Cuticular Hydrocarbons of Chagas Disease Vectors in Mexico

M Patricia Juárez/+, David A Carlson*, Paz María Salazar Schettino, Sergio Mijailovsky, Gloria Rojas****

Instituto de Investigaciones Bioquímicas de La Plata, Conicet-UNLP, Facultad de Ciencias Médicas, Calles 60 y 120, La Plata, 1900, Argentina *USDA, ARS, Medical and Veterinary Entomology Research Laboratory, Gainesville, Fl, USA **Laboratorio de Biología de Parásitos, Departamento de Microbiología y Parasitología, Facultad de Medicina, UNAM, Mexico DF, Mexico

Capillary gas-liquid chromatography was used to analyse the cuticular hydrocarbons of three triatomine species, Triatoma dimidiata, T. barberi *and* Dipetalogaster maxima, *domestic vectors of Chagas disease in Mexico. Mixtures of saturated hydrocarbons of straight and methyl-branched chains were characteristic of the three species, but quantitatively different. Major methylbranched components mostly corresponded to different saturated isomers of monomethyl, dimethyl and trimethyl branched hydrocarbons ranging from 29 to 39 carbon backbones. Sex-dependant, quantitative differences in certain hydrocarbons were apparent in* T. dimidiata.

Key words: *Triatoma* - *Dipetalogaster* - cuticular hydrocarbons - gas chromatography - chemotaxonomy - Mexico

Chagas disease (American trypanosomiasis), is widespread in the Americas, with the causative parasite, *Trypanosoma cruzi*, usually transmitted to humans in the faecal droppings of large blood-sucking insects of the subfamily Triatominae (Hemiptera, Reduviidae). In Mexico, over 30 species of Triatominae have been reported, mainly *Triatoma* species of the protracta and phyllosoma complex (Zeledón 1981, Zárate & Zárate 1985, Salazar Schettino et al. 1988, Schofield 2000). Most of them are actual or potential vectors of the disease, and Mexican authorities are currently carrying out surveys for Chagas disease vectors and implementing control trials in order to evaluate risk areas. As part of a larger study, the Latin American Network for Research on the Biology and Control of Triatominae is contributing to these efforts by evaluating the distribution and classification of domiciliated Triatominae using morphological, morphometric, molecular and biochemical techniques. Within this framework, we report here a study of the cuticular hydrocarbons of representative Mexican species of Triatominae, designed to evaluate the use of hydrocarbon profiles for species characterization and phylogenetic studies.

MATERIALS AND METHODS

Insects - We analyzed hydrocarbons from wings of adult male and female individual specimens of *T. dimidiata,* collected from domestic and peridomestic locations from the municipalities of Tempoal, Citlaltepec and Chontla, in the State of Veracruz. The *T. barberi* specimens were collected from domestic habitats in the village of Joaquín Herrera, municipality of Villa Corregidora, State of Querétaro, and the *Dipetalogaster maxima* specimens were collected from houses in Colonia Roma, municipality of La Paz, State of Baja California Sur (Table I). Wings of each specimen were wrapped in aluminum foil and stored at room temperature prior to analysis.

Hydrocarbon analysis - Cuticular hydrocarbons were extracted as previously described (Juárez & Blomquist 1993, Juárez et al. 2001). Wings from each specimen were washed with redistilled water to remove any water soluble contaminants, transferred to a glass vial with Teflon-lined caps, and submerged in redistilled hexane (6 ml/g) overnight, to extract total lipids. The solvent was transferred to another vial, reduced in volume under nitrogen, then hydrocarbons were separated from other components by adsorption chromatography performed on a mini-column of activated Biosil A (10 mm x 5 mm I.D.), eluting with redistilled hexane (6 ml/mg hydrocarbon). This final extract then was evaporated to an appropriate volume for gas chromatography. Capillary gas chromatographic (CGC) analysis was performed using a Hewlett-Packard (HP) Model 6890 gas chromatograph equipped with a cool on-column injector port and autoinjector system, fitted with a non-polar fused silica (0.2 µm) HP-5 capillary column (30 m x 0.32 mm I.D.), the carrier gas was H_2 at a linear velocity of 40 cm/sec. The oven temperature was programmed from 60°C (hold time 2 min) to 180°C at 20°C/min, then 180°C to 310°C at 3°C/min (hold 10 min). The flame ionization detector (FID) was held at 320°C. A PC based data system, Turbochrom 3 (Perkin Elmer, CA, USA) was used for data recording and quantification. Injection of *n*-alkane standards of 22 to 42 carbons was similarly performed for estimation of Kovat Indices (KI) (Kovats 1965). Numbers showed close agreement with the KI's calculated for other triatomines (Juárez & Blomquist 1993, Juárez et al. 2001), and prediction of methyl branching pattern was done as proposed by Carlson et al. (1998). The nomenclature used to describe hydrocarbons was (Cn) to describe the total number of carbons in the corre-

This work was partially supported by grants from the AVINA Foundation, and the European Commission, and benefited from international collaboration through the ECLAT network and through Mexican research project, Conacyt 30871-N and WHO/TDR ID-970854 to PMSS.
+Corresponding author. Fax: +54-221-4258988. E-mail: mjuarez@isis.unlp.edu.ar
Received 17 December 2001
Accepted 19 June 2002

TABLE I
Triatominae sp. evaluated

Insect	Locality	Municipality	State	N Latitude	W Longitud	Altitude (m)
T. dimidiata	Los Cerritos	Citlaltepetl	Veracruz	21°20'	97°53'	220
T. dimidiata	Tancolol	Chontla	Veracruz	21°18'	97°55'	260
T. dimidiata	El Cantarito	Tempoal	Veracruz	21°31'	98°23'	50
T. barberi	Joaquín Herrera	Villa Corregidora	Querétaro	20°32'	100°26'	1,940
D. maxima	Col. Roma	La Paz	Baja California Sur	24°09'	110°17'	20

T: *Triatoma; D*: *Dipetalogaster*

sponding hydrocarbon component; the location of methyl groups is indicated by (x- me) for monomethyl-alkanes, (x,x- dime) for dimethylalkanes and (x,x,x- trime) for trimethylalkanes.

