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Parasitic fauna of bats from Costa Rica

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ABSTRACT

Bats are important reservoirs and spreaders of pathogens, including those of zoonotic concern. Though Costa Rica hosts one of the highest bat species' diversity, no information is available about their parasites. In order to investigate the occurrence of vector-borne pathogens (VBPs) and gastrointestinal (GI) parasites of chiropterans from this neotropical area, ectoparasites ($n = 231$) and stools ($n = 64$) were collected from 113 bats sampled in Santa Cruz (site 1) and Talamanca (site 2). Mites, fleas and ticks were morphologically and molecularly identified, as well as pathogens transmitted by vectors (VBPs, i.e., *Borrelia* spp., *Rickettsia* spp., *Bartonella* spp.) and from feces, such as *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. were molecularly investigated. Overall, 21 bat species belonging to 15 genera and 5 families were identified of which 42.5% were infested by ectoparasites, with a higher percentage of mites (38.9%, i.e., *Cameronieta* sp. and *Mitonyssoides* sp.) followed by flies (2.6%, i.e., *Joblingia* sp.) and tick larvae (1.7%, i.e., *Ornithodoros* sp.). *Rickettsia* spp. was identified in one immature tick and phylogenetically clustered with two *Rickettsia* species of the Spotted Fever Group (i.e., *R. massiliae* and *R. rhipicephali*). The frequency of GI parasite infection was 14%, being 3.1% of bats infected by *Giardia* spp. (unidentified non-*duodenalis* species), 1.5% by *Eimeria* spp. and 9.4% by *Cryptosporidium* spp. (bat and rodent genotypes; one *C. parvum*-related human genotype). The wide range of ectoparasites collected coupled with the detection of *Rickettsia* sp., *Giardia* and *Cryptosporidium* in bats from Costa Rica highlight the role these mammals may play as spreaders of pathogens and the need to further investigate the pathogenic potential of these parasites.

1. Introduction

Bats represent one of the most diverse order of mammals, second only to rodents (Simmons, 2005), with one of the highest species' diversity occurring in the Neotropical Region (Willig and Selcer, 1989). Many of the species included in the order Chiroptera have a crucial ecological role since they contribute to the reduction of insect populations, pollination and seed dispersal (Kunz et al., 2011). In addition, bats may be reservoirs and spreaders of pathogens, as they inhabit a variety of habitats (e.g., caves, ravines, niches, human buildings), move from sylvatic to urban areas and share shelter with other animal species, including humans (Saoud and Ramadan, 1976; Santana Lima et al., 2018; Sándor et al., 2019).

Bats may be infested and harbor a large diversity of ectoparasites (Panti-May et al., 2021), such as flies (Diptera: Nycteribiidae and Streblidae), fleas (Siphonaptera: Ischnopsyllidae), mites (Mesostigmata:

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Spinturnicidae, Macronyssidae, Laelapidae and Spelaeorhynchidae) and ticks (e.g., *Argas* spp., *Carios* spp., *Ixodes* spp., and *Ornithodoros* spp.) (Sonenshine and Anastos, 1960; Marshall, 1982; Bruyndonckx et al., 2009; Labruna and Venzal, 2009; Hornok et al., 2015; Szentiványi et al., 2016). Although flies and mites have established a close relationship with their hosts (Marshall, 1982; Fritz, 1983) some of the pathogens they transmit are of zoonotic concern, raising questions about the role these mammals play as reservoirs (Allocati et al., 2016). Accordingly, *Borrelia burgdorferi* sensu lato was detected in the bat mite *Spinturnix americanus* from Canada (Banerjee et al., 2020) and in ticks feeding on cave-dwelling bats from Romania and Poland (Michalik et al., 2020). In addition, Hippoboscoidea flies collected from bats sampled in different countries worldwide (e.g., United Kingdom, Kenya, Guatemala, Costa Rica and Brazil) were found positive for *Bartonella* spp. (Concannon et al., 2005; Kosoy et al., 2010; Bai et al., 2011; Judson et al., 2015; Wray et al., 2016; do Amaral et al., 2018), whereas *Rickettsia* spp. were detected in both soft (e.g., *Argas vespertilionis*, *Ornithodoros hasei*) and hard (e.g., *Ixodes ricinus*) ticks infesting bats (Reeves et al., 2006; Socolovschi et al., 2012; Piksa et al., 2016; Dietrich et al., 2016; Tahir et al., 2016). Other *Rickettsia* spp. of the Spotted Fever Group (SFG) were also found in bat flies from the USA (Lack et al., 2011), Malaysia (Wilkinson et al., 2016) and Costa Rica (Moreira-Soto et al., 2017). Moreover, many species of gastrointestinal (GI) protozoa can be harbored by bats, with coccidia (e.g., Eimeriidae), and Gregarinomorphea (e.g., Cryptosporidiidae) (McAllister and Upton, 2009; Kváč et al., 2015) as the most common ones, including *Cryptosporidium parvum*, *Cryptosporidium hominis* (Kváč et al., 2015; Schiller et al., 2016), *Entamoeba* spp. and *Giardia* spp. (Santana Lima et al., 2018; Adhikari et al., 2020).

