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Original Article

Molecular detection of *Rickettsia* genus in chigger mites (Trombidiformes: Trombiculidae) collected on small mammals in southeastern brazilian

Detecção molecular do gênero *Rickettsia* em ácaros trombiculídeos (Trombidiformes: Trombiculidae) coletados em pequenos mamíferos do sudeste brasileiro

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Abstract

Chiggers are ectoparasites of vertebrates and may cause trombiculiasis or transmit pathogens to their hosts. Specimens collected from rodents and marsupials were morphologically identified as *Herpetacarus hertigi*, *Eutrombicula tinami*, *Kymocta* sp., *Quadraseta brasiliensis*, *Quadraseta falconensis*, *Quadraseta flochi*, *Quadraseta mackenziei*, *Quadraseta pazca*, *Quadraseta trapezoides*, *Quadraseta* sp., *Serratacarus* sp., and *Trombewingia bakeri*. These mites were submitted individually to molecular analyses for the detection of bacteria of the genus *Coxiella*, *Hepatozoon* and *Rickettsia*. Samples were positive to *Rickettsia* only. Obtained sequences for the *gltA* (350 pb) and *ompA* (488 pb) genes were identical to "*Candidatus* Rickettsia colombianensi", a species previously detected in ticks. In addition, molecular identification of mites based on 18S rDNA sequences are provided for *H. hertigi*, *Kymocta* sp., *Q. brasiliensis*, *Q. pazca*, *Q. trapezoides*, *Quadraseta* sp., and *T. bakeri* for the first time. This is the first report of the detection of a *Rickettsia* sp. in chigger mites collected on rodents in Brazil.

Keywords: Chiggers, "*Candidatus* Rickettsia colombianensi", ectoparasites, Rodentia, Didelphimorphia, Brazil.

Resumo

Os trombiculídeos são ectoparasitas de vertebrados e podem causar trombiculíase ou transmitir patógenos ao hospedeiro. Exemplares coletados em roedores e marsupiais foram identificados morfologicamente como *Herpetacarus hertigi*, *Eutrombicula tinami*, *Kymocta* sp., *Quadraseta brasiliensis*, *Quadraseta falconensis*, *Quadraseta flochi*, *Quadraseta mackenziei*, *Quadraseta pazca*, *Quadraseta trapezoides*, *Quadraseta* sp., *Serratacarus* sp. e *Trombewingia bakeri*. Estes ácaros foram submetidos individualmente à análise molecular para detecção de bactérias dos gêneros *Coxiella*, *Hepatozoon* e *Rickettsia*. Amostras foram positivas somente para *Rickettsia*. Sequências obtidas para os genes *gltA* (350 pb) e *ompA* (488 pb) foram idênticas à "*Candidatus* Rickettsia colombianensi", uma espécie anteriormente detectada em carrapatos. Além disso, foram fornecidas sequências de DNA 18S para identificação molecular de *H. hertigi*, *Kymocta* sp., *Q. brasiliensis*, *Q. pazca*, *Q. trapezoides*, *Quadraseta* sp. e *T. bakeri.* Este é o primeiro registro da detecção de *Rickettsia* em ácaros trombiculídeos coletados em roedores do Brasil.

Palavras-chave: Trombiculídeos, "*Candidatus* Rickettsia colombianensi", ectoparasitas, Rodentia, Didelphimorphia, Brasil.

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Introduction

Larval stages of trombiculid mites (Trombidiformes: Trombiculidae), also known as chiggers, typically parasitize terrestrial vertebrates and during feeding they inject digestive enzymes into the skin of their hosts. If infected, chiggers may transmit pathogens and/or cause severe skin reactions to the host, a disease commonly known as trombiculiasis (SANTIBÁÑEZ et al., 2015). In the Asia-Pacific region, chiggers are known as tsutsugamushi ("tsutsuga" = disease, "mushi" = bug), and are recognized vectors of *Orientia tsutsugamushi*, the agent of an acute febrile disease in humans, commonly known as Scrub typhus (RAPMUND et al., 1969; TAKAHASHI et al., 2004; KELLY et al., 2009; PHASOMKUSOLSIL et al., 2009).

