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Detection of *Rickettsia monacensis* and *Rickettsia amblyommatis* in ticks collected from dogs in Costa Rica and Nicaragua

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ABSTRACT

The neotropical climate of Central America provides ideal conditions for ticks, which may transmit several human pathogens, including spotted-fever group *Rickettsia*. Dogs may act as sentinels or reservoirs for human tick-borne diseases due to shared tick species. Here, ticks were collected from 680 client-owned dogs in Nicaragua and Costa Rica, and a total of 316 tick pools were investigated for *Rickettsia* infection by quantitative real-time PCR (qPCR) targeting the *gltA* gene. Subsequently, up to six further genomic targets (*16S* rDNA, *gltA*, *sca4*, *ompA*, *ompB* and the 23S-5S intergenic spacer) were investigated for *Rickettsia* species determination. The predominant tick species was *Rhipicephalus sanguineus* sensu lato (s.l.) (19.9% of dogs infested in Costa Rica, 48.0% in Nicaragua), followed by *Ixodes boliviensis* (3.1% in Costa Rica / none in Nicaragua) and *Amblyomma ovale* (4.8% in Costa Rica, 0.9% in Nicaragua). In total, 22 of 316 tick pools containing 60 of 1023 individual ticks were *Rickettsia*-positive as determined by qPCR, resulting in a minimum infection rate (MIR) of 2.2%. In detail, MIR in *Rh. sanguineus* s.l. was 0.7% (7/281 pools), in *I. boliviensis* 33.3% (12/13 pools) and in *A. ovale* 9.7% (3/22 pools). For 11 of 12 positive *I. boliviensis* pools and one of six positive *Rh. sanguineus* s.l. pools, the species could be determined as *R. monacensis*. *R. amblyommatis* was identified in one *Rh. sanguineus* s.l. pool from Costa Rica and one *A. ovale* pool from Nicaragua. Nine of 12 *R. monacensis*-positive tick pools were collected in San Rafael de Heredia, Costa Rica, indicating a high local occurrence in this area. This study supports recent evidence that *R. monacensis* is present on the American continent. Its high local occurrence among dog-associated *I. boliviensis*, which may also parasitize humans, in Costa Rica gives cause for concern, as *R. monacensis* is also pathogenic to humans.

1. Introduction

Rickettsioses are caused by worldwide distributed obligate intracellular bacteria of the genus *Rickettsia*, which are usually arthropod-associated. Members of the spotted-fever group (SFG) *Rickettsia*, which are mainly transmitted by ticks, may cause febrile disease in humans with symptoms ranging from mild to life-threatening (Parola et al., 2005). More than 20 species with known pathogenic potential for humans have been identified within this group to date, and multiple candidate species and subspecies have been proposed (Parola et al., 2013).

The neotropical climate of Central America provides ideal conditions for ticks. For example, more than 40 tick species are found in Panama, at least 15 of which parasitize humans (Esser et al., 2016). In contrast, only few tick-borne SFG *Rickettsia* with known pathogenic potential for humans have been detected in continental Central America so far. Until recently, *R. rickettsii*, the causative agent of Rocky Mountain spotted fever, was the only recognized agent of spotted fevers in Central America, responsible for cases in Mexico, Panama, Costa Rica (Hun et al., 2008; Tribaldos et al., 2011; Zavala-Castro et al., 2006) and presumably also Guatemala (Eremeeva et al., 2013). Recently, *Rickettsia* sp. strain Atlantic rainforest, which has caused cases of spotted fever in Brazil (Spolidorio et al., 2010), has been found in ticks in Belize (Lopes et al., 2016). *Rickettsia africae*, the causative agent of African tick-bite

Abbreviations: SFG, spotted-fever group; qPCR, quantitative real-time PCR; MIR, minimum infection rate

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fever, has been detected in ticks in Nicaragua (Vogel et al., 2018). Another pathogenic species, *Rickettsia parkeri*, has been reported to occur in ticks in Belize (Polsomboon et al., 2017). Moreover, *R. monacensis*, which may cause a Mediterranean spotted fever-like illness (Jado et al., 2007; Madeddu et al., 2012), has been identified in an *Ixodes boliviensis* specimen in Costa Rica (Campos-Calderón et al., 2016). Previously, the known distribution of this *Rickettsia* species, which was first isolated from *I. ricinus* in Germany (Simser et al., 2002), was restricted to Europe, northern Africa and Asia (Parola et al., 2013; Shin et al., 2013). However, this pathogen was recently also detected in a dog on the Cape Verde archipelago (Lauzi et al., 2016), and in an *Ixodes* sp. tick carried by a migratory songbird captured in Texas, United States of America (Cohen et al., 2015). These findings indicate a widespread geographic distribution and the capability of range-expansion via transport by migratory birds.

