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HIGH INCIDENCE OF *WOLBACHIA* INFECTION ON *SOLENOPSIS* (HYMEOPTERA: FORMICIDAE) POPULATIONS FROM URBANIZED AREAS

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Abstract *Solenopsis* ant genus has a worldwide distribution but species from *S. saevissima* species group native to South America, and popularly known as fire ants, have been introduced accidentally in several countries worldwide. Despite a high diversity species in the Neotropical region, their ecology and life history in Brazil are poorly known. These ants are highly aggressive and are responsible for accidents that can lead to anaphylactic shock and death. These ants are widely distributed in Brazil, including on urban areas. *Wolbachia* (Alphaproteobacteria, Rickettsiales) are intracellular bacteria inherited from the egg cytoplasm, found in large numbers of reproductive tissues in many arthropods. These bacteria can cause reproductive alterations in their hosts including cytoplasmic incompatibility, parthenogenesis and feminization of genetic males, and increase transmission to subsequent generations. There is widespread interest to use these endobacteria in biological control. Its incidence among ants of the genus *Solenopsis* is high but little is known about its occurrence on *Solenopsis* species associated to urban areas. In the present study we inferred the presence and distribution of these endosymbiont on populations of *Solenopsis* of such areas from Southern and Southeastern of Brazil, by means of PCR amplification of the *wsp* gene, and sequences of this fragment to infer a phylogenetic relationship of this endosymbiont associated with the urban pest. We found high frequency of *Wolbachia* among *Solenopsis* species analyzed. However, little genetic variability was found among the different *Wolbachia* strains suggesting possible events of horizontal transmissions of *Wolbachia*. Consequently, knowing the strain diversity of *Wolbachia* in natural populations of *Solenopsis* can be efficient on a future program of biological control of those pest ants.
Key Words Endosymbiont, fire ants, high infection incidence

INTRODUCTION

Ants of the genus *Solenopsis* occur worldwide, but relatively little is known about their ecology and life history in Brazil, where the genus is highly diverse. Native from South America, ants of the genus *Solenopsis* (*Solenopsis invicta* and *Solenopsis richteri*) were accidentally introduced in the United States in the beginning of the last century and have become a great public concern, causing damage to the local diversity by displacing native species, and to crops and public health (Wojcik et al., 2001). *S. invicta* invasions have also been reported in several countries such as Porto Rico, New Zealand, and Australia (Morrison et al., 2004).

The potential global range expansion of *S. invicta* has been correlated with temperature and precipitation, and abrupt variations of these factors may limit the success of the expansion (Morrison et al., 2004). Also, the presence of few natural enemies in areas invaded by this ant may be the cause of the abundance of individuals, since in its native range, the opposite scenario is observed. As a result of a fast expansion and interactions with several taxa, many ant species might have acquired several parasites, among them endosymbionts such as *Wolbachia* (Dedeine et al., 2005).

Wolbachia (Class Alphaproteobacteria, Order Rickettsiales) are intracellular bacteria inherited from the egg cytoplasm, found in large numbers in the reproductive tissues of many arthropods. Jeyaprakash and Hoy (2000) examined the presence of *Wolbachia* in 63 species of arthropods and found a frequency of 76%. Extrapolations of these estimates suggest that 10^6 insect species might be infected, making *Wolbachia* bacteria among the most widespread parasites of insects (Shoemaker et al., 2003; Hilgenboecker et al., 2008). These bacteria can cause reproductive alterations in their hosts to increase transmission to subsequent generations (O'Neill et al., 1992; Bandi et al., 1998; Stouthamer et al., 1999). Because of their effects on natural populations, there is a widespread

interest in using these endobacteria in biological control (Aeschilimann, 1990; Beard et al., 1993; Stouthamer, 1993; Girin and Bouletreau, 1995; Bourtzis, 2008). Reproductive alterations induced by *Wolbachia* in their hosts include cytoplasmic incompatibility, parthenogenesis induction, and feminization of genetic males (Werren, 1997). In social insects, however, the influence of *Wolbachia* in reproduction still remains unknown (Chapuisat and Keller, 1999; Keller et al., 2001, but see Wenseleers et al., 1998).

In the present study we inferred the presence and distribution of the endosymbiont *Wolbachia* on populations of *Solenopsis* from Southern and Southeastern of Brazil, by means of PCR amplification of the *wsp* gene, and sequences of this fragment to infer a phylogenetic relationship of this endosymbiont associated with the urban pest.

MATERIALS AND METHODS

Ant workers of several sizes were collected directly from nests and frozen in 80% ethanol to avoid DNA degradation. A total of 61 nests were analyzed. Total DNA was extracted out using a non-phenolic method. Five whole ant workers (pool) were used. The extraction method used was the same used in Martins et al. (2010). The material was identified using mitochondrial DNA, more specifically the cytochrome oxidase I (COI), for the identification of the species (Hebert et al., 2003a; Hebert et al., 2003b; Ratnasingham and Hebert, 2007). Based on the sequencing of part of the COI, fragments of the sampled populations were generated and compared using Blast searches (NCBI - National Center for Biotechnology Information).

