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Ticks and tick-borne *Rickettsia* in El Salvador

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Abstract
From May to November 2013, ticks were collected from wild and domestic hosts that were sampled by convenience in different localities of El Salvador. Among 48 localities, in total 1181 ticks were collected from 200 vertebrate animals, comprising 13 species of wild hosts (amphibian, reptiles, mammals) and five species of domestic mammals, plus four samples from humans and four samples from the environment. Through morphological analysis (corroborated by molecular analyses in a few cases), the following ten tick species were identified: *Amblyomma dissimile*, *Amblyomma mixtum*, *Amblyomma ovale*, *Amblyomma* cf. *parvum*, *Amblyomma sabanerae*, *Amblyomma scutatum*, *Dermacentor dissimilis*, *Dermacentor nitens*, *Rhipicephalus microplus*, and *Rhipicephalus sanguineus* sensu lato. Among a sample of 211 tick specimens tested for rickettsial infection by molecular methods, we identified: '*Candidatus* Rickettsia colombianensi' in 10% of the *A. dissimile* ticks and 11% of the *A. scutatum* ticks; *Rickettsia amblyommatis* in 77% of the *A. mixtum* ticks, 50% of the *A.* cf. *parvum* ticks, 8% of the *D. nitens* ticks, and 11% of the *Amblyomma* spp. nymphs; and *Rickettsia bellii* in 3% of the *A. dissimile* ticks and 17% of the *A. ovale* ticks. The tick fauna of El Salvador is currently represented by 12 reported species.

Keywords Tick fauna · Ixodidae · *Rickettsia* · Central America

Introduction

El Salvador in the smallest country in the American main land, occupying an area of 21,041 km^2. Despite its size, El Salvador bears a great diversity of ecosystems varying from coastal lands on the Pacific Ocean to mountainous and volcanic landscapes up to 2700 m above sea level, in addition to hydrographic basins composed by over 590 rivers

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and creeks (MARN 2017). The native fauna of El Salvador is currently composed by 36 species of amphibians, 103 of reptiles, 584 species of birds (native and migratory), and 159 of mammals (MARN 2018).

Previous reports related to bacteria of the genus *Rickettsia* in El Salvador have been restricted to a molecular detection of *Rickettsia bellii* in *Amblyomma sabanerae* (Barbieri et al. 2012) and seroepidemiological studies that indicated human exposure to *Rickettsia* spp. (reviewed by Bermúdez and Troyo 2018). This scarcity of data contrasts to the broad array of rickettsial organisms that have been reported infecting different tick species in Central America (Bermúdez and Troyo 2018).

In 2014, Navarrete-Abarca et al. (2014) published in a local journal a list of ticks that were collected in El Salvador. This list included 11 tick species collected from various host species. Ticks were identified only by morphological analysis; however, no information about the collected tick stages and their specific localities in the country was provided by the authors. Although Navarrete-Abarca et al. (2014) reported that some of these ticks were infected by three *Rickettsia* species—*Rickettsia amblyommatis* (reported as *R. amblyommii*), '*Candidatus* Rickettsia colombianensi' (reported as *Rickettsia* sp. strain Colombianensi) and *Rickettsia bellii*—no information about DNA sequences of rickettsiae was provided. Herein, we revised the taxonomic identification of the ticks reported by Navarrete-Abarca et al. (2014), including molecular analyses. We also provide detailed information on tick stages and locality of every tick species collected from the various host species. In addition, we tested the ticks and generated partial sequences of two rickettsial genes (*gltA* and *ompA*), and compared their similarities to sequences available in GenBank.

Materials and methods

From May to November 2013, ticks were collected from wild and domestic hosts that were sampled by convenience in different localities of El Salvador, through an active surveillance of the 'Ministerio de Agricultura y Ganadería' of El Salvador. Collections were performed with support of the University of El Salvador and Ministry of Agriculture and Livestock. Ticks were collected directly from the animals, put in plastic vials containing 70% ethanol, and transported to the Ministry of Agriculture and Livestock Laboratory, where they were subjected to taxonomic identification based on external morphology, following Arthur (1960), Fairchild et al. (1966), Voltzit (2007), and Nava et al. (2014).

