



Description of the diploid chromosome set of *Triatoma pintodiasi* (Hemiptera, Triatominae)

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ABSTRACT. *Triatoma pintodiasi* has been described and recently grouped in the Rubrovaria subcomplex. *T. pintodiasi* was initially compared to *T. carcavalloei* by staining and subsequently identified as *T. circummaculata*. However, after thorough examination, it was observed to be a cryptic species of *T. circummaculata*, and was described based on morphological features, morphometric data, and biochemical patterns of hemolymph. Thus, this paper aims to describe the karyotype of, and spermatogenesis in, *T. pintodiasi*, in order to elucidate the reproductive biology and taxonomy of the species. Sex chromosomes of *T. pintodiasi* formed a heteropyknotic chromocenter, and compaction of chromatin was observed during prophase. However, in contrast to observations in *T. carcavalloei* and *T. circummaculata*, in *T. pintodiasi* it was observed individualization of the sex chromosomes. The diploid chromosome set of the species $2n = 22$ (20A + XY) is described through analysis of metaphases I and II. Initial cytogenetic characteristics of *T. pintodiasi* are described and the observed differences in the chromocenter

are suggested as a possible cytotaxonomic tool. To gain a better understanding of the specific status of this cryptic species, however, we emphasize the need for further cytogenetic, molecular, biological, and biogeographical analysis, in addition to experimental hybrid crosses with other species of the Rubrovaria subcomplex.

Key words: Karyotype; Spermatogenesis; Rubrovaria subcomplex; *Triatoma pintodiasi*

INTRODUCTION

The triatomines are insects of great epidemiological interest. The 148 species currently described (Abad-Franch et al., 2013; Alevi et al., 2013a; Jurberg et al., 2013; Poinar Jr., 2013) are potential vectors of the protozoan *Trypanosoma cruzi*, the etiologic agent of Chagas disease. Triatomines are also of biological interest because their cells have several peculiarities in comparison to cells of other eukaryotes, such as holocentric chromosomes (Panzeria et al., 1996), inverted meiosis for sex chromosomes (Gómez-Palacio et al., 2008), and persistence of nucleolar material during meiosis (Tartarotti and Azeredo-Oliveira, 1999; Alevi et al., 2014a). In addition, triatomines are of evolutionary interest because their origins (monophyletic or polyphyletic) have not been conclusively determined (Tartarotti et al., 2006; Hwang and Weirauch, 2012).

For over 50 years, these hematophagous insects have been grouped into both complexes and specific subcomplexes (de Lucena, 1970; Dujardin et al., 2002; Schofield and Galvão, 2009; Justi et al., 2014). Although species complexes and subcomplexes are not formally recognized as taxonomic ranks, they should be monophyletic (Justi et al., 2014).

Schofield and Galvão (2009) proposed that the Rubrovaria subcomplex is composed of the species *Triatoma carcavalloei*, *T. circummaculata*, *T. klugi*, *T. limai*, *T. oliveirai*, and *T. rubrovaria*. The species of the Rubrovaria subcomplex were initially grouped through morphological analyses: *T. rubrovaria* and *T. carcavalloei* categorized in the Rubrovaria subcomplex; *T. circummaculata* and *T. limai* in the Circummaculata complex; and *T. klugi* in the Oliverai complex (Dujardin et al., 2002). Almeida et al. (2009) grouped *T. circummaculata* in subcomplex Rubrovaria based on phylogenetic and molecular data, and Gardim et al. (2013) and Justi et al. (2014) identified the subcomplex Rubrovaria as monophyletic. However, new approaches, such as experimental hybrid crosses and cytogenetic analyzes, are important and necessary to assist in understanding the evolutionary development of these vectors.

A new species, *T. pintodiasi*, has recently been described and grouped in Rubrovaria subcomplex (Jurberg et al., 2013). *T. pintodiasi* was initially compared to *T. carcavalloei* based on staining, but was found to be morphologically smaller. Subsequently, *T. pintodiasi* was classified as *T. circummaculata*. However, after thorough examination, it was observed to be a cryptic species of *T. circummaculata*, and was described based on morphological features, morphometric data, and biochemical patterns of hemolymph (Jurberg et al., 2013). The process of spermatogenesis and the karyotype of *T. pintodiasi* are described in the present study, with the aim of acquiring a detailed understanding of the reproductive biology and taxonomy of the species. In addition, a chromosomal review of all triatomine species is presented with karyotype descriptions.

