Research Note

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Morphological and molecular identification of ticks infesting *Boa constrictor* (Squamata, Boidae) in Manaus (Central Brazilian Amazon)

Identificação morfológica e molecular de carrapatos coletados de jiboias (*Boa constrictor*) (Squamata, Boidae) da zona urbana de Manaus

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Abstract

The *Boa constrictor* is one of the world's largest vertebrate carnivores and is often found in urban areas in the city of Manaus, Brazil. The morphological identification of ticks collected from 27 snakes indicated the occurrence of *Amblyomma dissimile* Koch 1844 on all individuals sampled. In contrast, *Amblyomma rotundatum* Koch was found on only two snakes. An analysis of the 16S rRNA molecular marker confirmed the morphological identification of these ectoparasites.

Keywords: *Amblyomma dissimile*, *Amblyomma rotundatum*, *Boa constrictor*, Manaus, Brazil.

Resumo

A jiboia (*Boa constrictor*), vertebrado carnívoro, tem sido encontrada em abundância na área urbana de Manaus. A identificação morfológica dos carrapatos coletados em 27 dessas serpentes verificou a ocorrência de *Amblyomma dissimile* Koch 1844, em todos os exemplares avaliados e a presença de *Amblyomma rotundatum* Koch 1844, em duas dessas serpentes. A análise do marcador 16S rRNA confirma a identificação morfológica das espécies *A. rotundatum* e *A. dissimile* e apresenta novas sequências destes organismos.

Palavras-chave: *Amblyomma dissimile*, *Amblyomma rotundatum*, *Boa constrictor*, Manaus, Brasil.

Introduction

The *Boa constrictor* is one of the largest snakes in the world and can reach a total length of over four meters. This predator with terrestrial and semi-arboreal habits consumes lizards, birds and mammals (CUNHA & NASCIMENTO, 1978; HENDERSON et al., 1995; BERNARDE, 2004; QUICK et al., 2005; PIZZATTO & MARQUES, 2007; PIZZATTO et al., 2009). *Boa constrictor* occur throughout Brazil and can be found in urban environments (MARTINS & OLIVEIRA, 1998; PIZZATTO & MARQUES, 2007; BERNARDE & ABE, 2006; BENTES, 2013). In fact, specimens are often caught in the city of Manaus, AM (central Brazilian Amazon), which has a population of almost two million (IBGE, 2012). In this region there are hundreds of rainforest fragments measuring 1 to 600 ha very close to urban areas. This combination of environments favors the parasitism of snakes by ticks (BENTES, 2013), which may be related to local parasitism dynamics (DAVIS et al., 2012). Despite this fact, only one earlier study has reported specimens of *A. dissimile* parasitizing a *Boa constrictor* captured in 1975 in an "INPA secondary forest" (ADIS, 1981).

The present study identifies tick species parasitizing *Boa constrictor* in urban areas of the city of Manaus, AM, with the aid of morphological and molecular tools.

Materials and Methods

All the ticks found attached to the body surfaces of *Boa constrictor* captured between September 2010 and November 2012 were collected by the Municipal Secretariat for Environment and

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Sustainability (SEMMAS) in urban areas in the city of Manaus, AM (3° 6' 7" S and 60° 1' 30" W). Most of the ticks were found attached to the head and the first 10 cm of the body. The ticks were removed manually using tweezers and stored in 70% alcohol for transfer to the laboratory, where males, females and nymphs were identified under a microscope.

The ticks were identified morphologically using dichotomous keys (GUIMARÃES et al., 2001; BARROS-BATTESTI et al., 2006). One hundred and one ticks were deposited in the Collection of Arthropods of Medical and Veterinary Importance at the Biological Institute of São Paulo, Brazil (accession number: 1313 AMB).

DNA was extracted from one male and two female ticks using a commercial kit (DNeasy Blood & Tissue Kit™ Qiagen®), following the manufacturer's instructions. Polymerase chain reaction (PCR) was performed following the manufacturer's protocol (2X DreamTaq Green PCR Master Mix), usig the primers listed in Table 1 to amplify the mitochondrial 16S rRNA gene fragment (MANGOLD et al., 1998). PCR products were purified and subjected to a commercially available standard semi-automated dideoxy Sanger sequencing method. The resulting sequences were aligned with each other and with other *Amblyomma* mitochondrial 16S rRNA sequences available in the GenBank nucleotide database.

Results

The number of ticks collected from each of the 222 boa constrictors sampled ranged from 0 to 354 (mean: 28.4; standard deviation: 46.2), with a total number of 5929 ticks, of which 62% were females, 32% males and 6% nymphs. The morphological identification of the ticks collected from 27 *Boa constrictor* revealed the occurrence of *A. dissimile* Koch, 1844 on all the sampled individuals, whereas *A. rotundatum* Koch, 1844 was found on only two of these snakes.

The sequence of mitochondrial 16S rRNA from *A. rotundatum* exhibited 99.7% identity with a fragment from the same species available in GenBank under accession number EU805569, differing in only one of the 324 base pairs sequenced. The sequence of mitochondrial 16S rRNA from *A. dissimile* exhibited 87.1% identity with the corresponding fragment from *A. rotundatum* (Figure 1) and over 90% identity with the same gene fragments from *A. longirostre* and *A. geayi* (GenBank accession numbers EU805565 and EU805566, respectively). Identity with other sequences ranged from 81.3% to 87%.

Table 1. Primers used for the PCR amplification and sequencing of the mitochondrial 16S rDNA sequence of *A. dissimile* and *A. rotundatum*.

