

Epidemiological study of ticks collected from the northern tamandua (*Tamandua mexicana*) and a literature review of ticks of Myrmecophagidae anteaters

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ABSTRACT

The family Myrmecophagidae contains three anteater species: *Tamandua mexicana* (Saussure, 1860), *Tamandua tetradactyla* (Linnaeus, 1758) and *Myrmecophaga tridactyla* (Linnaeus, 1758). These American anteater species currently face many conservation threats, among which road traffic accidents stand out. Parasitic studies on this family are scarce, and some of them include records of ectoparasites. Specifically for northern tamandua (*T. mexicana*), there is a lack of studies at population level. The objectives of the present research were to carry out an epidemiological study of tick species and its abundance on road-killed northern anteater specimens and, moreover, to perform a literature review of ticks collected from anteaters of Myrmecophagidae family. Five tick species were identified, including four *Amblyomma* spp. and *Rhipicephalus sanguineus* sensu lato, on 23 road-killed anteaters. Tick infestation prevalence was 43% (10/23), with a median tick infestation intensity of 3.5 per anteater (interquartile range 1–13.7). The bibliographic review highlighted the existence of twenty-nine ixodid species recorded on the three anteater species from 14 countries, mainly Brazil. The most common tick species on the Myrmecophagidae family are *Amblyomma nodosum*, *A. calcaratum*, *A. cajennense* sensu lato and *A. auricularium*. Some of these ixodids were also described as vectors of pathogens. Further studies are needed to evaluate the impact of ticks on anteater fitness, and to assess the role of these mammals as reservoirs of vector-borne diseases.

1. Introduction

The northern tamandua (*Tamandua mexicana*) is a mammal belonging to the Pilosa order, included in the superorder Xenarthra (Navarrete and Ortega, 2011). This superorder comprises armadillos, sloths and anteaters, which is the only group of mammals that evolved

exclusively in the New World (Delsuc et al., 2004). Anteaters are classified into two families, Cyclopedidae and Myrmecophagidae; the first includes the silky anteaters (*Cyclopes* Gray, 1821), and the later contains the giant anteater (*Myrmecophaga tridactyla*) and two tamanduas species (*Tamandua tetradactyla* and *T. mexicana*) (Miranda et al., 2017).

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Tamandua mexicana, which is distributed from northern Mexico to northern South America, is a solitary and diurnal species that feeds on ants and termites (Navarrete and Ortega, 2011). The International Union for Conservation of Nature (IUCN) categorizes this anteater species as “Least Concern”, because *T. mexicana* has a wide geographical distribution and can be found in various kinds of habitats, including severely disturbed areas (Superina et al., 2010). Nonetheless, the northern tamandua is under extinction risk in Mexico (Semarnat, 2010) and there is a lack of knowledge regarding basic biological aspects and parasitic infections and infestations affecting this species, particularly from a population perspective (Superina et al., 2010). Most parasitological studies in Myrmecophagidae anteaters have focused on ectoparasites, specifically ixodids of the genus *Amblyomma* (De Quadros et al., 2010), some of which are well-known vectors of bacterial and virus of medical and veterinary importance (Ogrzewalska et al., 2013). However, almost all publications about ticks on the family Myrmecophagidae lack information about host characteristics such as sex, age and body condition, limiting our understanding of epidemiological features of these ectoparasites. Moreover, these works are published in regional scientific journals whose register are scattered among different bibliographic databases and are difficult to obtain. Numerous tick records exist for *T. tetradactyla* and *M. tridactyla*, and some come from road-killed animals (De Quadros et al., 2010; Sangaletti-Lavina et al., 2011; Spolidorio et al., 2012; Forlano et al., 2013; da Costa-Pinheiro et al., 2015; Soares-Pereira et al., 2015). In contrast, there are few records of ticks from the northern tamandua, mainly case reports in which only a small number of tick species have been reported (Guzmán-Cornejo et al., 2006; Rodríguez-Vivas et al., 2016). Given these limitations, it is necessary to carry out ecological and epidemiological studies and assess the potential role of anteaters as tick reservoirs for other domestic and wild species, and also for humans. Unfortunately, sampling anteaters in the wild has important drawbacks.

The northern tamandua and the southern Myrmecophagidae species (*M. tridactyla* and *T. tetradactyla*) faces many conservation threats, like wildfires, hunting, capture for illegal trade, domestic dog attacks, habitat loss and road traffic accidents (De Quadros et al., 2010; Superina et al., 2010). In Mexico, the northern tamandua is a wild species frequently killed on road accidents (Nuñez-Perez et al., 2011), as occurs with other Xenarthra mammals. In fact, some researchers have made good use of samples from road-killed specimens, such as nine-banded armadillos (*Dasypos novemcinctus*) and giant anteaters (Loughry and McDonough, 1996; Richini-Pereira et al., 2014). These studies indicate that these animals are a useful source of data and biological samples, because often carcasses are in good condition and may represent a random sample of the species population (Loughry and McDonough, 1996; Richini-Pereira et al., 2014). Otherwise, it is not easy to sample rare and protected wild species as the northern tamandua, since field, ethical and legal difficulties exist for trapping and sampling individuals. And also because the capture of healthy specimens of endangered wild animal species has been linked to significant demographic declines (Minteer et al., 2014).

With the aim of improving knowledge about the northern tamandua tick species and facilitate access to available information on this issue for the three Myrmecophagidae anteater species, here we present (i) a study on tick species richness and tick infestation intensity from Mexican road-killed northern tamanduas, and (ii) a literature review of the tick species described on anteaters of Myrmecophagidae family, discussing the potential epidemiological role that these anteaters could play as reservoirs of vector-borne diseases.

