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Short Communication

First record of *Ornithodoros faccinii* (Acari: Argasidae) on toads of genus *Rhinella* (Anura: Bufonidae) in Brazil

Primeiro registro de *Ornithodoros faccinii* (Acari: Argasidae) em sapos do gênero *Rhinella* no Brasil

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Abstract

Although a group of soft ticks (Argasidae) associated with amphibians was recently discovered in Brazilian rainforests, parasitism by these ticks on cold-blooded animals remains less common than on mammal and bird species. In this study, we identified ticks that were collected from toads that had been caught in December 2016 and January 2017, at Itinguçú waterfall (22°54’05” S; 43°53’30” W) in the municipality of Itaguaí, state of Rio de Janeiro. Tick specimens were identified using a morphological and molecular approach. In total, twelve larvae of *Ornithodoros* ticks were collected from three individuals of *Rhinella ornata* and were identified as *Ornithodoros faccinii*. Our results include a longer 16S rRNA mitochondrial sequence for *O. faccinii* that supports its phylogenetic relatedness to *Ornithodoros saraivai*, and we report this tick species parasitizing *Rhinella* toads for the first time in Brazil.

Keywords: Ticks, Argasidae, Anurans, *Rhinella*, Brazil.

Resumo

Embora um grupo de carrapatos moles (Argasidae) associado a anfíbios tenha sido recentemente descoberto nas florestas brasileiras, o parasitismo por esses carrapatos em animais de sangue frio permanece menos comum do que nas espécies de mamíferos e aves. Neste estudo, identificamos carrapatos que foram coletados de sapos capturados em dezembro de 2016 e janeiro de 2017, na cachoeira de Itinguçú (22°54’05” S; 43°53’30” W) no município de Itaguaí, estado do Rio de Janeiro. Os espécimes de carrapatos foram identificados usando uma abordagem morfológica e molecular. No total, doze larvas de carrapatos *Ornithodoros* foram coletadas de três indivíduos de *Rhinella ornata* e foram identificadas como *Ornithodoros faccinii*. Nossos resultados incluem uma maior seqüência mitocondrial 16S rRNA para *O. faccinii* que suporta sua relação filogenética com *Ornithodoros saraivai* e relatamos esta espécie de carrapato parasitando sapos *Rhinella* pela primeira vez no Brasil.

Palavras-chave: Carrapatos, Argasidae, Anuros, *Rhinella*, Brasil.

Introduction

The fauna of anurans in Brazil is the largest in the world with more than 1000 extant species (IUCN, 2017; SBH, 2017). Of this total, less than 10% have been reported to present associations with ticks in this country. Amphibians of the families Bufonidae and Cycloramphidae have been found to act as the main associated hosts (LUZ & FACCINI, 2013; GUGLIELMONE et al., 2014; BARROS-BATTESTI et al., 2015; MUÑOZ-LEAL et al., 2017b). Ticks parasitizing anurans have been reported in the Brazilian biomes of the Cerrado, Caatinga, Mata Atlântica (Atlantic Forest) and Amazônia (Amazon region), and chiefly comprise hard ticks (Ixodidae) of the genus *Amblyomma* (WOEHL, 2002; DANTAS-TORRES et al., 2008; BARROS-BATTESTI et al., 2015; HORTA et al., 2015; LUZ et al., 2015). However, soft ticks (Argasidae) such as *Ornithodoros faccinii* Barros-Battesti, Landulfo & Luz, 2015, and *Ornithodoros saraivai* Muñoz-Leal & Labruna, 2017, have been recently added to the list, and have pointed towards the evolutionary possibility of a group that evolved in association with amphibians (MUÑOZ-LEAL et al., 2017b).

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Most Neotropical species of the family Argasidae have been recorded as parasitizing a wide variety of wild mammals, birds, reptiles and, to a lesser extent, amphibians (RAMOS et al., 2015; SPONCHIADO et al., 2015; LABRUNA et al., 2016; MUÑOZ-LEAL et al., 2016). Until now, only anurans of the families Bufonidae and Cycloramphidae have been recorded in association with soft ticks, restricted exclusively to species of the genus *Ornithodoros* (CAPRILES & GAUD, 1977; RIVAS et al., 2012; BERMÚDEZ et al., 2013; BARROS-BATTESTI et al., 2015; MUÑOZ-LEAL et al., 2017b). Of the 211 species of soft ticks that have been described to date, 129 belong to the genus *Ornithodoros*, and five of them, namely *Ornithodoros talaje* (Guérin-Méneville, 1849), *Ornithodoros puertoricensis* Fox 1947, *O. faccinii* and *O. saraivai*, and an additional species that is morphologically related to the *O. talaje* group (RIVAS et al., 2012), have been recorded parasitizing amphibians in the Americas.