Additionally, several tick-borne *Rickettsia* with unknown or unclear pathogenic potential occur in continental Central America: *Rickettsia amblyommatis* (Karpathy et al., 2016) has been detected in ticks from Costa Rica, Panama, Belize, Nicaragua, Honduras and Mexico (Bermúdez et al., 2011; Hun et al., 2011; Novakova et al., 2015; Polsomboon et al., 2017; Sánchez-Montes et al., 2016; Vogel et al., 2018), and *R. bellii* has been found in ticks in Costa Rica, El Salvador and Panama (Barbieri et al., 2012; Ogrzewalska et al., 2015; Bermúdez Castillero and Troyo Rodríguez, 2018). Furthermore, an uncultivated *Rickettsia* sp. closely related to *R. monacensis*, named strain IbR/CRC (Troyo et al., 2014) as well as another candidate species, *Candidatus* Rickettsia nicoyana (Moreira-Soto et al., 2017) and a *Rickettsia* sp. related to *R. asemboensis* (Troyo et al., 2016) have been detected in Costa Rican ticks. Similarly, *Rickettsia* sp. strain Colombianensi has been described from ticks in Honduras, Colombia and northern Brazil (Luz et al., 2018; Miranda et al., 2012; Novakova et al., 2015) and *Rickettsia* sp. ARAGOI from Nicaragua (Vogel et al., 2018).

Dogs live in intimate contact to humans, and a study has shown that pet ownership is significantly correlated with the risk of tick encounters (Jones et al., 2017). In fact, 10 of 21 tick species reported to parasitize dogs in Panama have also been found to bite humans (Esser et al., 2016). Thus, dogs may serve as sentinels for human tick-borne diseases, and, furthermore, might constitute a reservoir for certain pathogens, including some species of *Rickettsia* (Levin et al., 2011). Indeed, a previous study conducted in Costa Rica has shown that dogs living in areas associated with human spotted-fever outbreaks were more likely to be SFG *Rickettsia* seropositive compared to other dogs (Moreira-Soto et al., 2016). In the present study, ticks collected from client-owned dogs in Nicaragua and Costa Rica were analyzed for *Rickettsia* spp. in order to evaluate the potential risk for humans.

2. Materials and methods

Research and sample export permits were obtained from the Nicaraguan and Costa Rican authorities, respectively (permit no. 28927 and 00101785). From September to December 2013, dogs were presented to the veterinarian for various reasons in seven different cities in western Nicaragua and at 23 different locations in Costa Rica (Fig. 1, Table 1). Almost all dogs were presented to the veterinarian by their owners. Dogs at animal shelters were only included at one location in Nicaragua (Managua) and one location in Costa Rica (San Rafael, Heredia). During clinical examinations of dogs, tick infestation was assessed visually and ticks (adults, nymphs and larvae) were collected into sterile tubes. Ticks were conserved in 70% ethanol and identified based on morphological characteristics according to the guide of Barros-Battesti et al. (2006). Infestation rate for each tick species was compared between Nicaragua and Costa Rica using X^2-tests or Fisher Exact tests when appropriate.

From each tick-infested dog, a maximum of 3 adult tick pools (each containing max. 5 adult ticks of the same species) were selected for further analyses. DNA was extracted from pooled ticks using the Nucleospin® 8 Blood Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) as described previously (Tappe and Strube, 2013). Each pool was tested for *Rickettsia* DNA using two different quantitative real-time PCRs (qPCRs). First, a qPCR targeting the citrate synthase (*gltA*) gene based on a primer-probe combination by Stenos et al. (2005) was carried out at the Institute for Parasitology, University of Veterinary Medicine Hannover, as described by Tappe and Strube (2013). Second, positive samples were sent to the Bundeswehr Institute of Microbiology and subjected to another qPCR targeting the *gltA* gene, as described by Wölfel et al. (2008). Only tick pools with a positive result in both assays were regarded as confirmed positives. These were subjected to up to seven additional conventional PCRs and subsequent sequencing in order to achieve a *Rickettsia* species determination. The investigated targets, published in previous protocols, included partial sequences of *ompA*, *ompB* and the 23S-5S intergenic spacer. For three samples, additional targets were investigated for possible sequence variation (*16S* ribosomal DNA, *gltA*, *sca4*; Table 2). Subsequent Sanger sequencing was conducted by an external contractor (GATC Biotech, Konstanz, Germany). Sequences were analyzed using BioEdit Alignment Editor Version 7.1.1 (Hall, 1999) and compared with sequences deposited in the GenBank database of the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). Species determination based on the percentage nucleotide identity at each locus followed the criteria of Fournier et al. (2003).

