, pelo menos, duas possíveis espécies diferentes associadas a *T. sordida* sensu stricto, a saber, *T. sordida* Argentina e *T. sordida* La Paz. Alevi et al. (2020), com base em estudos de taxonomia integrativa, descreveram *T. rosai* Alevi et al., 2020 a partir de *T. sordida* Argentina.

O conceito de especiação críptica está pautado em espécies morfologicamente indistinguíveis (pelo menos, superficialmente) que foram classificadas dentro de apenas um táxon nominal (BICKFORD et al., 2007). Esse conceito foi erroneamente considerado como sinônimo de espécies irmãs, as quais conotam apenas ancestralidade comum e não necessariamente relação morfológica (SAEZ e LOZANO, 2005; BICKFORD et al., 2007). Além disso, espécie críptica também foi associada de forma errada com eventos recentes de especiação que não resultaram na fixação de diferenças morfológicas (KNOWLTON, 1993; GUSTAFSSON et al., 2014; STRUCK et al., 2018).

Espécies crípticas, de forma geral, são espécies pouco diferenciadas do ponto de

vista morfológico que, embora representem linhagens evolutivas distintas (isoladas reprodutivamente), são historicamente interpretadas como membros de uma única espécie (BICKFORD et al., 2007). O uso inadequado do termo “espécie críptica” gera ambiguidade taxonômica e evolutiva (STRUCK et al., 2018). O conceito mais utilizado [descrito por Bickford et al. (2007)] é considerado insatisfatório por apresentar problemas relacionados com questões biológicas ou taxonômicas que podem acarretar na caraterização equivocada de novas espécies (STRUCK et al., 2018). Além disso, Struck et al. (2018) destacam que a avaliação subjetiva das similaridades morfológicas e a falta de utilização de abordagens combinadas (muitos estudos caracterizaram esse evento evolutivo com base em apenas um marcador molecular) também resultam em problemas na caracterização de espécies críptica, pois alguns estudos assumem que nos estágios iniciais da especiação as características fisiológicas, imunológicas, reprodutivas ou comportamentais são as que atuam na diferenciação das espécies (BENSCH et al., 2004; DAMM et al., 2010; DERYCKE et al., 2016).

Do ponto de vista cromossômico, molecular, izoenzimático e pelo padrão de hidrocarbonetos cuticulares, *T. sordida* se diferencia em mais de um táxon (PANZERA et al., 1997, 2015; NOIREAU et al., 1998; CALDERÓN-FERNÁNDEZ e JUÁREZ, 2013; ALEVI et al., 2020). Contudo, embora todos os autores assumam que o processo de especiação que suporta essa diferenciação de espécies dentro de *T. sordida* seja resultado da especiação críptica, estudos morfológicos são necessários para comprovar esse evento evolutivo, pois a única comparação morfológica realizada em *T. sordida* proveniente de diferentes países (Brasil, Bolívia e Argentina) foi realizada por Nattero et al. (2017) que diferenciaram a população de *T. sordida* da Argentina [descrita como *T. rosai* (ALEVI et al., 2020)] dos exemplares provenientes do Brasil e da Bolívia a partir de dados morfométrico da asa e ressaltam a importância de estudos adicionais para confirmar a

validade específica do(s) novo(s) táxon(s). Além disso, com base na diferenciação fenotípica de *T. rosai* (caracterizada no manuscrito como *T. sordida* da Argentina) quando compara as outras localidades de *T. sordida*, Nattero et al. (2017) sugerem que a(s) nova(s) espécie(s) putativa de *T. sordida* pode(m) não ser críptica(s).

2. OBJETIVOS

2.1 Objetivo geral

Investigar e descrever a diversidade de *T. sordida* ao longo de sua distribuição geográfica na Bolívia, Brasil, Paraguai e Argentina (*T. rosai*), por meio de análises morfológicas e morfométricas.

2.2 Objetivos específicos

a) Analisar a morfologia comparativa do tórax e do abdômen, bem como da genitália feminina de *T. sordida* da Bolívia, Brasil, Paraguai e Argentina (*T. rosai*);

b) Avaliar os aspectos morfométricos comparativos da cabeça, do tórax e do abdômen de *T. sordida* da Bolívia, Brasil, Paraguai e Argentina (*T. rosai*).

3. RESULTADOS (apresentados na forma de artigo científico)

3.1 Artigo científico submetido para publicação na revista internacional *BMC Evolutionary Biology* (FI 3)

Triatoma sordida (Stål, 1859) (Hemiptera, Triatominae): Speciation or Cryptic speciation?

