

# Phylogeography and Genetic Variation of *Triatoma dimidiata*, the Main Chagas Disease Vector in Central America, and Its Position within the Genus *Triatoma*

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## Abstract

**Background:** Among Chagas disease triatomine vectors, the largest genus, *Triatoma*, includes species of high public health interest. *Triatoma dimidiata*, the main vector throughout Central America and up to Ecuador, presents extensive phenotypic, genotypic, and behavioral diversity in sylvatic, peridomestic and domestic habitats, and non-domiciliated populations acting as reinfestation sources. DNA sequence analyses, phylogenetic reconstruction methods, and genetic variation approaches are combined to investigate the haplotype profiling, genetic polymorphism, phylogeography, and evolutionary trends of *T. dimidiata* and its closest relatives within *Triatoma*. This is the largest interpopulational analysis performed on a triatomine species so far.

**Methodology and Findings:** Triatomines from Mexico, Guatemala, Honduras, Nicaragua, Panama, Cuba, Colombia, Ecuador, and Brazil were used. *Triatoma dimidiata* populations follow different evolutionary divergences in which geographical isolation appears to have had an important influence. A southern Mexican–northern Guatemalan ancestral form gave rise to two main clades. One clade remained confined to the Yucatan peninsula and northern parts of Chiapas State, Guatemala, and Honduras, with extant descendants deserving specific status. Within the second clade, extant subspecies diversity was shaped by adaptive radiation derived from Guatemalan ancestral populations. Central American populations correspond to subspecies *T. d. dimidiata*. A southern spread into Panama and Colombia gave the *T. d. capitata* forms, and a northwestern spread rising from Guatemala into Mexico gave the *T. d. maculipennis* forms. *Triatoma hegneri* appears as a subspecific insular form.

**Conclusions:** The comparison with very numerous *Triatoma* species allows us to reach highly supported conclusions not only about *T. dimidiata*, but also on different, important *Triatoma* species groupings and their evolution. The very large intraspecific genetic variability found in *T. dimidiata sensu lato* has never been detected in a triatomine species before. The distinction between the five different taxa furnishes a new frame for future analyses of the different vector transmission capacities and epidemiological characteristics of Chagas disease. Results indicate that *T. dimidiata* will offer problems for control, although dwelling insecticide spraying might be successful against introduced populations in Ecuador.

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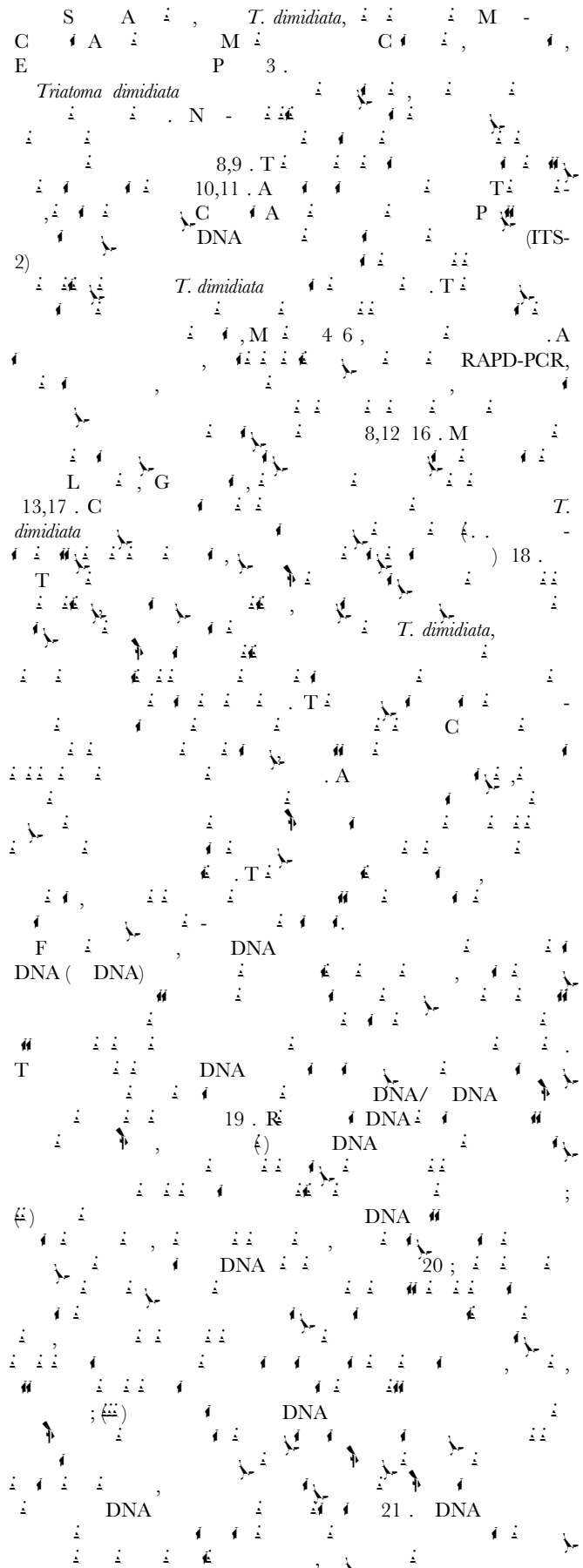
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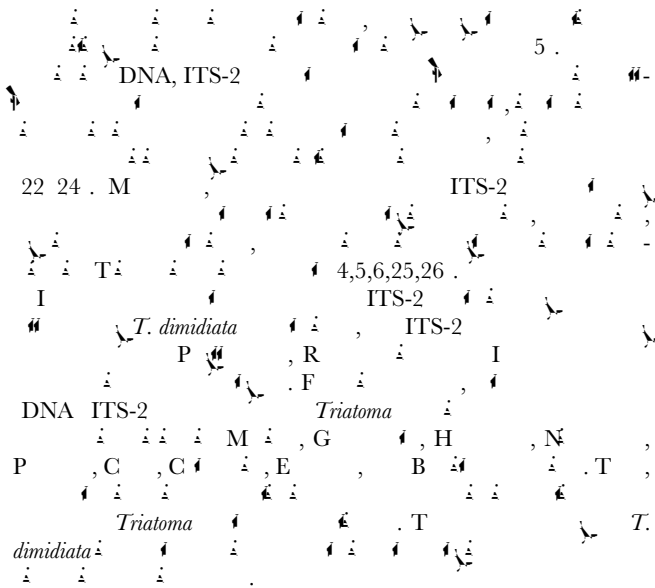
**Author Summary**

Chagas disease is a serious parasitic disease of Latin America. Human contamination in poor rural or periurban areas is mainly attributed to haematophagous triatomine insects. *Triatoma* includes important vector species, as *T. dimidiata* in Central and Meso-America. DNA sequences, phylogenetic methods and genetic variation analyses are combined in a large interpopulational approach to investigate *T. dimidiata* and its closest relatives within *Triatoma*. The phylogeography of *Triatoma* indicates two colonization lineages northward and southward of the Panama isthmus during ancient periods, with *T. dimidiata* presenting a large genetic variability related to evolutionary divergences from a Mexican-Guatemalan origin. One clade remained confined to Yucatan, Chiapas, Guatemala and Honduras, with extant descendants deserving species status: *T. sp. aff. dimidiata*. The second clade gave rise to four subspecies: *T. d. dimidiata* in Guatemala and Mexico (Chiapas) up to Honduras, Nicaragua, Providencia island, and introduced into Ecuador; *T. d. capitata* in Panama and Colombia; *T. d. maculipennis* in Mexico and Guatemala; and *T. d. hegneri* in Cozumel island. This taxa distinction may facilitate the understanding of the diversity of vectors formerly included under *T. dimidiata*, their different transmission capacities and the disease epidemiology. *Triatoma dimidiata* will offer more problems for control than *T. infestans* in Uruguay, Chile and Brazil, although populations in Ecuador are appropriate targets for insecticide-spraying.

**Introduction**

Latin America is home to several species of the genus *Triatoma*, including *Triatoma dimidiata*, *Triatoma maculipennis*, *Triatoma capitata*, *Triatoma hegneri*, and *Triatoma sp. aff. dimidiata*. *Triatoma dimidiata* is a major vector of Chagas disease in Central and Meso-America. The phylogeography of *Triatoma* indicates two colonization lineages northward and southward of the Panama isthmus during ancient periods, with *T. dimidiata* presenting a large genetic variability related to evolutionary divergences from a Mexican-Guatemalan origin. One clade remained confined to Yucatan, Chiapas, Guatemala and Honduras, with extant descendants deserving species status: *T. sp. aff. dimidiata*. The second clade gave rise to four subspecies: *T. d. dimidiata* in Guatemala and Mexico (Chiapas) up to Honduras, Nicaragua, Providencia island, and introduced into Ecuador; *T. d. capitata* in Panama and Colombia; *T. d. maculipennis* in Mexico and Guatemala; and *T. d. hegneri* in Cozumel island. This taxa distinction may facilitate the understanding of the diversity of vectors formerly included under *T. dimidiata*, their different transmission capacities and the disease epidemiology. *Triatoma dimidiata* will offer more problems for control than *T. infestans* in Uruguay, Chile and Brazil, although populations in Ecuador are appropriate targets for insecticide-spraying.





