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Original Article

Blankaartia sinnamaryi (Trombidiformes: Trombiculidae) parasitizing birds in southeastern Brazil, with notes on *Rickettsia* detection

Blankaartia sinnamaryi (Trombidiformes: Trombiculidae) parasitando aves no sudeste do Brasil, e notas sobre a detecção de *Rickettsia*

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Abstract

The larvae of the family Trombiculidae are ectoparasites of vertebrates, including birds. The bite of some species can cause deep lesions and severe skin reactions in the host, these can lead to dermatitis, popularly known as trombiculiasis. A morphological study of chiggers collected on birds from the state of Minas Gerais in Southeastern Brazil discovered *Blankaartia sinnamaryi*-infestation on Passeriformes birds. Molecular studies of the disclosed the 18S rDNA sequences of the mite, and the detection of a *Rickettsia* sp. in this chigger mite species.

Keywords: Chiggers, *Rickettsia*, passeriformes, dermatitis.

Resumo

As larvas da família Trombiculidae são ectoparasitas de vertebrados, incluindo aves. A picada de algumas espécies pode causam lesões profundas e reações cutâneas graves no hospedeiro, estas podem levar a dermatites, popularmente conhecidas como trombiculíases. Por meio de um estudo morfológico dos espécimes coletados parasitando aves do estado de Minas Gerais, Sudeste do Brasil relatou a infestação por *Blankaartia sinnamaryi* em aves Passeriformes. Além disso, nós fornecemos sequências de rDNA 18S desses ácaros e a detecção de uma espécie de *Rickettsia* sp. nesta espécie de trombiculídeo.

Palavras-chave: Trombiculídeos, *Rickettsia*, passeriformes, dermatite.

Introduction

Chigger mites of Trombiculidae family Ewing, 1944 are represented by ca. 3000 species (HOFFMANN, 1990). In Brazil, eight species of this family have been documented parasitizing birds (BRENNAN & GOFF, 1977; BASSINI-SILVA et al., 2017). Until now, *Parasecia thalurania* Brennan, 1969 was found parasitizing birds of the order Apodiformes (BRENNAN, 1969); *Apolonia tigipioensis* Torres & Braga, 1938 and *Eutrombicula batatas* (Linnaeus, 1758) were associated to some representatives of the order Galliformes (TORRES & BRAGA, 1938; EWING, 1925; CONFALONIERI & DE CARVALHO, 1973); *A. tigipioensis*, *Blankaartia sinnamaryi* (FLOCH & FAURAN, 1956), *Parasecia fundata* Brennan, 1969 and *Neoschoengastia esorhina* Brennan, 1971 were found parasitizing species of the order Passeriformes (ORNELAS-ALMEIDA et al., 2007; FLOCH & FAURAN, 1956; BRENNAN, 1969, 1971); *B. sinnamaryi* was found parasitizing species of the order Piciformes (FLOCH & FAURAN, 1956); *A. tigipioensis* was found parasitizing

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species of the order Struthioniformes (ORNELAS-ALMEIDA et al., 2007); *A. tigipioensis* and *Eutrombicula tinami* Oudemans, 1910 were found parasitizing species of the order Tinamiformes (ORNELAS-ALMEIDA et al., 2007; OUDEMANS, 1910); and only one species, *Blankaartia shatrovi* Bassini-Silva & Barros-Battesti, 2017, was collected on an unidentified bird (BASSINI-SILVA et al., 2017).

Some larvae species of the family Trombiculidae can cause deep lesions and severe skin reactions in the host because of bites (HASE et al., 1978), which can lead to dermatitis, popularly known as trombiculiasis (STEKOLNIKOV et al., 2014). One of the first cases of trombiculiasis documented on birds was reported by TORRES & BRAGA (1938) in Pernambuco, Brazil, who described chiggers of *A. tigipioensis* causing nodular lesions on the quill of domestic chickens. SPALDING et al. (1997), reported the species *B. sinnamaryi* caused dermatitis similar to *A. tigipioensis* in domestic chickens from the state of Florida, USA. Recently, MAKOL & KORNILUK (2017), reported the species *Blankaartia acuscutellaris* (Walch, 1923) causing lesions, when in large quantity, in the species *Gallinago media* (Charadriiformes: Scolopacidae), in Poland. They even noticed that the parasitized regions had a thick epidermis and no plumage. As is well known, chiggers are not host-specific, and several cases of human bites have also been reported (HEYNE et al., 2001; TAKAHASHI et al., 2004; RIPKA & STEKOLNIKOV, 2006; BURNS, 2010; SHATROV & STEKOLNIKOV, 2011).