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Abstract

Background: *Triatoma sordida* is an endemic species in South America, distributed in Brazil, Argentina, Bolivia, Paraguay, and Uruguay which is important from the epidemiological point of view. The Triatominae evolutionary processes were associated with disruptive evolution, allopatry, homoploid hybridization and cryptic speciation. The concept of cryptic species/speciation is broadly based on morphologically indistinguishable species (at least superficially) classified within only a nominal taxon. Chromosomal, molecular, isoenzimatic, and cuticular hydrocarbon pattern studies indicate cryptic speciation in *T. sordida*. Although all authors assume that the speciation process supporting these differentiations 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 [as in case of phenotypic differences were observed, the concept of cryptic species/speciation does not apply]. Based on this, morphological analyzes of thorax and abdomen using scanning electron microscopy and morphometric analyzes of the head, thorax, and abdomen in *T. sordida* from Brazil, Bolivia, Paraguay, and Argentina (recently described as *T. rosai*) were performed to assess whether the evolutionary process responsible for these variations is cryptic speciation. **Results:** 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. **Conclusions:** Based on this, the evolutionary process that supports the, so far, the divergences observed for *T. sordida* is not cryptic speciation. Thus, we draw attention to the necessity for morphological/morphometric studies to correctly apply the cryptic species/speciation terms in triatomines.

Keywords: Chagas disease vector, triatomines, morphology, morphometry, evolution

Background

Chagas disease is a neglected disease caused by the *Trypanosoma cruzi* (Chagas, 1909) (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 to 40% of chronic chagasic patients) [4].

Currently, there are 154 described species of triatomines [5]. Although there are species with a greater or lesser degree of importance in the transmission of the Chagas disease (highlighting *Triatoma infestans* (Klug, 1834), *Panstrongylus megistus* (Burmeister, 1835), *T. brasiliensis* Neiva, 1911, *T. pseudomaculata* Corrêa & Espínola, 1964 and *T. sordida* (Stål, 1859) that have greater vector skills in Brazil [6]), 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 [7,8].

Triatoma sordida is an endemic species in South America, distributed in Brazil, Argentina, Bolivia, Paraguay, and Uruguay [6]. Due to the high dissemination capacity of *T. sordida* [9], which inhabits sylvatic, peridomestic and domestic environments [8,10], and presents several food sources [11-16], this species has an appraisable ecological value [58]. Broadly, infection rates by *T. cruzi* are relatively low, ranging from 0.5% to 16.2% [11-16], although Lorosa et al. [17] observed an elevated rate of natural infection (41.9%) in specimens that mainly fed on opossums and/or rodents. This information associated with the habit of nymphs and adults of *T. sordida* to defecate during the blood meal [18], highlights the vectorial importance of this species in the transmission of the Chagas disease [16].

The Triatominae evolutionary processes initially were guided by disruptive evolution [19]. Furthermore, it is known that allopatric events, such as dispersion and/or vicariance, were important to the Triatominae speciation since they allowed diversification of the triatomine species [20]. However, homoploid hybridization events and cryptic speciation have also been associated with the differentiation of some taxa [21-34].

The concept of cryptic species/speciation, based on morphologically indistinguishable species (at least superficially) classified within only a nominal taxon [35], was mistakenly considered a synonymous of sister species, which connotes a common ancestry, and not necessarily morphological relationship [35,36]. Besides, the term cryptic species was wrongly associated to recent speciation events that have not resulted in the fixation of morphological differences [37-39], which can result in taxonomic and evolutionary ambiguity [39].

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. [24] pointed out that the authors performed an interspecific comparison between *T. sordida* and *T. garciabesi* [40], which resulted in an elevated genetic distance. Taking into account this initial hypothesis associated with cryptic speciation in *T. sordida*, chromosomal, molecular, isoenzimatic 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 [22,24,27]. Alevi et al. [59], based on studies of integrative taxonomy, described *T. rosai* Alevi et al., 2020 from *T. sordida* Argentina.

Although all authors assume that the speciation process which supports this differentiation in *T. sordida* is the result of cryptic speciation [21,22,24,27], further morphological and/or morphometric studies are necessary to prove the application of this evolutionary event (as in case of phenotypic differences were observed, the concept of cryptic

species/speciation does not apply [35]) since the only morphological comparison performed in *T. sordida* from different countries (Brazil, Bolivia, and Argentina) is based on geometric morphometry of the head, wings, and pronotum recently performed by Nattero et al. [41], and suggests 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 in *T. sordida* from Brazil, Bolivia, Paraguay, and *T. rosai* (previously considered as *T. sordida* from Argentina) were performed to assess whether the evolutionary process responsible for variations is the cryptic speciation phenomenon.

