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Original article

Genetic structure analysis of *Amblyomma mixtum* populations in Veracruz State, Mexico

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ABSTRACT

Amblyomma mixtum Koch, 1844 parasitizes livestock, humans, and wildlife in Mexico. However, information on population genetics for this tick species in the country is missing. Tick samples were collected from livestock in ten regions across the state of Veracruz (22°28′N, 17°09′S, 93°36′E, 98°39′W) to analyze the genetic structure of *A. mixtum* populations. Ticks were morphologically identified using taxonomic keys. In order to test the intraspecific variability of *A. mixtum* fragments of the mitochondrial gene 16S-rRNA and cytochrome oxidase subunit 1 (COI) were amplified. Ninety-six sequences were amplified from the 50 specimens' analyzed (96% amplification success). Eleven haplotypes were detected in 16S-rRNA gene and 10 more for COI. Neutrality tests showed negative results in most of the locations analyzed, which is indicative of an excess of recently derived haplotypes. However, these results were not statistically significant. Minimal union network analysis revealed that there is no separation of populations by geography, and that there is an overlap of several haplotypes among diverse populations. Significant genetic differentiation was not detected in the *A. mixtum* populations sampled in the state of Veracruz, Mexico, this may be due to the frequent movement of livestock hosts. This is the first report on the genetic structure of *A. mixtum* populations in Mexico.

1. Introduction

Ticks are obligate hematophagous ectoparasites, which can act as vectors of multiple pathogens such as bacteria, viruses, protozoa, and helminths that affect human, domestic animal, and wildlife populations (Baneth, 2014). Particularly in America, several members of the *Amblyomma cajennense* species complex represent a problem for livestock production and human public health, due to their adaptation to cattle and their anthropophilic habits (Estrada-Peña et al., 2004; Almazan et al., 2018; Labruna, 2018). Among them the species that exhibits the widest distribution is *Amblyomma mixtum* Koch, 1844, that has been registered from southwestern Texas to Colombia (Beati et al., 2013; Nava et al., 2014; Rivera-Páez et al., 2016, 2018). This species has adapted to diverse ecological niches, including semi-arid grasslands and subtropical secondary forests (Estrada-Peña et al., 2004).

This tick species parasitizes several domestic and wild mammals, in which it causes direct effects related to blood feeding such as weakness and weight loss. However, it also serves as a vector transmitting pathogens to animals (e.g. *Anaplasma marginale*) and human (e.g. *Rickettsia rickettsii*). In addition, other potentially zoonotic species of *Rickettsia* have been detected (e.g. *R. amblyommatis*) making *A. mixtum* one of the most important tick species in veterinary medicine and public health in Mexico (Estrada-Peña et al., 2004; Guzmán-Cornejo et al., 2011; Rodríguez-Vivas et al., 2016; Sánchez-Montes et al., 2016; Álvarez-Hernández et al., 2017; Piña et al., 2017).

However, information related to the genetic diversity of *A. mixtum* in Mexico is lacking. Genetic and demographic studies are essential to determine the magnitude of genetic exchange among tick populations (Araya-Anchetta et al., 2015; Esteve-Gassent et al., 2016). This knowledge has practical implications such as the ability to understand how the movement of livestock across agroecosystems may influence the gene flow between conspecific tick populations, and the possibility to predict the dispersion of genes that confer resistance to ixodicides (Loaiza et al., 2010). Moreover, these studies can yield critical

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information about the divergence of lineages, discrete populations and the timing of demographic processes that may influence the contribution of vectors in the transmission of several tick-borne pathogens.

Mitochondrial DNA (mtDNA) analysis is a powerful tool for the reconstruction of the phylogeny of species and their demographic history (Mirol et al., 2008). The advantages of mtDNA for studying closely related taxa and populations within species are its low recombination rate, maternal heredity, highly conserved structure, small effective population size and relatively fast mutation rate. In ticks, the 16S-rRNA and cytochrome oxidase subunit 1 (COI) genes have a high mutation rate, rendering them as very useful tools for intra-population studies (Esseghir et al., 1997; Ishikawa et al., 1999; Hodgkinson et al., 2003; de Lima et al., 2017). Therefore, we planned this study to investigate the genetic structure of *A. mixtum* populations infesting livestock in the state of Veracruz, Mexico. Veracruz is the state in Mexico with the largest cattle herd, which includes approximately four million head (Rodriguez-Vivas et al., 2017). The *A. mixtum* host diversity, as well as the variety of ecosystems and cattle movement in the state of Veracruz suggest small differences thus, ticks were sampled across ecological regions in the state to assess genetic divergence by the amplification of the 16S-rRNA and COI genes.

