Ticks and Tick-borne Diseases 10 (2019) 830–837

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

Hard tick (Acari: Ixodidae) survey of Oleoducto trail, Soberania National Park, Panama

Lillian Domínguez[a], Roberto J. Miranda[a], Sugeys Torres[a], Ricardo Moreno[b,c], Josué Ortega[b,c], Sergio E. Bermúdez[a,*]

[a] Departamento de Investigación en Entomología Médica, Instituto Conmemorativo Gorgas de Estudios de la Salud, Panama
[b] Fundación Yaguará Panamá, Panama
[c] Smithsonian Tropical Research Institute, Panama

ARTICLE INFO

Keywords:
Tick
Hosts
Beta diversity
Rainforest
Panama

ABSTRACT

Hard tick diversity was determined along the Oleoducto trail (OT), Soberania National Park, from February 2013 to September 2014. Ticks were surveyed at four sites of 500 m^2 each and with increasing forest cover gradient and decreasing disturbance. Tick collections were made by dragging and flagging vegetation, and traps and mist nets were used to capture mammals and birds. Animals confiscated from poachers were also examined. To obtain information about potential hosts along the trail, 20 camera traps were used. 1536 ticks were collected, representing 20 species; of these, 1089 were questing ticks (10 species) collected on flags. We examined 143 birds (30 species) and 59 mammals (10 species), of which 40 birds and 36 mammals had ticks. Site 1 presented the lowest number of species and also the lowest number of potential hosts. Artiodactyls were the most frequent mammals photographed in camera traps, and ticks that parasitize these animals were among the most abundant in sites 2–4. Of these, *Haemaphysalis juxtakochi* was the most abundant species. Differences among sites were consistent with the gradient of forest cover, disturbance along OT and distribution of potential hosts.

1. Introduction

Ticks are hematophagous mites that parasite all orders of terrestrial vertebrates, including semi aquatic vertebrates and sea birds (Guglielmone et al., 2014; Muñoz-Leal and González-Acuña, 2015). In general, their presence in a given area depends on suitable climatic and microclimatic environmental conditions, as well the presence of appropriate hosts (Guerra et al., 2002; Brunner and Ostfeld, 2008). In regards to the environment, since ticks are intermittent parasites that spend much of their life cycle off their hosts, vegetation constitutes an important factor that determines microclimatic conditions and refuge (Szabó et al., 2007; Alessio et al., 2012). Host specificity of ticks varies between species, and may be influenced by the sex or behavior of vertebrate hosts (Fairchild et al., 1966).

The relationship between ticks, vertebrates and their environment is of interest from the point of view of natural history. However, major studies drafted to understand these interactions are driven by the ability of the ticks to transmit pathogens to domestic animals and humans (Hoogstraal, 1985). In fact, tick-borne diseases (TBD) are among the most relevant zoonoses worldwide (Ogrzewalska et al., 2011). Therefore, in order to establish plans to prevent TBD, it is necessary to understand how many different ticks species are distributed in different regions; which species of vertebrates are reservoirs of pathogens, and determine which factors could favor the presence of tick vectors, in both wooded areas for eco-tourism activities and rural and urban environments (Randolph, 2004; Szabó et al., 2009; Dobson et al., 2011). While several studies of this kind have been undertaken in temperate regions, few similar studies have been published in tropical regions, especially in the Neotropics.

In the present survey we determined the composition of hard tick species along Oleoducto trail (OT), Soberania National Park (SNP) in Panama. This protected area represents one of the most important areas in terms of biodiversity in the country, and is used by people for various types of outdoor activities, including scientific research, bird watching, biking and trekking (Martínez, 2008; Bermúdez et al., 2012).

2. Material and methods

2.1. Sites

Soberania National Park (SNP) is located along the east bank of the Panama Canal basin (Fig. 1). The climate in SNP is equatorial and low

* Corresponding author at: Avenida Justo Arosemena y Calle 35, 0816-02593, Panama.
E-mail addresses: bermudezsec@gmail.com, sbermúdez@gorgas.gob.pa (S.E. Bermúdez).

https://doi.org/10.1016/j.ttbdis.2019.04.001
Received 16 November 2018; Received in revised form 28 March 2019; Accepted 3 April 2019
Available online 09 April 2019