GenBank: *T. phyllosoma* (AJ286881), *T. pallidipennis* (AJ286882), *T. longipennis* (AJ286883), *T. picturata* (AJ286884), *T. mazzotti* (AJ286885) (P); *T. barberi* (AJ293590) (R); *T. rubrovaria* H1 (AJ557258) 32, *T. infestans* CH1A (AJ576051), *T. sordida* (AJ576063) 25. *T. rhodnius prolixus* (T; R) (AJ286882) 6.

**Data deposition footnote**

GenBank (:// /G) ITS-2 DNA: *T. dimidiata* (AM286693-AM286723), *T. basolissae* (AM286724), *T. bolivari* (AM286725), 2, *T. hegneri* (AM286726, AM286727), *T. mexicana* (AM286728), 2, *T. pallidipennis* (AM286729, AM286730), *T. ryckmani* (AM286731), *T. flavida* (AM286732), *T. gerstaeckeri* (AM286734), *T. rubida* (AM286735), *T. nitida* (AM286733), *T. maculata* (AJ582027), *T. arthurneivai* (AM286736).

**Materials and Methods**

**Triatomine materials**

A total of 165 *Triatoma* specimens were analyzed, including 13 *T. dimidiata* and 64 *T. dimidiata* (T1; F1). *Triatoma* specimens were collected from various locations in Mexico and Central America.

**Sequencing of rDNA ITS-2**

Genomic DNA was extracted from individual specimens. PCR amplification was performed using specific primers for the ITS-2 region. The reaction conditions were as follows: 94°C for 30 s, 50°C for 1 min, 72°C for 30 s, 94°C for 7 s, 72°C for 30 s. The PCR products were purified and sequenced using ABI 3730 and ABI 3700 sequencers. The sequencing was performed at the University of California, San Diego (UCSD).

**Triatomine haplotype code nomenclature**

Haplotypes were identified based on the ITS-2 sequence. The nomenclature system used was (H) for *T. dimidiata* and (CH) for *T. dimidiata* haplotypes. A total of 25 haplotypes were identified.

**Sequence alignment**

Sequence alignment was performed using CLUSTAL-W and MEGA 3.1 software. The alignment was based on the ITS-2 region. The alignment was performed using the default parameters of the software.

**Phylogenetic inference**

Phylogenetic inference was performed using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The ML analysis was performed using PAUP\*4.0 with 10,000 bootstraps. The BI analysis was performed using MrBayes with 1,000,000 generations. The substitution model used was GTR+I+G4. The support values for the nodes are indicated in the tree.

**Genetic variation studies**

Genetic variation studies were performed using the haplotype network method. The network was constructed using TCS software. The network was based on the ITS-2 sequences of *T. dimidiata* and related species. The network shows the relationships between the different haplotypes and their geographic distribution.

**Table 1.** *Triatoma* species and samples studied, including ITS-2 sequence length and AT composition (in percentage).

Country	Map No.	Preliminary classification	Sampling sites	Haplotype code	Sequence length	% AT
<b>PHYLLOSOMA GROUP: PHYLLOSOMA COMPLEX</b>						
<b>1) TRIATOMA DIMIDIATA: 31 different haplotype sequences/137 specimens studied:</b>						
<b>MEXICO</b> n = 41	1	<i>T. dimidiata</i>	Atoyac Tlacorranchito, Veracruz	T.dim-H18	496	75.81
	2	<i>T. dimidiata</i>	Atoyac-Manzanillo, Veracruz	T.dim-H18	496	75.81
	3	<i>T. dimidiata</i>	Atoyac-Cordoba, Veracruz	T.dim-H18	496	75.81
	4	<i>T. dimidiata</i>	Ursulo-Galan, Veracruz	T.dim-H18	496	75.81
	5	<i>T. dimidiata</i>	Tanchahuil, San Luis Potosí	T.dim-H18	496	75.81
	6	<i>T. dimidiata</i>	Barrio Tzitzí, San Luis Potosí	T.dim-H18	496	75.81
	7	<i>T. dimidiata</i>	Huejutla, Hidalgo (3)	T.dim-H18	496	75.81
	8	<i>T. dimidiata</i>	Atlapexco, Hidalgo	T.dim-H18	496	75.81
	9	<i>T. dimidiata</i>	El Rosario, Tabasco	T.dim-H18	496	75.81
	10	<i>T. dimidiata</i>	Cozumel island, Quintana Roo	T.dim-H18	496	75.81
	11	<i>T. dimidiata</i>	Acomul, Hidalgo	T.dim-H18	496	75.81
	12	<i>T. dimidiata</i>	Mesa de Tlanchinol, Veracruz	T.dim-H19	494	75.71
	13	<i>T. dimidiata</i>	La Luz, Veracruz	T.dim-H19	494	75.71
	14	<i>T. dimidiata</i>	Emiliano Zapata, Veracruz	T.dim-H20	495	75.76
	15	<i>T. dimidiata</i>	Morelos	T.dim-H21	497	75.85
	16	<i>T. dimidiata</i>	Cajones, Morelos	T.dim-H21	497	75.85
	17	<i>T. dimidiata</i>	Huehuetla, Hidalgo	T.dim-H22	494	75.71
	18	<i>T. dimidiata</i>	Chalcatzingo, Morelos	T.dim-H23	496	75.60
	19	<i>T. dimidiata</i>	Santiago Cuixtla, Oaxaca	T.dim-H23	496	75.60
	20	<i>T. dimidiata</i>	Hierba Santa, Oaxaca	T.dim-H23	496	75.60
	21	<i>T. dimidiata</i>	Nopala, Oaxaca	T.dim-H23	496	75.60
	22	<i>T. dimidiata</i>	Alcaraces, Cuauhtemoc, Colima	T.dim-H24	496	75.40
	23	<i>T. dimidiata</i>	Paraíso, Yucatán (3)	T.dim-H25	493	75.66
	24	<i>T. dimidiata</i>	Palenque, Chiapas	T.dim-H25	493	75.66
	23	<i>T. dimidiata</i>	Paraíso, Yucatán	T.dim-H26	489	75.46
	23	<i>T. dimidiata</i>	Paraíso, Yucatán	T.dim-H27	494	75.51
	25	<i>T. dimidiata</i>	Yaxkukul, Yucatán	T.dim-H28	493	75.66
	26	<i>T. dimidiata</i>	Holbox island, Quintana Roo	T.dim-H28	493	75.66
	23	<i>T. dimidiata</i>	Paraíso, Yucatán	T.dim-H28	493	75.66
	27	<i>T. dimidiata</i>	Izamal, Yucatán	T.dim-H28	493	75.66
	28	<i>T. dimidiata</i>	Cozumel island, Quintana Roo (3)	T.dim-H28	493	75.66
23	<i>T. dimidiata</i>	Paraíso, Yucatán	T.dim-H28	493	75.66	
29	<i>T. dimidiata</i>	Chablekal, Mérida, Yucatán	T.dim-H31	489	75.25	
30	<i>T. dimidiata</i>	Mapastepec, Chiapas	T.dim-H1	497	76.06	
31	<i>T. dimidiata</i>	Tapachula, Chiapas	T.dim-H3	497	76.26	
<b>GUATEMALA</b> n = 37	32	<i>T. dimidiata</i>	Jutiapa, Jutiapa (4)	T.dim-H1	497	76.06
	33	<i>T. dimidiata</i>	Agua Zarca, Jutiapa	T.dim-H1	497	76.06
	34	<i>T. dimidiata</i>	Pueblo Nuevo Viñas, Santa Rosa	T.dim-H1	497	76.06
	35	<i>T. dimidiata</i>	Piedra Pintada, Jutiapa (3)	T.dim-H1	497	76.06
	33	<i>T. dimidiata</i>	Agua Zarca, Jutiapa	T.dim-H2	496	76.01
	36	<i>T. dimidiata</i>	Escuintla, Escuintla (3)	T.dim-H2	496	76.01
	37	<i>T. dimidiata</i>	San Andrés Sajcabaja, Quiché	T.dim-H2	496	76.01
	34	<i>T. dimidiata</i>	Pueblo Nuevo Viñas, Santa Rosa	T.dim-H2	496	76.01
	33	<i>T. dimidiata</i>	Agua Zarca, Jutiapa (2)	T.dim-H3	497	76.26
	36	<i>T. dimidiata</i>	Escuintla, Escuintla	T.dim-H3	497	76.26
	34	<i>T. dimidiata</i>	Pueblo Nuevo Viñas, Santa Rosa	T.dim-H3	497	76.26
	37	<i>T. dimidiata</i>	San Andrés Sajcabaja, Quiché	T.dim-H4	497	76.85
	34	<i>T. dimidiata</i>	Pueblo Nuevo Viñas, Santa Rosa	T.dim-H8	497	76.06