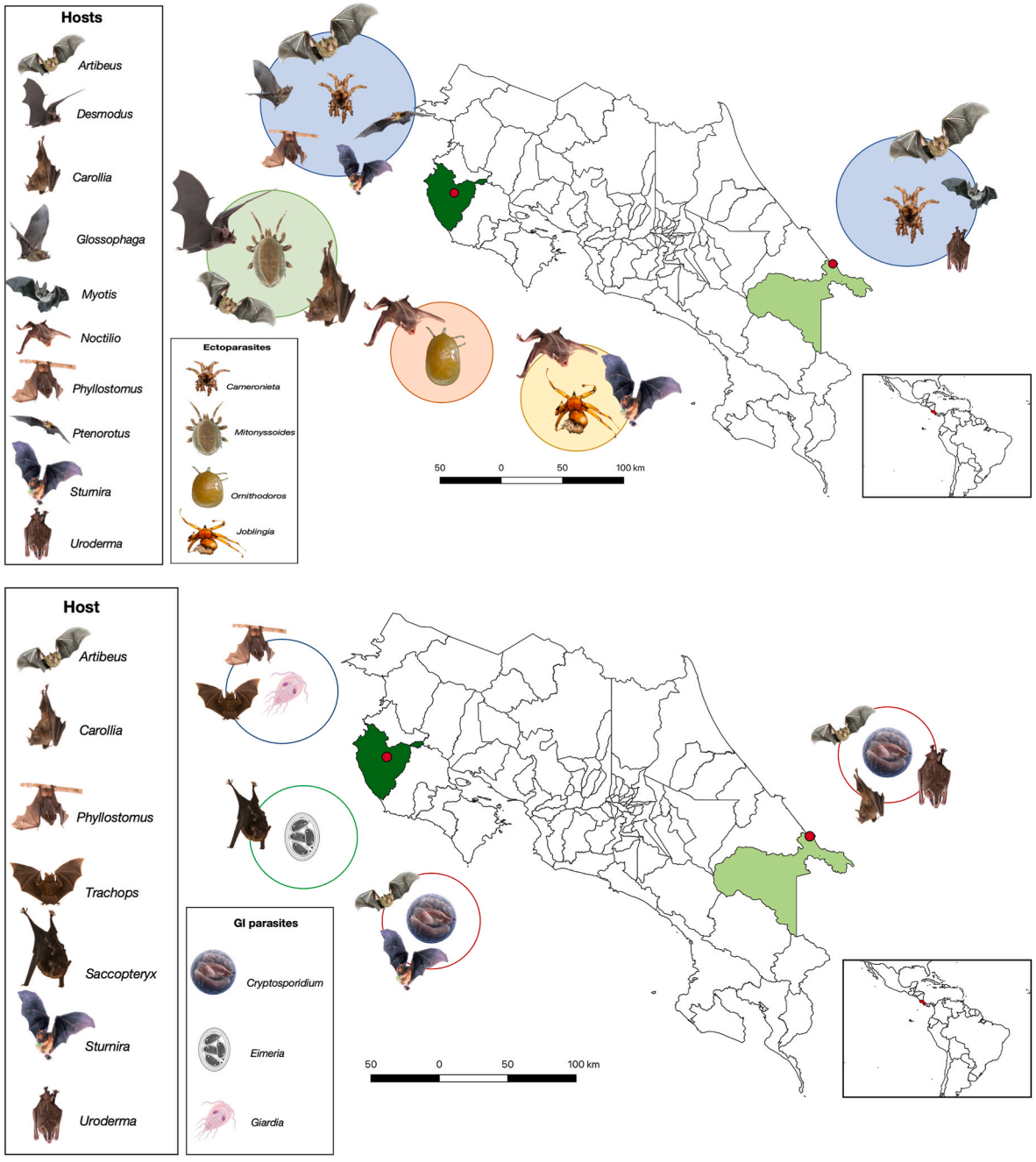

Fig. 1. Map showing the distribution of bats sampled from site 1 (Santa Cruz, dark green) and site 2 (Talamanca, light green), related to ectoparasites a) and GI parasites b). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Overall, data above suggest that bats may play a role as reservoirs of zoonotic vector-borne pathogens (VBPs) (Allocati et al., 2016) and spreaders of GI protozoa in the environment through guano (Allocati et al., 2016).

While some studies were conducted on endo and ectoparasite fauna of bats (Frank et al., 2014), no data are available for Costa Rica, a geographic region characterized by tropical forests edging anthropized areas and hosting one of the highest bat species' diversity (Willig and Selcer, 1989; Echeverri et al., 2022). Therefore, the occurrence of VBPs in bat ectoparasites from Costa Rica was herein investigated along with GI pathogens, in order to better understand the public health risk coming from these mammals.

2. Material and methods

2.1. Sample collection

Bats (n = 113) were collected in Santa Cruz (10°16′00"N - 85°39′00"W) (Northern Costa Rica, site 1) between August 2017 and April 2018, and in Talamanca (09°44′28"N – 82°50′46"W) (Southern Caribbean, site 2) between July and November 2018 (Fig. 1), using mist-netting, which were settled at sunset in rural areas close to the urban agglomerates. Bats were brought to the laboratory in individual cotton bags where they were identified to species level using the morphological keys by York et al. (2019). Gender was recorded for each individual, then, bats were euthanized via intramuscular anesthesia overdose using ketamine (10 mg/kg) plus xylazina (1 mg/kg) American Veterinary Medical Association (2020). During the post-mortem examination they were carefully examined for the presence of ectoparasites using an EyeMag magnifying glass (Zeiss). Moreover, approximately 2 g of feces were obtained from the rectum of 64 individuals using a sterile plastic spoon. Ectoparasites (n = 231) and fecal samples (n = 64) were both stored in individual tubes in 70% ethanol. The capture and sampling of bats were authorized by the National System of Conservation Areas of Costa Rica (SINAC ACT-PIM-070-17, R-SINAC-PNI-ACLAC-054-2018) and the protocol for euthanasia approved by the Institutional Committee for the Care and Use of Animals of the University of Costa Rica (CICUA-042-17).

