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Chromosomal variation and genome size support existence of cryptic species of *Triatoma dimidiata* with different epidemiological importance as Chagas disease vectors

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Summary The wide geographical distribution of *Triatoma dimidiata*, one of the three major vectors of Chagas disease, ranges from Mexico to northern Peru. Since this species occupies a great diversity of artificial and natural ecotopes, its eradication is extremely difficult. In order to assist control efforts, we used chromosome analyses and DNA amount as taxonomic markers to study genetic variability in populations of *T. dimidiata* from Mexico, Guatemala, El Salvador and Colombia. We differentiated three groups or cytotypes defined by characteristic chromosome C-banding patterns and genome size measured by flow cytometry. The three cytotypes are restricted to different geographic locations. Cytotype 1 occurs in Mexico (excluding Yucatán), Guatemala (excluding Petén), El Salvador and Colombia. Cytotype 2 occurs in Yucatán and cytotype 3 occurs in Petén. Cytotype 1, commonly associated with domestic and peridomestic environments but also inhabiting sylvatic ecotopes, is the most widespread and with major epidemiological significance. In contrast, the Yucatán cytotype inhabits wild ecotopes but increasingly enters houses, while the Petén cytotype appears exclusively sylvatic. We suggest that these cytotypes represent cryptic species of *T. dimidiata* with different epidemiological relevance as Chagas disease vectors. Poor ability to colonize human dwellings, together with their restricted geographic distribution, indicate that the Yucatán and Petén putative species probably have much less epidemiological significance than cytotype 1. Thus, the genetic markers we describe are powerful tools to differentiate cryptic species in *T. dimidiata* with different epidemiological significance, contributing to planning the most effective control measures.

keywords Chagas disease, chromosome variation, genome size, *Triatoma*

Introduction

Chagas disease extends from Mexico to Argentina and affects an estimated 16–18 million people throughout Latin America, with another 100 million at risk for the disease (Dias *et al.* 2002). The protozoan *Trypanosoma cruzi*, causative agent of Chagas disease, is mainly transmitted to humans by blood-sucking insects of the subfamily Triatominae (Hemiptera: Reduviidae). The three major vectors of Chagas disease are *Triatoma infestans, Rhodnius prolixus* and *T. dimidiata*. The latter is distributed from Mexico throughout all countries of Central America, with additional populations in parts of Colombia, coastal regions of Ecuador and northern Peru. It also occupies a wide diversity of habitats, including domestic, peridomestic and sylvatic environments (Lent & Wygodzinsky 1979; Zeledón 1981; Tabaru *et al.* 1998).

Despite substantial progress of the Central American Initiative against Chagas disease (IPCA)(Ponce 1999) in elimination of *R. prolixus*, main vector of Chagas disease in Central America, the control of *T. dimidiata* is more difficult due mainly to its capacity to reinfest treated houses from peridomestic and sylvatic populations (Zeledón 1981; Acevedo *et al.* 2000; Monroy *et al.* 2003; Nakagawa *et al.* 2005). Schofield (2002) suggests that this species should not be considered a feasible candidate for eradication using available methods, and recommends different strategic options for *T. dimidiata* control, depending on their degree of domesticity and the existence of sylvatic populations in the treated areas. The study of *T. dimidiata* population structure and of useful genetic and phenetic population markers are therefore considered a priority (Schofield 2002, 2005).

Triatoma dimidiata represents an assemblage of morphologically variable populations (Zeledón 1981; Schofield 2005). Usinger (1941, 1944) gave subspecific status for some populations, namely *T. dimidiata dimidiata* (Central American forms), *T. dimidiata capitata* (Colombian forms) and *T. dimidiata maculipennis* (some Mexican forms). However, Lent and Wygodzinsky (1979) synonymized these three subspecies, with the observation that analysis of enough specimens made it impossible to morphologically distinguish among them. Recently, population differentiation has been detected using genetic and phenetic markers including rDNA internal transcribed spacer 2 (ITS-2), RAPD-PCR, genital structures, metric variation of head characters, cuticular hydrocarbons and antennal phenotypes (Lent & Jurberg 1985; Marcilla *et al.* 2001; Bargues *et al.* 2002; Dorn *et al.* 2003; Bustamante *et al.* 2004; Calderon *et al.* 2004; Catalá *et al.* 2005; Fernandez *et al.* 2005; Lehmann *et al.* 2005). Even more, some of these data also suggest that *T. dimidiata* may represent a complex of cryptic species (i.e. morphologically indistinguishable, yet reproductively isolated taxa). The analysis of ITS-2 of several populations from Mexico to Ecuador reveals differences consistent with a specific status for populations from the Yucatán peninsula (Mexico) (Marcilla *et al.* 2001) and the cuticular hydrocarbon analyses suggest that a sylvatic population from Lankin (Guatemala) is undergoing a speciation process (Calderon *et al.* 2005).

In this study, we use chromosome analyses and DNA quantification as taxonomic markers to study genetic variability in natural populations of *T. dimidiata* from Mexico, Guatemala, El Salvador and Colombia. The karyotype of *T. dimidiata* is re-described and its genome size is reported for the first time. The methods applied here have been successfully used to detect cryptic species (Panzera *et al.* 1997) and intraspecific variation in other species groups among the Triatominae (Panzera *et al.* 1992, 2004).