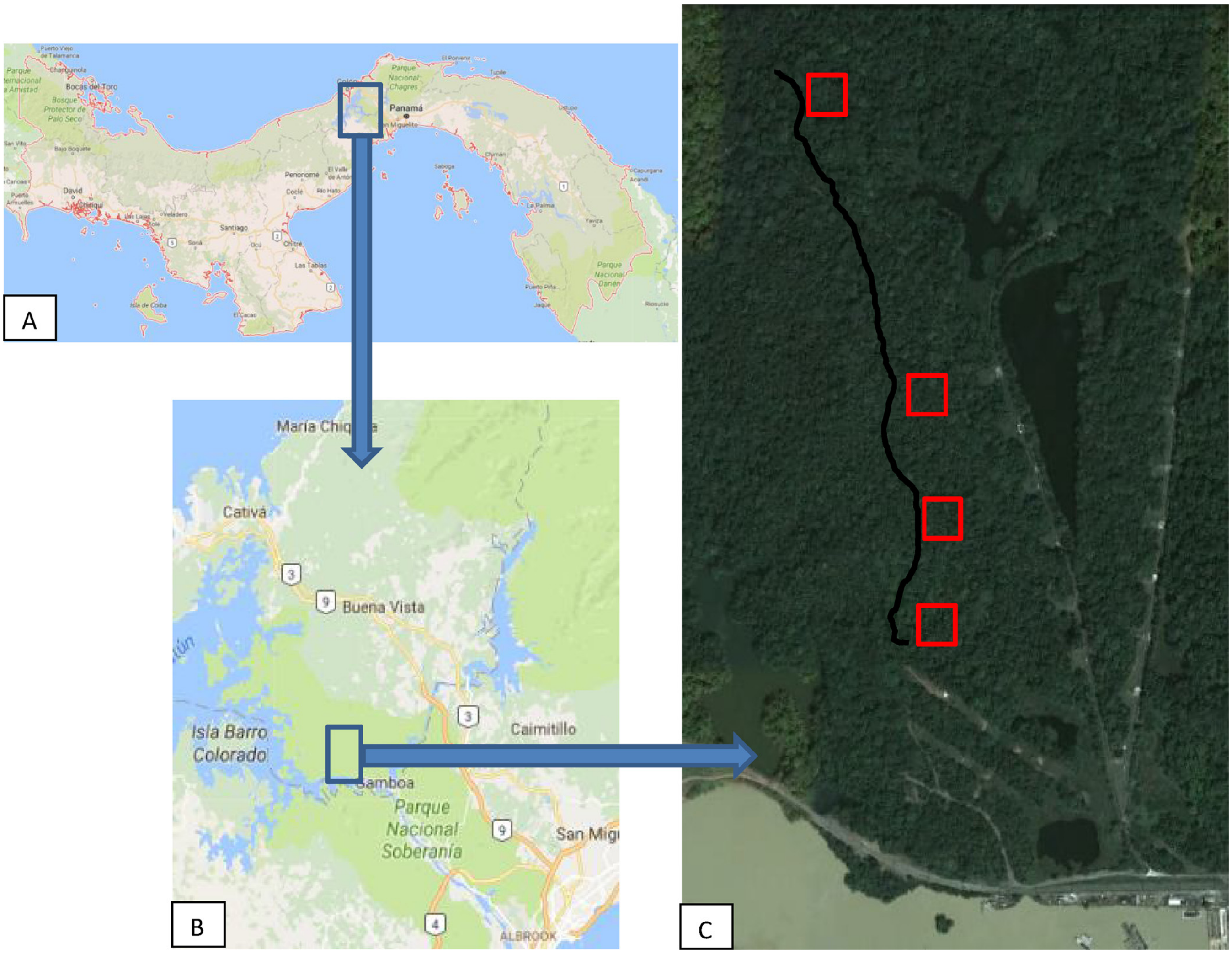

Fig. 1. Localization of sampling sites. A. General localization in Panama. B. Soberanía National Park. C. Sites along Oleoducto Trail.

elevation (≤ 300 m) with the rainy season extending from May to December, and the dry season from January-April; with 2500 mm of average of rain per year. This region presents temperatures with an average of 28 °C, and maximum and minimum temperatures of 35–20 °C, respectively; average of relative humidity is 60% in dry season and 98% in rainy season. High biodiversity has been reported in SNP, including more than 100 species of mammals, 525 species of birds, and close to 91 species of reptiles and amphibians (Miambiente, 2011).

Oleoducto trail (OT) extends for 17 km and is a continuous trail that is wooded on both sites. The physiognomy of this road is characterized by a tropical rainforest in a gradient of forest cover, which varies from mature forest to shrubs along the borders. Vegetation was previously described by Martínez (2008), who reported a canopy height varying between 10–35 meters.

2.2. Sample units

The study was conducted from February 2013 to September 2014 and the samples were obtained over 5 days each month. To determine the composition of the tick fauna, four plots of 500 m^2 were selected and characterized according to different types of forest cover. Each plot was distributed as follows: site 1 was 20 m before the entrance of OT and presented an area of low trees and shrubs separated from the other sites by a patch of 200 m; site 2 was 0.2 km from the entrance of OT and constituted a secondary forest; site 3 was 2 km of the entrance and was composed of secondary forest crossed by a creek; site 4 was 9 km from the entrance and constituted secondary-primary transition forests, and also was crossed by a creek.

2.3. Tick sampling

For each site, we collected ticks, both free-living and parasitic phases. Questing ticks were collected using a white cloth (45 x 45 cm) that was dragged along the leaf-litter and vegetation inside each plot; in addition, to collect ticks that did not attach to the cloth, both sides of leaves of the plants were visually checked. These collections were made between 7:00 and 11:00 am. Adults were fixed and preserved in 95% ethanol, whereas immature stages were kept alive in a controlled environmental incubator (78% RH, 26 °C). In the laboratory (Medical Entomology Department of Gorgas Memorial Institute for Health Research), larvae and nymphs were allowed to feed on guinea pigs to facilitate their molting (protocol 2015/03 of Institutional Animal Use and Care Committee of the Gorgas Memorial Institute). Bred nymphs were then allowed to feed again on guinea pigs to reach the adult stage.

2.4. Trapping hosts

In each plot, mammals and birds we captured using traps and mist nets, respectively. 35 Sherman and seven Tomahawk traps were set in linear transects and baited with a mixture of oats and honey or banana and cat food, respectively. We followed the protocol described by Bermúdez et al. (2010) for the trapping, handling and collecting of ticks of wild mammals. Two mist nets were used to capture birds, which were identified using Angehr and Dean (2010). Mammals and birds were released once the ticks were removed. Reptiles and toads were also examined if they were found in the plots. This study was approved by the Ministry of Environment (Approval No. SC/A-12-13).

Additional data on tick-host associations were obtained from mammals seized from poachers. Engorged female and immature ticks were kept alive and reared in the laboratory, similar to the methods applied to questing ticks. Information of potential hosts was complemented with data from 20 cameras-traps located throughout the OT. The methods used for these camera-traps (location, hours) were previously described by Meyer et al. (2015). All mammals (captured and in photographs) were identified using the guide of Reid (2009).

2.5. Tick identification

Adult ticks were identified morphologically using the taxonomic keys of Fairchild et al. (1966) and Bermúdez et al. (2018). The last key follows the taxonomic criteria proposed by Nava et al. (2014) for the designation of *Amblyomma mixtum* within the *Amblyomma cajennense* species group, and the proposal of Lopes et al. (2016) to denominate *Amblyomma* cf. *oblongoguttatum*, as a tentative different species from *A. oblongoguttatum* from Brazil. Some nymphs of *Amblyomma* were identified using the descriptions of Martins et al. (2010).

2.6. Data analysis

For each site, the species richness of ticks (S) and Simpson´s dominance index (D) were estimated. In addition, the Whitaker beta diversity index was applied to determine the continuity of the tick community among the 4 plots. This method shows how the ecological gradient of our plots reflects the turnover of species. This analyzes the presence/absence of species between two plots, which is expressed with values ranging from 0 to 1, where 0 indicates the same species richness, while values of 1 would indicate a total change in species richness (Koleff et al., 2003). Additionally, the Bray-Curtis similarity coefficient was applied to evaluate the similarity among plots, since this index takes into account the abundance of the species present. All these analyses were performed using PAST: Paleontological Statistics Software Package 2.17 (Hammer et al., 2001).

3. Results

Overall, 1570 ticks from the environment and animals were collected, belonging to three genera and 20 species. These ticks corresponded to 16 species of *Amblyomma*, two of *Haemaphysalis* and two of *Ixodes*. With the exception of *H. leporispalustris*, Table 1 shows the species by sites and hosts. In addition, in the laboratory, we reared *A. mixtum*, *A. naponense* and *A.* cf. *oblongoguttatum* from questing ticks, and *A. auricularium*, *A. calcaratum*, *A. dissimile*, *A. geayi*, *A. longirostre*, *A. naponense*, *A. nodosum*, *A.* cf. *oblongoguttatum*, *A. ovale*, *A. sabanerae*, *A. tapirellum*, *A. varium* from engorged immature.