2.2. Identification of ectoparasites

The ectoparasites were identified using morphological and molecular methods. For the morphological evaluation the material of each tube was placed into Petri dishes and identified by optical observation using a stereomicroscope. After separating mites, tick larvae (single stage collected) and flies, at least one ectoparasite (more than one in case of co-infestation and/or presence of both sexes) per each bat infested were mounted and cleared on a glass slide using Hoyer's medium. For fly identification, in order to better observe morphological features, given the minute size of specimens collected, each individual was mounted in a slide using Hoyer's medium following the Stern and Sucena (2012) protocol, given that Hoyer's solution is appropriate for delicate and small organisms (Whitakerjr et al., 2009). After 3 days at 25 °C, slides were investigated by light microscopy and dichotomous keys, as well as original descriptions, were used for the morphological identification at genera level of each ectoparasite mounted (Radovsky, 1967; Herrin and Tipton, 1975; Yunker et al., 1990; Hoskins, 1991; Graciolli and Dick, 2012; Barros-Battesti et al., 2013; Almeida et al., 2011; Szentiványi et al., 2019).

For the molecular identification and pathogen detection the DNA was extracted from 5 ectoparasites, selected randomly (i.e., ticks, mites and flies) (Banerjee et al., 2020; Concannon et al., 2005; Reeves et al., 2006), per each bat that was infested by more than 10 ectoparasites. On the other hand, 2 spinturnicid mites were randomly selected and molecularly identified, using a modified guanidine isothiocyanate protocol, as described elsewhere (Chomkzynski, 1993). Conventional PCR (cPCR) was performed for molecular identification of tick larvae (n = 10) as well as of mites (n = 7) and flies (n = 5), targeting 16S rRNA gene (300 bp) and 18S rRNA gene (464–490 bp), respectively (Burlini et al., 2010; Sourassou et al., 2015).

For tick larvae identification, the PCR protocol by Burlini et al. (2010) was slightly modified as follows: 95 °C for 10 min initial denaturation, followed by 35 cycles of 94 °C for 45 s, 58 °C for 1 min, and 72 °C for 1 min, and then 72 °C for 7 min for the final elongation.

Amplified PCR products were visualized by gel-electrophoresis in 2% agarose gel containing GelRed nucleic acid gel stain (VWR International PBI, Milan, Italy) and viewed on a GelLogic 100 gel documentation system (Kodak, New York, USA). Negative (i.e., ultra-pure sterile water) and positive controls (i.e., *Rhipicephalus sanguineus* s.l., *Caparinia* sp.) were included in all PCR runs.

2.3. Molecular detection of VBPs

Ectoparasite DNA samples (n = 20) were screened for *Borrelia* spp. and *Rickettsia* spp. by cPCR targeting the *flagelin* gene (482bp) and the *gltA* gene (401bp), respectively (Wójcik-Fatla et al., 2009; Sgroi et al., 2021). Positive samples for *Rickettsia* spp. were tested by a second cPCR targeting the *ompA* gene (632bp) present only in SFG rickettsiae (Sgroi et al., 2021).

For *Borrelia* spp. detection, the PCR protocol by Wójcik-Fatla et al. (2009) was slightly modified as follows: 95 °C for 5 min initial denaturation, followed by 40 cycles of 94 °C for 30 s, 54 °C for 1 min, and 72 °C for 45 s, and then 72 °C for 7 min for final elongation.

Amplified PCR products were visualized as described above. To detect *Bartonella* spp. DNA, transfer-mRNA ssrA specific qPCR was performed as previously described (Diaz et al., 2012). Negative (i.e., ultra-pure sterile water) and positive controls (i.e., *Rickettsia asembonensis*, *Rickettsia slovaca*, *Borrelia burgdorferi* s.l., *Bartonella clarridgeiae* and *Bartonella henselae*) were used in all PCR runs.

2.4. Molecular detection of protozoan pathogens

Bat fecal samples were centrifugated (13000 rpm × 10 min) to remove the ethyl alcohol (70%). After centrifugation DNA was extracted using a commercial kit (DNeasy PowerSoil Pro Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA quantity of 6 random selected samples was evaluated by the Qubit assay (Applied Biosystems, USA).

For *Giardia duodenalis* detection, qPCR was performed targeting the 18S rRNA gene (62 bp) (Nazeer et al., 2013; Giangaspero et al., 2019). This PCR was positive for two samples, which were then subjected to a duplex qPCR-HRM assay, for further identification, thus detecting and synchronously differentiating between *Giardia duodenalis* Assemblages A and B (Lamien-Meda et al., 2020). This PCR was run on a CFX Connect real-time PCR detection system (BioRad Laboratories, Inc., Singapore) with an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of 95 °C for 5 s, 57 °C for 3 s and 68 °C for 5 s. For high-resolution melting (HRM) curve analysis, the amplicons were denaturated at 95 °C for 1 min, cooled to 65 °C (held for 30 s) and continuously heated at 0.5 °C/s with fluorescence acquisition from 65 °C to 90 °C. As this PCR was negative for both samples, a nested PCR amplifying partial triosephosphate isomerase (*tpi*) gene (532 bp) was used, to detect also all other assemblages (Sulaiman et al., 2003). Conditions for the primary and secondary nested PCRs were identical and consisted of 35 cycles at 94 °C for 45 s, 50 °C for 45 s and 72 °C for 1 min and a final elongation at 72 °C for 7 min. As the samples were also negative at this PCR, a more universal and more sensitive nested PCR (shorter fragment) was performed amplifying a 147 bp fragment of the 18S rRNA gene by 35 cycles of 94 °C for 20 s, 59 °C for 20 s, and 72 °C for 30 s and a final elongation at 72 °C for 7 min (Hopkins et al., 1997).

For detection and identification of *Cryptosporidium* spp., the nested PCR described by Ryan et al. (2003) was used, targeting a fragment of

the 18S rRNA gene. This PCR is able to also detect other coccidians, including *Eimeria* spp. The conditions for the primary and secondary nested PCR were identical and consisted of 45 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s and a final elongation at 72 °C for 7 min.