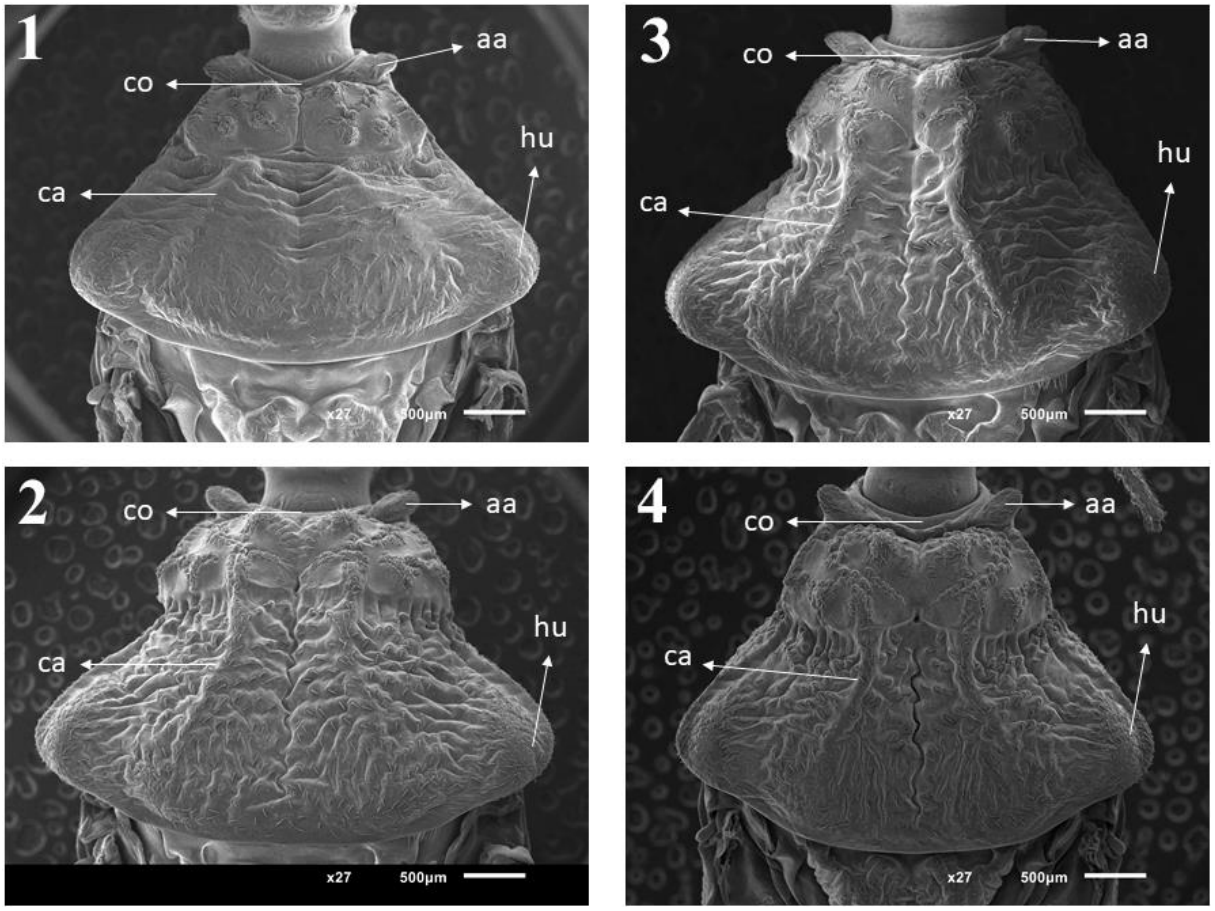
Results

Morphology remarks

Thorax

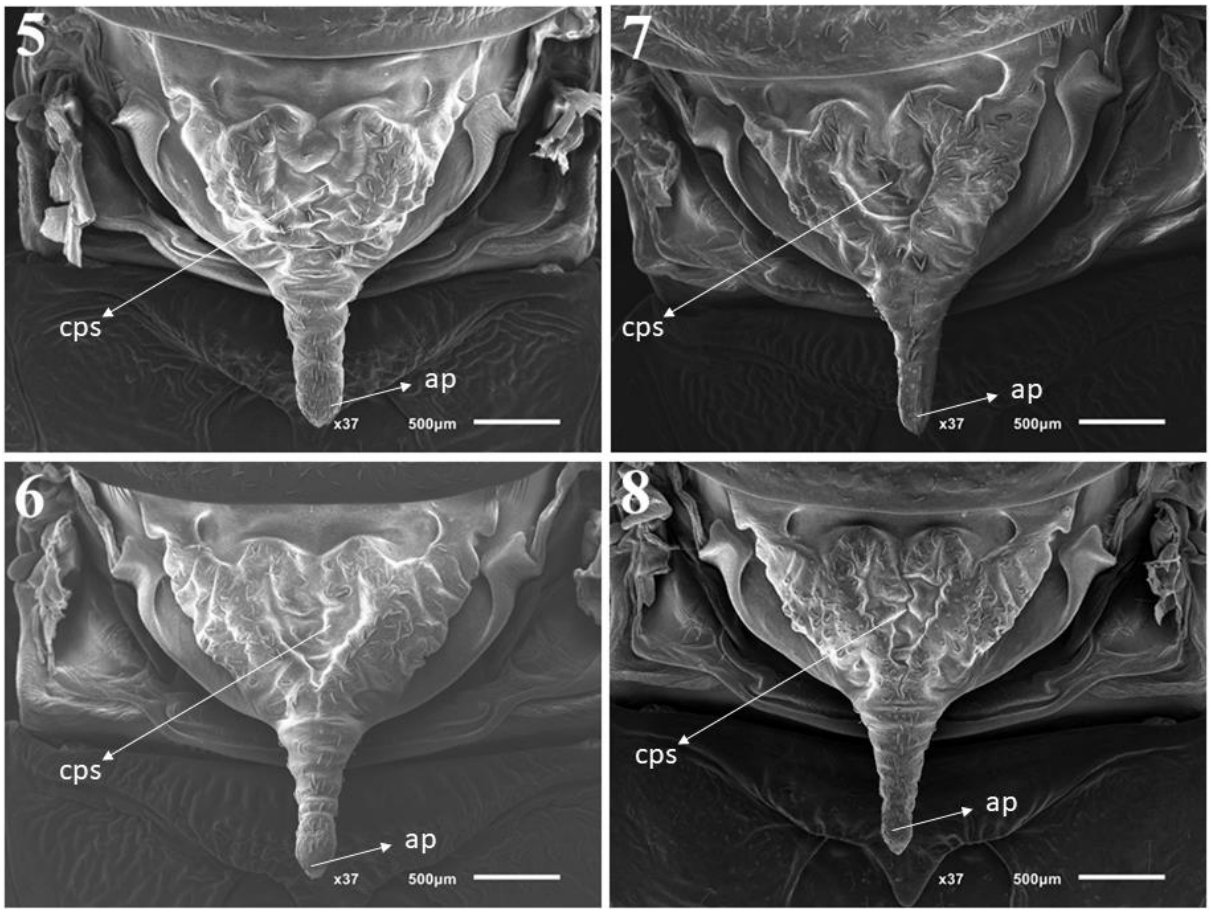
Examining the images in the dorsal position of the thorax of *T. rosai* (Figures 1 and 5), Bolivia (Figures 2 and 6), Brazil (Figures 3 and 7), and Paraguay (Figures 4 and 8), it was possible to find some differences in the pronotum (Figures 1-4) and scutellum (Figures 5-8). In the pronotum, each specimen presented a format for the anterolateral angle (Figures 1-4, aa): Argentina (*T. rosai*) (Figure 1, aa) is round and the tip is not tapered; Bolivia and Paraguay (Figures 2 and 4, aa, respectively) is round and long, and the tip is not tapered; Brazil (Figure 3, aa) is oval with tapered ends; The submedian carina is evident in Bolivia (Figure 2, ca), Brazil (Figure 3, ca) and Paraguay (Figure 4, ca), whereas in Argentina (*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 Argentina (*T. rosai*) and Paraguay (Figures 1 and 4, hu, respectively) is narrow at the lateral line level of the pronotum; in 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): *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 populations.



Figures 1-4. Details of pronotum of *T. rosai* and *T. sordida*: 1. *T. rosai* (Argentina); 2. *T. sordida* (Bolivia); 3. *T. sordida* (Brazil) and 4. *T. sordida* (Paraguay). aa: anterolateral angle, ca: submedian carina, co: collar, hu: humeral angles and circle: median pronotal

depression.

