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Chemotaxonomy of Five South American Species of the *Triatoma* genus (Hemiptera: Reduviidae: Triatominae) Based on Their Cuticle Hydrocarbon Pattern

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Abstract

The *Triatoma sordida* subcomplex traditionally included four triatomine species, *T. sordida*, *Triatoma garciabesi*, *Triatoma guasayana*, and *Triatoma patagonica*, distributed in the Southern Cone of South America. These species have a large intraspecific variability together with an overall similarity, making difficult to establish their taxonomic status. Many cytogenetic, morphometric, and molecular markers have been applied to address this. Recent studies have posed concerns on the inclusion of *T. guasayana* and *T. patagonica* within the subcomplex. Also, *T. sordida* from Argentina has been designed as a new species, *Triatoma rosai*. Using the cuticular hydrocarbon pattern as chemotaxonomic marker, the relationships among several populations of these species were analyzed by capillary gas chromatography and linear discriminant analysis along 25 collection sites in Argentina, Bolivia, Brazil, and Paraguay. *T. sordida* and *T. rosai* populations were differentially clustered in two CHC-based groups: "Group 1" included *T. sordida* from Eastern Brazil, Eastern Paraguay, and the Bolivian populations from La Paz and Izozog G1; "Group 2" included *T. rosai*, and *T. sordida* from Izozog G2 (Bolivia), and Western Paraguay. Whereas *T. garciabesi* remained closely related to *T. sordida* and *T. rosai*, *T. guasayana*, and *T. patagonica* were clearly separated from the species of the *T. sordida* subcomplex. Our results agree with those from other several techniques suggesting that the taxonomy of the *T. sordida* subcomplex should be revised.

Key words: Chagas disease, insect hydrocarbon, discriminant analysis, capillary gas chromatography

Triatomines (Hemiptera: Reduviidae: Triatominae) are blood-sucking insects, currently including 157 species (Justi and Galvão 2017, Alevi et al. 2020, Galvão 2020, Dale et al. 2021, Zhao et al. 2021). They are vectors of the parasite *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida: Trypanosomatidae), the causative agent of Chagas' disease, affecting from 6 to 7 million people worldwide (WHO 2020). Triatomine species were clustered in distinctive groups called complexes and subcomplexes, based on their similarity in morphology, geographical distribution, behavior, and ecology (Schofield and Galvão 2009). Based on the development of new study tools, it was proposed that complexes and subcomplexes should form natural groups (monophyletic), which resulted in the regrouping of several species (Gardim et al. 2014; Pita et al. 2016; Justi and Galvão 2017; Alevi et al. 2017, 2020; Belintani et al. 2020).

Initially, the *Triatoma sordida* subcomplex, included within the *Triatoma infestans* complex, was composed of four species, as follows (Schofield and Galvão 2009): *Triatoma sordida* (Stål, 1859) distributed from Central Brazil, throughout Paraguay, Central and Southern Bolivia, Uruguay, to Central Argentina; *Triatoma garciabesi* (Carcavalloet al., 1967) and *Triatoma patagonica* (Del Ponte, 1929) exclusively distributed in Argentina; and *Triatoma guasayana*

(Wygodzinsky and Abalos, 1949) distributed from most part of Bolivia and Paraguay to central Argentina.

The large phenotypic variability of these four species along with their geographic range and their overall similarity started to raise doubts about their taxonomic status, which has been questioned many times. Former morphological and chromosomal studies considered *T. garciabesi* as a dry-climate-adapted *T. sordida* variant (Lent and Wygodzinsky 1979, Panzera et al. 1997), however, it has been next revalidated as single species based on morphological characters of the head and genitalia, as well as chromosomal and isoenzymatic differences (Jurberg et al. 1998). Recently, the species status of *T. garciabesi* was confirmed by morphological, morphometric, molecular, and cytogenetic studies (Panzera et al. 2015; Belintani et al. 2020, 2021). Two distinct genotypes were early detected for *T. sordida* after enzymatic (*T. sordida* Group 1 and *T. sordida* Group 2, Noireau et al. 1998) and chromosomal (*T. sordida* Argentina and *T. sordida* Brazil, Panzera et al. 1997) studies. After that, new chromosomal analysis distinguished three taxa (named *T. sordida* sensu stricto, *T. sordida* Argentina, and *T. sordida* La Paz), and suggested that the *T. sordida* Group 2 detected by Noireau et al. (1998) was a *T. garciabesi* population, spreading out the distribution of this species to the Bolivian Chaco (Panzera et al. 2015). Morphometric studies carried out by Nattero et al. (2017) and Belintani et al. (2020) also showed significant differences among *T. sordida* samples from Argentina and those from Bolivia and Brazil. And recently, *T. sordida* from Argentina was defined as a new species, *T. rosai* (Alevi et al., 2020) based on morphological, crossbreeding, morphometric, and molecular analyses. The species status of *T. patagonica* has been questioned by morphometric analyses (Gorla et al. 1993), despite the typical black color in its legs and other body parts. However, several genetic markers (isoenzymes, chromosomal, morphometric, and molecular) differentiated this species from the other species of *T. sordida* subcomplex, although the species evidenced a very large variability even in its chromatic pattern (Panzera et al. 1997; Nattero et al. 2016; Belintani et al. 2020, 2021).

An extensive analysis based on morphometry of head, pronotum, and wings has shown that species differentiation may depend on the character being analyzed (Nattero et al. 2017). Similarly, different levels of species differentiation have been obtained by studying the female external genitalia and egg morphology (Belintani et al. 2021). On the other hand, chromosomal studies (Panzera et al. 2015, Pita et al. 2016), and very recent wing morphometry, female genitalia, egg morphology, and DNA analyses (Belintani et al. 2020, 2021), have questioned the integrity of the whole subcomplex, suggesting that *T. guasayana* and *T. patagonica* should be placed within the *Triatoma rubrovaria* subcomplex.

Cuticle hydrocarbons (CHC) have been one of the most widely studied chemotaxonomic characters in insects. Early papers by Carlson and co-authors were the first ones to recognize the role of CHC in chemical taxonomy (Carlson and Service 1979, 1980; Carlson and Walsh 1981). Since then, CHC have been used to infer the taxonomic relationships into several insect groups (Carlson et al. 1993, Bagnères and Wicker-Thomas 2010, Braga et al. 2013, Soares et al. 2017). In triatomines, the CHC pattern was successfully used to differentiate between the genera *Triatoma* (Laporte, 1832), *Rhodnius* (Stål, 1859) (Hemiptera: Reduviidae: Triatominae), and *Panstrongylus* (Berg, 1875) (Hemiptera: Reduviidae: Triatominae) (Juárez et al. 2000), and also assess the intraspecific variability of two of the most important vectors of Chagas disease, *Triatoma dimidiata* (Latreille, 1811) and *T. infestans* (Klug, 1834), along most of their geographic range (Calderón-Fernández et al. 2005, 2011, 2012). The CHC of the four species grouped by Schofield and Galvão (2009) in the *T. sordida* subcomplex (*T. sordida*, *T. guasayana*, *T. garciabesi*, and *T. patagonica*) have been previously identified by capillary gas chromatography (CGC) coupled to mass spectrometry (MS) (Calderón-Fernández and Juárez 2013). About 100 components from 21 to 43 atoms in the carbon backbone were described, comprising a complex mixture of straight and methyl-branched saturated chains with 1 up to 4 methyl substitutions. *n*-Alkanes formed a homologous series from nC21 to nC33 and represented 33–45% of the hydrocarbon fraction, whereas methyl-branched alkanes showed alkyl chains from 24 to 43 atoms in the carbon backbone.