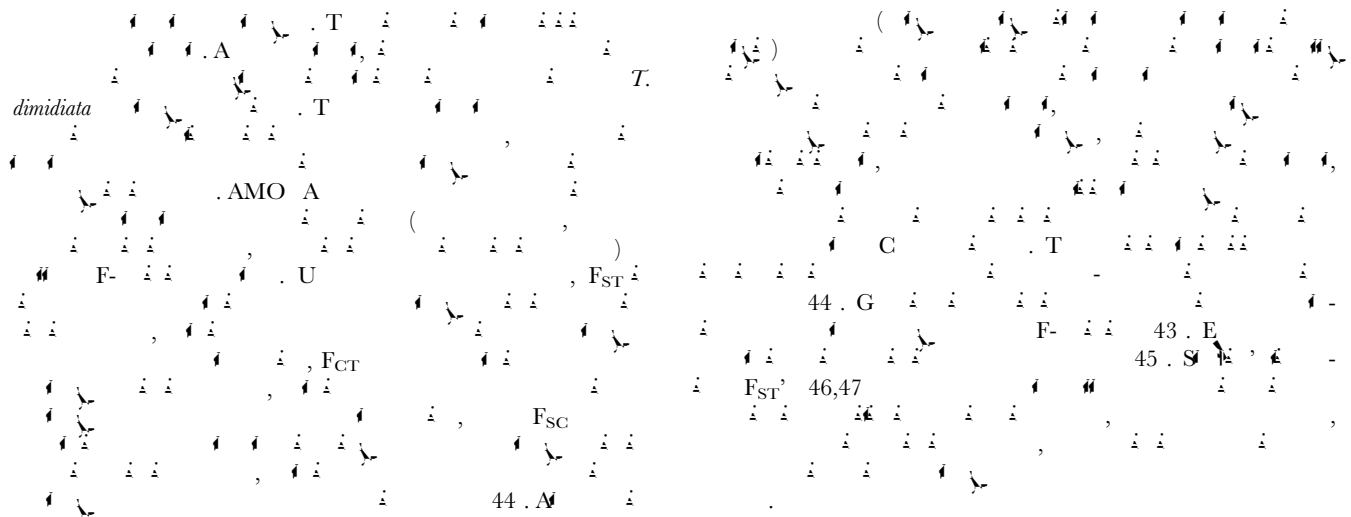
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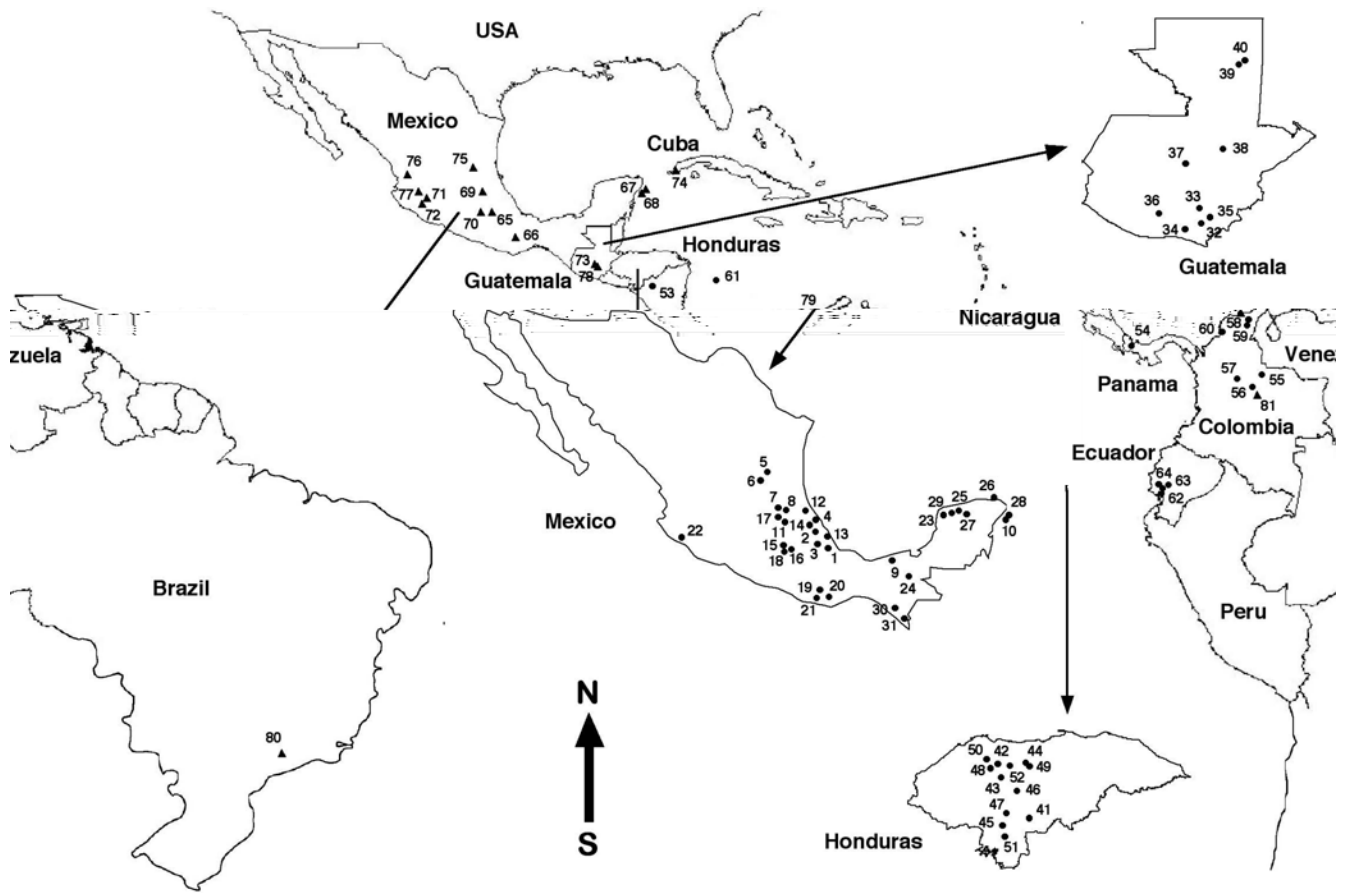
Country	Map No.	Preliminary classification	Sampling sites	Haplotype code	Sequence length	% AT
	35	<i>T. dimidiata</i>	Aldea Piedra Pintada, Jutiapa	T.dim-H8	497	76.06
	38	<i>T. dimidiata</i>	Lanquín, Alta Verapaz (4)	T.dim-H10	496	76.01
	39	<i>T. dimidiata</i>	Chultún, Yaxhá, Petén (2)	T.dim-H18	496	75.81
	37	<i>T. dimidiata</i>	San Andrés Sajcabaja, Quiché (2)	T.dim-H18	496	75.81
	40	<i>T. dimidiata</i>	Yaxhá, Petén	T.dim-H25	493	75.66
	40	<i>T. dimidiata</i>	Yaxhá, Petén (2)	T.dim-H28	493	75.66
	40	<i>T. dimidiata</i>	Yaxhá, Petén (3)	T.dim-H28	493	75.66
	40	<i>T. dimidiata</i>	Yaxhá, Petén	T.dim-H30	491	75.56
<b>HONDURAS</b>	41	<i>T. dimidiata</i>	Güinope, El Paraiso	T.dim-H1	497	76.06
n = 20	42	<i>T. dimidiata</i>	El Tablon, Yoro (2)	T.dim-H2	496	76.01
	43	<i>T. dimidiata</i>	El Zapote, Yoro	T.dim-H2	496	76.01
	44	<i>T. dimidiata</i>	El Salitre, Yoro	T.dim-H2	496	76.01
	45	<i>T. dimidiata</i>	El Cacao, Francisco Morazán (2)	T.dim-H2	496	76.01
	46	<i>T. dimidiata</i>	Orica, Francisco Morazán	T.dim-H2	496	76.01
	47	<i>T. dimidiata</i>	Tegucigalpa, Francisco Morazán (2)	T.dim-H2	496	76.01
	48	<i>T. dimidiata</i>	Corral Falso, Yoro (2)	T.dim-H2	496	76.01
	49	<i>T. dimidiata</i>	El Salitre, Montaña, Yoro	T.dim-H2	496	76.01
	50	<i>T. dimidiata</i>	Subirama, Yoro	T.dim-H2	496	76.01
	51	<i>T. dimidiata</i>	San José, Choluteca	T.dim-H6	496	76.01
	48	<i>T. dimidiata</i>	Corral Falso, Yoro	T.dim-H9	496	75.81
	43	<i>T. dimidiata</i>	El Zapote, Yoro	T.dim-H9	496	75.81
	50	<i>T. dimidiata</i>	Subirama, Yoro	T.dim-H9	496	75.81
	50	<i>T. dimidiata</i>	Subirama, Yoro	T.dim-H29	494	75.71
	52	<i>T. dimidiata</i>	El Paraiso, Yoro	T.dim-H29	494	75.71
<b>NICARAGUA</b>	53	<i>T. dimidiata</i>	Madriz	T.dim-H7	497	75.65
n = 1						
<b>PANAMA</b>	54	<i>T. dimidiata</i>	Boquete, Chiriqui (3)	T.dim-H16	497	76.06
n = 4	54	<i>T. dimidiata</i>	Boquete, Chiriqui	T.dim-H17	495	75.96
<b>COLOMBIA</b>	55	<i>T. dimidiata</i>	Pore, Casanare	T.dim-H11	497	75.85
n = 31	56	<i>T. dimidiata</i>	Boavita, Boyacá (13)	T.dim-H11	497	75.85
	57	<i>T. dimidiata</i>	San Joaquín, Santander (3)	T.dim-H11	497	75.85
	58	<i>T. dimidiata</i>	Com. Los Kuisés, SNSM Magdalena	T.dim-H12	495	75.76
	56	<i>T. dimidiata</i>	Boavita, Boyacá (4)	T.dim-H12	495	75.76
	59	<i>T. dimidiata</i>	Sierra Nevada, Santa Marta (4)	T.dim-H12	495	75.76
	56	<i>T. dimidiata</i>	Boavita, Boyacá	T.dim-H13	493	75.66
	60	<i>T. dimidiata</i>	San Onofre, Sucre (insectary) (2)	T.dim-H14	497	76.06
	56	<i>T. dimidiata</i>	Boavita, Boyacá	T.dim-H15	497	75.65
	61	<i>T. dimidiata</i>	Providencia island	T.dim-H1	497	76.06
<b>ECUADOR</b>	62	<i>T. dimidiata</i>	Guayas, Guayaquil	T.dim-H5	497	75.85
n = 3	63	<i>T. dimidiata</i>	Cerro del Carmen, Guayas, Guayaquil	T.dim-H5	497	75.85
	64	<i>T. dimidiata</i>	Pedro Carbo, Guayaquil	T.dim-H6	496	76.01
) <i>TRIATOMA BASSOLSAE</i> 1 sequence/ specimens studied:						
<b>MEXICO</b>	65	<i>T. bassolsae</i>	Acatlán, Puebla (2)	T.bas-H1	490	76.94
n = 2						
3) <i>TRIATOMA BOLIVARI</i> : 1 sequence/1 specimen studied:						
<b>MEXICO</b>	66	<i>T. bolivari</i>	Oaxaca, Oaxaca	T.bol-H1	501	76.85
4) <i>TRIATOMA HEGNERI</i> : sequences/5 specimens studied:						
<b>MEXICO</b>	67	<i>T. hegneri</i>	Ruinas S.Gervasio, Cozumel isl., Q. Roo	T.heg-H1	496	75.81