RESULTS

T. dimidiata - Typical gas chromatographic profiles of hydrocarbons extracted from individual male and female wings of *T. dimidiata* are shown in Fig. 1 A-B. The straight chain hydrocarbon components comprised saturated chains ranging from C22 up to 35 total carbons and accounted for 64.1% (males) and 50.4% (females) of the total wing hydrocarbon extract. Odd-chain components prevailed, with *n*- C31 accounting for 35.8% (males) and 20.9% (females) of the total hydrocarbon, followed by *n*- C29, *n*- C27 and *n*- C33; smaller amounts of even-numbered hydrocarbons, *n*- C22 through *n*- C30, were also detected (Table II).

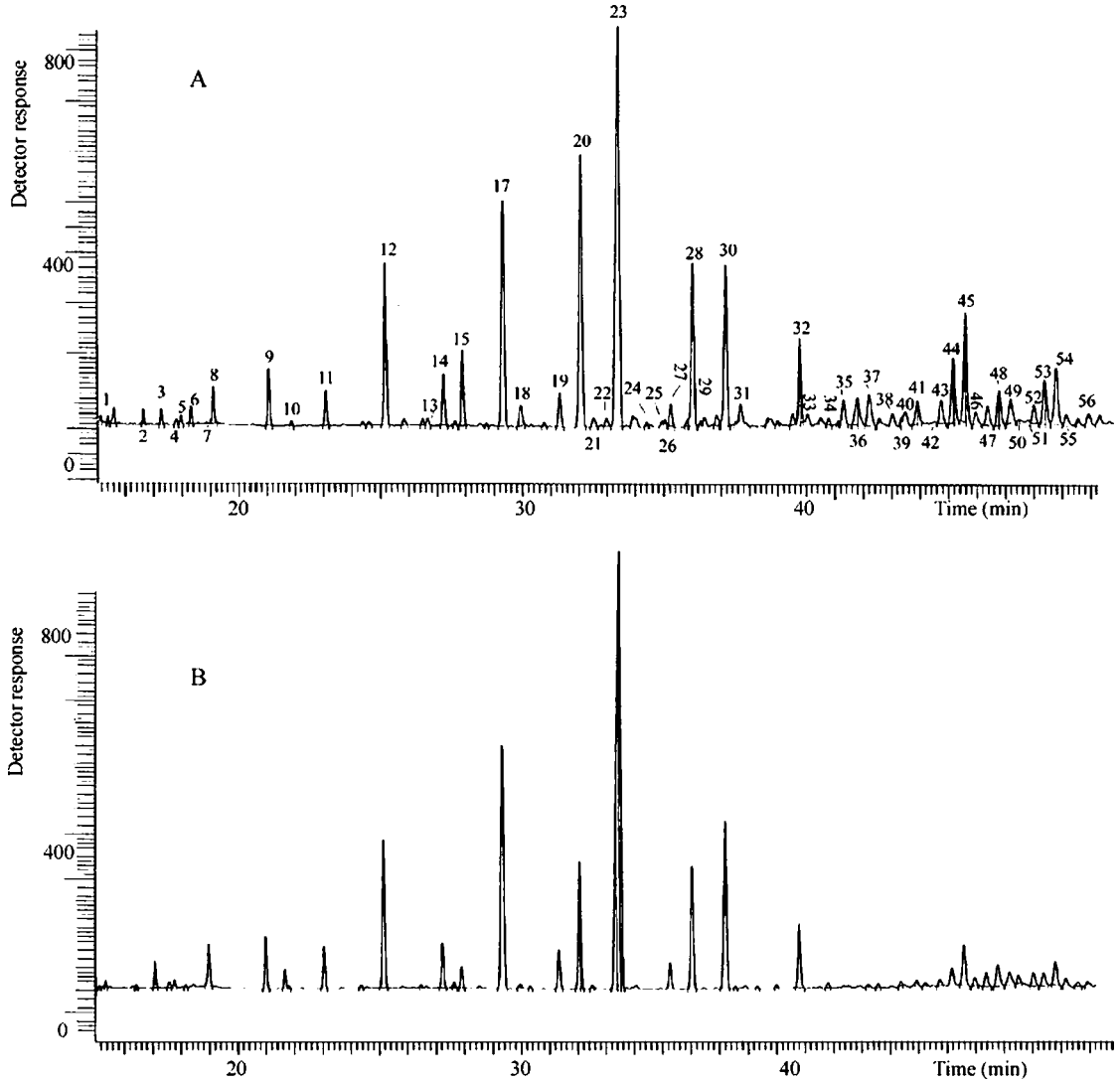

Fig. 1: capillary gas chromatography profiles of the cuticular hydrocarbons of *Triatoma dimidiata*. A: adult females; B: adult males. Numbers indicating each hydrocarbon peak are indicated in A, and correspond to peak numbers from Table II.

