Chapter 5

Transmitter Insect of Chagas Disease in Northwest Mexico: A Comparative Study of the Cuticular Hydrocarbons Profile of Three Populations of *Triatoma rubida*: Peridomestic, Domestic and Sylvatic

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Abstract

In México, biogeography data are available for species of triatomas called *Trypanosoma cruzi* transmitters; for example, the phyllosoma complex is distributed in several states of the south-central southeast of the country. In contrast, Northwestern Mexico species such as *Triatoma rubida* are considered sylvatic and in the process of domestication. The lack of research of these northern species of the country has generated an ignorance that contrasts with a growing number of alleged new cases of Chagas disease registered in health institutions in states such as Sonora. From the six species of triatomas that are potential transmitters of the *trypanosoma* in the state of Sonora, *Triatoma rubida* is the only one that has recent studies of distribution and transmission capacity. It is important then to know the degree of domesticity of the native species with the capacity of transmission of *Trypanosoma cruzi* and to define areas of risk. The process of adaptation of the sylvatic triatomines to the peridomestic and the domestic habitat has been understood in terms of environmental and biological variables. In this research, the profile of cuticular hydrocarbons of a peridomestic, domestic and sylvatic population of *Triatoma rubida* was analyzed and compared.

Keywords: *Triatoma rubida*, triatomines, cuticular hydrocarbons

1. Introduction

Chagas disease (CD) is caused in humans and animals by the parasite *Trypanosoma cruzi* (*T. cruzi*) and it is a major cause of mortality in the Americas. It is estimated that about 100 million people are at risk of infection from 6 million people who are infected, generating 56,000 new cases per year for all forms of transmission and 12,000 deaths annually [1–3]. In Mexico, the actual prevalence of CD is unknown and several epidemiological studies have demonstrated the presence of the disease in large urban and rural regions of the country [4]. Even so, it is estimated that there were approximately 1,100,000 infected individuals and 29,500,000 at risk of infection [5, 6]. The most important factors for this to happen are: (1) adaptability of triatomines to human dwellings and the circulation of *T. cruzi* among them and sylvatic and domestic animals; (2) the poverty situation in communities with poor housing and (3) the migration of people between communities and even distant countries where it did not exist [2, 7–10].

In Mexico 32 species are reported; 19 belong to the gender *Triatoma*, 6 to the gender Meccus, 2 to the gender *Panstrongylus* and 1 species of the genders *Belminus, Dipetalogaster, Eratyrus, Paratriatoma and Rhodnius. Triatoma barberi (Usinger), T. dimidiata (Latreille), T. pallidipennis, T. longipennis and T. mazzotti (Usinger)* are the main species found in our country, considered good transmitters of *Trypanosoma cruzi*, *T. barberi* (usinger), *T. dimidiata* (castreille), *T. pallidipennis*, *T. longipennis*, *T. mazzotii* (usinger), *P. picturata, T. mexicana (Herrich-Schaeffer) and T. gerstaeckeri (Stal) Rhodnius prolixus, Dipetalogaster maxima* and *Panstrongylus* spp. [11, 12]. Many of them are described and studied in the central and southern part of the country. However, to date, the factors that predispose the northern part of the country to CD are unknown despite the presence of transmitters in this part of Mexico [13]. The northern arid zones of Baja California Norte (BCN), Baja California Sur (BCS), Chihuahua, Sonora, Durango and Coahuila report a limited presence of domestic triatomines [6, 7]. In the state of Sonora, six species of triatomines have been described: *Triatoma rubida*, they belong to the subgroup *Rubrofasciata*, with five subspecies (*cochimiensis, jaegeri, rubida, sonoriana and uhleri), Triatoma protracta*, *Triatoma recurva*, *Paratriatoma hirsuta papagoensis*, *Triatoma sinaloenses* and *Triatoma incrassata* [14]. All these species are considered sylvatic with little epidemiological value. However, *T. rubida* and *T. recurve* have been associated with human dwellings and with high infection rates (90%) [15–17].

2. Bibliographic revision

2.1. *Triatoma rubida*

This insect has a wide geographic distribution in the Northwest of Mexico, Nayarit, Sinaloa and Sonora, and has been found in the Southwest of the United States in the states of Arizona, California, New Mexico and Texas. It is an established species throughout its range, and there is no information available on its dispersion [18, 19]. The populations of *T. rubida* have been divided into several subspecies based mainly on differences in the pattern and color of transverse spots on the connective border surrounding the abdomen (**Table 1**) [14]. Under laboratory conditions, *T. rubida* completes its life cycle in 130 days, developing 2 generations with

very low mortality during their shedding, so that more than 98% of their eggs hatch and 94% complete their development until adulthood. *T. rubida* is distinguished because the female manages to eat in less than 10 min and her time of defecation can be immediate or between 5 and 20 s after her blood intake. The insect behaves very persistently during feeding and has the ability to hang firmly onto the host until it completes its feeding. This is consistent with the information collected during the fieldwork, where people report having seen their pet such as dogs carrying the bugs attached to the body [20, 21]. According to Tropical Disease Research (TDR) and the World Health Organization (WHO), studies on the mobility of sylvatic and domesticated populations in endemic areas of types can potentially be adaptable to the human habitat [22, 23]. The process of adaptation is considered a dynamic and continuous phenomenon that varies from one species to another according to its degree of ecological adaptation to eco-modified man-made ecosystems. It should also be considered that transmission (which becomes important in some areas) can occur without necessarily occurring true habitat events but only cases of invasion in human environments by adult triatomines or human-vector contact in the sylvatic environment [24–26]. The destruction of the ecotopes can cause changes, and eventually the disappearance of sylvatic animals as natural blood resources for triatomines, resulting in the invasion of houses by the vector in the search for human blood and exposure of the population to the risk of contracting Chagas disease [27–29].

Currently in the anti-vectorial fight of CD, in endemic countries like Brazil, Argentina, Bolivia and Peru, morphometric, biochemical, molecular and genetic studies of vector species are being developed, that contribute in the decision-making for the eradication in their houses. One of these lines of work is the analysis of the cuticular hydrocarbons (CH) of the triatomines [30].

2.2. Cuticle of insects

The cuticle of the insect is secreted by a double layer of epidermal and hypodermic cells. The hypodermis is described as a functional syncytium and is formed as a base membrane particularly during deposition and expansion of the old cuticle. The cuticle is formed by an inner procuticle composed of chitin (*N*-acetylglucosamine) and protein and a thinner chitinous outer layer the epicuticle. The pro-cuticle in turn is divided into an inner layer and an outer layer called exocuticle (pre-mutated cuticle) which is formed by sclerosed protein. The inner endocutaneous layer (cuticle after shedding) has remnants of the same sclerosed protein [31, 32].

Subspecies	**Geographical Distribution**
Triatoma rubida (Uhler)	Baja California Sur, Sonora
Triatoma rubida cochomiensis Ryckman	Baja California
Triatoma rubida jaegeri Ryckman	Isla Estanque BC., Sonora
Triatoma rubida sonoriana Usinger	Sonora, Sinaloa y Nayarit
Triatoma rubida uhleri Usinger	Suroeste de USA, Sonora y Veracruz

*Lent et al., 1979 [14].

Table 1. Geographic distribution of sub-species of ***Triatoma rubida****.