Table 1. cont.

Country	Map No.	Preliminary classification	Sampling sites	Haplotype code	Sequence length	% AT
n=5	68	<i>T. hegneri</i>	Cedral, Cozumel isl., Quintana Roo (3)	T.heg-H1	496	75.81
	68	<i>T. hegneri</i>	Cedral, Cozumel isl., Quintana Roo	T.heg-H2	496	76.01
<b>5) TRIATOMA MEXICANA: 1 sequence/1 specimen studied:</b>						
MEXICO	69	<i>T. mexicana</i>	Itatlaxco, Hidalgo	T.mex-H1	492	75.61
<b>6) TRIATOMA PALLIDIPENNIS: 1 sequence/3 specimens studied:</b>						
MEXICO	70	<i>T. pallidipennis</i>	Chalcatzingo, Morelos	T.pal-H1	491	76.98
n=3	71	<i>T. pallidipennis</i>	San Gabriel, Jalisco	T.pal-H2	490	76.94
	72	<i>T. pallidipennis</i>	Tecalitlan, Jalisco	T.pal-H2	490	76.94
<b>7) TRIATOMA RYCKMANI: 1 sequence/ specimens studied:</b>						
GUATEMALA	73	<i>T. ryckmani</i>	El Progreso, El Progreso (2)	T.ryc-H1	500	76.00
n=2						
<b>PHYLLOSOMA GROUP: FLAVIDA COMPLEX</b>						
<b>8) TRIATOMA FLAVIDA: 1 sequence/4 specimens studied:</b>						
CUBA	74	<i>T. flavida</i>	Peninsula of Guanahacabibes (4)	T.fla-H1	493	78.70
n=4						
<b>RUBROFASCIATA GROUP: RUBROFASCIATA COMPLEX: LECTICULARIA SUBCOMPLEX</b>						
<b>9) TRIATOMA GERSTAECKERI: 1 sequence/1 specimen studied:</b>						
MEXICO	75	<i>T. gerstaeckeri</i>	Tanchahuil, S. Luis Potosí	T.ger-H1	483	76.81
<b>10) TRIATOMA RUBIDA: 1 sequence/ specimens studied:</b>						
MEXICO	76	<i>T. rubida</i>	Mocorito, Nayarit	T.rub-H1	516	77.71
n=2	77	<i>T. rubida</i>	San Martin, Jalisco	T.rub-H1	516	77.71
<b>RUBROFASCIATA GROUP: PROTRACTA COMPLEX</b>						
<b>11) TRIATOMA NITIDA: 1 sequence/1 specimen studied:</b>						
GUATEMALA	78	<i>T. nitida</i>	El Progreso, El Progreso	T.nit-H1	490	76.33
<b>INFESTANS GROUP: INFESTANS COMPLEX: MACULATA SUBCOMPLEX</b>						
<b>1) TRIATOMA MACULATA: 1 sequence/4 specimens studied:</b>						
COLOMBIA	79	<i>T. maculata</i>	Santa Marta, Magdalena (4)	T.mac-H1	488	78.28
n=4						
<b>INFESTANS GROUP: INFESTANS COMPLEX: RUBROVARIA SUBCOMPLEX</b>						
<b>13) TRIATOMA ARTHURNEIVAI: 1 sequence/ specimens studied:</b>						
BRAZIL	80	<i>T. arthurneivai</i>	Espirito Santo do Pinhal	T.art-H1	486	77.98
n=2			Sao Paulo (Fiocruz) (2)			

doi:10.1371/journal.pntd.0000233.t001





**Figure 1. Geographical distribution of the sampling sites furnishing the triatomine materials.** Numbers correspond to sampling sites listed in Table 1. ● = *Triatoma dimidiata*; ▲ = other *Triatoma* species studied.  
doi:10.1371/journal.pntd.0000233.g001

**Results**

**Sequence Analyses of *Triatoma dimidiata* Populations**

T 137 ITS-2  
*T. dimidiata* (T. -H1 T. -H31)  
 (T. 1 2 ). T.  
 489 497 ( ) ( , 495.10) AT-  
 75.25 76.85% (75.72%). S  
 31  
 1 (T. -H1 T. -H10); 2  
 (T. -H11 T. -H17); 3 (T. -H18 T. -  
 H24); 4 (T. -H25 T. -H31) (2).  
 T  
 C P I , , C  
 240 N . N  
 T 31 *T. dimidiata*  
 501 450 24  
 (AT)<sub>4</sub> 5  
 TTT (AT)<sub>5</sub> 7 47 73  
 T 51 2.  
 10.18% T  
 T. -H25 T. -H31  
 (2). T