PCR primer[a]	Primer sequence (5'-3')	Fragment Size (bp)	Position[b]
16S + 1	CTGCTCAATGAIIIIIITTAAATTGCTGTGG	456 (*A. dissimile*)	6957-7415
16S – 1	TCGGTITAAACTCAGATCATGT	449 (*A. rotundatum*)	6957-7415

[a] Modified primers described by Mangold et al. (1998). Some nucleotides were replaced with inosines to allow for the amplification of unknown genomic sequences; [b] Relative to the mitochondrial *A. cajennense* genome taken as reference, publicly available in GenBank under accession number JX573118.

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A. rotundatum       1 TAAGGCTTTAATTGGGTGCTAAAAGAATGAAATTACAAAAAAAGACTTTC     50
                      ||||..|||||||..|||||||.|||||||.||||||||||||.|||||||.
A. dissimile        1 TAAGATTTTAATTGAATGCTAAGAGAATGGAATTACAAAAAATGACTTTT     50

A. rotundatum      51 TTAATTTTAATAATTAAAATTATTTTTTTTTTGTGAAGAAACAAAAATAAA    100
                      ||||.||.||.||||.||.||||||||.||||||||||||||||.|||.||
A. dissimile       51 TTAAATTCAAAAATTGAATTTATTTTTATTTGTGAAGAAACAATAATTAA    100

A. rotundatum     101 AATTAAAGACAAGAAGACCCTAAGAATTTTC------TTTTTTACTTAAT    144
                      .||||||||||||||||||||||||||||||.      ||||.||.||.||
A. dissimile      101 TATTAAAGACAAGAAGACCCTAAGAATTTTAAAAGAATTTTATATTTGAT    150

A. rotundatum     145 ATAAAATATTTTATTTAAAAATTTAATTGGGGCGATTAATGAATATTAAT    194
                      ..|...||.|||.|||      ||||||||||||||||..||||||||.
A. dissimile      151 TAATTTTAATTTCTTT------TTAATTGGGGCGATTAAAAAATATTAAA    194

A. rotundatum     195 AACTTTATAAAGAAATAAAATGATCCATTATTAATGATTATTTGATTAAA    244
                      ||||||.|.|. ||.||||||||.||.|||.|:|.|..|...||||.|||
A. dissimile      195 AACTTTTTTAC-AATTAAAATGAACCGTTACTMACGGGTGGATGATAAAA    243

A. rotundatum     245 ATACTCTAGGGATAACAGCGTAATAATTTTTGATAGTTCTTATAGAAAAA    294
                      ||||||||||||||||||||||||||||||||||||||||||||||.|||
A. dissimile      244 ATACTCTAGGGATAACAGCGTAATAATTTTTGATAGTTCTTATAGACAAA    293

A. rotundatum     295 ATAGTTTGCGACCTCGATGTTGGATTAGGATTC                    327
                      |||||||||||||||||||||||||||||||.|
A. dissimile      294 ATAGTTTGCGACCTCGATGTTGGATTAGGATAC                    326
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Figure 1. Mitochondrial 16S rRNA pairwise alignment with EMBOSS MATCHER program (European Bioinformatics Institute); vertical lines = identical nucleotides; horizontal lines = gaps in sequence; dots = different nucleotides.

Discussion

The reliability of morphological features as criteria for the differentiation of *A. dissimile* from *A. rotundatum* was reinforced in this study by the analysis of a molecular marker. Mitochondrial 16S rRNA is used in the comparison of individuals from the same species or different species of the same genus (MANGOLD et al., 1998). The DNA sequences of this marker among species of *Amblyomma* are reported to have 6.4 to 20.4% identity rates. Labruna et al. (2009) and Nava et al. (2010) studied 24 sequences of 16S rRNA from *Amblyomma* and found identity ranging from 10 to 18%. The data presented herein broaden the variation range of this marker in *Amblyomma* species, as 28 sequences were analyzed (Table 2).

Variations in DNA sequences encoding 16S rRNA among individuals of the same species are also common (NAVA et al., 2010), which explains the polymorphism of this marker found in the present study in comparison to the corresponding *A. rotundatum* sequence available in GenBank under accession number EU805569. The analysis of this marker confirmed the identification of *A. rotundatum* and *A. dissimile* and detected new sequences in these organisms that are not found in the public database, which greatly improved our confidence in the identity of the individuals, based on morphological criteria.

This is the first record of *A. rotundatum* infesting *Boa constrictor* in the Amazon region. Future studies should be conducted to evaluate the ecological relationships involved in *Boa constrictor* populations as well as in other snake and lizard populations in the Amazon.

Table 2. List of publicly available mitochondrial 16S rRNA DNA sequences for pairwise alignments using the EMBOSS MATCHER program (European Bioinformatics Institute) to calculate percentage of identity.

Species	GenBank Accession Number
A. geayi	EU805566
A. longirostre	EU805565
A. brasiliense	FJ424399
A. incisum	FJ424405
A. pacae	JX141384
A. parkeri	JN573300
A. coelebs	FJ424408
A. naponense	FJ424406
A. auricularium	FJ627951
A. cajennense	FJ424404
A. maculatum	AY375442
A. parvum	EU543571
A. triste	AY498563
A. humerale	GQ891952
A. fuscum	JX141385
A. oblongoguttatum	FJ424407
A. americanum	L34314
A. calcaratum	JN573302
A. tigrinum	FJ965339
A. dubitatum	GU301912
A. aureolatum	JN573301
A. pseudoconcolor	AY628134
A. nodosum	FJ424403
A. goeldii	GQ891950
A. boeroi	JN828797
A. romitii	JX141383
A. rotundatum	EU805569
A. ovale	JN573304

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