The aim of this study is to provide an additional approach on the interspecific and the intraspecific variability of *T. sordida*, *T. rosai*, *T. garciabesi*, *T. guasayana*, and *T. patagonica* using the CHC as chemotaxonomic markers.

Materials and Methods

Insect Samples

Hemielytra and hindwings corresponding to 232 adult specimens of *T. sordida*, *T. rosai*, *T. garciabesi*, *T. guasayana*, and *T. patagonica* were used to extract CHC; sex-related qualitative differences in the CHC were not detected (Calderón-Fernández and Juárez 2013). Sampling sites in Argentina, Bolivia, Brazil, and Paraguay (25 sites) comprised an extensive area located between latitudes 11°37' S and 30°35' S and longitudes 46°19' W and 68°25' W. This heterogeneous area includes the Andean region to the West, with altitudes in the range of 1,850–2,900 m.a.s.l. and an altitude-depending climate, together with the Gran Chaco region with altitudes in the range of 100–650 m.a.s.l. and a hot and dry climate (Table 1 and Supp Fig. S1 [online only]).

Samples of *T. dimidiata* and *T. infestans* were used as outgroups; their CHC relative amounts were available from previous studies (Calderón-Fernández et al. 2011, 2012). *T. dimidiata* samples came from Cartago, Guanacaste, Heredia, and San José departments (Costa Rica) (16 specimens). *T. infestans* samples came from Cochabamba and Tarija (Bolivia), Arequipa (Peru), Presidente Hayes and San Pedro (Paraguay), and Chaco and Catamarca (Argentina) (113 specimens) (Supp Table S1 [online only]). Triatomine samples were classified by collectors according to Lent and Wygodzinsky (1979) and Jurberg et al. (1998). *T. sordida* specimens from Izozog (Bolivia) were analyzed already classified as members of genotypes G1 and G2 according to previous isoenzymatic analysis (Noireau et al. 1998, Table 1).

CGC Analysis of CHC

Wing lipids of each specimen were extracted with hexane, and the CHC fraction was eluted through silica columns, concentrated to appropriate volume, and analyzed by CGC in a Hewlett-Packard gas chromatograph 6890 (Hewlett Packard, Wilmington, DE) coupled to a flame ionization detector (FID) as previously described (Calderón-Fernández et al. 2011, 2012; Calderón-Fernández and Juárez 2013). Hydrocarbon peak areas were calculated for each chromatogram (HP Chem Station, Hewlett Packard) and expressed as a percentage of the total peak area. The chromatographic retention index (KI) was calculated for each hydrocarbon peak (Kovats 1965) after measuring the elution times of alkane standards run in similar conditions. Shorthand notation was used throughout text and tables to identify the hydrocarbons, as follows: CXX, for the total number of carbons in the straight chain; nCXX, for linear alkanes; nCXX:x, for unsaturated alkenes, where x indicates the number of unsaturations.

Multivariate Analyses

Hydrocarbon peaks were considered as characters and their relative amount values as character states. Hydrocarbon relative amount values were subjected to arcsine transformation prior to multivariate analysis

Table 1. Collection sites of the specimens analyzed in this study

Species[a]	Country	Department/province	Localities[b]	n[c]	Longitude (W)	Latitude (S)
T. rosai	Argentina [AR][d]	Formosa	Pirané (1)	13	59°07'12"	25°44'31"
T. sordida	Bolivia [BO]	La Paz	Apolo (2)	7	68°25'29"	14°43'11"
		Santa Cruz	Izozog (3)	14[e]	62°44'35"	19°25'30"
		Cochabamba	Quillacollo (4)	4	66°16'30"	17°26'14"
	Brazil [BR]	Goias	Posse (5)	4	46°19'38"	14°05'40"
		Rio Verde	Rio Verde (6)	16	50°57'58"	17°43'43'
		Mato Grosso	Rondonopolis (7)	8	54°42'48"	16°26'04"
		Tocantins	Sâo Valério (8)	11	48°14'00"	11°37'59"
	Paraguay [PA]	Boquerón	Mariscal Estigarríbia (9)	10	60°47'08"	22°01'47"
		Concepción	Itacurubí (10)	2	57°14'21"	23°19'12"
			Saladillo (11)	3	57°28'30"	23°19'12"
		Guairá	Zorrilla Cué (12)	3	56°15'38"	25°41'45"
		Paraguarí	Pirayú (13)	1	57°14'22"	25°28'33"
			Sapucai (14)	4	56°57'04"	25°40'41"
		Presidente Hayes	Pozo Colorado (15)	5	58°47'35"	23°29'44"
			Jope (16)	6	59°41'59"	23°01'03"
		San Pedro	Villa del Rosario (17)	5	57°05'44"	24°25'07"
			Itacurubí del Rosario (18)	5	56°50'12"	24°33'09"
T. garciabesi	Argentina	La Rioja	Castro Barros (19)	16	65°45'31"	30°35'56"
		Salta	General Güemes	5	65°00'34"	24°38'43"
		Santiago del Estero	Avellaneda (20)	19	63°28'35"	28°25'33"
T. guasayana	Argentina	Santiago del Estero	Avellaneda (21)	32	63°28'35"	28°25'33"
	Bolivia	Cochabamba	Mataral (22)	12	64°12'25"	18°07'53"
	Paraguay	Boquerón	Jericó (23)	5	59°48'38"	22°35'47"
		Presidente Hayes	Jope (24)	6	59°41'59"	23°01'03"
T. patagonica	Argentina	Santa Fé	9 de Julio (25)	21	61°41'42"	28°56'44"

[a]Classification by species made by the collectors according to Lent and Wygodzinsky (1979) and Jurberg et al. (1998). *T. sordida* from Argentina has been redescribed as a new species, *T. rosai* by Alevi et al. (2020).

[b]Numbers correspond to positions in the map (Supp Fig. 1 [online only]).

[c]Number of insects per locality.

[d]In brackets, country abbreviations used in tables and figures.

[e]*T. sordida* specimens from Izozog were sent to our lab already classified by the provider (Dr. François Noireau) as members of genotypes G1 (5 insects) or G2 (9 insects).

(Sokal and Rohlf 2001). Linear discriminant analysis (DA) was used to assess the population structure within each species as well as the relationship between species. CHC relative amount data of *T. dimidiata* and *T. infestans* samples were re-analyzed together with those from the other species. SPSS Version 15.0 (SPSS, Inc., Chicago, IL) and Statistica Version 6.0 (StatSoft, Inc., Tulsa, OK) were used to perform multivariate analyses. Backward-elimination stepwise method was applied to select those hydrocarbons that best contribute to discrimination. Wilks' lambda (λ) statistic (Wilks 1932) and its statistical significance were used as discrimination significance measures. The accuracy of the discriminant functions (DF) was tested by reclassifying the specimens using the cross-validation method (Lachenbruch and Mickey 1968). Mahalanobis distances obtained from DA were used to construct the trees that show the relationship and level of similarity between populations and species, using MVSP Version 3.13b (Kovach Computing Services, Anglesey, U.K.). CHC contributing the best to differentiate between species and populations were identified by analyzing the DF coefficients at sample's centroids together with the correlation values between the DF and the original variables (hydrocarbons).

