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Palm Tree Landscape as Risk Indicator for Chagas Disease in Western Venezuela

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Abstract
Ninety-six specimens of palm trees were sampled in 65 localities of 5 states of western Venezuela to estimate the potential risk for Chagas disease transmission in the study area. The analysis revealed 99% and 95% infestation and colonization indexes, respectively. *Rhodnius prolixus* resulted predominant species with 96.6% of the total collected triatomine-bugs. In 47 of the infested palms *T. cruzi*-infected triatomine bugs were detected, revealing a 49% palm *T. cruzi*-infection index. A total of 2146 bugs were collected in 95 dissected palm trees, showing a crowding index of 23±30 bugs/palm, being detected 193 bugs (9%) with *T. cruzi*-infection. Presence of triatomine in palms was recorded at any season. However, collection during rainy season was significantly higher than in dry season ($P<0.01$). In addition, in 24 (25%) dissected palm trees, vertebrate animals were recorded, showing a palm-*T. cruzi* infected bugs-susceptible animal association in 12 (50%) of them. A strong relationship between palm tree and *T. cruzi*-infected triatomine bugs association in close proximity to dwellings with acute chagasic patients was demonstrated. Gathering the present results together, we conclude that: i. palm tree is a favorable habitat to support maintenance and circulation of *T. cruzi*-bug association as a well-balanced biocoenosis in the wild, and ii. Anthropogenic activity provoking disturbance and passive flow of bug population from sylvatic to domestic environments, transform palm tree landscape in a risk for Chagas disease occurrence. Epidemiological implications of present results are discussed, and official intervention for a constant and efficient epidemiological surveillance and control of Chagas disease, is claimed.

1. Introduction

From long ago the presence of Reduviidae-triatomine bugs has been recorded on different species of palm trees widely distributed in rural areas of Central and South American regions [1-8]. For more than a half a century, researchers have systematically demonstrated the potential role of palm trees as suitable habitat in natural biotopes for wild triatomine bugs vectors of *Trypanosoma cruzi*, the etiological agent of Chagas disease, as well as for susceptible mammal hosts to maintain the cycle of the parasite in nature [2, 4, 9]. Palm trees belonging to 18 genera harboring more than 30 species of triatomine-bugs have been reported from rural localities of different American countries located from 41°N at the Southern USA to 56°S in Argentina, overlapping with the distribution of *T. cruzi* [7-10]. Years ago, Carcavallo et al. [4] provided information on the habitat of 111 species of triatomine bugs, 25 (22.5%) of which were associated to palm trees as natural shelters. In this referential study, 35 species of palm trees belonging to 14 genera were recognized harboring specimens of 8 genera of the hematophagous bugs, being *Attalea, Copernicia*, *Acrocomia* and *Mauritia* the most frequently bug infested palms with species of the genera *Rhodnius, Triatoma* and *Panstrongylus* with 44%, 20% and 12% of presence, respectively. The bugs were most frequently found associated to palms in sylvatic environments. This is particularly relevant for wild *R. prolixus* found in 15 species of palm trees of 11 genera, indicating its high capacity to adapt and reproduce in these sylvatic habitats.

In western Venezuela, especially in the states located in the "Llanos region" the abundance of palm trees is noticeable where specimens of the genus *Attalea* are predominant, although the presence of *Copernicia* and *Acrocomia* are also common. In most part of the Venezuelan western region, rural villages have been founded after families looking for places to settle down invaded the primary forest. To establish their dwellings, they intensively cut palm trees down using dissected leaves to build them. While doing that, they passively carry specimens of triatomine bugs, at any stage, from their natural niche to domestic environments, producing behavioral changes from the wild to indoor conditions, exposing human hosts to bites inflicted by these hematophagous insects when procuring blood as source of energy [11]. Once established in the chosen place, the irruption into the forest persist all around the dwellings, and palm trees are substituted for agricultural practices, tilling commercial species or establishing shelters for breeding animals. This invasion and practice changes the natural environment provoking in many occasions the occurrence of acute Chagas disease outbreaks with the consequent appearance of severe health effects on the invader population [12-15].

This article deals with the evaluation of the role of palm trees as favorable ecotope for *T. cruzi*-infected triatomine bugs and with the estimation of potential risks for the occurrence of acute Chagas disease outbreaks associated to human behavior in rural localities of western Venezuela.