2.3. Insects' cuticular hydrocarbons

The insect's cuticular lipids consist of aliphatic material, which forms a thin layer in its integument. These lipids or surface waxes are presented as highly stable complex mixtures with unique structural characteristics. Among its main compounds hydrocarbons (HCs), fatty alcohols and waxes of high molecular weight predominate. The main function of these lipids in the insect is to restrict water loss and avoid lethal drying. It has been shown that they also participate in the absorption of chemical substances that can affect the activity of microorganisms and intervene in various chemical communication processes [33–35].

Cuticular hydrocarbons (CH) are continuously synthesized in the insect's intrategumenal tissue, through the enzymatic action of fatty acid synthetase (FAS), an acetyl CoA for elongation, a reductase and a decarboxylase that produces hydrocarbons and CO_2. The epidermal cells responsible for its production are the oenocytes that lie beneath the hypodermis. Oenocytes transport hydrocarbons through tissues through a hemolytic lipoprotein called liporin. This lipid synthesis is considered dynamic and changes as the insect passes through its nymphal stages, stopping at the adult stage. De Renobales et al. [36], proposed that the hydrocarbons synthesized by the insect are stored inside their tissues until the next shedding. The insect needs a new layer of lipids as regulators of its permeability [35, 37].

Based on studies conducted in particular on nine species of triatomines of the genus *Triatoma*, *Pastrongylus* and *Rhodnius*, it has been found that such HCs are alkanes of 27–33 carbon atoms and chains of branched alkanes with 1–3 methyl groups inserted along a carbon skeleton from 29 to 41 carbon atoms [35]. The predominant linear components are nC27, nC29, nC31 and nC33, while in the branched fraction predominate isomers of dimethyl- C37, trimethyl-C37, trimethyl-C35 and trimethyl-C39 as reported by Juárez et al. [38] (**Figure 1**). Williams and Jackson [45] suggested that hydrocarbon differentiation may be an early evolution of specialization; in addition, geographic differentiation also led these authors to suggest that the phenotype may be differentiated prior to species divergence. Similarly, Juárez and Brenner [39, 40], considered that the composition of triatomine HC can be used as a taxonomic criterion to separate individual populations and

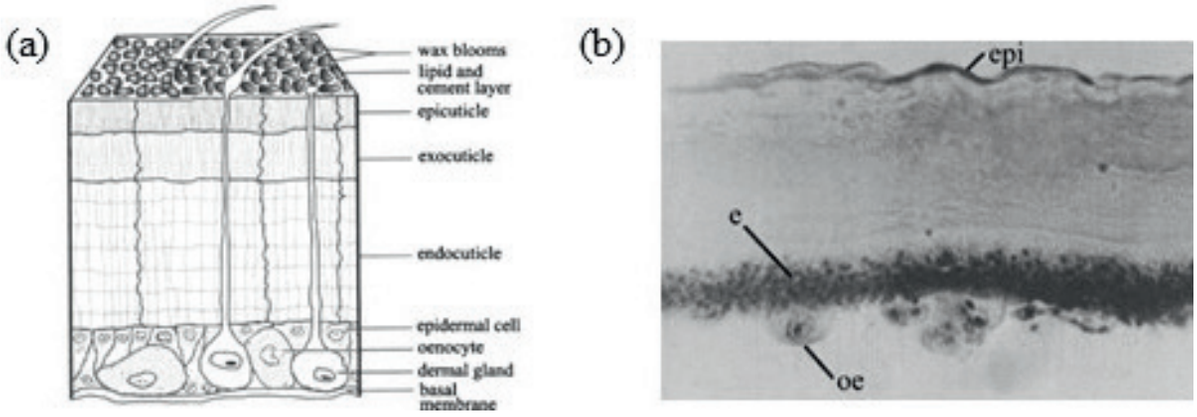

Figure 1. (a) Diagram of a cross-sectional area of the insect integument, illustrating the major layers of the cuticle and (b) cross-sectional view of *T. infestans* integument. Epi, epicuticle; e, epidermal cell layer; oe, oenocytes. Source: Juarez, M.P. 2007.

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specimens, based on the graphical comparison of their corresponding profiles (fingerprints) or through the quantitative calculation of numerical indicators such as the determination of HC [41].

Finally, understanding how insect HCs, together with other surface lipids, are involved in the absorption of chemicals is essential for the timely and adequate vector control measures to be applied in the future [42, 43].

This research analyzes and compares the profile of cuticular hydrocarbons of a peridomestic, domestic and a sylvatic population of *Triatoma rubida*; this is an insect that transmits the Chagas disease in the state of Sonora. The rationale for this proposal was to define the hydrocarbon profiles of *T. rubida* peridomestic, domestic and sylvatic, in order to obtain differences between each of the swarms and to be able to differentiate the three populations of insects by their HC profile. Having the knowledge that *T. rubida* participates in the vectorial transmission of *T. cruzi*, the study is very helpful and of high epidemiological value, to take measures to eradicate and/or control it in human dwellings.

3. Materials and methods

3.1. Sampling area

The city of Guaymas Sonora was chosen because it is considered, in this study, to be an endemic area of the CD. The port is situated at 110°53′34″ North latitude and 27°55′30″ West Greenwich, at a height of 15 m. It has a desert or hot climate, with a maximum monthly temperature of 30–35°C in the months from July to August and a minimum average monthly temperature of 18°C. Its average annual temperature is 28°C. Its vegetation is xerophytic type, where mesquite (*Prosopis velutina*), pitahaya (*Stenocereus thurberi*), palo fierro (*Olneya tesota*), palo verde (*Parkinsonia aculeata*), jito (*Forchammeria* watsonii) and scrub subinerme abound [44].

3.2. Sampling and capture of insects

Three districts of the City of Guaymas were monitored to collect the batch of peridomestic and domestic insects, where the epidemic was known: El Rastro, Cerro Gandareño and Yucatán. In these areas, the existence of triatomines was known, particularly *Triatoma rubida sonoriana*, which was the subspecies chosen for this research and had entomological indices indicating infestation in 63%, colonization of 68.4% and density of 8.5% [15].

The sylvatic insects were collected from the surrounding hills, a hill in the northern part was chosen 1400 m away from the city where domestic and peridomestic insects were collected. *Neotoma* spp. were observed in this area and confirmed during visits. Four nests of *Neotoma* were distributed in a radius of 60 m all placed at the base of pitahayas (*Stenocereus thurberi*).

Once collected, the two pairs of main and secondary wings were extracted from the adult insects which were wrapped in foil and transported to the laboratory of parasitology at the University of Sonora, North Caborca Unit. For the identification of the morphological characteristics that define *rubida*, the keys described by Lent and Wygodzinsky [14] were used.

3.3. Analysis and extraction of hydrocarbons

Cuticular hydrocarbons (CHs) were extracted following the methods of Juárez and Blomquist [45], and Juárez et al. [46]. Each pair of specimen wings were given a washing treatment with 2 mL of distilled water twice to remove any contaminants such as feces or soil particles. They were then transferred to a 4 mL glass vial with a screw cap, Teflon septum and properly labeled. A 1 mL of high-performance liquid chromatography (HPLC) grade hexane was added with 99% purity (Sigma-Aldrich, México). With this solvent they were kept for 1 day, at a temperature of 28°C for the extraction of the cuticular lipids.

3.4. Isolation of hydrocarbons

The next step consisted of separating the hydrocarbons from the other cuticular components (lipids, waxes, etc.) present in the extract. For this purpose, the lipid mixture was reconstituted from each vial with hot hexane and then applied to a mini glass column (10 × 5 mm ID) with 1.74 g of silica gel, 60% pore and 70-230 (Sigma-Aldrich, México; cat. 288,624). Previously equilibrated with hexane, the elution was carried out with 4 mL of hexane for each sample (4 mL/mg). The silica from the column was renewed every three samples (**Figure 2**).