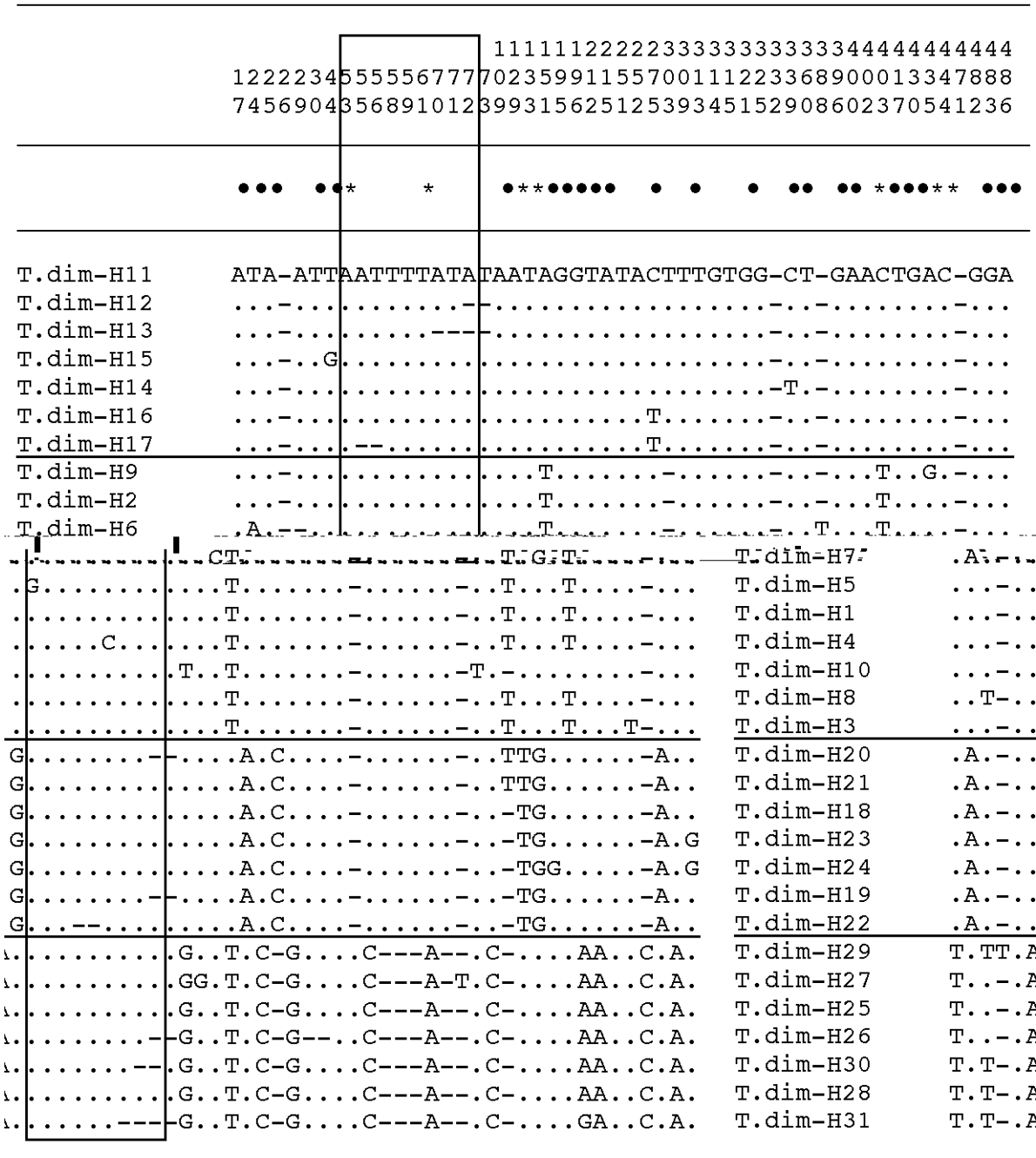
T. -H1 T. -H24  
 5.62% 498- 6 (1.20%)  
 ( ), 13 (2.61%) ( ) 9 (1.81%)  
 / ( );  
 T. -H25  
 T. -H31 (M : I  
 , C , C I H I ; G -  
 : P ; H ) 2.42%  
 495- , 12  
 2 (0.40%) 10 (2.02%).

**Sequence Analyses in the Phyllosoma and Rubrofasciata Groups**

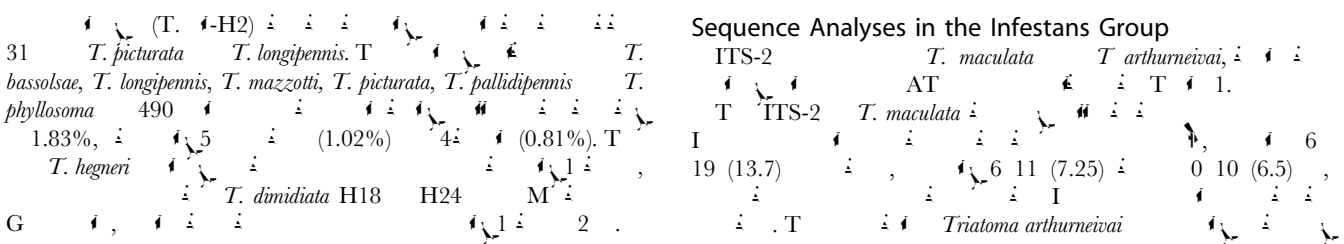
ITS-2 *T. bassolsae*, *T. bolivari*, *T. hegneri*, *T. mexicana*,  
*T. pallidipennis*, *T. ryckmani*, *T. flavida*, *T. nitida*, *T. gerstaeckeri*, *T.*  
*rubida*, AT  
 T 1. T ITS-2  
 P R  
 ( G B ) 48 T  
 365 99  
 T. *dimidiata*  
 P , F , P R  
*Triatoma bassolsae*  
 489 *T. pallidipennis* M , M  
 (AJ286882). T *T. pallidipennis*





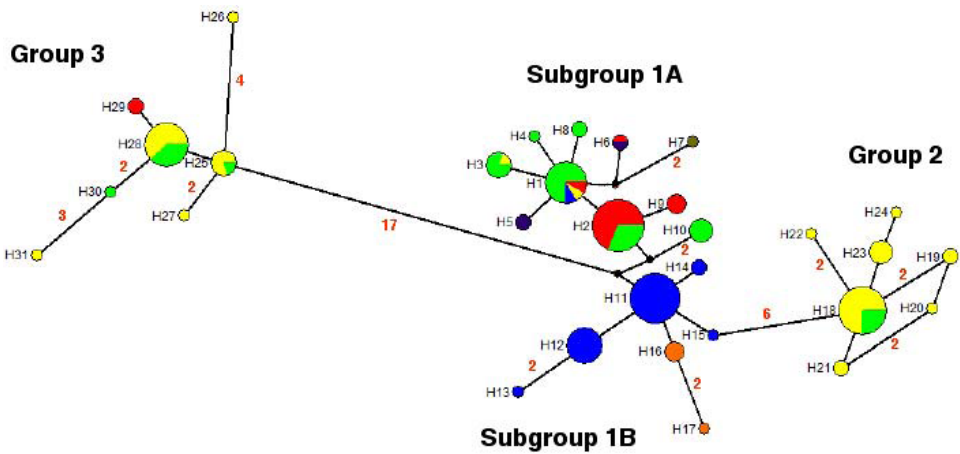


**Figure 1. Interhaplotype sequence differences found in the rDNA ITS-2 of the *Triatoma dimidiata* populations analyzed.** Numbers (to be read in vertical) refer to positions obtained in the alignments made with CLUSTAL-W 1.8 and MEGA 3.3. .= identical; \*= singleton sites (7); ● = parsimony informative positions (24); - = insertion/deletion. Rectangled area = microsatellite region. Horizontal lines separate the four major *T. dimidiata* haplotype groupings according to sequence analyses.  
doi:10.1371/journal.pntd.0000233.g002



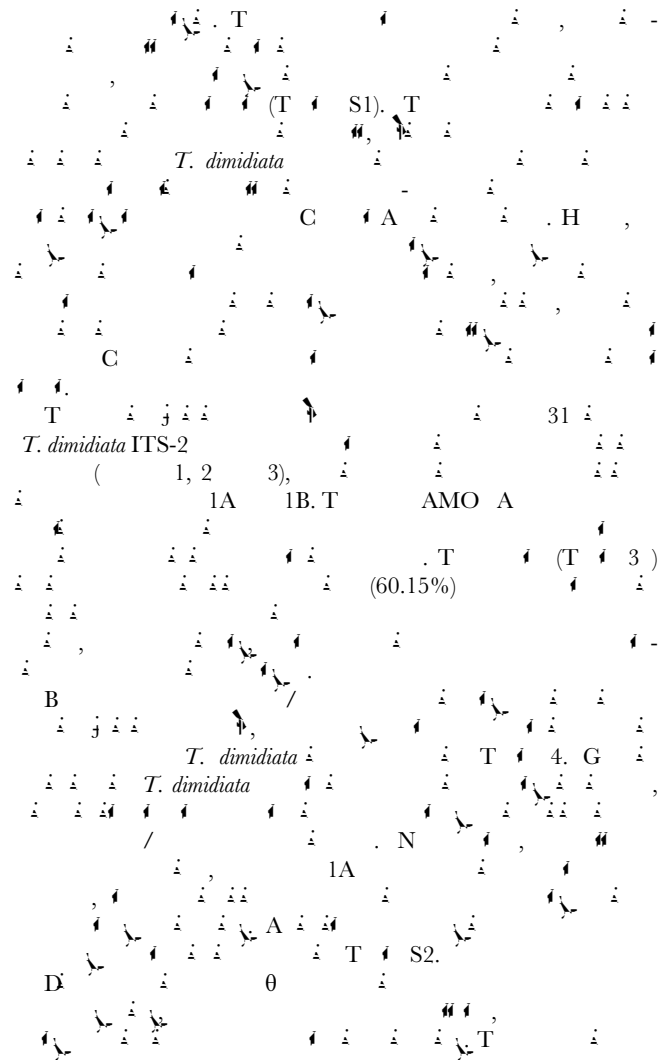
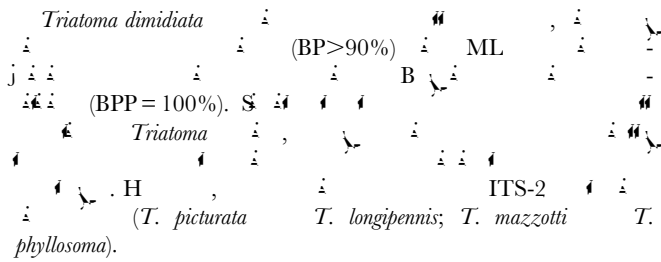
*T. rubrovaria* H1 (AJ557258), (1.22%), 6  
 Phylogenetic Analyses  
 31 *T. dimidiata* AIC, GTR+I  
 (-L = 887.089), 0.166. T  
 (M, G) 3 (M, G)  
 31 *T. dimidiata* ML, G 1  
 T 2  
 M, G, H, T 3, 1  
 3, 1  
 IA, C, P  
 C, E, G, H, M (S)  
 T ITS-2  
*Triatoma* T 562-  
 T 53 ITS-2 *Triatoma*