2. Material and methods

2.1. Study area and road-killed northern tamanduas collection

Twenty-three road-killed *T. mexicana*, hereinafter referred to as Mexican road-killed northern tamanduas, were collected from the main

highways of Chiapas, Guerrero and Tabasco states (Mexico) between 2009 and 2014. All animals were collected within 12 h of death as evidenced by the absence of blowfly eggs or maggots typically observed when the post-mortem interval is greater (Reibe and Madea, 2010), and were frozen at -20°C , until analyzed. The sex of each animal was ascertained except from those in which as a result of the accident, the intra-abdominal gonads were not found. Animals were age-classified as adult or juvenile according to the morphometric criteria based on weight and total length, as described by Navarrete and Ortega (2011) and, in the case of the only juvenile identified, its age category was confirmed using a digital radiological study of the thoracic limbs in order to look for the physal growth plates, using a Rotanode™ E7238X-125 kV high frequency equipment (Toshiba Electron Tubes & Devices Co., LTD, Otawara-shi, Tochigi, 324–8550, Japan), set at 65–69 kVp, 1/120 s and 1.7 mA, controlled by the Canon CXDI-55 G digital radiography system (Canon USA Inc, Lake Success, New York, 11042, United States of America).

The collection of Mexican road-killed northern tamanduas was carried out under authorization of the “Secretaría de Medio Ambiente y Recursos Naturales” (Semarnat) of Mexico (permit references: SGPA/DGVS/03663/11, SGPA/DGVS/04726/13 and SGPA/DGVS/07303/14).

2.2. Tick collection and identification

At the laboratory, all animals were visually inspected and, when found, ticks were collected with fine forceps and placed into a glass tube with 70% ethanol. A further examination for tick collection was carried out by brushing the animals coat with a flea comb for dogs (Hartz®) over a piece of white paper. Brushed material was put in a Petri dish with a shallow layer of tap water and examined under a stereoscopic microscope (Leica® EZ4). Ticks collected after this step (mainly nymphs and larvae) were similarly stored in 70% ethanol. Subsequently, adult ticks were counted and identified using morphological keys (Robinson, 1926; Barros-Battesti et al., 2006; Guzmán-Cornejo et al., 2011) under a stereoscopic microscope (Nikon® SMZ645). After species identification, ticks were deposited at the “Colección del Laboratorio de Acarología, Facultad de Ciencias, Universidad Nacional Autónoma de México”, in Mexico City (accession numbers: LAFC000257-267).

The collected nymphs and larvae were identified molecularly. DNA was extracted individually from each tick. Briefly, each specimen was placed in a 1.5-mL Eppendorf tube and crushed with the help of a sterile pistil. Then 500 μL of 5% Chelex® 100 Chelating Resin (Biorad, USA) solution and 20 μL of Proteinase K (SIGMA life sciences, USA) were added per sample, and allowed to incubate at 56°C during twelve hours. After that, samples were centrifuged at 14,000 rpm for 15 min, and the supernatant was collected in new tubes and subsequently stored at -20°C until further use (Ballados-González et al., 2018). A partial fragment of the mitochondrial 16S rDNA gene (≈ 440 bp) was amplified using the protocols of Norris et al. (1996). The reaction mixture consisted of 12.5 μL PCR master mix solution (Quiagen), 100 pg of each primer, 6.5 μL nuclease-free water and 100 ng DNA in a final volume of 25 μL (Sánchez-Montes et al., 2016). Amplicons were submitted for sequencing to “Laboratorio de Biología Molecular de la Biodiversidad y la Salud, Universidad Nacional Autónoma de México”. Recovered sequences were compared to those deposited in GenBank using the Basic Local Alignment Search Tool (BLAST).

2.3. Tick records and related information search

A literature search was conducted using the scientific electronic databases PubMed, Web of Science, Google Scholar, Research Gate, Redalyc and Scielo, without restriction of language (English, Portuguese and Spanish), publication date and knowledge area. The last search was conducted on 9 March 2019. Duplicate entries were

identified and removed. For this purpose, the following keywords combinations were chosen: “Amblyomma” and “anteater”, “tamandua” and “carrapato”, “anteater” and “ectoparasites”, “oso hormiguero” and “garrapata”, “anteater” and “ixodid”, “anteater” and “tick”, “Myrmecophaga” and “carrapato”, “Myrmecophaga” and “ectoparasites”, “Myrmecophaga” and “garrapata”, “Myrmecophaga” and “ixodid”, “Myrmecophaga” and “tick”, “Tamandua” and “ectoparasites”, “Tamandua” and “garrapata”, “Tamandua” and “ixodid”, “Tamandua” and “tick”, “xenarthra” and “carrapato”, “xenarthra” and “ectoparasites”, “xenarthra” and “garrapata”, “xenarthra” and “ixodid”, and “xenarthra” and “tick”. Full-text research articles that contain information about anteater species, tick species, developmental stage and gender, number of ticks collected per anteater and country of origin were included. The animal host sources were classified into three categories: 1) road-killed, 2) kept in captivity, which includes zoo animals, captured in the wild for scientific purpose, translocation or in rehabilitation, and 3) without origin description, assigned when the anteater was of unknown origin or unmentioned source.

2.4. Statistical data analysis of Mexican road-killed northern tamanduas

Tick infestation prevalence, tick species richness and tick infestation intensity were calculated. Tick infestation prevalence was defined as the number of individuals with at least one tick divided by the number of individuals examined, richness was the number of tick species on the anteater population, and tick infestation intensity was defined as the number of specimens of a particular ticks species per infested host. Differences in tick infestation prevalence according to host gender were analyzed with Fisher’s exact test using Epidat® 3.1 software. Statistical analyses by year and season were not done because Mexican road-killed northern tamanduas were collected opportunistically (field trips were not done under a structured schedule). Significance was considered for $p < 0.05$ for a two-tailed test.

2.5. Statistical data analysis of Myrmecophagidae family review

Tick species frequency, tick species richness and tick infestation intensity were calculated on the basis of the information found in the reviewed articles. Tick species frequency was defined as the number of individuals of one anteater species with at least one specimen of certain tick species divided by the total number of individuals of that anteater species found in this literature search. For intensity, distributions were summarized with the mean, median and range. Differences in median tick infestation intensity according to animal host sources were analyzed with Mann-Whitney test using Past® 3.14 software. Significance was considered for $p < 0.05$ for a two-tailed test.