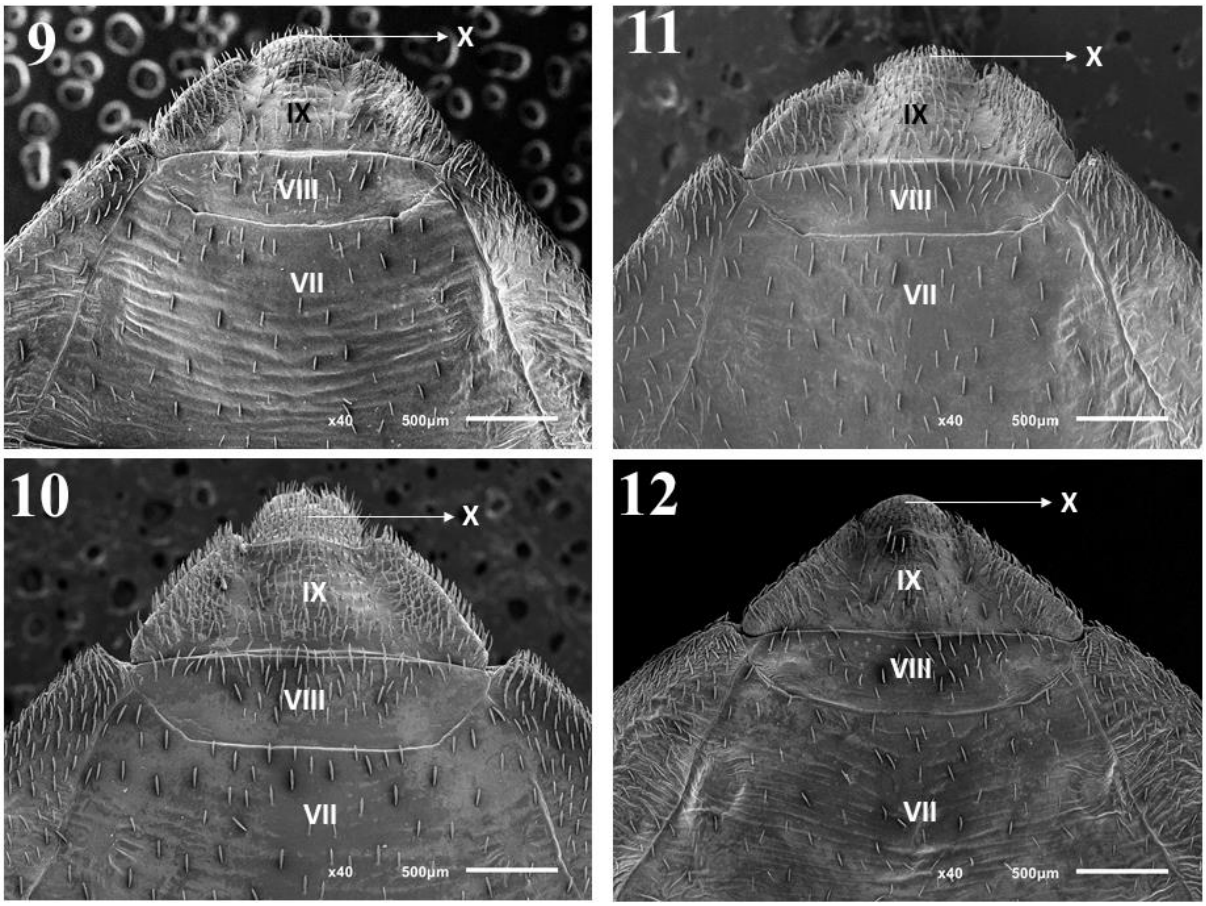


Figures 5-8. Details of scutellum of *T. rosai* and *T. sordida*: 5. *T. rosai* (Argentina); 6. *T. sordida* (Bolivia); 7. *T. sordida* (Brazil) and 8. *T. sordida* (Paraguay). ap: scutellum apex, cps: central portion of the scutellum.

Female external genitalia

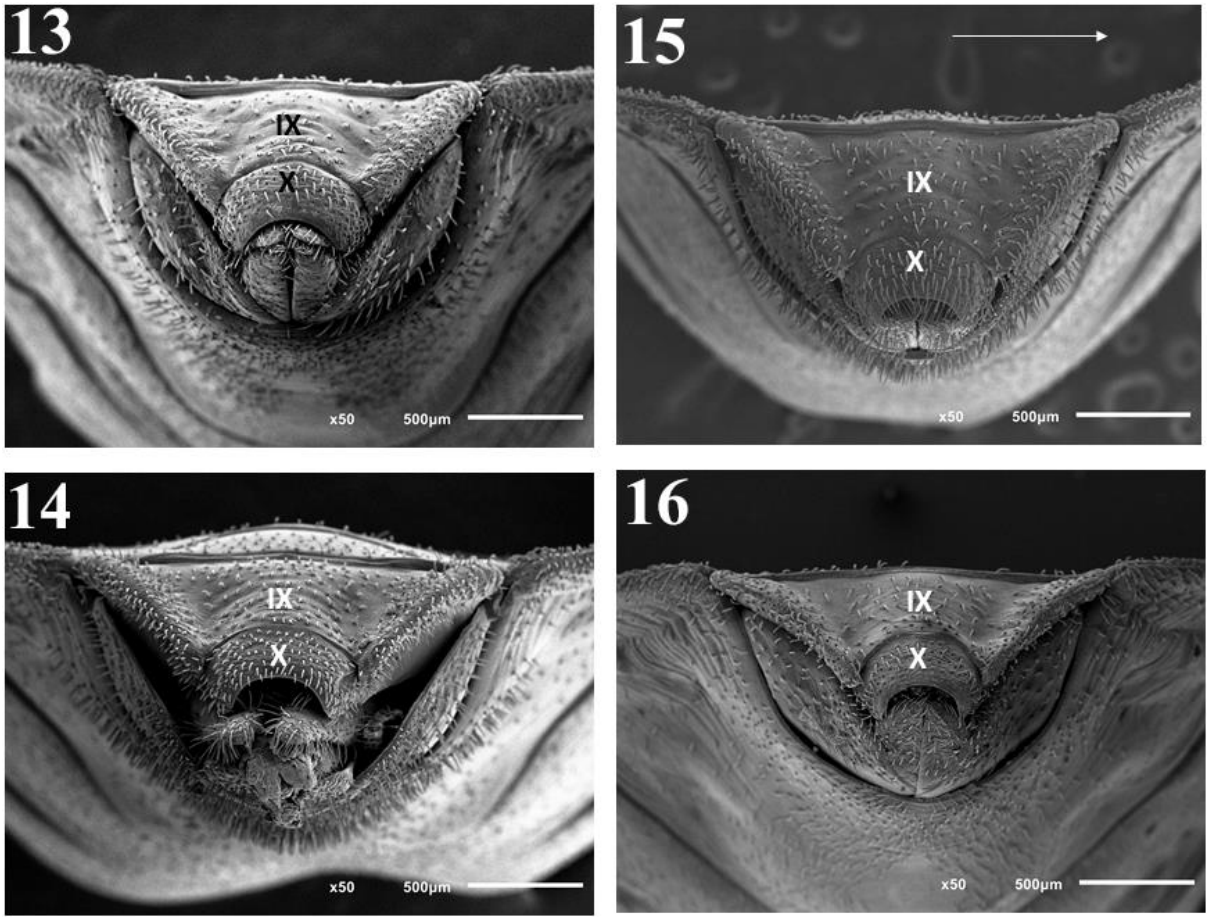
Differences in the female external genitalia of *T. rosai* from Argentina (Figures 9, 13 and 17), and *T. sordida* specimens from Bolivia (Figures 10, 14 and 18), Brazil (Figures 11, 15 and 19), and Paraguay (Figures 12, 16 and 20), were found. The analyses of the dorsal view showed that the shape of the eighth segment is trapezoidal in *T. sordida* Bolivia (Figure 10, VIII) and Brazil (Figure 11, VIII), and subtly oval in *T. rosai* (Argentina) (Figure 9, VIII) and Paraguay (Figure 12, VIII). Besides that, the line that separates the seventh from the