2. Materials and Methods

2.1. Study Area

The present work was carried out in 65 different localities of 5 states of western Venezuela, including Barinas, Apure, Cojedes and Portuguesa, located at the Llanos region, and Trujillo at the foothills of the Andean region. The 55 places selected in Barinas were located from 7°42'13''N-70°11'29''W to 8°28'33''N-68°22'8''W. The remaining 10 localities were located in Apure (3) at 8°2'56''N-69°19'58''W, Cojedes (2) at 9°49'34''N-68°26'20''W, Portuguesa (1) at 8°2'5''N-62°38'46''W and Trujillo (4) at 9°34'36''N-70°42'49''W. In general, the study areas were located at altitudes between 100 and 1000 m. a. s. l, with temperatures from 28°C to 32°C, relative humidity >75% and a mean rainfall over 1500mm/year, and characterized by a dry season (September to March) and a rainy season (April-August). During the study, 34 localities were selected to be sampled taking into consideration: i. the detection of previous occurrence of Chagas disease acute cases, ii. the local practice of cutting palm trees down and use palm leaves to build dwellings, and iii. the record and collection of triatomine bugs in dwellings' indoor. The other 31 places were chosen considering the abundance of palm trees located far from the inhabited dwellings and, at the same time, the need to compare them with those located in close proximity to dwellings where acute chagasic events have occurred.

2.2. Palm Tree Selection and Triatomine Bugs Sampling, Collection and Identification

Ninety-six palm trees were selected to be sampled from the previously described places. Criteria used for selection included, firstly, palm specimens located in close proximity to dwellings where acute Chagas disease cases occurred (N=41). This part of the study was carried out in 34 places of Barinas state, close to houses of similar number of acute chagasic patients (ACP) previously diagnosed and treated by our research team. Secondly, palm trees located either far from the above mentioned domiciles or from places where acute chagasic cases have not been reported in Barinas (N=45) and other places in Trujillo, Apure, Cojedes and Portuguesa (N=10).

Once selected the palm, it was cut down using a chain saw machine and then each of the leaves were dissected out and carefully examined for the presence and collection of triatomine bugs. The insects were collected by two trained field workers for each of the leaf, who placed specimens into a marked flask previously prepared to transport the bugs to our laboratory at the Faculty of Sciences, University of Los Andes in Merida, Venezuela, to be identified and individually examined for parasites. In addition, the presence of mammals or other vertebrates was recorded. This included nests or any evidence of co-occurrence of animals and triatomine-bugs at the same habitat.

From the total 96 selected palms, 49 (51%) of them were dissected out during the dry season from September to March, and the remaining 47 (49%) were dissected during the period April-August corresponding to the rainy season at the study area.

In the laboratory, the bugs collected from each of the dissected palms were counted and separated by stage, identifying date, place and palm of collection. This also included the identification of the family representative, and/or the patient code, when bugs came from a palm close to the domicile where a chagasic case occurred. Species identification of collected triatomine bugs was carried out at the "Herman Lent" Laboratory, Faculty of Sciences

University of Los Andes, Merida, Venezuela, following Lent and Wygodzinsky [3]. Palm trees were identified using keys previously reported [16, 17].

2.3. Processing of Bugs and Isolation, Culture and Genetic Typing of *Trypanosoma cruzi* Isolates

After identification, the bugs were individually examined for *T. cruzi* infection following microscopic observation of fresh and Giemsa stained fecal samples. Both fresh and stained samples were examined under an Axioscop microscope (Zeiss-Germany) connected to a Noticam 480 camera in interphase with a Laptop-HP to record the activity and morphology of the parasites, respectively. Once confirmed the infection, the bugs were dissected out and the flagellates, mostly metacyclic forms, were inoculated into young healthy mice. When the mice showed patent parasitemia, they were sampled by cardio-puncture and the blood placed into tubes containing NNN culture medium. After established in culture, the parasites were grown in mass, collected and used for genetic typing, following protocols previously reported [18, 19]. The obtained isolates from the infected bugs were processed for DNA extraction using the classical phenol-chloroform method. Previous to genotyping, parasite identification was carried out using a *T. cruzi*-specific PCR based on the kDNA minicircle molecule, and performed with primers (S35/S36) and conditions previously developed [20]. For PCR amplification of the divergent domain of the 24S-α-ribosomal RNA gene, primers D71 and D72 were used as previously described [21] to generate 110/125 bp DNA bands. For the intergenic region of the mini-exon gene, a pool of primers Tc, Tc1 and Tc2 were used to generate a 350/300 bp DNA bands following Souto et al [21]. The PCR amplified products from both reactions were separated electrophoretically in 3% agarose gels stained with ethidium bromide. In all cases reference strains of *T. cruzi* were used as control, including G and Y isolates for identification.