3. Results

3.1. Epidemiological study of ticks on Mexican road-killed northern tamanduas

Twenty-three Mexican road-killed northern tamanduas were collected during the study period (Fig. 1A). Two adult males and one adult female were from Chiapas, one adult male from Guerrero, and ten adult females, six adult males, two adults of undetermined sex and one juvenile of undetermined sex from Tabasco. A total of 113 ixodid ticks were collected from four *Amblyomma* species, mostly *A. nodosum* males, as well as one *Rhipicephalus sanguineus* s.l. (Table 1, Figs. 1B and C). The five tick species were found in Tabasco and *A. nodosum* was the only species also found in Guerrero and Chiapas, on a single animal in each state. Moreover, six immature specimens of *A. mixtum* (that exhibited an identity of 99% with a sequence of *A. mixtum* from Texas [KM519935.1]), two larvae and two nymphs were detected on one juvenile anteater of undetermined sex, and one larvae and one nymph on one adult male, both from Tabasco state. The sequences were

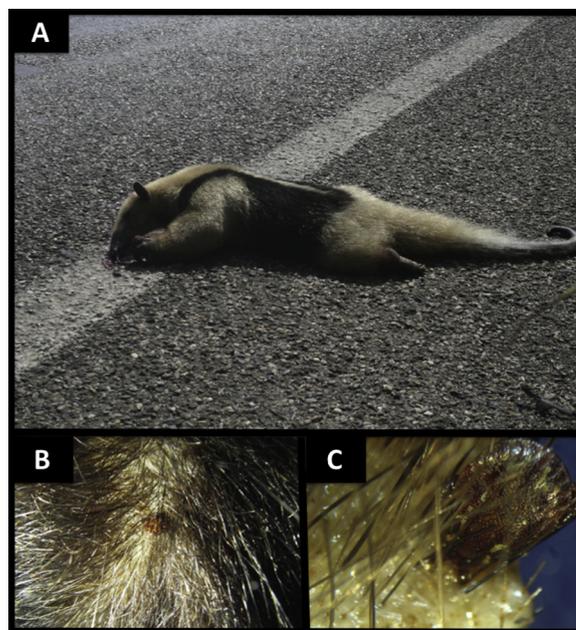


Fig. 1. Road-killed *Tamandua mexicana* (A), *Amblyomma nodosum* attached to the skin of *T. mexicana* (B, C).

deposited in GenBank (no. MG938670 and MG938671). Most tamanduas presented single species tick infestations, except one pregnant female parasitized by *A. nodosum* and *A. auricularium*, and one non-pregnant adult female which bore *A. auricularium* and *A. mixtum*. Only one *R. sanguineus* s.l. specimen was identified, infesting an adult female.

The overall tick infestation prevalence in Mexican road-killed northern tamanduas was 43% (10/23), with a median \pm SD (range) tick infestation intensity of 3.5 ± 18.6 (1 to 56). The tick infestation prevalence by host sex was 44% (4/9) for males and 45% (5/11) for females ($p > 0.05$), and the tick infestation intensity was 3 ± 2.5 (1–7) in female tamanduas, and 18 ± 26.7 (1–56) in males ($p > 0.05$). The most heavily infested animals were two adult males with 56 and 34 *A. nodosum* adult specimens, respectively, while the other infested tamanduas had each from one to four ticks.

3.2. Bibliographic review of ticks on Myrmecophagidae anteaters

The review about ticks on the three anteater species generated a total of 71 research articles, 61 of which also provide some data related to tick infestation intensity on the studied hosts. The ten studies without infestation data or without information about the number of examined hosts were those reported by Keirans (1985); Need et al. (1991); Bechara et al. (2000); De Campos Pereira et al. (2000); Guglielmone et al. (2004); Basu et al. (2012); Barbieri et al. (2013); Coelho (2016); Bermúdez et al. (2018) and Tarragona et al. (2018).

Only ten articles referred to *T. mexicana*, 33 to *M. tridactyla* and 49 to *T. tetradactyla*, 17 of the aforementioned studies reported data on two anteater species and two reported data on three anteater species. Studies were carried out in 14 countries: Brazil (47), Argentina (6), Panama (6), Mexico (2), Peru (2), Venezuela (2), Belize (1), Colombia (1) Costa Rica (1), French Guiana (1), Guiana (1), Nicaragua (1), Paraguay (1), Trinidad and Tobago (1) and one article did not indicate the collection country site. Some studies reported data on more than one country (for detailed literature references see Supplementary material).

The number of anteaters analyzed per study varied between one to 72 individuals; in those about *T. mexicana* the average number of anteaters analyzed was 1.7 (range 1–4; median 1), for *T. tetradactyla* it was 4.3 (1–37; 2), and for *M. tridactyla* it was 7.1 (1–72; 3). The number

Table 1

Tick species and stages, infestation prevalence and intensity, and host origin of *Tamandua mexicana* specimens road-killed in Mexico from 2009 to 2014.

Tick species	No. of animals	Prevalence ¹	Number of ticks					Intensity ² range	Mexican state of origin ³
			Total	Adults		Nymphs	Larvae		
				males	females				
<i>Amblyomma auricularium</i>	2	9	6	6	0	0	0	2 - 4	Tab
<i>A. calcaratum</i>	1	4	1	1	0	0	0	–	Tab
<i>A. nodosum</i>	5	22	98	89	9	0	0	1- 56	Tab, Chi, Gue
<i>A. mixtum</i>	3	13	7	1	0	3	3	1 - 4	Tab
<i>Rhipicephalus sanguineus</i> s.l.	1	4	1	0	1	0	0	–	Tab
Total	10*		113	97	10	3	3		

¹ Percentage of infested animals divided by the number of individuals examined.

² Number of ticks per infested animal.

³ Chiapas (Chi), Guerrero (Gue), Tabasco (Tab).

* two tamanduas had mixed infestations.