eighth segment in the central portion is straight in Bolivia (Figure 10) and Brazil (Figure 11), and slightly curved in *T. rosai* (Argentina) (Figure 9) and Paraguay (Figure 12). Analyses of the posterior view demonstrated that the shape and length of the tenth segment brings an elongated and wide aspect in *T. rosai* (Figure 13, X) and 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 Brazil (Figure 19, Gc8 and Gp8), while in Bolivia (Figure 18, VII, Gc8, and Gp8, respectively) the sides are elevated, and the central portion is straight, and in Paraguay (Figure 20, VII, Gc8, and Gp8, respectively) only the central portion is elevated.

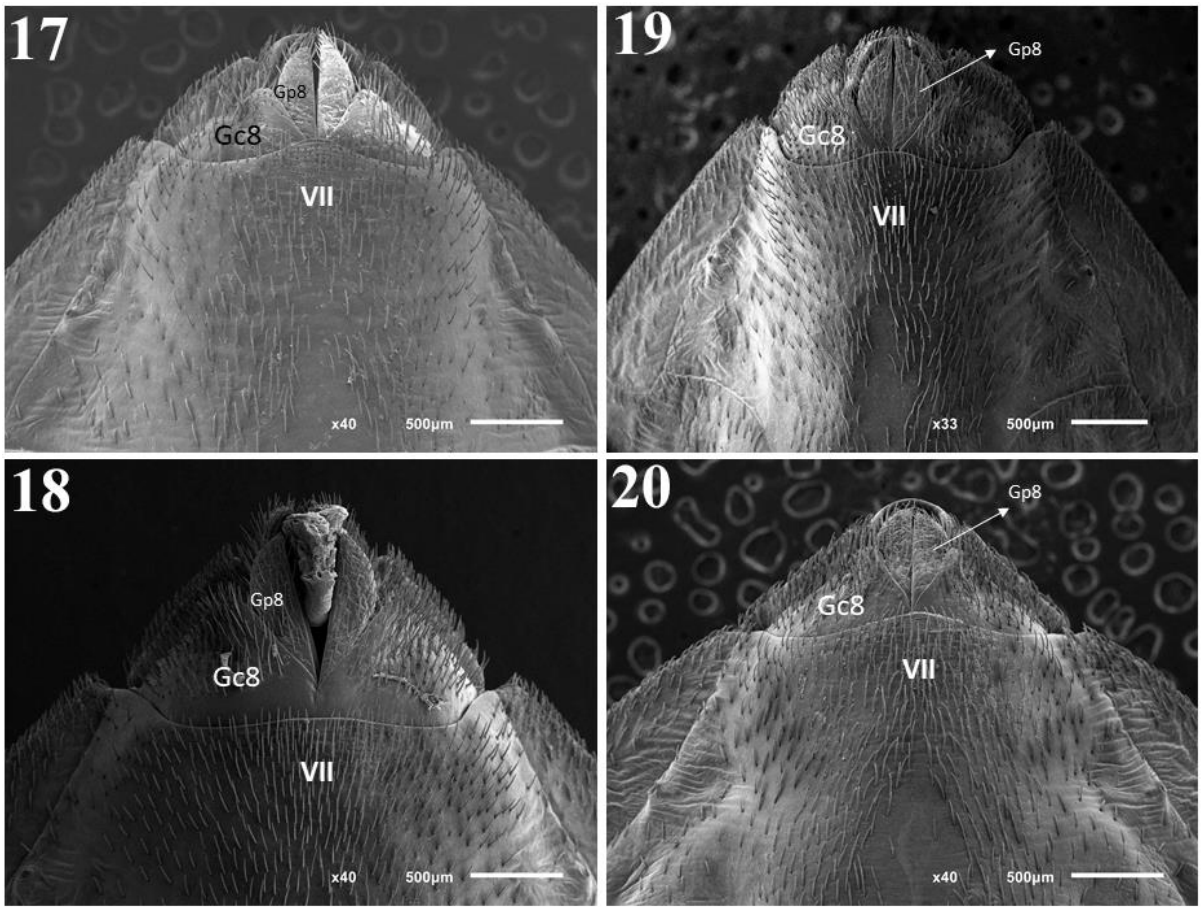


Figures 9-12. Details of female external genitalia by dorsal view *T. rosai* and *T. sordida*: 9. *T. rosai* (Argentina); 10. *T. sordida* (Bolivia); 11. *T. sordida* (Brazil) and 12. *T. sordida*

(Paraguay). VII: seventh segment; VIII: eighth segment; IX: ninth segment, and X: tenth segment.



Figures 13-16. Details of female external genitalia by posterior view *T. rosai* and *T. sordida*: 13. *T. rosai* (Argentina); 14. *T. sordida* (Bolivia); 15. *T. sordida* (Brazil) and 16. *T. sordida* (Paraguay). IX: nineth segment, and X: tenth segment.



Figures 17-20. Details of female external genitalia by ventral view of *T. rosai* and *T. sordida*: 17. *T. rosai* (Argentina); 18. *T. sordida* (Bolivia); 19. *T. sordida* (Brazil) and 20. *T. sordida* (Paraguay).

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. sordida* male and/or female (Table 2). In males, approximately 46% of the structures showed significant 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).

Table 1. Measurement averages of *T. rosai* (Argentina) and *T. sordida* from Bolivia, Brazil, and Paraguay.

T. sordida	**Country**	**Structures**						
		HL	**IE**	**PHW**	**PRO**	**SCU**	**THO**	**ABD**
Male	*T. rosai* (ARGENTINA)	5,174	0,951	1,490	4,717	3,077	6,704	15,117
	BOLIVIA	5,254	1,058	1,485	4,457	3,077	6,498	15,202
	BRAZIL	4,857	0,966	1,381	4,107	2,816	6,093	14,084
	PARAGUAY	5,273	1,062	1,463	4,564	3,243	6,622	14,070
Female	*T. rosai* (ARGENTINA)	5,055	1,003	1,486	4,429	3,215	6,531	16,418
	BOLIVIA	5,417	1,132	1,570	4,603	3,158	6,632	17,118
	BRAZIL	5,208	1,066	1,510	4,221	3,214	6,459	16,167
	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.

Table 2. Meaningfulness of the comparative parameters of *T. rosai* (Argentina) and *T. sordida* from Bolivia, Brazil, and Paraguay.

	Comparison	**Structures**							
		ABD	**HLN**	**HL**	**IE**	**PHW**	**PRO**	**SCU**	**THO**
Male	BRA x ARG	**0,01**	0,18	**0,01**	0,34	**0,01**	**0,0002**	**0,02**	**0,003**
	BRA x PAR	0,49	0,20	**0,02**	**0,05**	0,07	0,08	**0,0001**	**0,04**
	BRA x BOL	**0,01**	0,11	**0,01**	**0,01**	**0,01**	**0,03**	**0,01**	**0,05**
	ARG x PAR	**0,004**	0,09	0,22	**0,04**	0,26	0,28	0,05	0,35
	ARG x BOL	0,40	0,25	0,21	**0,001**	0,44	0,06	0,50	0,12
	PAR x BOL	**0,006**	0,19	0,45	0,47	0,30	0,35	**0,02**	0,31
Female	BRA x ARG	0,32	0,21	0,18	**0,02**	0,3	0,18	0,5	0,39
	BRA x PAR	0,18	0,4	0,10	**0,05**	0,11	**0,02**	0,22	0,12
	BRA x BOL	**0,04**	0,18	0,10	**0,03**	0,10	**0,05**	0,32	0,25
	ARG x PAR	0,23	0,23	**0,05**	**0,01**	0,07	0,06	0,21	0,15
	ARG x BOL	0,40	0,25	0,21	**0,001**	0,44	0,06	0,50	0,12
	PAR x BOL	0,40	0,38	0,30	0,47	0,29	0,22	0,15	0,23

BRA: *T. sordida* from Brazil; ARG: *T. rosai* from Argentina; PAR: *T. sordida* from Paraguay; BOL: *T. sordida* from 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.