2. Materials and methods

2.1. Tick collection and mapping

A total of 50 (23 female, 27 male) *A. mixtum* from cattle and equine from ten regions were collected and analyzed during August-November 2015 and February-April 2016 (Table 1) in a convenience sampling. The ranch locations sampled across the ten regions in the state of Veracruz include diverse ecosystems that range from tropical coastal plains up to 1,520 m above sea level. Hosts were physically restrained and inspected for the presence of ticks, particularly in the neck, limbs and inguinal region. All ticks were recovered from the hosts using forceps and were fixed and preserved in ethanol. All experimental protocols were approved by the animal care and use committee of the School of Veterinary Medicine, University of Veracruz, Veracruz, Mexico.

2.2. Morphological identification

Morphological characters of each tick were observed under a Motic® stereoscope microscope. These were identified according to the morphological keys from Guzmán-Cornejo et al. (2011) and Nava et al. (2014).

2.3. DNA extraction

Tick DNA extraction was performed individually. Every specimen was transferred to a 1.5-mL Eppendorf tube, which was placed on the surface of a container with liquid nitrogen. Subsequently the sample was crushed with the help of a sterile pistil. Then, 500 µL of 5% Chelex® 100 Chelating Resin (Biorad, USA) solution and 20 µL of Proteinase K (SIGMA life sciences, USA) were added per sample, and allowed to incubate at 56 °C for two hours. The samples were then centrifuged at 25,000 × *g* for 15 min and the supernatant was collected in new tubes and stored at −20 °C until further use (Ballados-González et al., 2018).

2.4. Polymerase chain reaction (PCR)

To analyze the intra-specific variability of *A. mixtum*, we amplified a fragment of ~400 bp from the 16S-rRNA gene using a pair of oligonucleotides and conditions reported by Norris et al. (1996), and a fragment of ~379 bp from the cytochrome oxidase subunit 1 (COI) with the conditions of Hafner et al. (1994). PCR was performed in a volume of 25 µL, which contained 300–500 ng of genomic DNA, 1 µL of each primer (2 µM), 12.5 µL of green GoTaq of Promega, and 8.5 µL of free nuclease sterile water. A negative control (reaction mix without DNA), and a positive control (reaction mix and *Rhipicephalus sanguineus* s. l. DNA) were both included. PCR products were resolved in 2% agarose gels using 1 kb molecular weight marker (nucleic acid markers, Axygen) in 1x TBE buffer. Amplicons of the expected size were submitted in the purification and sequencing to Laboratorio de Biología Molecular y de la Salud, Universidad Nacional Autónoma de México.

2.5. Population genetics analysis

Sequence data were edited, and global alignments were done using Clustal W algorithm in Mega 6.0. Sequences generated in this study were submitted to GenBank using the Bankit tool. In order to ascertain genetic diversity, we calculated the number of haplotypes, number of unique haplotypes, number of mutations, number of segregating sites, number of unique sites, haplotypic diversity, and nucleotide diversity in DNAsp 5.10 (Librado and Rozas, 2009). Additionally, we analyzed selective neutrality estimating Tajimas D, Fu and Lis D tests using DnaSP v.5.10 software (Librado and Rozas, 2009). Genetic divergence between population pairs were calculated with the Fixation index (*Fst*), and the average number of nucleotide substitutions per site (*Dxy*) determined with DNAsp. To identify the relationship among haplotypes, minimal union networks were constructed using the program PopArt.