Results

CHC Variability in *T. sordida*, *T. rosai*, and *T. guasayana*

Ten CHC (KIs 2857, 2975, 3000, 3175, 3183, 3258, 3375, 3383, 3581, 3981) (Table 2) were used to classify *T. sordida* and *T. rosai* samples (Wilk's λ of 0.32, $P < 0.001$). The specimens correctly classified in the original and cross-validated classification were 83.5% and 75.2%, respectively, supporting the usefulness of the discriminant functions to assess the population structure (Supp Table S2 [online only]). The DA (Fig. 1A) showed a high intraspecific variability, and a clear subdivision of the species' populations into two CHC-based groups. One of them, named here as "Group 1", included *T. sordida* populations from Brazil (Goias, Rio Verde, Mato Grosso, and Tocantins), two populations from Bolivia (La Paz and Izozog G1), and samples from Eastern Paraguay (Concepción, Paraguarí, and San Pedro). The other main group, "Group 2", included *T. rosai* samples from Argentina (Formosa); and *T. sordida* specimens from Bolivia (Izozog G2), and from Western Paraguay (Boquerón and Presidente Hayes). Within each group, populations clustered mostly according to their geographic proximity and country of origin. The *T. sordida* specimens from Cochabamba (Bolivia) were not included in any of those groups (Fig. 1A). CHC KI 3175, KI 3183, KI 3258, KI 3383, KI 3581, and KI 3981 were those which contributed the best to differentiate between Group 1, Group 2, and the Cochabamba population. The other four CHC in the DA contributed additionally to differentiate the remaining *T. sordida* and *T. rosai* populations.

Regarding *T. guasayana*, 94.5% and 90.9% of the specimens were correctly classified in the original and cross-validated classification, respectively. Four CHC (KIs 3175, 3678, 3727, and 3805) (Table 2) were used to classify the species' populations. The specimens were grouped mostly according to their country of origin,

Table 2. Relative amounts of the CHC used in the discriminant analyses of the species and populations analyzed in this study

Species and populations	KI 2775	KI 2857	KI 2975	KI 2993
Tros Formosa [AR]	1.17 ± 0.15	0.37 ± 0.04	4.97 ± 0.54	tr
Tsor La Paz [BO]	0.24 ± 0.09	0.35 ± 0.07	1.17 ± 0.25	nd
Tsor Santa Cruz (IzozogG1) [BO]	0.43 ± 0.08	2.44 ± 0.29	0.85 ± 0.08	nd
Tsor Santa Cruz (IzozogG2) [BO]	0.32 ± 0.06	0.13 ± 0.04	1.34 ± 0.14	nd
Tsor Cochabamba [BO]	1.55 ± 0.16	1.10 ± 0.16	2.84 ± 0.25	nd
Tsor Goias [BR]	0.55 ± 0.02	0.22 ± 0.05	0.90 ± 0.25	nd
Tsor Rio Verde [BR]	0.22 ± 0.03	0.25 ± 0.05	0.70 ± 0.09	nd
Tsor Mato Grosso [BR]	0.22 ± 0.06	0.80 ± 0.15	1.18 ± 0.15	tr
Tsor Tocantins [BR]	0.20 ± 0.04	0.10 ± 0.03	0.44 ± 0.07	nd
Tsor Boquerón [PA]	0.66 ± 0.21	tr	1.67 ± 0.34	nd
Tsor Concepción [PA]	1.72 ± 0.43	0.33 ± 0.10	4.24 ± 1.57	nd
Tsor Paraguarí [PA]	1.48 ± 0.49	0.14 ± 0.09	3.83 ± 0.45	nd
Tsor Pte. Hayes [PA]	0.83 ± 0.21	tr	1.24 ± 0.27	tr
Tsor San Pedro [PA]	1.24 ± 0.29	0.25 ± 0.03	4.62 ± 0.49	nd
Tgar La Rioja [AR]	4.40 ± 0.50	0.96 ± 0.07	2.07 ± 0.12	0.90 ± 0.10
Tgar Sgo. del Estero [AR]	1.14 ± 0.16	0.55 ± 0.02	1.18 ± 0.14	0.71 ± 0.07
Tgar Salta [AR]	4.17 ± 0.40	0.47 ± 0.05	6.22 ± 1.22	0.45 ± 0.04
Tgua Sgo. del Estero [AR]	0.57 ± 0.04	0.23 ± 0.02	1.00 ± 0.05	nd
Tgua Cochabamba [BO]	0.23 ± 0.03	tr	0.24 ± 0.03	nd
Tgua Boquerón [PA]	1.48 ± 0.66	tr	0.15 ± 0.07	nd
Tgua Pte. Hayes [PA]	0.76 ± 0.26	tr	0.34 ± 0.07	nd
Tpat Santa Fé [AR]	0.37 ± 0.03	0.11 ± 0.02	0.29 ± 0.03	nd
Tinf Tarija [BO]	0.18 ± 0.01	0.41 ± 0.03	4.29 ± 0.23	nd
Tinf Cochabamba [BO]	0.16 ± 0.01	0.26 ± 0.03	7.50 ± 0.32	nd
Tinf Catamarca [AR]	0.18 ± 0.01	0.33 ± 0.02	6.44 ± 0.39	nd
Tinf Chaco [AR]	0.16 ± 0.01	0.39 ± 0.03	5.98 ± 0.33	nd
Tinf San Pedro [PA]	0.20 ± 0.03	0.46 ± 0.10	4.66 ± 0.81	nd
Tinf Pte. Hayes [PA]	0.24 ± 0.03	0.47 ± 0.10	4.53 ± 0.70	nd
Tinf Arequipa [PE]	0.18 ± 0.02	0.42 ± 0.03	8.34 ± 1.02	nd
Tdim Costa Rica	tr	nd	tr	nd
Species and populations	KI 3000	KI 3087	KI 3175	KI 3183
Tros Formosa [AR]	0.64 ± 0.03	nd	4.72 ± 0.47	3.14 ± 0.41
Tsor La Paz [BO]	1.08 ± 0.07	nd	0.85 ± 0.18	0.11 ± 0.06
Tsor Santa Cruz (IzozogG1) [BO]	1.85 ± 0.12	nd	0.93 ± 0.13	nd
Tsor Santa Cruz (IzozogG2) [BO]	0.92 ± 0.06	nd	11.70 ± 1.32	1.40 ± 0.24
Tsor Cochabamba [BO]	0.63 ± 0.06	nd	13.69 ± 0.58	nd
Tsor Goias [BR]	0.98 ± 0.04	nd	1.15 ± 0.23	nd
Tsor Rio Verde [BR]	1.13 ± 0.09	nd	0.50 ± 0.08	nd
Tsor Mato Grosso [BR]	0.79 ± 0.05	nd	0.54 ± 0.09	tr
Tsor Tocantins [BR]	0.80 ± 0.03	nd	0.75 ± 0.07	nd
Tsor Boquerón [PA]	1.36 ± 0.08	nd	5.40 ± 0.77	1.05 ± 0.32
Tsor Concepción [PA]	0.72 ± 0.05	nd	1.20 ± 0.23	0.15 ± 0.07
Tsor Paraguarí [PA]	0.93 ± 0.12	nd	2.04 ± 0.32	tr
Tsor Pte. Hayes [PA]	1.24 ± 0.07	nd	2.85 ± 0.34	0.98 ± 0.38
Tsor San Pedro [PA]	0.86 ± 0.05	nd	1.82 ± 0.29	0.18 ± 0.15
Tgar La Rioja [AR]	0.53 ± 0.05	nd	5.70 ± 0.61	0.24 ± 0.03
Tgar Sgo. del Estero [AR]	0.65 ± 0.03	nd	3.70 ± 0.35	0.38 ± 0.05
Tgar Salta [AR]	0.18 ± 0.01	nd	6.30 ± 1.15	tr
Tgua Sgo. del Estero [AR]	1.04 ± 0.06	nd	4.75 ± 0.20	nd
Tgua Cochabamba [BO]	1.03 ± 0.09	nd	1.64 ± 0.41	nd
Tgua Boquerón [PA]	1.89 ± 0.15	nd	0.92 ± 0.28	tr
Tgua Pte. Hayes [PA]	1.27 ± 0.08	nd	2.04 ± 0.76	tr
Tpat Santa Fé [AR]	0.88 ± 0.03	nd	3.16 ± 0.16	nd
Tinf Tarija [BO]	2.64 ± 0.09	nd	0.69 ± 0.05	tr
Tinf Cochabamba [BO]	2.32 ± 0.13	nd	1.90 ± 0.10	tr
Tinf Catamarca [AR]	2.44 ± 0.06	nd	0.59 ± 0.03	tr
Tinf Chaco [AR]	2.05 ± 0.06	nd	0.65 ± 0.04	tr
Tinf San Pedro [PA]	2.71 ± 0.18	nd	0.49 ± 0.10	0.10 ± 0.06
Tinf Pte. Hayes [PA]	2.39 ± 0.20	nd	0.53 ± 0.06	tr
Tinf Arequipa [PE]	2.93 ± 0.32	nd	1.46 ± 0.20	tr
Tdim Costa Rica	1.23 ± 0.06	1.67 ± 0.15	nd	nd