Discussion

The terms “cryptic species” and “cryptic speciation” have already been used several times in the Triatominae subfamily [21-31,33]. Nevertheless, as proposed for *T. sordida* [41],

the use of these terms possibly occurred erroneously, since interspecific morphological/morphometric differences invalidate the application of these concepts [35]. Our results clearly show morphological/morphometric differences between *T. rosai* and *T. sordida* populations from Brazil, Bolivia and Paraguay, which confirms that the event that underlies the divergences observed by chromosomal [22,24], molecular [24], isoenzimatic [22] and cuticular hydrocarbon pattern [27] studies and possibly differentiate this species in more than one taxon [24] is speciation and not cryptic speciation.

Conceptual problems associated with the terms cryptic species/speciation may lead to a misconceived characterization of new species [39]. Struck et al. [39] 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 [24,31]. It is acceptable that polymorphisms (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* (Latreille, 1811)] [42,43]. However, after further studies and morphological and/or morphometric differences are observed, an application of these terms becomes inadequate.

Rhodnius robustus Larrousse, 1927 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, IV) [25]. 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* Rosa et al. (2012) (lineage II) and *R. marabaensis* Souza et al., 2016 (lineage III) [44-50], which makes it impossible to apply the term cryptic speciation.

Triatoma pintodiasi Jurberg et al. (2013) is a related species of *T.*

circummaculata (Stål, 1859) [31]. Based on the great morphological similarity of these taxa, *T. pintodiasi* was presented as a *T. circummaculata* cryptic species [31]. Yet, when describing *T. pintodiasi*, the authors brought morphometric and morphological differences that make the application of this term unviable [31]. Likewise, Alevi et al. [51] analyzed *T. vitticeps* (Stal, 1859) 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 [29,30], morphometry [26], cytogenetics [23], cuticular hydrocarbons [27] and reproductive isolation [33] analyses suggested the existence of *T. dimidiata* cryptic species [42,43]. However, morphological [29,30] and morphometric [26] divergences observed since 2004 for different *T. dimidiata* populations demonstrated that the term cryptic species/speciation has always been erroneously used [22,26,29,30].

Recently, based on integrative taxonomy, two new species closely related to *T. dimidiata* were characterized: *T. mopan* Dorn et al. (2018) and *T. huehuetenanguensis* Lima-Cordón et al. (2019) [52,53]. Even though the term cryptic species/speciation had already been used several times for *T. dimidiata* [22,26,29,30], Dorn et al. [52] and Lima-Cordón et al. [53] do not, at any time, use these terms in the description of the new taxa, since morphological and/or morphometric differences were observed among species related to *T. dimidiata* (which, have already been considered as *T. dimidiata* [42,43]), emphasizing that the authors were based correctly on the application of evolutionary terms (which should be applied only when no interspecific morphological/morphometric divergence is observed [35]).

Panzera et al. [24], considering the species *T. sordida*, *T. patagonica* Del Ponte, 1929, *T. guasayana* Wygodzinsky & Abalos, 1949 and *T. garciabesi*, suggested cryptic speciation for the *T. sordida* complex proposed by Schofield and Galvão [54]. However, even

if subtle, there are morphological and/or morphometric differences between all studied species [28, 40,, 41, 55-57]. Besides, our results point out significant morphological/morphometric differences between the different *T. sordida* populations (considered as, possibly, new taxa by Panzera et al. [24]), confirming the geometric morphometry data presented by Naterro et al. [41]. Therefore, the term, once more, was incorrectly used in Triatominae.

Conclusions

Based on this, the evolutionary process that supports the, so far, these divergences observed for *T. sordida* 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.

Methods

Insects

The specimens used (*T. rosai* from Province of Corrientes/Argentina, *T. sordida* from Corumbá/MS/Brazil, Department San Miguel/, Apolo/La Paz/Bolivia, and Paraguayan Chaco/Paraguay) 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 Scanning Electron Microscopy (SEM)

For morphological characterization of the triatomines in SEM (according to Rosa et al. [44]), 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 45º for 20 minutes, and then fixed in small aluminum cylinders with colorless enamel. Afterward, they were metalized by sputtering for two 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. rosai* from Argentina (7 males and 7 females), *T. sordida* from Brazil (9 males and 9 females), Bolivia (7 males and 10 females), and Paraguay (3 males and 3 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.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the conclusions of this article are included within the article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KCCA, JO, CG and JAR contributed to the study design. JAR, CG, NEGB and HJC contributed to specimens analysed. ACCG, JO, DCC, LMGD and IFB performed the experimental work and analysed the data. ACCG and KCCA wrote the manuscript. All authors read and approved the final manuscript.

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