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Synanthropic mammals in transmission cycle of Trypanosoma cruzi in Yucatán, Mexico

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ABSTRACT

OBJECTIVE

Trypanosoma cruzi comprises highly heterogeneus populations classified within six Discrete Typing Unit (DTU´s) named Tc-I to Tc-VI ans TcBat. Evolutionary history of *T. cruzi* has a very strong association with their mammal hosts and some phylogenetic and ecolobiologically studies suggest that ecotopes, hosts and vectors are factors that determine the different lineages of *T. cruzi*.

METHODS

Herein we characterized *T. cruzi* isolates from synanthropic individuals of *Didelphis virginiana* and *Rattus rattus* captured in the village of Molas, Yucatan.

RESULTS

Forty households were selected and traps were placed in the yard during January to May of 2014. Sixty six opossum (*Didelphis virginiana*) and twenty five rats (*Rattus rattus*) were captured and 13 were diagnosed as *T. cruzi* infected by microhematocrit and blood culture. Ten isolates of *T. cruzi* were obtained for phylogenetic analysis with SSU rDNA, gGAPDH and Cytochrome B genes to describe the relationships between them and classify them into the different DTU´s.

CONCLUSIONS

This study demonstrated the participation of synanthropic animals *D. virginiana* and *R. rattus* as a reservoirs of *T. cruzi* in Yucatan_Mexico and the different isolates of the parasite belonged to Tc-I. The proximity of these species to the domestic environment favor the contact of the trypanosome with the human population in domestic environment.

DESCRIPTORS

Opossum. Rats. DTU´s. Phylogeny. Chagas Disease.

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INTRODUCTION

The zoonotic disease with the name of American Trypanosomiasis or Chagas' disease whose distribution extends with a distribution from the south of the United States to South America. It is currently considered a parasitic pathology that causes havoc in public health and the economy in Latin America[1-3].

Phylogenetic analysis of *T. cruzi* based on a series of nuclear and mitochondrial markers indicate that the genus *Trypanosoma* is *monophyletic*[4,5]. The monophyly hypothesis suggests that an ancestral parasite originated the trypanosomes of mammals in Africa, America and Australia. The first forms of *T. cruzi* were associated with the marsupials of the Didelphidae family 80 million years ago[6, 7]. With the passing of time, *T. cruzi* adapted to different mammals and began the hematophagous habits of the vectors[8] to develop the digenetic cycle in which different selective pressures acted and gave rise to an extensive variety of populations of this parasite[9].

For years, studies of electrophoretic variants of isoenzymes[10, 11] and other molecular techniques were carried out to finally identify six discrete typing units (DTU) of genetically related *T. cruzi*, named as Tc- I to Tc-VI and TcBat which are present in different ecotopes that determine the evolutionary ecology of the parasite in a region and associates biological characters with the clinical manifestations of the disease[12-14].

All DTUs are considered infective for humans, some studies suggest that Tc-II, Tc-III and Tc-IV are associated with domestic environments and with patients with chronic manifestations of the disease, Tc-III and Tc-IV with environments wild and Tc-I with both environments[14-16].

In Mexico, studies have been carried out since 1998 on various techniques on the molecular and biological characterization of *T. cruzi* obtained from triatomines, host mammals and human cases, which reveal the presence of *T. cruzi* group I mainly in southeastern Mexico and *T. cruzi* II in the center of this country with variations in biological behavior in murine models at the level of parasitaemia, mortality and tropism[17-22].

However, studies in this regard are very scarce but have revealed that populations of *T. cruzi* circulate among mammalian hosts, vectors and ecotopes in a selective manner depending on the association (host-parasite). From this, the present study was conducted in the town of Molas, Yucatan, where the closeness of wild species, domestic animals and triatomines may present a risk for the transmission of *T. cruzi* to humans.

Here in we phylogenetically characterized the isolates of *T. cruzi* that circulate in the synanthropic population of *D. virginiana* and *R. rattus* from the rural town of Molas, Yucatán, Mexico.