2.4. Description of Entomological Indexes

From the data collected in the present study entomological indexes were applied according to the following description: i. Infestation index as the ratio of infested to total sampled palms (i.e. the number of palms harboring triatomine bugs/number of sampled palms X 100). ii. Colonization index (number of palms harboring any of the developmental stages of triatomine bugs/number of infested palm X 100). iii. Palm *T. cruzi*-infection index (number of palms with *T. cruzi*-infected bugs/number of infested palms X 100). iv. Density index as the proportion of number of bugs per sampled palm trees (i.e. number of collected bugs/number of sampled palms). v. Clustering index (number of collected bugs/number of infested palms). vi. Crowding index as the average ±SD of triatomine bugs/palm. vii. Infection index (number of infected bugs/total collected bugs X100).

2.5. Statistical Analysis

Statistical comparison between species of bugs considering the number of collected and *T. cruzi*-infected specimens was estimated using the Chi-square test (X^2) contingency table. In addition, a similar comparison between palms associated to acute Chagas disease cases and those selected far from them, considering together number of dissected palms, collected and *T. cruzi*-infected bugs was estimated using X^2 contingency tables with 2 rows and C columns following the Brand and Snedecor's formula [22].

3. Results

3.1. Palm Tree as Shelter of Wild Triatomine Bugs

A total of 96 specimens of palm trees were dissected out in 65 localities of 5 states of western Venezuela. These included palms of the genera *Attalea* (88), *Acrocomia* (6) and *Copernicia* (2) dissected in Barinas (86), Trujillo (4), Apure (3), Cojedes (2) and Portuguesa (1). Ninety-five of the total dissected palms were found harboring triatomine bugs, revealing 99% palm infestation index (Infested/dissected X100). In addition, 95% colonization index was estimated as product of collecting instar-nymphs in 90 of the 95 infested palms, in which all the bug's developmental stages were recorded, including 35% of them with first instar nymphs, 52% palms with II, 60% with III stage and collection of IV, V and adult stages in 67, 56 and 63% of the dissected palms, respectively. The palm *T. cruzi*-infection index was estimated in 49% after detecting *T. cruzi*-infected triatomine bugs in 47 of the 95 infested palms. During the study, a total of 2146 bugs were collected in 95 dissected palm trees, showing an average (crowding index) of 23±30 bugs/palm (range: 1-191), being detected 193 bugs (9%) infected with *T. cruzi.* Table 1 describes the obtained results showing palm trees acting as shelter of wild triatomine bugs, indicating its age structure and association with *T. cruzi* in areas where Chagas disease is endemic.

Table 1. *Palm tree as shelter of wild triatomine bugs in western Venezuela.*

	N° Dissected palm tree			**N° (%)**
State	***Attalea***	***Acrocomia***	***Copernicia***	**Palm tree harboring bugs**
Barinas	84	2	-	85
Trujillo	-	4	-	4
Apure	3	-	-	3
Cojedes	-	-	2	2
Portuguesa	1	-	-	1
TOTAL:96	88	6	2	95
(%)	(92)	(6)	(2)	(99)*

Table 1. *Continue.*

State	N° (%) palm trees harboring different stages of triatomine bugs** I	II	III	IV	V	Adult	N° (%) Palm tree with *T. cruzi* infected bugs	N° (%) Collected bugs	N° (%) *T. cruzi* infected bugs
Barinas	32	46	50	56	45	51	41	1915	174
Trujillo	-	-	2	3	3	4	3	58	9
Apure	-	2	3	3	3	2	1	46	1
Cojedes	-	-	1	1	1	2	1	52	7
Portuguesa	1	1	1	1	1	1	1	75	2
TOTAL:96	33	49	57	64	53	60	47	2146	193
(%)	(35)	(52)	(60)	(67)	(56)	(63)	(49)***		(9)