TABLE II

Percent hydrocarbons from selected components of *Triatoma dimidiata*

Peak	Hydrocarbon [a]	Kovats Indices	Male % [b]	Male S.E.	Female %	Female S.E.
1	*n*- C22	2200	0,1	0,0	traces	0,0
2	monome- C22	2259	0,2	0,0	0,2	0,1
3	*n*- C23	2300	0,4	0,1	0,2	0,0
4	internally branched monome C23	2322	0,2	0,0	traces	0,0
5	internally branched monome C23	2332	0,2	0,0	0,1	0,1
6	5- me C23	2354	0,2	0,1	0,2	0,1
7	unknown	2390	0,2	0,1	traces	0,0
8	*n*- C24	2400	0,6	0,2	0,3	0,2
9	*n*- C25	2500	1,4	0,2	1,9	0,2
10	internally branched monome C25	2527	0,2	0,1	0,1	0,1
11	*n*- C26	2600	0,8	0,2	0,7	0,0
12	*n*- C27	2700	5,4	0,4	7,4	1,3
13	x,x,- dime C27	2759	0,3	0,1	traces	0,0
14	*n*- C28	2800	1,6	0,1	1,4	0,1
15	3,x- dime C27	2815	1,9	0,2	2,6	0,1
16	internally branched monome C28	2832	traces	0,0	traces	0,0
17	*n*- C29	2900	13,3	0,7	12,8	2,0
18	internally branched monome C29	2924	0,1	0,0	0,5	0,2
19	*n*- C30	3000	1,2	0,2	1,0	0,1
20	3,x,x- trime C29 + monome C30	3034	11,1	1,8	13,8	0,7
21	x,x- dime C30	3058	0,1	0,0	0,3	0,1
22	unknown	3085	traces	0,0	0,2	0,2
23	*n*- C31	3100	31,7	2,7	20,8	1,4
24	5-me C31	3150	traces	0,0	0,1	0,1
25	x,x- dime C31	3170	traces	0,0	0,2	0,2
26	x,x- dime C31 + x,x,x,- trime C31	3180	traces	0,0	0,2	0,2
27	x,x,x,- trime C31	3190	0,7	0,1	0,5	0,0
28	3,x,x- trime C31 + monome C32	3237	8,9	1,4	7,8	0,5
29	unknown	3250	traces	0,0	0,1	0,1
30	*n*- C33	3300	7,5	1,3	3,8	0,1
31	internally branched monome C33	3329	traces	0,0	0,2	0,1
32	3,x,x- trime C33	3447	4,2	0,7	4,3	0,3
33	4- me C34	3458	traces	0,0	0,1	0,1
34	*n*- C35	3500	0,1	0,1	traces	0,0
35	internally branched monome C35	3530	traces	0,0	0,2	0,2
36	5- me C35	3553	traces	0,0	0,2	0,2
37	x,x- dime C35 + x,x,x- trime C35	3580	traces	0,0	0,9	0,7
38	internally branched monome C36	3628	traces	0,0	0,2	0,2
39	x- me C36	3645	0,2	0,0	traces	0,0
40	x- me C36 (?)	3651	traces	0,0	0,1	0,0
41	x,x,x- trime C36	3680	0,1	0,1	0,6	0,3
42	unknown	3713	0,1	0,1	traces	0,0
43	internally branched monome C37	3728	0,3	0,1	0,4	0,2
44	5- me C37	3753	0,3	0,2	2,8	0,4
45	x,x- dime C37	3777	1,8	0,4	5,0	0,5
46	3,x- dime C37	3800	0,2	0,1	0,6	0,1
47	internally branched monome C38	3825	0,4	0,1	0,6	0,2
48	x,x- dime C38	3852	1,4	0,2	2,2	0,2
49	x,x- dime C38	3874	0,4	0,1	0,1	0,0
50	x,x,x- trime C38	3900	0,6	0,2	traces	0,0
51	unknown	3915	0,1	0,1	traces	0,0
52	internally branched monome C39	3930	0,5	0,2	0,5	0,2
53	5- me C39	3952	0,4	0,2	1,9	0,9
54	x,x- dime C39 + x,x,x- trime C39	3976	1,1	0,2	1,3	0,5
55	unknown	4000	traces	0,0	0,2	0,1
56	unknown	4053	traces	0,0	0,3	0,3
	branched/normal		0,6		1,0	

a: hydrocarbon and peak numbers are the same as reported in Fig. 1; *b*: means were compared by the unpaired t test, differences between males and females were extremely significant for peaks 2815 and 3777 ($P < 0.0001$), very significant for 3100 and 3852 KI ($P < 0.005$), and significant for peaks 3300, 3851 and 3952 KI ($P < 0.03$) n = 4 for females, n = 9 for males. n = 4 for females, n = 9 for males