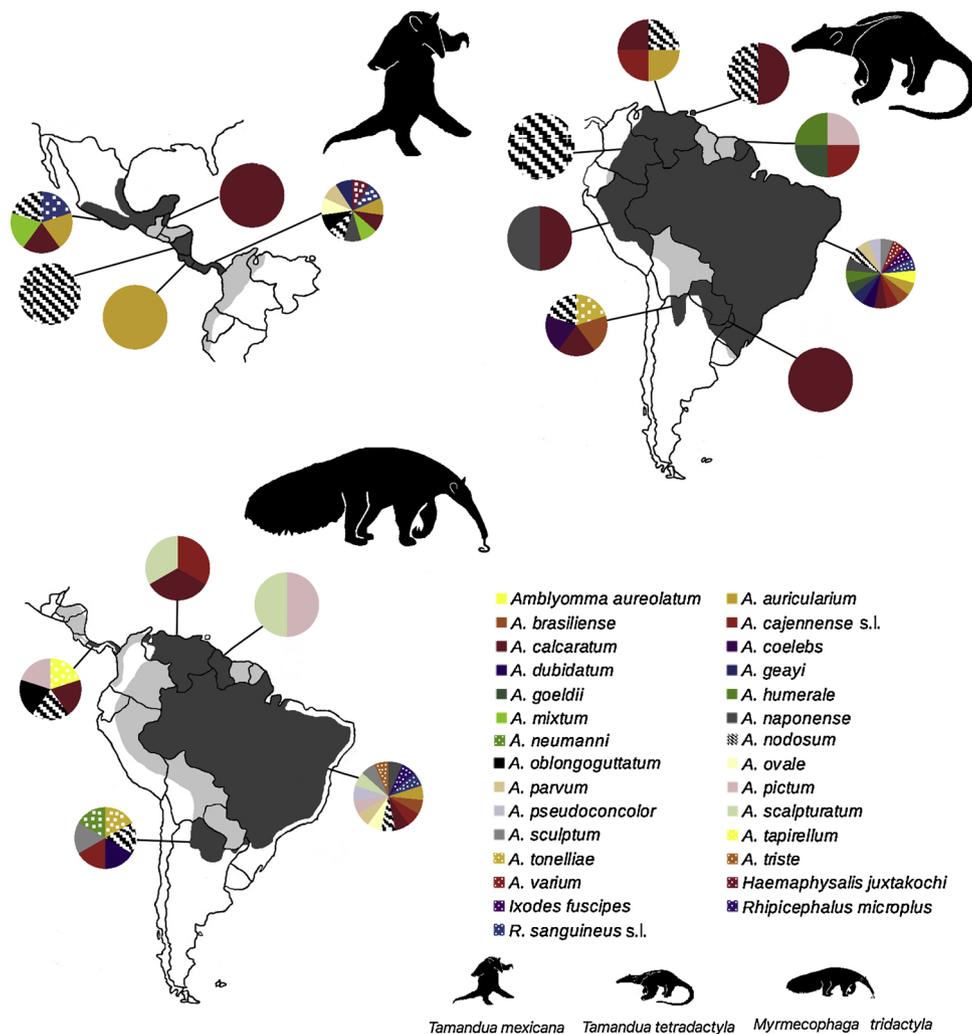


Fig. 2. Tick species richness and distribution by anteater species and country. Light grey color indicates the natural distribution of the anteater species and the dark grey indicates that the country has at least one tick record. Tick species are represented by colors within each anteater species. Natural distribution information for *Tamandua mexicana* was obtained from Navarrete and Ortega (2011), for *T. tetradactyla* was obtained from Hayssen (2011) and for *Myrmecophaga tridactyla* was obtained from Porini et al. (2006), Portillo-Reyes et al. (2010) and Gaudin et al. (2018).

of anteaters examined with tick infestation intensity data was 433 (including those from our study). Details of countries with tick records per anteater species are depicted in Fig. 2.

Twenty-nine ixodid species belonging to *Amblyomma* (n = 25), *Rhipicephalus* (n = 2), *Ixodes* (n = 1) and *Haemaphysalis* (n = 1) genera were reported, the most common being *A. nodosum* (mentioned in 45

articles), followed by *A. calcaratum* (34), *A. cajennense* sensu lato (s.l.) (22) and *A. auricularium* (10) (for details see Supplementary material). Specifically, the only tick species that were detected on the three anteater species are *A. nodosum*, *A. calcaratum*, *A. auricularium* and *A. parvum*. The southern anteaters *T. tetradactyla* and *M. tridactyla* had the highest tick species richness (20 tick species each one), while *T.*

Table 2
Frequency by ticks species, number of specimens by stage and intensity of infestation on Myrmecophagidae anteaters.