The nested PCRs were run on an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany) with an initial hot start at 94 °C for 10 min. Amplicons were visualized by 2% agarose gel electrophoresis using GelRed™ (BioTrend, Cologne, Germany), and purified with the PCR and Gel Band Purification kit (illustra GFX, GE Healthcare, Austria).

2.5. Sequencing and phylogenetic analysis

All the positive cPCR products were purified and sequenced in both directions using the same forward and reverse primers, employing the Big Dye Terminator v.3.1 chemistry in a 3130 Genetic analyzer (Applied Biosystems, California, USA) in an automated sequencer (ABI-PRISM 377). Nucleotide sequences were edited, aligned and analyzed using the Geneious platform version 9.0 (Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012), and compared with available sequences in the GenBank database, using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi) for species identification.

The consensus sequences obtained in this study and those retrieved from GenBank were aligned using the Clustal/W software (Thompson et al., 1994) via Bioedit v. 7.0.5.3 (Hall, 2004). Phylogenetic inference was based on Bayesian Inference (BI) and Maximum Likelihood (ML) methods. The ML analysis was inferred with the W-IQ-Tree tool available online (http://iqtree.cibiv.univie.ac.at/) using 8000 bootstrapping replicates (Trifinopoulus et at., 2016). The best evolution model was selected by the program jModelTest2 (version 2.1.6) on XSEDE (Santorum et al., 2014), under the Akaike Information Criterion (AIC). All trees were examined in Treegraph 2.0 beta-software (Stover and Muller, 2010).

2.6. Statistical analysis

Frequency, mean abundance (number of ectoparasites per total number of hosts) and mean intensity (number of ectoparasites per number of infested hosts) of bats were calculated using Quantitative Parasitology software, version 3.0 (Rózsa et al., 2000). Co-occurrence of two or more ectoparasite species on the same bat individual was descriptively analyzed.

3. Results

A total of 113 bats ($n = 61$ from site 1, $n = 52$ from site 2) belonging to the families Emballonuridae, Mormoopidae, Noctilionidae, Phyllostomidae and Vespertilionidae were examined (Table 1). Of the bats above, 57% were females ($n = 37$ from site 1, $n = 27$ from site 2) and 43% males ($n = 24$ from site 1, $n = 25$ from site 2) (Fig. 1). Out of the 113 animals, 42.5% were infested (48/113; 95% CI: 0.34–0.52), with a significantly higher frequency from site 1 (57%, 95% CI: 0.44–0.68) than site 2 (25%, 95% CI: 0.15–0.38). Specifically, 91.6% of infested bats (44/48; 95% CI: 0.8–0.97) presented at least one mite, 4.2% (2/48; 95% CI: 0.01–0.14) one tick larva and 6.2% one fly (3/48; 95% CI: 0.02–0.17). Solely one bat from site 1 was co-infested with tick larvae and flies. Overall, 135 mites, 71 tick larvae and 25 flies were detected with different ectoparasitic loads (Table 2) and infestation rates (Table 1).

Cameronieta sp. (Spinturnicidae) and *Mitonyssoides* sp. (Macronyssidae) (Fig. 2a and b) were the two genera of mites morphologically detected, along with the fly genus *Joblingia* sp. (Streblidae) (Fig. 2c), and the tick genus *Ornithodoros* sp. (Argasidae) (Fig. 2d). Genus and number of ectoparasites identified according to the host species and collection sites (Table 3) are depicted in Fig. 1a.

The consensus sequences of *Cameronieta* sp. detected (97% nucleotide identity with the GenBank sequence FJ911861) grouped with other sequences of the same genus, with high bootstrap support (100%) (Fig. 3), while the sequences of *Mitonyssoides* sp. (96.8% nucleotide identity with the GenBank sequence FJ911824) clustered with those of the Macronyssidae family (*Steatonyssus furmani*) but also the Rhinonyssidae family (*Sternostoma porter*). The phylogenetic analysis of *Joblingia* sp. sequences (94% nucleotide identity with the GenBank sequence OU612099), depicted in Fig. 4, showed a close relationship with those of other species of *Thricobious* spp. (Streblidae).

Of the ectoparasites screened for the detection of VBPs, only one tick larva, *Ornithodoros* sp., collected from a *Noctilio leporinus* bat (site 1), scored positive for *Rickettsia* sp. (93.8% nucleotide identity with the GenBank sequence MT178338), clustering with *Rickettsia rhipicephali* from Brazil and *Rickettsia massiliae* from Spain (Fig. 5). The *Ornithodoros* sp. sequences (95.7% nucleotide identity with the GenBank sequence MZ773898) clustered with high bootstrap support (97%) with *Ornithodoros hasei* (Fig. 6).

The overall frequency of GI parasite infection was 14% (9/64, 95% CI: 0.08–0.25), being 3.1% (2/64, 95% CI: 0.01–0.11) of bats infected by

Table 1
Species of bats examined in the study area and their infestation rates (no. infested/no. examined).