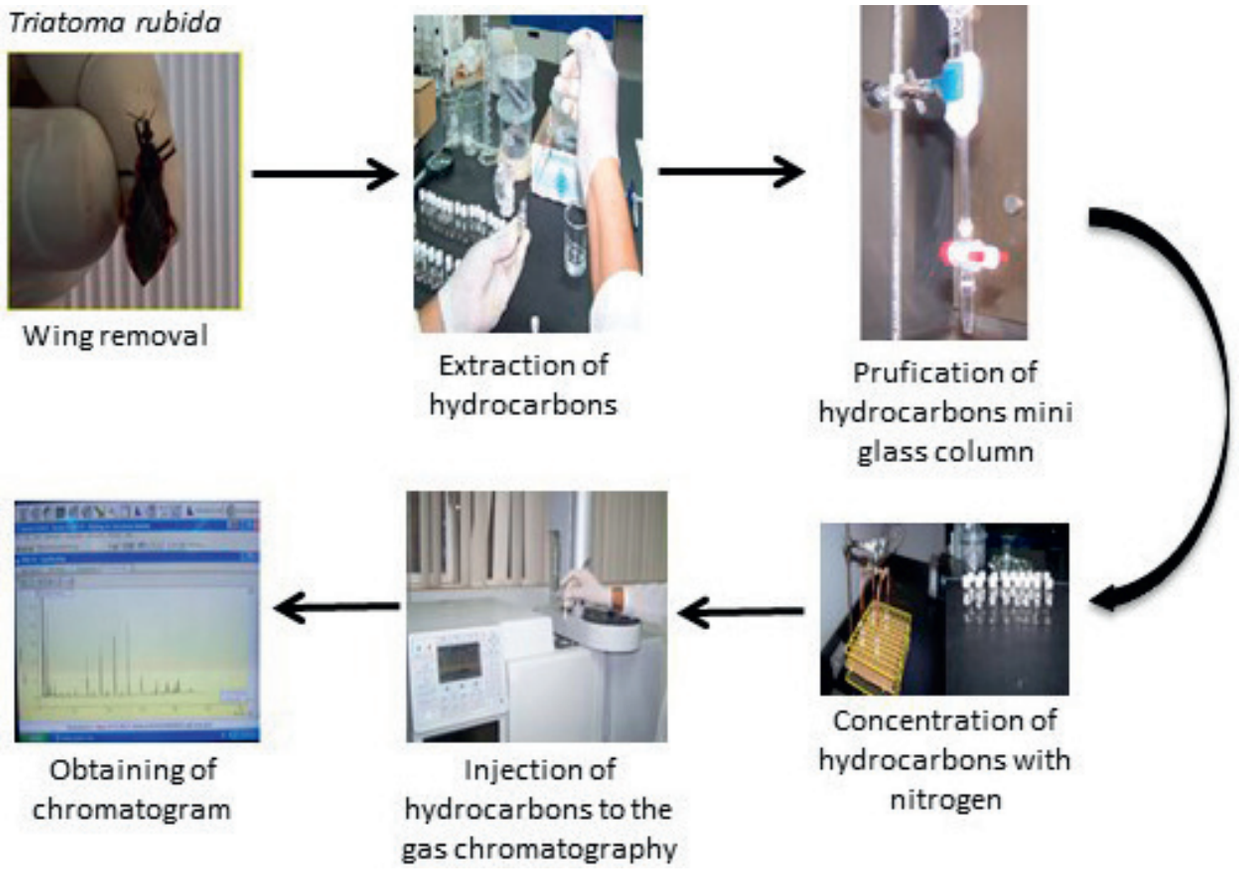

Figure 2. Diagram for the extraction of cuticular hydrocarbons.

3.5. Obtaining the chromatographic profiles of each population

Once the methodology was adjusted, 3 μl of each of the hydrocarbon samples extracted from *Triatoma rubida* was injected into the chromatograph. To do this, 7 μL of hot hexane was taken and poured into the vial containing the hydrocarbons of each specimen, until the largest amount possible from the sample was mixed by circular movements in the bottom of the container, where the required amount was injected in the GC.

3.6. Identification of HC by GC-MS

For the identification of the linear hydrocarbons, an HP 6890 chromatograph coupled to an Agilent 5975C VL mass spectrometer was used. GC conditions were HP-5MS nonpolar column of 30 × 0.25 mm ID × 0.25 μm film; helium carrier gas at 1.5 mL/min constant flow; oven temperature programmed 50°C (1 min) to 200–50°C /min, then to 320–3°C/min (25 min) and the injector was operated in split-less mode at 320°C. The conditions of the MS detector were ionization energy of 70 eV; transfer line at 320°C; the ionization chamber at 230°C and the quadrupole at 150°C. For the analysis of the collected data, an MSD ChemStation Agilent Technologies Inc. was used.

4. Statistical analysis

We estimated central tendency measures and compared the relative means of abundance of hydrocarbons between genera; the significance was tested by a nonpaired t (Excel 2006 package), after normalization of the data with arcosene. Relative means (Tukey's post hoc test) were compared between the three populations through a one-way analysis of variance (ANOVA). The data were presented in tables and graphs. All tests were estimated at one tail and values of $p < 0.05$ were considered as statistically significant. For these analyses the statistical package BioStat 2007 was used.

5. Results

5.1. Collection of insects

A total of 120 peridomestic, 50 domestic and 50 sylvatic specimens were collected. Of the 220 insects, there were nymphs of second stage (NII; 1.4%), nymphs of the third stage (NIII; 11.4%), nymphs of the fourth instar (NIV; 17.3%), 53.6% were nymphs of the fifth instar (NV; 53.6%), 6.8% were adult females (AF) and 9.5% were adult males (AM). No specimens of the first nymphal period were found in all three populations.

5.2. Analysis of the hydrocarbons: Retention times obtained

The gas chromatographic standardization process allowed to obtain the retention times of 14 commercial hydrocarbons standards, AccuStandard Brand, Inc. USA (purity 99%), which were used to estimate Kovats indexes for each sample analyzed (**Table 2**).

Standard	Name	Retention Time (Minutes)
C19	Nonadecane	6.31
C20	Eicosane	7.18
C21	Heneicosane	8.23
C22	Docosane	9.51
C23	Tricosane	10.99
C24	Tetracosane	12.67
C25	Pentacosane	14.49
C26	Hexacosane	16.44
C28	Octacosane	20.45
C30	Triacontane	24.46
C32	Dotriacontane	26.36
C36	Hexatriacontane	35.71
C38	Octatriacontane	38.78
C40	Tetracontane	41.25

Table 2. Retention time of injected standards.

5.3. Identification of the cuticular hydrocarbons profile of *Triatoma rubida*

The 35 components of *T. rubida* were detected (**Figure 3**); however, for this study, only 14 major peaks were considered (**Figure 4**). Five linear hydrocarbons were identified in the three populations of *T. rubida* in both females and males, corresponding to the first five selected peaks of the chromatogram: pentacosano, heptacosano, nonacosano, hentriacontano and tritriacontano. It is important to note that these five hydrocarbons are present in all three populations (**Figure 5**).