Pathogenic microorganisms other than *O. tsutsugamushi* also can be found in trombiculid mites. Frank (1977) detected the presence of the protozoan *Hepatozoon erhardovae* in chiggers of the species *Hirsutiella zachvatkini* (Schluger 1948) in Austria, and *Coxiella burnetii*, the causative agent of Q-fever, was detected once in African chiggers (DANIEL, 1961; KEPKA, 1965). Furthermore, Blanc et al. (1952) successfully infected *Neotrombicula autumnalis* (Shaw 1790) with *C. burnetii* under laboratory conditions, and Le Gac et al. (1953) reported a Q-fever case in a laboratory technician who was handling chiggers from an endemic area in Equatorial Africa. On the other hand, bacteria of genus *Rickettsia* were detected in chiggers from Ukraine (VYSOTSKAYA & SCHLUGER, 1953), Korea (CHOI et al., 2007) and China (HUANG et al., 2017). In particular, *Rickettsia monacensis* and *Rickettsia helvetica* were detected in mites from Slovakia (MIŤKOVÁ et al., 2015).

In Brazil, Fonseca (1932) emphasized the importance of mites as potential vectors in the epidemiological cycle of rickettsial diseases. Recently, Bassini-Silva et al. (2018a) detected a *Rickettsia felis*-like agent in the trombiculid *Blankaartia sinnamaryi* (Floch & Fauran, 1956) parasitizing birds, which corresponded to the first report of a rickettsial agent in Brazilian mites.

Here, we are contributing with the knowledge on molecular studies of chiggers parasitizing small mammals. In addition, we report the molecular detection of a *Rickettsia* sp. in three species of chiggers collected from three hosts.

Materials and Methods

Molecular analyses

Chiggers collected in São Paulo state, preserved in ethyl alcohol PA 100%, stored in a -80ºC freezer, housed in the Acari Collection of the Instituto Butantan (IBSP) of São Paulo City, Brazil, were individually submitted to DNA extraction using the Guanidine Isothocianate (GT) lysis protocol following Chomczynski (1993). Each mite was placed into a plastic 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 using Hoyer's medium in order to perform morphological identifications according to Walter & Krantz (2009).

Conventional PCRs targeting a partial fragment of the mite 18S ribosomal gene and a section of the mite mitochondrial cytochrome oxidase I (COI) gene were initially performed using thermal conditions and employed primers listed in Table 1. All reactions included a positive (DNA extracted from *B. sinnamaryi*) and a negative (DNA-free ultrapure water) control. As 18S rRNA and COI gene-PCRs were used as endogenous controls, negative samples were excluded from further analyses.

Positive samples were then screened for agents in the genera *Coxiella*, *Hepatozoon* and *Rickettsia* through conventional and heminested PCR protocols. All the reactions included a negative

Table 1. Oligonucleotide primers used for the amplification of the genes 18S rRNA and COI (endogenous controls), *gltA* and *ompA* (*Rickettsia*), 16S rRNA (*Coxiella*) and 18S rRNA (*Hepatozoon*).