Family	Species	Site 1	Site 2	Tota no. infested
Emballonuridae	*Saccopteryx bilineata*	–	0/1	–
	Saccopteryx leptura	0/3	–	–
Mormoopidae	*Ptenorotus mesoamericanus*	1/3	–	1
Noctilionidae	*Noctilio albiventris*	1/1	–	1
	Noctilio leporinus	1/2	–	1
Phyllostomidae	*Artibeus jamaicensis*	18/20	4/14	22
	Artibeus lituratus	2/3	1/2	3
	Artibeus phaeotis	1/3	–	1
	Artibeus watsoni	–	1/1	1
	Carollia castanea	–	0/6	–
	Carollia perspicillata	2/3	0/7	2
	Chiroderma trinitatum	0/2	–	–
	Desmodus rotundus	1/2	0/1	1
	Glossophaga soricina	1/5	0/8	1
	Lonchophylla robusta	–	0/1	–
	Phyllostomus discolor	1/2	–	1
	Sturnira parvidens	6/11	–	6
	Trachops cirrhosus	0/1	–	–
	Uroderma convexum	–	6/9	6
Vespertilionidae	*Myotis nigricans*	–	1/1	1
	Rhogeessa io	–	0/1	–
Total		35/61	13/52	48

Table 2
Frequency (F), mean intensity (MI) and mean abundance (MA) of ectoparasite infestations.

	F (%), (95% IC)	MI (%), (95% CI)	MA (%), (95% CI)
Mites	38.9, 0.3–0.48	3, 2–5.6	1.2, 0.7–2.1
Ticks	1.8, 0–0.06	35.5, 29–35.5	0.63, 0-2
Flies	2.6, 0.01–0.08	8.3, 1–15.6	0.22, 0.01–1

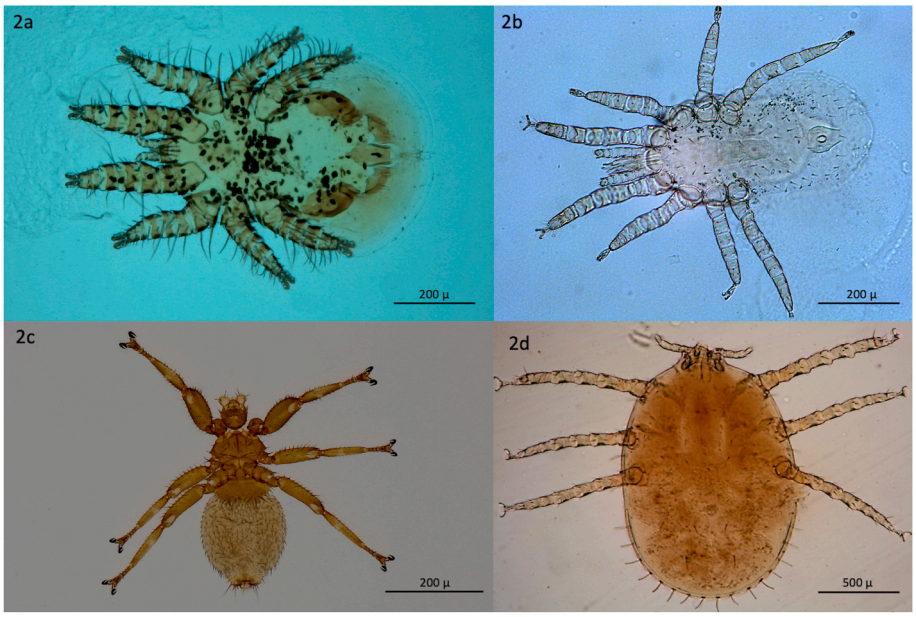

Fig. 2. Ectoparasite genera found on bats from Cost Rica a) *Cameronieta* sp. female specimen b) *Mitonyssoides* sp. male specimen c) *Joblingia* sp. female specimen d) *Ornithodoros* sp.

Table 3
Genera and number of ectoparasites identified on different host species and collection sites.

Order	Family	Genera (tot no.)	Host	Collection site
Mesostigmata	Spinturnicidae	*Cameronieta* (100)	*Artibeus jamaicensis*	Site 1 and Site 2
			Artibeus lituratus	Site 1 and Site 2
			Artibeus watsoni	Site 2
			Glossophaga soricina	Site 1
			Myotis nigricans	Site 2
			Phyllostomus discolor	Site 1
			Ptenorotus mesoamericanus	Site 1
			Sturnira parvidens	Site 1
			Uroderma convexum	Site 2
	Macronnysidae	*Mitonyssoides* (35)	*Desmodus rotundus*	Site 1
			Artibeus phaeotis	Site 1
			Carollia perspicillata	Site 1
Ixodida	Argasidae	*Ornithodoros* (71)	*Noctilio albiventris*	Site 1
			Noctilio leporinus	Site 1
Diptera	Streblidae	*Joblingia* (25)	*Noctilio leporinus*	Site 1
			Sturnira parvidens	Site 1

Giardia spp., 1.5% (1/64, 95% CI: 0–0.08) by *Eimeria* spp. and 9.4% (6/64, 95% CI: 0.04–0.19) by *Cryptosporidium* spp. Specifically, the 2 bats positive for *Giardia* spp. were infected with a non-*duodenalis* genotype (98% nucleotide identity in a fragment of the 18S rRNA gene with two *Giardia* sp. isolates from pigs: GenBank MK430921, MK430923); the *Eimeria* sp. sequence was 98.3% identical to the GenBank sequence MG770470; 3 *Cryptosporidium* sp. sequences and 1 sequence were 99.6% and 96.5% respectively identical to the bat genotype (MH553322), 1 sequence 94.26% to a rodent genotype (MH912997) and the last sequence was 97.3% identical to a *Cryptosporidium* sp. isolated from humans (KJ506854).

All sequences generated in this study were deposited in the Gene Bank database (*Cameronieta* sp. OP739496 and OP739497, *Mitonyssoides* sp. OP739498, *Ornithodoros* sp. OP748358 and OP748359, *Joblingia* sp. OP739499, *Rickettsia* sp. OP819942, *Eimeria* sp. OQ123727, *Cryptosporidium* sp. OQ132820, OQ132821, OQ132822 and OQ132823, *Giardia* sp. OQ134923).