Birds are known spreaders of ectoparasites including ornithophylic chigger mites. When mites are attached, their hosts can transport them remarkable distances, and eventually move infected chiggers from one place to another, which directly contributes to the dissemination of associated bacteria (VARMA, 1964). In the past, public health departments were faced with the need for cataloging and understanding the biology of these mites due to their potential role as *Rickettsia* vectors (WHARTON & FULLER, 1952). Recently in Slovakia, *Rickettsia monacensis* and *Rickettsia helvetica* were molecularly detected in *Hirsutiella zachvatkini* (Schluger, 1948), and in addition, *R. monacensis* was found associated with *Kepkatrombicula storkani* (Daniel) (MIŤKOVÁ et al., 2015). In particular, the current role of chigger mites in the epidemiology of rickettsiosis is still unknown in the Neotropics (AZAD & BEARD, 1998; POINAR & POINAR, 1998). Recently WEITZEL et al. (2016) found the presence of the bacterium, *Orientia tsutsugamushi,* in humans, suggesting the possible transfer by a bite from chiggers in these patients. In Brazil, FONSECA (1932) emphasized the importance of bloodsucking mites as potential vectors in the epidemiological cycle of rickettsial diseases. Nevertheless, the role of trombiculid mites as vectors has never been confirmed.

The purpose of this study is to conduct morphological and molecular studies of chiggers collected on birds from the state of Minas Gerais in Southeastern Brazil and determine if *Rickettsia* could be present in these chiggers.

Material and Methods

Collection of mites

Thirty-four field trips, each lasting five days, were made during March 2013 and December 2015 at the Botanical Garden of the Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais (21° 43'S, 43° 22' W, elevation 852 m), with the authorization of the System of Authorization and Information on Biodiversity (SISBIO, protocol number 29268) and The National Center for Bird Conservation (CEMAVE, protocol number 3954). Birds were captured using 10 ornithological mist nets (12 m long × 3 m wide, 16 mm mesh) between 06.00 h and 16.00 h each day. Trapped birds were weighed, measured and photographed, with identifications established following (RIDGELY & TUDOR, 2009). Birds were examined for the presence of mites by checking their entire body. In the laboratory, the specimens were counted and separated for slide-mounting for morphological studies using a microscope, scanning electron microscopy studies, and DNA extraction with the help of a stereomicroscope Nikon SMZ745T (Sao Paulo, Brazil).

Morphological identification of mites

The material was slide-mounted and deposited into the Acari Collection of the Instituto Butantan, São Paulo, Brazil (IBSP). Chiggers were identified based on the original descriptions of all species of the genus *Blankaartia* (EWING, 1926; FLOCH & ABONNENC, 1941; BOSHELL & KERR, 1942; MICHENER, 1946; FLOCH & FAURAN, 1956; RADFORD, 1957; VERCAMMEN-GRANDJEAN, 1960; TAUFFLIEB & MOUCHET, 1959; BRENNAN & JONES, 1961; BRENNAN, 1965; BRENNAN & YUNKER, 1966; CROSSLEY & ATYEO, 1972; TAUFFLIEB, 1972; NADCHATRAM & GOFF, 1980; BASSINI-SILVA et al., 2017) and by comparison with type and material slides that were deposited at the USNM (Appendix 1), which are housed at the Systematic Entomology Laboratory (USDA-ARS, BARC). Some specimens were also prepared for Scanning Electron Microscopy (SEM) according to WALTER & KRANTZ (2009) with a Digital Scanning Electron Microscope FEI (Hillsboro, Oregon USA), Quanta 250, at the Laboratory of Cell Biology, Instituto Butantan. The terminology of GOFF et al. (1982), with adaptations proposed by STEKOLNIKOV (2008) and STEKOLNIKOV & DANIEL (2012) concerning general nomenclature of larval stages was followed with the terminology for specialized setae using KETHLEY (1990), WOHLTMANN et al. (2006, 2007) and BASSINI-SILVA et al. (2017). Voucher specimens have been deposited at the IBSP collection, under the accession numbers found on table 1.