*: Infestation index; **: Colonization index; ***: Palm *T. cruzi*-infection index

3.2. Triatomine Species, Number of Specimens, Age Structure and *T. cruzi*-Infection in Bugs Collected from Dissected Palm Trees

Four triatomine species were recorded from the total 2146 bugs collected in palm trees dissected in localities of western Venezuela. These included 2073 (96.6%) specimens of *Rhodnius prolixus* collected in Barinas (1897), Trujillo (58), Apure (43) and Portuguesa (75), 18 (0.8%) *Triatoma maculata* (15 in Barinas and 3 in Apure), 3 (0.1%) *Eratyrus mucronatus* (Barinas) and 52 (2.4%) *Rhodnius pictipes* caught at Cojedes state. All the six developmental stages were detected during the study. The recorded number of bugs comprising the age structure was made up of 306 (14%) first instar nymphs, 486 (23%) second stage, 388 (18%) third instar, 354 (16%) fourth and 299 (14%) and 313 (15%) of fifth instar and adult stages, respectively. Infection with *T. cruzi* was detected in 193 of the total collected bugs (9%), including 173 specimens of *R. prolixus* (90%), 13 (7%) *T. maculata* and 7 (3%) *R. pictipes*. Interestingly, *T. cruzi* infection in collected bugs was recorded in all the states where dissection of palm trees was carried out, including 174 infected out of 1915 bugs (9%) in Barinas, 16% (9/58) in Trujillo, 7 infected of 52 collected in Cojedes (13%), and 3% and 2% of infection in bugs from Portuguesa and Apure, respectively. Regarding the proportion of infected bugs considering the collected species, the major level was detected in specimens of *T. maculata*, showing 72% *T. cruzi* infection, resulting 13 infected bugs out of the 18 collected specimens in palms from Barinas (15) and Apure (3), followed by *R. pictipes* from Cojedes with 13% (7/52) and *R. prolixus* with 8% product of detecting 173 *T. cruzi*-infected bugs out of 2073 collected bugs from palm trees dissected in localities of Barinas, Trujillo, Apure and Portuguesa. Statistical comparison among the species of bugs considering the number of collected and *T. cruzi*-infected specimens, revealed highly significant differences (P<0.01). Details on the aspects referred above are shown in Table 2.

Table 2. *Age structure and Trypanosoma cruzi infection in species of triatomine bugs collected from palm trees in western Venezuela.*

State	Species of bugs	N° of Collected bugs	N° (%) Age structure of collected bugs I	II	III	IV	V	Adult	N° (%) *T. cruzi* infected bugs
Barinas	*R. prolixus*	1897	297 (15)	471 (25)	337 (18)	313 (16)	248 (13)	231 (12)	161 (8.5)
	T. maculata	15	-	-	-	3 (20)	9 (60)	3 (20)	13 (86.7)
	E. mucronatus	3	-	-	-	-	1 (33)	2 (67)	-
Trujillo	*R. prolixus*	58	-	-	7 (12)	11 (19)	18 (31)	22 (38)	9 (15.5)
Apure	*R. prolixus*	43	-	5 (12)	21 (49)	4 (9)	5 (12)	8 (18)	1 (2.3)
	T. maculata	3	-	-	-	-	-	3 (100)	-
Cojedes	*R. pictipes*	52	-	-	3 (6)	8 (15)	17 (33)	24 (46)	7 (13.5)
Portuguesa	*R. prolixus*	75	9 (12)	10 (13)	20 (27)	15 (20)	1 (1)	20 (27)	2 (2.6)
Total	4 species	2146	306 (14)	486 (23)	388 (18)	354 (16)	299 (14)	313 (15)	193 (8.9)

3.3. Palm Tree, Vertebrate Animals and *T. cruzi*-Infected Bugs Association in Chagas Disease Endemic Areas

In 24 (25%) of the dissected palm trees, the presence of vertebrate animals was recorded. The observation included mammals highly susceptible to infection by *T. cruzi* (opossums, bats and mice) and other animals such as lizards, snakes and frogs, which may act as alternative blood source to maintain developmental stages at the ecological niche. In addition, in those palm trees acting as natural shelters for wild vertebrate animals, 691 triatomine bugs were collected which represents a 32% of the total collected bugs. From this number of bugs, 49 (7%) were detected bearing infection by *T. cruzi* in the intestinal tract. The association palm tree, *T. cruzi*-infected triatomine bugs and mammals susceptible to *T. cruzi* infection, was recorded in 12 (12.5%) of the total dissected palms representing 50% of palms harboring vertebrate animals. For details see Table 3.