Minimum infection rates were calculated for each tick species by dividing the number of *Rickettsia*-positive tick pools by the total number of ticks represented in all tested tick pools.

3. Results

In total, 680 dogs were examined (329 in Nicaragua, 351 in Costa Rica), of which 638 were client-owned, whereas 12 dogs from Managua, Nicaragua, and 30 dogs from San Rafael, Heredia, Costa Rica, were sampled at animal shelters. Ticks were found on 37.4% of the examined dogs, with significantly lower infestation prevalences in Costa Rica than in Nicaragua, as detailed below. A total of 1023 adult ticks were collected from 159 dogs in Nicaragua and 96 dogs in Costa Rica. The predominant tick species was *Rhipicephalus sanguineus* s.l. (956 adult specimens, 19.9% of dogs infested in Costa Rica, 48.0% in Nicaragua [X^2-test, $P < 0.001$]), followed by *I. boliviensis* (36 adult specimens, 3.1% of dogs infested in Costa Rica, none in Nicaragua [Fisher Exact test, $P < 0.001$]) and *Amblyomma ovale* (31 adult specimens, 4.8% in Costa Rica, 0.9% in Nicaragua [Fisher Exact test, $P = 0.002$]).

In total, 22/316 tick pools (containing 60 of the total 1023 individual ticks) originating from 20 different dogs were *Rickettsia*-positive as determined by qPCR, resulting in an overall MIR of 2.2%. In detail, 7/281 *Rh. sanguineus* s.l. pools, 12/13 *I. boliviensis* pools and 3/22 *A. ovale* pools were *Rickettsia*-positive. The corresponding MIRs were 0.7%, 33.3% and 9.7%, respectively. The distribution of tick species, numbers of *Rickettsia*-positive pools and MIRs per country are presented in Table 3.

For 11 of 12 positive *I. boliviensis* pools and one of six positive *Rh. sanguineus* s.l. pools, the species could be determined as *R. monacensis*, based on evaluation of up to six genetic loci as shown in Table 4. A comparison to recently described rickettsiae from Central American *Ixodes* ticks (e.g. *Rickettsia* sp. IbR-CRC1, *Rickettsia* sp. Barva1) was only possible for the partial *gltA*-gene due to different genomic regions of the used targets. In particular, the comparison of the partial *gltA*-gene showed identities ranging from 98.3 to 100% to these strains, as well as 100% identity to the *R. monacensis* reference strain IrR/Munich (*Rickettsia* sp. IbR-CRC1: KJ507211–KJ507214: 100%, KJ507215: 99.1%, KJ507216: 98.3%; uncultured *Rickettsia* sp. [KU001172]: 100%; *Rickettsia* sp. Barva1 [KF702332]: 100%; reference strain IrR/Munich [LN794217.1]: 100%). Nine of the *R. monacensis*-positive pools were