The aims of the current study were to provide the first report of *O. faccinii* parasitizing anurans of the family Bufonidae in Brazil and to reassess its phylogenetic position by means of a longer partial sequence of 16S mitochondrial rRNA.

Materials and Methods

Study site and toad-catching

Ticks were collected from toads between December 2016 and January 2017, at Itinguçú waterfall in the municipality of Itaguaí, state of Rio de Janeiro, distant 30 meters (22°54'05" S; 43°53'30" W) from the type locality of *O. faccinii*.

Anurans were caught manually through an active search, at dusk on alternate days, over a total six days, comprising 19 hours of sampling effort. All the animals thus caught were examined for tick infestation, and they were released at the same location where they had been caught, in order to cause minimal impact on the population of these hosts. The taxonomic nomenclature for amphibians that was used followed the system of the Brazilian Society of Herpetology (SBH, 2017), and the animals were caught and manipulated in accordance with the recommendations of the Brazilian Institute for the Environment and Renewable Natural Resources - Chico Mendes Institute for Biodiversity Conservation (IBAMA-ICMBio - number 36164-1).

Collection and morphological identification of ticks

Larval stages of soft ticks were removed with tweezers, kept in vials containing RNAlater (Sigma-Aldrich) and transported to the laboratory. For morphological and morphometric analyses, three ticks were slide-mounted in Hoyer's medium, and were photographed using an Olympus DP70 camera that was coupled to an Olympus BX40 optical microscope (Olympus Optical Co. Ltd., Japan). Specimens were identified to genus level as described by Barros-Battesti et al. (2013), and species-level diagnosis was performed by comparing the morphological traits of the slide-mounted ticks with those of other Neotropical Ornithodorinae (ENDRIS et al., 1989; BARROS-BATTESTI et al., 2015; MUÑOZ-LEAL et al., 2017b). Type species of *O. faccinii* from the Acari collection of the Butantan Institute (IBSP 10316) were examined in order to compare the number of dorsal setae.

Molecular tools

For molecular analyses, larval DNA was extracted using the bead-beater/phenol-chloroform method (SANTOLIN et al., 2013). Subsequently, conventional PCR as described by Mangold et al. (1998) was performed using the primers 3'- CCGGTCTCAACTCAGATCAAGT-5' (forward) and 3'-GCTCAATGATTTTTTAAATTGCTGT-5' (reverse), targeting a fragment of approximately 460 bp from the mitochondrial sequence encoding 16S rRNA. Sequencing of PCR products was performed by combining the same amplification primers, purified amplicons and BigDye Ready Reaction mix (ABI Corp) in an automated genetic analyzer (model 3500; ABI Corp). The sequences were assembled using Sequencher (Version 5.3, Genecodes Corporation, CA, USA). We used the BLAST search algorithm (ALTSCHUL et al., 1990) to determine closest gene identities.

Phylogenetic analysis

The sequences obtained for the mitochondrial 16S rRNA gene were aligned with another 60 Argasidae sequences retrieved from GenBank using Clustal X (THOMPSON et al., 1997), and were manually adjusted using the GeneDoc software. Sequences from *Ixodes holocyclus* Neumann, 1899 and *Ixodes uriae* White, 1852 were used as outgroups (accession numbers of all sequences are shown in the phylogenetic tree). The alignment was used to construct a phylogenetic tree using maximum parsimony, as implemented in PAUP version 4.0b10 (SWOFFORD 2002), with 500 bootstrap replicates, random stepwise addition to start trees (with random addition sequences) and TBR branch swapping. Bayesian analysis was performed using MrBayes v3.1.2 (HUELSENBECK & RONQUIST, 2001) with four independent Markov chain runs for 1,000,000 metropolis-coupled MCMC generations, in which one tree was sampled every 100th generation. The first 25% of the trees represented burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities.

Results

We caught 15 anurans (seven specimens of *Rhinella ornata* (Spix, 1824) and eight of *Thoropa miliaris* (Spix, 1824), and a total of 12 larvae of the genus *Ornithodoros* were collected from three specimens of *R. ornata* (20%) (Figure 1). Nine ticks were collected from the dorsal region of the hind limbs and three from the abdomen.