Table 3. *Palm trees, vertebrate animals and Trypanosoma cruzi-infected triatomine bugs association in Chagas disease endemic area in western Venezuela.*

Vertebrate specimens collected in dissected palm trees					
Total Dissected Palm trees	**Palm trees harboring vertebrate N° (%)**	***Didelphis* N° (%)**	**Bat N° (%)**	**Mouse N° (%)**	**Other vertebrates* N° (%)**
96	24 (25)	11 (46)	6 (25)	1 (4)	16 (67)

Table 3. *Continue.*

Vertebrate specimens collected in dissected palm trees		
Collected bugs in palm trees harboring vertebrates N° (%)	***T. cruzi*-infected bugs collected in palm trees with vertebrates N° (%)**	**Total palm trees harboring vertebrates and infected bugs N° (%)**
691 (32)	49 (7)	12 (50)

*: Snake: 6 (25); Frog: 3 (13); Lizard: 7 (29)

3.4. *Trypanosoma cruzi*-Infected Bugs in Palm Trees Associated to Chagas Disease Acute Patients in Barinas, Venezuela

A total of 1253 triatomine bugs were collected from 41 dissected palm trees located in close proximity to dwellings where 34 acute chagasic patients (ACP) were diagnosed in Barinas state of western Venezuela, where Chagas disease is highly endemic. The average bugs collected per palm tree was 30±39 (range: 0-191). From the total collected bugs, 112 (9%) specimens captured in 22 dissected palms were found infected by *T. cruzi* when examined, indicating that 54% of palms served as successful niche for the maintenance of the parasite-vector association in close proximity to houses where chagasic episodes occurred. Statistical comparison between the 41 dissected palm trees associated to the 34 acute chagasic cases detected in Barinas and the 55 palms dissected in the other selected places indicated above, revealed no significant differences (P>0.05) when considering the number of collected bugs. However, when the variable infected bugs was included in the analysis a significant difference was detected (X^2=9.2; P<0.05). In addition, a similar comparison between palms associated to acute Chagas disease cases and those selected far from them, in the same state of Barinas (N=45), considering together number of dissected palms, collected and *T. cruzi*-infected bugs, revealed differences statistically significant (P<0.01) suggesting close relationship between the presence of palm trees harboring infected bugs and the occurrence of acute Chagas disease episodes in the study area. Interestingly, comparing the number of collected bugs between palms in close proximity to ACP, those palms harboring *T. cruzi*-infected bugs (N=22) showed a highly significant number of triatomine (P<0.001) than palms where infected bugs were not detected (N=19). Details on ACP, number of dissected palms, number collected and *T. cruzi*-infected bugs are presented in Table 4.

Table 4. *Trypanosoma cruzi-infected bugs in palm trees in close proximity to acute chagasic patient in Barinas, Venezuela.*