2.6. Phylogenetic tree reconstruction

The best nucleotide substitution model was selected based on the Akaike information criterion (AlCc) calculated in Mega 6.0. Phylogenetic trees were created using the unique haplotypes detected in this study (GenBank Accession Numbers MG930053-MG930063 for 16S-rRNA gene, and MH374165-MH374174 for COI), sequences of *A. mixtum* from the US (GenBank Accession Numbers L34317.1, KM519935.1 and JX573118), one sequence of *A. mixtum* from Colombia (GenBank Accession Number MF353122.1) and sequences of several other hard tick genera (*Haemaphysalys parva*, *Rhipicephalus appendiculatus*, and *Ixodes scapularis*) and a soft tick species (*Argas persicus*) as an outgroup. For phylogenetic reconstruction, we used the software MrBayes v.3.2. (Ronquist et al., 2012), with four Markov Chain Monte Carlo chains that were run for 10,000,000 generations (sampled every 1000 generations) to allow adequate time for convergence (=0.003). The first 25% of the sampled trees were considered burn in. The final tree was visualized and edited by ITOL software.

3. Results

Ninety-six sequences were amplified from the 50 specimens analyzed (96% amplification success). Two samples, one from the Capital and another one from the Olmeca regions could not be amplified. Fragments of 380–400 base pairs from the 16s-rRNA gene were obtained, and 350–370 bp in COI.

3.1. Population genetics analysis

3.1.1. s-rRNA gene

For population genetics analysis, we constructed an alignment with the 48 sequences recovered in this study. The final alignment consisted of 380 base pairs, with 370 conserved and 10 variable sites, three singletons (in positions 30, 164, and 247), and seven parsimony informative sites(in positions 107, 114, 164, 247, 107, 114, 129, 137, 216, 293, and 311).

We detected the presence of 11 haplotypes. The most frequent haplotype detected was H3, with 24 sequences (50%), followed by haplotypes H4 with five (10%) and H5 with four sequences (8%). The least frequent haplotypes were H6, H7, H8 and H11, which were recorded once each (Table 1). Haplotype diversity (Hd) was 0.7323, and

Table 1
Sampling sites, hosts and haplotypes of specimens collected in this study. NA, Not amplified.

ID	Region	Municipality	Host	Latitude	Longitude	16S-rRNA	COI
1	Huasteca Alta	Pánuco	Cattle	22.0719	−98.1822	H1	H1
2	Huasteca Alta	Pánuco	Cattle	22.0719	−98.1822	H1	H1
3	Huasteca Alta	Pánuco	Equine	22.0250	−98.1764	H2	H1
4	Huasteca Alta	Pánuco	Equine	22.1174	−98.2047	H2	H1
5	Huasteca Alta	Pánuco	Equine	22.1174	−98.2047	H3	H1
6	Huasteca Baja	Tuxpan	Cattle	20.9404	−97.4116	H3	H1
7	Huasteca Baja	Tuxpan	Cattle	20.9909	−97.3967	H3	H2
8	Huasteca Baja	Naranjos	Equine	21.3356	−97.7633	H3	H3
9	Huasteca Baja	Naranjos	Equine	21.3356	−97.7633	H3	H1
10	Huasteca Baja	Naranjos	Equine	21.3565	−97.7316	H3	H1
11	Totonaca	Tihuatlán	Cattle	20.5875	−97.5283	H4	H4
12	Totonaca	Tihuatlán	Cattle	20.6181	−97.6144	H5	H1
13	Totonaca	Tecolutla	Equine	20.3711	−96.9167	H5	H1
14	Totonaca	Tecolutla	Cattle	20.4753	−97.0364	H6	H5
15	Totonaca	Gutiérrez Zamora	Cattle	20.4133	−97.0419	H5	H5
16	Nautla	Nautla	Cattle	20.1819	−96.8242	H4	H1
17	Nautla	Nautla	Cattle	20.1094	−96.8011	H4	H1
18	Nautla	Nautla	Cattle	20.1533	−96.8281	H4	H6
19	Nautla	Nautla	Equine	20.1819	−96.8242	H4	H2
20	Nautla	Nautla	Equine	20.1819	−96.8242	H3	H2
21	Capital	Xalapa	Cattle	19.5467	−96.8681	H7	H2
22	Capital	Xalapa	Equine	19.5467	−96.8681	H8	H6
23	Capital	Xalapa	Equine	19.5467	−96.8681	H2	H1
24	Capital	Coatepec	Equine	19.4619	−96.9881	NA	NA
25	Capital	Xalapa	Equine	19.5467	−96.8506	H3	H1
26	Montaña	Yanga	Equine	18.8025	−96.7592	H3	H1
27	Montaña	Orizaba	Equine	18.8711	−97.0897	H3	H1
28	Montaña	Coscomatepec	Cattle	19.0886	−97.0531	H3	H1
29	Montaña	Coscomatepec	Cattle	19.0886	−97.0531	H3	H1
30	Montaña	Coscomatepec	Equine	19.0886	−97.0531	H3	H1
31	Sotavento	Medellín	Cattle	19.0844	−96.1808	H1	H1
32	Sotavento	Medellín	Equine	19.0328	−96.1756	H3	H6
33	Sotavento	Paso de Ovejas	Cattle	19.2011	−96.3839	H3	H1
34	Sotavento	Paso de Ovejas	Cattle	19.2733	−96.5017	H3	H1
35	Sotavento	Puente Nacional	Cattle	19.2283	−96.6861	H9	H1
36	Papaloapan	Tierra Blanca	Cattle	18.4519	−96.3822	H5	H1
37	Papaloapan	Tierra Blanca	Equine	18.6319	−96.1717	H3	H7
38	Papaloapan	Tlacotalpan	Equine	18.5544	−95.7283	H3	H7
39	Papaloapan	Tlacotalpan	Cattle	18.5814	−95.7489	H9	H1
40	Papaloapan	Tlacotalpan	Cattle	18.4067	−95.5589	H9	H8
41	Tuxtlas	Catemaco	Equine	18.5564	−94.9919	H3	H8
42	Tuxtlas	Catemaco	Cattle	18.3956	−95.1211	H3	H8
43	Tuxtlas	Santiago Tuxtla	Equine	18.4542	−95.2711	H10	H8
44	Tuxtlas	Santiago Tuxtla	Equine	18.3644	−95.4797	H10	H4
45	Tuxtlas	San Andrés Tuxtla	Cattle	18.6747	−95.2842	H3	H1
46	Olmeca	Acayucan	Cattle	18.0706	−94.9861	H3	H10
47	Olmeca	Acayucan	Cattle	18.0183	−95.0467	H3	H1
48	Olmeca	Acayucan	Equine	18.0614	−94.8844	NA	NA
49	Olmeca	Jesús Carranza	Equine	17.5219	−95.0747	H11	H10
50	Olmeca	Jesús Carranza	Cattle	17.3542	−94.9658	H3	H6