METHODS

Study area and mammals caught

The study was conducted in the town of Molas (20 ° 48'00''N and 89 ° 38'00''W), located 16 km south of the city of Mérida, Yucatán, Mexico. This town has a population of 2,014 inhabitants and is within the limits of the Cuxtal Ecological Reserve. It has a subhumid warm climate with summer rains with an average annual temperature of over 26°C. Surrounding the conurban zone, fragments of secondary vegetation predominate in different stages of succession composed of native species of low deciduous forest (Ceiba aesculifolia Bombaceae, Bursera Simaruba Burceraceae and Talisia Olivaeformis Sapindaceae, among others) and small fragments with characteristics of this jungle whose height varies from 6 to 15 m.

Sampling was carried out in a total of 40 dwellings (5m to houses) in Molas municipality (Figure 1) with 20 Tomahawk Inc.® traps, 20 Sherman® traps were placed in each yard using seasonal fruit, and oatmeal with vanilla as baits, respectively, for three consecutive days of each month in the period January to May 2014. The traps were marked at the time of their placement with a label at the top with the following information: date, number of individual captured and the assigned home number.

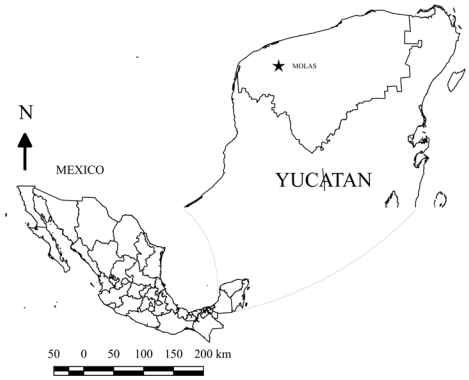

Figure 1. Geographic location of the town of Molas, Yucatan, Mexico.

Animals were sedated with 1–5 mg/kg of Ketamine and made aseptic with solution of 70% ethanol before collecting samples. Two aliquots of blood were collected by cardiac puncture, a part of the blood was transferred to sterile tubes containing alcohol to PCR analysis and the other to blood culture.

For microhaematocrit test, two heparinized capillary tubes werefilled with 50 L of cord blood each one at delivery; tubes were centrifuged at 3000g for 1 min and after centrifugation. The buffy coat was placed in a slide and coveredwith a coverslip, and the complete coverslip area was analyzed under the light.

All procedures for handling animals were performed according to the Ethics Committee on Animal Use of the Faculty of Veterinary Medicine, University of São Paulo, Brazil

Trypanosome isolation

To detect trypanosomatid parasites, blood samples from wild obtained through were inoculated into Vacutainer tubes containing a biphasic medium consisting of 15% sheep red blood cells as the solid phase (blood agar base), overlain by liquid liver infusion tryptose (LIT) medium supplemented with 20% fetal bovine serum[23]. The culture was incubated at 28ºC and grown in LIT medium for DNA preparation, and the isolates were cryopreserved in liquid nitrogen in the Brazilian Trypanosomatid Collection (Coleção Brasileira de Tripanossomatídeos, CBT), in the Department of Preventive Veterinary Medicine and Animal Health, Faculty of Veterinary Medicine, University of São Paulo, Brazil. Blood samples were fixed in ethanol (primary samples) for molecular detection.

Extraction and amplification of DNA from blood and culture

The extraction of DNA for culture samples was extracted from the trypanosome cultures using the phenol-chloroform method. The extraction of DNA from blood was performed according to the protocol established by Purelink kit (Thermo Fisher Scientific Inc, 2012, USA). The DNA samples were subjected to the conventional polymerase chain reaction (PCR) for trypanosomatids barcoding on a fragment of ~ 900 bp of the V7V8 SSU rDNA[12, 23], gGAPDH (~600bp)[24] and cytochrome B (~400pb)[12]. PCR products of the expected size were purified and sequenced in an automated sequencer (ABI Prism 310, Applied Biosystems, Foster City, CA).

Phylogenetic analysis

The sequences obtained were aligned with sequences previously determined for other trypanosomatid species available in GenBank (Table 1) using ClustalX[25] and were adjusted manually using GeneDoc[26]. The barcoding sequences were used to construct a phylogenetic tree using maximum parsimony, as implemented in PAUP version 4.0b10[27] with 500 bootstrap replicates. Bayesian analysis was performed using MrBayes v3.1.2[28] with 1,000,000 replicates. The first 25% ofthe trees represented burn-in, and the remaining trees were used to calculate Bayesian posterior probability.