*R. prolixus* ML  
 (-L = 2648.5129) HK +G = 0.58.  
 AIC  
 T (E 4)  
 • 31 *T. dimidiata* ML/NJ/BPP, (99/97/100), 1A, 1B, 2, 3  
 1 = T. -H1 T. -H10) (H, G  
 C A (H, M, E, P  
 I); T. -H10  
 L, G  
 2 = T. -H11 T. -H17) IB  
 C P  
 3 = T. -H18 T. -H24) 2  
 (91/92/100) M (G  
 C G  
*T. hegneri* 3  
 (97/96/100)  
 4 = T. -H25 T. -H31)  
 C C (M),  
 H G  
 • *T. bassolsae* *T. phyllosoma*, *T. mazzotti*, *T. longipennis*, *T. picturata* *T. pallidipennis* (99/91/100) ML/NJ/BPP) *T. dimidiata*; *T. pallidipennis*  
 • *T. mexicana* *T. gerstaeckeri* *T. dimidiata* *T. phyllosoma* (100/99/100) *T. mexicana*, *T. gerstaeckeri*, *T. phyllosoma* *T. dimidiata*;  
 • *T. barberi*, *T. nitida*, *T. rubida*, *T. ryckmani* *T. bolivari* *T. ryckmani* *T.*

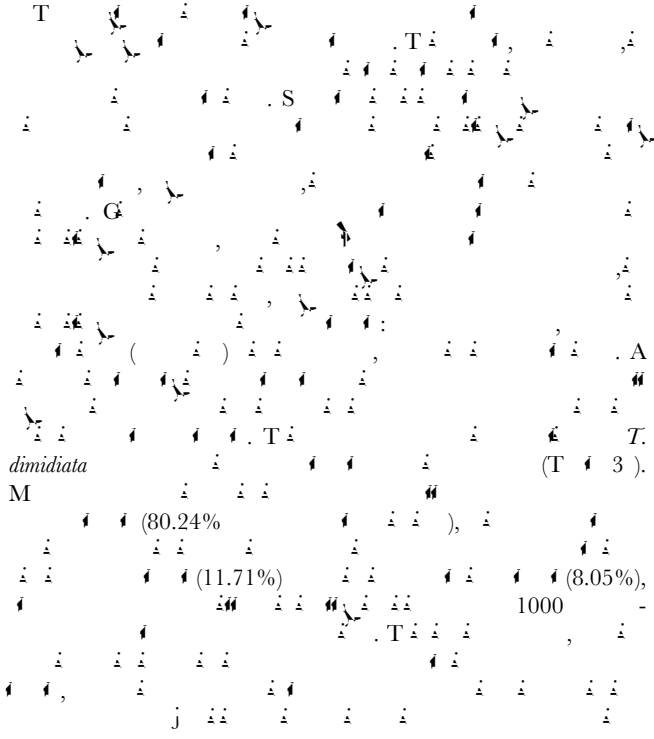


**Figure 3. Median network for *Triatoma dimidiata* haplotypes based on rDNA ITS-2 sequences.** The area of each haplotype is proportional to the total sample. Small black-filled circles represent haplotypes not present in the sample. Mutational steps between haplotypes are represented by a line. More than one mutational step is represented by numbers. H = haplotype. Blue: Colombia; orange: Panama; yellow: Mexico; red: Honduras; lilac: Ecuador; ocher: Nicaragua; green: Guatemala.  
 doi:10.1371/journal.pntd.0000233.g003





Genetic Variation Analyses



**Table 3.** Summary of analysis of molecular variance for *Triatoma dimidiata* populations.

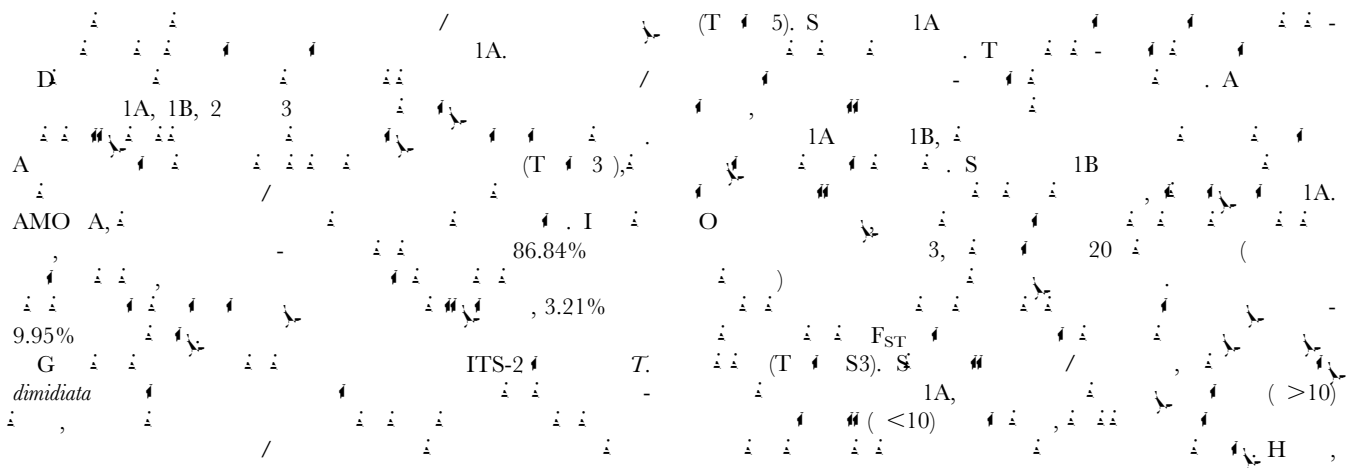
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	Fixation Indices
a)					
Among groups	2	528.273	6.732 Va	80.24	$F_{CT} = 0.802^{***}$
Among populations within groups	10	86.820	0.982 Vb	11.71	$F_{ST} = 0.920^{***}$
Within populations	123	83.047	0.675 Vc	8.05	$F_{SC} = 0.593^{***}$
Total	135	698.140	8.389		
b)					
Among groups	1	68.257	1.4785	60.15	$F_{CT} = 0.602^*$
Among populations within groups	6	15.547	0.3007	12.23	$F_{ST} = 0.724^{***}$
Within populations	77	52.267	0.6788	27.62	$F_{SC} = 0.307^{***}$
Total	84	136.071	2.4580		
c)					
Among groups	3	596.530	5.890	86.84	$F_{CT} = 0.868^{***}$
Among populations within groups	9	18.563	0.218	3.21	$F_{ST} = 0.900^{***}$
Within populations	123	83.047	0.675	9.95	$F_{SC} = 0.244^{***}$
Total	135	698.140	6.783		

(a) Three groups (1, 2, and 3), (b) two subgroups (1A vs 1B), and (c) four groups/subgroups (1A, 1B, 2 and 3) were considered as indicated in the text. Populations within groups correspond to countries of sampling. \*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ . d.f. = degrees of freedom.  
doi:10.1371/journal.pntd.000233.t003

**Table 4.** Summary of population genetic variation parameters from ITS-2 haplotypes in the *Triatoma dimidiata* populations.

