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DETECTION OF *WOLBACHIA* (ALPHAPROTEOBACTERIA: RICKETTSIALES) IN THREE SPECIES OF TERRESTRIAL ISOPODS (CRUSTACEA: ISOPODA: ONISCIDEA) IN BRAZIL

Bianca Laís Zimmermann[1]*, Maurício Pereira Almerão[1], Didier Bouchon[2], Paula Beatriz Araujo[1]

[1]Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil; [2]Laboratoire Ecologie, Evolution, Symbiose, Université de Poitiers, Poitiers, France.

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ABSTRACT

Terrestrial isopods are widely infected with *Wolbachia*. However, little is known about the presence of bacteria in the Neotropical species. The objective of this study was to test the hypothesis of presence of *Wolbachia* infection in the native species of terrestrial isopods, *Atlantoscia floridana* and *Circoniscus bezzii*, and in the introduced species *Burmoniscus meeusei*.

Key words: *Wolbachia*, terrestrial isopods, 16S rDNA, Brazil.

Wolbachia are Alphaproteobacteria that infect a wide variety of arthropods and nematodes (26). These bacteria use different strategies of symbiosis in their hosts, acting as reproductive parasites in arthropods and as mutualists in nematodes (6). *Wolbachia* has attracted considerable interest, not only because of its pandemic nature (13), but also because of the reproductive phenotypes observed as a consequence of its reproductive parasitism (26). Moreover, the potential of *Wolbachia* as a biocontrol agent has been explored in recent years, with studies that suggest their use in controlling pests and disease vectors (5, 9, 16).

Terrestrial isopods are important representatives of the soil fauna, because they take part in soil formation and also in nutrient recycling, in addition to being a food source for a variety of animals (22, 23, 25). This group has developed structural, physiological and behavioral characteristics in order to become fully independent of the aquatic medium for reproduction, which has enabled them to occupy a variety of environments, from the coastal zone to deserts (21).

Terrestrial isopods are widely infected with *Wolbachia*; however, the majority of studies of these associations have been conducted in Europe (8). The first molecular identification of *Wolbachia* was in 1992, in populations of *Armadillidium vulgare* and *Porcellio dilatatus* from France (20). The presence of the bacterium was detected in *Chaetophiloscia elongata* and *Porcellionides pruinosus*, also in France, in 1994 (14). Later, *Wolbachia* was found in more than 14 European species of terrestrial isopods (7) and in *Hyloniscus riparius*, *Trachelipus rathkii* and *T. ratzeburgi*i in Hungary (17). More recently, the infection was detected in 11 species from Tunisia (4) and in two species of *Philoscia* sp. occurring in Thailand (28). Little is known about the presence of bacteria in terrestrial isopods in Brazil, which has a rich isopod fauna of more than 120 species (21).

***Corresponding Author. Mailing address:** Avenida Bento Gonçalves, nº 9500, Bairro Agronomia, Porto Alegre, RS, Brasil. Cep.: 91501-970.; Tel.: (+5551) 3308-7698 Fax: (+5551) 3308-7696.; E-mail: bia.lais@gmail.com

The objective of the present study was to test the hypothesis of presence of *Wolbachia* infection in the native species *Atlantoscia floridana* and *Circoniscus bezzii*, and in the introduced species *Burmoniscus meeusei*. *Atlantoscia floridana* occurs in the United States, Ascension Island and St. Helena, and from northern Brazil to La Plata in Argentina (2). It is a generalist species in terms of habitat (19), occurring in diverse environments, often in abundance (1, 3). *Circoniscus bezzii* is a little-studied species that occurs in Brazil (in the states of Minas Gerais and Pará) and Paraguay (15). *Burmoniscus meeusei* is an introduced species from Asia, with records in England, Hawaii, Brazil and Taiwan (21).

The specimens of *A. floridana* and *B. meeusei* examined were collected in Porto Alegre in Rio Grande do Sul, and the individuals of *C. bezzii* were collected in the municipality of Presidente Olegário in Minas Gerais. The specimens were collected by hand, fixed in absolute ethanol, and transported to the Carcinology Laboratory at Federal University of Rio Grande do Sul, where they were stored in a freezer at -20°C for subsequent DNA extraction.