Three larvae were slide-mounted and were morphologically identified as *O. faccinii*, based on the following characters: idiosome with 11 pairs of dorsolateral setae and three pairs of central dorsal setae; seven (instead of six) pairs of dorsal anterolateral and four pairs of dorsal posterolateral setae; dorsal plate smooth, elongated, almost rectangular, with anterior and posterior margins rounded; ventral idiosome provided with seven pairs of setae

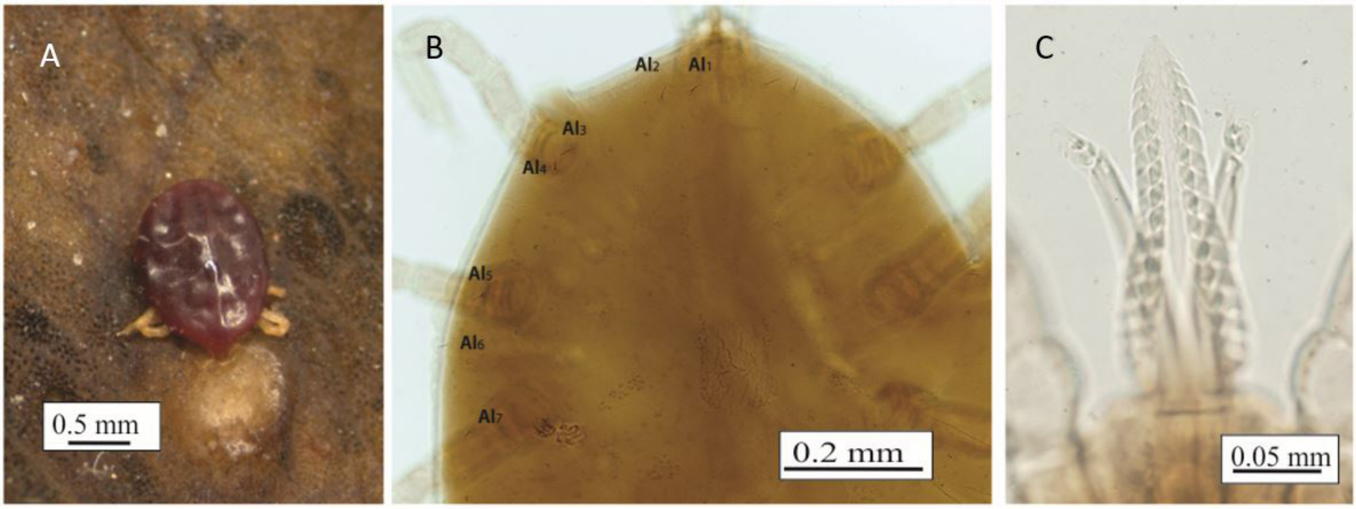

Figure 1. Larva of *Ornithodoros faccinii*: (A) larva parasitizing *Rhinella ornata,* note the subcutaneous abscess like at the attachment site; (B) dorsal view pointing seven pairs of dorsal anterolateral (Al) setae, and (C) hypostome.

(three pairs sternal, three circumanal and one postcoxal), plus one anal pair; posteromedian setae absent; hypostome pointed; and dental formula 3/3 in the anterior third, then 2/2 towards base (BARROS-BATTESTI et al., 2015) (Figure 1). Slide-mounted ticks were deposited in the "Danilo Gonçalves Saraiva" National Tick Collection (CNC) of the School of Veterinary Medicine of the University of São Paulo, São Paulo, Brazil, under accession number CNC-3514.

Morphological identifications of ticks were confirmed by means of molecular analyses, since DNA extracted from nine ticks retrieved a mitochondrial 16S rRNA consensus sequence of 428 bp that matched the shorter and sole 366-bp sequence of *O. faccinii* available in GenBank (KP861242), in which the nucleotides were 100% identical. The 16S sequence obtained in this study was deposited in GenBank under the accession number KY661385.

The phylogenetic relationships between this tick and other Neotropical Argasidae were inferred from alignment of a partial fragment of the mitochondrial 16S rDNA gene, including the longer sequence for *O. faccinii* that was obtained in the present study. This alignment showed that *O. saraivai* was a sister taxon clustering together within a larger clade composed of six other species of *Ornithodoros* (Figure 2).

