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






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# Contribution to the knowledge of *Parichoronyssus bakeri* Morales-Malacara and Guerrero, 2007 (Mesostigmata: Macronyssidae): new locality and host-association records with additional molecular data

Victor de Souza Mello-Oliveira <sup>a</sup>, Fernando de Castro Jacinavicius <sup>b</sup>, Mariane Guerre-de-Souza <sup>a</sup>, Leormando Fortunato Dornelas Júnior <sup>c,d</sup>, Ana Carolina Castro-Santiago <sup>e</sup>, Ana Cláudia Calchi <sup>a</sup>, Elizabete Captivo Lourenço <sup>f</sup>, Ashley P. G. Dowling <sup>g</sup>, Katia Maria Famadas <sup>h</sup>, Marcos Rogério André <sup>a</sup>, Luis Marcelo Aranha Camargo <sup>c,d,i,j</sup>, Darci Moraes Barros-Battesti <sup>a</sup>, Ricardo Bassini-Silva <sup>a,b</sup>

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## Original research

### ABSTRACT

The mite genus *Parichoronyssus* Radovsky (Mesostigmata: Macronyssidae) comprises twelve known species found parasitizing bats in the New World. This study aimed to identify morphologically and molecularly macronyssid mites collected from bats and kept as backlog in the tissue bank (-80C) at the Acarological Collection of the Butantan Institute (IBSP). After the examination, the material was listed and identified as *Parichoronyssus bakeri* Morales-Malacara and Guerrero. Here, we were able to provide diagnostic microscopic images for the females of this species, as well as molecular information (18S rRNA and 16S rRNA) that were deposited in Genbank for reference. New locality records were also provided for *P. bakeri* in Rio de Janeiro and Rondônia States, Brazil.

**Keywords** mites; bats; 16S rRNA; 18S rRNA; Brazil

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## Introduction

The family Macronyssidae Oudemans, 1936 stands out among mites of medical-veterinary importance since some species can cause dermatitis and even more severe injuries in animals and humans, as well as can carry pathogens, playing a role as putative vectors in the epidemiological cycles of illnesses (Reeves 2007; Hornok *et al.* 2012; Bassini-Silva *et al.* 2019; 2022a). Despite that, this family still needs studied since little is known about species diversity, host-parasite associations, biology, and associated microbiome.

Macronyssidae is distributed worldwide and includes 35 genera and over 200 species (Radovsky 2010; Orlova *et al.* 2017; Bassini-Silva *et al.* 2021; 2022b). Most species parasitize vertebrates, with approximately 75% of the described species having bats as their primary hosts (Radovsky 2010). In Brazil, 15 genera and 29 species of Macronyssidae have been recorded (Bassini-Silva *et al.* 2021; 2022b), with about half the diversity recorded parasitizing bats (Bassini-Silva *et al.* 2021; 2022b).

The genus *Parichoronyssus* Radovsky, 1966 currently includes 12 species described in New World bats (Morales-Malacara and Guerrero 2020). Four of the 12 species are known to occur on phyllostomid bats in Brazil: *Parichoronyssus bakeri* Morales-Malacara and Guerrero, 2007; *Parichoronyssus crassipes* Radovsky, 1967; *Parichoronyssus euthyesternum* Radovsky, 1967; and *Parichoronyssus sclerus* Radovsky, 1966 (Whitaker and Mumford 1977; Azevedo *et al.* 2002; Almeida *et al.* 2011; Moras *et al.* 2013).

So far, the distribution of these four species is restricted to the southeastern region of Brazil, with *P. bakeri*, *P. crassipes*, and *P. sclerus* only recorded in the state of Minas Gerais (Whitaker and Mumford 1977; Azevedo *et al.* 2002; Moras *et al.* 2013) and *P. euthyesternum* in the state of Rio de Janeiro (Almeida *et al.* 2011). Host records in Brazil are also few, with *P. bakeri*, *P. crassipes*, and *P. euthyesternum* collected on the little yellow-shouldered bat, *Sturnira lilium* (É. Geoffroy, 1810) (Azevedo *et al.* 2002; Almeida *et al.* 2011; Moras *et al.* 2013), *P. sclerus* on the tailed tailless bat, *Anoura caudifer* (É. Geoffroy, 1818) (Whitaker and Mumford 1977), and *P. bakeri* on the Seba's short-tailed bat, *Carollia perspicillata* (Linnaeus, 1758) (Moras *et al.* 2013).