Beta diversity was evaluated in two aspects: species turnover in a vegetation gradient (1) and similarity between tick communities present in plots (2) (Table 2). Whitaker's index showed more species turnover between plots 1 and 3 and the lowest between plots 2 and 4 (Table 3). However, the Bray-Curtis similarity index showed a higher degree of identity among the tick communities present in plots 2 and 3, while the lowest similarity was observed between plots 1 and 3.

Altogether, 1089 free-living ticks were collected along OT: *H. juxtakochi* (n = 430, 39% of total) was the most abundant species, followed by *A. naponense* (209, 19%), *A.* cf. *oblongoguttatum* (77, 7%), *A. tapirellum* (46, 4.2%), *I. affinis* (35, 3%), *A. pecarium* (21, 2%), *A. mixtum* (9, < 1%), *A. ovale* (7, < 1%), *A. pacae* (2, < 1%), *A. coelebs* and *A. dissimile* both with (1, < 1%) and 251 (23%) immature of undetermined *Amblyomma* spp. (Table 4).

Table 1
Tick species collected along Oleoducto trail, Soberania National Park, Panama (2013–2014).

Species	Site 1	Site 2	Site 3	Site 4	Total
Amblyomma sp.	30	52 (2[b])	16 (1[b])	281 (9[b])	**379 (12[b])**
Amblyomma auricularium	0	0	1	0	**1**
Amblyomma calcaratum	0	1	6	0	**7**
Amblyomma coelebs	0	0	0	1	**1**
Amblyomma dissimile	3	8	0	2	**13**
Amblyomma geayi	0	0	3	0	**3**
Amblyomma longirostre	0	6	2	3	**11**
Amblyomma mixtum	12 (2[b])	0	0	1	**13 (2[b])**
Amblyomma naponense	0	46	34 (1[b])	144	**224 (2[b])**
Amblyomma nodosum	0	2	2	0	**4**
Amblyomma cf. *oblongoguttatum*	2	28	47 (2[b])	14	**91 (2[b])**
Amblyomma ovale	0	9	10	1	**20**
Amblyomma pacae	0	1	0	1	**2**
Amblyomma pecarium	0	1	5	15	**21**
Amblyomma sabanerae	0	0	1	1	**2**
Amblyomma tapirellum	0	12	12	22	**46**
Amblyomma varium	0	0	4	0	**4**
Haemaphysalis juxtakochi	0	134 (2[b])	109 (4[b])	228 (3[b])	**471 (9[b])**
Ixodes affinis	0	3	10	22	**35**
Ixodes luciae	0	5	1	1	**7**
Ixodes sp.	0	0	3	0	**3**
Total	**47 (2[b])**	**308 (4[b])**	**266 (8[b])**	**737 (12[b])**	**1358 (27[b])**

[a]Excluded ticks collected from poached.
[b] Larval cluster considered as a larval unit.

Table 2
Beta diversity of tick of Oleoducto trail, Soberania National Park, Panama (2013–2014).

Sites	Beta diversity (Whitaker)			
	Site 1	Site 2	Site 3	Site 4
Site 1	0	0.64706	0.78947	0.55556
Site 2	0.64706	0	0.21429	0.18519
Site 3	0.78947	0.21429	0	0.31034
Site 4	0.55556	0.18519	0.31034	0

Table 3
Biodiversity of tick species of Oleoducto trail, Soberania National Park, Panama (2013–2014).

	Biodiversity Indexes			
	Site 1	Site 2	Site 3	Site 4
Taxa_S	4	13	15	14
Individuals	18	236	246	456
Dominance_D	0.4877	0.3585	0.2592	0.3565
Simpson_1-D	0.5123	0.6415	0.7408	0.6435

Altogether, 143 birds belonging to five orders, 11 families and 30 species were examined. Passeriformes was the most abundant order with 134 of birds (93.7%); other orders were Apodiformes (2%), Galbuliformes (2%), Caraciiformes (1%) and Columbiformes (< 1%). We collected 78 ticks from 40 birds, which were identified as one *H. juxtakochi* and 77 *Amblyomma* spp. Of these, nine species of *Amblyomma* were identified: *A. calcaratum* (5 larvae, 2 nymphs), *A. dissimile* (1 nymph), *A. geayi* (3 nymphs), *A. longirostre* (1 larva, 10 nymphs), *A. nodosum* (1 larva, 3 nymphs), *A. naponense* (2 larvae, 3 nymphs), *A.* cf. *oblongoguttatum* (1 nymph), *A. sabanerae* (1 nymph), *A. varium* (2 larvae, 2 nymphs) and 30 larvae and 10 nymphs that were not identified (Table 5).

Thirty-six of 59 examined mammals, including seven confiscated

Table 4
Ticks collected from vegetation along Oleoducto trail, Soberania National Park, Panama (2013–2014)[a].

	Species	Site 1	Site 2	Site 3	Site 4	Total
Larvae (clusters)	*Amblyomma* sp.	0	2[a]	1[a]	9[a]	**12**
	Amblyomma mixtum	2[a]	0	0	0	**2**
	Amblyomma naponense	1[a]	0	1[a]	0	**2**
	Amblyomma cf. *oblongoguttatum*	0	0	2[a]	0	**2**
	Haemaphysalis juxtakochi	0	2[b]	4[a]	3[a]	**9**
Nymphs	*Amblyomma* sp.	0	7	0	244	**251**
	Amblyomma naponense	0	18	6	20	**44**
	Amblyomma cf. *oblongoguttatum*	0	8	12	0	**20**
	Amblyomma ovale	0	4	2	1	**7**
	Amblyomma tapirellum	0	0	0	2	**2**
	Haemaphysalis juxtakochi	0	43	48	108	**199**
Adults	*Amblyomma coelebs*	0	0	0	1	**1**
	Amblyomma dissimile	0	0	0	1	**1**
	Amblyomma mixtum	8	0	0	1	**9**
	Amblyomma naponense	0	20	22	123	**165**
	Amblyomma cf. *oblongoguttatum*	1	14	28	14	**57**
	Amblyomma pacae	0	1	0	1	**2**
	Amblyomma pecarium	0	1	5	15	**21**
	Amblyomma tapirellum	0	12	12	20	**46**
	Haemaphysalis juxtakochi	0	60	52	119	**231**
	Ixodes affinis	0	3	10	22	**35**
Total		**9 (3[a])**	**191 (4[a])**	**197 (8[a])**	**692 (12[a])**	**1089 (27[a])**

[a] Larval cluster considered as a larval unit.

from poachers, were parasitized by ticks (61%) (Tables 6 and 7). Ticks collected were *A. auricularium, A. dissimile, A. mixtum, A. naponense, A.* cf. *oblongoguttatum, A. ovale, A. pacae, A. pecarium, A. sabanerae, H. juxtakochi, H. leporispalustris, I. luciae* and unidentified immatures of *Amblyomma* spp. No ticks were found on *Metachirus nudicaudatus*. In addition to warm-blood vertebrates, three males, three females and four nymphs of *A. dissimile* were found on two toads *Rhinella marina*.