Discussion

The record of *O. faccinii* larvae parasitizing anurans of the family Bufonidae is the first report of this in Brazil. Out of the 211 valid species of soft ticks worldwide, only representatives of the genus *Ornithodoros* have been recorded in association with amphibians. Indeed, early records of soft ticks on anurans in the Neotropical region documented larvae of a species of *Ornithodoros* that was morphologically related to the *O. talaje* group, and *O. puertoricensis* parasitizing *Eleutherodactylus cooki* Grant, 1932, in Puerto Rico (CAPRILES & GAUD, 1977). Much more recently, Rivas et al. (2012) reported that they had collected approximately 200 larvae of an unidentified species of *Ornithodoros* from a single specimen of *Rhinella arenarum* (Hensel, 1867) in Argentina. Additionally, larval stages of *O. puertoricensis* were also reported by Bermúdez et al. (2013), collected from the axillae of a specimen of *Rhinella marina* (Linnaeus, 1758) in Panama. Lastly, soft ticks corresponding to the recently described *O. faccinii* and *O. saraivai* were recorded as parasites of *T. miliaris* and *Cycloramphus boraceiensis* Heyer, 1983, in the states of Rio de Janeiro and São Paulo respectively (BARROS-BATTESTI et al., 2015; SÁ-HUNGARO et al., 2016; MUÑOZ-LEAL et al., 2017b). To date, these two species are the sole amphibian-associated soft ticks in Brazil. The frogs *T. miliaris* and *C. boraceiensis* are nidicolous endemic species of the Atlantic rainforest that frequent moist fissures in rocky environments near waterfalls (COCROFT & HEYER, 1988; FEIO et al., 2006; AMPHIBIAWEB, 2017). However, specimens of *T. miliaris* were also observed beyond its usual habitat in rocky environments near waterfalls during the fieldwork, under damp leaves in the forest understory, and often sharing the same space with specimens of *R. ornata*. Occurrences of both amphibian species in the same microhabitat might explain the records of *O. faccinii* larvae on *R. ornata*. In turn, it cannot be ruled out that *O. faccinii* might also occur not only in association with lotic water environments, but also in the moisture of the Atlantic rainforest soil, where *Rhinella* toads live.

Although, in the original description of *O. faccinii*, Barros-Battesti et al. (2015) used the same primers as in the current study in order to sequence an approximately 460-bp fragment of mitochondrial 16S rRNA, the sequence available for this species did not exceed 366 bp, and was 62 bp smaller than the sequence presented in the current study, which was composed of 428 bp. Barros-Battesti et al. (2015) constructed a phylogenetic tree in which *O. faccinii* clustered in a clade with *Ornithodoros capensis* Neumann, 1901, and *Ornithodoros sawaii* Kitaoka & Susuki, 1973. The first species, except Antarctica, occurs in all zoogeographic regions of the world, including the coasts of the Atlantic and Pacific oceans in the Neotropical Region, while