The chromatographic profile was similar for the three populations, and the hydrocarbons corresponded to n-alkanes with a continuous series of C25, C27, C29, C31, C33 and C35. In addition, the Kovats indices identified C35.52, C36.00, C37.74, C37.75, C38.00, C39.41, C39.60 and C39.83, which are likely representations of branched isomers of alkanes. The location of the methyl branches of these hydrocarbons, by the proposal of Katritzky et al. [47], was estimated. The Kovats indexes: IK3552, IK3600, IK3774, IK3775, IK3800, IK3941, IK3960 and IK3983 therefore correspond to the hydrocarbons described in **Table 3**.

5.4. Quantification in percent of area of hydrocarbons analyzed

The relative amounts in the percent of area of each linear and branched hydrocarbon analyzed for each population were obtained. In **Table 4**, the data for the peridomestic, domestic and sylvatic *Triatoma rubida* population are presented.

In **Table 5**, the total amounts for linear and branched hydrocarbons are presented for each of the three populations of *rubida;* also the relative percentage of the majority hydrocarbon is

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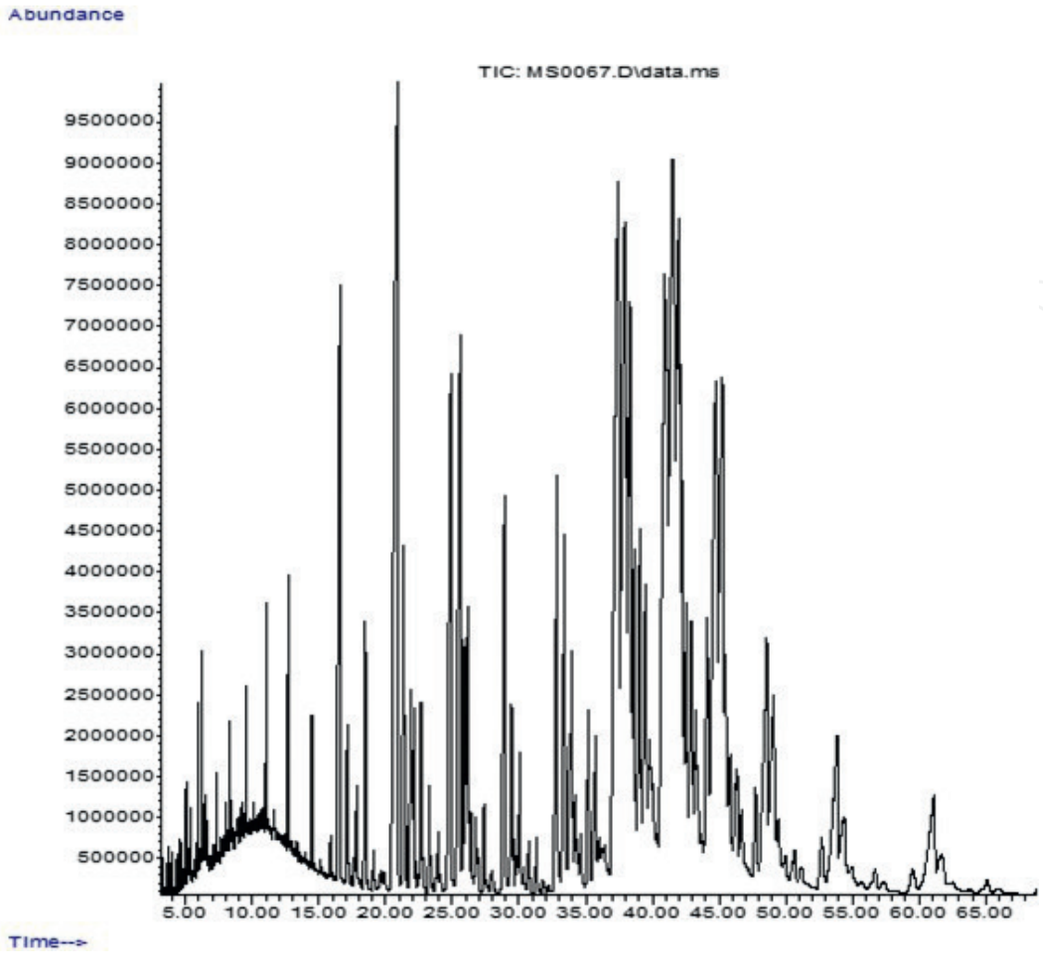

Figure 3. Ion chromatogram total of *Triatoma rubida*.

presented. Using statistical analysis and using the unpaired "t" test, when comparing females and males for each population, a significant difference was found between males and domestic females for the IK2700 peak. When comparing peridomestic males and females, significant differences were found for the IK3100 peak. Regarding the comparison of sylvatic males and females, significant differences were observed for the IK3100 and IK3300 peaks, in addition to the peaks IK2500 and IK2700 (**Figure 6**).

5.5. Comparison of the three populations of *Triatoma rubida*

To analyze the differences between *rubida* species, the one-way parametric analysis of variance (ANOVA) was used. When comparing the three populations of females, significance was found for the relative average abundance of the C27 hydrocarbon. Tukey's post hoc test estimated the differences among the three female populations, finding a significant difference between domestic and sylvatic ones ($p = 0.00001$).

In the comparison of groups of males, significant differences were also found between domestic males and peridomestic males in HC27 ($p = 0.01$) and HC29 ($p = 0.03$), whereas when comparing domestic with sylvatic males, there were significant differences in HC33 ($p = 0.002$). On the other hand, when comparing the populations of females with males, significant differences were observed in the population of domestic females compared to that of peridomestic and sylvatic

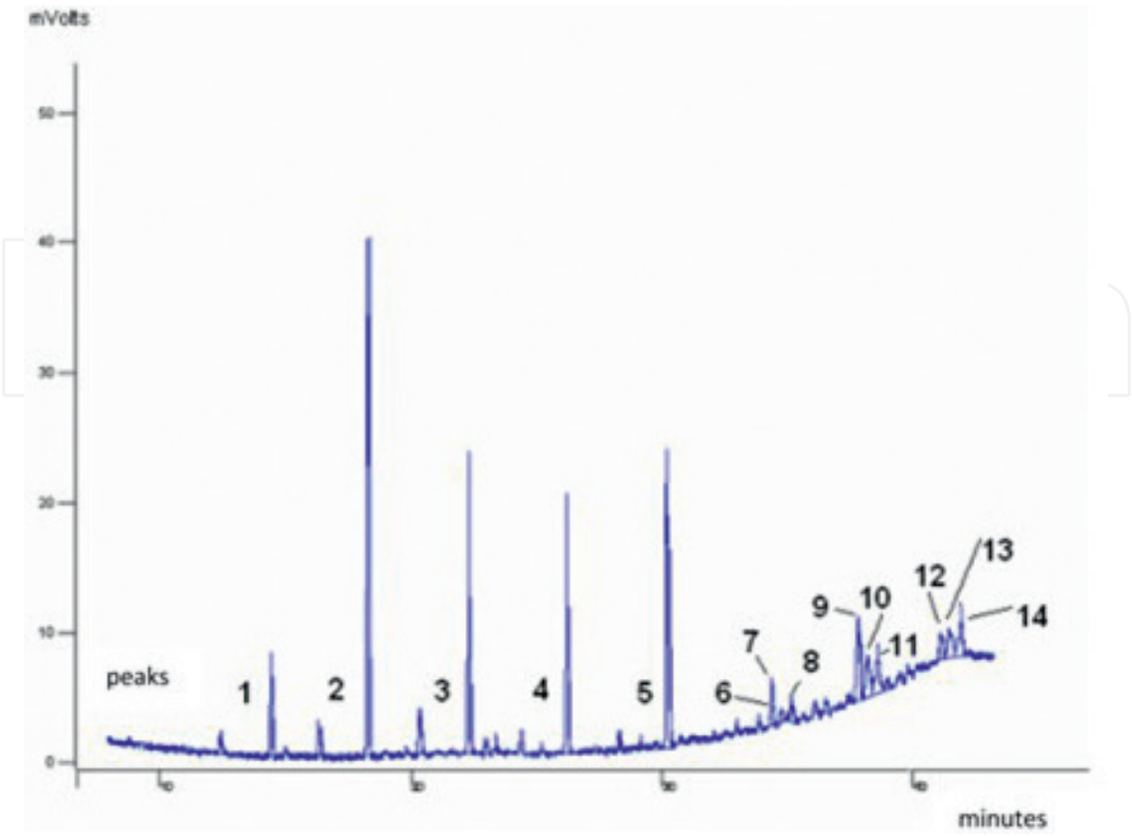

Figure 4. Selection of 14 hydrocarbon peaks, majority in *Triatoma rubida*.