Organisms	Gene	Sequence 5'-3'	Size (pb)	Reference
Rickettsia spp.	*gltA*	CS-78 (GCAAGTATCGGTGAGGATGTAAT) CS-323 (GCTTCCTTAAAATTCAATAAATCAGGAT)	401	Labruna et al. (2004)
Rickettsia spp. (SPF)	*ompA* (1st round)	Rr190.70 (ATGGCGAATATTTCTCCAAAA) Rr190.701R2 (GTTCCGTTAATGGCAGCATCT)	632	Regnery et al. (1991) and Roux et al. (1996)
Rickettsia spp. (SPF)	*ompA* (hemi-nested)	Rr190.70 (ATGGCGAATATTTCTCCAAAA) Rr190.602 (AGTGCAGCATTCGCTCCCCCT)	532	Regnery et al. (1991)
Coxiella spp.	16S	16SrRNA F (GGGGAAGAAAGTCTCAAGGGTAATATCCTT) 16SrRNA R (TGCATCGAATTAAACCACATGCTCCACCGC)	532	Almeida et al. (2012)
Hepatozoon spp.	18S	HEP142-169-F (GCTTGAAACACTCTARTTTTCTCAAAG) HEP743-718-R (ACAATAAAGTAAAAAACAYTTCAAAG)	574	Almeida et al. (2013)
Mite	COI	COI 772 (TGATTTTTTGGTCACCCAGAAG) COI 773 (TACAGCTCCTATAGATAAAAC)	408	Navajas et al. (1994) adapted by Soller et al. (2001)
Mite	COI	bcdF01 CATTTTCHACTAAYCATAARGATATTGG bcdR04 TATAAACYTCDGGATGNCCAAAAAA	560-680	Dabert et al. (2008, 2010)
Mite	COI	LCO1490 GGTCAACAAATCATAAAGATATTGG LCO2198 TAAACTTCAGGGTGACCAAAAAATCA	710	Folmer et al. (1994)
Mite	18S	18S-1F (ATATTGGAGGGCAAGTCTGG) 18S-1R (TGGCATCGTTTATGGTTAG)	500	Otto & Wilson (2001)

SPF: Spotted Fever Group rickettsiae.

control (DNA-free Milli-Q water) and an appropriate positive control, which consisted of DNA of *Coxiella* sp., extracted from infected *Ornithodoros* ticks; *Hepatozoon canis,* extracted from blood of an infected dog; and *Rickettsia vini* strain Breclav, extracted from an infected Vero cell culture. Reactions yielding amplicons of the expected size were treated with ExoSAP-IT (USB Corporation®, OH) following the manufacturer instructions (3 µl of ExoSAP with 7.5 µl of the amplified DNA). Sanger sequencing of the amplicons was performed at the "Centro de pesquisa sobre Genoma Humano e Células Tronco do Instituto de Biociências da USP". Obtained sequences were assembled and trimmed with Geneious R9 (KEARSE et al., 2012), and then submitted to BLASTn analyses (NCBI, 2019) in order to infer closest similarities with other homologous sequences (ALTSCHUL et al., 1990).

Morphological tools

The slide-mounted voucher specimens were identified to the genus level using the key by Brennan & Goff (1977), and to the species level based on the original descriptions cited in the Brazilian checklist of chiggers (JACINAVICIUS et al., 2018b). Additional comparisons were made with type series and other specimens deposited in the mite collection of the United State National Museum (USNM), currently housed at the Systematic Entomology Laboratory, Beltsville, Maryland, USA (BARC-USDA-ARS).

Results

The 317 chiggers examined at the IBSP were collected from nine cricetid rodents (*Akodon montensis*, *Akodon* sp., *Delomys sublineatus*, *Euryoryzomys russatus*, *Hylaeamys megacephalus*, *Necromys lasiurus*, *Nectomys squamipes*, *Oligoryzomys* sp. and *Thaptomys nigrita*), one echimyid rodent (*Thrichomys fosteri*), and four marsupials (*Didelphis aurita*, *Gracilinanus agilis*, *Monodelphis americana*, *Monodelphis domestica* and *Thylamys macrurus*). After DNA extraction, mites were slide mounted and morphologically identified generating the following vouchers: *Herpetacarus hertigi* (Brennan, 1970) (22), *Eutrombicula tinami* (Oudemans, 1910) (14), *Kymocta* sp. (7), *Quadraseta brasiliensis* Goff and Gettinger, 1989 (175), *Quadraseta falconensis* Goff and Brennan, 1977 (6), *Quadraseta flochi* (Brennan and Jones, 1960) (5), *Quadraseta mackenziei* (Yunker and Brennan, 1964) (4), *Quadraseta pazca* (Brennan and Jones, 1964) (54), *Quadraseta trapezoides* (Brennan and Jones, 1964) (11), *Quadraseta* sp. (9), *Serratacarus* sp. (1) and *Trombewingia bakeri* (Fonseca, 1955) (9).