Materials and methods

Material analyzed

All specimens came from natural populations. The origin and number of individuals studied for each population as well as the technique applied (cytogenetic or flow cytometry) are shown in Table 1 and Figure 1. In several cases, the same individual was analyzed with both techniques. Besides domestic and peridomestic populations (Table 1) we studied several sylvatic populations from Yucatán (Mexico), Petén (Guatemala), Santa Marta and El Carmen from Colombia. The Yucatán specimens were collected in and around Mérida in the Northern region of the peninsula. The Petén insects were collected from tropical rain forest within the Yaxhá archaeological site in Guatemala. The Colombian sylvatic specimens were collected from *Attalea butyracea* palm trees (Santa Marta) and in domestic environments attracted to the light (El Carmen). Specimens were captured by manual collection and by the use of light tramps.

Cytogenetic studies

Gonads (testes and ovaries) were removed from freshly killed adults, fixed in an ethanol–acetic acid mixture (3:1) and stored at −20 °C. C-banding treatment was carried out on air-dried squashes as described by Pérez *et al.* (1992). This technique was used to observe the distribution and behaviour of C-heterochromatin during mitosis and meiosis. One hundred and sixteen *T. dimidiata* specimens were examined by C-banding (Table 1).

The C-banding pattern for each specimen was determined by analysing at least 100 cells. In males, both mitotic (spermatogonial prometaphase) and meiotic (first and second metaphases) plates were observed. For females, only oogonial prometaphases were studied because no meiotic stages can be detected. In order to describe the chromosomal variation, we considered several cytogenetic markers previously used to differentiate species and populations of triatomines (Panzera *et al.* 1995, 1997, 2004; Pérez *et al.* 1992, 2002). These markers include the relative chromosomal size and the localization, distribution and meiotic behaviour of C-heterochromatin regions in autosomes and sex chromosomes. In order to quantify the autosomal C-heterochromatin, we estimated the relative amount of C-heterochromatin presented in the autosomal complement. For each specimen, at least 3–5 images of gonial metaphases plates were analysed by means of the IPPLUS measurement software (Media Cybernetics Inc., USA). At least five specimens for each cytotype were quantified.

Table 1 Collection sites, ecotope and number of individuals for *T. dimidiata* studied in this paper

Country	Department/ state	Municipality/ locality	Ecotope	Number of individuals	
				Chromosomal studies	Flow cytometry studies
Mexico	San Luis de Potosí	San Antonio	D	10	4
Mexico	San Luis de Potosí	Tancahuitz de Santos	D	1	1
Mexico	Hidalgo	Huejutla	D	10	7
Mexico	Hidalgo	Molango	D	1	2
Mexico	Veracruz	Misantla	P	1	1
Mexico	Veracruz	Atoyac	D	4	3
Mexico	Veracruz	Pajapan	D,P	3	1
Mexico	Veracruz	Tuxpan	P	2	1
Mexico	Veracruz	Jalapa	D	2	2
Mexico	Veracruz	Tepetzintla	P	1	0
Mexico	Veracruz	Panuco	P	2	1
Mexico	Veracruz	Atotac	D	1	1
Mexico	Veracruz	Tlalixcoyan	D	1	0
Mexico	Veracruz	Citlaltepetl	D	1	0
Mexico	Yucatán	Progreso	S	11	2
Mexico	Yucatán	Mérida	S	4	3
Mexico	Yucatán	Mérida	D	0	1
El Salvador	Santa Ana	Monte Largo/ Comecayo	D	13	6
El Salvador	La Unión	El Farito	D	1	0
Guatemala	Petén	Yaxhá, Melchor de Mencos	S	11	6
Guatemala	Jutiapa	San José Acatempa	D,P	3	6
Guatemala	Jutiapa	Carrizal/El Tule	D	10	3
Guatemala	Quiché	San Andrés Sajcabajá,	D,P	7	5
Guatemala	Quiché	Canillá	D	3	3
Colombia	Santa Marta	Tarapacá	S	7	3
Colombia	Sucre	San Onofre	P	3	3
Colombia	Boyacá	Boa Vita	P	0	2
Colombia	Santander	San Joaquín	D	2	3
Colombia	Santander	El Carmen	S	1	4

DNA quantification and statistical analysis

Flow cytometry was used to measure nuclear DNA content in gonad cells from 74 male insects (Table 2) using the procedures described by Panzera *et al.* (2004). For the evaluation of absolute DNA content, normal human lymphocytes fixed in ethanol/acetic acid were used as internal references. To translate relative DNA cell content into picograms of DNA, standard human lymphocytes were considered to have 6.436 pg of DNA per diploid nucleus (2C) (International Human Genome Sequencing Consortium 2001). For calculation of base pair (bp) number, 1 pg of DNA was assumed to represent 0.978×10^9 bp (Dolezel *et al.* 2003).

Means and standard deviations of DNA measurements were calculated (Table 2). One-way ANOVA tests were used for grouping the DNA values for cytotype 1 and for comparing the DNA contents among the three cytotypes. Statistical analyses were performed using SPSS 10.00 software (SPSS Inc., Chicago, IL, USA).

