CytoKey: Identification Key for the Chagas Disease Vectors of the Largest Brazilian Urban Center (São Paulo State), Based on Cytogenetic Data

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Abstract. Chagas disease is caused by the protozoan *Trypanosoma cruzi*. This disease is distributed in 21 Latin American countries, where it is mostly vector-borne. In Brazil, there are 68 triatomine species. To date, the epidemiological surveys indicate that the state of São Paulo presents 11 species of triatomines, and most of these species have already been collected in a home environment and found to be infected with *T. cruzi*. Problems in correctly identifying species can lead to incorrect panorama of distribution of Chagas disease vectors. Thus, we developed an identification key for the triatomines of the state of São Paulo, based on cytogenetic data. With the exception of *Panstrongylus diasi* that does not present cytogenetic data available in the literature, all species were differentiated by cytogenetic characteristics. We emphasize the importance of using this key as a simple and objective tool in the entoepidemiological surveys conducted by the vector control programs.

Chagas disease, also known as American trypanosomiasis, is a potential life-threatening illness caused by the protozoan *Trypanosoma cruzi* (Chagas, 1909).¹ It is estimated that 8 million people are infected worldwide, mostly in Latin America; more than 10,000 people die every year from clinical manifestations of Chagas disease; and more than 25 million people risk acquiring the disease.¹

This neglected disease is distributed in 21 Latin American countries, where it is mostly vector-borne.¹ The main vector involved in the transmission of the parasite to humans is a triatomine bug, also known as “kissing bug,” and vector control remains the most useful method to prevent infection.¹

Presently, there are 153 species of triatomines^{2–4} and all species are considered as potential vectors of the etiologic agent of Chagas disease. In Brazil, there are 68 species distributed in all Brazilian states,^{5–8} grouped in the genera

Panstrongylus (Berg, 1879), *Rhodnius* (Stål, 1859), *Psammolestes* (Bergroth, 1911), *Triatoma* (Laporte, 1832), *Cavernicola* (Usinger, 1944), *Eratyrus* (Stål, 1859), *Microtriatoma* (Prosen and Martínez, 1952), *Alberprosenia* (Martínez and Carcavallo, 1977), and *Belminus* (Stål, 1859). To date, the epidemiological surveys indicate that the state of São Paulo, the largest Brazilian urban center with more than 11 million people,⁹ presents 11 species of triatomines,^{5,10} being that most of the species have already been collected in a home environment and found to be infected with *T. cruzi* (Table 1).

In the past few years, several species of triatomines have been registered in the urban perimeter of the state of São Paulo¹³ and the main form of identification of these vectors occurred on the basis of morphological data.^{13,14} However, problems in correctly identifying species can lead to an incorrect panorama of distribution of Chagas disease vectors as

TABLE 1

List of triatomine species present in the state of São Paulo with information on *Trypanosoma cruzi* infection and collection sites

Species	Infected by <i>T. cruzi</i>	Habitat
<i>Panstrongylus diasi</i> (Pinto and Lent, 1946)	–	Peridomiciliary and intradomiciliary*
<i>Panstrongylus geniculatus</i> (Latreille, 1811)	Yes†	Peridomiciliary and intradomiciliary*†
<i>Panstrongylus megistus</i> (Burmeister, 1835)	Yes†	Peridomiciliary and intradomiciliary†
<i>Psammolestes tertius</i> (Lent and Jurberg, 1965)	–	Wild*
<i>Rhodnius domesticus</i> (Neiva and Pinto, 1923)	No‡	Wild*
<i>Rhodnius neglectus</i> (Lent, 1954)	Yes†	Peridomiciliary and intradomiciliary†
<i>Triatoma infestans</i> (Klug, 1834)	Yes§	Peridomiciliary and intradomiciliary†§
<i>Triatoma rubrofasciata</i> (De Geer, 1773)	–	Peridomiciliary and intradomiciliary*
<i>Triatoma sordida</i> (Stål, 1859)	Yes*	Peridomiciliary and intradomiciliary*
<i>Triatoma tibiamaculata</i> (Pinto, 1926)	Yes‡	Wild*
<i>Triatoma wygodzinskyi</i> (Lent, 1951)	Yes*	Peridomiciliary and intradomiciliary*

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† Rodrigues et al.¹¹

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TABLE 2
Key for the triatomines of the São Paulo state based on cytogenetic data

Identification key (cytokey)	
1. Karyotype with $2n = 21$ chromosomes ($18A + X_1X_2Y$)	<i>Panstrongylus megistus</i>
2. Karyotype with $2n = 25$ chromosomes ($22A + X_1X_2Y$)	<i>Triatoma rubrofasciata</i>
3. Karyotype with $2n = 23$ chromosomes ($20A + X_1X_2Y$)	4
4a. Prophase with heterochromatic blocks dispersed inside the nucleus	<i>Triatoma tibiamaculata</i>
4b. Prophase without heterochromatic blocks dispersed inside the nucleus	<i>Panstrongylus geniculatus</i>
5. Karyotype with $2n = 22$ chromosomes ($20A + XY$)	6
6a. Prophase with heterochromatic blocks dispersed inside the nucleus	7
6b. Prophase without heterochromatic blocks dispersed inside the nucleus	8
7a. Heterochromatin in 3–4 large pairs of autosomes	<i>Triatoma infestans</i>
7b. Heterochromatin in all autosomes	<i>Triatoma sordida</i>
8a. Chromocenter formed by three heterochromatic corpuscles	9
8b. Chromocenter formed by only one heterochromatic corpuscle	10
9a. Absence of heterochromatin in autosomes	<i>Rhodnius neglectus</i>
9b. Presence of heterochromatin in some autosomes	<i>Rhodnius domesticus</i>
10a. 45S rDNA probe located in autosomes	<i>Triatoma wygodzinskyi</i>
10b. 45S rDNA probe located in X and Y chromosomes	<i>Psammolestes tertius</i>

it happened, for example, with *Triatoma arthurneivai* (Lent and Martins, 1940): for more than 40 years, it was believed that this species was present in the state of São Paulo, and Carbajal de la Fuente et al.¹⁵ demonstrated that the species that exists in São Paulo is *Triatoma wygodzinskyi* (Lent, 1951) that was incorrectly identified as *T. arthurneivai* (endemic to the state of Minas Gerais).⁵

Cytogenetic tools are contributing to the taxonomy of triatomines; for example, Alevi et al.¹⁶ recently characterized the prophase cells of *T. arthurneivai* and *T. wygodzinskyi* and differentiated the species easily, highlighting the applicability of the cytological analyzes in the correct identification of the species. Based on this information, we developed an identification key for the triatomines of the state of São Paulo, based on cytogenetic data.

Cytogenetic data available in the literature^{17–23} were grouped and used for the construction of the identification key (which we call CytoKey). From the cytogenetic characteristics (karyotype, constitutive heterochromatin pattern in chromatin and chromosomes, formation of chromocenter, and localization of 45S rDNA probes), identification key was created for triatomines of the São Paulo state (with the exception of *Panstrongylus diasi* (Pinto and Lent, 1946) that does not present cytogenetic data available in the literature) (Table 2).

Thus, from the cytogenetic data, we developed, for the first time, a key identification for the species of the state of São Paulo and we emphasize the importance of using this key as a simple and objective tool in the entomological surveys conducted by the vector control programs.

Received August 20, 2018. Accepted for publication October 24, 2018.

Published online May 13, 2019.

Acknowledgments: This work was financed by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (process number 2013/19764-0), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES) (Finance Code 001).

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