Camera traps contributed about 612 night/traps during the observations in this study. This represented 18 species of mammals, in addition to domestic dogs and humans (Table 8). A complete analysis of the density of mammals of this data will be presented in subsequent publications.

Voucher specimens of each species were deposited in the "Dr. Eustorgio Méndez" Zoological Collection of ICGES.

4. Discussion

Significantly, our study recorded ≈54% of the 37 species of hard tick species reported in Panama, particularly for the genera *Amblyomma* and *Haemaphysalis* (Fairchild et al., 1966; Bermúdez et al., 2018). Although the distribution of ticks varied considerably among site 1 versus the other sites, these findings represent a high sustainability for tick communities, both environmentally and with respect to host availability. Moreover, the differences among sites are consistent with forest cover gradients and disturbance along OT, which influences distribution of potential hosts, local weather and microenvironment. In this sense, a greater number of tick-hosts interactions were observed in sites 2, 3 and 4 than in site 1, and the similarity between the populations of ticks was higher between sites 2 and 4.

Site 1 had low tick diversity including few individuals, and also few potential hosts. This was expected since degraded areas present poor conditions for vertebrate populations, particularly mid and large-size vertebrates (Ostfeld and Keesing, 2000; Meyer et al., 2015). On the other hand, it is known that the abundance of vertebrates influences the abundance of ticks, in that greater numbers and abundance of hosts increases the probability that the ticks will find them and vice versa (Randolph, 2004; Rizzoli et al., 2009).

Previous reports have indicated that the species present in site 1, both ticks and vertebrates, are adapted to inhabit different types of environments, including disturbed areas (Fairchild et al., 1966; Adler et al., 1997; Bermúdez et al., 2010, 2016). Although immatures of *A. mixtum* and *A.* cf. *oblongoguttatum* parasitize rodents, opossums and other small mammals, adults depend of mid or large sized mammals for their reproduction (Fairchild et al., 1966; Guglielmone et al., 2014). Therefore, the absence of suitable hosts for adults could be a limiting factor for the establishment of these species in site 1. This could not only explain the few individuals of these species, but also the absence of other species of ticks that were found in sites 2–4 (García et al., 2014a).

Sites 2–4 showed more similarity among tick communities and also had a higher diversity of potential hosts compared to site 1. Additionally, these sites supported rare species of ticks. This is understandable if it is considered that less degraded environments, characterized by a higher canopy and more plant species in the understory, not only present more resources for different vertebrate species, but also refuge sites for ticks (Bechara et al., 2000; Meyer et al., 2015). In addition, some species of mammals and birds require large wooded areas to survive, and cannot adapt to small fragments of forests (Ogrzewalska et al., 2011; Meyer et al., 2015; 2016). Consequently, in these sites, there were more interactions between ticks and vertebrates.

According to the camera traps census, along sites 2–4 there are viable populations of deer (*Odocoileus virginianus*, *Mazama temama*) and peccaries (*Pecari tajacu*), which showed both night and day activity (data not shown). Their abundance in SNP has been reported recently by Meyer et al. (2015, 2016), who attribute this abundance to the low presence or absence of natural predators such as jaguars or pumas. Artiodactyla are the main hosts of the adults of *H. juxtakochi*, *A. naponense* and *A. pecarium* and, to a lesser extent, to *A. tapirellum* and *A.* cf. *oblongoguttatum* (Fairchild et al., 1966; Guglielmone et al., 2014). These species crawl actively in the underbrush (e.g. leaf-litter and plants), and consequently were the most abundant species collected by flagging. In fact, *H. juxtakochi* represented ≈40% of all ticks collected. In addition to abundant adults, immatures of *A. naponense*, *A.* cf. *oblongoguttatum* and *H. juxtakochi* also parasitized small mammals or birds in these sites. Thus, we interpret that a large number of hosts for adults and immatures, in addition to adequate environmental conditions, allows the persistence of these species of ticks in sites 2–4.

Ixodes affinis was collected from vegetation during the present study but not from animals. This species is a parasite of carnivores and deer, but other groups of mammals are also recorded as hosts (Fairchild et al., 1966; Guglielmone et al., 2014; Nadolny et al., 2016). In SNP, ocelots have been recorded with mating pairs of *I. affinis* (Bermúdez et al., 2015), which could suggest that they are its main host in this park.

Table 5
Ticks collected from birds along Oleoducto trail, Soberania National Park, Panama (2013–2014).

Sites	Bird species	n/N[b]	Tick species	Stages
2	*Ceratopipra mentalis*	4/13	*Amblyomma* sp.	1 L
			Amblyomma longirostre	1 L[c], 3N[c]
	Hylophylax naevioides	1/4	*Amblyomma nodosum*	1N[c]
	Lepidothrix coronate	3/5	*Amblyomma* sp.	2N
			Amblyomma calcaratum	1 L[c]
			Amblyomma sp.	3L
			Amblyomma naponense	2 L[c]
			Amblyomma nodosum	1N[c]
	Platyrinchus coronatus	¼	*Amblyomma longirostre*	2N[c]
			Amblyomma sp.	4 L
	Thamnophilus atrinucha	2/3	*Amblyomma* sp.	7L
3	*Baryphethegus martii*	2/2	*Amblyomma* sp.	5L
			Amblyomma geayi	3N[c]
			Amblyomma longirostre	1N[c]
	Ceratopipra mentalis	2/4	*Amblyomma calcaratum*	2 L[c]
			Amblyomma varium	2N[c]
	Dendrocicla fuliginosa	2/3	*Amblyomma naponense*	2N
			Amblyomma varium	2 L[c]
	Formicarius analis	2/3	*Amblyomma* sp.	6L
	Gymnopithys leucaspis	1/6	*Amblyomma nodosum*	1N[c]
			Amblyomma sp.	1N
	Hylophylax naevioides	1/8	*Amblyomma* sp.	1 L
	Lepidothrix coronate	3/5	*Amblyomma calcaratum*	2 L[c]
			Amblyomma nodosum	1 L[c]
			Amblyomma longirostre	1N[c]
			Amblyomma sp.	1 L, 1N
	Malacopsila panamensis	1/3	*Amblyomma calcaratum*	2N[c]
	Thamnophilus atrinucha	2/2	*Amblyomma sabanerae*	1N[c]
			Amblyomma cf. *oblongoguttatum*	1N
4	*Ceratopipra mentalis*	6/15	*Amblyomma* sp.	1N[d], 2 L,1N
			Amblyomma naponense	1N[c,e]
			Amblyomma longirostre	2N[c]
	Cnipodectes subbrunneus	¼	*Haemaphysalis juxtakochi*	1N
	Gymnopithys leucapsis	2/3	*Amblyomma* sp.	1N[d], 1N[e]
	Hylophylax naevioides	1/5	*Amblyomma longirostre*	1N
	Mymotherula axillaris	2/4	*Amblyomma* sp.	1N[d]
			Amblyomma dissimile	1N[c]
	Thamnophilus atrinucha	1/3	*Amblyomma* sp.	1N[d]

[a] Habit of foraging or feeding.
[b] infested (n)/captured (N).
[c] Molted in laboratory.
[d] Engorged.
[e] Partially engorged.