This study aimed to identify morphologically and molecularly previously unidentified macronyssid specimens stored in the tissue bank (-80C) at the Acarological Collection of the Butantan Institute (IBSP). Both morphological and molecular data were used to support identification.

## Material and methods

The specimens examined in the present study were stored in ethyl alcohol (100%) at (-80C) in the tissue bank at the Acarological Collection of the Instituto Butantan (IBSP). In order to identify these mites, while some specimens were used for DNA extraction, others were slide-mounted in Hoyer's medium, following the protocols by Walter and Krantz (2009). The morphological identification until the genus level followed Radovsky (2010), while the key previously described by Morales-Malacara and Guerrero (2020, 2023) was used for species identification. The original description was consulted to support the identification of the material (Morales-Malacara and Guerrero 2007).

Slide-mounted specimens were examined using a Leica DFC 500 digital camera and a phase-contrast Leica DM4000B optical microscope. Extended focal range images were composed using Leica Application Suite version 2.5.0. The images were prepared with Adobe Photoshop v.13.0 software.

Each mite pulled for DNA analysis was individually placed into a 1.5 µL Eppendorf® microtube. DNA extraction was performed following the manufacturer's QIAGEN DNeasy Blood & Tissue Kit protocols. After DNA extraction, the remaining cuticles were slide-mounted as vouchers and identified following the protocols described above.

Three molecular markers (18S rRNA, 16S rRNA, and COI) were targeted for molecular characterization. The following primer pairs were used to target each gene fragment: 18S rRNA using the primers Mite18S-1F (5'-ATATTGGAGGGCAAGTCTGG-3') and Mite18S-1R (5'-TGGCATCGTTTATGGTTAG-3') (Otto and Wilson 2001); 16S rRNA using the primers 16S+1 (5'-CTGCTCAATGATTTTTTAAATTGCTGTGG-3') and 16S-1 (5'-CCGGTCTGAACTCAGATCAAGT-3') (Mangold *et al.* 1998); and COI using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.* 1994). PCR reagents concentration and thermal cycler conditions followed the abovementioned works. Negative (ultrapure water type I) and positive controls (pool of *Tyrophagus* sp.) were used for each reaction. All positive products were purified with ExoSap-IT (GE Healthcare® Pittsburgh, PA).

Sanger sequencing was performed at the “Centro de Pesquisa sobre Genoma Humano e Células Tronco do Instituto de Biociências”, University of São Paulo, São Paulo State. The obtained sequences were assembled using Sequencing Analysis 5.3.1 software and submitted to BLAST analysis (Altschul *et al.* 1990) to infer similarities with other mite sequences available in GenBank. Different haplotypes were visually discriminated after an alignment using the CLUSTAL W algorithm (Thompson *et al.* 1994) implemented in Geneious R11 (Kearse *et al.* 2012).