From the collected ticks, we selected 211 specimens to be tested by molecular analyses. The remaining specimens were deposited as voucher specimens in the 'Tick collection from Ministry of Agriculture and Livestock, El Salvador'. For molecular analysis, ticks were individually submitted to DNA extraction by High Pure PCR Template Preparation Kit following manufacturer's instructions for isolation of nucleic acids from mammalian tissue. The taxonomic identification of 13 tick specimens was verified by molecular analysis through PCR amplification of a ca. 460-bp fragment of the tick mitochondrial 16S rRNA gene, as previously described (Mangold et al. 1998). PCR amplicons of the expected size were submitted to direct DNA sequencing in an automated ABI automated sequencer (Applied Biosystems/Thermo Fisher Scientific, model ABI 3500 Genetic Analyser, Foster City, CA, USA) according to the manufacturer's protocol. The partial sequences were subjected to BLAST analyses (ncbi.nlm.nih.gov/blast) to determine the closest similarities to other tick species available in GenBank.

Extracted DNA of ticks was tested individually by PCR using the primers CS-78 (forward) and CS-323 (reverse), which amplify a 401-bp fragment of the citrate synthase gene (*gltA*) of all known *Rickettsia* species (Labruna et al. 2004). If an expected product was observed following gel electrophoresis, the tick was tested using two other PCR protocols: one targeting an 834-bp overlapping fragment of the *gltA* gene, with primers CS-239 and CS-1069 (Labruna et al. 2004), and the other targeting a ca. 635-bp fragment of the rickettsial 190-kDa outer membrane protein gene (*ompA*), using primers Rr190.70F and Rr190.701R, as described by Eremeeva et al. (2006). In each set of reactions, negative control tubes containing water were included, and also a positive control tube containing DNA of *Rickettsia parkeri* strain NOD. Amplicons were DNA sequenced as described above.

Results

Among 48 localities of El Salvador (Table 1), in total 1181 ticks were collected from 200 vertebrate animals, comprising 13 species of wild hosts (amphibian, reptiles, mammals) and five species of domestic mammals, plus four samples from humans and four samples from the environment. Through morphological analysis (corroborated by molecular analyses in a few cases described below), the following ten tick species were identified: *Amblyomma dissimile* (49 males, 23 females, 1 nymph), *A. mixtum* (15 males, 13 females, 1 nymph), *A. ovale* (8 males, 8 females), *A. parvum* (50 males, 31 females, 1 nymph), *A. sabanerae* (31 males, 6 females), *A. scutatum* (77 males, 46 females), *Dermacentor dissimilis* (2 males, 6 females), *D. nitens* (39 males, 38 females, 28 nymphs, 10 larvae), *Rhipicephalus microplus* (67 males, 237 females, 25 nymphs, 1 larva), and *Rh. sanguineus* sensu lato (109 males, 119 females, 30 nymphs). In addition to these ten species, 84 nymphs and 26 larvae were morphologically identified only to genus level, and were retained as *Amblyomma* spp. Tick species according to hosts and localities are shown in Table 2.

Molecular identification of ticks was performed on 13 specimens, as shown in Table 3. Three *Amblyomma* nymphs were molecularly identified as *A. dissimile*, *A. mixtum*, and *A. parvum.* Morphological identifications of adult ticks were corroborated by molecular analyses on one *A. dissimile*, two *A. mixtum*, one *A. ovale*, four *A. parvum*, and one *D. nitens*; i.e., the 16S rDNA partial sequences of these ticks were 98.6–100% identical to conspecific sequences from GenBank. On the other hand, one *A. scutatum* specimen yielded a 16S rDNA partial sequence that was at most 96.1% identical to any sequence from GenBank (e.g., *A. dissimile*), as there was no 16S rDNA sequence of *A. scutatum* available in GenBank.