RESULTS

A total of 91 mammals, between *Rattus novergicus* (n=25) and *Didelphis virginiana* (n=66) were sampled in the area and examined. Wild mammals overall rate of infection were evaluated by microhematocrit test and blood culture and 11.83% (13/91) were positive by and 9.1% (10/91) of positive cultures was established.

The sequences of SSU rDNA, gGAPDH and cytochrome B (CytB) genes were obtained for ten isolates and compared with sequences retrieved from GenBank and congruent topologies were obtained by maximum parsimony and Bayesian analysis.

The isolates were grouped as *T. cruzi* (100% of boostrap/1.0 a posteriori probability). All isolates of *T. cruzi* obtained are belonging to TCI Group (100% boostrap/ 100% a posteriori probability) (Fig. 2A;2B). Intraspecific analysis were performed by Cyt B gene (Fig. 2C) when compared to the GenBank reference strains from Mexican isolates from different states and hosts (*Triatoma dimidiata, Homo sapiens, Dipetalogaster maxim, Triatoma pallidipennis*)[29] they did not show differences between them (100%).

Table 1: Trypanosome isolates, host and geographic origin and sequences of SSU rDNA, gGAPDH and cytochrome B used for phylogenetic analysis

Trypanosomatids species	Isolate code	Host	Geographic oringin		Acession Number[a]		
					SSU rDNA	gGAPDH	CytB
Trypanosoma rangeli	TCC643	*Platyrrhinus lineatus*	Miranda	MS	EU867803		
T. dionisii	CBT58	*Sturnira lillium*	Pinheiros	ES	KF557744	KF557735	
	CBT59	*Lophostoma braziliense*	Pinheiros	ES	KF557745	KF577736	
	PJ	*Pipistrellus pipistrellus*	Belgium		AJ009152		
	P3	*Pipistrellus pipistrellus*	England		AJ009151		
	TCC211	*Eptesicus brasiliensis*	São Paulo	SP		AJ620271	
	TCC289	*Eptesicus brasiliensis*	São Paulo	SP	FJ001651		
	TCC495	*Carollia perspicillata*	Porto Velho	RO		GQ140363	
	TCC633	*Sturnira lillium*	Miranda	MS	EU867812		
T. erneyi	TCC1293	*Tadarida sp.*	Mozambique		JN040987	JN040964	
	TCC1294	*Tadarida sp.*	Mozambique		JN040988		
	TCC1932	*Mops condylurus*	Mozambique		JN040990		
	TCC1934	*Mops condylurus*	Mozambique		JN040991	JN040968	
T. cruzi marinkellei	CBT3	*Phyllostomus hastatus*	Confresa	MT	JX845151		
	CBT7	*Phyllostomus hastatus*	Confresa	MT	JX845154		
	CBT8	*Carollia perspicillata*	Confresa	MT	JX845155		
	CBT10	*Trachops cirrhosus*	Poconé	MT	JX845157		
	CBT67	*Myotis nigricans*	Pinheiros	ES		KF557743	
	B7	*Phyllostomus discolor*	São Felipe	BA	AJ009150		
	TCC420	*Chrotopterus auritus*	Barcelos	AM	FJ001636		
	TCC501	*Carollia perspicillata*	Porto Velho	AM		GQ140361	
	TCC708	*Chrotopterus auritus*	Barcelos	AM	FJ001648		
	CBT95	*Phyllostomus* sp.	Riachão	MA	KP197159	KP197169	
	CBT99	*Phyllostomus hastatus*	Riachão	MA	KP197160	KP197170	
T. cruzi	Y	*Homo sapiens*	São Paulo	SP	AF301912	GQ140353	
	CLBR	*Triatoma infestans*	São Paulo	SP	AF245383		
	Peru	*Homo sapiens*	Peru		X53917		
	JJ	*Homo sapiens*	Barcelos	AM	AY491761	GQ140355	
	TCC463	*Cebus albifrons*	Barcelos	AM	EU755224		
	NRcl3	*Homo sapiens*	Chile		AF228685	GQ140357	
	TCC186	*Homo sapiens*	Bolivia		FJ001630		
	TCC862	*Euphractus sexcinctus*		RN	FJ183397		
	TCC863	*Euphractus sexcinctus*		RN	FJ549376		
	MT3663	*Panstrongylus geniculatus*		AM	AF288660	GQ140355	
	TCC129	*Proechimys iheringi*	São Paulo	SP	FJ555652		
	TCC793	*Myotis levis*	São Paulo	SP	FJ001634	GQ140358	