A total of 20 samples yielded expected size amplicons for the mite 18S rRNA gene. Obtained sequences corresponded to a unique haplotype for each of the following seven chigger species. The GenBank accession numbers are: one *H. hertigi* (MG817637), two *Kymocta* sp. (MG817642), four *Q. brasiliensis* (MG817643), two *Q. pazca* (MG817644), seven *Q. trapezoides* (MG817645), one *Quadraseta* sp. (KY934461), and three *T. bakeri* (MG817646). One amplified sample of *Q. pazca* did not generate a good quality sequence and was not deposited in GenBank. Attempts to amplify fragments of the COI gene from all samples were unsuccessful. After BLASTn analyses, consensus sequences of *H. hertigi*, *Kymocta* sp., *Q. brasiliensis*, *Q. pazca*, *Q. trapezoides*, *Quadraseta* sp., and *T. bakeri* were 98.63% (431/437-pb), 99.51% (408/410-pb), 99.50% (397/399-pb), 99.75% (404/405-pb), 98.77% (400/405-pb), 98.39% (428/435-pb), and 99.50% (397/399-pb) identical with a homologous sequence of *Eutrombicula splendens* (Ewing, 1913) (KY922159).

All 20 samples positive for 18S rDNA PCR assays were tested for *Coxiella*, *Hepatozoon* and *Rickettsia*. A total of 13 samples yielded rickettsial *gltA* amplicons, but only six were successfully sequenced. In addition, one sample also yielded rickettsial *ompA* amplicons. PCR targeting 16S rDNA gene of *Coxiella* sp. and 18S rDNA gene of *Hepatozoon* sp. were negative. Sequences of *Rickettsia* were submitted to BLASTn analysis. Six identical *gltA* sequences were 100% (350/350 bp) identical to "*Candidatus* Rickettsia colombianensi" (MG970682). These sequences came from one larva of *H. hertigi*, three larvae of *Q. trapezoides* and two of *T. bakeri*. Besides that, one of the successfully-sequenced *ompA* samples was 100% (488/488 bp) identical to "*Ca.* Rickettsia colombianensi" (MG970683) as well. Sequences of *Rickettsia* generated in the current study were deposited in GenBank under the accession numbers MG906649, MG906650, MG906652, MG906653, MG906654, MG906656.

Overall, one species of *H. hertigi* collected in Fontes do Ipiranga State Park (PEFI), São Paulo municipality, parasitizing an *Oligoryzomys* sp.; three specimens of *Q. trapezoides* from Morro Grande, Cotia municipality, collected on *Nectomys squamipes*; and two specimens of *T. bakeri* collected in Campos do Jordão municipality, on *Akodon montensis*, were positive for "*Ca.* Rickettsia colombianensi".

Discussion

The successful amplification rates for the mite 18S rRNA gene upon individual chiggers, was 13.63% (3/22) for *H. hertigi*, 28.57% (2/7) for *Kymocta* sp., 2.28% (4/175) for *Q. brasiliensis*, 5.55% (3/54) for *Q. pazca*, 63.64% (1/11) for *Q. trapezoides*, 11.11% (1/9) for *Quadraseta* sp., and 33.33% (3/9) for *T. bakeri*. Amplification of this same locus was unsuccessful for *E. tinami*, *Q. falconensis*, *Q. flochi*, *Q. mackenziei* and *Serratacarus* sp. We were successful in amplifying only 7.26% (20/317) of all individually tested species, while Bassini-Silva et al.(2018a) obtained a 72.5% (29/40) of success in amplifying the same gene for *B. sinnamaryi*. Park et al. (2015) successfully amplified the 18S rRNA gene in 50% (38/76) for *Helenicula miyagawai* (Sasa, Kumada and Miura, 1951) mites, and in 87.5% (7/8) for *Leptotrombidium scutellare* (Nagayo, Miyagawa, Mitamura, Tamiya and Tenjin, 1921).