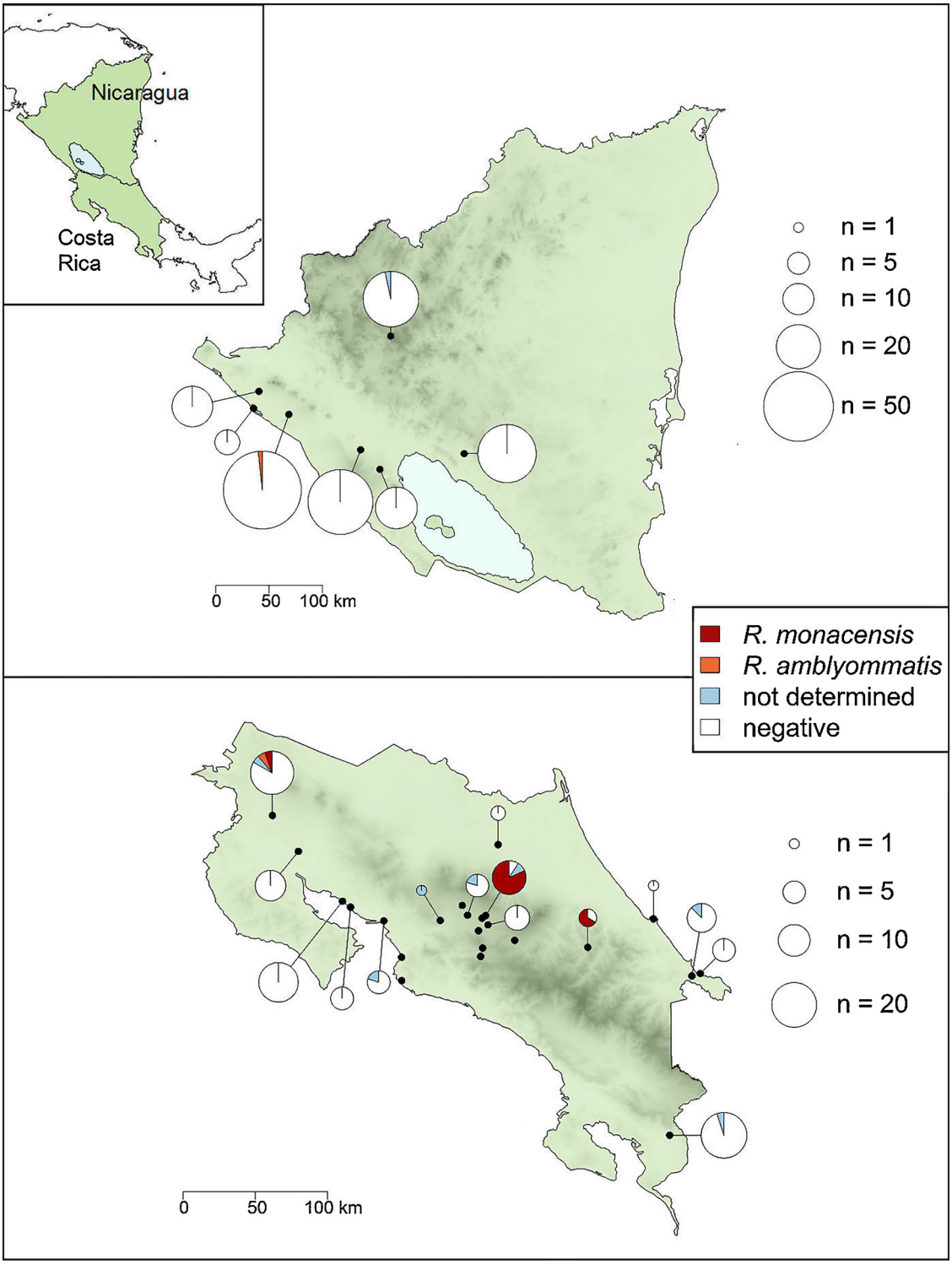

Fig. 1. Proportion of *Rickettsia*-infected tick pools and results of *Rickettsia* species determination per sampling site in Nicaragua (top panel) and Costa Rica (bottom panel). Pie chart size is proportional to the number of tick pools analyzed per site. Sampling sites where no ticks were found are indicated by black dots without pie charts. Not determined = qPCR positive for *Rickettsia* spp., but species identification unsuccessful.

collected in San Rafael, province Heredia, Costa Rica, and two in the village of Grano de Oro, province Cartago, Costa Rica. *Rickettsia amblyommatis* was identified in one of 18 *Rh. sanguineus* s.l. pools from Liberia, province Guanacaste, Costa Rica, and in one of two *A. ovale* specimens from the city of Léon, province Léon, Nicaragua (Fig. 1). For the remaining nine positive pools, determination of the *Rickettsia* species was not successful due to low DNA concentration.

4. Discussion

In this study, tick parasitism of client-owned dogs in Costa Rica and Nicaragua was assessed and collected ticks were screened for *Rickettsia* infection. Almost 40% of dogs carried ticks, predominantly *Rh. sanguineus* s.l. In addition, *I. boliviensis* and *A. ovale* were found. All three tick species also parasitize humans and have been reported as the most

Table 1
Tick infestation of dogs and tick species collected at the different sampling locations in Costa Rica and Nicaragua.