TCC480	*Noctilio albiventris*	Miranda	MS	EU867804		
TCC947	*Myotis nigricans*	São Paulo	SP	FJ001626		
TCC949	*Myotis nigricans*	São Paulo	SP	FJ001627		
TCC1122	*Myotis levis*	São Paulo	SP		GQ140359	
G	*Didelphis marsupialis*		AM	AF239981	GQ140351	
TCC642	*Carollia perspicillata*	Montenegro	RO			
TCC711	*Didelphis marsupialis*	Barcelos	AM	EU755229		
TCC1456	*Monodelphis brevicaudata*	Pará	PA	FJ555623		
AnitaII	*Triatoma dimidiata*	Campeche	MX			JX431272
Cam6	*Triatoma dimidiata*	Campeche	MX			JX431273
Cristy	*Homo sapiens*	San Luiz Potosi	MX			JX431274
Mich1	*Triatoma dimidiata*	Michoacan	MX			JX431275
Ninoa	*Homo sapiens*	Oaxaca	MX			JX431276
PLI	*Dipetalogaster máxima*	Baja California Sur	MX			JX431277
QROI	*Triatoma Barberi*	Queretaro	MX			JX431278
TQI	*Triatoma pallidipenis*	Morelos	MX			JX431279
XAL1	*Triatoma dimidiata*	Veracruz	MX			JX431280
CBT169	*Rattus rattus*	Molás	MX	**MK652762**	**MK681431**	**MK681141**
CBT170	*Rattus rattus*	Molás	MX	**MK652763**	**MK681432**	**MK681142**
CBT171	*Rattus rattus*	Molás	MX	**MK652764**	**MK681433**	**MK681143**
CBT172	*Didelphis virginiana*	Molás	MX	**MK652765**	**MK681434**	**MK681143**
CBT173	*Didelphis virginiana*	Molás	MX	**MK652766**	**MK681435**	**MK681144**
CBT183	*Didelphis virginiana*	Molás	MX	**MK652767**	**MK681436**	**MK681145**
CBT184	*Didelphis virginiana*	Molás	MX	**MK652768**	**MK681137**	**MK681146**
CBT185	*Didelphis virginiana*	Molás	MX	**MK652769**	**MK681138**	**MK681147**
CBT186	*Didelphis virginiana*	Molás	MX	**MK652770**	**MK681139**	**MK681148**
CBT187	*Didelphis virginiana*	Molás	MX	**MK652671**	**MK681140**	**MK681149**

[a] Sequences determined in this study and deposited in GenBank are underlined and bold.Mexico (MX) and brazilian states: Espirito Santo (ES); São Paulo (SP); Rondônia (RO); Mato Grosso (MT); Bahia (BA); Mato Grosso do Sul (MS); Amazonas (AM); Rio Grande do Norte (RN); Maranhão (MA).