				***T. cruzi* isolates from**		***T. cruzi* genotypes from**	
Chagas disease acute patients Code	**Dissected palm tree in close proximity to acute chagasic patients N°**	**Collected bugs N°**	***T. cruzi* infected bugs N°**	**Acute patient N°**	**Triatomine bug N°**	**Acute patient N°**	**Triatomine bug N°**
02-89	2	56	-	-	-	-	-
03-89	1	191	4	-	1	-	DTU I
05-89/06-89	2	31	-	1	-	DTU I	-
01-90	1	20	-	1	-	DTU I	-
03-91/04-91	1	28	-	1	-	DTU I	-
14-91	1	6	1	1	-	DTU I	-
04-92	1	96	-	1	-	DTU I	-
05-93/07-93	1	7	-	1	-	DTU II	-
07-94	2	19	2	1	2	DTU I	DTU I (2)
10-94	1	127	8	1	1	DTU I	DTU I
11-94/12-94	2	66	2	2	1	DTU I (2)	DTU I
08-95	1	34	-	1	-	DTU I	-
24-95	1	17	2	1	-	DTU I	-
17-96	1	1	-	-	-	-	-
20-96	1	69	-	-	-	-	-
24-96*	1	17	-	-	-	-	-
25-96	1	5	-	-	-	-	-
26-96	3	32	5	-	-	-	-
07-97	1	53	-	-	-	-	-
JA-97	1	-	-	1	-	DTU I	-
07-98	1	88	-	1	-	DTU I	-
01-99*	1	14	1	1	-	DTU I	-
IM-00/YM-00	3	98	61	2	-	DTU II (2)	-
VAR-06*	1	20	5	-	1	-	DTU I

				T. cruzi isolates from		*T. cruzi* genotypes from	
Chagas disease acute patients Code	**Dissected palm tree in close proximity to acute chagasic patients N°**	**Collected bugs N°**	***T. cruzi* infected bugs N°**	**Acute patient N°**	**Triatomine bug N°**	**Acute patient N°**	**Triatomine bug N°**
ER-07*	1	60	6	-	-	-	-
CAL-10	1	11	1	1	-	DTU II	-
14-10	1	2	1	1	-	DTU I	-
02-11	3	59	13	1	13	DTU I	DTU I (8)
02-13	3	26	-	-	-	-	-
TOTAL: 34	41	1253	112 (9%)	20	19	20 16 DTU I 4 DTU II	14 DTU I

*: Fatal cases

3.5. Potential Risk of Palm Trees as Tool to Build Dwellings in Rural Areas Where Chagas Disease Is Endemic

In the study region, the most abundant palm tree was *Attalea humboltiana*, known by local people as "yagua palm", specimens commonly used to build houses after cutting them down carrying dissected leaves to make the roof (Figure 1). A mature palm, in average, is made up by 20 leaves ranging from 15 to 25 leaves. According to local dwelling builders, most popular dwellings size in the region are built of $50m^2$ (5m width x10m long) using 500-700 palm leaves, equivalent to 10-14 palms to cover $1m^2$ roof. Considering the results obtained in the present study, 1 palm equivalent to 20 leaves harbored 23 bugs (1.1bug/leaf), being 9% the resulting proportion of *T. cruzi*-infected bugs; therefore, each palm may theoretically harbor 1.8 infected bugs. So, the estimation of potential risk indicates that under conditions described in the present study some bugs, including infected ones, may be incorporated to each built house (model in preparation). The analysis revealed a directly proportional risk to the size of built dwellings. Figure 2 gives details of a typical dwelling built in the study area, showing the internal part of the thatched palm roof and an improvised bedroom where infected triatomine bugs were collected.

Figure 1. Palm trees in close proximity to a poor rural dwelling in Western Venezuela. Note a man preparing a leaf to be carried as indicated in the text.

A B C

Figure 2. Thatched palm roof dwelling (A) showing the internal part of the roof and the arrangement of palm leaves (B). Note an improvised bed close to the roof (arrowed) and engorged triatomine bugs collected inside and around a mosquito net (C).

3.6. Detection of *Trypanosoma cruzi* - Infection in Palm Tree Collected Bugs and Estimation of Infective Forms

From 193 palm-tree-collected triatomine bugs, naturally infected with *T. cruzi*, 19 (10%) were selected to estimate the proportion of infective metacyclic forms detected by microscopically examined Giemsa stained fecal samples. The selected sample was made up of 14 specimens of *R. prolixus* (9 adults and 5 instar nymphs) and 5 *T. maculata* (1 adult and 4 instar nymphs). For each sample, 100 microscopic fields (1000X) were examined, showing an average ± SD 4±2 flagellates/field (range 0-5 flagellates) revealing 40%±17% (range 15-78%) of the infective metacyclic forms (Figure 3). In addition, from 112 *T. cruzi*-infected bugs collected in close proximity to ACP, 19 isolates were obtained after established them in culture medium. Isolates identification was corroborated by PCR assays, being genotyped 14 of them as DTU I which matched with most isolates detected in ACPs (Table 4).