Table 6
Ticks collected from trapped mammals in Oleoducto trail, Soberania National Park, Panama (2013–2014).

Sites	Mammal species	n/N[a]	Ticks species	Stages
1	*Didelphis marsupialis*	1/1	*Amblyomma* sp.	5L
			Amblyomma dissimile	1N[b]
			Amblyomma mixtum	4 L[b]
	Oryzomys sp	2/2	*Amblyomma* sp.	25L
			Amblyomma cf. *oblongoguttatum*	1N[b]
2	*Oryzomys* sp	5/13	*Amblyomma* sp.	2 L, 1N
			Amblyomma naponense	2 L[b], 4N[b]
	Didelphis marsupialis	4/6	*Amblyomma* sp.	17L
			Amblyomma cf. *oblongoguttatum*	1N[b]
			Haemaphysalis juxtakochi	14 L, 2N
			Ixodes luciae	2 L, 1♂, 2♀
	Proechimys semispinosus	4/6	*Amblyomma* sp.	8L
			Amblyomma ovale	3L[b], 2N[b]
			Amblyomma cf. *oblongoguttatum*	4 L[b], 1N[b]
			Haemaphysalis juxtakochi	15L
3	*Didelphis marsupialis*	2/2	*Amblyomma ovale*	1N
			Haemaphysalis juxtakochi	7L
			Ixodes sp.	2 L
			Ixodes luciae	1♀
	Marmosa sp	½	*Haemaphysalis juxtakochi*	2 L
			Ixodes sp.	1 L
	Oryzomys sp	½	*Amblyomma ovale*	3N[b]
	Proechimys semispinosus	5/5	*Amblyomma* sp.	1 L
			Amblyomma auricularium	1N[b]
			Amblyomma naponense	4 L[b]
			Amblyomma ovale	4 L[b]
			Amblyomma cf *oblongoguttatum*	6 N[b]
4	*Didelphis marsupialis*	5/10	*Amblyomma* sp.	1 L, 28N
			Amblyomma sabanerae	1N[b]
			Ixodes luciae	1♀

[a] No. infested (n)/No. captured (N).
[b] Molted in laboratory.

Besides the presence of deer, camera traps showed various mid-sized carnivores such as ocelots, coatis, raccoons, foxes and coyotes, which are also hosts for *I. affinis* (Fairchild et al., 1966; Nadolny et al., 2016). During our sampling we observed that *I. affinis* was found at different heights on plants and was less active compared to other species found on vegetation (data to be published in another study). This behavior could be associated with nocturnal behavior of their hosts, but more observations would be needed to confirm this.

In addition to the species mentioned above, other species of ticks were found in smaller numbers in sites 2–4. This low presence may be explained by low populations of suitable hosts, unfavorable environment or inadequate sampling methods (e.g. flagging or dragging). For example, *A. coelebs* was collected once during this study. Tapirs are the main hosts of *A. coelebs* in Panama (Fairchild et al., 1966), but none were evident in the camera trap records despite a previously report by Meyer et al. (2015) in SNP. If ticks that parasitize wandering animals are usually collected on vegetation (Szabó et al., 2009; Terrasini et al., 2010), the finding of a single *A. coelebs* adult could indicate that the lack or rarity of tapirs in OT is a limiting factor for this species along the OT.

On the other hand, differences in questing behavior or hours of activity could influence the capture of tick species that do not actively crawl on the ground or that have an arboreal or nidicolous behavior (Randolph, 2008; Labruna et al., 2007; Terrasini et al., 2010). In this sense, some species may go unnoticed. As for other Neotropical ticks, several aspects of the natural history of these species remain unknown, and further research will be necessary to understand their behavior. Nevertheless, based on the behavior of vertebrates, general ideas about the presence of certain tick species can be suggested. For example, during this study, no adults of *A. auricularium* were collected on vegetation and all were collected from hosts. In this sense, camera traps revealed the presence of a considerable number of armadillos and other potential hosts along sites 2–4; thus, it is possible that flagging is not the best method for collecting *A. auricularium* or at least, is less effective than collecting directly from the animals or their burrows.

These explanations also support our findings for *A. pacae, H. leporispalustris* and *I. luciae* which were found on their main hosts: rodents (*Cuniculus paca*), wild rabbits (*Sylvilagus gabbii*) and opossums (*D. marsupialis* and *Marmosa* sp.), respectively. These three species of mammals are mainly nocturnal and, with the exception of *D. marsupialis*, seem to maintain a greater preference for forests than intermediate environments (e.g. site 1). Pacas and wild rabbits are terrestrial and dig burrows while opossums are arboreal or scansorial (Reid, 2009). Therefore, tick behavior is expected to influence the collection of these tick species if only flagging is used for sampling.

Table 7
Ticks collected from poached mammals in Oleoducto trail, Soberania National Park, Panama (2013–2014).

Mammal species	n/N[a]	Ticks species	Stages
Cuniculus paca	1/1	*Amblyomma* sp.	4 L, 2N
		Amblyomma pacae	1♂, 2♀
Dasypus novencinctus	1/1	*Amblyomma auricularium*	4 L, 6 N, 11♂, 14♀
Tayassu tajacu	3/3	*Amblyomma* sp.	2 L
		Amblyomma naponense	5♂, 2♀
		Amblyomma cf. *oblongoguttatum*	7♂
		Amblyomma pecarium	22♂, 17♀
		Haemaphysalis juxtakochi	3♂, 9♀
Odocoileus virginianus	1/1	*Amblyomma naponense*	4♂, 2♀
		Amblyomma cf. *oblongoguttatum*	4♂
		Haemaphysalis juxtakochi	21♂, 19♀
Sylvilagus gabbii	1/1	*Haemaphysalis leporispalustris*	4N, 6♂, 7♀

[a] No. infested (n)/No. captured (N).