Results

Chromosome complement of *T. dimidiata*

All specimens of *T. dimidiata* have a diploid chromosome number (2n) constituted by 10 pairs of autosomes plus sex chromosomes (X_1X_2Y in males, Figure 2a, and $X_1X_1X_2X_2$ in females, Figure 2b). The karyotype presents little variation in the size of the autosomes, although two or three autosomal pairs are slightly larger than the rest. The Y chromosome is always C-heterochromatic and the X

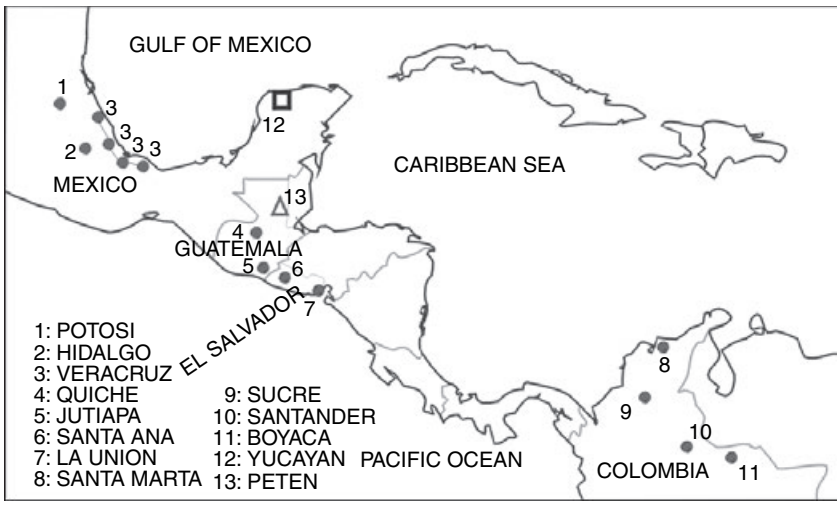

Figure 1 Map of Mexico, Central America and Colombia showing collection sites for *T. dimidiata* populations studied in this paper (see also Table 1).

Table 2 Haploid DNA content (C-value) expressed in picograms (mean values and standard deviation) in several natural populations of *T. dimidiata*, measured by flow cytometry, discriminated by their chromosomal pattern or cytotype and ecotope

Country	State/department	Ecotope	No.	Mean	SD
Cytotype 1					
México	San Luis de Potosí	D	5	0.975	0.037
México	Hidalgo	D	9	0.990	0.035
México	Veracruz	D,P	10	1.011	0.064
Guatemala	Jutiapa	D,P	9	0.974	0.052
Guatemala	Quiché	D,P	8	0.978	0.066
El Salvador	Santa Ana	D	6	0.973	0.061
Colombia	Santander/Boyacá/ Sucre/Santa Marta	D,P,S	15	0.980	0.060
Total cytotype 1			62	0.985	0.055
Cytotype 2					
México	Yucatán	D,S	6	0.844	0.029
Cytotype 3					
Guatemala	Petén	S	6	0.900	0.064

No., number of individuals analysed; SD, standard deviation; D, domestic; P, peridomestic; S, sylvatic.

chromosomes are the smallest of the complement (Figure 2a).

Chromosomal variation in *T. dimidiata*

The following three chromosomal patterns or cytotypes are observed in individuals of *T. dimidiata* from different geographic locations.

Cytotype 1 occurs in Mexican (excluding Yucatán), Guatemalan (excluding Petén), El Salvadorian and Colombian specimens (Figures 2a and 3a–c). It is characterized by the presence in most autosomes of C-heterochromatic dots localized in one or both telomeres (arrows Figure 2a). The C-heterochromatin content comprises around 10% of the total autosomal complement. During male meiotic prophase, the C-dots appear scattered in the prophase nuclei and the three sex chromosomes are associated to form a main heteropycnotic chromocenter (arrowhead Figure 3a). Terminal location of C-dots is clearly seen on the 10 autosomal bivalents during diplotene (arrows Figure 3b). Due to the small size of these heterochromatic dots, they are not clearly detected in the more compact meiotic metaphase chromosomes (Figure 3c) but are observed in gonial mitotic metaphase (arrows Figure 2a). During the second meiotic metaphase, the sex chromosomes appear in the centre of a ring formed by the 10 half bivalents. The Y chromosome is heterochromatic while both X chromosomes are completely euchromatic (Figure 3c).