Table 2. Continued

Species and populations	KI 3202	KI 3258	KI 3287	KI 3375
Tros Formosa [AR]	0.38 ± 0.03	0.79 ± 0.09	nd	2.03 ± 0.19
Tsor La Paz [BO]	0.71 ± 0.13	0.44 ± 0.09	nd	3.30 ± 0.46
Tsor Santa Cruz (IzozogG1) [BO]	0.72 ± 0.08	1.83 ± 0.31	nd	0.69 ± 0.07
Tsor Santa Cruz (IzozogG2) [BO]	0.40 ± 0.04	1.44 ± 0.15	nd	2.77 ± 0.23
Tsor Cochabamba [BO]	0.20 ± 0.01	0.99 ± 0.10	nd	1.65 ± 0.23
Tsor Goias [BR]	0.59 ± 0.13	0.73 ± 0.07	nd	3.86 ± 0.18
Tsor Rio Verde [BR]	0.96 ± 0.05	0.16 ± 0.05	nd	1.12 ± 0.16
Tsor Mato Grosso [BR]	1.25 ± 0.16	0.61 ± 0.06	nd	1.07 ± 0.18
Tsor Tocantins [BR]	1.36 ± 0.11	0.18 ± 0.04	nd	1.06 ± 0.13
Tsor Boquerón [PA]	0.60 ± 0.05	1.61 ± 0.17	nd	2.43 ± 0.38
Tsor Concepción [PA]	1.01 ± 0.17	tr	nd	0.78 ± 0.12
Tsor Paraguarí [PA]	0.56 ± 0.05	0.15 ± 0.07	nd	1.40 ± 0.28
Tsor Pte. Hayes [PA]	0.59 ± 0.03	1.02 ± 0.11	nd	1.52 ± 0.22
Tsor San Pedro [PA]	0.58 ± 0.05	0.14 ± 0.06	nd	1.09 ± 0.12
Tgar La Rioja [AR]	0.15 ± 0.03	0.41 ± 0.04	nd	1.32 ± 0.12
Tgar Sgo. del Estero [AR]	0.20 ± 0.02	0.25 ± 0.03	nd	0.88 ± 0.04
Tgar Salta [AR]	0.37 ± 0.04	0.68 ± 0.13	nd	1.43 ± 0.21
Tgua Sgo. del Estero [AR]	0.44 ± 0.02	0.28 ± 0.02	nd	5.97 ± 0.31
Tgua Cochabamba [BO]	0.53 ± 0.05	0.39 ± 0.10	nd	5.03 ± 0.53
Tgua Boquerón [PA]	0.92 ± 0.10	tr	nd	5.08 ± 0.27
Tgua Pte. Hayes [PA]	0.62 ± 0.08	0.19 ± 0.04	nd	3.98 ± 0.43
Tpat Santa Fé [AR]	0.36 ± 0.02	0.20 ± 0.02	nd	7.63 ± 0.35
Tinf Tarija [BO]	0.45 ± 0.01	1.52 ± 0.06	nd	0.21 ± 0.01
Tinf Cochabamba [BO]	0.58 ± 0.02	1.47 ± 0.09	nd	0.83 ± 0.06
Tinf Catamarca [AR]	0.27 ± 0.01	2.00 ± 0.11	nd	0.19 ± 0.01
Tinf Chaco [AR]	0.32 ± 0.01	1.53 ± 0.08	nd	0.16 ± 0.01
Tinf San Pedro [PA]	0.34 ± 0.03	1.69 ± 0.23	nd	0.29 ± 0.05
Tinf Pte. Hayes [PA]	0.48 ± 0.06	1.13 ± 0.22	nd	0.22 ± 0.04
Tinf Arequipa [PE]	0.54 ± 0.04	2.34 ± 0.23	nd	0.48 ± 0.06
Tdim Costa Rica	0.36 ± 0.04	nd	1.00 ± 0.10	nd