## Results

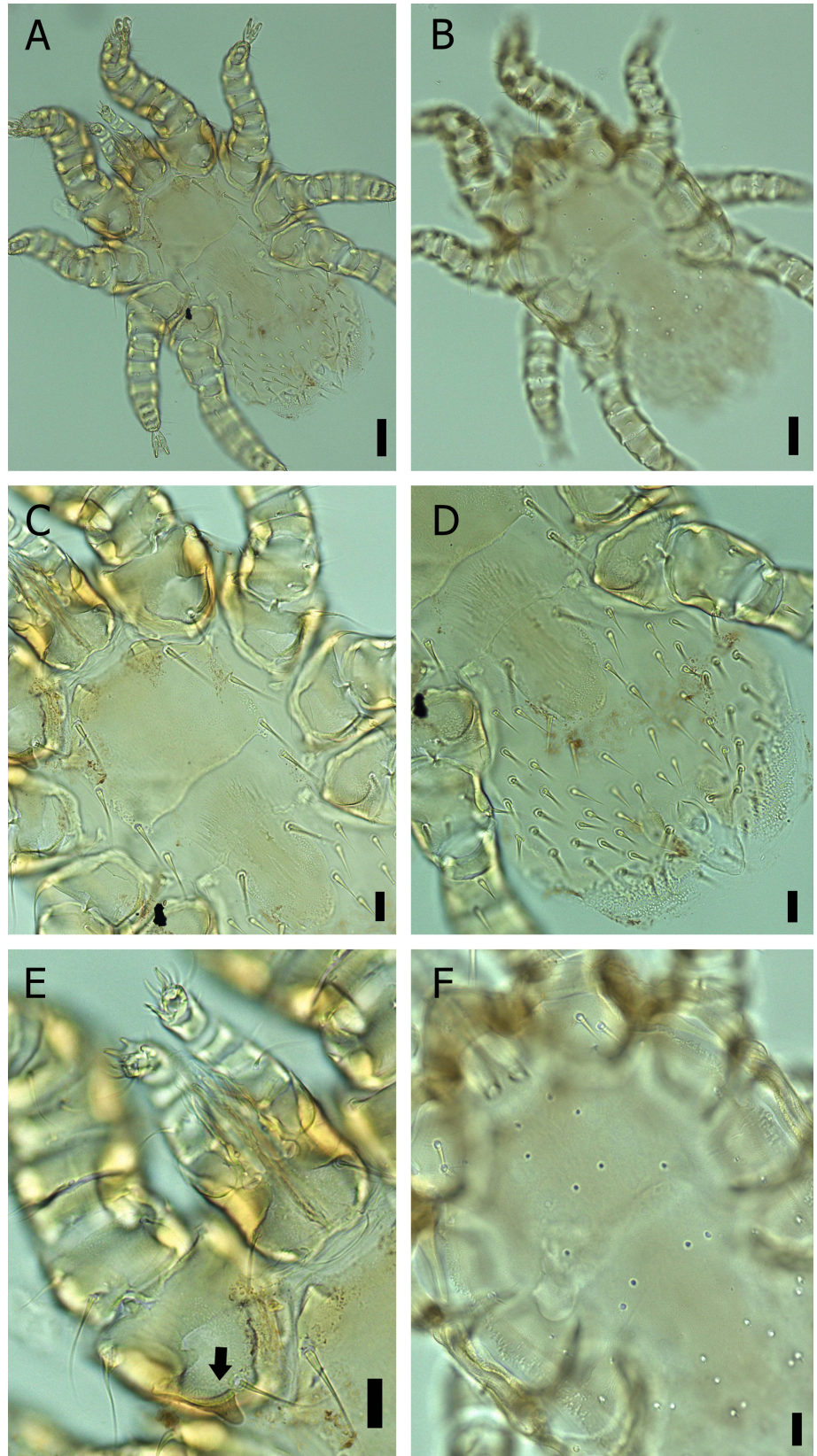
A total of 85 macronyssid specimens collected from the greater spear-nosed bat, *Phyllostomus hastatus* (Pallas, 1767) (Chiroptera: Phyllostomidae) were examined and identified as *Parichoronyssus bakeri* (Figure 1). Sixty-five of the total number of specimens were used in the molecular analysis, and only three 18S rRNA and six 16S rRNA sequences were obtained from the females of the mites. The obtained sequences were deposited in Genbank as reference sequences for the genus and species. COI was not successfully amplified from any specimen.

The females of *P. bakeri* (Figure 1) can be separated from other *Parichoronyssus* by having the dorsal shield with 13 pairs of setae; trichopores present in the place of setae *j4* to *j6*, and *z5*; seta *r2* absent; more than 24 pairs of setae on the integument of the ventral opisthosoma; entire anal shield without a line interrupting the perianal zone in the anterior region; coxal field I with a spine-like projection with rounded tip in the posterior region (Figure 1E).