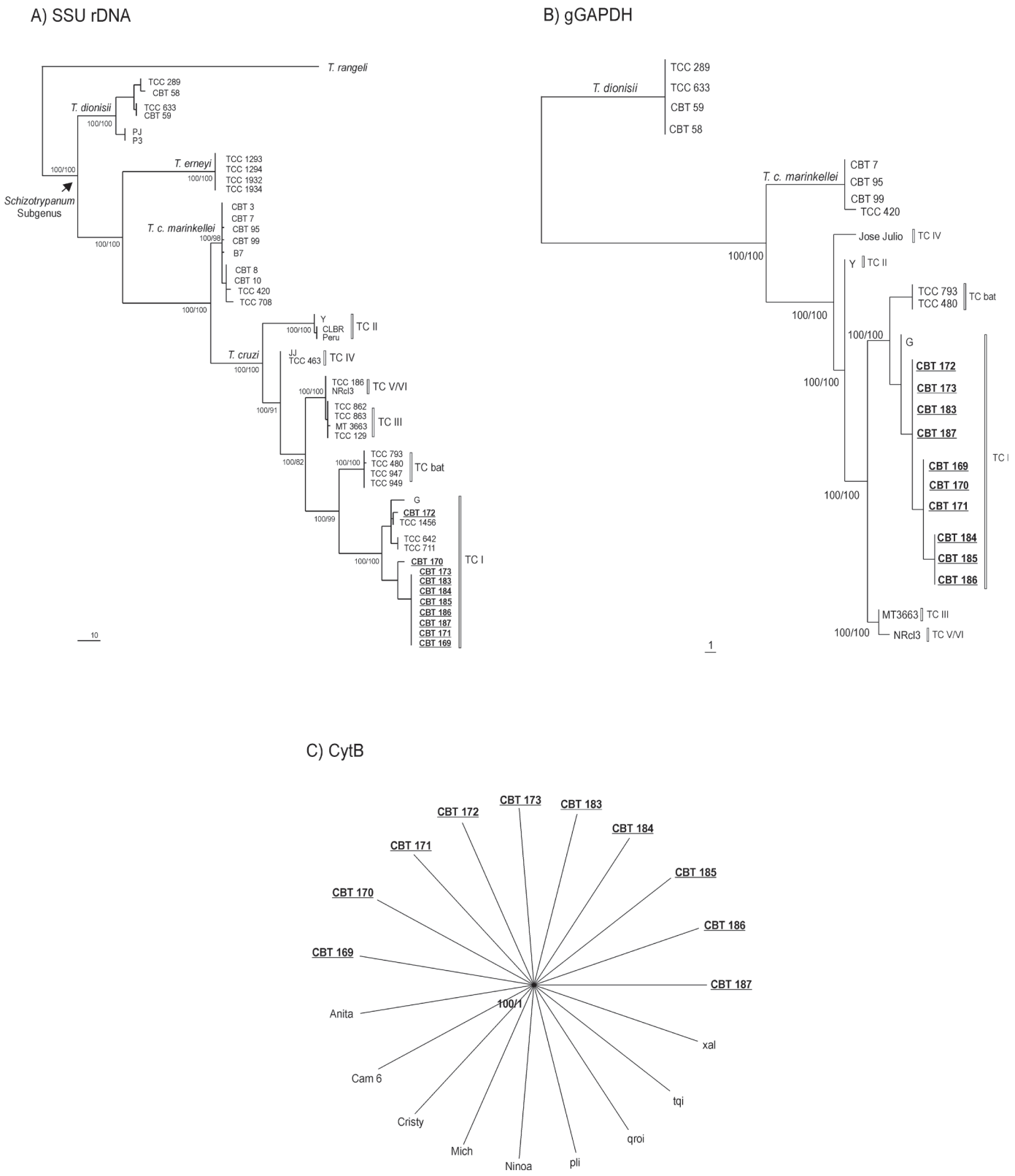

Figure 2. Phylogenetic trees inferred by maximum parsimony and Bayesian inference. A. SSU rDNA gene with 48 sequences of trypannosomatids from GenBank with T. rangeli as an external group. The numbers of the nodes are the support values for the main branches (Boostrap/posterior probability); B. gGAPDH gene with 20 trypanosomatid sequences from GenBank with T. dionisii as an external group. The numbers of the nodes are the support values for the main branches (Boostrap/posterior probability); C. Cyt B gene with 19 GenBank trypanosomatid sequences. The numbers of the nodes are the support values for the main branches (Boostrap/posterior probability). The isolates of the study were underlined.

DISCUSSION

The occurrence of *T. cruzi* infection in synanthropic mammals was 11,83% by microhematocrit and blood. The results of the our study show similarities to others studies carried in *Didelphis* [30-32], demonstrating the importance of opossums of the genus *Didelphis*, several geographic zones in Yucatan, as reservoir of *T. cruzi*.

Phylogenetic analyzes of *T. cruzi* from the *D. virginiana* and *R. rattus* isolates that were obtained from Molas were grouped in Tc-I, the most common DTU in Mexico. The positivity of *T. cruzi* in sinantropic mammals from Molas was 11,83%. The results of the our study show similarities with past and recent studies[30-32].