Among the methyl-branched alkanes, the major components consisted of a mixture of different isomers of terminally branched trimethyl odd chains together with internally branched monomethyl of even numbered chain, eluting at 3034 KI (3,x,x- trimethylC29 plus x- methyl C30) and 3237 KI (3,x,x- trimethyl C31 plus x- methyl C32), followed by 3,x,x- trimethyl C33 (3447 KI), 3,x- dimethyl C27 (2815 KI), and internally branched x,x- dimethyl C37 (3777 KI), x,x- dimethyl C38 (3852 KI) and at 3976 KI eluted a mixture of x,x- dimethyl and x,x,x- trimethyl C39. Sexual differences evaluated by the unpaired t test were very significant for the peak eluting at 3100 KI, the major hydrocarbon component *n*-C31, extremely significant ($P < 0.005$) for peaks of 3777 and 2815 KI ($P < 0.0001$) corresponding to x,x- dimethyl C37 and 3,x- dimethyl C27, respectively. Significant differences were found for peaks eluting at 3300, 3852 and 3952 KI ($P = 0.02$) (Table II). Four peaks were selected for peak ratios (R values) to evaluate sex-dependant, quantitative differences in hydrocarbon components, and the comparison of three R values separated *T. dimidiata* males and females at the 95% level of confidence (Table III). In addition, females had higher amounts of methyl-branched components than males with a branched to normal ratio of 0.98 for females and 0.56 for males. Peaks at 3150, 3170, and 3180 KI corresponding to mono-, di- and trimethyl C31 as well as mono-, di-, and trimethyl C35 eluting at 3530, 3553 and 3580 KI, and monomethyl C36 (3628 KI) were detecttable in females, but less apparent in males.

T. barberi - Fig. 2A, B shows the gas chromatographic traces for adult males and females of *T. barberi*. The major n-alkanes are n-C29, n-C31, together with n-C33 and n-C27, plus minor amounts of straight chains from 22 to 37 carbons representing 57.9-52.5% respectively of the total hydrocarbons. Major methyl-branched components corresponded to mono-, di- and trimethyl derivatives of C33, C35 and C37 chains, with a branched to normal ratio of 0.90 for males and 0.73 for females. The KI values agreed well with one methyl group internally and subterminally located, dimethyl isomers with at least one methyl inserted internally, subterminal or terminally, and when three methyl groups were present, they were located at internal positions. Minor amounts of mono-, di- and trimethyl derivatives of C32, C34, C36 were observed as well as mono- and dimethyl- C25, C27, C29 and C31 chains (Table IV); differences between males and females were not significant.

D. maxima - *D. maxima* hydrocarbon profiles showed a predominance of normal chains with a branched to normal ratio of 0.67 for males and 0.50 for females (Table V, Fig. 3), and no sexual dimorphism was evident. The major alkane was *n*- C31, followed by *n*- C29 and *n*- C33 which together accounted for 52.8% of total hydrocarbon for males and 61.1% for females. In the methylbranched fraction, three isomer series of 35, 37 and 39 atoms in the carbon backbone together with minor amounts of 4-me C34 and two series of 36 and 38 carbons were the prevailing branched structures.

DISCUSSION

Analysis of insect cuticular hydrocarbons was shown to be useful for discriminating members of a number of insect complexes, among them the *Anopheles gambiae* complex (Carlson & Service 1979, Milligan et al. 1986), *A. maculipennis* complex (Phillips et al. 1988), and G*lossina* species (Carlson et al. 1993). Within the Triatominae, cuticular hydrocarbon analysis can help in differenciating species of the *infestans* complex (Juárez & Brenner 1985), and also provides phylogenetic markers for comparing the main genera, *Triatoma*, *Rhodnius* and *Panstrongylus* (Juárez et al. 2000). The hydrocarbon structures of the Mexican species *T. pallidipennis* and *T. mazzotti*, both included in the *phyllosoma* complex, were previously determined by CGC coupled to mass spectrometry (MS) (Juárez & Brenner 1987, Juárez & Blomquist 1993).

Gas chromatographic analysis of adults of *T. dimidiata*, *T. barberi* and *D. maxima* showed characteristic hydrocarbon profiles, with carbon number ranging from 22 to more than 40, with a mixture of saturated straight and methyl-branched chains, with one, two, and three methyl groups, external and internally located. Methylbranched chains exhibited methyl-branching patterns consistent with previous data from other Triatominae (Juárez & Blomquist 1993, Juárez et al. 2000, 2001). However, when *T. dimidiata* was compared to closely related species *T. pallidipennis* and *T. mazzotti* (phyllosoma complex), differences were evident. *T. dimidiata* showed larger amounts of branched chains, with terminally mono, di- and trimethyl derivatives of C29, C31 and C33 accounting for 26% of the total hydrocarbon content, whereas these components were present in minor amounts in the other species (Juárez & Blomquist 1993). The major components for both species eluted quite closely at 37.9 ECL (corresponding to 3790 KI), the mass spectral identification showed large amounts of a mixture of x,x-dimethyl C37 together with x-me C38 for *T. pallidipennis* (Juárez & Brenner 1987) and at 37.7 ECL (ca. 3770 KI) for *T. mazzotti* (Juárez & Blomquist 1993); *T. dimidiata* showed a peak eluting at 3770 KI (x,x- dimethyl C37), although quantitatively less relevant. Sex-dependant, quantitative differences in certain hydrocarbons were found in *T. dimidiata*. The comparison of three selected peak ratios (R values)

TABLE III

R values for selected peaks in *Triatoma dimidiata* adult insects

T. dimidiata	3034/3100 ± SE [a]	3300/3777 ± SE	3777/3100 ± SE
Males	0.36[b] ± 0.11	4.14 ± 0.14	0.06 ± 0.04
Females	0.67 ± 0.12	0.78 ± 0.12	0.24 ± 0.08

a: peak ratios calculated from data shown in Table II; *b*: means differed significantly at the 95% level of confidence; n = 4 for females, n = 9 for males