MATERIAL AND METHODS

At least three adult males each of *T. pintodiasi*, *T. carcavalloei*, and *T. circummaculata* were analyzed. The specimens of *T. pintodiasi* were provided by the National Laboratory and International Reference on Taxonomy of Triatominae, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil, and the species of *T. carcavalloei* and *T. circummaculata* were provided by Insectarium of Triatominae, FCFAR/UNESP, Araraquara, São Paulo, Brazil. Seminiferous tubules of *T. pintodiasi*, *T. carcavalloei*, and *T. circummaculata* were isolated, shredded, smashed, and set on a slide in liquid nitrogen. They were then stained by the cytogenetic technique using lacto-acetic orcein as outlined by De Vaio et al. (1985), with modifications according to Alevi et al. (2012a). The biological material was analyzed by a Jenaval light microscope (Zeiss) coupled to a digital camera and an image analyzer Axio Vision LE 4.8 (Copyright© 2006-2009 Carl Zeiss Imaging Solutions GmbH). The images were subjected to 1000X magnification. All karyotypes described in the literature are presented in Table 1.

RESULTS

The early stages of meiosis in spermatogenesis were described by means of classical cytogenetic analysis. Compaction of chromatin (Figure 1A-C and 2A) was observed during prophase, with the formation of chiasma between autosomes (Figure 1C).

The sex chromosomes of *T. pintodiasi* formed a heteropyknotic chromocenter (Figure 1A-C and 2A); however, individualization remained throughout prophase. This observation was not consistent with that for *T. carcavalloei* (Figure 2B) and *T. circummaculata* (Figure 2C). The diploid chromosome set of the species $2n = 22$ ($20A + XY$) (Figure 1C and D, respectively), was determined by analysis of metaphases I and II. In both metaphases I and II, the Y sex chromosomes of *T. pintodiasi* were larger and more heteropyknotic (Figure 1D and E; arrows).

Analysis of the species presented in Table 1, revealed that two species have 21 chromosomes ($2n = 18 + X_1X_2Y$); 51 species have 22 chromosomes ($2n = 20 + XY$); 30 species have 23 chromosomes ($2n = 20 + X_1X_2Y$); four species have 24 chromosomes ($2n = 20 + X_1X_2X_3Y$); and only one species has 25 chromosomes ($2n = 22 + X_1X_2Y$).

DISCUSSION

In cryptic speciation, the resulting species show great morphological similarity. Specific tools are necessary to differentiate between cryptic species. It was possible to distinguish the sex chromosomes in a heteropyknotic chromocenter during meiotic prophase in *T. pintodiasi*. This feature is quite peculiar in the subfamily Triatominae, since most triatomines have only one heteropyknotic chromocenter, as observed in *T. carcavalloei* and *T. circummaculata* (Panzera et al., 1998).

T. pintodiasi presented a karyotype consisting of 22 chromosomes, consistent with that observed for all species of the Rubrovaria subcomplex (Ueshima, 1966; Panzera et al., 1996; Alevi et al., 2013a). Although this number can vary from 21 to 25 chromosomes, 22 has been described as the modal number of chromosomes for the Triatominae subfamily (Ueshima, 1966) (Table 1). Ueshima (1966) also proposed that Triatominae has 22 chromosomes ($20A + XY$).

Table 1. Review of all triatomine species with karyotype descriptions.