Among the 211 tick specimens tested for rickettsial infection, rickettsial DNA was amplified from 27 (12.8%) specimens (Table 4). Three *A. dissimile* (2 adults, 1 nymph) and two adults of *A. scutatum* generated a 1083-bp partial sequence of the *gltA* gene, and a 489-bp partial sequence of the *ompA* gene. This *gltA* sequence was 99.7% (1080/1083 bp) identical to an uncharacterized *Rickettsia* sp. from *Amblyomma sculptum* from Brazil (MH158234); however, a smaller portion of this fragment was 100% (372/372 bp) identical to '*Candidatus* Rickettsia colombianensi' from *A. dissimile* from Brazil (MG563768); i.e., there were no larger *gltA* sequences of '*Ca.* R. colombianensi' in GenBank. The *ompA* partial sequences of these *A. dissimile* and *A. scutatum* ticks were 100% (489/489 bp) identical to '*Ca.* R. colombianensi' from *A. dissimile* from Colombia (JF905458) and Brazil (MG970683). One *A. dissimile* adult and one *A. ovale* adult generated a *gltA* partial consensus sequence that was 100% (1088/1088 bp) identical to *Rickettsia bellii* type strain

Table 1 Localities in El Salvador where ticks were collected during 2013

Localities		Elevation (m a.s.l.)	Geographical coordinates (North, West)
No.	Name		
1	Apanta, Santa Ana	672	14°11′10.89″, 89°20′43.51″
2	Apastepeque, San Vicente	596	13°40′03.28″, 88°46′49.07″
3	Arcatao, Chalatenango	506	14°05′37.14″, 88°44′53.26″
4	Cancasque, Chalatenango	233	13°58′26.49″, 88°51′05.43″
5	Candelaria de Ftra, Santa Ana	707	14°06′56.04″, 89°38′56.94″
6	Candelaria, San José, Cancasque, Chalatenango	233	13°57′49.24″, 88°52′11.22″
7	Cerron Grande, Jutiapa, Cabañas	258	14°00′28.90″, 89°03′11.28″
8	Ciudad Delgado, San Salvador	487	13°46′16.59″, 89°09′34.06″
9	Cojutepeque, Cuscatlan	868	13°43′15.72″, 88°56′00.82″
10	Colima, Suchitoto, Cuscatlan	294	14°02′59.82″, 89°08′00.81″
11	Comalapa, San Juan Talpa, El Salvador	30	13°26′33.78″, 89°03′20.22″
12	Cton. Las anonas. Tecoluca, San Vicente	277	13°32′15.99″, 88°46′51.75″
13	El Gramal, San Antonio Los Ranchos, Chalatenango	497	13°59′59.35″, 88°54′59.73″
14	El Marquesado, San Vicente	252	13°34′15.67″, 88°42′20.27″
15	El Pedregal, San Pedro Masahuat, La Paz	20	13°28′06.34″, 89°00′55.36″
16	Ilobasco, Cabañas	690	13°50′29.81″, 88°51′34.73″
17	Jutiapa, Cabañas	413	13°53′14.53″, 88°54′04.37″
18	La Reina, Chalatenango	412	14°11′36.90″, 89°09′01.96″
19	Las Pilas, San Ignacio Chalatenango	1,085	14°20′28.95″, 89°10′21.79″
20	Matazano, Soyapango, San Salvador	640	13°41′21.63″, 89°08′26.29″
21	Mejicanos, San Salvador	716	13°43′47.27″, 89°12′37.49″
22	Merliot,Santa Tecla, La Libertad	890	13°40′43.02″, 89°16′02.57″
23	Nejapa, San Salvador	477	13°48′29.62″, 89°13′43.91″
24	Nueva Concepción, Chalatenango	323	14°07′35.82″, 89°17′05.63″
25	Parque Zoologico Nacional, San Salvador	655	13°41′04.09″, 89°11′42.88″
26	Potonico, Chalatenango	257	15°57′54.81″, 88°53′34.02″
27	Puerto de La Libertad,La Libertad	15	13°29′24.07″, 89°19′13.16″
28	Quezaltepeque, La Libertad	427	13°50′08.57″, 89°16′32.72″
29	San Fernando, Chalatenango	1,040	14°18′31.23″, 89°01′36.63″
30	San Isidro Cabañas	362	13°49′56.81″, 88°42′57.23″
31	San Isidro Labrador, Chalatenango	270	14°00′34.06″, 88°50′33.07″
32	San Isidro Lempa, San Pablo Tacachico, La Libertad	288	14°02′27.83″, 89°21′13.39″
33	San Juan Opico, La Libertad	424	13°51′28.39″, 89°21′40.48″
34	San Luis Talpa, La Paz	54	13°28′33.20″, 89°05′58.85″
35	San Martín, San Salvador	575	13°42′37.22″, 89°07′10.86″
36	San Miguel	128	13°27′37.44″, 88°10′58.19″
37	San Pablo Tacachico, La Libertad	314	13°38′13.44″, 89°20′28.90″
38	San Rafael, Chalatenango	362	14°08′04.56″, 89°01′40.52″
39	San Ramon, Sonsonate	432	13°44′27.76″, 89°39′03.47″
40	San Salvador	656	13°41′01.34″, 89°11′43.08″
41	San Salvador	758	13°40′36.34″, 89°12′25.57″
42	San Vicente, San Vicente	433	13°38′35.34″, 88°45′38.42″