However the results on the genotype of *T. cruzi* that is present in the synanthropic population of *D. virginiana* and and *R. rattus* from the town of Molas, is concordant with the reports made since 1992 of the Yucatecan strains of both vectors and wild animals of humans[33, 34] as well as other regions of Mexico[34, 17, 19, 35, 36] reporting that lineage 1 (old classification) is circulating in different cycles of transmission of this parasite.

Bosseno and collaborators[19] obtained 56 isolates (*T. longipennis, T. pallidipennis, Humans, T. barberi, D. virginiana, T. phyllostoma, T. picturata, D. marsupialis and Philander opossum*) from nine states of the Mexican Republic (Colima, Guanajuato, Jalisco, Morelos, Nayarit, Oaxaca, Veracruz, Yucatan and Zacatecas) according to the analyzes that were reported, all the isolates corresponded to lineage I except 2.4% of these, because they were grouped with lineage II; that corresponded to the isolates located in Veracruz of *D. virginiana* and *Philander opossum*. In 2012, the first report was made in Mexico detecting different DTU circulating in the same transmission cycles, this study was carried out by capturing 300 triatomines (*T. dimidiata*) in homes and peridomiciles of the localities of Orizaba and Cordova in Veracruz , finding a natural *T. cruzi* infection of 13.7% (41/300) and the following UDTs were detected by several molecular methods: Tc-I: 27% (9/33), Tc-II: 12% (4/33)), Tc-III: 15% (5/33), Tc-IV: 18% (6/33) and Tc-V: 27% (9/33), indicating a clear diversity with high frequencies of the different DTUs of *T. cruzi* despite the limited number of samples[22].

Despite the different studies that show different DTU distributed in Mexico, the predominance of Tc-I in our study may be due to the fact that it has been shown that vectors can act as biological filters, selecting the populations of the parasite to be transmitted through specific mechanisms such as intestinal immune factors, digestive enzymes and lytic factors among others[37, 38] in this way, determines the etiology of *T. cruzi* strains involved in the transmission cycle[39].

On the other hand, it must be mentioned that our results are compatible with the studies of Roelling and collaboratos[40, 41] which demonstrated the genotype-host association in the southeastern United States with two species of marsupials through experimental infection (*Monodelphis domestic*a and *D. virginiana*), where the association of *Didelphis* with DTU I, suggesting that the infection dynamics of the different strains of *T. cruzi* can vary considerably in different wildlife hosts.

In the state of Yucatan, only 42 human cases have been documented with chagasic cardiomyopathy of 1970-1985[43, 44], a predominant characteristic of the DTU I. Recent studies have shown In Yucatan, seroprevalence varies between 1% in urban areas and up to 4% in rural localities[45, 46]. In this study, it is possible that the genetic flow of the populations of *T. cruzi* is the same that *D. virginiana* and *R. rattus* introduces from the sylvatic ecotope to the domestic one due to its synanthropic behavior to establish shelters inside and outside the human dwellings, forcing the vectors (*T. dimidiata*) to the domiciliary adaptation, where they find shelter and sufficient food of both human blood and domestic animals, as a consequence, the human population could be exposed to *T. cruzi* and develop Chagas Disease.

The transmission cycles of *T. cruzi* in this area are mainly linked by synanthropic populations and yours movement patterns. In USA, *D. virginiana* features family behavior and have constant movement within their territory[47-50]. In the sylvatic cycles of transmission of *Trypanosoma cruzi* are maintained by terrestrial and triatomine wild mammals[12]. In Brazil, domestic populations of *R. rattus* are responsible for maintaining intradomiciliar colonies of Triatoma species[12]. *D. virginiana* is a wild species that has synanthropic habits for the search of food, on the other hand, *R. rattus* is a totally synanthropic species and always associated with human occupations. The proximity of these species to the domestic environment favor the contact of the trypanosome with the human population and the domicilation of triatomine species to the domestic environment.

CONCLUSION

This study demonstrates the participation of synanthropic mammals of high population density and actively participating in the cycle of transmission of Chagas disease in Molas municipality, Mérida, Yucatán, Mexico

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