Anteater species	Tick species	No. of animals	Frequency ¹	Number of ticks			Intensity ²			Reference				
				Total Adults			Nymphs	Larvae	mean ± SE		median	range		
				all	males	females								
<i>Myrmecophaga tridactyla</i> n = 216	<i>Amblyomma nodosum</i>	84	38.9	479	476 [†]	316	93	0	0	5.7 [*]	1	1–58	Acosta et al., 2016; Bechara et al., 2002; da Costa-Pinheiro et al., 2015; Dantas-Torres et al., 2010a; DeBárbora et al., 2012; Frank et al., 2012; Guglielmine and Nava, 2006; Labruna et al., 2002; Machado et al., 2018; Martins et al., 2004; Martins et al., 2015b; Szabó et al., 2019; Garcia et al., 2013; Witter et al., 2016; Cançado et al., 2017; Machado et al., 2018; Martins et al., 2015b, 2016; Nava et al., 2014; Szabó et al., 2019; Witter et al., 2016	
	<i>A. sculptum</i>	92	42.6	1472	1071 [†]	669	264	382	7	16 [*]	*	1–105	Cançado et al., 2017; Machado et al., 2018; Martins et al., 2015b, 2016; Nava et al., 2014; Szabó et al., 2019; Witter et al., 2016	
	<i>A. cajemense</i> s.l.	40	18.5	605 [§]	236	150	86	349	0	0	15.1 [*]	*	1–79	Bechara et al., 2002; Botelho et al., 1989; Labruna et al., 2002; Machado et al., 2018; Martins et al., 2004; Garcia et al., 2013
	<i>A. calcaratum</i>	41	18.9	451	450	300	149	1	0	0	11 [*]	*	1–45	Arzua et al., 2005; Botelho et al., 1989; Cutolo et al., 2000; Esser et al., 2016b; Guglielmine and Nava, 2006; Jones et al., 1972; Labruna et al., 2005a; Martins et al., 2015b; Szabó et al., 2019
	<i>A. parvum</i>	18	8.3	103	103 [†]	21	65	0	0	0	5.7 [*]	*	1–23	Cançado et al., 2017; Martins et al., 2004; Szabó et al., 2019
	<i>A. dubidatum</i>	5	2.3	69	13	3	10	39	17	17	13.8 ± 8.3	11	6–28	DeBárbora et al., 2012
	<i>Rhipicephalus microplus</i>	6	2.8	150	50	22	28	97	3	3	25 [*]	*	1–122	Martins et al., 2015b; Szabó et al., 2019
	<i>A. pseudoconcolor</i>	4	1.8	5	5	3	2	0	0	0	1.5 ± 0.7	1.5	1–2	Botelho et al., 1989
	<i>A. cajemense</i> s.s.	3	1.4	42	42	3	39	0	0	0	14 ± 18.3	6	1–35	Martins et al., 2016; Nava et al., 2014; Witter et al., 2016
	<i>A. sculpturatum</i>	3	1.4	5	5	5	0	0	0	0	1.7 ± 1.1	1	1–3	Labruna et al., 2005b
	<i>A. auricularium</i>	2	0.9	7	7	7	0	0	0	0	3.5 ± 3.5	3.6	1–6	Guglielmine et al., 2003; Szabó et al., 2019
	<i>A. pictum</i>	2	0.9	3	3	2	1	0	0	0	1.5 ± 0.7	1.5	1–2	Esser et al., 2016b; Fairchild et al., 1966
	<i>A. ovale</i>	2	0.9	2	2 [†]	0	1	0	0	0	1 ± 0	1	1	Cançado et al., 2017; Szabó et al., 2019
	<i>A. triste</i>	2	0.9	2	2	1	1	0	0	0	1	1	1	Labruna et al., 2002; Szabó et al., 2019
	<i>A. brasiliense</i>	1	0.5	1	0	0	0	1	0	1	1	1	1	Martins et al., 2015b
	<i>A. neumanni</i>	1	0.5	4	1	1	0	3	0	0	4	4	4	Guglielmine and Nava, 2006
	<i>A. oblongoguttatum</i>	1	0.5	1	1 [†]	0	0	0	0	0	1	1	1	Esser et al., 2016b
	<i>A. tapirellum</i>	1	0.5	6	6	3	3	0	0	0	6	6	6	Fairchild et al., 1966
<i>A. tonellae</i>	1	0.5	2	2	1	1	0	0	0	2	2	2	Nava et al., 2014	
<i>A. nodosum</i>	8	33.3	128	122	104	18	0	6	6	16 ± 20.1	4	1–56	Bermúdez et al., 2010; Fairchild et al., 1966; Rodríguez-Vivas et al., 2016	
<i>A. auricularium</i>	5	20.8	42	39	19	20	0	3	3	8.4 ± 10.6	4	2–27	Bermúdez et al., 2010; Fairchild et al., 1966; Tonn et al., 1963	
<i>A. calcaratum</i>	4	16.7	25	25	18	7	0	0	0	6.2 ± 4.6	6.5	1–11	Bermúdez et al., 2010; Fairchild et al., 1966; Guzmán-Cornejo et al., 2006	
<i>A. mixtum</i>	4	16.7	11	5	3	2	3	3	3	2.75 ± 1.5	3	1–4	Rodríguez-Vivas et al., 2016	
<i>Tamandua mexicana</i> n = 24	<i>R. sanguineus</i> s.l.	3	12.5	3 [§]	2	0	2	0	0	1 ± 0.0	1	1	Bermúdez et al., 2010, 2016	
	<i>A. naponense</i>	1	4.2	1	1	0	1	0	0	1	1	1	Fairchild et al., 1966	
	<i>A. parvum</i>	1	4.2	1	1	0	1	0	0	1	1	1	Esser et al., 2016b	
	<i>Haemaphysalis juxtacochi</i>	1	4.2	1	0	0	0	1	0	1	1	1	Esser et al., 2016a	
	<i>A. nodosum</i>	77	39.9	787	787 [†]	576	160	0	0	0	10.2 [*]	*	1–100	Almeida et al., 2013; Arzua et al., 2005; Bastos et al., 2016; Bitencourth et al., 2007; Forlano et al., 2013; Gomes-do Nascimento et al., 2017; Guglielmine and Nava, 2006; Ivancovich, 1987; Labruna et al., 2002, 2005a; Luque, 1948; Luz et al., 2018; Martins et al., 2004, 2015b, 2017a; Moerbeck et al., 2018; Soares-Pereira et al., 2015; Szabó et al., 2019; Garcia et al., 2013; Witter et al., 2016
<i>tetradactyla</i>	<i>A. godditi</i>	66	34.2	602	602 [†]	421	162	0	0	9.1 [*]	*	2–177	Binetruy et al., 2019; Giamzella et al., 2018; Gomes-do Nascimento et al., 2017; Martins et al., 2015a; Soares-Pereira et al., 2015	

(continued on next page)

Table 2 (continued)