Based on camera trap records and examined animals, the main hosts of *A. pacae* and *H. leporispalustris* are numerous along sites 2–4, but other potential hosts such as opossums, rats and birds were also abundant (Fairchild et al., 1966; Camil and Drenner, 1978; Vogel, 1979; Ogrzewalska and Pinter, 2016; Zeringotá et al., 2016; Esser et al., 2016). For *H. leporispalustris*, George (1964, 1971) demonstrated that detachment from the host occurs mainly during the afternoon, responding to circadian rhythms when rabbits are resting. Hence, it is possible that most of the individuals of *H. leporispalustris* are confined to places or hours where their hosts rest and that we did not adequately sample the underbrush around them.

With respect to opossums and *I. luciae*, it was interesting to note that *D. marsupialis* was the only vertebrate captured in all sites, but only those captured from sites 2–4 were parasitized by this species of tick. In addition, this tick parasitized *Marmosa* sp. in site 3. It is possible that site 1 did not provide suitable environmental conditions for *I. luciae*, since its main hosts were present. This is consistent with a study by Diaz et al. (2009) in Peruvian Amazonia, who pointed out that forest might be an environmental requirement for this species rather than disturbed areas. Since macroclimatic parameters did not vary much among the sites 1–4, more observations should be made about what types of microenvironments this species prefers.

Sites 2–4 were notable for a high diversity and abundance of small mammals and birds which are hosts to immature of several tick species. It is known that larvae and nymphs of certain species are less host-specific to vertebrate groups, and instead typically show a greater preference towards certain habitats (Fairchild et al., 1966; Esser et al., 2016). In this sense, nymphs of *A. ovale* were collected from rodents and opossums, as previously reported in Panama (Fairchild et al., 1966; Murgas et al., 2013). Cameras traps showed an abundance of potential hosts for immature and adults, in particular coatis, which seem to be the preferred hosts for adults (Fairchild et al., 1966; Bermúdez et al., 2015). Similar to other ticks, it is possible that *A. ovale* has different search or ambush behaviors for hosts during different phases of its life cycle. According to Szabó et al. (2007, 2009), immatures of *A. ovale* quest for hosts on the ground or from leaf litter, while adults have been reported to quest from vegetation (30–40 cm), which corresponds to the size of its hosts in both stages.

Birds were parasitized by immatures of 10 species of ticks in sites 2–4, but by no ticks in site 1, which has also been observed in studies in Brazil (Ogrzewalska et al., 2011). The majority of these birds forages on the ground or perch on branches at low altitudes, which favor parasitism by tick species that are active on the ground (Labruna et al., 2007; Ogrzewalska et al., 2011). For at least five species of ticks, birds represent opportunistic hosts, since the principal hosts of these ticks are mammals (*A. naponense*, *A.* cf. *oblongoguttatum*, *H. juxtakochi*), reptiles or amphibians (*A. sabanerae* and *A. dissimile*) (Scott et al., 2001; Guglielmone and Nava, 2010; Guglielmone et al., 2014; Miller et al., 2016).

Birds seem to be the main hosts for immatures of *A. longirostre*, *A. nodosum*, and to a lesser extent of *A. calcaratum*, *A. geayi* and *A. varium* (Labruna et al., 2007; Ogrzewalska and Pinter, 2016). The adults of these species of tick are parasites of scansorial mammals such as porcupines (*A. longirostre*), sloths (*A. geayi* and *A. varium*) and, with the exception to terrestrial *Myrmecophaga tridactyla*, semi-arboreal ant-eaters (*A. calcaratum* and *A. nodosum*) (Guglielmone et al., 2014; Ogrzewalska and Pinter, 2016). No adults or immatures of these ticks were identified in our samples from underbrush or hosts, and their presence was only evident based on immatures collected from birds, which is similar to other reports in Panama (Fairchild et al., 1966; Miller et al., 2016; Esser et al., 2016) and other countries (Ogrzewalska and Pinter, 2016). Some authors have proposed that *A. longirostre* might be an arboreal or nest-associated tick (Labruna et al., 2007).

From the point of view of public health, several of the tick species found in this study have been reported as human parasites (Bermúdez et al., 2012; Guglielmone and Robbins, 2018), and *A. mixtum* stands out

Table 8
Mammals recorded by camera along Oleoducto trail, Soberania National Park, Panama (2013–2014).

Order	Family	Species	S 1	S 2	S 3	S 4
Artiodactyla	Cervidae	*Odocoileus virginianus*		■	■	■
		Mazama temama			■	■
	Tayassuidae	*Pecari tajacu*		■	■	■
Carnivora	Canidae	Dog	■		■	
		Cerdocyon thous			■	
		Canis latrans		■	■	
	Felidae	*Leopardus pardalis*			■	■
	Mustelidae	*Eira Barbara*			■	
	Procionidae	*Nasua narica*		■	■	■
		Procyon cancrivorus			■	
Cinculata	Dasypodidae	*Dasypus novencinctus*		■		■
Didelphimorphia	Didelphidae	*Didelphis marsupialis*	■	■	■	■
Lagomorpha	Leporidae	*Sylvilagus gabbii*			■	
Pilosa	Myrmecophagidae	*Tamandua mexicana*			■	
Primates	Cebidae	*Cebus capicinus*		■		
	Hominidae	Human	■		■	
Rodentia	Caviidae	*Hydrochoerus isthmius*			■	
	Cuniculidae	*Cuniculus paca*		■		■
	Dasyproctidae	*Dasyprocta punctata*		■	■	■
	Sciuridae	*Sciurus granatensis*		■		

White cells represent absent or non-recorded mammals; black cells represent recorded mammals.

for its importance as a vector of *Rickettsia rickettsii*, the most important pathogenic agent transmitted by ticks in the Neotropics (Rodaniche, 1953; Labruna et al., 2011). Recently in Gamboa, a locality close to SNP, García et al. (2014b) reported *A. mixtum* on ponchos (lesser capybaras) (*Hydrochoerus isthmus*), and Domínguez et al. (2016) on terrestrial turtles (*Trachemys scripta*). Ponchos are the largest rodents in Panama and inhabit riparian habitats. In OT, camera traps recorded ponchos in site 3 and one of us (RM) saw them in streams close to site 4, which could explain the capture of one *A. mixtum* at this site. Although *A. mixtum* is widely distributed in Panama, the type of environment that it prefers seems to be paddocks and dry forests, instead of secondary or primary forests such as those present in sites 2–4 (Fairchild et al., 1966; Bermúdez et al., 2016). Therefore, it is possible that this species benefits from modifications that alter the natural landscape.