Molecular tools

DNA extraction

Forty field-collected larvae (Table 1) were individually subjected to DNA extraction using the Guanidine Isothocianate (GT) lysis protocol following CHOMCZYNSKI (1993). Each mite was placed in an Eppendorf microtube, and punctured in the idiosomal region with a sterile needle (1.20*40-18G). After DNA extraction, exoskeletons of the specimens were recovered and slide-mounted for observations in a microscope in order to perform morphological identifications.

PCR

PCR technology was used initially performed following OTTO & WILSON (2001), SIMON et al. (1994) and NAVAJAS et al. (1994) to target a partial fragment of the mite 18S ribosomal gene and two PCR protocols were used to amplify a section of the mite mitochondrial cytochrome oxidase I (COI) gene. All the reactions were performed using DNA extracted from the trombiculid *Quadraseta brasiliensis* Goff & Gettinger (1989) as positive and DNA-free Milli-Q water as negative controls. 18S gene PCR was used as endogenous control, and negative samples were excluded from further analyses. The presence of *Rickettsia* DNA was detected by, through the use of PCR, targeting partial fragments of the rickettsial genes *gltA* (LABRUNA et al., 2004), *ompA* (REGNERY et al., 1991), and *ompB* (ROUX & RAOULT, 2000) genes. Primers used in the reactions are listed in Table 2. Reactions yielding amplicons of expected size were purified using the Qiagen (Sao Paulo, Brazil) MinElute kit following the manufacturer instructions. Sanger sequencing of the samples was performed at the "Centro de pesquisa sobre Genoma Humano e Células Tronco do Instituto de Biociências da USP". Sequences that were obtained were assembled and the primer trimmed with Geneious R9 (KEARSE et al., 2012), and then submitted to a BLAST analysis (www.ncbi.nlm.nih.gov/blast) in order to infer closest similarities with other homologous sequences (ALTSCHUL et al., 1990).

Results

Bird captures and collection of mites

A total of 95 mites were collected on six bird species (Table 1). Two specimens of the species *Chiroxiphia caudata* (Passeriformes: Pipridae) were captured and four chiggers were collected in these hosts; five specimens of the species *Tachyphonus coronatus* (Passeriformes: Thraupidae) were captured and twenty seven chiggers were collected in these hosts; two specimens of the

Table 1. Specimens of *Blankaartia sinnamaryi* collected on birds from March 2013 to May 2016, in the Botanical Garden of the Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil.

Host Family	Host Species	IBSP accession number	No. of *B. sinnamaryi* specimens collected (No. specimens tested by PCR)
Pipridae	*Chiroxiphia caudata*	12331	2 (2)
	Chiroxiphia caudata	12336	2 (2)
	Manacus manacus	12338	4 (2)
Thraupidae	*Tachyphonus coronatus*	12323A	10 (4)
	Tachyphonus coronatus	12343	7 (2)
	Tachyphonus coronatus	12340	4 (2)
	Tachyphonus coronatus	12342	4 (2)
	Tachyphonus coronatus	12351	2 (2)
Troglodytidae	*Troglodytes musculus*	12344A	20 (2)
	Troglodytes musculus	12345A	36 (16)
Turdidae	*Turdus rufiventris*	12350	2 (2)
Tyrannidae	*Lathrotriccus euleri*	12348	2 (2)
Total			95 (40)

Table 2. Primers for endogenous control and detection of *Rickettsia* spp.

Target	Genes	Names and DNA sequences (5'- 3') of the primers	Fragment size	Reference
Chiggers	18S	18S–1R: ATATTGGAGGGCAAGTCTGG	~650-bp	Otto and Wilson (2001)
		18S-1R: TGGCATCGTTTATGGTTAG		
	COI-1	CI-J-175I: GGWGCWCCWGAYATRGCWTTYCC	950-bp	Simon et al. (1994)
		CI-N-219I: GGWARAATTAAAATATAWACTTC		
		772: TGATTTTTTGGTCACCCAGAAG	408-bp	Navajas et al. (1994)
		773: TACAGCTCCTATAGATAAAAC		
Rickettsia spp.	*gltA*	CS 78 F: GCAAGTATCGGTGAGGATGTAAT	401-bp	Labruna et al. (2004)
		CS 323 R: GCTTCCTTAAAATTCAATAAATCAGGAT		
		CS-239 F: GCTCTTCTCATCCTATGGCTATTAT	830-pb	Labruna et al. (2004)
		CS-1069 R: CAGGGTCTTCGTGCATTTCTT		
	*ompA**	Rr 190.70 F: ATGGCGAATATTTCTCCAAAA	532-bp	Regnery et al. (1991)
		Rr 190.701 R: GTTCCGTTAATGGCAGCATCT		
	ompB	120.M59 F: CCGCAGGGTTGGTAACTGC	862-bp	Roux and Raoult (2000)
		120-807 R: CCTTTTAGATTACCGCCTAA		