The terrestrial isopods were dissected according to the methodology proposed by Bouchon *et al*. (7). The DNA extractions were performed from reproductive tissue (ovaries of females and utricles of males), part of the nerve cord, and the muscle at the base of the pereiopods, using the Chelex (Bio-Rad) protocol. The PCR assays to detect the presence of *Wolbachia* were performed under conditions adapted from Bouchon *et al.* (7), targeting the 16S rDNA gene (18). The use of this gene is due to the fact that it has proven to be the most efficient to detect *Wolbachia* in Neotropical terrestrial isopods (data not shown). Part of 16S rDNA gene was amplified using the *Wolbachia* specific primers 99F 5'- TTG TAG CCT GCT ATG GTA TAA CT - 3' and 994R 5' – GAA TAG GTA TGA TTT TCA TGT - 3', which produced fragments of approximately 900 bp (18).

The PCRs were carried out in volumes of 25 µl, using 1.0 µl of DNA, 0.16 µl of Platinum® Taq (5 U/µl) (Invitrogen), 2.5 µl of 10X buffer (Invitrogen), 1.6 µl $MgCl_2$ (50 mM) (Invitrogen), 0.5 µl of forward primer (20 µM), 0.5 µl of reverse primer (20 µM), 0.5 µl of dNTPs (10 mM) (Invitrogen) and 18.18 µl of ultrapure water. The amplifications were carried out under the following conditions: 35 cycles (1 min at 95°C, 1 min at 50.6°C, 1 min at 72°C), including an initial denaturation step of 95°C for 2 min and a final extension step of 72°C for 5 min. As a positive control for the PCR reaction, DNA extracted from an individual of the terrestrial isopod *Balloniscus glaber* was used, in which infection by *Wolbachia* was previously detected (unpublished data).

PCRs were confirmed by electrophoresis in 1% agarose gels. Possible failures in the amplifications with the 16S rDNA primers could occur for the following reasons: (i) absence of *Wolbachia*; (ii) a flaw in the DNA extraction process; and/or (iii) an incorrect concentration of DNA solution (27). In order to control the last two possibilities, we tested samples assumed to be negative, with primers of subunit I of the Cytochrome Oxidase mitochondrial gene (*COI*) (11). Samples that generated a product of the expected size were considered to be truly negative for the presence of *Wolbachia*.

The amplified DNA fragments of positive samples were sent to the company MACROGEN Advancing through Genomics for purification and sequencing. This company uses the BigDye™ Terminator protocol, and sequencing was conducted on a 3730xl DNA analyzer. The resulted sequences were compared by the Blastn algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with sequences deposited in the GenBank database. All sequences were subsequently submitted in the Genbank-EMBL database (http://www.ncbi.nlm.nih.gov/) under accession numbers: JF799948, JF799949, JF799950 and JF799951. Alignment of sequences was performed using the BioEdit program (12). The *Wolbachia* strain names were based on the nomenclature system proposed by Zhou *et al.* (29) and specified by Charlat *et al.* (10). Each strain's name is defined by w (in italics) denoting *Wolbachia*. This is followed by three letters coming

from the first three letters of the species name. Multiple strains present in a given species are distinguished by numbers added at the end.

Positive samples were detected in the three species *A. floridana*, *B. meeusei* and *C. bezzii*, representing the first record of *Wolbachia* infection in these species. The infection was observed in 30 individuals: 25 females of *A. floridana*, 2 females of *B. meeusei* and 3 females of *C. bezzii* (Table 1). A greater number of individuals of *A. floridana* were tested because this species usually occurs in abundance in nature (1), which facilitates their collection. However, *B. meeusei* and *C. bezzi* are difficult to collect. In addition, this is the first record of the presence of *B. meeusei* in the state of Rio Grande do Sul; until now, this species has been known only from Santa Catarina.

A single 16S rDNA sequence from *Wolbachia* was identified for *A. floridana* (*w*Flo) and *B. meeusei* (*w*Mee). For *C. bezzii*, two very different 16S rDNA sequences (*w*Bez1 and *w*Bez2) were found in two different individuals (Figure 1). Thus, multiple infections were not detected. Natural multiple infections in a single host have never been demonstrated in terrestrial isopods species (24), and this work supports this statement.