Anteater species	Tick species	No. of animals	Frequency ¹	Number of ticks				Intensity ²			Reference		
				Total Adults		Nymphs		Larvae	mean ± SE	median		range	
				all	males	all	males	all	SE	range			
n = 193	<i>A. calcaratum</i>	46	23.8	194	193	139	53	1	0	4.2*	*	1 – 29	Arzua et al., 2005; Moraes-Barros and Baggio, 1992; Brum et al., 2003; Cáceres et al., 2002; Evans et al., 2000; Jones et al., 1972; Labruna et al., 2005a; Lamattina et al., 2018; Lima-Gonzalez et al., 2017; Martins et al., 2015b, 2017a, 2017b; Sangaletti-Lavina et al., 2011; Soares-Pereira et al., 2015; Spolidorio et al., 2012; Witter et al., 2016; Zimmer et al., 2002
	<i>A. sculptum</i>	14	7.2	151	17†	3	7	134	0	10.8*	*	1 – 59	Cançado et al., 2017; Martins et al., 2015b; Nava et al., 2014; Szabó et al., 2019; Witter et al., 2016
	<i>A. humerale</i>	7	3.6	30	2	1	1	28	0	4.3*	*	1 – 23	Binetruy et al., 2019; Gianizella et al., 2018; Gomes-do Nascimento et al., 2017; Soares-Pereira et al., 2015; Witter et al., 2016
	<i>A. cajiennense</i> s.l.	6	3.1	41	7†	2	4	34	0	6.8*	*	1 – 6	Labruna et al., 2002; Spolidorio et al., 2012; Garcia et al., 2013
	<i>A. parvum</i>	6	3.1	13	12†	1	6	1	0	2.7*	*	1 – 6	Cançado et al., 2017; Dantas-Dantas-Torres et al., 2010b; Martins et al., 2004; Witter et al., 2016
	<i>A. auricularium</i>	3	1.5	43	16	10	6	20	7	14.3 ± 12.0	15	2 – 26	Dantas-Torres et al., 2010b; Guglielmo et al., 2003; Oliveira et al., 2017
	<i>A. brasiliense</i>	3	1.5	5	0	0	0	5	0	1.7*	*	1 – 4	Lamattina et al., 2018; Martins et al., 2015b
	<i>A. geayi</i>	3	1.5	19	19	12	7	0	0	6.3 ± 4	4	4 – 11	Gianizella et al., 2018
	<i>R. sanguineus</i> s.l.	3	1.5	9	8	2	6	1	0	3 ± 1.7	2	2 – 5	Martins et al., 2017a; Oliveira et al., 2017; Szabó et al., 2019
	<i>A. cajiennense</i> s.s.	2	1.0	2	2	0	2	0	0	1	1		Binetruy et al., 2019; Nava et al., 2014
	<i>A. coelebs</i>	2	1.0	6	0	0	0	6	0	3*	*		Lamattina et al., 2018
	<i>A. aureolatum</i>	1	0.5	1	1	0	1	0	0	1			Arzua et al., 2005
	<i>A. dubitatum</i>	1	0.5	302	0	0	0	1	301	302			Dantas-Torres et al., 2010b
	<i>A. naponense</i>	1	0.5	4	0	0	0	4	0	4			Gomes-do Nascimento et al., 2017
	<i>A. pseudoncolor</i>	1	0.5	1	1	0	1	0	0	1			Guimarães et al., 2001
	<i>A. rotundatum</i>	1	0.5	1	1	0	1	0	0	1			Moraes-Barros and Baggio, 1992
	<i>A. tonelliae</i>	1	0.5	1	1	0	1	0	0	1			Nava et al., 2014
	<i>A. varium</i>	1	0.5	1	1	0	1	0	0	1			Gianizella et al., 2018
	<i>Ixodes fuscipes</i>	1	0.5	2	0	0	0	2	0	2			Gianizella et al., 2018



¹ Percentage of infested animals: number of individuals of one anteater species with at least one specimen of certain tick species divided by the total number of individuals examined of that anteater species.

² Number of ticks per infested animal.

† Some ticks of this stage were recorded only as adults, but gender were unspecified.

‡ Tick presence was recorded but stage was unspecified.

* Standard deviation and median cannot be calculated because some records were reported summarizing the results of several individuals, and so, individual records were not available in those cases.

♦ Include the ten tick-positive road-killed individuals from this study.

mexicana had the lesser (11 tick species). Individual tick species richness were recorded only on 276 of the 433 anteaters (64%), of which 187 individuals had a richness of one tick species (43%; 187/433), 72 had two tick species (17%; 72/433), 16 had three tick species (4%; 16/433), and only one host had four species (< 1%; 1/433). Tick species richness data from 157 anteaters were not included because infestations were not presented individually for each anteater.

Tick species frequencies within each anteater species and infestation intensity on the three Myrmecophagidae anteaters are shown in Table 2. The majority of anteaters with tick infestation intensity records were described on *M. tridactyla* (216 individuals), followed by *T. tetradactyla* (193), and *T. mexicana* (24). According to the literature search, the source of the 433 anteaters analyzed included 202 individuals kept in captivity (three *T. mexicana*, 96 *T. tetradactyla*, and 103 *M. tridactyla*), 137 animals that were road-killed (14 *T. mexicana*, 45 *T. tetradactyla*, and 78 *M. tridactyla*) and 94 without origin description (seven *T. mexicana*, 52 *T. tetradactyla*, and 35 *M. tridactyla*). Among anteaters without origin description, kept in captivity and road-killed, median tick intensities (interquartile range) were 3 (1–7), 6 (1–20) and 18 (13–18), respectively. When tick intensities were compared by origin of anteater, differences were found between those kept in captivity and those without origin description (Mann-Whitney, $p = 0.035$), also between those without origin description and road-killed ($p = 0.000$), and between kept in captivity and road-killed ($p = 0.000$).

Tick infestation intensity data on *A. pictum* from *T. tetradactyla* (Fairchild et al., 1966), and on *A. geayi*, *A. oblongoguttatum* and *A. ovale* from *T. mexicana* (Fairchild et al., 1966; Esser et al., 2016b) were not presented, because no data were found or intensities were recorded without the number of examined hosts. A single record of *A. maculatum* on *M. tridactyla* from Brazil was found (Botelho et al., 1989), but it was ruled out because do not correspond with the geographic range of this tick species (Estrada-Peña et al., 2005). Twenty-four tick species combinations were reported in individuals infested with two or more tick species, and the tick species most frequently associated with other tick species were *A. nodosum* and *A. calcaratum*. For details about ticks combinations see Table 3.

4. Discussion

The study of ticks from *T. mexicana* here presented is the first carried out from a population perspective and, also, the one with the highest number of northern tamanduas analyzed. Five tick species were identified, and although some of these tick species have been previously reported on the species by Guzmán-Cornejo et al. (2006) and Rodríguez-Vivas et al. (2016) in Mexico, and by Bermúdez et al. (2010) in Panama, this is the first time that *A. auricularium* and *R. sanguineus* s.l. are cited on this host species in Mexico. Moreover, our study analyses for the first time the relationship between tick infestation and host sex.