Cytotype 2 is found in the Yucatán (Mexico) specimens (Figure 3d–f). It is characterized by the absence of

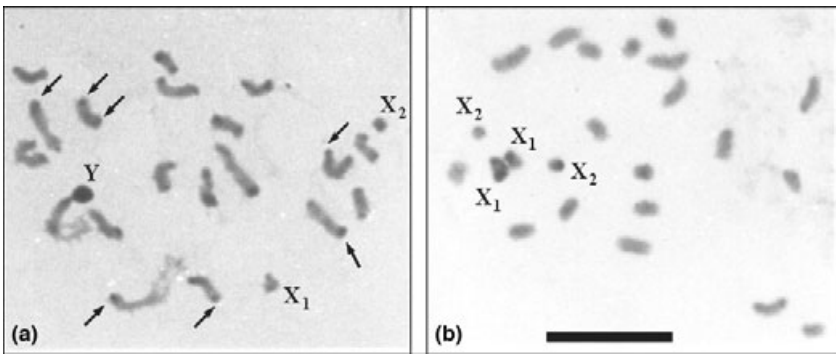

Figure 2 *Triatoma dimidiata*. Gonial mitosis. C-banding technique. Bar = 10 μm. Spermatogonial prometaphase. Cytotype 1. The male has 23 chromosomes (2n = 20 autosomes plus X_1X_2Y sex chromosomes). Most autosomes have C-dots in one or both chromosomal ends (arrows). The Y chromosome is entirely heterochromatic, while the X chromosomes are euchromatic. Oogonial prometaphase. Cytotype 3. The female has 24 chromosomes (2n = 20 autosomes plus $X_1X_1X_2X_2$ sex chromosomes). The autosomes not have C-dots. The X chromosomes in this individual are polymorphic. One X_1 chromosome has two telomeric heterochromatic blocks while the other has a single one. One X_2 chromosome has one telomeric heterochromatic block while the other is completely euchromatic.

heterochromatic regions in all autosomal pairs and in both X chromosomes. During male meiotic prophase there is a single heteropycnotic chromocenter formed by the association of the three sex chromosomes (arrowhead Figure 3d and e). From diplotene to second metaphase it can be clearly observed that only the Y chromosome is heterochromatic (Figure 3e and f).

Cytotype 3 is found in Petén (Guatemala) specimens. It is characterized by the presence of a conspicuous C-block in one or both telomeres of the larger X chromosome (X_1) (Figures 2b and 3g–i). This character differentiates this cytotype from the others and allows its easy identification during any meiotic stage. During early meiotic prophase, a characteristic 'parachute-like' chromocentre, composed by one large heterochromatic region from the Y chromosome and another one from the X_1, is observed (arrowhead Figure 3g). With the exception of one individual that presented a C-block in one autosomal pair (arrow Figure 3i), all individuals from this cytotype show completely euchromatic autosomes (Figure 3h). The X_2 chromosome can be euchromatic (seven individuals)(Figure 3h) or have an evident C-dot at one end (three specimens) (Figure 3i). The heterochromatic polymorphism detected in both X chromosomes is confirmed in the female karyotype shown in Figure 2b.

DNA quantification

DNA measurements were performed on the three identified cytotypes (Table 2). The mean haploid DNA content measured by flow cytometry in 62 specimens of cytotype 1 is 0.985 with a standard deviation (SD) of 0.055 pg. Variance analysis (ANOVA test) of cytotype 1 samples from different geographic regions do not show significant differences ($P > 0.05$) ($F = 0.532$; df = 6,55; $P = 0.781$). The six Yucatán specimens (cytotype 2) have 0.844 ± 0.029 pg. The mean value obtained in six insects of the Petén population (cytotype 3) is 0.900 ± 0.064 pg of DNA per haploid nucleus. The differences among the three cytotypes are statistically significant by ANOVA test ($P < 0.05$) ($F = 23.563$; df = 2,71; $P = 0.0001$).

Discussion

T. dimidiata karyotype

Schreiber and Pellegrino (1950) reported the male karyotype of *T. dimidiata* as constituted by 20 autosomes plus an XY sex chromosomes. However, Panzera *et al.* (1996) described, in insectary material from Mexico, that this species presents 20 autosomes plus X_1X_2Y in males and $X_1X_1X_2X_2$ in females. This number and sex system were confirmed in all individuals analysed here (Figure 2).

All *Triatoma* species from North America (except *T. lecticularia*) have multiple sex chromosomes, while almost all South American species possess an XY system (Dujardin *et al.* 2002). The chromosome number of *T. dimidiata* confirms its closer relationship with North American *Triatoma* species complexes, such as phyllosoma, protracta and flavida. The small amount of autosomal heterochromatin observed in the *T. dimidiata* karyotype is similar to that seen in the species of the phyllosoma and flavida complexes. In contrast, species of the protracta complex have a larger proportion (around 25%) of heterochromatin (unpublished data).