No.	Triatomines	Karyotype	Described by:
	Tribe Alberproseniini		
	Genus <i>Alberprosenia</i>		
1	<i>Alberprosenia goyovargasi</i>	Not described	
2	<i>Alberprosenia malheiroi</i>	Not described	
	Tribe Bolboderini		
	Genus <i>Belminus</i>		
3	<i>Belminus corredori</i>	Not described	
4	<i>Belminus costaricensis</i>	Not described	
5	<i>Belminus ferroae</i>	Not described	
6	<i>Belminus herreri</i>	Not described	
7	<i>Belminus laportei</i>	Not described	
8	<i>Belminus peruvianus</i>	Not described	
9	<i>Belminus pittieri</i>	Not described	
10	<i>Belminus rugulosus</i>	Not described	
	Genus <i>Bolbodera</i>		
11	<i>Bolbodera scabrosa</i>	Not described	
	Genus <i>Microtriatoma</i>		
12	<i>Microtriatoma borbai</i>	Not described	
13	<i>Microtriatoma trinidadensis</i>	Not described	
	Genus <i>Parabelminus</i>		
14	<i>Parabelminus carioaca</i>	Not described	
15	<i>Parabelminus yurupucu</i>	Not described	
	Tribe Cavernicolini		
	Genus <i>Cavernicola</i>		
16	<i>Cavernicola lenti</i>	Not described	
17	<i>Cavernicola pilosa</i>	Not described	
	Tribe Linshcosteimi		
	Genus <i>Linshcosteus</i>		
18	<i>Linshcosteus carnifex</i>	Not described	
19	<i>Linshcosteus chota</i>	Not described	
20	<i>Linshcosteus confimus</i>	Not described	
21	<i>Linshcosteus costalis</i>	Not described	
22	<i>Linshcosteus kali</i>	Not described	
23	<i>Linshcosteus karupus</i>	Not described	
	Tribe Rhodniini		
	Genus <i>Psammolestes</i>		
24	<i>Psammolestes arthuri</i>	Not described	
25	<i>Psammolestes coreodes</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
26	<i>Psammolestes tertius</i>	2n = 22 (20A + XY)	Panzera et al., 1998
	Genus <i>Rhodnius</i>		
27	<i>Rhodnius amazonicus</i>	Not described	
28	<i>Rhodnius barretti</i>	Not described	
29	<i>Rhodnius brethesi</i>	2n = 22 (20A + XY)	Panzera et al., 1998
30	<i>Rhodnius colombiensis</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
31	<i>Rhodnius dalessandroi</i>	Not described	
32	<i>Rhodnius domesticus</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
33	<i>Rhodnius ecuadoriensis</i>	2n = 22 (20A + XY)	Panzera et al., 1998
34	<i>Rhodnius milesi</i>	2n = 22 (20A + XY)	Panzera et al., 2010
35	<i>Rhodnius montenegrensis</i>	2n = 22 (20A + XY)	Alevi et al., 2015a
36	<i>Rhodnius nasutus</i>	2n = 22 (20A + XY)	Pérez et al., 1992
37	<i>Rhodnius neglectus</i>	2n = 22 (20A + XY)	Barth, 1956
38	<i>Rhodnius neivai</i>	2n = 22 (20A + XY)	Koshy, 1979a
39	<i>Rhodnius pallescens</i>	2n = 22 (20A + XY)	Panzera et al., 1996
40	<i>Rhodnius paraensis</i>	Not described	
41	<i>Rhodnius pictipes</i>	2n = 22 (20A + XY)	Koshy, 1979b
42	<i>Rhodnius prolixus</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
43	<i>Rhodnius robustus</i>	2n = 22 (20A + XY)	Koshy, 1979b
44	<i>Rhodnius stali</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
45	<i>Rhodnius zeledoni</i>	Not described	

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Table 1. Continued.