Parameter	Group1	Subgroup1A	Subgroup1B	Group	Group3
Gene copies	85	51	34	27	24
Haplotypes	17	10	7	7	7
Polymorphic sites	23	13	9	7	11
Hap. diversity	0.8782	0.797	0.686	0.6353	0.6775
Std. error	0.0178	0.040	0.065	0.0972	0.0902
Pairwise diff. mean	3.2398	1.707	1.524	1.1510	1.6377
Std. error	1.6872	1.014	0.938	0.7670	1.0007
Nucleot diversity	0.0065	0.003	0.003	0.0023	0.0033
Std. error	0.0037	0.002	0.002	0.0017	0.0023
$\theta$ (Het)	6.0371	3.105	1.668	1.3162	1.5990
S.D. $\theta$ (Het)	1.1075	0.822	0.523	0.5710	0.6892
$\theta$ (k)	6.1156	3.444	2.385	2.7281	2.9510
95 % C.I. for $\theta$ (k)	3.476,10.432	1.668,6.785	1.009,5.308	1.134,6.223	1.213,6.838
$\theta$ (S)	3.1911	2.445	1.223	0.5189	0.8034
S.D. $\theta$ (S)	1.1040	0.976	0.636	0.3844	0.5094
$\theta$ ( $\pi$ )	3.2398	1.707	1.524	1.1510	1.6377
S.D. $\theta$ ( $\pi$ )	1.8694	1.125	1.043	0.8553	1.1155
Tajima's D	-1.261ns	-1.572*	-1.553*	-0.536ns	-0.6435ns
Ewens-Watterson	0.132ns	0.219ns	0.334ns	0.388ns	0.3507ns
Fu's Fs	-3.401ns	-2.601ns	-1.111ns	-2.426*	-1.4665ns

$\theta$  = effective mutation rate estimated from equilibrium heterozygosity [ $\theta$ (Het)], number of alleles [ $\theta$ (k)], number of polymorphic sites [ $\theta$ (S)] and nucleotide diversity [ $\theta$ ( $\pi$ )]. The last 3 rows correspond to different statistics of neutrality at the population level. S.D. = standard deviation; C.I. = confidence interval. NS:  $P > 0.05$ ; \* =  $P < 0.05$ . doi:10.1371/journal.pntd.0000233.t004



**Table 5.** Population average pairwise differences in *Triatoma dimidiata* populations.

	Group 1	Subgroup1A	Subgroup1B	Group	Group3
Group 1	3.240	-	-	9.953	20.719
Subgroup1A	-	1.707	4.922	10.325	21.118
Subgroup1B	-	3.307	1.524	9.397	20.120
Group2	7.758	8.896	8.059	1.151	26.875
Group3	18.280	19.446	18.539	25.481	1.638

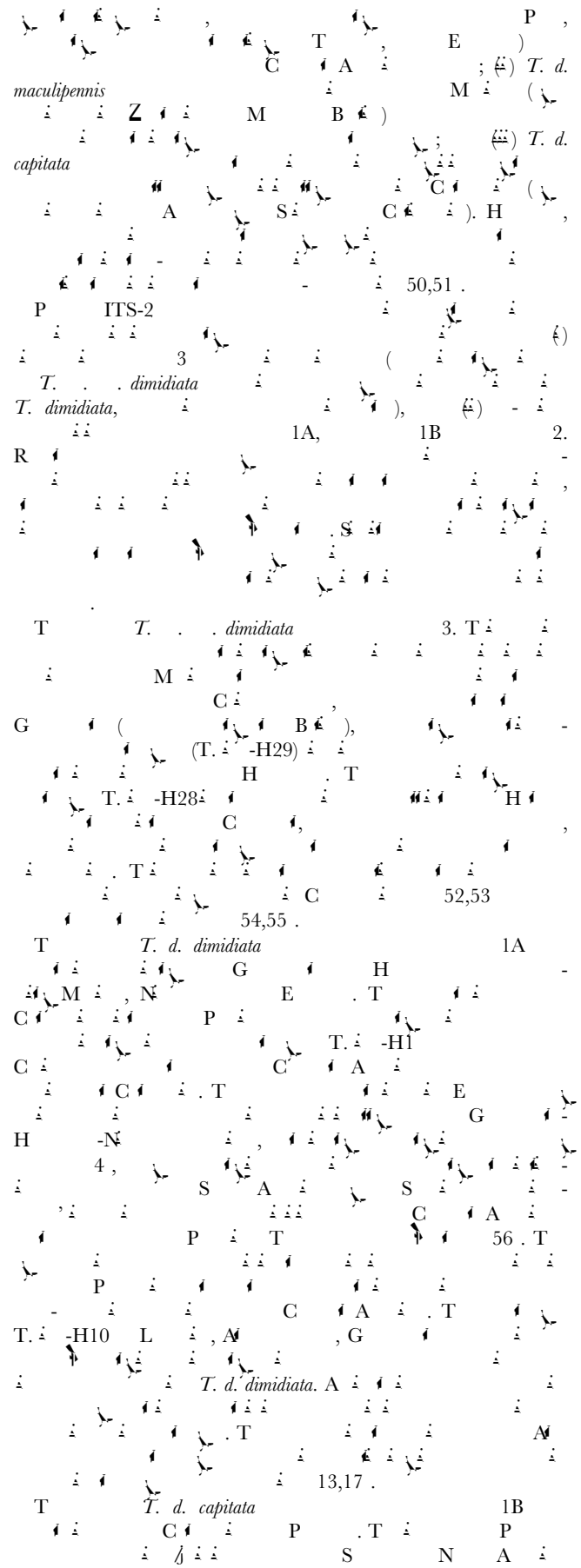
Above diagonal: Average number of pairwise differences between populations ( $\pi_{xy}$ ). Diagonal elements: average number of pairwise differences within population ( $\pi_x$ ). Below diagonal: corrected average pairwise difference ( $(\pi_{xy} - (\pi_x + \pi_y)/2)$ ). doi:10.1371/journal.pntd.0000233.t005

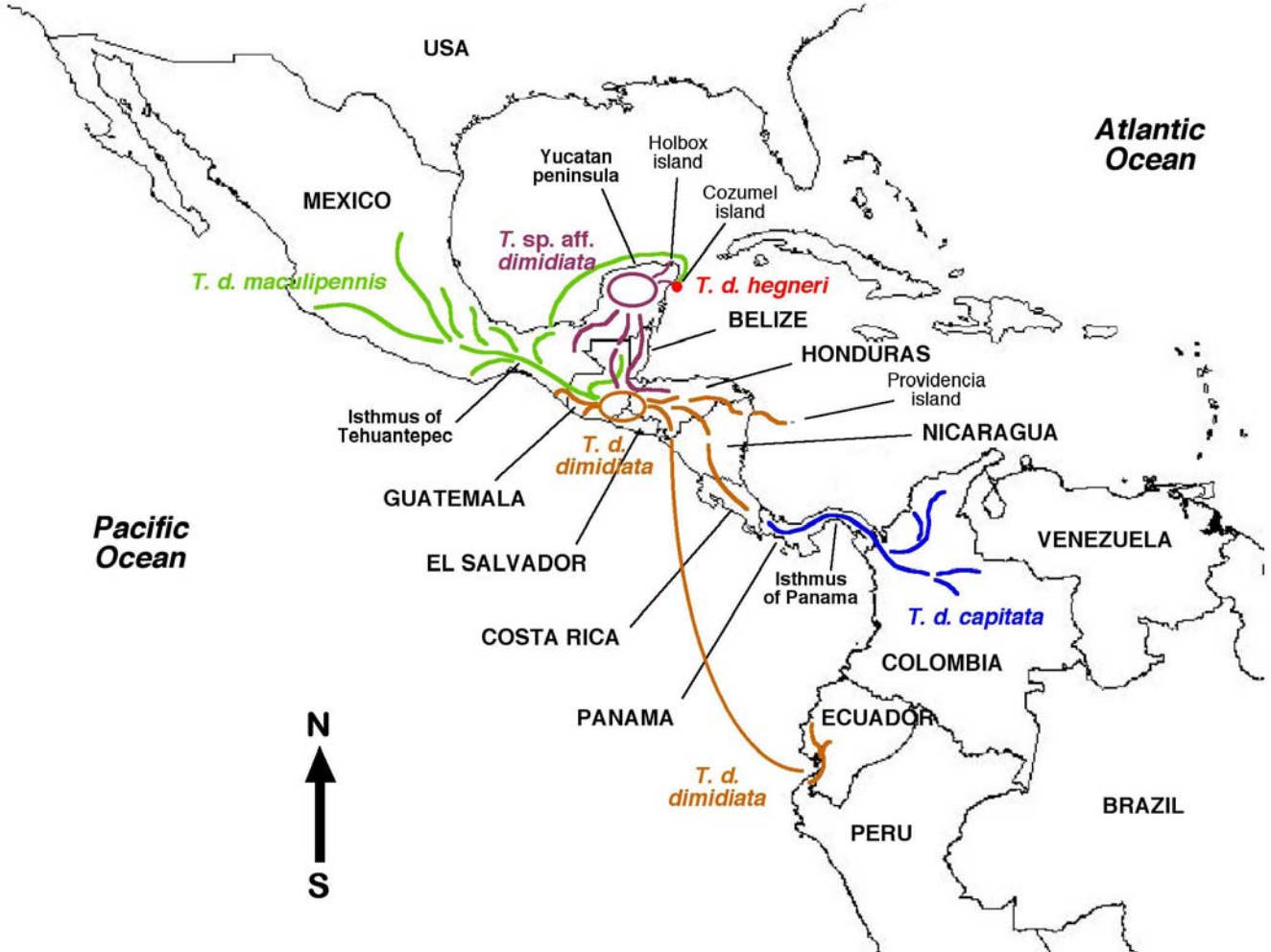
1B (C 2, =30, P =4)  
 T 2 (M 2, =23, G 2, =4).  
 H 3 (=2) G 3 (=7),  $F_{ST}=0.529$ ,  
 $P<0.05$ . N  
 M 3 (=15). S 1A 1 (=26),  
 H 1 (=18) G 1 (=26),  
 $F_{ST}=0.193$ ,  $P<0.001$ . A  
 2.1  
 C M 1, G 1 H E 1  
 N P S /  
 $F_{ST}$  (T S4).