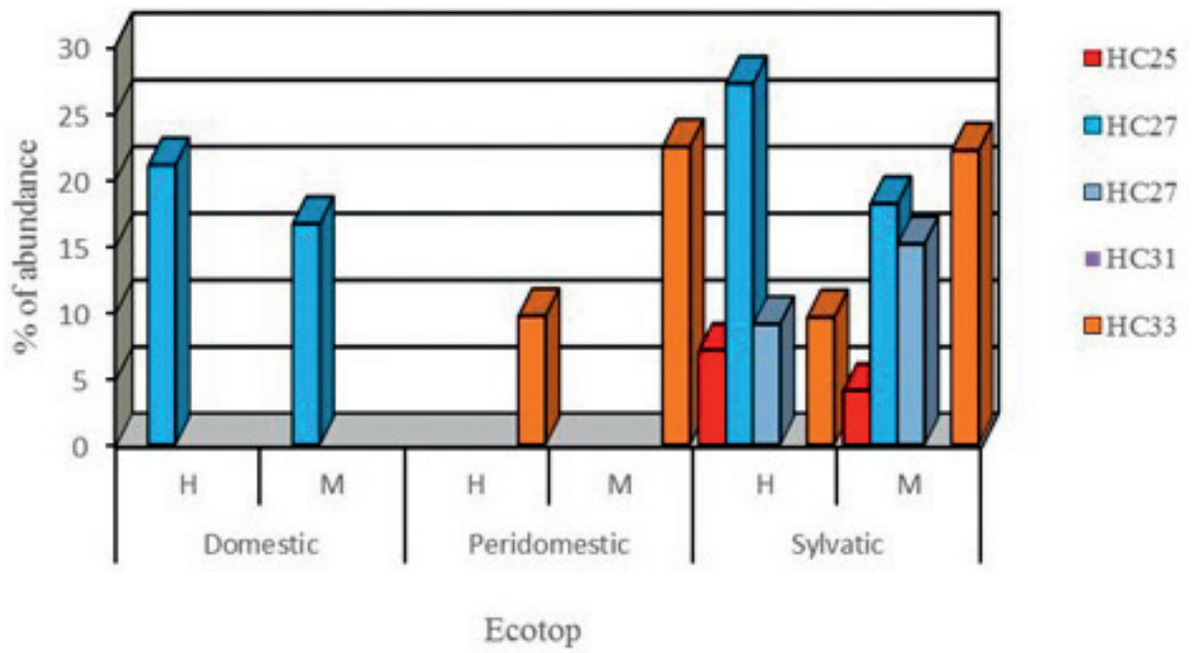

Figure 5. Quantitative variation of cuticular hydrocarbons of *T. rubida* considering its generous.

males in the HC 29 ($p = 0.01$), 31 ($p = 0.03$) and 33 ($p = 0.001$). Meanwhile, the population of peridomestic females, when compared to domestic males and sylvatic males, had significant differences in HC27 ($p = 0.007$), HC29 ($p = 0.01$) and HC33 ($p = 0.0009$). Finally, in the comparison

Retention rate	Type of hydrocarbon
3574	03 Methyl Pentacontane
3600	3x Dimethyl Pentacontane
3752	13, 23 Dimethyl Heptatriacontane
3775	15,19,23 Dimethyl Heptatriacontane
3800	3x Dimethyl Octariacontane
3941	x Trimethyl Nonatriacontane
3960	xx Dimethyl Nonatriacontane
3983	15.19, 23 Trimethyl Nonatriacontane

*Katritzky et al., 2000 [47].

Table 3. Qualitatively identified branched hydrocarbons.

Peak	Hydrocarbon*	Kovats Index	Domestic[1]		Peridomestic[2]		Sylvatic[3]	
			Male	Female	Male	Female	Male	Female
1	n-25	2500	6.59 ± 2.3	5.50 ± 2.3	4.50[a] ± 0.09	7.56[b] ± 0.9	5.54 ± 1.3	6.18 ± 1.0
2	n-27	2700	16.50[a] ± 1.3	20.90[b] ± 2.0	19.00 [a] ± 3.2	27.88[b] ± 0.8	23.00 ± 1.5	26.64 ± 2.6
3	n-29	2900	9.33 ± 1.2	10.80 ± 2.0	12.82 ± 1.6	13.83 ± 0.8	15.29 ± 0.9	16.10 ± 1.9
4	n-31	3100	11.86 ± 1.8	10.00 ± 1.4	15.69[a] ± 1.2	9.41[b] ± 0.9	14.08 ± 0.7	12.17 ± 2.3
5	n-33	3300	14.06 ± 2.7	12.00 ± 1.8	22.29[a] ± 1.7	9.61[b] ± 0.6	15.72[a] ± 1.3	12.29[b] ± 1.7
6	n-35	3500	5.19 ± 1.7	5.20 ± 0.4	2.98 ± 0.3	3.82 ± 0.5	2.59 ± 1.3	3.03 ± 0.5
7	03 Methyl Pentacontane	3574	2.74 ± 0.3	2.90 ± 0.2	1.55 ± 0.3	1.81 ± 0.3	1.84 ± 1.0	2.00 ± 0.3
8	3x Dimethyl Pentacontane	3600	2.76 ± 0.6	2.00 ± 0.4	1.53 ± 0.1	1.47 ± 0.3	1.70 ± 0.5	1.40 ± 0.3
9	13, 23 Dimethyl Heptatriacontane	3572	6.55 ± 0.2	7.40 ± 1.0	5.61 ± 0.9	7.73 ± 1.2	5.77 ± 0.3	6.00 ± 0.3
10	15,19,23 Dimethyl Heptatriacontane	3775	7.07 ± 0.8	6.80 ± 1.3	4.84 ± 2.7	4.73 ± 1.2	4.13 ± 1.0	4.04 ± 1.4
11	3x Dimethyl Octariacontane	3800	5.63 ± 1.1	4.90 ± 0.5	3.02 ± 1.3	4.00 ± 0.2	3.38 ± 0.6	3.00 ± 1.6
12	x Trimethyl Nonatriacontane	3941	2.14 ± 0.3	1.90 ± 0.3	1.96 ± 0.3	2.22 ± 0.6	1.90 ± 0.2	1.70 ± 1.2
13	xx Dimethyl Nonatriacontane	3960	4.79 ± 0.8	4.50 ± 0.9	3.16 ± 0.9	3.57 ± 0.8	3.18 ± 0.6	3.00 ± 1.1
14	15.19, 23 Trimethyl Nonatriacontane	3983	4.79 ± 1.9	5.20 ± 0.9	2.56 ± 2.2	3.50 ± 0.9	3.07 ± 0.7	3.00 ± 2.2

*The hydrocarbons and peak number are the same as reported in **Figures 6-7**. The means were compared with the unpaired *t* test. [a, b] The differences between males and females were significant among the hydrocarbons in each row. Population analyzed: [1.2] n = 12 females and n = 9 males; [3] n = 9 females and n = 12 males.