Table 2
Summary statistics for polymorphisms and neutrality tests of 16S-rRNA gene from *Amblyomma mixtum* from Veracruz. **Abbreviations**: **N**, number of individuals; **S**, Number of polymorphic sites; **h**, number of haplotypes; **hd**, haplotype diversity; π, nucleotide diversity.*$p < 0.05$.

Locality	N	S	h	Hd	π	Tajima's D	Fu and Li's D	Fu and Li's F	Fu's Fs
Huasteca Alta (Ha)	5	5	2	0.6666	0.0088	2.1249	2.1249*	2.0080	3.153
Huaseca Baja (Hb)	5	0	1	0.0000	0.0000	(-)	(-)	(-)	(-)
Totonaca (To)	5	2	3	0.7000	0.0021	−0.9726	−0.9726	−0.9544	−0.829
Nautla (Na)	5	2	2	0.4000	0.0021	−0.9726	−0.9726	−0.9544	1.040
Capital (Ca)	4	3	4	1.0000	0.0040	−0.7545	−0.7545	−0.6747	−2.367
Montaña (Mo)	5	0	1	0.0000	0.0000	(-)	(-)	(-)	(-)
Sotavento (So)	5	4	3	0.7000	0.0058	0.9571	0.9571	0.9742	0.804
Papaloapan (Pa)	5	4	3	0.8000	0.0058	0.9571	0.9571	0.9742	0.804
Tuxtlas (Tu)	5	2	2	0.6000	0.0032	1.4588	1.4588	1.4316	1.688
Olmeca (Ol)	4	2	2	0.5000	0.0026	−0.7099	−0.7099	−0.6043	1.099
Total	48	10	11	0.7323	0.0045	−0.7024	−0.4249	−0.6074	−3.703

Table 3
Summary statistics for polymorphisms and neutrality tests of COI gene from *Amblyomma mixtum* from Veracruz. **Abbreviations**: **N**, number of individuals; **S**, Number of polymorphic sites; **h**, number of haplotypes; **hd**, haplotype diversity; π, nucleotide diversity.*p < 0.05.