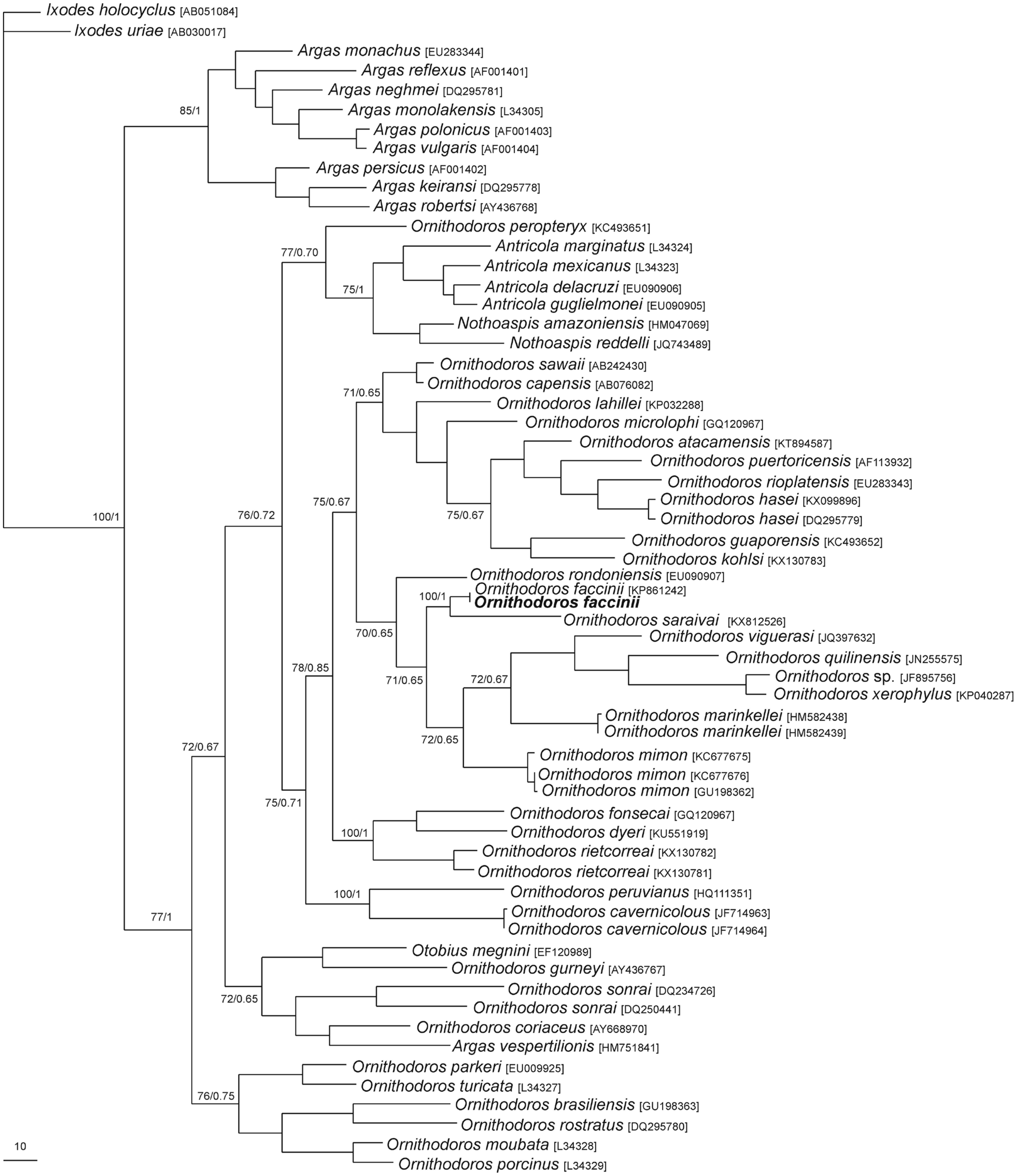

Figure 2. Phylogenetic tree inferred from 16S rDNA gene partial sequences, including the new sequence of *Ornithodoros faccinii* that was generates in the current study.

the latter was restricted to the Japanese islands (KIM et al., 2015; MUÑOZ-LEAL et al., 2017a). In this same analysis, it could be seen that these three species were grouped within a larger clade that included *Ornithodoros marinkellei* Kohls, Clifford & Jones 1969, *Ornithodoros fonsecai* (Labruna & Venzal, 2009), *Ornithodoros rioplatensis* Venzal, Estrada-Peña & Mangold, 2008, and *O. puertoricensis*. In turn, using the longer 16S mitochondrial rRNA sequence (428 bp) that was obtained through the current study, our phylogenetic tree showed that *O. faccinii* forms a monophyletic group with *O. saraivai* and that it clusters within a larger clade that rather excludes *O. capensis* and *O. sawaii* from the group. In this way, the present study corroborates the phylogeny of Argasidae using partial sequences of this mitochondrial gene that was presented by Muñoz-Leal et al. (2017b).

Although, in the current study, larval stages of *O. faccinii* collected from *R. ornata* presented shared identity of at least 366 bp with the sequence of 16S mitochondrial rRNA gene that was characterized in the original description of this species (BARROS-BATTESTI et al., 2015), we observed one noteworthy difference in the dorsal setae, in comparing our material with the dorsal chaetotaxy reported in the original description of this species: seven instead of six pairs of dorsal anterolateral setae. In *Ornithodoros* larvae, dorsolateral setae appear arranged in one anterior group, chiefly composed by seven pairs, and one posterior group with a variable number. Therefore, presence of six pairs of dorsal anterolateral setae is a morphological characteristic that rapidly separates *O. faccinii* from every other Neotropical species of the genus *Ornithodoros*. Since the larva of *O. faccinii* was described exclusively from field-collected material, morphological analyses on laboratory-reared larvae that had not been exposed to tegumentary attrition caused by their microhabitat or by their host would need to be performed to evaluate whether this morphological dissimilarity is a naturally polymorphic characteristic.

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