	Province	Location	Altitude	Tick-infested dogs/examined dogs (%)	Ticks subjected to further analyses (no. of ticks/no. of pools [species])
Nicaragua	Chinandega	Corinto	10 m	5/24 (20.8%)	25/6 [*Rh. sanguineus*]
		Chinandega	70 m	14/31 (45.2%)	44/16 [*Rh. sanguineus*]
	León	León	86 m	40/55 (72.7%)	213/58 [*Rh. sanguineus*], 2/2 [*A. ovale*]
	Managua	Managua	100 m	36/83 (43.4%)	123/41 [*Rh. sanguineus*]
	Masaya	Masaya	234 m	14/36 (38.9%)	70/17 [*Rh. sanguineus*]
	Jinotega	Jinotega	1000 m	23/50 (46%)	110/29 [*Rh. sanguineus*], 1/1 [*A. ovale*]
	Chontales	Juigalpa	115 m	27/50 (54%)	115/33 [*Rh. sanguineus*]
Costa Rica	San José	Tibás	1150 m	4/13 (30.8%)	26/6 [*Rh. sanguineus*]
		Jorco	1300 m	0/2 (0%)	–
		Escazú	1090 m	0/2 (0%)	–
		Tarbaca	1700 m	0/9 (0%)	–
	Alajuela	Alajuela City	950 m	5/11 (45.5%)	15/5 [*Rh. sanguineus*]
		Atenas	692 m	1/8 (12.5%)	1/1 [*A. ovale*]
		Poás	1150 m	0/2 (0%)	–
	Cartago	Cartago City	1430 m	0/20 (0%)	–
		Grano de Oro	1115 m	2/2 (100%)	3/2 [*I. boliviensis*], 1/1 [*A. ovale*]
	Heredia	Heredia City	1140 m	0/3 (0%)	–
		Sarapiquí	40 m	2/11 (18.2%)	4/2 [*A. ovale*]
		San Rafael	1260 m	9/30 (30%)	33711 [*I. boliviensis*]
	Guanacaste	Liberia	150 m	17/62 (27.4%)	43/18 [*Rh. sanguineus*]
		Bagatsí	20 m	7/8 (87.5%)	40/9 [*Rh. sanguineus*]
	Puntarenas	Tárcoles	10 m	0/3 (0%)	–
		Jacó Beach	10 m	0/1 (0%)	–
		El Roble	13 m	4/16 (25%)	18/5 [*Rh. sanguineus*]
		Punta Morales	17 m	3/22 (13.6%)	22/5 [*Rh. sanguineus*]
		Ciudad Neily	54 m	15/41 (36.6%)	37/11 [*Rh. sanguineus*], 12/9 [*A. ovale*]
		Costa de Pajarós	20 m	13/37 (35.15)	36/15 [*Rh. sanguineus*]
	Limón	Limón City	5 m	1/5 (20%)	2/1 [*Rh. sanguineus*]
		Limón Puerto Viejo	5 m	5/22 (22.7%)	13/5 [*Rh. sanguineus*]
		Kéköldi	100 m	8/21 (38.1%)	5/2 [*Rh. sanguineus*], 10/6 [*A. ovale*]

Rh. sanguineus = *Rhipicephalus sanguineus*, *A. ovale* = *Amblyomma ovale*, *I. boliviensis* = *Ixodes boliviensis*.

common dog-associated ticks in Central America (Esser et al., 2016; Troyo et al., 2012).

In total, at least 2.2% of individual ticks were positive for DNA of *Rickettsia* spp. Depending on the *Rickettsia* sp., ticks may become infected when feeding on an infected host. Thus, since ticks were collected directly from dogs, the presence of rickettsial DNA might have been due to infected blood ingested by these ticks, and not necessarily due to pre-existing infection of the unfed ticks. However, this is highly unlikely as blood samples from three dogs which carried positive ticks were tested for *Rickettsia* DNA by the same *gltA*-qPCR as the tick samples with a negative result, i.e. no measurable cycles threshold (Ct)-values, whereas Ct-values of positive ticks ranged between 18.67 and 39.99 (median: 35.86; unpublished results).

Rhipicephalus sanguineus s.l., found on approximately one third of all dogs, showed a low *Rickettsia* MIR (0.7%), which is in line with results of previous studies in Costa Rica (Troyo et al., 2016) and Argentina (Cicuttin et al., 2014). In contrast, 12.3% of adult *Rh. sanguineus* s.l. specimens were tested *Rickettsia*-positive in an urban setting in Panama (Bermúdez et al., 2011), and approximately 30% tested positive in Mexicali, Mexico, in an investigation following an outbreak of Rocky Mountain spotted fever in this area (Eremeeva et al., 2011). However, in these studies adult ticks were analyzed individually rather than in pools, which may contribute to the observed difference. Still, it is possible that occurrence varies on a local scale, and hyperendemic foci of *Rickettsia*-positive *Rh. sanguineus* s.l. exist (Parola et al., 2013). In this study, local occurrence varied between 0 and 25% of tested *Rh. sanguineus* s.l. pools; however, sample size varied considerably between sites, ranging from 1 to 59 tick pools. For the majority of sites, larger sample sizes would be necessary to accurately estimate local occurrences.