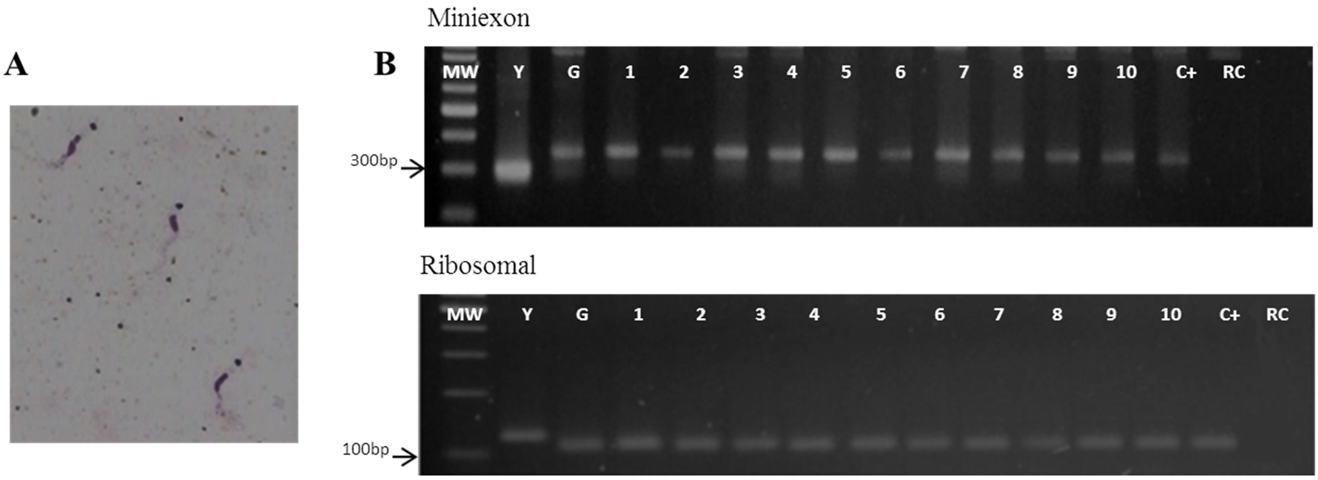

Figure 3. *Metacyclic infective forms detected in Giemsa stain fecal samples of infected wild triatomine bugs collected in palm trees (A) and its molecular characterization (B). PCR assay performed in isolates from T. cruzi-infected bugs collected in close proximity to ACP: Lanes 1-10: selected DNA samples from 14 isolates. C+: T. cruzi DNA control. RC: Reaction control. MW: Molecular weight. Y-G: Genotype reference DNA. PCR product in 3% agarose gel.*

4. Discussion

The analyses of data recorded in the present study demonstrate that palm tree is one of the most favorable natural habitats for wild triatomine bugs in western Venezuela. The observed 99% infestation index in dissected palms, which harbored bugs belonging to four species (*R. prolixus, R. pictipes, T. maculata, and E. mucronatus),* together with the high colonization index (95%), indicated by the presence of all the components of the bug's age structure detected in 2146 collected bugs, appear to corroborate the above statement. Although most of this study was done in Barinas state where 86 palms were dissected and triatomine specimens (N=1915) predominating *R. prolixus* were collected, our observations revealed that in the remaining 10 palms sampled in other places, different species of bugs colonizing same wild shelters, were also detected. These figures support previous reports considering palms as the most important sylvatic ecotope for *R. prolixus* at Los Llanos region in central Venezuela [23], and reporting frequency of palm infestation harboring triatomine breeding colonies in rural areas and its proximity to houses, which increase the risk of contact between bugs and people [7, 8].

Interestingly, in the present study bugs were frequently found in a similar number of dissected palms at any of the two characteristic seasons throughout the years of sampling. However, collection of bugs during rainy season (N=1610) resulted higher than those caught during dry season (536), showing a 3:1 proportion rainy to dry seasons (P<0.01). Further, comparative analysis regarding the amount of instar nymphs and adults, revealed a major number of the immature or juvenile stages over the adult one, when considered its presence by dissected palms and/or season of collection (P<0.01). In addition, statistical comparison among bug's developmental stages considering dissected palms and collected bugs, also revealed significant differences (X^2=15.9, P<0.01, DF:5 and X^2=36, P<0.001, DF:5, respectively). These results statistically support the high infestation and colonization indexes recorded in the present study. Indeed, this ecotope seems to guarantee ecological success to different bug's species providing them blood, as source of energy, by the co-occurrence of diverse vertebrate animals, as well as protection from predation and conducive of bio-climatic conditions, including temperature and humidity, to keep them able for reproduction.