Species and populations	KI 3383	KI 3581	KI 3678	KI 3727
Tros Formosa [AR]	2.31 ± 0.24	0.76 ± 0.06	0.90 ± 0.08	3.69 ± 0.26
Tsor La Paz [BO]	0.45 ± 0.09	2.88 ± 0.29	1.55 ± 0.22	3.45 ± 0.23
Tsor Santa Cruz (IzozogG1) [BO]	nd	0.63 ± 0.06	0.82 ± 0.18	3.68 ± 0.44
Tsor Santa Cruz (IzozogG2) [BO]	1.12 ± 0.20	0.65 ± 0.06	0.71 ± 0.08	3.30 ± 0.25
Tsor Cochabamba [BO]	0.25 ± 0.03	2.38 ± 0.21	2.36 ± 0.46	5.49 ± 0.26
Tsor Goias [BR]	0.13 ± 0.13	2.80 ± 0.25	1.25 ± 0.15	4.47 ± 0.47
Tsor Rio Verde [BR]	0.40 ± 0.10	2.71 ± 0.38	1.67 ± 0.24	4.60 ± 0.33
Tsor Mato Grosso [BR]	0.90 ± 0.14	3.05 ± 0.25	1.48 ± 0.08	3.83 ± 0.36
Tsor Tocantins [BR]	0.30 ± 0.05	3.04 ± 0.22	1.86 ± 0.18	4.23 ± 0.22
Tsor Boquerón [PA]	3.89 ± 0.34	0.82 ± 0.06	0.40 ± 0.05	1.50 ± 0.14
Tsor Concepción [PA]	0.20 ± 0.15	1.64 ± 0.20	1.39 ± 0.19	2.64 ± 0.36
Tsor Paraguarí [PA]	0.22 ± 0.07	1.62 ± 0.25	1.18 ± 0.14	2.53 ± 0.44
Tsor Pte. Hayes [PA]	3.68 ± 0.61	1.20 ± 0.08	0.67 ± 0.10	1.85 ± 0.16
Tsor San Pedro [PA]	0.33 ± 0.26	1.07 ± 0.10	0.92 ± 0.07	2.38 ± 0.20
Tgar La Rioja [AR]	0.13 ± 0.04	1.08 ± 0.06	1.08 ± 0.10	1.50 ± 0.10
Tgar Sgo. del Estero [AR]	tr	0.95 ± 0.05	1.19 ± 0.13	1.37 ± 0.18
Tgar Salta [AR]	0.11 ± 0.07	0.90 ± 0.10	0.89 ± 0.11	2.53 ± 0.37
Tgua Sgo. del Estero [AR]	tr	1.41 ± 0.09	1.19 ± 0.07	0.65 ± 0.04
Tgua Cochabamba [BO]	tr	1.26 ± 0.14	0.40 ± 0.07	2.23 ± 0.10
Tgua Boquerón [PA]	tr	1.01 ± 0.18	0.67 ± 0.03	0.52 ± 0.04
Tgua Pte. Hayes [PA]	tr	0.94 ± 0.19	0.56 ± 0.12	0.47 ± 0.06
Tpat Santa Fé [AR]	nd	0.98 ± 0.05	0.72 ± 0.04	0.63 ± 0.05
Tinf Tarija [BO]	nd	1.47 ± 0.07	0.38 ± 0.02	1.62 ± 0.06
Tinf Cochabamba [BO]	nd	0.90 ± 0.05	0.59 ± 0.03	1.47 ± 0.09
Tinf Catamarca [AR]	nd	1.33 ± 0.09	0.35 ± 0.02	1.68 ± 0.07
Tinf Chaco [AR]	nd	1.52 ± 0.10	0.37 ± 0.02	1.85 ± 0.07
Tinf San Pedro [PA]	nd	1.84 ± 0.44	0.61 ± 0.12	1.37 ± 0.16
Tinf Pte. Hayes [PA]	nd	1.51 ± 0.15	0.41 ± 0.07	1.65 ± 0.25
Tinf Arequipa [PE]	nd	0.77 ± 0.09	0.31 ± 0.03	1.96 ± 0.10
Tdim Costa Rica	nd	0.38 ± 0.04	0.59 ± 0.14	1.37 ± 0.10

Table 2. Continued

Species and populations	KI 3761	KI 3805	KI 3827	KI 3877
Tros Formosa [AR]	tr	1.91 ± 0.17	0.35 ± 0.03	0.39 ± 0.08
Tsor La Paz [BO]	0.21 ± 0.03	2.16 ± 0.20	0.78 ± 0.07	0.17 ± 0.06
Tsor Santa Cruz (IzozogG1) [BO]	0.29 ± 0.05	0.93 ± 0.11	1.19 ± 0.10	0.30 ± 0.04
Tsor Santa Cruz (IzozogG2) [BO]	tr	1.98 ± 0.16	0.48 ± 0.08	0.38 ± 0.05
Tsor Cochabamba [BO]	0.86 ± 0.10	2.49 ± 0.29	2.06 ± 0.23	0.28 ± 0.03
Tsor Goias [BR]	nd	2.91 ± 0.11	0.88 ± 0.11	0.50 ± 0.08
Tsor Rio Verde [BR]	0.19 ± 0.06	2.68 ± 0.27	0.71 ± 0.06	0.13 ± 0.04
Tsor Mato Grosso [BR]	tr	2.16 ± 0.09	0.71 ± 0.08	0.22 ± 0.05
Tsor Tocantins [BR]	0.20 ± 0.07	2.06 ± 0.12	0.80 ± 0.11	0.20 ± 0.05
Tsor Boquerón [PA]	nd	1.63 ± 0.20	0.82 ± 0.05	0.22 ± 0.06
Tsor Concepción [PA]	0.14 ± 0.09	1.32 ± 0.16	0.90 ± 0.07	tr
Tsor Paraguarí [PA]	nd	1.71 ± 0.42	0.65 ± 0.10	tr
Tsor Pte. Hayes [PA]	nd	1.70 ± 0.17	0.89 ± 0.07	0.25 ± 0.07
Tsor San Pedro [PA]	nd	1.20 ± 0.20	0.72 ± 0.11	tr
Tgar La Rioja [AR]	tr	1.68 ± 0.12	0.45 ± 0.03	0.37 ± 0.04
Tgar Sgo. del Estero [AR]	0.58 ± 0.40	1.67 ± 0.09	0.68 ± 0.07	0.42 ± 0.03
Tgar Salta [AR]	0.68 ± 0.14	2.42 ± 0.12	0.40 ± 0.03	0.86 ± 0.07
Tgua Sgo. del Estero [AR]	3.44 ± 0.23	0.88 ± 0.04	0.22 ± 0.03	tr
Tgua Cochabamba [BO]	1.33 ± 0.16	0.88 ± 0.10	0.82 ± 0.06	0.12 ± 0.03
Tgua Boquerón [PA]	2.13 ± 0.24	1.91 ± 0.09	0.68 ± 0.05	0.31 ± 0.05
Tgua Pte. Hayes [PA]	2.00 ± 0.35	1.67 ± 0.20	0.67 ± 0.07	0.37 ± 0.06
Tpat Santa Fé [AR]	1.42 ± 0.09	1.58 ± 0.08	0.27 ± 0.04	tr
Tinf Tarija [BO]	nd	0.63 ± 0.03	tr	0.23 ± 0.02
Tinf Cochabamba [BO]	nd	1.23 ± 0.05	tr	0.37 ± 0.02
Tinf Catamarca [AR]	nd	0.60 ± 0.04	tr	0.17 ± 0.01
Tinf Chaco [AR]	nd	0.50 ± 0.03	tr	0.19 ± 0.01
Tinf San Pedro [PA]	nd	0.62 ± 0.13	tr	0.36 ± 0.08
Tinf Pte. Hayes [PA]	nd	0.57 ± 0.13	tr	0.27 ± 0.09
Tinf Arequipa [PE]	nd	1.01 ± 0.08	tr	0.19 ± 0.02
Tdim Costa Rica	nd	0.77 ± 0.09	1.05 ± 0.13	0.54 ± 0.09