Of the 73 chiggers species reported from Brazil, 18S rDNA sequences were previously available for only three species: *B. sinnamaryi* (MG783391) (BASSINI-SILVA et al., 2018a), *Eutrombicula daemoni* Bassini-Silva and Jacinavicius, 2018 (MG707783, MG70778) (BASSINI-SILVA et al., 2018b), and *Q. brasiliensis* (MF113413, MF113412, KY934462, KY934463, KY934464) (JACINAVICIUS et al., 2018a). In the present study, we provide new 18S rDNA sequences for *H. hertigi*, *Kymocta* sp., *Q. pazca*, *Q. trapezoides*, and *T. bakeri.* According to Hillis & Dixon (1991) and Cruickshank (2002), the 18S rRNA gene

has a slow rate of evolution and it is suitable to infer family and subfamily level phylogenies. As the availability of sequences of this locus for chiggers from South America is still scarce, the creation of a South American chigger gene bank may afford the inclusion of these taxa in future phylogenetic studies dealing with the relationships between mites in higher groupings.

The mitochondrial COI gene has a faster rate of sequence divergence if compared with nuclear ribosomal genes (OTTO & WILSON, 2001). Kampen et al. (2004), Moniuszko et al. (2015) and Kumlert et al. (2018) successfully amplified this gene for some chigger species of the genera *Ascoschoengastia*, *Blankaartia*, *Hirsutella*, *Leptotrombidium*, *Neotrombicula*, *Schoengastia*, *Schoutedenichia*, and *Walchia*, from Europe and Asia. Although in the current study we employed this same pairs of primers, none of our samples yielded amplicons, which is in the line with previous studies in Brazil (BASSINI-SILVA et al., 2018a; JACINAVICIUS et al., 2018a). We highlight then the need for testing new sets or designing new primers that will successfully amplify this gene for South American chiggers.

Rickettsial DNA identical to two "*Ca.* Rickettsia colombianensi" genes was detected in three chigger species for the first time, all from the state of São Paulo. "*Ca.* Rickettsia colombianensi" was originally characterized from the tick *Amblyomma dissimile* Koch, 1844 in Colombia (MIRANDA et al., 2012). In Brazil, this species of *Rickettsia* sp. was recently detected in *A. dissimile* from Amazon biome of Amapá state (LUZ et al., 2018), and the current finding extends its occurrence to further Acari representatives, in particular, to trombiculid mites in the Atlantic rainforest biome of the São Paulo state. "*Candidatus* R. colombianensi" belongs to the spotted fever group of rickettsiae, and its isolation in Vero cells has demonstrated a marked cytopathic effect (MIRANDA et al., 2019). Although infection in cattle and iguanas has been suggested in Colombia (MIRANDA et al., 2012), the role of "*Ca.* Rickettsia colombianensi" as a pathogenic agent is still obscure.

The single record of *Rickettsia* in chiggers from Brazil was documented by Bassini-Silva et al. (2018a), and correspond to a *Rickettsia felis*-like agent detected in *B. sinnamaryi*, collected on *Tachyphonus coronatus* (Aves: Passeriformes) from the state of Minas Gerais. However, *Rickettsia* spp. have been reported from various mites collected on birds and mammals (VYSOTSKAYA & SCHLUGER, 1953; HASE et al., 1978; CHOI et al., 2007; TAKAHASHI et al., 2004; MIŤKOVÁ et al., 2015; HUANG et al. 2017; BASSINI-SILVA et al., 2018a), which suggest that our finding is not an isolated case. On the other hand, we had no success in detecting the presence of *Coxiella* sp. and *Hepatozoon* sp. in the analyzed chiggers.

This is the first report of *Rickettsia* detection in chiggers collected on rodents in Brazil. As studies on chiggers as possible vectors of *Rickettsia* are scarce in the Neotropical region, more prospections are needed to determine if these mites are, in fact, involved in the epidemiological cycles of rickettsial diseases.

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