No.	Triatomines	Karyotype	Described by:
	Tribe Triatomini		
	Genus <i>Dipetalogaster</i>		
46	<i>Dipetalogaster maxima</i>	2n = 22 (20A + XY)	Ueshima, 1966
	Genus <i>Eratyrus</i>		
47	<i>Eratyrus cuspidatus</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
48	<i>Eratyrus mucronatus</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
	Genus <i>Hermanlentia</i>		
49	<i>Hermanlentia matsunoi</i>	Not described	
	Genus <i>Meccus</i>		
50	<i>Meccus bassolsae</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
51	<i>Meccus longipennis</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 1996
52	<i>Meccus mazzottii</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 1996
53	<i>Meccus pallidipennis</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
54	<i>Meccus phyllosomus</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
55	<i>Meccus picturatus</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 1996
	Genus <i>Mepraia</i>		
56	<i>Mepraia gajardoii</i>	2n = 23 (20A + X ₁ X ₂ Y)	Frias et al., 1998
57	<i>Mepraia parapatrica</i>	2n = 23 (20A + X ₁ X ₂ Y)	Frias-Lasserre, 2010
58	<i>Mepraia spinolai</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 1998
	Genus <i>Nesotriatoma</i>		
59	<i>Nesotriatoma bruneri</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 2010
60	<i>Nesotriatoma flavida</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
61	<i>Nesotriatoma obscura</i>	Not described	
	Genus <i>Paratriatoma</i>		
62	<i>Paratriatoma hirsuta</i>	2n = 22 (20A + XY)	Ueshima, 1966
	Genus <i>Panstrongylus</i>		
63	<i>Panstrongylus chinai</i>	2n = 23 (20A + X ₁ X ₂ Y)	Crossa et al., 2002
64	<i>Panstrongylus diasi</i>	Not described	
65	<i>Panstrongylus geniculatus</i>	2n = 23 (20A + X ₁ X ₂ Y)	Crossa et al., 2002
66	<i>Panstrongylus guentheri</i>	Not described	
67	<i>Panstrongylus hispaniolae</i>	Not described	
68	<i>Panstrongylus howardi</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 2010
69	<i>Panstrongylus humeralis</i>	Not described	
70	<i>Panstrongylus lenti</i>	Not described	
71	<i>Panstrongylus lignarius</i>	2n = 23 (20A + X ₁ X ₂ Y)	Crossa et al., 2002
72	<i>Panstrongylus lutzii</i>	2n = 24 (20A + X ₁ X ₂ X ₃ Y)	Santos, 2010
73	<i>Panstrongylus megistus</i>	2n = 21 (18A + X ₁ X ₂ Y)	Schreiber and Pellegrino, 1950
74	<i>Panstrongylus mitarakaensis</i>	Not described	
75	<i>Panstrongylus rufotuberculatus</i>	2n = 23 (20A + X ₁ X ₂ Y)	Crossa et al., 2002
76	<i>Panstrongylus tupyngambai</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzer et al., 1998
	Genus <i>Triatoma</i>		
77	<i>Triatoma amicitiae</i>	Not described	
78	<i>Triatoma arthurneivai</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
79	<i>Triatoma baratai</i>	2n = 22 (20A + XY)	Alevi et al., 2015b
80	<i>Triatoma barberi</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
81	<i>Triatoma bolivari</i>	Not described	
82	<i>Triatoma boliviana</i>	Not described	
83	<i>Triatoma bouvieri</i>	Not described	
84	<i>Triatoma braiilovskyi</i>	Not described	
85	<i>Triatoma brasiliensis</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
86	<i>Triatoma b. macromelanosoma</i>	2n = 22 (20A + XY)	Panzer et al., 2000
87	<i>Triatoma breyeri</i>	Not described	
88	<i>Triatoma carcavalloii</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
89	<i>Triatoma carrioni</i>	Not described	
90	<i>Triatoma cavernicola</i>	Not described	
91	<i>Triatoma circummaculata</i>	2n = 22 (20A + XY)	Panzer et al., 1998
92	<i>Triatoma costalimai</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
93	<i>Triatoma deaneorum</i>	Not described	
94	<i>Triatoma delponteii</i>	2n = 22 (20A + XY)	Ueshima, 1966

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Table 1. Continued.