Table 4. Relative percent of majority hydrocarbons of ***Triatoma rubida*** peridomestic, domestic and sulvatic (%).

Population /Hydrocarbon	*Triatoma rubida*					
	Domestic		Peridomestics		Sylvatic	
	Female	Male	Female	Male	Female	Male
Linear	56.76	51.75	76.41	76.22	72.11	77.28
Branched	43.24	48.25	23.59	23.78	27.89	22.72
2700*	37.40		49.64		46.88	

*Major component of hydrocarbon detected.

Table 5. Content of cuticle hydrocarbons in ***Triatoma rubida*** (%).

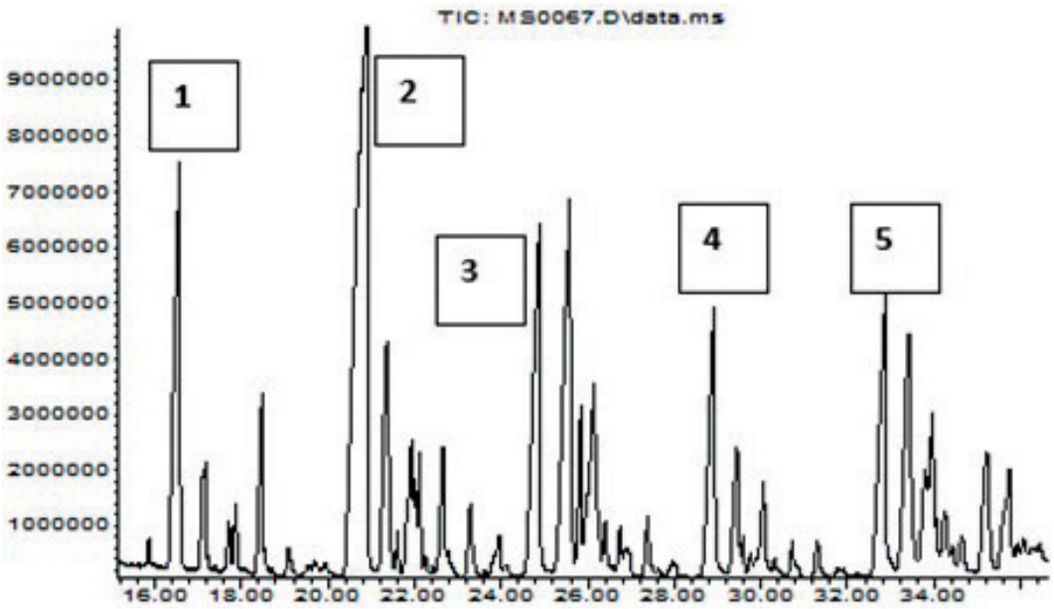

Figure 6. Confirmed linear hydrocarbon mass spectra.

of populations of sylvatic versus domestic males and peridomestic males, significant differences were observed for HC27 ($p = 0.0001$) and HC29 ($p = 0.002$).

6. Discussion

6.1. Domestic population

The *GC–MS* analysis showed that in the population of *Triatoma rubida*, domestic, odd chains of HC prevailed, and C27, C29, C31 and C33 predominated, which together represented 56.8% of HC in females and 51.8% for the males. The branched hydrocarbons represented 32.4% in males and 36.5% in females, of total hydrocarbons. The relative amount of pentacosane hydrocarbon was 37.40%. The characteristics of the typical chromatogram of females and males showed that the chromatographic profiles of the cuticular hydrocarbons in domesticated *Triatoma rubida* were qualitatively very similar for both genera. However, their relative amounts were different, as demonstrated in the pentacosane hydrocarbon, where the female had 20.9% and the male had 16.50%.

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Based on the graphical representation of the chromatographic profiles, the identification of five of their linear hydrocarbons by mass spectrometry and the statistical analysis when comparing the relative means between genders, we suggest how the typical profile of domestic Triatoma rubida is described in **Figure 7**.

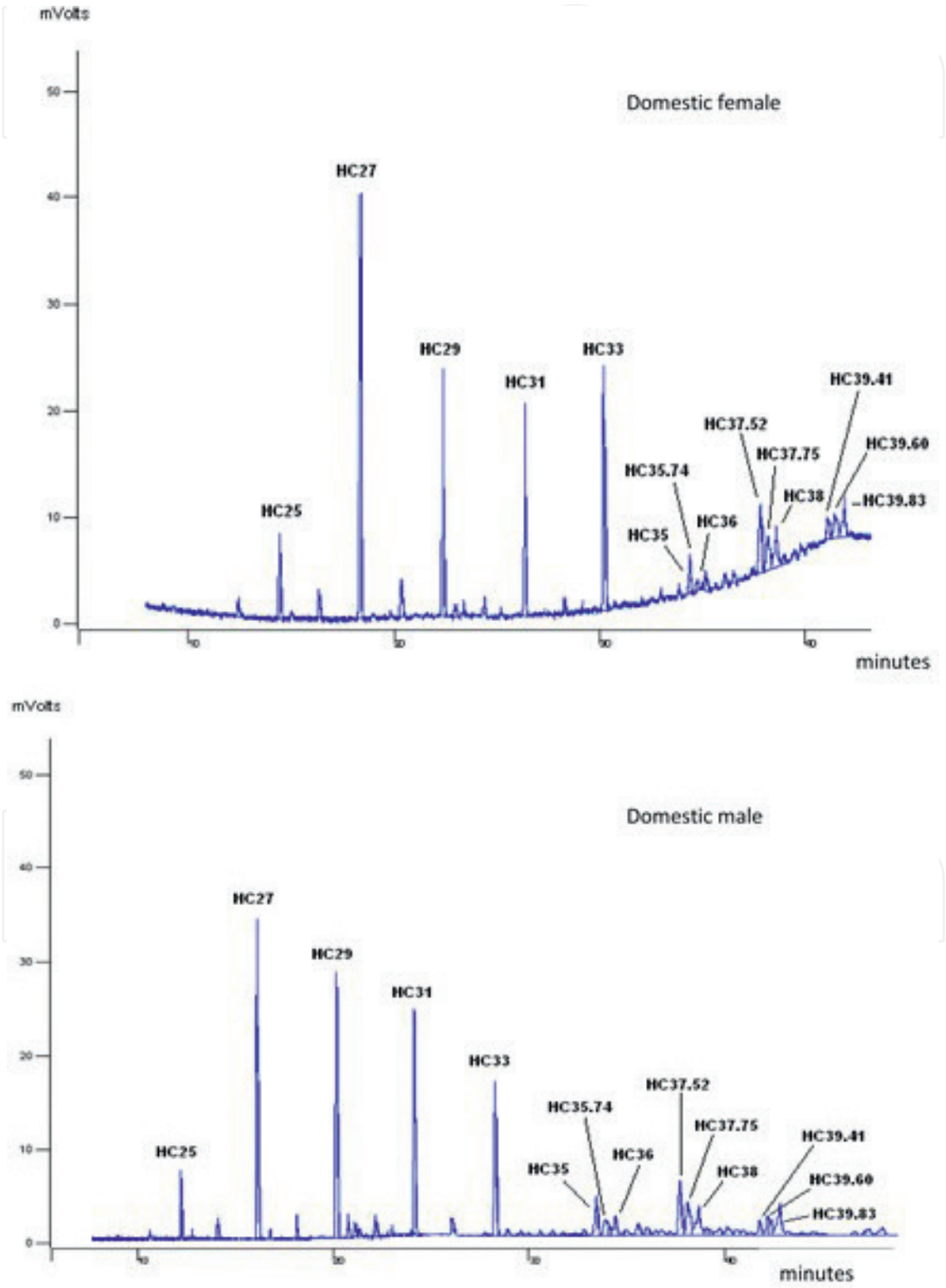

Figure 7. Typical chromatogram of female and domestic male of *Triatoma rubida*.