In contrast to *Rh. sanguineus* s.l., a high *Rickettsia* MIR of 33.3% was found for *I. boliviensis*. These infections could almost exclusively be assigned to *R. monacensis*. Another *Rickettsia* sp., closely related to *R. monacensis*, named strain IbR/CRC (Troyo et al., 2014) as well as two genetically similar, unnamed *Rickettsia* sp. (Lopes et al., 2016; Ogrzewalska et al., 2015) have been detected in *Ixodes* ticks in Central America. However, in the present study, even the comparison of the conserved genomic region of partial *gltA* revealed differences in some of the IbR/CRC strains mentioned above, while the similarity of the obtained *gltA* sequences to the reference strain IrR/Munich was 100%. Therefore, there is no indication that the sequences found in this study should be related more closely to the IbR/CRC strains than to *R. monacensis*. Moreover, criteria to propose a new candidatus species as published by Fournier et al. (2003) were not met. Thus, our findings confirm a recent report of *R. monacensis* occurrence in the province of Heredia, Costa Rica (Campos-Calderón et al., 2016), whereas neither *R. monacensis* nor *I. boliviensis* were found in the neighbouring country of Nicaragua. *Ixodes boliviensis* is known to occur at higher altitudes and in more rural areas than *Rh. sanguineus* s.l. (Bermúdez and Miranda, 2011). Here, all sampling locations in Nicaragua were situated at low altitudes (max. 1000 m above sea level [asl]), so that the absence of *I. boliviensis* from these localities was not surprising. The Costa Rican *I. boliviensis* specimens were collected at only two different locations, namely in San Rafael de Heredia, which belongs to the Greater Metropolitan Area of Costa Rica, at approximately 1200 m asl, and in the remote village of Grano de Oro, province Cartago, at approximately 1115 m asl. Nine of 11 *R. monacensis*-positive pools were collected in San Rafael de Heredia which indicates that this area may represent a local “hotspot” of *R. monacensis*-positive *I. boliviensis*, similar to the hyperendemic foci of *R. rickettsii*-infected *Rh. sanguineus* s.l. in Mexico described above (Parola et al., 2013). In Italy, a high *R. monacensis*-MIR of 27.5% in *I. ricinus* has been described (Scarpulla et al., 2016), while MIRs in other European countries are usually below 2% (Biernat et al., 2016; Eshoo et al., 2014; Lommano et al., 2012).

Rickettsia monacensis has most often been found in ticks of the genus *Ixodes* (e.g. Benredjem et al., 2014; Schreiber et al., 2014; Shin et al., 2013), but occurrence in *Rh. sanguineus* s.l. has also recently been reported (Estrada-Peña et al., 2017; Pennisi et al., 2015). Here, *R.*

Table 2
Primers and probes used for molecular investigation of rickettsiae.

Target (partial)	Primers and probes	Product size	Reference
real-time PCR			
gltA	CS-F: 5'-TCGCAAATGTTCACGGTA-3' CS-R: 5'-TCGTGCATTTCTTTCCATTGTG-3' CS-P: 5'-6-FAM-TGCAATAGCAAGAACCGTAGGCTGGATG-BHQ-3'	74bp	Stenos et al. (2005)
gltA	PanRick_gltA_2_for: 5'-ATAGGACAACCGTTTATTT-3' PanRick_gltA_2_rev: 5'-CAAACATCATATGCAGAAA-3' PanRick_3_taq: 5'-6FAM-CCTGATAATTCGTTAGATTTTACCG-TMR-3'	70bp	Wölfel et al. (2008)
conventional PCR			
gltA	RH314: 5'-AAACAGGTTGCTCATCATTC-3' RH654: 5'-AGAGCATTTTTTATTATTGG-3'	321bp	Nilsson et al. (1999)
16S rDNA	Ric: 5'-TCTAGAACGAACGCTATCGGTAT-3' RicRt: 5'-TTTCATCGTTTAACGGCGTGGACT-3'	757bp	Nilsson et al. (1997)
sca4	Rsca4_1707f: 5'- CTCTGAATTAAGCAATGCGG-3' Rsca4_2837r: 5'- CCTgATACTACCCTTACATC-3'	1130bp	Matsumoto and Inokuma (2009)
ompA II	RR-3588: 5'-AACAgTgAATgTAggAgCAg-3' cRR-4406: 5'-ACTATACCCTCATCgTCATT-3'	818bp	Fournier et al. (1998)
ompA IV	RR-5125: 5'-gCggTTACTTTAgCCAAAgG-3' cRR-6013: 5'-TCTTCTgCgTTgCATTACCg-3'	888bp	Fournier et al. (1998)
ompB	120-2788: 5'- AAACAATAATCAAGGTACTGT-3' 120-3599: 5'-TACTTCCGGTTACAGCAAAGT-3'	811bp	Roux and Raoult (2000)
23S-5S intergenic spacer	Rick 23 s for: 5´- GATAGGTCGGGTGTGGAAGCAC Rick 23 s rev: 5´- GGGATGGGATCGTGTGTTTCAC	300-550bp (species-dependent)	Chitimia-Dobler et al. (2018)