Although appearing to be a biological contradiction, the excellent conditions offered by palm tree to maintain the association *T. cruzi*-wild triatomine bugs, at the same time; may be interpreted as a risky habitat from the epidemiological viewpoint. Indeed, the frequent findings of this association convert palm trees in a risky landscape for human after making irruption into such well-balanced natural niche. Such association represents a public health concern, as an essential risk factor to *T. cruzi*-human infection in Latin America, in general, and at the here study area in particular [10, 13, 24-27].

The above statement is supported in the present study based on the estimated infestation, colonization, palm *T. cruzi*-infection and bug infection indexes, as well as by the recorded association palm-infected bugs-mammal hosts. These figures remark the findings of *T. cruzi* infections in 3 of 4 species of bugs detected in palms from all the five federal entities studied in western Venezuela, and confirm previous opinion that palms and bugs particularly *Attalea* and *R. prolixus*, may be considered ecological risk indicators for Chagas disease [3, 5, 25, 28]. The above conclusion is strongly supported by the results obtained on the relationships between palm tree and *T. cruzi*-infected triatomine bugs association found in close proximity to dwellings where ACPs were detected in localities of Barinas state. In this case the risk was estimated taking into consideration dissection of 22 palms (55%) close to dwellings inhabited by 16 ACPs (47%), where 112 *T. cruzi*-infected bugs (16%) were detected. Therefore, the high incidence of acute Chagas disease observed in people living at the study area, may be consequence of the presence of *T. cruzi*-infected triatomine bugs inside poor quality rural dwellings built with palm trees found all around the localities and houses, as previously reported [12, 13].

The above analyzed information, together with the detected high number of metacyclic infective forms (±700/mm^3) observed in wild dissected bugs, and the similar *T. cruzi* genotype (DTU I) identified in 14 isolates from bugs and 16 from ACPs (details will be published elsewhere), add evidences enough to demonstrate the risky life of inhabitants at palm tree landscapes of this part of western Venezuela.

The overall analysis of the here detected risk factors associated to the palm tree landscape, together with the burden of infective forms recorded in dissected indoor-caught bugs, lead us to recognize how *T. cruzi* is maintained as a constituent of the Chagas disease complex system, both in nature and at domestic shelters.

Finally, gathering together the here obtained results allow us to consider palm tree as an ecotope able to support the maintenance and circulation of *T. cruzi* in natural foci at rural localities. In addition, anthropogenic action producing foci disturbance and passive movement of bug population from sylvatic to domestic habitats, lead us to conclude that palm tree landscape is an ecological risk indicator for Chagas disease in the study area, corroborating previous observations in other latitudes [7, 25, 29, 30].

The above conclusion makes mandatory to take into consideration the presence of bug sylvatic populations in their natural habitats, if the health national authorities think to re-launch a new campaign to control Chagas disease in Venezuela. The proven close relationships between *T. cruzi* and bug populations, both at sylvatic and domestic shelters, associated to anthropological behavior are, by itself, an essential risk for human infection as demonstrated here, and it obligates establishing State policies for a constant and efficient epidemiological surveillance to avoid transformation of natural biocoenosis in severe public health problems.

5. Conclusion

From the analysis of the present results we reach to the following conclusions: i. Palm tree is a favorable habitat to support maintenance and circulation of *T. cruzi*-bug association as a well-balanced biocoenosis in the wild, ii. Anthropogenic activity provoking disturbance and passive flow of bug population from sylvatic to domestic environments, transform palm tree landscape in a risk for Chagas disease occurrence, iii. There is a strong relationship between palm tree and *T. cruzi*-infected triatomine bugs association in close proximity to dwellings with acute chagasic patients, iv. The official intervention for a constant and efficient epidemiological surveillance and control of Chagas disease in the study area is mandatory.

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