Table 1 (continued)

Localities		Elevation (m a.s.l.)	Geographical coordinates (North, West)
No.	Name		
43	Santa Ana	691	13°58′25.84″, 89°32′44.52″
44	Santa Rosa, Santa Ana	436	14°12′20.81″, 89°21′06.75″
45	Santa Tecla, La Libertad	949	13°40′53.42″, 89°16′57.22″
46	Sonsonate	215	13°42′46.11″, 89°43′44.55″
47	Suchitoto, Cuscatlan	389	13°56′12.00″, 89°01′33.89″
48	Zacatecoluca, La Paz	164	13°30′23.96″, 88°51′51.18″

369-C^T from the USA (CP000087). Finally, ten *A. mixtum* (9 adults, 1 nymph), eight *A. parvum* (7 adults, 1 nymph), one *D. nitens* adult and one *Amblyomma* sp. nymph generated *gltA* (1054 bp) and *ompA* (588 bp) partial sequences that were 100% identical to *Rickettsia amblyommatis* from Panama (HM582435) and the type strain WB-8-2^T from the USA (CP003334), respectively.

Among the tick species tested by PCR, '*Ca.* R. colombianensi' was detected in 10% of the *A. dissimile* ticks and 11% of *A. scutatum*; *R. bellii* was detected in 3% of *A. dissimile* and 17% of *A. ovale*; and *R. amblyommatis* was detected in 77% of *A. mixtum*, 50% of *A. parvum*, 8% of *D. nitens*, and 11% of *Amblyomma* spp. nymphs (Table 4). No rickettsial DNA was detected in *A. sabanerae*, *D. dissimilis*, *R. microplus* and *R. sanguineus* s.l.

GenBank nucleotide sequence accession numbers for the partial mitochondrial 16S rDNA sequences obtained in the present study are MW369631 (*A. dissimile*), MW369632 (*A. parvum*), MW369633 (*A. scutatum*), MW369634 (*A. mixtum*), MW369635 (*A. ovale*), MW369636 (*D. nitens*), and MW384861 and MW384862 for partial sequences of '*Candidatus* R. colombianensi' (*gltA* and *ompA* genes, respectively), MW384863 and MW384864 for partial sequences of *R. amblyommatis* (*gltA* and *ompA* genes, respectively), and MW384865 for partial sequences of *R. bellii* (*gltA* gene).