Finally, these results represent a first attempt to interpret some relationships between the environment, ticks, and their vertebrate hosts in a forested area in Panama, allowing interpretations for future changes in species composition according to further environmental modifications in the canal basin. In this sense, further studies are necessary to understand how regional changes may influence the distribution of *A. mixtum*, and other possible vectors and their reservoirs, to prevent new human cases of TBD.

Acknowledgments

We sincerely thank to the Ministry of Environment for the permits and special thanks to Lance Durden (Georgia Southern University, Statesboro, US) for reviewing and commenting on the manuscript.

References

Adler, G., Arboledo, J., Travi, B., 1997. Population dynamics of *Didelphis marsupialis* in Northern Colombia. Stud. Neotrop. Fauna E 32, 7–11.

Alessio, F., Dantas-Torres, F., Siqueira, D., Lizee, M., Marvulo, M., Martins, T., Labruna, M., Silva, J., Mauffrey, J., 2012. Ecological implications on the aggregation of *Amblyomma fuscum* (Acari: Ixodidae) on *Thrichomys laurentius* (Rodentia: Echimyidae), in northeastern Brazil. Exp. Appl. Acarol. 57, 83–90.

Angehr, G., Dean, R., 2010. The Birds of Panama. A Field Guide, first ed. Cornell University Press, Ithaca, New York, US.

Bechara, G., Szabó, M., Duarte, J., Mutushima, E., Pereira, M., Rechav, Y., Keirans, J., Fielden, L., 2000. Ticks associated with wild animals in the Nhecolândia Pantanal, Brazil. Ann. N. Y. Acad. Sci. 916, 289–297.

Bermúdez, S., Miranda, R.J., Smith, D.E., 2010. Ticks (Ixodida) species in the Summit Municipal Park and adjacent areas, Panama City, Panama. Exp. Appl. Acarol. 52, 439–448.

Bermúdez, S., Castro, A., Esser, H., Liefting, Y., García, G., Miranda, R., 2012. Ticks (Ixodida) on humans from central Panama, Panama (2010-2011). Exp. Appl. Acarol. 58, 81–88.

Bermúdez, S., Esser, H., Miranda, R., Moreno, R., 2015. Wild carnivores as hosts for ticks (Ixodida) in Panama. Syst. Appl. Acarol. 20, 13–19.

Bermúdez, S., Castro, A., Trejos, T., García, G., Gabster, A., Miranda, R., Zaldívar, Y., Paternina, L., 2016. Distribution of spotted Fever Group Rickettsiae in hard ticks (Ixodida: Ixodidae) from Panamanian urban and rural environments. EcoHealth 13, 274–284.

Bermúdez, S., Apanaskevich, D., Domínguez, L., 2018. Garrapatas Ixodidae De Panamá. ISBN 978-9962-699-25-5. .

Brunner, J.L., Ostfeld, R.S., 2008. Multiple causes of variable tick burdens on small-mammal hosts. Ecology 89, 2259–2272.

Camil, J., Drenner, R., 1978. Climbing behavior and host-finding of larval rabbit ticks (*Haemaphysalis leporispalustris*). J. Parasitol. 64, 905–909.

Diaz, M., Nava, S., Guglielmone, A., 2009. The parasitism of *Ixodes luciae* (Acari: Ixodidae) on marsupials and rodents in Peruvian Amazon. Acta Amazon. 39, 997–1002.

Dobson, A., Taylor, J., Randolph, S., 2011. Tick (*Ixodes ricinus*) abundance and seasonality at recreational sites in the UK: hazards in relation to fine-scale habitat types revealed by complementary sampling methods. Ticks Tick-borne Dis. 2, 67–74.

Domínguez, L., Hernández, A., Estrada, E., Correa, N., Cleghorn, J., Bermúdez, S., 2016. Inusual parasitismo de *Amblyomma mixtum* (Ixodida: ixodidae) en *Trachemys scripta* (Testudines: emydae) en Panamá. Rev Ib Aracnol 28, 137–139.

Esser, H., Herre, E., Blüthgen, N., Loaiza, J., Bermúdez, S., Jansen, P., 2016. Host specificity in a diverse Neotropical tick community: an assessment using quantitative network analysis and host phylogeny. Parasit. Vectors 9, 372.

Fairchild, G., Kohls, G., Tipton, V., 1966. The ticks of Panama (acarina: Ixodoidea). In: Wenzel, R., Tipton, V. (Eds.), Ectoparasites of Panama. Field Museum of Natural History Chicago, Illinois, pp. 167–219.

García, G., Castro, A., Bermúdez, S., Nava, S., 2014a. Some ecological aspects of free-living *Haemaphysalis juxtakochi* Cooley, 1946 (Acari: Ixodidae) in Panama. Rev Med Vet Zootec 19, 3984–3989.

García, G., Castro, A., Rodríguez, I., Bermúdez, S., 2014b. Ixodid ticks of *Hydrochoerus isthmius* Goldman 1912 (Rodentia: Caviidae) in Panama. Syst. Appl. Acarol. 19, 404–408.

George, J., 1964. The circadian rhythm of "drop-off" engorged rabbit ticks, *Haemaphysalis leporispalustris* from rabbits. Proc. 1st International Congress Acarology, vol. 6, 343–349.

George, J., 1971. Drop-off rhythms of engorged rabbit ticks, *Haemaphysalis leporispalustris* (Packard 1896) (Acari: Ixodidae). J. Med. Entomol. 8, 461–479.

Guerra, M., Walker, E., Jones, C., Paskewitz, S., Cortinas, M., Stancil, A., Beck, L., Bobo, M., Kitron, U., 2002. Predicting the risk of lyme disease: habitat suitability for ixodes scapularisin the North Central United States. Emerg. Infect. Dis. 8, 289–297.

Guglielmone, A., Nava, S., 2010. Hosts of *Amblyomma dissimile* Koch, 1844 and *Amblyomma rotundatum* Koch, 1844 (Acari: Ixodidae). Zootoxa 2541, 27–49.

Guglielmone, A., Robbins, R., 2018. Hart ticks (Acari: Ixodida: Ixodidae) parasitizing humans. A global review. Springer Nature.

Guglielmone, A., Robbins, R., Apanaskevich, D., Petney, T., Estrada-Peña, A., Horak, I., 2014. The Hard Ticks of the World. Springer.

Hammer, Ø., Harper, D., Ryan, P., 2001. Past: paleontological statistics software package for education and data analysis. Palaeontol. Electronica 4, 1–9.

Hoogstraal, H., 1985. Argasid and Nuttalliellid ticks as parasites and vectors. Adv. Parasitol. 24, 135–238.

Koleff, P., Gaston, K., Lennon, J., 2003. Measuring beta diversity for presence-absence data. J. Anim. Ecol. 72, 367–382.