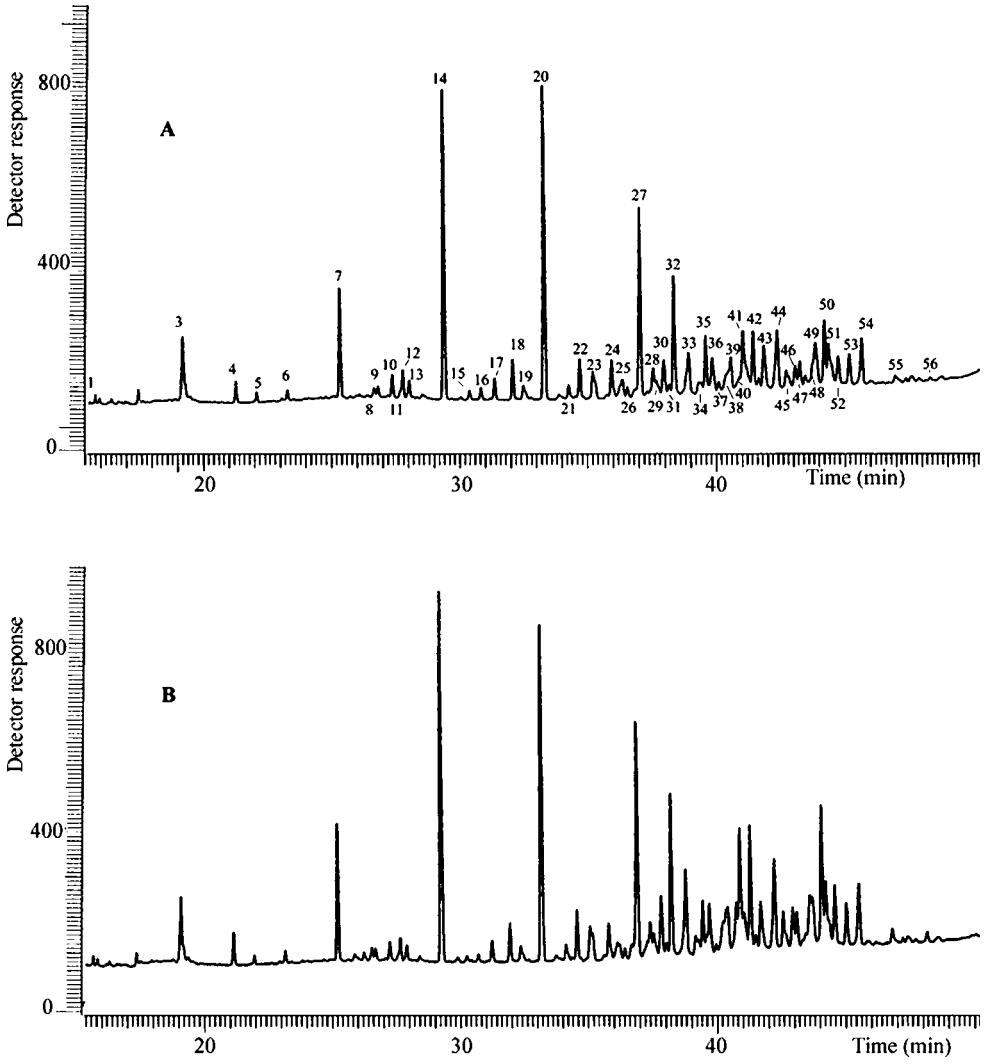

Fig. 2: capillary gas chromatography profiles of the cuticular hydrocarbons of *Triatoma barberi*. A: corresponds to adult females; B: adult males. Numbers indicating each hydrocarbon peak are indicated in A, and correspond to peak numbers from Table IV.

separated *T. dimidiata* males and females at the 95% level of confidence (Table III) however, both sexes were not distinguishable for *T. barberi* and *D. maxima.*

Analysis at genus level based on the complete 18S rRNA gene showed the impossibility of distinguishing the genera *Dipetalogaster*, *Panstrongylus* and *Triatoma* (Bargues et al. 2000), however the rather simple hydrocarbon pattern of *Dipetalogaster* is easily differentiated from both Triatominii (Juárez et al. 2000). The analysis of triatomine wings by CGC is reliable and thus enables comparison of these data to those obtained by other techniques for specimen identification on the same insect, and collaborative work is presently addressing this subject. Among more than 130 species reported for Triatominae, the cuticular hydrocarbon structure and CGC profiles for species with major epidemiological significance, *T. infestans, T. brasiliensis, Rhodnius prolixus* and a number of related species, were previously reported (Juárez & Blomquist 1993, Juárez et al. 2000, 2001). A pattern of primarily quantitative rather than qualitative differences reinforces the idea that epicuticular hydrocarbons represent relatively primitive characters for these insects. *T. dimidiata* showed a simpler hydrocarbon fingerprint and is easily separated from species of the *phyllosoma* complex (Juárez & Brenner 1986, Juárez & Blomquist 1993). Within each genera examined there was an indication that species from drier regions present more complex cuticular hydrocarbon profiles than their congenerics from wetter regions. Although interspecific variations might be more relevant, the complexity of the hydrocarbon pattern of *T. dimidiata* was intermediate between those reported for *T. tibiamaculata* and *T. vitticeps* from high relative humidity (RH) coastal regions, and that for *T. infestans* from varying RH areas (Juárez et al. 2000). *T. barberi* of drier regions, showed a more complex pattern. *D. maxima* surface hydrocarbon mixture showed the largest amounts of straight chains from 60% to 67.8% of the total hydrocarbon mixture, for males and females respectively; the relative abundance of *n*-alkanes might be related to exposure to warm conditions.