Species and populations	KI 3897	KI 3940	KI 3981
Tros Formosa [AR]	nd	0.99 ± 0.14	1.68 ± 0.22
Tsor La Paz [BO]	nd	0.98 ± 0.09	1.76 ± 0.20
Tsor Santa Cruz (IzozogG1) [BO]	nd	1.74 ± 0.20	2.87 ± 0.14
Tsor Santa Cruz (IzozogG2) [BO]	nd	1.04 ± 0.22	1.83 ± 0.08
Tsor Cochabamba [BO]	nd	1.37 ± 0.01	3.99 ± 0.34
Tsor Goias [BR]	nd	1.23 ± 0.12	1.97 ± 0.20
Tsor Rio Verde [BR]	nd	1.92 ± 0.30	3.33 ± 0.35
Tsor Mato Grosso [BR]	nd	1.00 ± 0.15	2.25 ± 0.36
Tsor Tocantins [BR]	nd	2.26 ± 0.35	3.06 ± 0.37
Tsor Boquerón [PA]	nd	1.39 ± 0.09	2.25 ± 0.38
Tsor Concepción [PA]	nd	1.86 ± 0.30	3.47 ± 0.65
Tsor Paraguarí [PA]	nd	1.76 ± 0.25	4.48 ± 0.70
Tsor Pte. Hayes [PA]	nd	1.65 ± 0.09	2.56 ± 0.39
Tsor San Pedro [PA]	nd	1.59 ± 0.20	2.21 ± 0.25
Tgar La Rioja [AR]	nd	1.19 ± 0.06	4.12 ± 0.30
Tgar Sgo. del Estero [AR]	nd	1.19 ± 0.06	5.26 ± 0.38
Tgar Salta [AR]	nd	1.31 ± 0.17	4.08 ± 0.28
Tgua Sgo. del Estero [AR]	nd	1.16 ± 0.05	5.52 ± 0.45
Tgua Cochabamba [BO]	nd	3.16 ± 0.31	10.22 ± 1.30
Tgua Boquerón [PA]	nd	1.19 ± 0.17	7.46 ± 0.80
Tgua Pte. Hayes [PA]	nd	1.15 ± 0.17	7.66 ± 1.32
Tpat Santa Fé [AR]	nd	1.24 ± 0.07	4.61 ± 0.40
Tinf Tarija [BO]	nd	nd	1.32 ± 0.09
Tinf Cochabamba [BO]	nd	nd	1.61 ± 0.09
Tinf Catamarca [AR]	nd	nd	0.85 ± 0.04
Tinf Chaco [AR]	nd	nd	0.84 ± 0.03
Tinf San Pedro [PA]	nd	nd	1.18 ± 0.22
Tinf Pte. Hayes [PA]	nd	nd	1.37 ± 0.31

Table 2. Continued

Species and populations	KI 3897	KI 3940	KI 3981
Tinf Arequipa [PE]	nd	nd	1.33 ± 0.07
Tdim Costa Rica	1.58 ± 0.34	1.05 ± 0.16	1.09 ± 0.15

Values are mean relative abundance ± standard error of the mean.

Uppercase initials in brackets indicate population's country of origin.

Abbreviations: Tros, *T. rosai*; Tsor, *T. sordida*; Tgar, *T. garciabesi*; Tgua, *T. guasayana*; Tpat, *T. patagonica*; Tinf, *T. infestans*; Tdim, *T. dimidiata*; KI, Kovats index; nd, not detected; tr, traces (relative abundance < 0.10)

CHC identity according to Calderón-Fernández et al. (2011, 2012) and Calderón-Fernández and Juárez (2013), as follows: KI 2775, 3-methyl C27; KI 2857, 4-methyl C28; KI 2975, 3-methyl C29; KI 2993, 7,13,17-trimethyl C29; KI 3000, nC30; KI 3087, nC31:1; KI 3175, 3-methyl C31; KI 3183, 9,13,19- + 11,15,19- + 11,15,21-trimethyl C31; KI 3202, nC32; KI 3258, 4-methyl C32; KI 3287, nC33:1; KI 3375, 3-methyl C33; KI 3383, 11,15,21- + 11,17,21-trimethyl C33; KI 3581, 13,17,23- + 13,17,21- + 11,15,21-trimethyl C35; KI 3678, 6,10-dimethyl C36; KI 3727, 2,6,10-trimethyl C36 + 11- + 13- + 15-methyl C37; KI 3761, 13,17- + 11,15- + 9,13- + 15,19-dimethyl C37; KI 3805, 3,11- + 3,15- + 3,13- + 3,17-dimethyl C37; KI 3827, 12- + 14- + 16-methyl C38; KI 3877, 6,10-dimethyl C38 + 14,18,22-trimethyl C38; KI 3897, 6,10,14-trimethyl C38; KI 3940, 4,8,12,16-tetramethyl C38; KI 3981, 5,15- + 5,17-dimethyl C39 + 13,17,21- + 13,17,23 + 15,19,23-trimethyl C39.

with some mixing between insects from the Paraguayan populations (Supp Table S3 [online only]).

Interspecific Variability

Sixteen out of more than 100 CHC (KIs 2775, 2857, 2975, 2993, 3087, 3175, 3202, 3287, 3383, 3727, 3761, 3827, 3877, 3897, 3940, and 3981) were used to classify all the species and populations analyzed in this study (Wilk's λ of 0.11, $P < 0.001$). The specimens correctly classified in the original and cross-validated classification were 87.5% and 78.6%, respectively (Supp Table S4 [online only]). The classification results showed that there was no overlapping between most of the species; however, 4% of the *T. patagonica* specimens were misclassified as *T. guasayana* from Argentina. Some CHC contributed the most for the differentiation between species and species' populations, as follows: KI 3087 and KI 3897 for *T. dimidiata*; KI 3940 for *T. infestans*; KI 2993 and KI 2775 for *T. garciabesi*; KI 3981, KI 3375, and KI 3761 for *T. guasayana* and *T. patagonica*, together; KI 3827, KI 3175, and KI 3383 for *T. sordida* and *T. rosai* (Table 2). The most unexpected result of the analysis was that *T. guasayana* and *T. patagonica* were placed outside the *T. sordida* subcomplex (Fig. 1B). In addition, *T. patagonica* did not cluster separately as a different species, instead it was grouped as another *T. guasayana* population, closer to *T. guasayana* specimens from Central Argentina.

Discussion

Within triatomines, *T. infestans* and *T. dimidiata* are currently the two species of higher sanitary relevance. Our previous work using the CHC pattern as a chemotaxonomy tool has provided valuable information on their population structure throughout most of their distribution range (Calderón-Fernández et al. 2011, 2012). These studies showed a large intraspecific variability and led to suggest the existence of different subspecies for both species, in agreement with other molecular, cytogenetic, and phenotype markers (Bargues et al. 2006, 2008; Panzera et al. 2006, 2014). Additionally, significant CHC differences were detected in *T. infestans* between insecticide-resistant and insecticide-susceptible populations (Calderón-Fernández et al. 2012).

Early studies of the triatomine CHC have given preliminary evidence of the potential of this technique; *T. sordida* was easily differentiated from *T. infestans*, *T. guasayana* and other triatomines (Juárez and Brenner 1986). And more recently, the description of the CHC identity in the four species initially grouped in the *T. sordida* subcomplex has suggested significant differences between species as well as between *T. sordida* populations from Argentina and Brazil (Calderón-Fernández and Juárez 2013). In this work, the use of multivariate techniques to analyze the CHC profiles of *T. sordida*, *T. rosai*, *T. garciabesi*, *T. guasayana* and *T. patagonica* along most of their distribution range provides a more comprehensive view of the relationship between their populations and between the species themselves.