**Rickettsia* of the Spotted Fever Group.

species *Troglodytes musculus* (Passeriformes: Troglodytidae) were captured and fifty six chiggers were collected in these hosts; one specimen of the species *Manacus manacus* (Passeriformes: Pipridae), *Turdus rufiventris* (Passeriformes: Turdidae), and *Lathrotriccus euleri* (Passeriformes: Tyrannidae) were captured and two mites were collected in each of these hosts species.

The chiggers were identified as *Blankaartia sinnamaryi* (FLOCH & FAURAN, 1956) by the following combination of external characters: genu of the leg I with three *sigma*, tarsus of the leg III with one mastisetae, on the C row of dorsal idiosomal setae with eight setae, palp tarsus with seven branched setae, galealae branched, odontus trifurcated (Figure 1). These mites were collected from different regions of the birds, including the flank and upper body, which exhibited a deep skin lesion in the area where mites were attached (Figure 2).

Molecular tools

Only 29 of 40 samples were positive to the 18S gene via PCR and attempts to sequencing expected size amplicons were successful only for three samples that yielded an identical haplotype of 456-bp (GenBank accession numbers: MG783391). Attempts

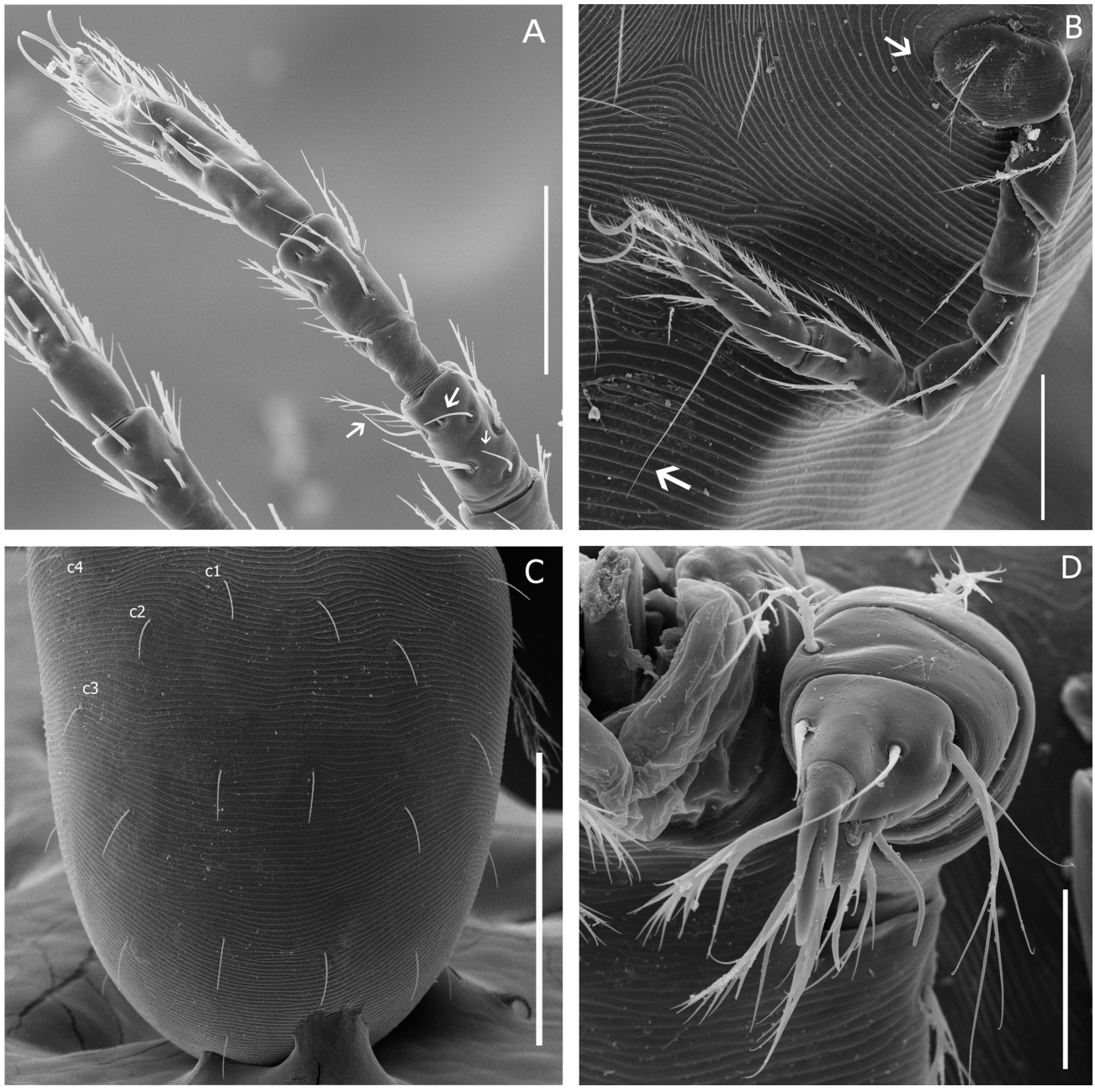

Figure 1. Morphological details of *Blankaartia sinnamaryi*. A - leg I; B - leg III; C - dorsal opisthosomal setae; D - palpal genu, tibia and tarsus. The white arrows highlight the specialized setae of the segments of the legs I and III and the striate coxae III; *c1-c4*= setae of the C row; The Scale bars: A and B 50µm, C 300µm, D 20µm.