Discussion

This study reports ten tick species and three *Rickettsia* species infecting ticks in El Salvador. Although the same tick specimens here evaluated were previously reported in a local journal by Navarrete-Abarca et al. (2014), these authors reported *Amblyomma auricularium* on *Dasypus novemcinctus* (armadillos), sheep (*Ovis aries*), and black iguana (*Ctenosaura similis*). Herein, the specimens on *D. novemcinctus*, *O. aries* and *C. similis* were classified as *A. parvum*, and no *A. auricularium* was identified. Navarrete-Abarca et al. (2014) had reported *A. parvum* only on *Herpailurus yaguarondi* (jaguarundi). Here, we confirmed by molecular analyses that the ticks collected on *D. novemcinctus* and *H. yaguarondi* were the same species, as they yielded the same 16S rDNA haplotype (Table 3); therefore, they were all classified as *A. parvum.* On the other hand, a recent phylogeographical study on *A. parvum* indicated that the specimens from Central America probably represent a taxon different from South American populations of *A. parvum* (Lado et al. 2016). For this reason, the *A. parvum* specimens from El Salvador should be provisionally classified as *Amblyomma* cf. *parvum* until further studies elucidate the taxonomic status of this taxon in Central

Table 2 Ticks (*M* males, *F* females, *N* nymphs, *L* larvae) according to host species and localities in El Salvador, during 2013

Tick species[a]	No. specimens	Host species (no. individuals)	Localities[b]
Amblyomma dissimile	5M	*Agkistrodon bilineatus* (1)	37
	7M, 5F	*Boa constrictor* (5)	4,25,26,40
	2M	*Crotalus durissus* (1)	14
	1M, 4F	*Ctenosaura similis* (2)	4,26
	24M, 3F, 1N[c]	*Iguana iguana* (6)	4,12,24,26,48
	5M, 7F	*Rhinella marina* (6)	4,17,25,26,40
	5M, 4F	*Rhinoclemmys pulcherrima* (8)	1,9,24,26
A. mixtum	1M, 1F, 1N[c]	*Bos taurus* (3)	3
	14M, 12F	*Equus caballus* (10)	4,6,16,18,19,29
A. ovale	2M, 3F	*Canis familiaris* (4)	20,35,40
	3F	*Capra hircus* (1)	43
	6M, 2F	*Urocyon cinereoargenteus* (1)	25
A. parvum	47M, 30F, 1N[c]	*Dasypus novemcinctus* (12)	4,10,14,17,24,26,36
	2M	*Herpailurus yaguarondi* (1)	11
	1M	*C. similis* (1)	10
	1F	*Ovis aries* (1)	8
A. sabanerae	1M	*B. constrictor* (1)	26
	30M, 6F	*R. pulcherrima* (15)	4,6,13,22,24–26,28,36,37
A. scutatum	77M, 45F	*C. similis* (17)	4,6,7,8,10,24,26,37,40,42
	1F	*R. marina* (2)	24
Dermacentor dissimilis	2M, 6F	*E. caballus* (2)	19,29
D. nitens	39M, 38F, 28N, 10L	*E. caballus* (13)	3,15,19,23,26,31,33,42,44
Rhipicephalus microplus	57M, 210F, 21N, 1L	*Bos taurus* (36)	2–5,16,19,23,24,26,30,32,34,37–39,48
	2M, 8F, 4N	*C. familiaris* (2)	6,24
	1M, 5F	*C. hircus* (1)	5
	4M, 12F	*E. caballus* (4)	24,26,39
	2M	*Homo sapiens* (2)	24,37
	1M, 2F	*Odocoileus virginianus* (2)	10
R. sanguineus	99M, 109F, 29N	*C. familiaris* (34)	4,9,16,20,21,24,26,27,30–33,36,37,40–42,45–48
	5M, 2F	*Canis latrans* (1)	40
	1N	*H. sapiens* (1)	37
	5M, 8F	Environment (4)	4,27,36,37