T. sordida

T. sordida has been early recognized as a highly variable single species (Lent and Wygodzinsky 1979). However, some studies performed in the '90s started to point out the possible existence of at least two cryptic species within *T. sordida*. Significant differences in the autosomal C-heterochromatin and isoenzymatic markers, like those reported for recently diverging species, were detected between Argentinean and Brazilian populations (Panzera et al. 1997). In addition, isoenzymatic analysis carried out in Bolivian populations revealed the existence of two putative cryptic species, which coexist in the Chaco region (Noireau et al. 1998); hybrid specimens of these two groups were also detected. CGC-MS analysis of CHC obtained from two samples, a population from Argentina and another from Brazil, revealed significant qualitative differences in their CHC profiles (Calderón-Fernández and Juárez 2013). Studies based on chromosomal, genetic, morphologic features, and morphometric measures (Gonzalez-Britez et al. 2014, Panzera et al. 2015, Nattero et al. 2017, Belintani et al. 2020) gave further support to the hypothesis of significant differences between *T. sordida* specimens from Argentina and those from Bolivia, Brazil, and Paraguay, leading to integrative taxonomic analyses that described *T. sordida* from Argentina as a new species named *T. rosai* (Alevi et al. 2020).

Our current work analyzing samples of *T. sordida* and *T. rosai* shows that most of the specimens formed two CHC-based groups, as follows:

- Group 1 includes *T. sordida* specimens distributed in Brazil, Central and Eastern Paraguay, and part of the Bolivian Chaco; it shows some coincidence with the so-called *T. sordida* Eastern Paraguay (Gonzalez-Britez et al. 2014), *T. sordida* sensu stricto (Panzera et al. 2015), *T. sordida* from Brazil (Belintani et al. 2020), and *T. sordida* Brazil and Bolivia (Nattero et al. 2017).
- Group 2 includes *T. rosai*, and *T. sordida* specimens from Western Paraguay and some parts of Bolivia; it shows some coincidence with the taxa *T. sordida* Western Paraguay

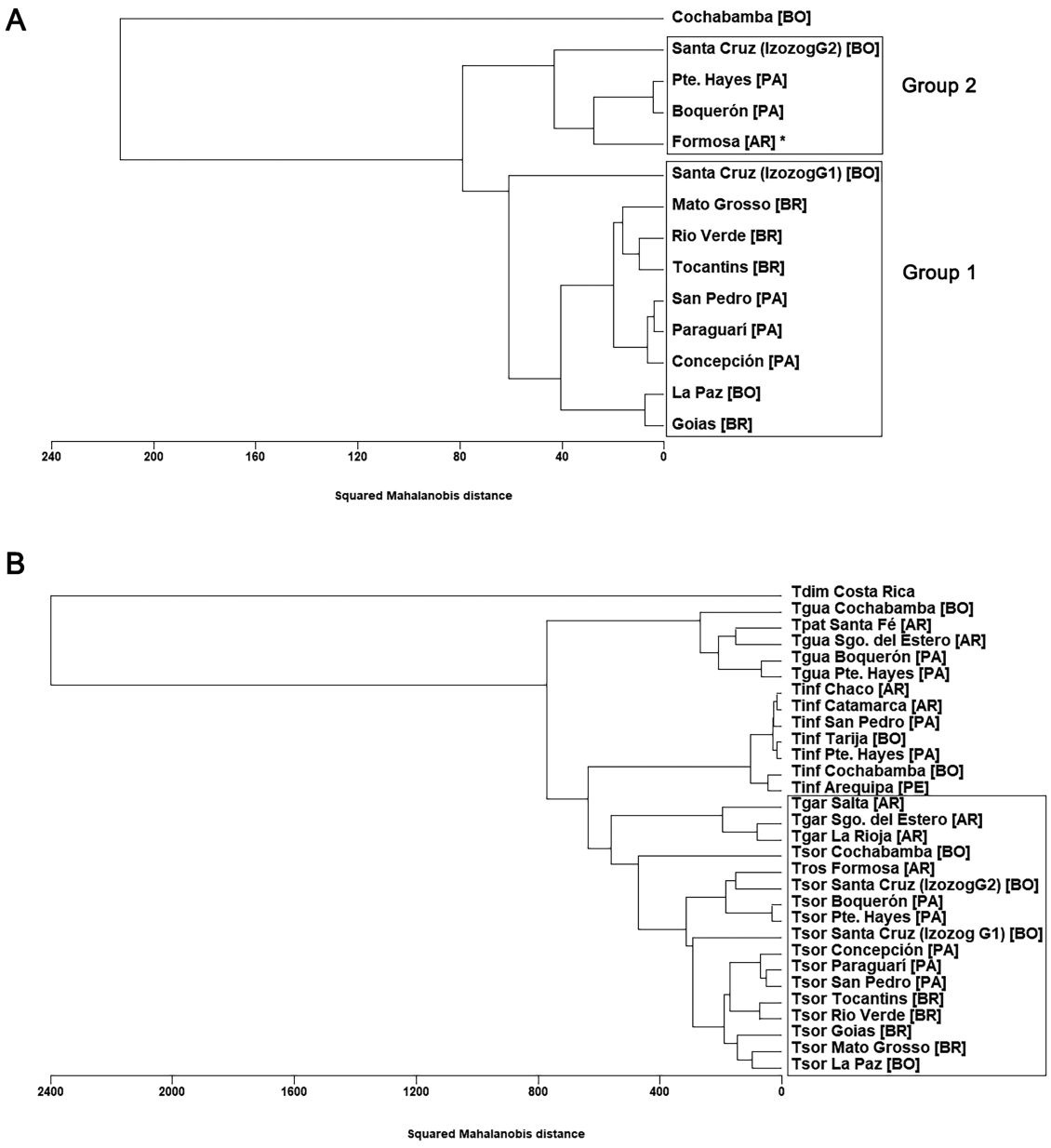

Fig. 1. Mahalanobis distance unweighted pair-group method with arithmetic average tree based on discriminant analyses of *T. sordida* and *T. rosai* populations (A), all the species and populations analyzed in this study (B). Rectangles in (A): Most of *T. sordida* populations clustered in two major CHC-based groups, Group 1 and Group 2; group names are given following the original nomenclature by Noireau et al. (1998). *T. rosai* from Formosa, Argentina (*) is included in Group 2. Rectangle in (B): *T. sordida* subcomplex. Uppercase initials in brackets indicate population's country of origin (AR, Argentina; BO, Bolivia; BR, Brazil; PA, Paraguay; PE, Peru). Abbreviations: Tros, *T. rosai*; *Tsor*, *T. sordida*; Tgar, *T. garciabesi*; Tgua, *T. guasayana*; Tpat, *T. patagonica*; Tinf, *T. infestans*; Tdim, *T. dimidiata*; Pte., Presidente; Sgo., Santiago.

(Gonzalez-Britez et al. 2014), *T. sordida* from Bolivia (Belintani et al. 2020), and *T. sordida* Argentina (Panzera et al. 2015, Nattero et al. 2017, Belintani et al. 2020), the latter now described as a new species, *T. rosai* (Alevi et al. 2020).