Locality	N	S	h	Hd	π	Tajima's D	Fu and Li's D	Fu and Li's F	Fu's Fs
Huasteca Alta (Ha)	5	0	1	1.000	0.00,000	(-)	(-)	(-)	(-)
Huaseca Baja (Hb)	5	4	3	0.700	0.00533	−1.20106	−1.09380	−1.11335	0.276
Totonaca (To)	5	2	3	0.800	0.00333	0.24314	0.24314	0.23860	−0.475
Nautla (Na)	5	3	3	0.800	0.00600	1.57274	1.57274	1.57783	0.469
Capital (Ca)	4	3	3	0.833	0.00556	0.16766	0.16766	0.14992	−0.133
Montaña (Mo)	5	0	1	0.000	0.00,000	(-)	(-)	(-)	(-)
Sotavento (So)	5	1	2	0.400	0.00133	−0.81650	−0.81650	−0.77152	0.090
Papaloapan (Pa)	5	4	3	0.800	0.00733	0.95707	0.95707	0.97418	0.804
Tuxtlas (Tu)	5	4	3	0.700	0.00600	−0.41017	−0.41017	−0.41751	0.469
Olmeca (Ol)	4	4	3	0.833	0.00833	1.36522	1.36522	1.26092	0.461
Total	48	11	10	0.670	0.00488	−1.20106	0.30228	−0.22730	−3.458

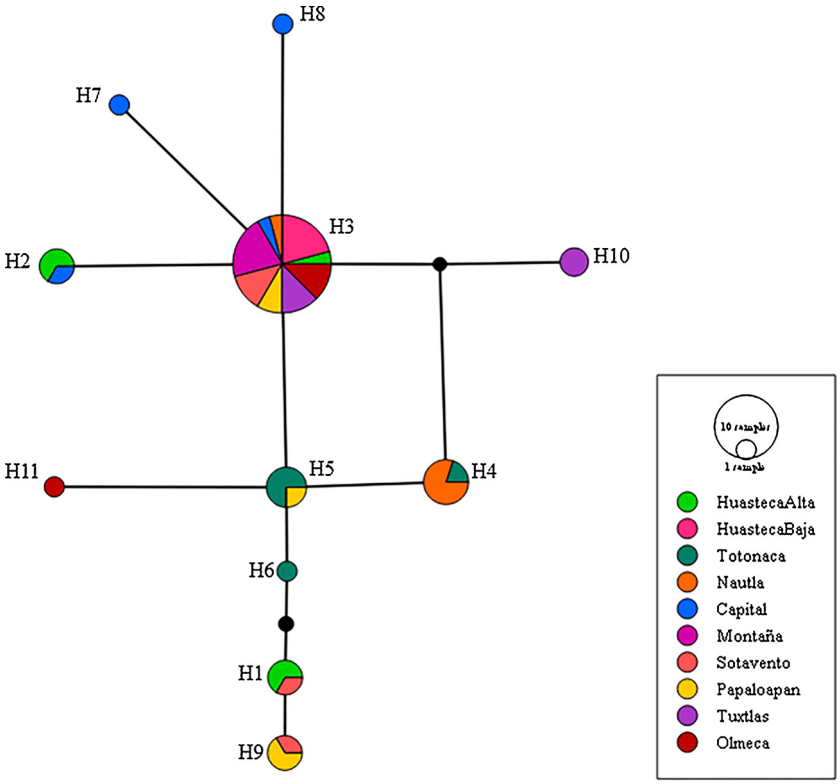

Fig. 1. Minimal union network of *A. mixtum* from ten different populations in Veracruz, Mexico, based on 16S rRNA sequences.

Table 4
16S-rRNAGenetic distances *Fst* (below diagonal) and *Dxy* (above diagonal) between pairs of populations of *A. mixtum* from Veracruz.