This study reports one of the first records of *Wolbachia* infection in species of terrestrial isopods in Brazil, in particular in the native species *A. floridana* and *C. bezzii* and the introduced species *B. meeusei*. Although these bacteria are widely studied, very few investigations of their interactions with terrestrial isopods and other organisms have been carried out in the Neotropical region. It is hoped that further studies will be undertaken to expand knowledge of the *Wolbachia* bacteria and their Neotropical hosts.

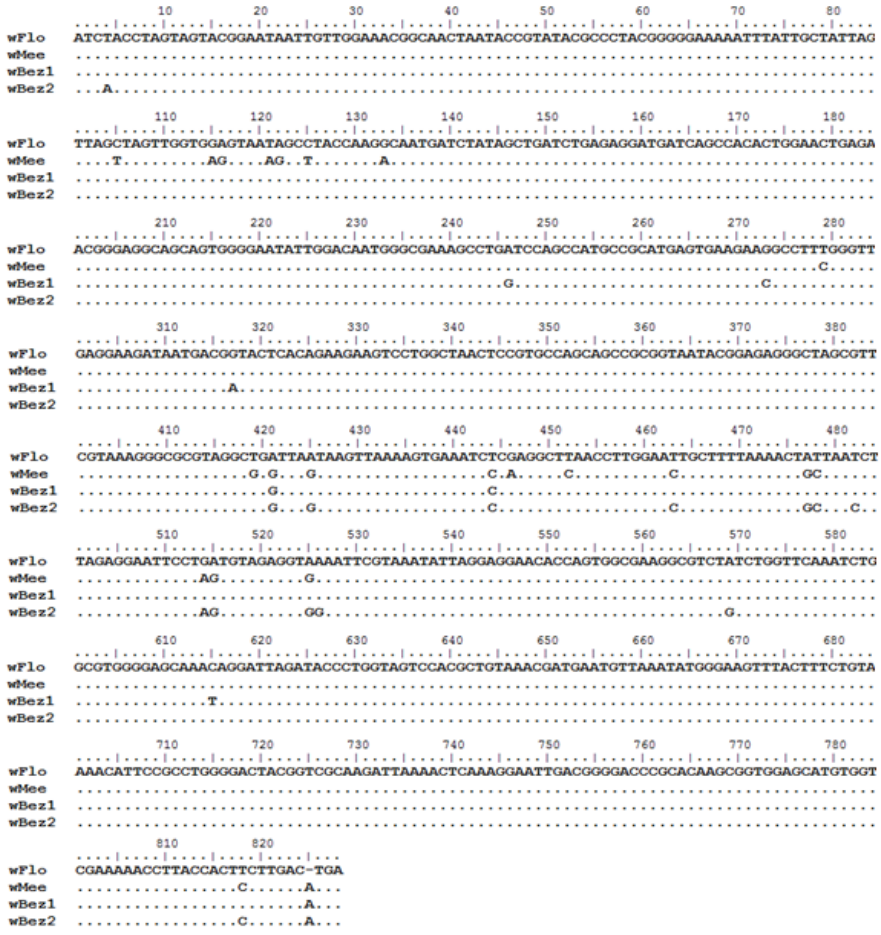

Figure 1. Alignment of *Wolbachia* 16S rDNA sequences found in *A. floridana* (*w*Flo), *B. meeusei* (*w*Mee) and *C. bezzii* (*w*Bez1 and *w*Bez2).

Table 1. Total of individuals of each species tested for the presence of *Wolbachia*. FT: females tested; MT: males tested; FI: females infected; MI: males infected; RS: Rio Grande do Sul; MG: Minas Gerais.

Species	FT	MT	FI	MI	Locality	Geographical Coordinates
Atlantoscia floridana	45	3	25	0	Porto Alegre/RS	30°04'76"S/51°07'28"W
Burmoniscus meeusei	2	0	2	0	Porto Alegre/RS	30°04'49"S/51°07'31"W
Circoniscus bezzii	7	0	3	0	Presidente Olegário/MG	18°24'02"S/46°25'50"W

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