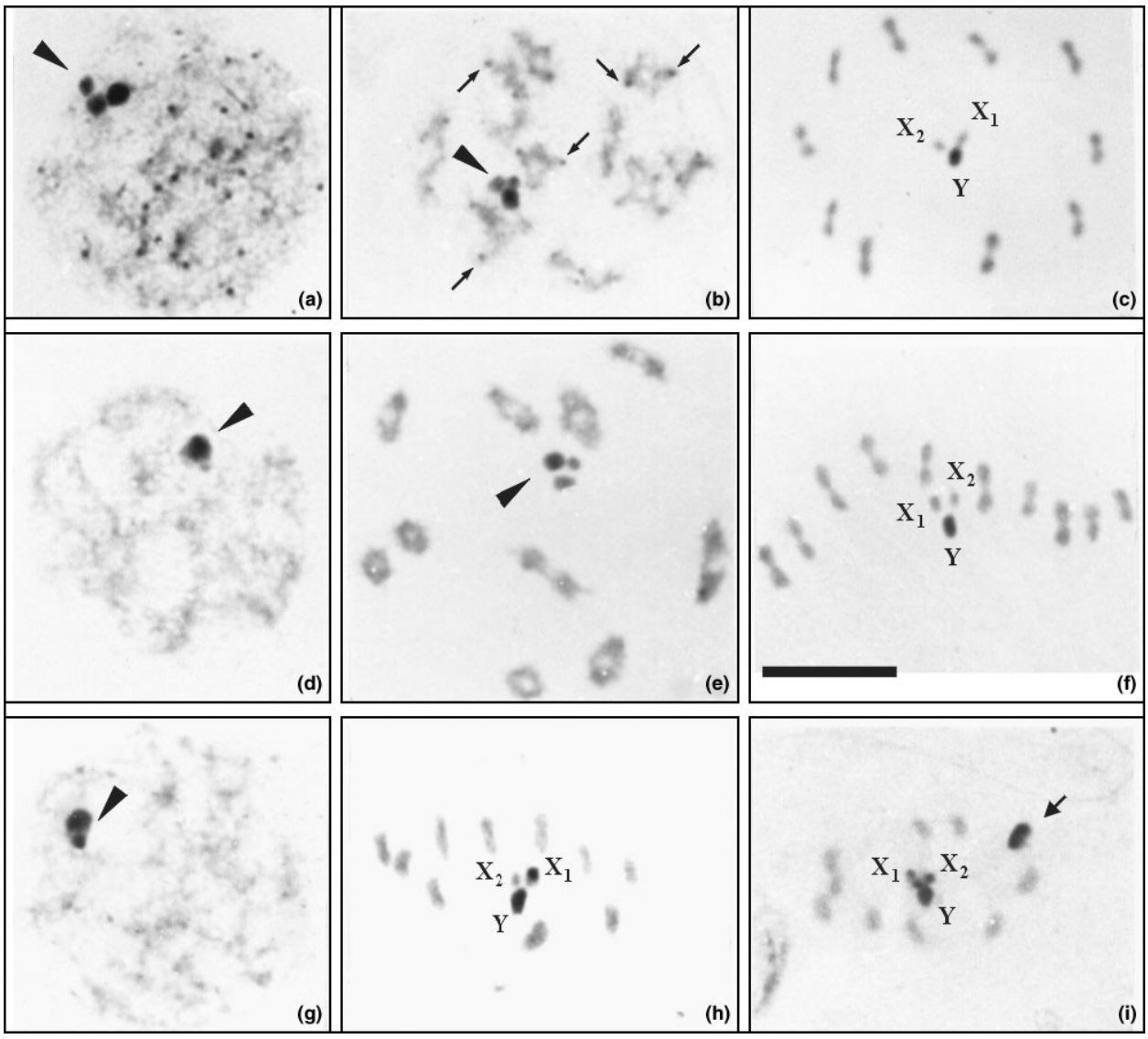

Figure 3 *Triatoma dimidiata*. Male meiosis. C-banding technique. Bar = 10 μm. Cytotype 1. (a): Diffuse stage. One main heterochromatic chromocentre is formed by the association of the three sex chromosomes (arrowhead). Several C-positive dots are also observed scattered throughout the nucleus. (b) Early diplotene stage. Small terminal C-dots are present on some autosomal bivalents (arrows). (c) Second metaphase. The 10 half bivalents form a ring-shape configuration with the three sex chromosomes in the centre. This spatial disposition is characteristic of all triatomine species. The sex chromatids are associated at this stage forming a 'pseudobivalent', which allow them to segregate at anaphase. The euchromatic X_1 and X_2 chromatids will move to one pole and the Y to the other. Cytotype 2. (d): Diffuse stage. The three sex chromosomes are associated with each other forming a single heterochromatic chromocenter (arrowhead). The rest of the nucleus is euchromatic. (e) Diplotene stage. Sex chromosomes remain associated. (f) Second metaphase. Lateral view. Cytotype 3. (g): Diffuse stage. A characteristic chromocentre, like parachute, formed by a big and small darker dot is observed (arrowhead). The autosomal chromatin do not presents C-dots. (h) Second metaphase. All half bivalents (10) are euchromatic. The Y chromosome is heterochromatic, the X_1 chromosome has a conspicuous C-block in one chromosomal end and the X_2 chromosome is euchromatic. (i) Second metaphase: Nine half bivalents are euchromatic and the other has a C-block (arrow). The three sex chromosomes (X_1, X_2 and Y) appear heterochromatic. The X_1 has a C-block in both chromosomal ends while the X_2 chromosome has a C-dot in only one chromosomal end.

The present data are consistent with results of molecular analyses using ITS-2 (Marcilla *et al.* 2001; Bargues *et al.* 2002) and mitochondrial fragments of 12S and 16S rDNA (Hypsa *et al.* 2002; Sainz *et al.* 2004; Paula *et al.* 2005), which supported the North American origin of *T. dimidiata* as well as its relationship with the *T. phyllosoma* complex. This is in disagreement with Carcavallo *et al.* (2000), who argued that *T. dimidiata* differs morphologically from other species in Mexico and the United States and therefore established a separate *T. dimidiata* complex with putative origin in northern South America.