Locality	Ha	Hb	To	Na	Ca	Mo	So	Pa	Tu	Ol
Ha		0.007	0.007	0.009	0.008	0.007	0.007	0.007	0.009	0.007
Hb	0.333		0.004	0.004	0.002	0.000	0.004	0.004	0.002	0.001
To	0.235	0.714		0.003	0.006	0.004	0.006	0.006	0.005	0.004
Na	0.374	0.750	0.259		0.006	0.004	0.007	0.007	0.005	0.004
Ca	0.194	0.000	0.465	0.511		0.002	0.006	0.006	0.004	0.003
Mo	0.333	0.000	0.714	0.750	0.000		0.004	0.004	0.002	0.001
So	0.107	0.214	0.330	0.440	0.140	0.214		0.005	0.006	0.005
Pa	0.109	0.214	0.330	0.440	0.140	0.214	0.250		0.006	0.005
Tu	0.313	0.250	0.510	0.432	0.129	0.250	0.227	0.227		0.003
Ol	0.212	0.000	0.357	0.471	0.000	0.000	0.111	0.111	0.154	

Table 5
COI Genetic distances *Fst* (below diagonal) and *Dxy* (above diagonal) between pairs of populations of *A. mixtum* from Veracruz. NC. Not calculable.

Locality	Ha	Hb	To	Na	Ca	Mo	So	Pa	Tu	Ol
Ha	–	0.003	0.002	0.005	0.003	0.000	0.001	0.005	0.004	0.006
Hb	0.000	–	0.005	0.005	0.005	0.003	0.003	0.006	0.006	0.008
To	0.167	0.071	–	0.007	0.005	0.002	0.003	0.007	0.006	0.008
Na	0.357	NC	0.300	–	0.005	0.005	0.005	0.008	0.008	0.010
Ca	0.167	NC	0.167	NC	–	0.003	0.003	0.007	0.007	0.008
Mo	0.000	0.000	0.167	0.357	0.167	–	0.001	0.005	0.004	0.006
So	0.000	NC	0.125	0.191	NC	0.000	–	0.005	0.004	0.006
Pa	0.214	NC	0.200	0.138	0.033	0.214	0.097	–	0.007	0.009
Tu	0.250	0.115	0.222	0.237	0.133	0.250	0.167	0.020	–	0.008
Ol	0.286	0.163	0.255	0.246	0.167	0.286	0.216	0.145	0.044	–

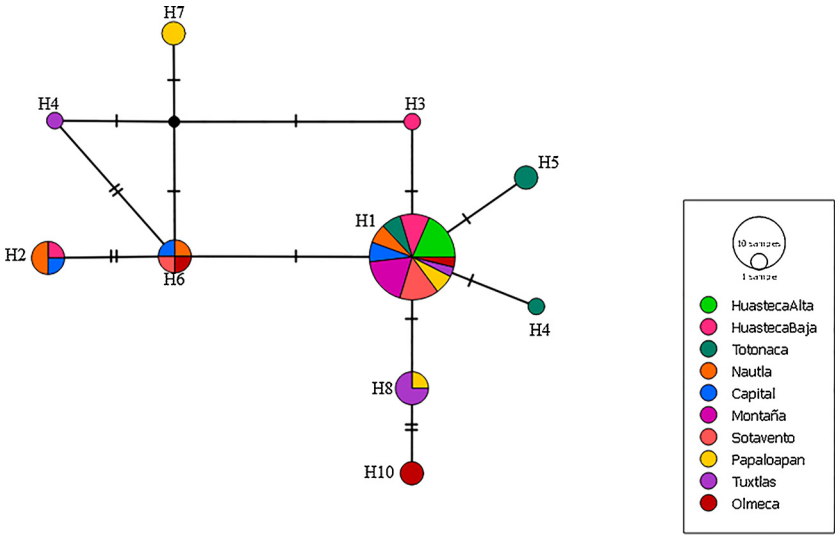

Fig. 2. Minimal union network of *A. mixtum* from ten different populations in Veracruz, Mexico, based on COI sequences.