No.	Triatomines	Karyotype	Described by:
95	<i>Triatoma dimidiata</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzera et al., 1996
96	<i>Triatoma dispar</i>	Not described	
97	<i>Triatoma eratyrsiformis</i>	2n = 24 (20A + X ₁ X ₂ X ₃ Y)	Ueshima, 1966
98	<i>Triatoma garciabesi</i>	2n = 22 (20A + XY)	Panzera et al., 1997
99	<i>Triatoma gerstaeckeri</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
100	<i>Triatoma gomeznunezi</i>	Not described	
101	<i>Triatoma guasayana</i>	2n = 22 (20A + XY)	Panzera et al., 1996
102	<i>Triatoma guazu</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
103	<i>Triatoma hegneri</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
104	<i>Triatoma incrassata</i>	Not described	
105	<i>Triatoma indictiva</i>	Not described	
106	<i>Triatoma infestans</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
107	<i>Triatoma i. melanosoma</i>	2n = 22 (20A + XY)	Panzera et al., 1996
108	<i>Triatoma jatai</i>	Not described	
109	<i>Triatoma juazeirensis</i>	2n = 22 (20A + XY)	Panzera et al., 2000
110	<i>Triatoma jurbergi</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
111	<i>Triatoma klugi</i>	2n = 22 (20A + XY)	Costa et al., 2008
112	<i>Triatoma lecticularia</i>	2n = 22 (20A + XY)	Ueshima, 1966
113	<i>Triatoma lenti</i>	2n = 22 (20A + XY)	Alevi et al., 2012b
114	<i>Triatoma leopoldi</i>	Not described	
115	<i>Triatoma limai</i>	Not described	
116	<i>Triatoma maculata</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
117	<i>Triatoma matogrossensis</i>	2n = 22 (20A + XY)	Crossa et al., 1992
118	<i>Triatoma melanica</i>	2n = 22 (20A + XY)	Panzera et al., 2000
119	<i>Triatoma melanocephala</i>	2n = 24 (20A + X ₁ X ₂ X ₃ Y)	Alevi et al., 2012b
120	<i>Triatoma mexicana</i>	2n = 23 (20A + X ₁ X ₂ Y)	Panzera et al., 2010
121	<i>Triatoma migrans</i>	Not described	
122	<i>Triatoma neotomae</i>	Not described	
123	<i>Triatoma nigromaculata</i>	Not described	
124	<i>Triatoma nitida</i>	2n = 21 (18A + X ₁ X ₂ Y)	Schreiber and Pellegrino, 1950
125	<i>Triatoma oliveirai</i>	Not described	
126	<i>Triatoma patagonica</i>	2n = 22 (20A + XY)	Ueshima, 1966
127	<i>Triatoma peninsularis</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
128	<i>Triatoma petrochiae</i>	2n = 22 (20A + XY)	Panzera et al., 2000
129	<i>Triatoma pintodiasi</i>	2n = 22 (20A + XY)	Present study
130	<i>Triatoma platensis</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
131	<i>Triatoma protracta</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
132	<i>Triatoma pugasi</i>	Not described	
133	<i>Triatoma pseudomaculata</i>	2n = 22 (20A + XY)	Schreiber et al., 1972
134	<i>Triatoma recurva</i>	Not described	
135	<i>Triatoma rubida</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
136	<i>Triatoma rubrofasciata</i>	2n = 25 (22A + X ₁ X ₂ Y)	Manna, 1950
137	<i>Triatoma rubrovaria</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
138	<i>Triatoma ryckmani</i>	2n = 23 (20A + X ₁ X ₂ Y)	Dujardin et al., 2002
139	<i>Triatoma sanguisuga</i>	2n = 23 (20A + X ₁ X ₂ Y)	Payne, 1909
140	<i>Triatoma sherlocki</i>	2n = 22 (20A + XY)	Panzera et al., 2010
141	<i>Triatoma sinaloensis</i>	2n = 23 (20A + X ₁ X ₂ Y)	Ueshima, 1966
142	<i>Triatoma sinica</i>	Not described	
143	<i>Triatoma sordida</i>	2n = 22 (20A + XY)	Schreiber and Pellegrino, 1950
144	<i>Triatoma tibiamaculata</i>	2n = 22 (20A + XY)	Panzera et al., 1998
145	<i>Triatoma vandae</i>	2n = 22 (20A + XY)	Panzera et al., 2010
146	<i>Triatoma venosa</i>	Not described	
147	<i>Triatoma vitticeps</i>	2n = 24 (20A + X ₁ X ₂ X ₃ Y)	Schreiber and Pellegrino, 1950
148	<i>Triatoma williami</i>	2n = 22 (20A + XY)	Dujardin et al., 2002
149	<i>Triatoma wygodzinskyi</i>	2n = 22 (20A + XY)	Panzera et al., 2012
150	<i>Triatoma dominicana</i>	Not described	