TABLE IV

Percent hydrocarbons from selected components of *Triatoma barberi*

			Male		Female	
Peak	Hydrocarbon [a]	Kovats Indices	% [b]	S.E.	%	S.E.
1	*n*- C22	2200	0,3	0,1	0,3	0,0
2	*n*- C23	2300	0,5	0,1	0,3	0,0
3	*n*- C24	2400	1,1	0,1	1,1	0,2
4	*n*- C25	2500	1,1	0,1	1,1	0,2
5	x- me + x,x- dime C25	2540	0,3	0,1	0,3	0,0
6	*n*- C26	2600	0,4	0,0	0,5	0,1
7	*n*- C27	2700	3,7	1,0	5,5	0,5
8	5- me C27	2750	0,3	0,1	0,4	0,1
9	x,x- dime C27	2773	0,2	0,1	0,4	0,1
10	*n*- C28	2800	0,8	0,1	1,0	0,1
11	unknown	2809	traces	0,0	0,5	0,1
12	unknown	2820	0,3	0,2	1,2	0,3
13	internally branched monome C28	2831	0,2	0,1	0,7	0,1
14	*n*- C29	2900	17,0	1,3	19,6	2,6
15	x- me + x,x- dime C29	2941	traces	0,0	0,3	0,0
16	x,x- dime C29	2972	0,1	0,1	0,4	0,0
17	*n*- C30	3000	0,9	0,1	1,0	0,1
18	internally branched monome C30	3033	1,6	0,6	0,9	0,4
19	4- me + x,x- dime C30	3058	1,1	0,8	traces	0,0
20	*n*-C31	3100	16,3	0,6	15,0	0,4
21	5- me C31	3149	0,4	0,1	0,5	0,0
22	x,x- dime C31	3171	1,6	0,3	1,1	0,3
23	*n*- C32	3200	0,5	0,1	0,7	0,2
24	x- me C32	3238	1,2	0,5	1,1	0,3
25	x,x- dime C32	3262	traces	0,0	0,4	0,0
26	3- me + x,x- dime C32	3275	0,2	0,1	0,4	0,0
27	*n*- C33	3300	8,7	0,5	7,2	1,3
28	x- me C33	3333	0,2	0,1	1,1	0,2
29	x- me + x,x- dime C33	3339	traces	0,0	0,4	0,0
30	5- me + x,x- dime C33	3357	1,6	0,2	1,2	0,4
31	x,x- dime C33	3369	traces	0,0	0,4	0,0
32	5,x- dime + x,x,x- trime C33	3381	6,3	1,0	2,4	1,1
33	3,x- dime C33	3412	3,3	0,8	2,5	0,4
34	x- me C34	3432	0,1	0,0	0,3	0,0
35	x- me C34	3446	1,0	0,3	1,5	0,5
36	4- me + x,x- dime C34	3461	1,5	0,4	1,4	0,2
37	x,x- dime C34	3473	traces	0,0	0,3	0,0
38	4,x- dime + x,x,x- trime C34	3489	0,3	0,2	0,5	0,2
39	*n*- C35	3500	1,2	0,4	1,1	0,2
40	4,x,x- trime C34	3519	1,0	0,3	0,7	0,0
41	internally br- monome C35	3527	1,3	0,2	1,7	0,6
42	5- me C35	3550	3,0	0,8	1,8	0,4
43	3- me + x,x- dime C35	3572	2,5	0,4	1,6	0,6
44	3,x- dime C35	3600	5,8	0,5	5,2	0,6
45	unknown	3623	0,3	0,1	0,4	0,0
46	6- me C36	3646	0,6	0,2	0,7	0,2
47	4- me + x,x- dime C36	3656	0,9	0,3	0,9	0,1
48	x,x,x- trime C36	3687	0,5	0,2	1,4	0,3
49	*n*- C37	3700	0,6	0,4	1,8	0,7
50	4,x,x- trime C37	3716	4,3	0,9	2,5	0,2
51	x- me C37	3726	0,6	0,1	0,8	0,1
52	5- me C37	3747	1,3	0,1	0,8	0,2
53	3- me + x,x- dime C37	3774	1,1	0,4	1,4	0,2
54	3,x- dime C37	3800	1,9	0,6	2,7	0,2
55	x,x,x- trime C38	3887	traces	0,0	0,3	0,1
56	3,x- dime C39	4004	traces	0,0	0,3	0,0
	branched/normal		0,9		0,7	

a: hydrocarbon and peak numbers are the same as reported in Fig. 2; *b*: differences between means were not significant; n = 4 for females, n = 5 for males