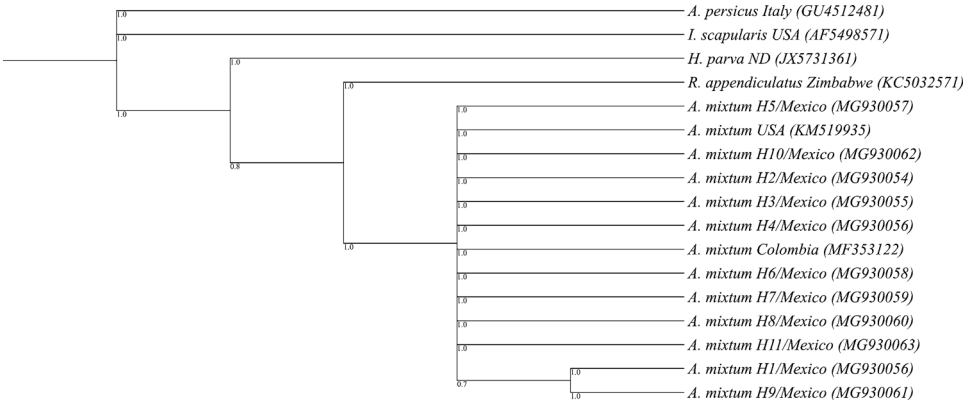

Fig. 3. Bayesian inference reconstruction of partial sequences of 380 bp of the 16S-rRNA gene of *A. mixtum* inferred using Hasegawa, Kishino and Yano substitution model (HKY). Numbers on each branch represent posterior probabilities.

it varied between populations between 0 and 1. The variance of haplotype diversity was 0.00405, and the standard deviation of 0.064. Nucleotide diversity was 0.00448 and varied between populations among 0-0.0088. Sampling variance of Pi was 0.0000004, and the standard deviation of Pi of 0.00064 (Table 2).

Neutrality tests showed negative results in most of the locations analyzed, which is indicative of an excess of recently derived haplotypes. However, these results were not statistically significant (Table 2). These data did not support the null hypothesis that populations are not genetically different because *Dxy* tests showed low values that go through 0.001–0.009 (Table 3), and the same pattern was observed with F_{ST}, which showed low but non-significant values (0.1 $p > 0.001$) (Table 3).

Minimal union network analysis revealed that there is no separation of populations by geography, and that there is an overlap of several haplotypes among diverse populations. Notably, haplotype 3 is the most widely distributed in eight of the 10 analyzed populations (80%). However, this haplotype was not recorded in the Huasteca Alta and Totonaca regions. Haplotypes H6, H1 and H9 are related to each other, but do not share a relationship with most of the other haplotypes that are derived from Haplotype 3. The Capital region included a larger number of unique haplotypes (H6 and H7) that are not shared with any other population (Fig. 1).

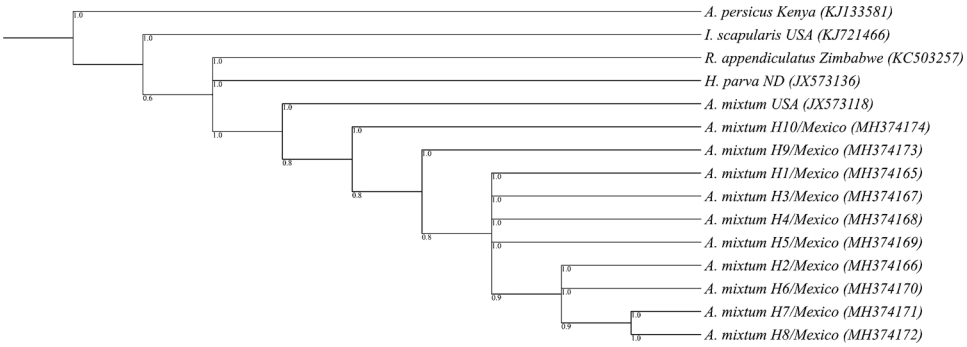

Fig. 4. Bayesian inference reconstruction of partial sequences of 350 bp of the COI gene of *A. mixtum* inferred using Kimura two parameters substitution model (K2). Numbers on each branch represent posterior probabilities.

3.1.2. Cytochrome oxidase subunit 1 (COI)

The final alignment consisted of 300 base pairs, with 289 conserved and 11 variable sites, two singletons (in positions 111, and 193), and nine parsimony informative sites (in positions 5, 8, 14, 20, 36, 46, 55, 179, and 182) (Table 4).