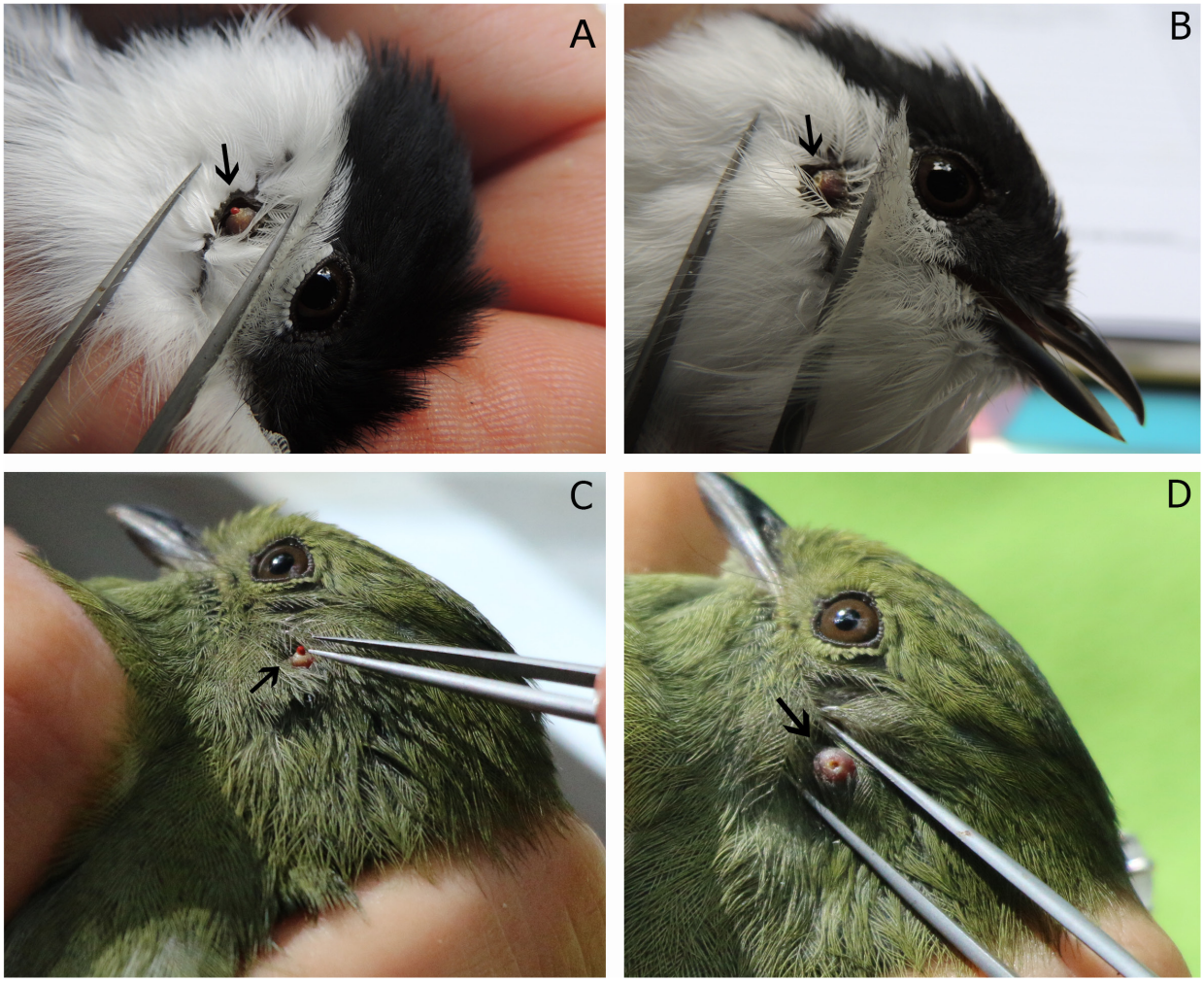

Figure 2. *Blankaartia sinnamaryi* parasitizing birds. A - *Manacus manacus* (♂) parasitized by one chigger; B - Injury on *Manacus manacus* (♂) after removal of the mite; C - *Manacus manacus* (♀) parasitized by one chigger; D - Injury on *Manacus manacus* (♀) after removal of the mite.

to amplify fragments of the COI gene were unsuccessful. After a BLAST analysis, the consensus of these three 18S sequences was 98.9% (451/456-bp) identical to the corresponding sequence of *Eutrombicula splendens* (Ewing, 1913) (KP325057), and 98.7% (450/456-bp) identical to *Quadraseta brasiliensis* Goff & Gettinger, 1989 (KY934464, MF113412, and MF113413). Three out of the 29 18S-positive samples were positive for rickettsial *gltA* gene; however only one of them yielded a clean (but short) sequence after sequencing attempts. After a BLAST analysis, the sequence shown to be 100% (1011/1011-bp) identical with *Rickettsia felis* (JQ674484, JN375498 and CP000053). Attempts to amplify fragments of the *ompA* and *ompB* genes were unsuccessful. The *Rickettsia* sp. sequence obtained in this study has been deposited in GenBank under accession number MG783574.

Discussion

Blankaartia sinnamaryi has already been collected on birds; however, three new host associations are added by the results of this study: *Chiroxiphia caudata*, *Lathrotriccus euleri* and *Troglodytes musculus* (Table 1). Captured birds were visually examined in search for mites, Figure 2 shows a local dermal lesion by only one mite. Similar injuries caused by trombiculid mites have already been noted by TORRES & BRAGA (1938) and SPALDING et al. (1997).

All collected mites were morphologically identified as *B. sinnamaryi* and were able to be genetically characterized using the 18S rRNA gene. The nuclear small ribosomal subunit gene has been pointed to be slow evolving, which favors the use of universal primers to amplify sequences in genetically unidentified species (HILLIS & DIXON, 1991). However, this slow rate of evolution precludes a good separation of taxa to the genera level and would be phylogenetically more informative in higher groupings among eukaryotes (HILLIS & DIXON, 1991). Although partial fragments were compared the 18S sequences from *B. sinnamaryi* are highly similar (<99%) to other trombiculid species (i. e. *E. splendens* and *Q. brasiliensis*), which reflects that all three species belong to a same family of mites. Unfortunately, no sequences of less-conserved COI gene were obtained, which in turn would be certainly more informative in separating the morphological identity of *B. sinnamaryi* from a genetic point of view.

JACINAVICIUS et al. (2018) and this study was successful in amplifying the 18S gene for chiggers. The same pair of primers for the gene COI used by KAMPEN et al. (2004) was used to amplifying successfully the sequences for the species *Neotrombicula autumnalis* (SHAW & NODDER, 1790). Because this was successful, it was expected that *B. sinnamaryi* samples would amplify the COI gene, however none were detected.