Labruna, M., Sanfilippo, L., Demetrio, C., Menezes, A., Pinter, A., Guglielmone, A., Silveira, L., 2007. Ticks collected on birds in the state of São Paulo. Brazil. Exp. Appl. Acarol. 43, 147–160.

Labruna, M., Mattar, S., Nava, S., Bermúdez, S.E., Venzal, J., Dolz, G., Abarca, K., Romero, L., Souza, R., Oteo, J., Zavala-Castro, J., 2011. Rickettsioses in Latin America, Caribbean, Spain and Portugal. Rev Med Vet Zoot 16, 2435–2457.

Lopes, M., May, J., Foster, R., Harmsen, B., Sanchez, E., Martins, T., Quigley, H., Marcili, A., Labruna, M., 2016. Ticks and rickettsiae from wildlife in Belize, Central America. Parasit. Vectors 9, 62. https://doi.org/10.1186/s13071-016-1348-1.

Martínez, R., 2008. Cobertura vegetal, uso del suelo y tasa de deforestación en la cuenca hidrográfica del Canal de Panamá, 2008. In: de Panamá, Canal (Ed.), Agua y Bosques en la Cuenca del Canal: Tendencias a largo plazo.

Martins, T., Onofrio, V., Barros-Battesti, D., Labruna, M., 2010. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. Ticks Tick-borne Dis. 1, 75–99.

Meyer, N., Esser, H., Moreno, R., Van Langevelde, F., Liefting, Y., Ros Oller, D., Vogels, C., Carver, A., Nielsen, C., Jansen, P.A., 2015. An assessment of the terrestrial mammal communities in forests of central Panama, using camera-trap surveys. J. Nat. Conserv. 26, 28–35.

Meyer, N., Moreno, R., Sanches, E., Ortega, J., Brown, E., Jansen, P., 2016. Do protected areas in Panama support intact assemblages of ungulates? Theria 7, 65–76.

Miambiente, 2011. Parque Nacional Soberanía. http://www.anam.gob.pa/soberania/index.

Miller, M.J., Esser, H.J., Loaiza, J.R., Herre, E.A., Aguilar, C., Quintero, D., Álvarez, E., Birmingham, E., 2016. Molecular ecological insights into Neotropical bird–tick interactions. PLoS One 11, e0155989. https://doi.org/10.1371/journal.pone.0155989.

Muñoz-Leal, S., González-Acuña, D., 2015. The tick *Ixodes uriae* (Acari: Ixodidae): hosts, geographical distribution, and vector roles. Ticks Tick-Borne Dis. 6, 843–868.

Murgas, I., Castro, A., Bermúdez, S.E., 2013. Current status of *Amblyomma ovale* Koch 1844 (Acari: Ixodidae) in Panama. Ticks Ticks-Borne Dis. 4, 164–166.

Nadolny, R., Gauthier, D., Gaff, H., Bermúdez, S., 2016. Preliminary assessment of the population genetics of *Ixodes affinis* (Ixodida: Ixodidae) in North and Central America. Syst. Appl. Acarol. 21, 1300–1308.

Nava, S., Beati, L., Labruna, M., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp., and *Amblyomma patinoi* n. sp., and the reinstatement of *Amblyomma mixtum* Koch, 1844 and *Amblyomma scultum* Berlese, 1888 (Ixodida, Ixodidae). Ticks Ticks-Borne Dis 5, 252–276.

Ogrzewalska, M., Pinter, A., 2016. Ticks (Acari: Ixodidae) as ectoparasites of Brazilian wild birds and their association with rickettsial diseases. Braz. J. Vet. Res. Anim. Sci., São Paulo. 53, 1–31.

Ogrzewalska, M., Uezu, A., Jenkins, C., Labruna, M., 2011. Effect of forest fragmentation on tick infestations of birds and tick infection rates by *Rickettsia* in the Atlantic Forest of Brazil. EcoHealth 8, 320–331.

Ostfeld, R., Keesing, F., 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. Can. J. Zool. 78, 2061–2078.

Randolph, S., 2004. Tick ecology: processes and patterns behind the epidemiology risk posed by ixodid ticks as vectors. Parasitol 129 (Suppl), S37–65.

Randolph, S., 2008. The impact of ticks ecology on pathogen transmission dynamic. In: Bowman, A., Ticks, Nuttall P. (Eds.), Biology, Disease and Control. Cambridge Univ. Press, pp. 40–72.

Reid, F., 2009. A Field Guide to the Mammals of Central America and Southeast Mexico, second edition. .

Rizzoli, A., Hauffe, H., Tagliapietra, V., Neteler, M., Rosá, R., 2009. Forest structure and roe deer abundance predict tick-borne encephalitis risk in Italy. PLoS One 4, e4336.

Rodaniche, E., 1953. Natural infection of the tick, *Amblyomma cajennense*, with *Rickettsia rickettsii* in Panama. Am. J. Trop. Med. Hyg. 2, 696–699.

Scott, J., Fernando, K., Banerjee, S., Durden, L., Byrne, S., Banerjee, M., Mann, R., Morshed, M., 2001. Birds disperse Ixodid (Acari: Ixodidae) and *Borrelia burgdorferi* infected ticks in Canada. J. Med. Entomol. 38, 493–500.

Szabó, M., Castro, M., Ramos, H., García, M., Castagnolli, C., Pinter, A., Veronez, V., Magalhaes, G., Duarte, J., Labruna, M., 2007. Species diversity and seasonality of free-living ticks (Acari: Ixodidae) in the natural habitat of wild marsh deer (*Blastocerus dichotomus*) in Southeastern Brazil. Vet. Parasitol. 143, 147–154.
Szabó, M., Labruna, M., Garcia, M., Piner, A., Castagnolli, K., Pacheco, R., Castro, M., Veronez, V., Magalhaes, G., Vogliotti, A., Duarte, J., 2009. Ecological aspects of the free-living ticks (Acari: Ixodidae) on animal trails within Atlantic rainforest in south-eastern Brazil. Ann. Trop. Med. Parasitol. 103, 57–72.
Terrasini, F., Barbieri, F., Albuquerque, S., Szabó, M., Camargo, M., Labruna, M., 2010. Comparison of two methods for collecting free-living ticks in the Amazonian forest. Ticks Tick-Borne Dis. 1, 194–196.
Vogel, G., 1979. Climbing behavior of Haemaphysalis leporispalustris. J. Kans. Entomol. Soc. 52, 437.
Zeringotá, V., Maturano, R., Santolin, Í., McIntosh, D., Famadas, K., Daemon, E., Faccini, J., 2016. New host records of *Haemaphysalis leporispalustris* (Acari: Ixodidae) on birds in Brazil. Parasitol. Res. 115, 2107–2110.