When comparing this profile of hydrocarbons obtained with studies of other triatomines, a clear differentiation of species can be seen. For example, the literature reports that *T. barberi*, one of the habitat triatomines, is considered to be a transmitter of CD in Mexico, has major alkanes such as C29, C31 along with C33 and C27, respectively and most of its branched components correspond to mono-, di- and trimethyls of C33, C35 and C37 [43]. On the other hand, *T. dimidiata*, considered one of the most important domestic triatomines in Mexico, presents a profile of cuticular hydrocarbons, formed by saturated hydrocarbon chains ranging from C22 to C35. Of these, the odd one strands like C31 followed by C29, C27 and C33 and small amounts of the C22 and C30 hydrocarbons prevail. It also has branched alkanes, most of them mixtures of different isomers: mono-, di- and trimethyl in their internal chains [43]. *Triatoma longipennis* insect belonging to the phyllosoma complex, widely distributed particularly in Central and Southern Mexico, is considered to be a *Triatoma* with a high degree of habitation [48] and has a saturated hydrocarbon profile ranging from C23, C25, C27, C29, C31 to C33, with the majority being C29 (16%) [49].

6.2. Peridomestic population

The GC–MS analysis showed that the chromatographic profiles of the HC in *Triatoma rubida* peridomestic are qualitatively very similar for both genders. As in the domestic population, they corresponded to n-alkanes with a continuous series of C25, C27, C29, C31, C33 C35. In addition, C35.52, C36.00, C37.74, C37.75, C38.00, C39.41, C39.60 and C39.83 were identified, were likely to be representative of branched isomers of alkanes. The location of the methyl branches of these HCs, by the proposal of Katritzky et al. [47], was estimated. The proposal made it possible to estimate that the hydrocarbons IK3552, IK3600, IK3774, IK3775, IK3800, IK3941, IK3960 and IK3983 correspond to the same hydrocarbons identified in the domestic population.

For this population, odd strands prevailed predominantly, C27, C29, C31 and C33, which together represented 76.41% in females and 76.22% in males. The total relative amount of branched hydrocarbons in males and females was 23.59% and 23.78%, respectively. The relative amount of the pentacosane hydrocarbon was 49.64%. Based on the graphical representation of the peridomestic rubida species chromatographic profiles, in their identification by mass spectrophotometry and in the statistical analysis when comparing the relative means between genders. We can suggest as typical profile of Triatoma rubida peridomestic described in **Figure 8**.

6.3. Sylvatic population

The analysis of *GC–MS* showed that sylvatic-type *Triatoma rubida* HC is qualitatively very similar for both sexes. These HCs corresponded to n-alkanes with a continuous series of C25, C27, C29, C31, C33 and C35. In addition, C35.52, C36.00, C37.74, C37.75, C38.00, C39.41, C39.60 and C39.83 were identified, which are likely representations of branched isomers of alkanes. Through the proposal of Katritzky et al. [47], the location of the methyl branches for the identified hydrocarbons was estimated, resulting as qualitatively equal to the two previous populations. Odd numbers, predominantly C27, C29, C31 and C33, chains prevailed in this population, which together represented 72.1% of the relative percentage in females and 72.3% for males. The total relative amount of branched hydrocarbons in males and females

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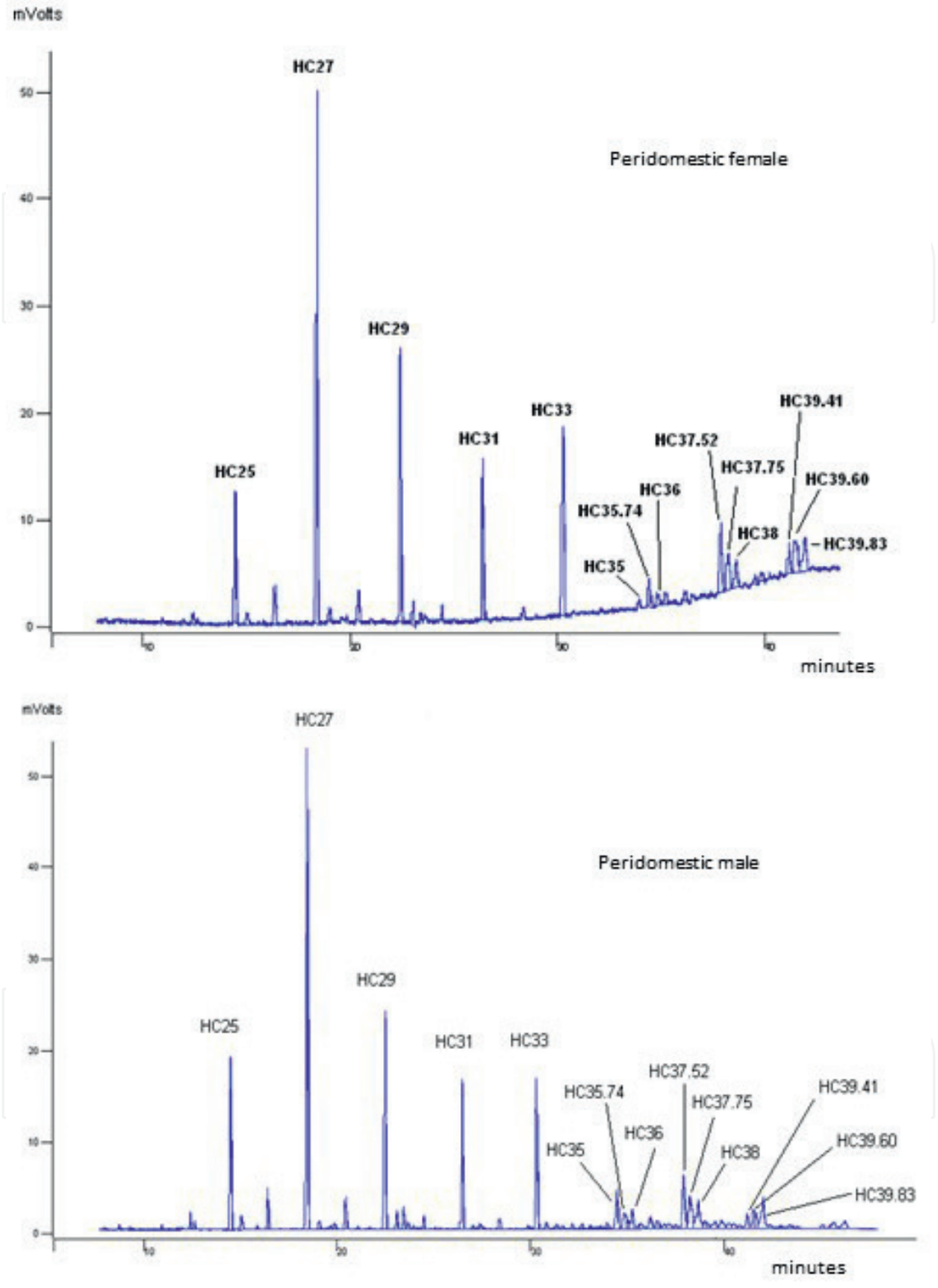

Figure 8. Typical chromatogram of female and peridomestic male of *Triatoma rubida*.

was 27.9 and 22.7%, respectively. The relative amount of the pentacosane hydrocarbon was 46.88%. The pentacosane hydrocarbon of females was present in 27.88% while in the males they presented 19%, similarly the hentriacontano hydrocarbon was 15.59% in males with

respect to 9.41% of the females. Likewise, there were differences in the tritriacontano hydrocarbon of females, 9.61% with respect to 22.29% in males.