monacensis was identified based on 99% identity in the *ompAIV* locus in a single *Rh. sanguineus* s.l. pool from Costa Rica, which was collected in a different region of the country than the *R. monacensis*-positive *I. boliviensis* pools. The high occurrence in *I. boliviensis* pools confirms the predominant association of this *Rickettsia* species with the genus *Ixodes*, but occurrence in *Rh. sanguineus* s.l. illustrates that the range of this pathogen is not necessarily restricted to suitable habitats for *Ixodes* ticks.

Outbreaks of spotted fever have occurred in various locations in Costa Rica, usually to the north of the central Costa Rican mountain range and on the Caribbean coast (Hun et al., 2008). However, in the recent past autochthonous cases have also occurred in the densely populated Greater Metropolitan Area (Argüello et al., 2012). All cases described so far have been attributed to infection with *R. rickettsii* (Argüello et al., 2012; Hun et al., 2008), while autochthonous human *R. monacensis* infections have not been described on the American continent so far. *Rickettsia rickettsii* was not identified in this study, although *Rh. sanguineus* s.l., the most abundant tick species in our sample, is regarded as an epidemiologically important vector for this species in Central America (Parola et al., 2013). However, *Rickettsia* species determination was only successful for one of seven positive *Rh. sanguineus* s.l. pools, so it remains possible that *R. rickettsii* was present among the samples, but not identified.

The detected MIR of *Rickettsia* spp. in *A. ovale* (9.7%) is comparable

Table 3
Distribution of tick species, *Rickettsia*-positive tick pools and minimum infection rates (MIR) in Costa Rica and Nicaragua. n. d. = no differentiation possible.

	Costa Rica				Nicaragua			
Tick species	Infested dogs	*Rickettsia*-positive tick pools	MIR	Identified *Rickettsia* sp.	Infested dogs	*Rickettsia*-positive tick pools	MIR	Identified *Rickettsia* sp.
Rhipicephalus sanguineus	70/351 (19.9%)	6/82 (7.3%)	2.3%	*R. amblyommatis* (1/6 pools), *R. monacensis* (1/6 pools)	158/329 (48.0%)	1/199 (0.5%)	0.1%	n. d.
Ixodes boliviensis	11/351 (3.1%)	12/13 (92.3%)	33.3%	*R. monacensis* (11/12 pools)	0/329 (0.0%)	–	–	–
Amblyomma ovale	17/351 (4.8%)	2/19 (10.5%)	7.1%	n. d.	3/329 (0.9%)	1/3 (33.3%)	33.3%	*R. amblyommatis* (1/1 pool)
Total	96/351 (27.3%)	20/114 (17.5%)	6.2%		159/329 (48.3%)	2/202 (1.0%)	0.3%	

Table 4
Results of successful multi-locus sequencing of rickettsial DNA amplified from ticks collected in Nicaragua and Costa Rica. Percentage nucleotide identity as determined by NCBI BLAST analysis in comparison to reference strains CP01012 *R. amblyommatis* An13 and LN794217 *R. monacensis* IrR/Munich is indicated in brackets. CR = Costa Rica; N = Nicaragua; neg = negative; nd = not done.