4. Discussion

The wide range of mites, ticks and flies collected from bats in Costa Rica coupled with the detection of VBPs and GI protozoa suggest that they may play a role as amplifiers of pathogens. The variety in the number of bat species herein identified (i.e., 21 bat species belonging to 15 genera and 5 families) confirms the high biodiversity described in the Neotropical Region (Willig and Selcer, 1989). Though an overall high ectoparasite infestation rate (42.5%) was recorded in sampled animals, the higher frequency of ectoparasites from Santa Cruz was probably due to the to the higher number of female bats captured from this area (37 *vs* 27), being the latter more prone to the parasitism (Patterson et al., 2008; Presley and Willig, 2008), but also to the drier and milder climate of Santa Cruz that provides increased ectoparasite reproduction rates, as well as mammal infestations (Adler et al., 2003; Rui and Graciolli, 2005; Lourenço and Palmerim, 2008; de Mendonça et al., 2020; Cordes et al., 2022). In addition, the higher detection of mites (91.6%, 44/48) than flies (6.2%, 3/48), along with the presence of co-infestation (i.e., tick larvae and flies) in a single individual, differ from previous reports, in

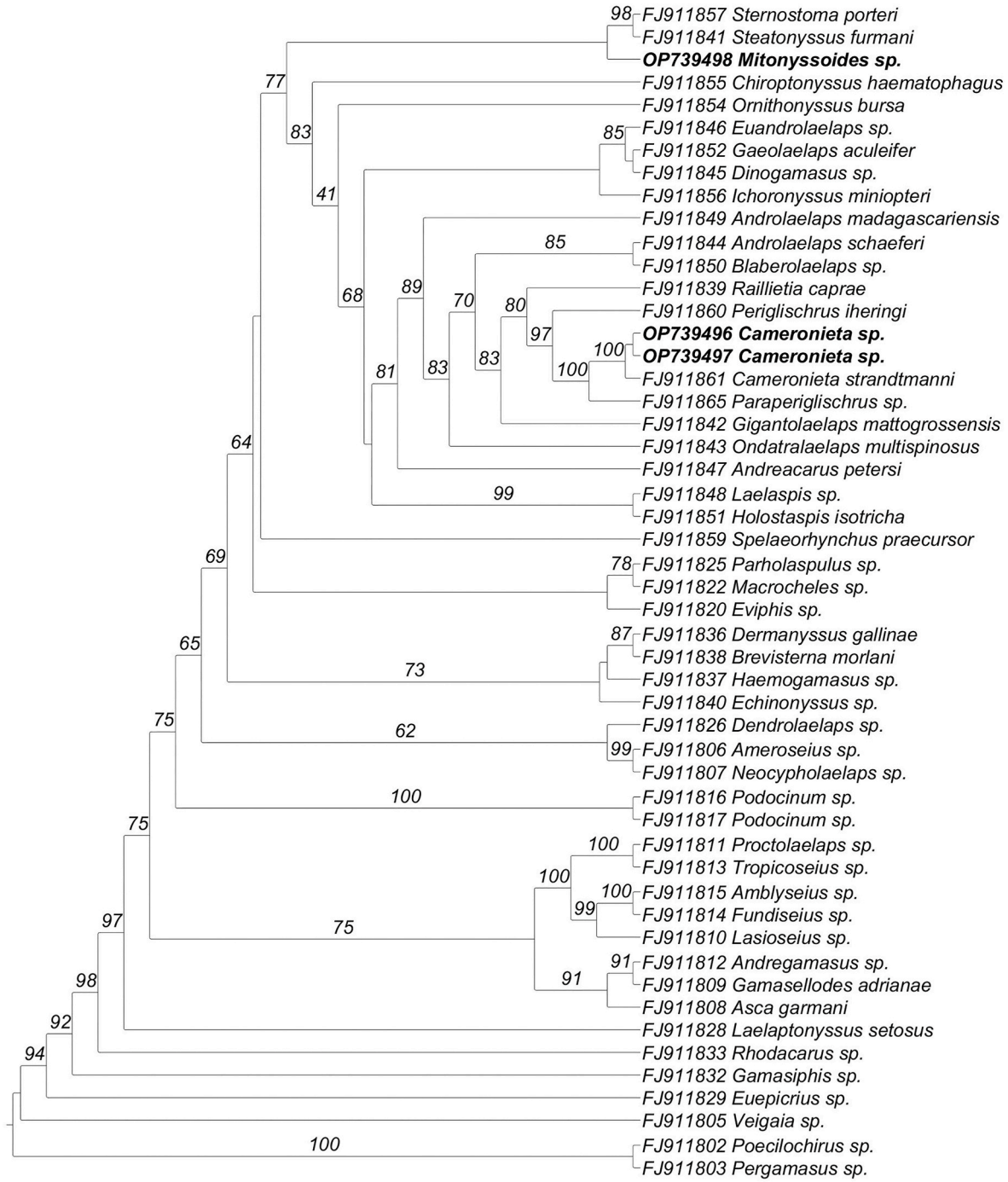

Fig. 3. Phylogenetic tree based on maximum likelihood analysis of the 18S rRNA sequences obtained from bat mites (consensus sequences of this study are bold) and other sequences available in the GenBank database. *Poecilochirus* sp. (FJ911802) and *Pergamasus* sp. (FJ911803) were used as outgroup.

which flies were the most abundant ectoparasites, often co-infesting bats, with the association streblid-spinturnicid species as the most common, especially in the Neotropical Region (Szentiványi et al., 2019; Lourenço et al., 2020). The difference in the results above may be related to the ectoparasite collection carried out in the laboratory and not in the field, therefore representing a higher abundance of mites, that remained attached on the bat surface, than flies.