We detected the presence of 10 haplotypes. The most frequent haplotype detected was H1, with 27 sequences (56.3%), followed by haplotypes H2, H6, and H8 with four (8.3%). The least frequent haplotype was H3, which was recorded once (Table 1). Haplotype diversity (Hd) was 0.6702, and it varied between populations between 0 and 1. The variance of haplotype diversity was 0.00524, and the standard deviation of 0.072. Nucleotide diversity was 0.005 and varied between populations among 0-0.0083. Sampling variance of Pi was 0.0000007, and the standard deviation of Pi of 0.00085 (Table 3).

As in the case of 16S-rRNA, neutrality tests showed negative results, confirming the excess of recently derived haplotypes (Table 3). *Dxy* and *FST* tests presented the same pattern observed with 16S-rRNA. None of the values have been significant ($0.1 p > 0.001$) (Table 5).

Also as the minimal union network of 16S-rRNA, there is no separation of populations by geography. Haplotype 1 is the most widely distributed in all the analyzed populations. Haplotypes H8, and H10 are related to each other, but do not share a relationship with most of the other haplotypes that are derived from Haplotype 1. The Totonaca region exhibited a larger number of unique haplotypes (H4 and H5) that are not shared with any other population (Fig. 2).

3.2. Phylogenetic reconstruction

3.2.1. s-rRNA gene

The phylogenetic tree grouped the sequences obtained in the present study with those of reference of *A. mixtum* from the United States and Colombia deposited in GenBank in a clade with a very high support value (1.0) (Fig. 3). Additionally, the analysis revealed the presence of a single subgroup: the clade integrated by the haplotypes H1, and H10 (Fig. 3).

3.2.2. Cytochrome oxidase subunit 1 (COI)

As in the case of 16S-rRNA analysis, the phylogenetic tree grouped our ten haplotypes with the only sequence of COI from *A. mixtum* deposited in GenBank, in a monophyletic clade, with a strong support of 1.0, confirming the identification of ticks as *A. mixtum* (Fig. 4).

4. Discussion

This is the first report on the genetic structure of *A. mixtum* populations in Mexico. The entire state of Veracruz, which borders the Gulf of Mexico to the east, is part of the known geographic range for *A. mixtum* in Mexico. However, information on gene flow between *A. mixtum* populations inhabiting the diverse ecosystems in Veracruz was lacking.

Haplotype network analysis of 16S-rRNA and COI sequences from *A. mixtum* indicated that there is no genetic structure between populations infesting livestock across the state of Veracruz. Haplotype H3 of the 16S-rRNA sequences seems to be the ancestral haplotype because it is the most abundant occurring in 10 of the populations, and it is at the center of the network. The same pattern was detected in Haplotype 1 from COI. The frequent overlap of haplotypes among populations indicates narrow reproductive contact. The presence of 11 haplotypes of 16S-rRNA and 10 from COI, in the populations sampled throughout the state suggests high genetic diversity and gene flow. Populations that have not suffered a drastic reduction in size within evolutionary time, and in which the effects of genetic diversification are reduced, appear to be able to retain an ancestral polymorphism (Avise et al., 1984; Hamarsheh et al., 2007).

An apparent lack of genetic differentiation in the *A. mixtum* populations analyzed may be due to the continuous mobilization of livestock hosts. In the state of Veracruz there are around 4 million head of cattle, which tend to be moved from south to north throughout the state. Unlike *Rhipicephalus microplus* infestations in livestock, *A. mixtum* is not a surveilled species in Mexico (Rodriguez-Vivas et al., 2017; Almazan et al., 2018). The frequent movement of cattle infested with this tick species across Veracruz apparently is a process promoting the genetic homogeneity of *A. mixtum* populations along the Gulf of Mexico. Further research is needed to determine if a similar situation occurs in the other Mexican states bordering the Gulf of Mexico.

A. mixtum has been able to adapt to different climatic conditions across Mexico and infestations are found commonly in equines, but the tick can parasitize other livestock (Estrada-Peña et al., 2004; Coronel-Benedett et al., 2018). The movement of horses has been identified a frequent path for the dispersal of *A. mixtum* (Labruna et al., 2002; Alvarez and Bonilla, 2007). Additionally, resistance to acaricides was reported in populations of *A. cajennense* sensu lato infesting cattle Veracruz state (Alonso-Díaz et al., 2013). Further studies encompassing samples from the entire geographic range of this tick species in Mexico will enhance our understanding of the genetic basis of acaricide resistance in this tick species of veterinary and public health importance.

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