Chromosome variation in *T. dimidiata*

Cytogenetic studies on Triatominae have been performed in order to compare and discriminate species of the same and different genera (Panzera *et al.* 1995, 1998; Pérez *et al.* 1992, 2002) as well as for the detection of intraspecific variation or polymorphism (Panzera *et al.* 1992, 1997, 2004; Pérez *et al.* 2002, 2004). The main source of chromosome polymorphism in Triatominae is the variation in amount, behaviour and position of highly repetitive DNA regions, identified as heterochromatin by C-banding. In some cases, variation of this C-heterochromatin leads to the identification of cryptic species such as *T. sordida* and *T. garciabesi* (Panzera *et al.* 1997; Jurberg *et al.* 1998). In other cases, the taxonomic status of populations with striking chromosomal variation (e.g. *Panstrongylus geniculatus*) remains to be resolved (Pérez *et al.* 2002). Chromosomal variation can also be observed at the intraspecific level. We detected changes in heterochromatin among populations of *T. infestans* that involve autosomes and sex chromosomes (Panzera *et al.* 1992, 2004). However, the chromosomal variation detected herein in *T. dimidiata* is different since we observed the presence or absence of autosomal heterochromatin and a polymorphism in both X chromosomes.

These chromosomal data can be used to identify three discrete groups (cytotypes 1–3) that are restricted to particular geographic areas (Figures 2 and 3). These cytotypes are clearly differentiated in meiotic prophase (compare Figure 3a, d, g). These cell stages are the most abundant in the testes, which allow a rapid diagnosis. More condensed stages (e.g. meiotic metaphases) cannot be used to distinguish between cytotypes 1 and 2. The three cytotypes are distinguished by using three criteria: (1) the presence or absence of C-dots in the autosomes, (2) the C-banding on the X_1 sex chromosome and (3) the nuclear DNA content (Figures 2 and 3, Table 2).

Our results suggest that *T. dimidiata* probably encompasses several cryptic taxa with different epidemiologic relevance. In the following sections we summarize the molecular, phenetic and ecological data that support our hypothesis.

T. dimidiata from Mexico (excluding Yucatán), Guatemala (excluding Petén), El Salvador and Colombia

All individuals of *T. dimidiata* grouped in the cytotype 1 have two distinctive characteristics: they have a high capacity to colonize human environments and are present across a wide geographical area, from Mexico to Colombia.

This cytotype includes domestic, peridomestic and sylvatic individuals characterized by the presence of autosomal C-dots dispersed in most autosomes (Figures 2a and 3a,b). The presence of this autosomal heterochromatin correlates with the higher DNA content observed in individuals from this cytotype (Table 2). The occurrence of cytotype 1 in sylvatic and domestic populations suggests the existence of gene flow between both ecotopes. This hypothesis is supported by RAPD-PCR in Colombian specimens (Ramírez *et al.* 2005), which detect the existence of high gene flow among domestic, peridomestic and sylvatic individuals inhabiting the same area, suggesting that they essentially function as a single panmictic unit.

Cytogenetic features among domestic populations also exhibit a remarkable level of homogeneity. Previous genetic and phonetic studies on these populations indicate a clinal variation along a north-south axis compatible with a subpopulation status (Marcilla *et al.* 2001; Calderon *et al.* 2005; Fernandez *et al.* 2005).

T. dimidiata from Yucatán (Mexico)

Phenetic and genetic markers indicate that the highest levels of divergence appear in insects from the Yucatán State. The analysis of head morphometry (Lehmann *et al.* 2005) and cuticular hydrocarbons (Calderon *et al.* 2005) show significant divergence between the Yucatán population and any other domestic population tested. The Yucatán specimens have the smallest body size observed in all populations of *T. dimidiata* (Lehmann *et al.* 2005). Moreover, analysis of rDNA ITS-2 sequence shows a level of variation compatible with a separate species status. *T. dimidiata* from Yucatán show 21-27 nucleotide differences when compared to other populations from Mexico, Honduras and Ecuador (Marcilla *et al.* 2001; Bargues *et al.* 2002).

The absence of C-heterochromatin both in the autosomes as well as in the X chromosomes is characteristic of this population, and readily permits their distinction from any other populations, including sylvatic populations of Petén (Figure 3). The absence of C-heterochromatin is

positively correlated with their total DNA content measured by flow cytometry. The population from Yucatán has 15% less DNA content than populations from Mexico and others countries (cytotype 1)(Table 1).

Biological parameters related to the vector capacity of these populations also show significant differences. The defecation rates in adults and nymphs are significantly more extended in Yucatán populations than in *T. dimidiata* from Costa Rica (Guzmán-Marín *et al.* 1992). A strong seasonal invasion of houses by flying adults and the low rate of colonization of domestic environments are biological characteristics of the Yucatán population that are very different from the ones observed in other domestic populations from Mexico (Dumonteil *et al.* 2002, 2004; Dumonteil & Gourbière 2004).