**Discussion**

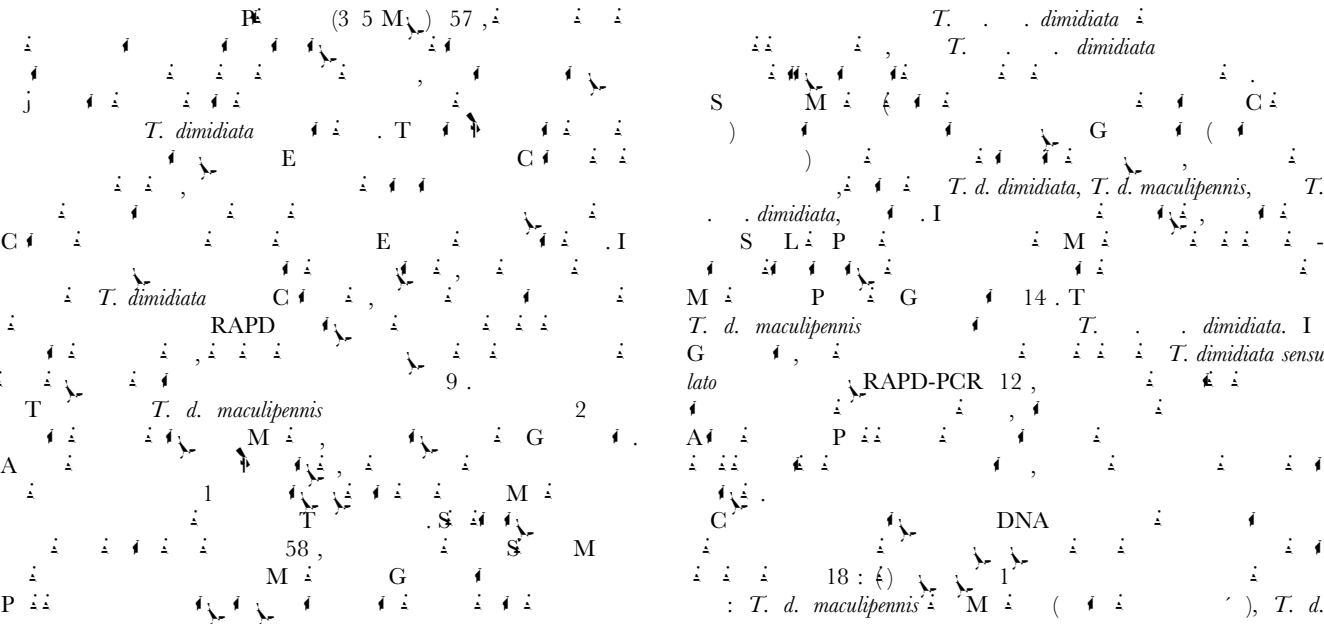
*Triatoma dimidiata*, *T. sp. aff. dimidiata* and *T. hegneri*

*T. dimidiata* ITS-2  
 2.70% (13/482) *T. infestans*  
 10.18% (=51/501) *T. dimidiata* (E 2)  
*Triatoma* G 3 2.42%  
 (T 5)  
 H I), G (P) H ( )  
 T 3 *T. dimidiata*  
 (M) 5.9 10.5 DNA  
 T (E 2) 5.62% 24 ITS-2  
 E H M  
 1A, 1B  
 3 (T 5),  
 T : 2.61% 1A,  
 2.41% 1B, 2.01% *T. dimidiata* 2.  
 R *T. dimidiata* (E 5). A  
 10. M  
*T. dimidiata* 13.  
 T 48,49 : ( ) *T. d. dimidiata*  
 P ( )





**Figure 5. Phylogeography of *Triatoma dimidiata sensu lato*.** Distribution and spreading routes of *T. d. dimidiata*, *T. d. capitata*, *T. d. maculipennis*, *T. d. hegneri* and *Triatoma* sp. aff. *dimidiata* in Mesoamerica, Central America and the northwestern part of South America are represented according to network analyses and genetic variation studies based on rDNA ITS-2 sequences. doi:10.1371/journal.pntd.0000233.g005



*dimidiata* G (P) E T  
 S ; *T. d. capitata* C ; 2  
 M (P C) M ,  
 M *T. d. dimidiata* 5 ,  
 (T. -H25, T. -H26, T. -H27, T. -H28  
 T. -H31); 3 , P , G  
*T. d. maculipennis* (T. -H18) T.  
*dimidiata* (T. -H25, T. -H28 T. -H30)  
 S 18  
 3 T.  
*dimidiata* T. -H28 T. -H30. C  
 2 3 T.  
*dimidiata*.  
 T *T. hegneri* 3  
*T. d. maculipennis*. T  
*T. hegneri*  
*T. dimidiata*, 2 (E 4),  
 T.  
*T. d. maculipennis*.  
 O *T. d. hegneri* C 3,  
*Triatoma hegneri*,  
*T. dimidiata* 50,  
 T.  
*dimidiata* (R.E. R. I  
*T. d. maculipennis* (T. -H18) T.  
*dimidiata* (T. -H28)  
 T *T. d. dimidiata* (1A), *T. d.*  
*capitata* (1B), *T. d. maculipennis* (2),  
*dimidiata* (3), *T. d. hegneri* /  
 F  
 T. *dimidiata*,  
 T. *dimidiata*  
 G *capitata* P C  
*maculipennis* (E 5). T  
*dimidiata* T.  
 ITS-2 50  
 6.  
 M C  
*T. dimidiata sensu lato*  
 M  
 P 11. R *T. dimidiata*  
 M  
 90% C 59. T  
*T. dimidiata*  
*T. dimidiata*  
 T. *dimidiata*.

*T. d. dimidiata*  
 E *T. infestans*  
 U C B  
 S C I C 60. T  
*T. d. dimidiata* E  
 61. U  
 C T. d.  
*capitata* *T. d. dimidiata* E  
 C T.  
 9. S C , *T. dimidiata*  
 C A  
*T. infestans* S C  
**The other Meso- and Central American *Triatoma* Species**  
*Triatoma bassolsae* *T. pallidipennis*  
 5  
 P : *T. longipennis*, *T. mazzotti*, *T. picturata*, *T.*  
*pallidipennis* *T. phyllosoma*. T  
 ( ),  
 S  
 ITS 62. T 63,64  
 49. T  
 0.74 2.28 M  
 DNA 4, ITS-2  
 5,6,65. B  
 64,  
 66,67. R  
 T 67. U  
 P P  
 ITS-2 *Triatoma bassolsae*  
 65. T  
 M  
 3  
 G P  
 DNA  
 ITS-2 16S 68, C B  
 65,69, COI 69. T  
 DNA  
 ITS-2. M



C, B COI  
 P  
 )  
*T. picturata* 69, *T. pallidipennis*  
 A  
 DNA  
 DNA  
 70,  
 DNA  
 71, T  
 P  
 T  
 ITS-2 DNA  
*Triatoma mexicana*  
 P  
 DNA COI 69, S *T. mexicana*, *T. gerstaeckeri*  
 (R )  
 P  
*T. phyllosoma* (*phyllosoma*, *longipennis*,  
*mazzotti*, *picturata*, *pallidipennis* *bassolsae*), *T. dimidiata* (*dimidiata*, *capitata* *maculipennis*, *hegneri*  
), *T. dimidiata*, *T. mexicana* *T. gerstaeckeri*  
*Meccus*,  
72, 50  
73, P  
ITS-2 74  
DNA 16S 68,  
*Meccus*  
T *Meccus*, *Nesotriatoma*  
H  
*T. flavida* P  
7, T  
*T. bolivari*  
*T. ryckmani* P  
*T. rubida* R  
(83 96 ML BPP, A *T. rubida*

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