TABLE V

Percent hydrocarbons of selected components of *Dipetalogaster maxima*

Peak	Hydrocarbon [a]	Kovats Indices	Male % [b]	Male S.E.	Female %	Female S.E.
1	*n*- C22	2200	0,1	0,1	traces	0,0
2	*n*- C23	2300	0,2	0,1	0,1	0,0
3	internally branched monome C23	2338	0,1	0,1	traces	0,0
4	*n* -C24	2400	0,2	0,1	0,1	0,0
5	*n* -C25	2500	0,6	0,1	0,5	0,1
7	*n* -C26	2600	0,3	0,1	0,3	0,0
8	internally branched monome C26	2635	0,3	0,2	traces	0,0
9	*n* -C27	2700	2,1	0,1	3,7	0,9
10	internally branched monome C27	2730	1,4	0,8	0,3	0,1
11	5- me C27	2749	0,1	0,1	traces	0,0
13	*n* -C28	2800	0,4	0,1	0,5	0,1
14	unknown	2820	0,2	0,2	traces	0,0
15	internally branched monomeC28	2830	0,1	0,0	0,2	0,0
16	*n* -C29	2900	11,7	1,0	15,3	0,5
17	internally branched monome C29	2926	0,1	0,0	traces	0,0
18	5-me C29	2955	0,4	0,3	traces	0,0
19	*n*-C30	3000	1,3	0,1	1,8	0,1
20	internally branched monome C30	3034	0,5	0,4	0,9	0,2
21	*n*-C31	3100	25,6	2,8	28,3	2,0
22	unknown	3119	0,1	0,0	0,2	0,1
23	5-me C31	3145	0,5	0,5	0,1	0,1
24	x,x,x- trime C31	3186	1,3	0,2	1,9	0,1
25	3,x,x- trime C31 + internally branched me C32	3230	1,8	1,2	2,5	1,0
26	*n* -C33	3300	15,4	3,3	17,5	1,0
27	5- meC33	3356	traces	0,0	0,1	0,0
28	3,x- dimeC33	3406	1,8	0,3	1,3	0,3
29	unknown	3421	0,0	0,0	0,2	0,1
30	4- me C34	3450	2,5	0,9	3,1	1,0
31	x,x- dime C34	3461	traces	0,0	0,1	0,0
32	*n* -C35	3500	1,7	0,3	1,4	0,1
33	internally branched monome C35	3531	4,2	1,8	1,6	0,2
34	x- mono + x,x- dime C35	3540	1,4	0,4	1,0	0,4
35	5- me C35	3550	1,0	0,3	0,8	0,1
36	x,x,- dime C35	3572	0,2	0,1	0,2	0,1
37	3,x- di + x,x,x- trime C37	3600	0,7	0,2	0,2	0,0
38	internally branched monome C36	3627	0,2	0,1	0,3	0,1
39	internally branched monome C36	3634	0,6	0,5	0,4	0,1
40	x- me C36	3644	1,0	0,2	0,7	0,2
43	internally branched monome C37	3731	5,3	0,9	3,8	0,9
44	5- me C37	3749	3,5	1,8	3,0	0,8
45	3,x- dime C37 + unknown	3771	1,8	0,8	0,7	0,2
46	*n* -C38	3800	0,4	0,3	0,1	0,1
47	internally branched monome C38	3826	1,8	0,6	0,5	0,2
48	5- me C38	3847	0,5	0,4	0,6	0,2
49	3- me + x,x- dime C38	3870	0,2	0,2	0,3	0,0
50	internally branched monome C39	3930	2,1	0,2	1,7	0,2
51	5- me C39	3949	2,1	1,2	2,7	0,4
52	x,x- dime C39	3969	1,8	0,9	1,3	0,3
	branched/normal		0,7		0,5	

a: hydrocarbons and peak number are the same as in Fig. 3, minor component peaks 6, 12, 41, 42 and 53 corresponding to 2530 KI (internally br monome- C25), 2730 KI (x,x- dime C27), 3670 KI (x,x- dime C36), 3700 KI (n- C37), and 4050 KI (3,x- di plus 5,x,x- trime C39) are not included; *b*: n = 4 for males, n = 5 for females