T. dimidiata population from Petén (Guatemala)

All reports, including this paper, regarding the *T. dimidiata* population from the Petén, involved wild individuals collected in the same area: the archaeological site of Yaxhá. So far, only one genetic marker was used to study this population. By RAPD-PCR, Calderon *et al.* (2004) show that the sylvatic population from the Petén is genetically distinct from domestic populations from Guatemala. It shows a higher Nei's genetic distance when compared to the other populations, which is not correlated with the geographic distance. The analysis of phenetic markers, such as head morphometry (Fernandez *et al.* 2005; Lehmann *et al.* 2005), cuticular hydrocarbons (Calderon *et al.* 2005) and antennal phenotypes (Catalá *et al.* 2005) clearly separated the Petén population from any other domestic population including those from Guatemala, Mexico (Veracruz-Hidalgo), Honduras and Colombia. With morphometry, Bustamante *et al.* (2004) show a partial overlapping of the Petén population with a domestic population from Mexico (Veracruz), although this was not confirmed by Lehmann *et al.* (2005). Although some of these studies established that the Petén population is different from some domestic populations of *T. dimidiata*, none of them detected differences between Petén and Yucatán populations. This study is the first report of a genetic marker that distinguishes these two sylvatic populations.

The presence of C-heterochromatin in the X_1 chromosome observed in the Petén population is very distinctive from that observed in any other population of *T. dimidiata* (Figures 2 and 3). The heterochromatic X_1 chromosome is not detected in any other species related to *T. dimidiata*, such as species from the phyllosoma or flavida complexes. Until now, only some species of infestans and protracta complexes show this characteristic. This fact suggests that the population of Petén is genetically isolated and support the identification of Petén population as a different entity. Furthermore, the very low capacity of this population to colonize the domestic habitat (Monroy *et al.* 2003) suggests that the Petén population is basically sylvatic and very different from that of the Yucatán or other domestic populations of *T. dimidiata*. To date, systematic searches of domestic habitats in 19 villages of the Petén have not revealed domestic colonies of *T. dimidiata* in this region (Tabaru *et al.* 1999).

Origin of species complex of *T. dimidiata*

The genetic differentiation reported here correlates with geographic region but not with a specific ecotope. Cytotypes 1 and 2 were detected in domestic and peridomestic environments, while all three detected cytotypes comprised sylvatic populations (Santa Marta/El Carmen, Yucatán and Petén). The chromosome and genomic differences detected here appear to be the result of genetic divergence in allopatry, suggesting population fragmentation from a once more-widespread ancestor. We hypothesized that this ancestor was distributed over a large region of Mexico and Guatemala (including central Mexico, Isthmus of Tehauntepec and the Yucatán peninsula). The severe climatic and vegetation changes that occurred in these regions during Pleistocene (Lee 1996), would have led to the geographical isolation of the different cytotypes.

According to this hypothesis, cytotype 1 inhabited the gulf coast of Mexico from where it could have spread to Central America and then to Colombia using southern migration via the Isthmus of Tehuantepec and the Soconosco region. Such a scenario of dispersal would undoubtedly have involved not only some active dispersal by adult flight but also, and may be more important, passive dispersal in association with human or migrant hosts such as didelphid opossums. This cytotype retained its ability to invade both sylvatic and domestic habitats (Schofield 2002), which could also explain the observed clinal variation along a north-south axis and the occurrence of gene flow among domestic, peridomestic and sylvatic populations in Colombia (Ramírez *et al.* 2005).

On the other hand, cytotype 2 (Yucatán) and cytotype 3 (Petén) remain rather isolated in their particular territories. The Yucatán peninsula is relatively isolated by the existence of physical barriers as well as by ecological characteristics that maintain its particular climatic environment apart from the west of Mexico. In the same way, the region of Petén is relatively inaccessible since it is separated from the Yucatán peninsula by marsh areas prone to flooding, and from other border regions of Guatemala and Mexico by hills and large rivers.

Relevance of the chromosomal studies in vector control of *T. dimidiata*

Our cytogenetic analysis can be used as a genetic marker to identify three cytotypes which probably have different epidemiological significance according to their capacity to colonize human environments. Cytotype 1 is present in Mexico, Guatemala, El Salvador and Colombia, and probably also predominates in the intervening countries such as Honduras, Nicaragua, Costa Rica and Panama. It is commonly associated with domestic and peridomestic environments but also inhabits sylvatic ecotopes. This cytotype appears to be the most widespread and with major epidemiological significance. By contrast, the Yucatán cytotype (2) inhabits wild habitats but increasingly enters houses, while the Petén cytotype (3) appears exclusively sylvatic. The low capacity to colonize human dwellings, together with their restricted geographic distribution, indicate that the Yucatán and Petén forms probably have much less epidemiological significance than cytotype 1. Thus, the new markers described herein may be useful for effective control planning.