Panzera et al. (2015) suggested that *T. sordida* samples from Western Paraguay and those identified as Group 2 by isoenzymatic studies (Noireau et al. 1998) were misclassified specimens of *T. garciabesi*. Thus, they extended the distribution of this species to Western Paraguay and the Bolivian Chaco. However, our analysis did not show agreement with that result, as many significant differences were detected in the CHC profile between *T. sordida* from Western Paraguay and Bolivia, and *T. garciabesi* (Table 2), and this is reflected in the classification results and the dendrogram derived from our multivariate analyses. Interestingly, one of these controversial specimens was further classified as *T. sordida* by Belintani et al. (2020). A multidisciplinary study using the same samples may help elucidate this discrepancy.

The CHC series of internally branched odd chain trimethyl isomers from C31 to C39 (KI 3183, KI 3383, KI 3581, and KI 3981), which starts from the same single precursors (internally branched trimethyl isomers of C29), seems to have differentially evolved to distinguish between these two CHC-based groups. The CHC 3-methyl C31 (KI 3175) also contributes to this differentiation (Table 2, Fig. 1A). The *T. sordida* population from Cochabamba showed a large differentiation (Fig. 1A) compared to the other populations of the

species, suggesting it might be part of another Group to be identified. This differentiation is based mostly on the relative amounts of the internally branched trimethyl isomers of C33 (KI 3383), 3-methyl C31 (KI 3175), and 4-methyl C32 (KI 3258) hydrocarbons, but it is also based on its overall more complex hydrocarbon pattern, with larger relative abundance of high molecular weight methyl-branched hydrocarbons than other *T. sordida* populations. The CHC pattern of this population is somewhat similar to that of *T. garciabesi*, a species associated with a dry climate, and molecular studies on the biological role of methyl-branched hydrocarbons in triatomines have shown that they are related to an increased desiccation tolerance (Moriconi et al. 2019, Dulbecco et al. 2020). Thus, the CHC pattern of this population could be an evolutive adaptation to the higher altitude and drier climate of Western Bolivia. Interestingly, a third putative cryptic species was proposed after studying the chromosomal features of nine specimens collected in La Paz, Bolivia (Panzera et al. 2015).

Our CHC analysis based on Mahalanobis distances failed in separating *T. rosai* from the other *T. sordida* populations clustered in Group 2 (Fig. 1A and B). This could be related to using a single population of this recently described species for CHC analysis, as something somewhat similar happened when we analyzed the interspecific relationship of *T. patagonica* and *T. guasayana* (see below).

T. garciabesi

After its original description (Carcavallo et al. 1967), *T. garciabesi* was synonymized with *T. sordida* by Lent and Wygodzinsky (1979), being considered as a darker and smaller form found in Northwestern Argentina. However, the species was later revalidated based on morphological and cytogenetic characters (Jurberg et al. 1998). *T. garciabesi* showed a more complex hydrocarbon profile in comparison to the other species of the subcomplex, especially in its larger number of hydrocarbon components (Calderón-Fernández and Juárez 2013). The discriminant analysis of the whole subcomplex reflects that this species has a level of differentiation in their CHC compatible with its classification as a distinct species (Fig. 1B). This differentiation is based mostly on the relative abundance of the 7,13,17-trimethyl C29 (KI 2993), 3-methyl C27 (KI 2775), 2,6,10-trimethyl C36 (KI 3727), and trimethyl isomers of C39 (KI 3981) hydrocarbons (Table 2). In addition, species reclassification using the cross-validation method evidenced some overlapping between *T. garciabesi* populations from Santiago del Estero and La Rioja, but no overlapping with specimens of the other species was observed (Supp Table S4 [online only]), which gives support to its specific status. Recent chromosomal, molecular, morphological, and morphometric analysis have contributed to clearly differentiate *T. garciabesi* from *T. sordida*, *T. rosai*, and other species of the *T. sordida* subcomplex (Panzera et al. 2015; Nattero et al. 2017; Alevi et al. 2020; Belintani et al. 2020, 2021).

T. guasayana and *T. patagonica*

Our previous study on CHC identification showed that both species have a very similar hydrocarbon profile, and almost no qualitative differences are evident between them (Calderón-Fernández and Juárez 2013). Current multivariate analysis, using a larger sample size and more populations, shows a complete inclusion of *T. patagonica* within the group formed by *T. guasayana* populations (Fig. 1B). Furthermore, after reclassifying the specimens by cross-validation, the single overlapping between species was detected for *T. guasayana* and *T. patagonica* (Supp Table S4 [online only]). These results might suggest that the taxonomic distinction of these two species should be reconsidered. Early studies based on body morphometrics and antennal sensilla pattern had shown an incomplete separation between *T. patagonica* and *T. guasayana* (Gorla et al. 1993), and more recent head-and-wing morphometric analyses resulted in about 6% to 14% of the *T. patagonica* specimens misclassified as *T. guasayana* (Nattero et al. 2017). However, our results should be taken cautiously as only a single *T. patagonica* population has been analyzed, which might affect the usefulness of CHC to discriminate between species. In addition, a recent comprehensive work made on this species showed that it has a large phenotypic variation along its distribution (Nattero et al. 2016). Early chromosomal analyses provided a good differentiation of these two species, although they showed a closer relationship of *T. patagonica* with *T. guasayana* than with *T. sordida* (Panzera et al. 1997), and more recent molecular, morphometric, and morphological studies of insects and their eggs clearly identified *T. patagonica* as a distinct species (Belintani et al, 2020, 2021).

Regarding the relationship of these two species with *T. sordida* and *T. garciabesi*, previous CHC identification has shown that they have a much simpler hydrocarbon profile, characterized by the lack of several components detected in the latter two species (Calderón-Fernández and Juárez 2013). Present work shows that *T. guasayana* and *T. patagonica* do not have a close relationship with *T. sordida* and *T. garciabesi*. The 5,15- and 5,17-dimethyl isomers of C39 (within the peak KI 3,981), 3-methyl C33 (KI 3375), and dimethyl isomers of C37 (KI3761), are the hydrocarbons that best contribute to this result (Table 2), although the overall CHC profile is very different in these species, compared to that of *T. sordida*, *T. rosai*, and *T. garciabesi*. In contrast, there is a qualitative similarity between *T. sordida*, *T. rosai*, *T. garciabesi* and *T. infestans*, based on the amounts of mono, di, tri, and tetramethyl components with the first methyl group inserted in position 4 of the linear carbon chain together with other components, suggesting a common ancestor for these four species (Juárez et al. 2007). Recent work from our lab has shown that the presence of specific hydrocarbons in the triatomine's cuticle depends on the expression of several integument genes (Moriconi et al. 2019, Dulbecco et al. 2020), reflecting the genetic basis of the triatomine's CHC pattern. Thus, *T. guasayana* and *T. patagonica* should not be included in the *T. sordida* subcomplex together with *T. sordida* and *T. garciabesi*, as it has been reflected by recent results applying other taxonomic markers (Panzera et al. 2015, Pita et al. 2016, Belintani et al. 2020). In fact, based on phylogenetic systematics, it is currently considered that the *T. sordida* subcomplex is a monophyletic group composed of the species *T. sordida*, *T. garciabesi*, *T. rosai*, *T. jurbergi* (Carcavallo et al., 1998), *T. matogrossensis* (Leite and Barbosa, 1953), and *T. vandae* (Carcavallo et al., 2002).

A multidisciplinary study using biochemical, molecular, chromosomal, and morphometric analyses on the same samples may help clarify the questions that still need answers about some aspects of the classification of these species.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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