The low percentages of nucleotide identity encountered in the ectoparasite sequences are probably due to the limited resolution of the 18S rRNA gene in their molecular delineation (Black and Piesman, 1994; Otto and Wilson, 2001; Klompen et al., 2007), as well as the high frequency in identification of new species (Autino et al., 2009; Dantas-Torres et al., 2012; Orlova and Zhigalin, 2015; Morales-Malacara and Guerrero, 2020) and the lack of sequences deposited in the GenBank database. For instance, no sequences of the fly *Joblingia* sp. (Streblidae) and the mite *Mitonyssoides* sp. (Macronyssidae) were available in GenBank, preventing an identification at species level. Nonetheless, the phylogenetic relationship at family level and the morphological key characters were fundamental to identify *Joblingia* sp. (i.e., palps with rounded apex, lateroverticles and occipital lobes differentiated, mesonotum strongly convex) (Graciolli and Dick, 2012) and *Mitonyssoides* sp. (i.e., entire and ornamented dorsal plate; anal plate triangular and peritreme reaching to coxae I) (Yunker et al., 1990). Conversely, the high phylogenetic correlation between the 18 rRNA sequence of

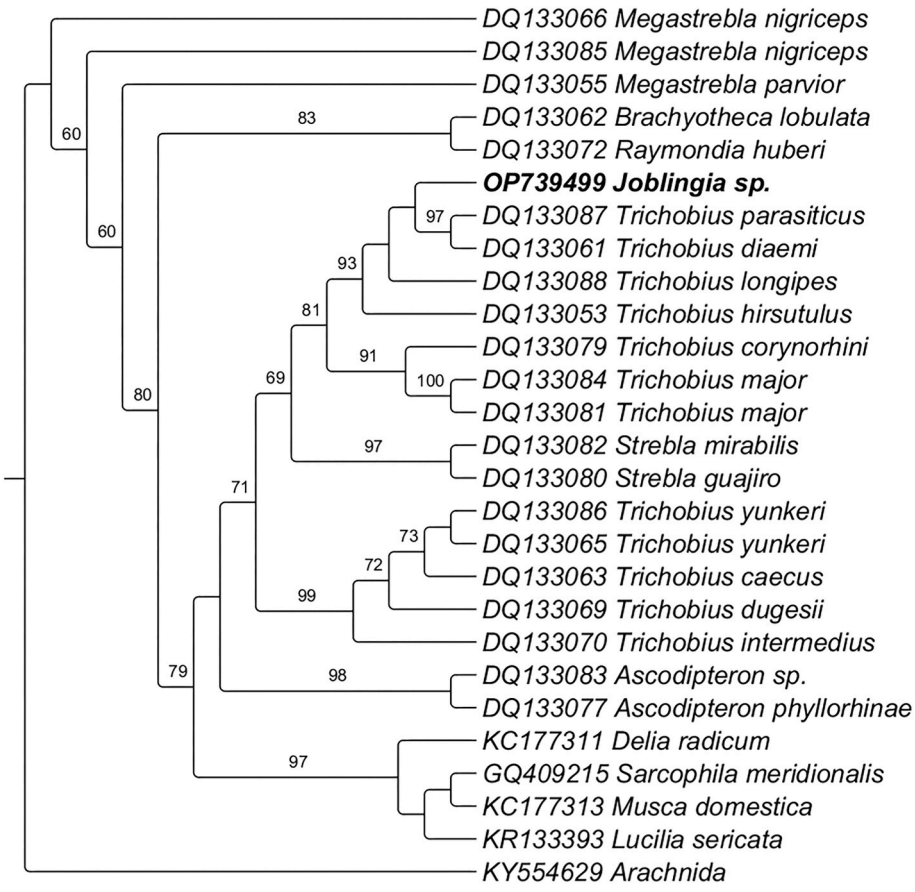

Fig. 4. *Joblingia* sp. tree based on 18S rRNA sequences here generated (in bold) with those available from GenBank database by using maximum likelihood analysis. Arachinida (KY554629) was used as outgroup.

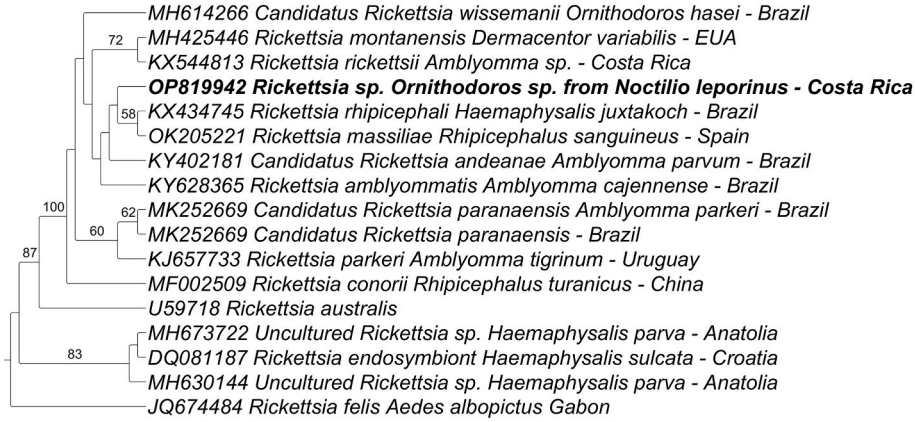

Fig. 5. Phylogenetic relationships between the sequences of the *Rickettsia* sp. detected from the positive immature tick and those available in the GenBank database, using maximum likelihood analysis on the *gltA* gene. *Rickettsia felis* (JQ674484) was used as outgroup.

Ornithodoros sp. herein detected and that of *Ornithodoros hasei* is suggested by the neotropical distribution of this tick species (e.g., Brazil, Guyana, Colombia, Costa Rica, Dominica, Guadeloupe, Guatemala, Martinique, Mexico, Nicaragua, Panama etc.) (de la Cruz, 2001), and further supported by its host association with bats of the genus *Noctilio* spp. (Kohls et al., 1965; Muñoz-Leal et al., 2016; Tahir et al., 2016).