			Closest identity in GenBank						
Sample	Sampling location	Tick host	23S-5S	*ompAII*	*ompAIV*	*ompB*	*16S* rDNA	*sca4*	*gltA*
N55	Léon, N	*A. ovale*	neg	neg	*R. amblyommatis* (99.9%)	neg	nd	nd	nd
C86	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.6%)	neg	neg	neg	nd	nd	nd
C87	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.3%)	neg	*R. monacensis* (99.0%)	*R. monacensis* (99.5%)	nd	nd	nd
C88	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.8%)	neg	*R. monacensis* (99.0%)	neg	nd	nd	nd
C89	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.6%)	*R. monacensis* (99.5%)	*R. monacensis* (99.0%)	*R. monacensis* (99.5%)	nd	nd	nd
C90	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.6%)	neg	*R. monacensis* (99.0%)	neg	nd	nd	nd
C91	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.8%)	*R. monacensis* (99.5%)	*R. monacensis* (99.0%)	*R. monacensis* (99.5%)	*R. monacensis* (99.4%)	*R. monacensis* (99.4%)	*R. monacensis* (100%)
C92	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.6%)	neg	*R. monacensis* (99.0%)	neg	nd	nd	nd
C93	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.6%)	neg	*R. monacensis* (99.0%)	*R. monacensis* (99.5%)	nd	nd	nd
C95	San Rafael, Heredia, CR	*I. boliviensis*	*R. monacensis* (98.3%)	neg	*R. monacensis* (99.0%)	neg	nd	nd	nd
C110	Liberia, Guanacaste, CR	*Rh. sanguineus*	*R. amblyommatis* (99.4%)	*R. amblyommatis* (100%)	*R. amblyommatis* (100%)	*R. amblyommatis* (99.7%)	nd	nd	nd
C113	Liberia, Guanacaste, CR	*Rh. sanguineus*	neg	neg	*R. monacensis* (99.0%)	neg	nd	nd	nd
C115	Grano de Oro, Cartago, CR	*I. boliviensis*	*R. monacensis* (98.3%)	*R. monacensis* (99.5%)	*R. monacensis* (99.0%)	*R. monacensis* (99.5%)	*R. monacensis* (99.4%)	*R. monacensis* (99.4%)	*R. monacensis* (100%)
C116	Grano de Oro, Cartago, CR	*I. boliviensis*	*R. monacensis* (98.3%)	*R. monacensis* (99.5%)	*R. monacensis* (99.0%)	*R. monacensis* (99.5%)	*R. monacensis* (99.4%)	*R. monacensis* (99.4%)	*R. monacensis* (100%)

to results of a previous study conducted in Costa Rica, which reported a minimum *Rickettsia* infection rate of 9.3% for this tick species (Troyo et al., 2016). *Rickettsia* species previously detected in *A. ovale* in Central America include *R. amblyommatis* and *R. asemboensis* (Troyo et al., 2016), *R. africae* (Vogel et al., 2018) and an unnamed *Rickettsia* species (Lopes et al., 2016). Here, species determination of *Rickettsia* was only successful for one *A. ovale* pool, and revealed the presence of *R. amblyommatis*. Additionally, this species was identified in one *Rh. sanguineus* s.l. pool. *Rickettsia amblyommatis* has been identified in a variety of tick species in Central America (Bermúdez et al., 2011; Lopes et al., 2016; Ogrzewalska et al., 2015; Vogel et al., 2018), and was the most common *Rickettsia* species identified in a previous study in Costa Rica, which tested ticks collected from various hosts (Troyo et al., 2016). In the study by Troyo et al. (2016), the prevalence of *R. amblyommatis* was especially high in *Amblyomma mixtum*; however, this tick species was not present in our study, which might explain the discrepancy in *R. amblyommatis* prevalence between both studies.

The pathogenic potential of *R. amblyommatis* remains unclear, as it has not been isolated from any clinical human specimen (Parola et al., 2013). Nevertheless, it has been suggested as a potential cause of tick bite rash in humans (Billeter et al., 2007), and its involvement in a spotted-fever outbreak in the United States of America, based on epidemiological data, was considered likely (Apperson et al., 2008). Furthermore, *R. amblyommatis* may be of epidemiological importance as it potentially confers cross-protective immunity against *R. rickettsii* (Rivas et al., 2015).

In contrast to previous studies conducted in Costa Rica, we did not identify *R. bellii* in the present study. However, in Central America *R. bellii* has so far only been found in *Amblyomma sabanerae* collected from birds and reptiles (Barbieri et al., 2012; Ogrzewalska et al., 2015; Troyo et al., 2016). This tick species was not present in our sample and, to our knowledge, has not been reported to parasitize dogs.

5. Conclusions

This study supports recent evidence that *R. monacensis* occurs in ticks on the American continent. The high local occurrence among dog-associated *I. boliviensis* in Costa Rica gives cause for concern from a public health perspective, as *R. monacensis* may also infect humans. In contrast, *R. rickettsii*, the causative agent of previous spotted fever outbreaks in Central America, has not been identified in this study.

Whether or not dogs are an effective reservoir for the detected *Rickettsia* spp. is unknown and should be the subject of future research. Likewise, it remains unknown whether *R. monacensis* and *R. amblyommatis* are able to cause clinical symptoms in dogs. Nevertheless, pet owners should be made aware of the risk associated with tick parasitism, and the possibility of reducing their own exposure by controlling tick infestation of their dogs.

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