Only diagnostic characteristics of the females are being highlighted here since we have yet to obtain sequences of males and nymphal stages (deutonymph and protonymph) to confirm the species. According to the literature, these other stages were morphologically identified, but it is worth noting that different species of *Parichoronyssus* can parasitize the same host.

The three partial 18S rRNA sequences were obtained from two different lots (IBSP 18475 and 18513) from Rio de Janeiro State and were identical among them, constituting a single haplotype (GenBank access number OR242724). Compared with the sequences available in Genbank, this sequence showed 96.15% identity (query cover: 99%; e-value: 0.0) with *Chiroptonyssus haematophagus* (GenBank access number: FJ911855). While the six partial 16S rRNA sequences were obtained from three different lots (IBSP 18475, 18513, and 18514), also from the same state. Five sequences were identical between them, constituting a single haplotype, and the other sequence with a difference when compared to the haplotype (GenBank access number OR242725 and OR242726). The two haplotypes of 16S rRNA partial sequences showed 89.44% and 88.89% identity (query cover: 98% and 99%; e-value: 6e-14 and 4e-137, respectively) with *Chiasmnyssus cavernicola* (GenBank access number: MW522530).

**Material examined. BRAZIL** — 14♀ and 8♂ (IBSP 18370); Monte Negro Municipality, Rondônia State (63°14'2"W, 10°17'42"S); 20 May 2021; ex. *Phyllostomus hastatus* (Pallas, 1767) (Chiroptera: Phyllostomidae); Dornelas L. F. Jr. coll. 8♀ and 4♂ (IBSP 18376); same data. 14♀, 2♂ and 7PN (IBSP 18475); Mário Takume Farm, Tinguá Neighborhood, Nova Iguaçu Municipality, Rio de Janeiro State; 17 Nov. 2011; same host; Lourenço E.C. *et al.* coll.



**Figure 1** Light Microscopy images of the female *Parichoronyssus bakeri*. A – General view of the ventral idiosoma; B – General view of the dorsal idiosome; C – Close up of sternal shield; D – Close up of genital and anal shields; E – Gnathosoma and coxa of the Leg I, with the black arrow pointed out the spine-like projection; F – Close up of the dorsal shield. Scales: A and B 50 $\mu$ m, C-F 20 $\mu$ m.

5♀ and 12PN (IBSP 18514); same locality and host; 14 Apr. 2012. 11♀ (IBSP 18513); same locality and host; 17 Nov. 2011.

## Discussion

Four species of *Parichoronyssus* have been recorded from Brazil: *P. bakeri*, *P. crassipes*, *P. euthysternum*, and *P. sclerus*. Based on known host records in the Neotropics (Azevedo *et al.* 2002; Morales-Malacara and Guerrero 2020), the previous reports from Brazil for some of these species might need to be revised. This might have happened due to possible contamination during collection and handling, accidental transmission of mites to the wrong hosts, or even misidentification. For example, the records of *P. bakeri* from *C. perspicillata* and *S. lilium* in Minas Gerais State (Moras *et al.* 2013) are problematic because elsewhere, this species has only been associated with the genus *Phyllostomus*. Additionally, *P. crassipes* from *Sturnira lilium* (Azevedo *et al.* 2002) is also questionable since *P. crassipes* is a mite species that primarily parasitizes *Carollia* spp. Specimens from those studies should be reexamined to validate species identification and document the natural distribution of these species in the Brazilian territory.

Regarding the molecular analysis, the only *Parichoronyssus* sequence available in Genbank is for a fragment of the 28S rRNA (Dowling and O'Connor 2010) without species identification. The present study contributed to future molecular identification of *Parichoronyssus* spp. by providing sequences for two molecular markers, namely 18S rRNA and 16S rRNA, aiming at improving the genetic representation of *P. bakeri* in Genbank. Also, we provide diagnostic images and additional information about *P. bakeri* females, as well as new locality records for this species in Rio de Janeiro and Rondônia States, all associated with *Ph. hastatus*.

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