The detection of *Rickettsia* sp. from the tick larva *Ornithodoros* sp. corroborates the role of bats as reservoirs of these bacteria (D'Auria et al., 2010; Tahir et al., 2016) and supports previous findings, including the isolation of novel *Rickettsia* species in *Ornithodoros knoxjonesi* and in *Ornithodoros hasei* from bats of Costa Rica and French Guiana respectively (Tahir et al., 2016; Moreira-Soto et al., 2017). The phylogenetic analysis showed the relationship among *Rickettsia* sp. herein detected and two rickettsiae of the SFG (i.e., *R. massiliae* and *R. rhipicephali*), which are associated with *Rhipicephalus sanguineus* sensu lato (Burgdorfer et al., 1975, 1978; Parola et al., 2013). *Rickettsia massiliae* is the causative agent of spotted fever and febrile SENLAT (Scalp Eschar and Neck Lymph Adenopathy After a Tick Bite) syndrome (Vitale et al., 2006; Cascio et al., 2013), while the pathogenic potential of *R. rhipicephali* in humans is still unknown (Parola et al., 2013).

The overall rate of GI protozoa (14%) was lower than in previous studies ranging from 100% to 43.5% (Saoud and Ramadan, 1976; Lord

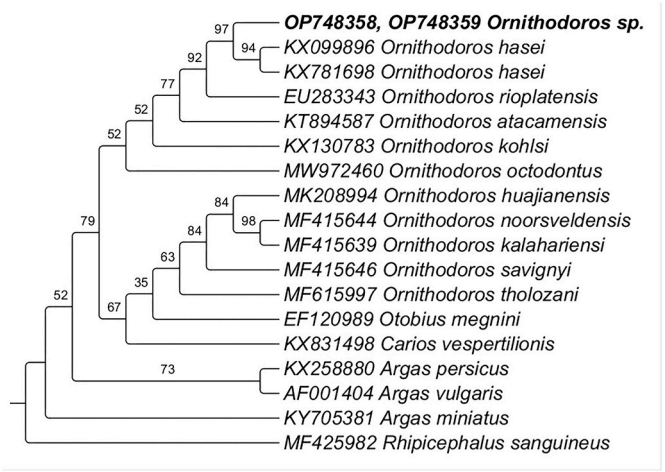

Fig. 6. Phylogenetic tree based on maximum likelihood analysis of the 16S rRNA sequences obtained from bat ticks (consensus sequences of this study are bold) and other sequences available in the GenBank database. *Rhipicephalus sanguineus* (MF425982) was used as outgroup.

et al., 2012; McAllister et al., 2012; Afonso et al., 2014; Adhikari et al., 2020) probably due to the sampling and analyzing methods employed. Conversely, the frequency of *Giardia* infection (3.1%) is similar to that described in bats from Southcentral Nepal (i.e., 3.3%; Adhikari et al., 2020). These data are supported by the documented large circulation of *Giardia* sp. in dogs, cats and pre-school children from Costa Rica (Vasquez Rojas and Zumbado, 1980; Scorza et al., 2011).

The finding of only one bat positive for *Eimeria* sp., accidently detected by using a PCR specific for *Cryptosporidium* spp., differs from previous reports describing higher rates of infections (McAllister and Upton, 2009; 2012; Afonso et al., 2014; Adhikari et al., 2020) but confirms bats as harboring hosts for this coccidian parasite (Santana Lima et al., 2018).

The 9.4% frequency of *Cryptosporidium* sp. infection agrees with the range described in literature (i.e., 2.8–9.5%) (Ziegler et al., 2007; Wang et al., 2013; Kváč et al., 2015) and the detection of several genotypes is expected, as many new *Cryptosporidium* genotypes were identified in wildlife hosts (Ziegler et al., 2007; Ryan and Power, 2012). In addition, the finding of high nucleotide identity of one isolate with *Cryptosporidium* human isolates provides evidence for these mammals as wildlife spillover of potential zoonotic pathogens (Schiller et al., 2016).

5. Conclusions

Bats play a crucial ecological role and fulfil important ecosystem functions since they are involved in the control of insect populations, pollination and seed dispersal but, at the same time, they may represent a health risk to other animals and potentially humans, as they harbor a large diversity of parasites (Allocati et al., 2016). Accordingly, this study provides data on the occurrence of mites, ticks larvae and flies parasitizing chiropterans from Costa Rica, along with the detection of *Rickettsia* sp. and several GI protozoa, spotting the importance of these mammals as a source of pathogens and the need to further investigate the pathogenic potential of the parasites here in found.

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Author's contribution

Mariaelisa Carbonara: Formal analysis; Investigation; Methodology; Writing - original draft; Writing - review & editing. Jairo Alfonso Mendonza-Roldan: Investigation; Methodology; Writing - review & editing; Supervision. Lívia Perles: Investigation; Formal analysis; Methodology; Writing - review & editing. Alejandro Alfaro-Alarcon: Methodology; Writing - review & editing. Luis Mario Romero: Methodology; Writing - review & editing. Daniel Barrantes Murillo: Methodology; Writing - review & editing. Marta Piche-Ovares: Methodology; Writing - review & editing. Eugenia Corrales-Aguilar: Methodology; Writing - review & editing. Roberta Iatta: Writing - review & editing. Julia Walochnik: Formal analysis; Methodology; Writing - review & editing. Mario Santoro: Investigation; Methodology; Writing - review & editing. Domenico Otranto: Conceptualization; Investigation; Methodology; Project administration; Supervision; Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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