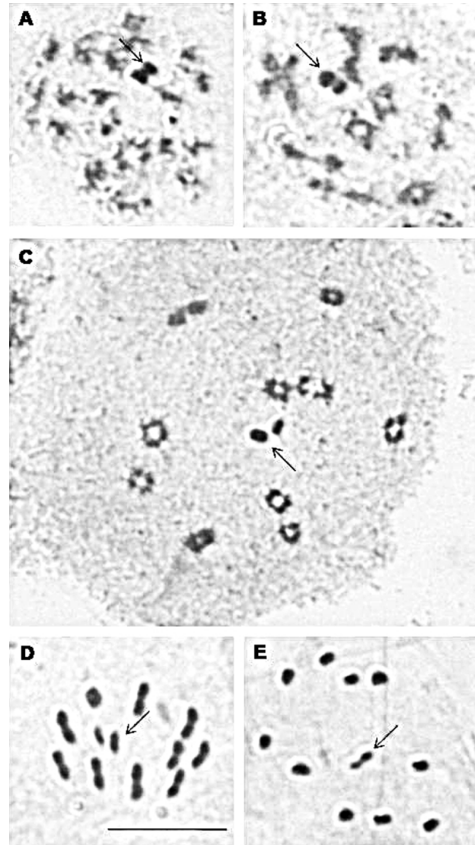


Figure 1. Meiosis of *Triatoma pintodiasi*. Diffuse intermediate stage (zygotene/pachytene) (A and B); diffuse final stage (diplotene) (C) with chromocenter that made it possible to distinguish the two sex chromosomes (arrows). Metaphase I (D) and metaphase II (E) with 22 chromosomes (20 autosomes + XY). Note the more heteropyknotic Y sex chromosome (arrows). Bar: 10 μ m.

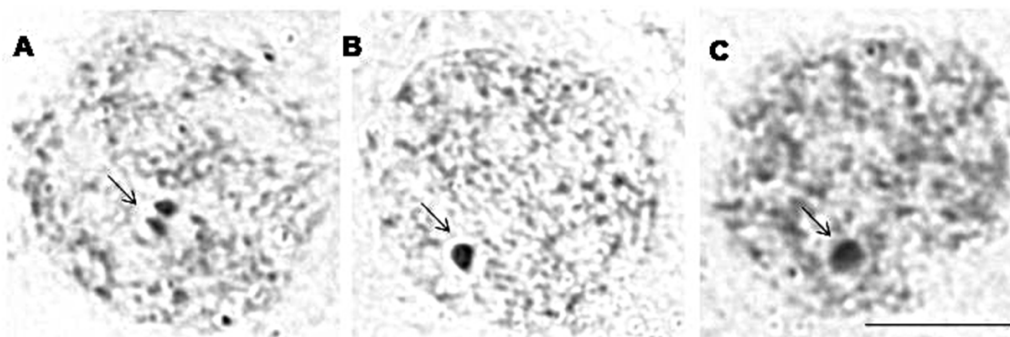


Figure 2. Prophase (initial diffuse stage) of *Triatoma pintodiasi* (A), *T. carvalhoi* (B), and *T. circummaculata* (C). Note that *T. pintodiasi* presented a chromocenter that made it possible to distinguish the two sex chromosomes (A; arrow), while *T. carvalhoi* (B; arrow), and *T. circummaculata* (C; arrow) showed a single chromocenter formed by the sex chromosomes. Bar: 10 μ m.

Ueshima (1979) suggested that the common ancestor of the triatomines showed 22 chromosomes. In addition, Nokkala and Nokkala (1983, 1984) believe that the common ancestor of the Hemiptera order had system sex determination of type XY. Thus, based on the principle that the common ancestor has 22 chromosomes, we suggest that major events that occurred during the karyotype evolution of these insects included mainly agmatoploidy (fission) and simploidy (fusion).

Cytogenetic analyses of triatomines are of great importance, because chromosome data, characteristics of spermatids, and meiotic features can be applied to determine cytotaxonomy of these vectors. Analysis of early prophase for example, can aid in differentiation between *T. sordida* and *T. guasayana* (Rebagliati et al., 1998). Cytogenetic data have also assisted in the revalidation of *T. garciabesi* (Jurberg et al., 1998); in the description of *Mepraia parapatrica* (Frias-Lasserre, 2010); and more recently, by analysis of spermatids, in the differentiation of morphologically related species (Alevi et al., 2013b, 2014b).

The present study describes initial cytogenetic characteristics of *T. pintodiasi* and suggests differences in the chromocenter, as a possible cytotaxonomic tool. The authors emphasize the need for further cytogenetic, molecular, biological, and biogeographical analysis, in addition to experimental hybrid crosses with other species of the Rubrovaria subcomplex.

Conflicts of interest

The authors declare no conflict of interest.

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