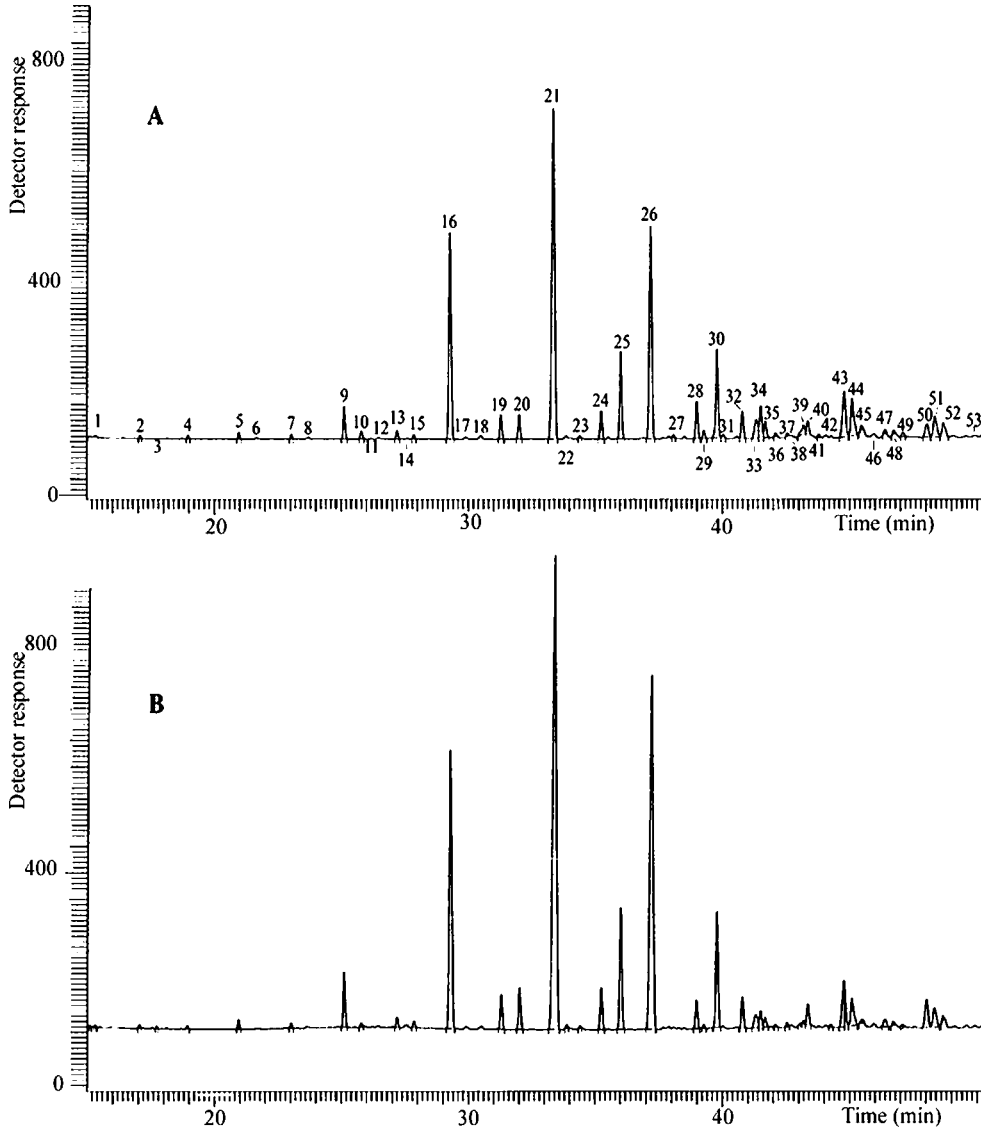

Fig. 3: capillary gas chromatography profiles of the cuticular hydrocarbons of *Dipetalogaster maxima*. A: corresponds to adult females; B: adult males. Numbers indicating each hydrocarbon peak are indicated in A, and correspond to peak numbers from Table V.

ACKNOWLEDGEMENTS

To CJ Schofield for his critical and helpful comments, editing and review of the manuscript.

REFERENCES

Bargues M, Marcilla A, Dujardin JP, Mas-Coma S 2000. Triatominae vectors of Chagas disease: a molecular perspective based on nuclear ribosomal DNA markers. In J Alvar, *Molecular Epidemiology and Diagnosis of Parasitic Diseases*, Book Homage to Prof Douglas C Barker, Cambridge University Press, Cambridge.

Carlson DA, Service MW 1979. Differentiation between species of the *Anopheles gambiae* giles complex (Diptera: Culicidae) by analysis of cuticular hydrocarbons. *Ann Trop Med Parasitol 73*: 589-592.

Carlson DA, Bernier UR, Sutton BC 1998. Elution patterns from capillary GC for methyl-branched alkanes. *J Chem Ecol 24*: 1845-1865.

Carlson DA, Milstrey SK, Narang SK 1993. Classification of tsetse flies *Glossina* spp. (Diptera: Glossinidae) by gas chromatographic analysis of cuticular components. *Bull Entomol Res 83*: 507-515.

Juárez MP, Blomquist GJ 1993. Cuticular hydrocarbons of *Triatoma infestans* and *T. mazzottii*. *Comp Biochem Physiol 106 B*: 667-674.

Juárez MP, Brenner RR 1985. The epicuticular lipids of *Triatoma infestans*. II. Hydrocarbon dynamics. *Comp Biochem Physiol 82B*: 793-803.

Juárez MP, Brenner RR 1986. Biochemistry of the evolution of *Triatoma infestans*. IX. Composition of cuticular hydrocarbons compared to other Triatominae. *Acta Physiol Pharmacol Latinoam 36*: 47-57.

Juárez MP, Brenner RR 1987. Hydrocarbons of *Triatoma pallidipennis* insect. *Comp Biochem Physiol 87 B*: 233-239.

Juárez MP, Blomquist GJ, Schofield CJ 2001. Hydrocarbons of *Rhodnius prolixux*, a Chagas disease vector. *Comp*

Biochem Physiol 129B: 733-746.

Juárez MP, Fernández R, Dujardin JP, Schofield CJ 2000. Intergeneric comparison of cuticular hydrocarbons in Triatominae. *Res Rev Parasitol 60*: 121-127.

Kovats E 1965. Gas chromatographic comparison of organic substances in the retention index system. *Adv Chromat 1*: 229- 247.

Milligan PJN, Phillips A, Molyneux DH, Subbarao SK, White GB 1986. Differentiation of *Anopheles culicifacies* Giles (Diptera: Culicidae) sibling species by analysis of cuticular components. *Bull Entomol Res 76*: 529-537.

Phillips A, Milligan PJM, Broomfield G, Molyneux DH 1988. Identification of medically important Diptera by analysis of cuticular hydrocarbons. In MW Service, *Biosystematics of Hematophagous Insects*, Systematics Association, Special vol. 37, Clarenden Press, Oxford, p. 39-59.

Salazar Schettino PM, Haro I de, Uribarren T 1988. Chagas disease in Mexico. *Parasitol Today 4*: 348-352.

Schofield CJ 2000. Challenges of Chagas disease vector control in Central America. Position paper WHO/CDS/WHOPES/GCDPPH/2000.1. WHO, Communicable Diseases Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme, Geneva, Switzerland.

Zárate LG, Zárate RJ 1985. A checklist of the Triatominae (Hemiptera: Reduviidae) of Mexico. *Int J Entomol 27*: 102-127.

Zeledón R 1981. *El Triatoma dimidiata (Latreille, 1811) y su Relación con la Enfermedad de Chagas*, Editorial Universidad Estatal a Distancia, San José, Costa Rica, 146 pp.