T. dimidiata as a complex of cryptic species

Data discussed above strongly suggest that *T. dimidiata*, long regarded as a single species, probably encompasses al least two additional cryptic species (Yucatán and Petén cytotypes) with different geographical distributions and epidemiological importance. In order to confirm if Yucatán and Petén insects should be considered as separate species, it will be advisable to study these with several additional markers such as isoenzymes, rDNA and mitochondrial sequences. Furthermore to confirm a discrete rather than a clinal variation, it will be important to include different populations over all of the extended area including the Petén, Belize, the northern states of Guatemala close to the Petén, the southern states of the Yucatán peninsula and Tabasco/Northern Chiapas, the area encompassing the Isthmus of Tehauntepec and central Mexico. We also suggest experimental crosses among these cryptic species in order to understand the origin and divergence of these taxonomic groups (Pérez *et al.* 2005).

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Variation chromosomique et taille du génome soutiennent l'existence d'espèces cryptiques de *Triatoma dimidiate* avec une épidémiologie d'importance variable des vecteurs de la maladie de Chagas

La vaste distribution géographique de *Triatoma dimidiate*, un des trois principaux vecteurs de la maladie de Chagas, sévi de Mexico au nord du Pérou. Parce que cette espèce occupe une grande diversité d'habitat composé de terrain artificiel et d'ecotopes naturels, son éradication est extrêmement difficile. Dans le but de contribuer aux efforts de contrôle, nous avons utilisé des analyses chromosomiques et les quantités d'ADN comme marqueurs taxonomiques pour étudier la variabilité génétique dans les populations de *T. dimidiate* de Mexico, Guatemala, Salvador et Colombie. Nous avons distingué trois groupes ou cytotypes définis par les profils caracteristiques en bandes C du chromosome et la taille du génome mesurée par flow cytometrie. Les trois cytotypes sont confinés dans des locations géographiques différentes. Le cytotype 1 sévi à Mexico (à l'exception du Yucatán), au Guatemala (à l'exception de Petén), au Salvador et en Colombie. Le cytotype 2 sévi dans le Yucatan et le cytotype 3 à Peten. Le cytotype 1, communément associé à l'environnement domestique et péri domestique mais aussi à des ecotopes sylvatiques habités, est le plus prévalant avec une épidémiologie majeure significative. Par contre, le cytotype du Yucatán sévi dans les ecotopes sauvages mais de plus en plus entre dans les maisons, alors que le cytotype de Petén semble être essentiellement sylvatique. Nous suggérons donc que ces cytotypes représentent des espèces cryptiques de *T. dimidiate* avec différentes importances epidemiologiques en tant que vecteurs de la maladie de Chagas. Le peu d'habilité à coloniser les habitations humaines ainsi que leur distribution géographiques restreintes indiquent que les putatives espèces du Yucatán et de Petén ont probablement peu de signification epidemiologique par rapport au cytotype 1. Les marqueurs génétiques que nous décrivons sont donc de puissants outils pour différencier des espèces cryptiques de *T. dimidiate*, pouvant ainsi contribuer à planifier des mesures de contrôle plus efficaces.

mots clés Maladie de Chagas, variations du chromosome, taille du génome, *Triatoma*

La variación cromosómica y el tamaño del genoma apoyan la existencia de una especie críptica de *Triatoma dimiditaa,* con importancia epidemiológica variable, como vectores de la enfermedad de Chagas

La amplia distribución del *Triatoma dimidiata*, uno de los tres vectores principales de la enfermedad de Chagas, se extiende desde Méjico al norte de Perú. Puesto que esta especie ocupa una gran diversidad de ecotopos artificiales y naturales, su erradicación es extremadamente difícil. Con el fin de ayudar en los esfuerzos de control, utilizamos el análisis cromosómico y la cantidad de ADN como marcadores taxonómicos para estudiar la variabilidad genética en poblaciones de *T. Dimidiata* provenientes de Méjico, Guatemala, El Salvador y Colombia. Diferenciamos tres grupos o citotipos, definidos por bandeo cromosómico y patrón de bandas C característicos y por el tamaño de su genoma, medido por citometría de flujo. Los tres citotipos estaban restringidos a diferentes áreas geográficas. El citotipo 1 ocurría en Méjico (excluyendo Yucatán), Guatemala (excluyendo Petén), El Salvador y Colombia. El citotipo 2 ocurría en Yucatán y el citotipo 3 en Petén. El citotipo 1, comúnmente asociado con ambientes domésticos y peridomésticos, pero que también habita ecotipos selváticos, es el más extendido y el que mayor significado epidemiológico tiene. En contraste, el citotipo de Yucatán habita ecotopos salvajes, pero cada vez con mayor frecuencia entra en las casas, mientras que el citotipo Petén es exclusivamente selvático. Sugerimos que estos citotipos representan la especie críptica de *T. Dimidiata* con diferente relevancia epidemiológica como vectores de la enfermedad de Chagas. Su escasa habilidad para colonizar viviendas humanas, junto con su distribución geográfica restringida, indican que las especies putativas de Yucatán y Petén probablemente tienen un significado epidemiológico muchísimo menor que el citotipo 1. Por lo tanto, los marcadores genéticos que describimos son herramientas poderosas para diferenciar entre especies crípticas de *T. dimidiata,* contribuyendo a planear medidas de control más efectivas.

palabras clave Enfermedad de Chagas, variación cromosómica, tamaño del genoma, *Triatoma*