This study also shows, based on our results and those from other authors, that the northern tamandua has the lowest tick richness (11) of the three Myrmecophagidae anteaters. This could be partly due to differences in the research effort dedicated to anteater species, since *T. mexicana* has been the least anteater studied, based on the number of published papers on the species (10) and the mean number of individuals analyzed per study (1.7). Moreover, all previous publications in this species are incidental reports and none were epidemiological and population-based studies (Guzmán-Cornejo et al., 2006; Esser et al., 2016a, b; Rodríguez-Vivas et al., 2016). In contrast, the present study was performed with a comparatively large number of northern tamanduas, so our findings may provide an accurate estimate of tick infestation prevalence, species richness and tick infestation intensity on the species distributed in Mexico. Notwithstanding this, we cannot rule out that our study underestimates tick infestation on *T. mexicana* and maybe we have lost some tick specimens if they detached from the

carcass immediately after the animals were killed. However, ticks are known to remain attached to the dead host's for a long time (Petney et al., 2017). In this respect, it is unlikely that the use of road-killed animals was a significant source of bias in the present study, and in fact, median tick intensity on roadkilled anteaters was significantly greater than on captive animals and on those of unknown origin. However, the cause for this is unclear. In addition, several reasons may explain the low research effort dedicated to the northern tamandua, which has already been mentioned by Muñoz-García et al. (2019), including: since this is a non-threatened species by the IUCN (Superina et al., 2010) maybe it is not a species of worldwide concern, or, even it is threatened by Mexican laws (Semarnat, 2010), it is difficult to obtain funding resources to carry out studies on wildlife species in Mexico (Muñoz-García et al., 2018).

The literature review had revealed some resemblances between tick infestations in the southern anteater species (*T. tetradactyla* and *M. tridactyla*), since both show the same tick species richness (20 each one). And other relevant findings are the noticeable host association of *A. nodosum*, *A. calcaratum* and *A. auricularium* to the three Myrmecophagidae anteaters. Moreover, *A. nodosum* was the predominant over other tick species on the three anteater species. This finding supports previous ones of the host preference of adult *A. nodosum* for *T. tetradactyla* and *M. tridactyla* (De Quadros et al., 2010; Forlano et al., 2013; da Costa-Pinheiro et al., 2015), and now it is also proved the preference for *T. mexicana*. Importantly, *A. nodosum* ticks have been reported infected with *Rickettsia* spp. (Almeida et al., 2013). Also *Rickettsia* spp. characterization at species level has occurred from immature *A. nodosum* specimens collected from Passeriformes birds, and among the two species found, *R. parkeri* and *R. bellii*, the former is zoonotic and the pathogenicity of the latter is unknown (Ogrzewalska et al., 2011). More recently, a *Rickettsia* sp. phylogenetically close to strain NOD was isolated from an adult *A. nodosum* specimen collected from a Brazilian *T. tetradactyla* (Moerbeck et al., 2018). More studies should be conducted to elucidate if Myrmecophagidae anteaters are involved in *Rickettsia* spp. transmission cycles.

Other frequently recorded tick species on the three Myrmecophagidae anteaters is *A. calcaratum*, whose adult stage is considered with high-affinity to this family (Bloemer et al., 1987). This tick species has a wide distribution, ranging from the USA (Kentucky) to northern Argentina (Bloemer et al., 1987). Immature stages of *A. calcaratum* have been reported infesting humans and Momotidae birds (da Costa-Pinheiro et al., 2015), and Thraupidae birds (Ogrzewalska et al., 2013); but also have been reported in the southern anteaters, a nymph on a *T. tetradactyla* from Venezuela (Jones et al., 1972) and a nymph on a *M. tridactyla* from Brazil (Arzua et al., 2005). Interestingly, the *Rickettsia* sp. strain NOD aforementioned, detected by Moerbeck et al. (2018) in the *A. nodosum* specimen, was also detected by Ogrzewalska et al. (2013) in four *A. calcaratum* nymphs collected from three Brazilian tanagers (*Ramphocelus carbo*). So, due to its probable role as *Rickettsia* sp. vector, it is important to search for this and other pathogens in *A. calcaratum* specimens collected from anteaters.

Finally, *A. auricularium* was also recorded on the three anteater species, being the second most frequent ixodid on the Mexican road-killed northern tamanduas. This tick species is a primary parasite of Xenarthra mammals and, also, is commonly found on armadillos (Dasypodidae), specifically the nine-banded armadillo (Guglielmine et al., 2003; Bermúdez et al., 2010; Dantas-Torres et al., 2010b; Friciello-Teixeira et al., 2013). This tick has also been identified as carrier of *Rickettsia* sp., specifically of the species *R. amblyommatis* (formerly “*Candidatus R. amblyommii*”) (Saraiva et al., 2013). *Rickettsia amblyommatis* has been isolated in Brazil by Saraiva et al. (2013) from an adult *A. auricularium* collected from a skunk (*Conepatus semistriatus*), and also was molecularly identified from eight adult specimens collected from two yellow armadillos (*Euphractus sexcinctus*).

Some other *Amblyomma* species that also seem to be common on Myrmecophagidae anteaters are *A. cajennense* s.l. and *A. parvum*. The

Table 3
Tick species combinations recorded on Myrmecophagidae anteaters.