[a]In addition to the ten tick species in this table, 84N and 26L were morphologically identified only to genus level, and were retained as *Amblyomma* spp. These 84N were collected on *A. bilineatus* (locality no. 37, see Table 1), *B. constrictor* (24,26), *Bos taurus* (3), *D. novemcinctus* (4,10,24,36), *Didelphis virginiana* (26), *C. similis* (6,8,10,26), *E. caballus* (29), *H. sapiens* (6,40), *I. iguana* (6,24,48), *R. marina* (25), and *R. pulcherrima* (22,24); the 26L were collected on *C. similis* (8,40), *R. pulcherrima* (24) and *D. novemcinctus* (4)

[b]Numbers refer to localities in Table 1

[c]This nymph was identified to species through molecular analysis (see text)

Table 3 Data of the mitochondrial 16S rDNA partial sequences generated from 13 tick specimens from El Salvador in the present study

Tick species	Tick stage	Host species	Locality[a]	Closest identity in GenBank			
				% query cover	% identity	Tick species, Country	Accession nr
Amblyomma dissimile	Nymph	*Iguana iguana*	4	100	99.2	*A. dissimile*, Brazil	MG023155
	Male	*Rhinella marina*	26	100	99.2	*A. dissimile*, Brazil	MG023155
A. mixtum	Male	*Equus caballus*	18	100	100	*A. mixtum*, Ecuador	KT820359
	Nymph	*Bos taurus*	3	100	100	*A. mixtum*, Ecuador	KT820359
	Male	*E. caballus*	6	100	100	*A. mixtum*, Ecuador	KT820359
A. ovale	Female	*Capra hircus*	43	100	98.6	*A. ovale*, Belize	KU001157
A. parvum	Male	*Dasypus novemcinctus*	10	98	100	*A. parvum*, El Salvador	KT820314
	Nymph	*D. novemcinctus*	24	98	100	*A. parvum*, El Salvador	KT820314
	Male	*D. novemcinctus*	24	98	100	*A. parvum*, El Salvador	KT820314
	Female	*D. novemcinctus*	26	98	100	*A. parvum*, El Salvador	KT820314
	Male	*Herpailurus yaguarondi*	11	98	100	*A. parvum*, El Salvador	KT820314
A. scutatum	Female	*Ctenosaura similis*	37	100	96.1	*A. dissimile*, Panama[b]	MK026013
Dermacentor nitens	Male	*E. caballus*	19	100	100	*D. nitens*, Cuba	MN880396

[a]Numbers refer to localities in Table 1

[b]At the time of the present study, there was no 16S rDNA sequence of *A. scutatum* in GenBank

Table 4 Results of molecular analyses for rickettsial infection in ticks collected in El Salvador during 2013

Tick species	Total no. tested ticks	No. PCR-positive ticks for *Rickettsia* (%)	*Rickettsia* species identified by DNA sequencing	Localities of the *Rickettsia*-infected ticks[a]
Amblyomma dissimile	31	3 (10)	'*Candidatus* R. colombianensi'	4,12,26
		1 (3)	*R. bellii*	37
A. mixtum	13	10 (77)	*R. amblyommatis*	3,6,18,19,29
A. ovale	6	1 (17)	*R. bellii*	43
A. parvum	16	8 (50)	*R. amblyommatis*	10,11,14,24,26
A. sabanerae	16	0 (0)		
A. scutatum	18	2 (11)	'*Ca.* R. colombianensi'	37
Amblyomma spp.	9	1 (11)	*R. amblyommatis*	3
Dermacentor dissimilis	2	0 (0)		
D. nitens	13	1 (8)	*R. amblyommatis*	19
Rhipicephalus microplus	47	0 (0)		
R. sanguineus	40	0 (0)		
TOTAL	211	27 (12.8)		

[a]Numbers refer to localities in Table 1

America. We also confirmed by morphological and molecular analyses that the specimens reported as *Amblyomma cajennense* by Navarrete-Abarca et al. (2014) represents the taxon *A. mixtum*, which is the only representative of the *A. cajennense* species complex that has been reported in Central America (Nava et al., 2014).