Based on the graphical representation of the chromatograms of sylvatic *rubida* species, their identification by mass spectrophotometry and statistical analysis when comparing the relative means between sexes, the typical profile of sylvatic Triatoma rubida can be suggested as described in **Figure 9**.

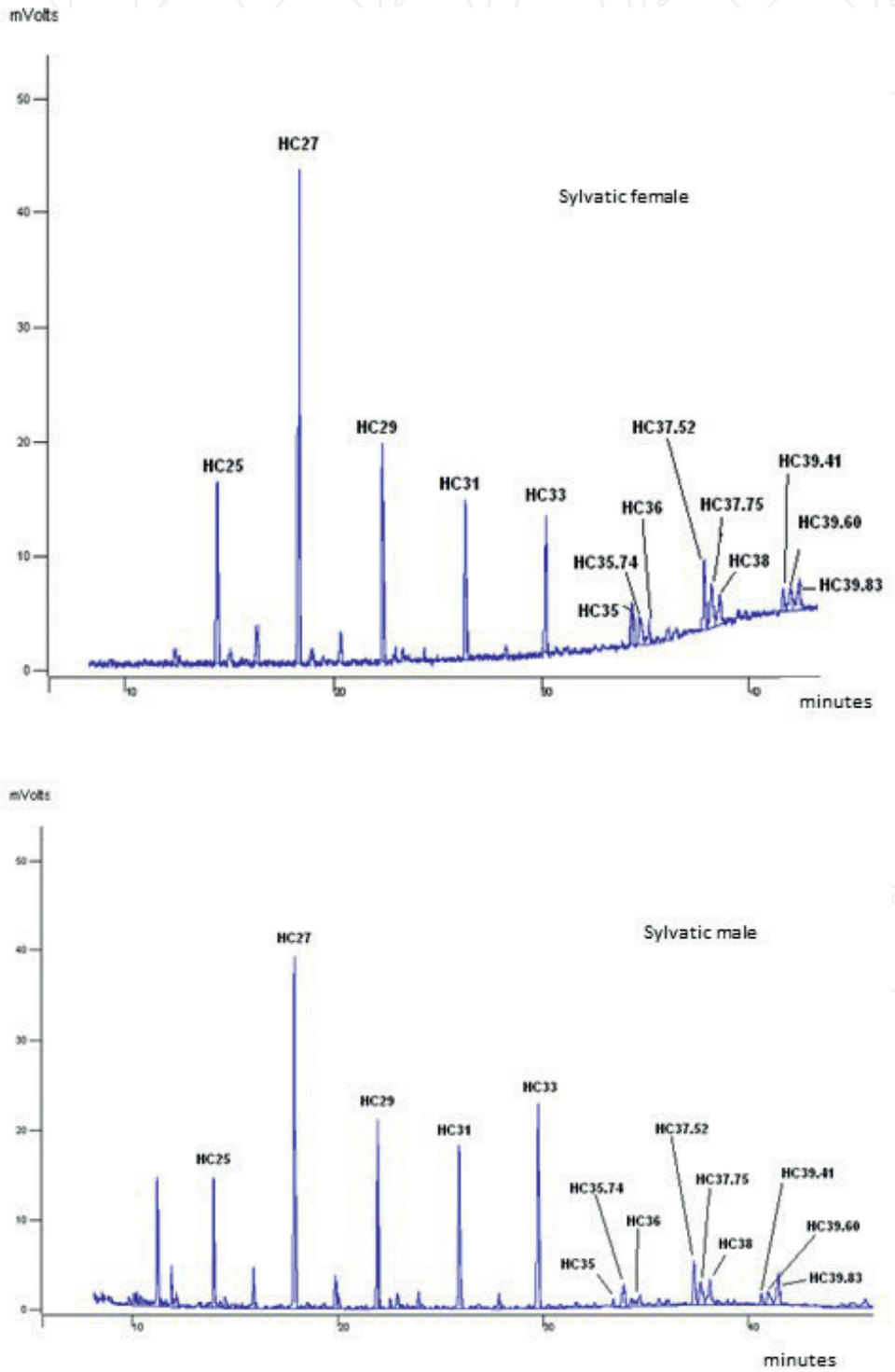

Figure 9. Typical chromatogram of female and sylvatic male of *Triatoma rubida*.

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Juárez et al. [38], suggested that quantitative rather than qualitative differences support the idea that cuticular hydrocarbons represent primitive characteristics for *Triatomas*. For example, the study of the tribes *Rhodnius* and *Triatomini* found few common traits among them, however, they converge in their hematophagous habits, even though they come from very different habitats. This led them to suggest that the HC profile obeys an ancestral base set by the selection, favoring the presence of certain hydrocarbons associated with conditions of dry habitats and humid ecotypes such as that of the City of Guaymas.

Therefore, species of triatomines of dry regions present their cuticular profiles as more abundant and complex than their congeners of humid regions. Among Triatoma, *T. brasilinesis* and *T. pseudomaculata* from arid regions of Northeastern Brazil present a more complex profile than *T. infestans* from less dry regions of Central Brazil and Argentina, and in turn the three species mentioned present larger complexity when compared to *T. bimaculata* and *T. vitticeps* from coastal regions [50].

In Mexico, *T. barberi* that lives in dry regions presents a more complex HC model. The population area of *Dipetalogaster maximus* that has been collected from Baja California Sur presents abundant and longer saturated chains and constitutes 60–67.8% of the total hydrocarbon mixture for females and males, respectively. Thus, the relative abundance of n-alkanes may be related to the exposure of adverse conditions [51].

7. Conclusions

This research provides basic knowledge on the cuticular lipids of Triatoma rubida. A unique and very different profile of cuticula hydrocarbons was obtained from *Triatoma barberi*, *Triatoma dimidiata* and *Dipetalogaster maximus*, species are considered as transmitters of *Trypanosoma cruzi* in Mexico. No characteristic profile in the cuticular hydrocarbons was found in the collected population of females and males of *Triatoma rubida*. However, the cuticular hydrocarbons profile of the peridomestic, domestic and sylvatic populations of the insect is qualitatively similar, but were identified by significant quantitative differences, so it is possible to state that distinctions can be made between populations. The profile of cuticular hydrocarbons, identified in this study, can be used as a reliable chemotaxonomic tool to identify the populations of *T. rubida*, considering the expression of hydrocarbons as the chemical phenotype of the vector that responds to environmental and biological factors of the insect.

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