Tick combinations	Number of individuals registered			References	
	Total	<i>Myrmecophaga tridactyla</i> 	<i>Tamandua tetradactyla</i> 		<i>T. mexicana</i> 
<i>Amblyomma nodosum</i> – <i>A. sculptum</i>	32	27	5	Szabó et al., 2019; Witter et al., 2016	
<i>A. calcaratum</i> – <i>A. sculptum</i>	11	10	1	Martins et al., 2015b	
<i>A. calcaratum</i> – <i>A. nodosum</i>	9	3	6	Guglielmone and Nava, 2006; Labruna et al., 2005a; Martins et al., 2015b; Szabó et al., 2019; Witter et al., 2016	
<i>A. cajennense</i> s.l. – <i>A. nodosum</i>	5		5	Labruna et al., 2002; Garcia et al., 2013	
<i>A. cajennense</i> s.l. – <i>A. parvum</i>	5	5		Martins et al., 2004	
<i>A. cajennense</i> s.l. – <i>A. calcaratum</i>	1	1		Botelho et al., 1989	
<i>A. calcaratum</i> – <i>Rhipicephalus microplus</i>	1	1		Martins et al., 2015b	
<i>A. auricularium</i> – <i>A. mixtum</i>	1			1	Recorded in the present study
<i>A. mixtum</i> – <i>A. nodosum</i>	1			1	Rodríguez-Vivas et al., 2016
<i>A. auricularium</i> – <i>A. nodosum</i>	1			1	Recorded in the present study
<i>A. nodosum</i> – <i>A. parvum</i>	1	1		Martins et al., 2004	
<i>A. nodosum</i> – <i>R. sanguineus</i> s.l.	1		1	Szabó et al., 2019	
<i>A. cajennense</i> s.l. – <i>A. sculptum</i>	1	1		Machado et al., 2018	
<i>A. sculptum</i> – <i>A. triste</i>	1	1		Szabó et al., 2019	
<i>A. geayi</i> – <i>A. varium</i>	1		1	Gianizella et al., 2018	
<i>A. cajennense</i> s.s. – <i>A. cajennense</i> s.l. – <i>A. sculptum</i>	1	1		Martins et al., 2016	
<i>A. calcaratum</i> – <i>A. nodosum</i> – <i>A. sculptum</i>	8	6	2	Martins et al., 2015b; Szabó et al., 2019; Witter et al., 2016	
<i>A. nodosum</i> – <i>A. parvum</i> – <i>A. sculptum</i>	2	1	1	Szabó et al., 2019; Witter et al., 2016	
<i>A. auricularium</i> – <i>A. nodosum</i> – <i>A. sculptum</i>	1	1		Szabó et al., 2019	
<i>A. nodosum</i> – <i>A. cajennense</i> s.l. – <i>A. sculptum</i>	1	1		Machado et al., 2018	
<i>A. calcaratum</i> – <i>A. nodosum</i> – <i>R. sanguineus</i> s.l.	1		1	Martins et al., 2017a	
<i>A. calcaratum</i> – <i>A. nodosum</i> – <i>A. ovale</i>	1	1		Szabó et al., 2019	
<i>R. microplus</i> – <i>A. nodosum</i> – <i>A. sculptum</i>	1	1		Szabó et al., 2019	
<i>A. calcaratum</i> – <i>A. nodosum</i> – <i>A. sculptum</i> – <i>A. humerale</i>	1		1	Witter et al., 2016	
Total	89	62	24	3	

first currently represents a complex of distinct taxonomic units (Beati et al., 2013), distributed in the Nearctic and Neotropical regions, while the latter has been recorded only in the Neotropics (Guglielmone and Nava, 2006). Both ticks are host-generalists found on domestic and wild mammals (Beati et al., 2013; Guglielmone and Nava, 2006). *Amblyomma cajennense* s.l. is the main vector of the Brazilian spotted fever caused by *R. rickettsii* (Krawczak et al., 2014), and *A. parvum* has been reported in Mexico infected with *R. rickettsii*, but its importance as disease vector is unknown (Dzul-Rosado et al., 2013). Since Myrmecophagidae anteaters are commonly infested by this tick species, its possible role as hosts of spotted fever group rickettsiae should be studied.

Lastly, other tick species recorded on the three anteaters was *R. sanguineus* s.l. - the brown dog tick (Bermúdez et al., 2010, 2016; Coelho, 2016; Martins et al., 2017a; Oliveira et al., 2017; Szabó et al., 2019) that is the most widespread tick in the world (Dantas-Torres, 2010). The existence of this species together with *R. microplus*, detected on *M. tridactyla* (Martins et al., 2015b; Szabó et al., 2019), could be linked to the presence of anteaters in anthropized habitats, since are ticks commonly reported on dogs and cattle, respectively. This is not surprising given that the natural habitat occupied by anteaters has been drastically altered (Superina et al., 2010), and highlights the risk of becoming parasitized by pathogens of domestic animals, particularly ectoparasites with low host specificity such as some tick species (Nava and Guglielmone, 2013).

The impact of ticks in anteaters health, populations, fitness and the host role on tick ecology needs to be further studied. Moreover, since ticks are specialized vectors of microorganisms causing diseases, the

potential role of anteaters ticks as vector-borne pathogens needs to be investigated. Among the tick species recorded in the present, *R. sanguineus* s.l. and *R. microplus* are possibly dangerous for anteaters because are involved in the transmission of many human and animal pathogens. Concretely, *R. sanguineus* s.l. is vector of *R. rickettsii*, *R. conorii*, *Ehrlichia canis* and *Coxiella burnetii* (Dantas-Torres et al., 2010b), while *R. microplus* transmits *Babesia* spp. and *Anaplasma marginale* (Rodríguez-Vivas et al., 2013). On the other hand, *Amblyomma* species with affinity to anteaters have been recently found as carriers of *Rickettsia* spp., for example: Almeida et al. (2013) found by PCR a 30.7% (4/13) frequency of *Rickettsia* spp. in *A. nodosum* specimens collected on two *T. tetradactyla* from Brazil; and most recently, *R. parkeri* NOD and *R. bellii* were detected by PCR and sequencing from five and three *A. nodosum* specimens, collected from five *M. tridactyla* and two *T. tetradactyla*, respectively, in Brazil (Szabó et al., 2019). Also *R. parkeri* NOD was detected from two *A. sculptum* collected from a *M. tridactyla* in Brazil (Szabó et al., 2019). Intriguingly, some other studies have searched for *Rickettsia* spp. in tick specimens collected from two Brazilian *T. tetradactyla*, one *A. nodosum* and eight *A. cajennense* s.l., without success (Spolidorio et al., 2012; Soares-Pereira et al., 2015). Until now, *Rickettsia* spp. or other pathogens have never been searched in tick specimens collected from *T. mexicana*.

5. Conclusions

Myrmecophagidae anteaters are hosts to a wide range of tick species, with a predominance of *Amblyomma* spp. However, more research is needed in *Tamandua mexicana* because is the least studied species.

Generally, there is a scarcity of population-based studies that attempt to identify risk factors for infestation, impact on anteaters health and the role of these hosts as reservoirs of pathogens of veterinary and Public Health importance. It is further important that new studies on ectoparasites of anteaters should also take into account ecological, biogeographical and evolutionary approaches.

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Appendix A. Supplementary data

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