The detection of *Rickettsia* DNA in *B. sinnamaryi* is not surprising, since bacteria of this genus have already been found in chiggers parasitizing birds and rodents in Europe and Asia (HASE et al., 1978; TAKAHASHI et al., 2004; MIŤKOVÁ et al., 2015). Apart from early suppositions for the American Continent, chiggers infesting rodents would be harboring *Rickettsia* spp. (FONSECA, 1932), however, no current evidence has been published. The larvae of *B. sinnamaryi* collected on the bird *Tachyphonus coronatus* was found to be positive for *Rickettsia gltA* gene via PCR. Though a BLAST comparison it can be inferred that the obtained sequence perfectly (100%) matched the *Rickettsia felis* sequence. A cautious presumption should be adopted, and consider the bacterium found in *B. sinnamaryi* as a *R. felis*-like agent, because further attempts to amplify *ompA* and *ompB* genes were unsuccessful. Notwithstanding, primers used for *gltA* amplification are specific for the *Rickettsia* genus (LABRUNA et al., 2004), which confirms that *B. sinnamaryi* could be indeed carrying a rickettsial agent which would require additional molecular characterization. It is worthy to mention that chiggers were collected which appeared to be engorged, which raises the possibility that the detection of *Rickettsia* might be the result of a rickettsaemic bird. However, this supposition becomes hardly possible, since current evidence has demonstrated that birds are immune to *Rickettsia* infection, possibly due to their higher body temperature (>40 °C), since it is practically impossible to grow *Rickettsia* in such temperature under *in vitro* conditions (OGRZEWALSKA & PINTER, 2016).

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Appendix 1. Type material and allotments examined at the USNM Acari Collection

Blankaartia alleei **(**Ewing, 1926**) -** Material: PANAMA - 10 larvae (RML 40415) in Cerro Pirre, Darién, 7-II-1961, *Hydrochoerus hydrochaeris*; 2 larvae (#160), in Juan Mima, Canal Zone, 29-II-1944, *Crotophaga ani*; 1 larva (#130), in same locality, 3-II-1944, *Egretta caerulea* (= *Florida caerulea caerulea*); 1 larva (#34), near the Chagres River, Canal Zone, 29-X-1945, "drift log"; Charles D. Michener coll.

Blankaartia amersoni **(**Brennan, 1965**) -** Material: TRINIDAD - 2 larvae (RML 45755), Soldado Rock, 9-V-1965, *Anous stolidus*; USA: 1 larva (RML 45767), at the Sand Island, Johnston Atoll, 6-I-1965, *Anous stolidus*; 1 larva (RML 46205), same locality, 11-VII-1965, *Onychoprion fuscatus* (= *Sterna fuscata*); 3 larvae (RML 46207), same locality and host, 12-VII-1963; 2 larvae (RML 46208), same locality and host, 30-VII-1965; 1 larvae (RML 46206), same locality, VIII-1965, *Bulweria bulwerii*, Thomas H. G. Aitken coll.

Blankaartia arremonops **(**Brennan & Jones,1961**) -** HOLOTYPE: larva (RML 35280), at the Coco Plantation, Panama, 4-V-1955, *Arremonops conirostris*, Gordon Field coll.

Blankaartia marui **(**Brennan & Yunker, 1966**) -** HOLOTYPE: larva (RML 40982), in Cerro Azul, Panama, 29-V-1961; *Nycticorax nycticorax*. PARATYPES: 2 larvae (RML 40983), same data.