All *Amblyomma* nymphs of the present study were reported as *Amblyomma* sp. by Navarrete-Abarca et al. (2014). Herein, we identified three of these nymphs by molecular analysis as *A. dissimile*, *A. mixtum* and *A.* cf. *parvum.* In addition, we generate for the first time a DNA sequence of the tick *A. scutatum.* The 16S rDNA partial sequence of *A. scutatum* was closest (96.1% identity) to *A. dissimile*, and second closest to *Amblyomma rotundatum* (93% to MG023149; data not shown). Our records of *A. scutatum* were mostly on the black iguana *C. similis*, and in a lesser extent on the cane toad *Rhinella marina*. In fact, *A. scutatum* is a tick species that has been reported mostly from ectothermic tetrapods, similarly to *A. dissimile* and *A. rotundatum* (Guglielmone et al. 2014).

The two *Dermacentor* species reported here, *D. nitens* and *D. dissimilis*, were collected exclusively from horses, which have been reported as major hosts for these tick species (Arthur 1960; Fairchild et al. 1966; Bermúdez et al. 2015). Interestingly, the present records of *D. dissimilis* were from two localities above 1000 m, whereas eight of the nine localities of our *D. nitens* records were < 510 m (Tables 1 and 2). These different altitudinal records agree with previous studies from other Central American countries, in which *D. nitens* was reported from areas < 500 m (Fairchild et al. 1966), and *D. dissimilis* from areas above 900 m (Bermúdez et al. 2015).

Based on DNA sequences of one or two rickettsial genes (*gltA* and *ompA*), we confirm the presence of three *Rickettsia* species in ticks from El Salvador: '*Ca.* R. colombianensi', *R. amblyommatis*, and *R. bellii*. These three agents were superficially reported by Navarrete-Abarca et al. (2014), who did not provide any DNA sequence or infection rates. Our findings of '*Ca.* R. colombianensi' in 10% of the *A. dissimile* ticks agrees with previous reports of this agent at variable infection rates among *A. dissimile* ticks in Colombia,

Honduras, Brazil, and Mexico (Miranda et al. 2012; Novakova et al. 2015; Ogrzewalska et al. 2019; Sánchez-Montes et al. 2019). Interestingly, a similar infection rate (11%) was detected in *A. scutatum* ticks; however, no other country has reported rickettsiae in *A. scutatum* ticks.

Our finding of *R. amblyommatis* at high infection rate (77%) in *A. mixtum* ticks agrees with several previous studies that have reported this rickettsia to be common in *A. mixtum* populations from Mexico, Costa Rica, Cuba, Honduras, Panama, and Colombia (Novakova et al. 2015; Bermúdez et al. 2016; Noda et al. 2016; Troyo et al. 2016; Merino et al. 2020). Similarly, our findings of *R. bellii* in 3% of the *A. dissimile* and in 17% of the *A. ovale* ticks is supported by previous studies that have reported this rickettsia infecting a great variety of neotropical ticks, including *A. dissimile* and *A. ovale* (Krawczak et al. 2018; Binetruy et al 2020). Until now, none of the three *Rickettsia* species detected in ticks in this study is known to cause human disease (Bermúdez and Troyo 2018). However, there has been serological evidence of human or animal exposure to *R. amblyommatis* and/or *R. bellii* (Delisle et al. 2016; Costa et al. 2017), suggesting that they might be at least transmitted to vertebrates during tick feeding. The significance of these findings for the ecology of tick-borne diseases in El Salvador remains to be investigated.

Previous studies reported the tick fauna of El Salvador to be represented by 12 species, being two argasids (*Ornithodoros dyeri* and *Ornithodoros yumatensis*) and ten ixodid species (*A. dissimile*, *A. cajennense*, *A. ovale*, *A. parvum*, *A. sabanerae*, *A. scutatum*, *D. dissimilis*, *D. nitens*, *R. microplus*, and *R. sanguineus* s.l.) (Guglielmone et al. 2003; Navarrete-Abarca et al. 2014; Bermúdez et al. 2015). Herein, we confirm the presence of ten ixodid species; however, we have reclassified *A. cajennense* as *A. mixtum*, and *A. parvum* as *A.* cf. *parvum.*

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