Blankaartia sinnamaryi **(**Floch & Fauran, 1956**) -** Material: JAMAICA: 3 larvae (AP23876), St. Thomas, Corn Puss Gap, 14-XI-1946, *Myadestes genibarbis*; PANAMA - 2 larvae (RML 44025), Gamboa, Canal Zone, 26-X-1961, *Baryphthengus ruficapillus*; 5 larvae (RML 35309), Coiba Island, 3-I-1956 to 13-II-1956, "Bird"; 1 larva (RML 35330), same locality, 3-I-1956 to 13-II-1956, "Bird"; 1 larva (RML 35313), same locality, 3-I-1956 to 13-II-1956, "Bird"; 4 larvae (RML 40638), Almirante, Bocas del Toro, 23-I-1961, *Myiozetetes* sp.; 16 larvae (RML 40639), same locality and host, 24-I-1961; 1 larva (RML 40640), same data; 1 larva (RML 40641), same data; 2 larvae (RML 40642), same locality and host, 25-I-1961; 1 larva (RML 40647), same locality and host, 3-II-1961; 2 larvae (RML 40644), same locality, 27-I-1961, *Piranga rubra*; 22 larvae (RML 40646), same locality, 2-II-1961, *Sporophila corvina*; 3 larvae (RML 40648), same locality, 14-II-1961, *Aramides cajaneus* (=*Aramides cajanea*); 1 larva (RML 40649), same locality, 16-II-1961, Bucconidae; 7 larvae (RML 40636), same locality, 9-I-1961, *Taraba major*; 2 larvae (RML 40645), same locality, 27-I-1961, *Aratinga finschi*; 1 larva (RML 43831), same locality, 25-IX-1961, *Geothlypis formosa* (= *Oporornis formosus*); 1 larva (RML 43284), same locality, 4-VIII-1961, *Xiphorhynchus guttatus*; 1 larva (RML 40650), same locality, 3-II-1961, *Zentrygon lawrencii* (= *Geotrygon lawrencii*); 1 larva (RML 43767), Rio Changena, Bocas del Toro, 22-IV-1961, *Catharus ustulatus* (= *Hylocichla ustulata*); 1 larva (43975), same locality, IX-1961, *Trogon massena*; 1 larva (RML 43702), same locality, 20-IX-1961, *Zentrygon lawrencii* (= *Geotrygon lawrencii*); TRINIDAD: 4 larvae (#33810), Melajo Forest, Sangre Grande, I-1956, *Pitangus sulphuratus*; 1 larva (#33898), same locality and host, 24-IV-1954; 1 larva (#33831), same locality, 20-XII-1955, *Thamnophilus doliatus* (= *Thamnophilus fraterculus*); 1 larva (#33839), same data; 6 larvae (#33883), same locality, 28-III-1956, *Tachyphonus rufus*; 2 larvae (#34137), same locality, 10-VII-1956, *Crotophaga ani*; 1 larva (#33840), same locality, 29-XI-1956, *Tyrannus melancholicus*; 6 larvae (#33842), same locality, 4-I-1956, *Galbula ruficauda*; 1 larva (#33843), same locality, 6-XII-1956, *Myiopagis gaimardii*; 5 larvae (#33802), same locality, 1-III-1956, *Amazona amazonica*; 3 larvae (#33839), same locality, 20-XII-1956, *Thamnophilus doliatus*; 1 larva (#34047), same locality and host, 3-VII-1956; 1 larva (#34135), same locality, 10-VII-1956, *Myiodynastes maculatus*; 1 larva (#34669), same locality, 24-XII-1959, *Xiphorhynchus guttatus*; 1 larva (#37051), Churchill-Roosevelt Highway, 29-XI-1956, *Tyrannus savana* (= *Muscivora tyrannus*); 1 larva (#35073), William Tr. Foster Rd., 1-I-1959, *Myiophobus fasciatus*; 2 larvae (#36681), Elgin Trace, Vega de Oropouche, 2-II-1960, *Tolmomyias flaviventris*; 2 larvae (#36674), same locality, 29-XII-1959, *Myiophobus fasciatus*; 2 larvae (#36684), same locality and host, 2-II-1960; 1 larva (#36673), same locality, 29-XII-1959, *Tolmomyias flaviventris*; 2 larvae (#36681), same locality and host, 2-II-1960; 1 larva (#38214), Vega de Oropouche, 10-I-1961, C*ampephilus melanoleucos* (= *Phloeceastes melanoleucos*); 1 larva (#35052), same locality, 7-X-1959, *Cyclarhis gujanensis*; 2 larvae (#36771), same locality, 15-XII-1959, *Pipra erythrocephala*; 3 larvae (#36688), same locality and host, 8-XII-1959; 1 larva (#36690), Fort Reid, 16-II-1960, *Myiophobus fasciatus*; 1 larva (#33660), same locality, 26-V-1955, *Tyrannus melancholicus*; 1 larva (#35092), 3-V-1959, *Columbina talpacoti* (= *Columbigallina talpacoti*); 1 larva (#36690), same locality, 2-III-1960, *Thamnophilus doliatus*; 3 larvae (#34682), Granville, 12-III-1956, *Ciccaba virgata*; USA: 2 larvae (#45011), Cameron Co., Texas, 7-V-1962, *Dumetella carolinensis*; 1 larva (#38715), same locality, 23-IV-1963, *Hylocichla mustelina*, Richard B. Eards coll.

Blankaartia wetmorei **(**Brennan & Yunker, 1966**) -** HOLOTYPE: larva (RML 43234), at Fort Kobbe, Canal Zone, Panama, 20-VII-1961, *Nyctanassa violacea*. PARATYPES: 2 larvae (RML 43234), same data, Frank Todd coll.