Mitochondrial DNA fragments from COI gene of approximately 920 bp were amplified by PCR. The thermal cycler was programmed as proposed by Ross and Shoemaker (1997). The primers used were: C1-J-2195 (COI-RLR) (5' – TTGATTTTTTGGTCATCCAGAAGT - 3') and DDS-COII-4 (5' – TAAGATGGTTAATGAAGAGTAG - 3') (Ross and Shoemaker, 1997; Ahrens et al., 2005).

The *Wolbachia* gene isolation were done using the pair of primers that amplifies the variable fragment of a gene that decodes a surface protein of the bacteria of approximately 600 pb, named *wsp*81F (5' – TGGTCCATTAAGTGATGAAGAAAC - 3') and *wsp*691R (5' – AAAAATTAAACGCTACTCCA – 3') (Braig et al., 1998; Zhou et al., 1998). A second pair was used as a control: EF1α-532F (5' - AGGCAAATGTCTTATTGAAG – 3') and EF1α-610R (5' – GCGGGTGCGAAGGTAACAAC – 3') (Shoemaker et al., 2000) that amplify a fragment of 400 pb of the nuclear gene EF1α (elongation factor).

The amplifications were carried out with final volume of 25 μL, with 250 to 500 ng of DNA template, 0.2-0.4 μM (5-10 pmol) of each primer, using the Ready-to-go kit (Amersham Pharmacia Biotech). The thermal cycler was programmed according to Braig et al. (1998) and Zhou et al. (1998).

The presence of the *wsp* gene was confirmed by the presence of two bands (the *wsp* gene with approximately 600 pb and the elongation factor or positive control with 400 pb) visualized in 1.5% agarose gel. After infection detection, amplification by PCR was carried out containing only the pair of primers *wsp*81F and *wsp*691R, followed by sequencing of the target fragment. Whenever the sequenced fragment showed more than two fragments cloning the fragments were required to separate the strains using the CloneJET PCR Cloning Kit (Fermentas Life Sciences). The direct products of PCR were used, which were inserted in the cloning vector, according to the protocol provided by the manufacturer.

Data Analysis

The *wsp* gene sequences from the endobacteria were initially analyzed separately with the software BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), aligned using the software Clustal (Higgins et al., 1992) followed by manual modifications. After the alignment, the data set of the *wsp* gene was analyzed with the software DAMBE (Xia and Xie, 2001). The reconstruction of the phylogeny based on the Bayesian analysis was carried out using the software MrBayes (Huelsenbeck and Ronquist, 2001) with the model GTR. In the absence of a suitable outgroup for rooting the inferred threes (see Lo et at., 2002), the evolutionary rate was assumed to be approximately uniform to all branches. Based on this premise, trees were rooted at midpoint.

RESULTS AND DISCUSSION

From the 61 analyzed nests we found 23 different haplotypes of *wsp* gene. From those 61 analyzed nests, only 15 showed no infection by the endosymbiont *Wolbachia*, which indicates a high infection rate of *Wolbachia* in *Solenopsis* ant genus (75%).

Phylogenetic analysis of different strains of *Wolbachia* found (Figure 1) clearly indicates the separation between the A and B supergroups of *Wolbachia* found in insects and already discussed in several other studies, such as in Ahrens and Shoemaker (2005).

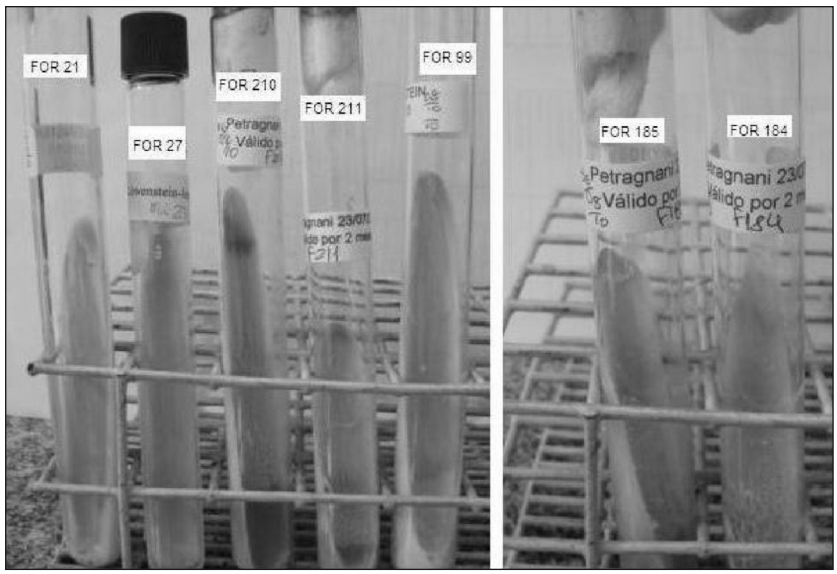

Figure 1. Phylogenetic tree based on *wsp* sequence gene from ants of Brazilian genera *Solenopsis*. Numbers in branches indicate posterior probability values from Bayesian analyses. H40 H43 H44 H45 H46: *S invicta*: RJ, RS, SP, SC. PR: Paraná State; SC: Santa Catarina State; SP: São Paulo State; RJ: Rio de Janeiro State.

Six *Wolbachia* strains of the supergroup A were found in S. *invicta* and three in *S. saevissima* with the last one forming a basal clade from the first one. At the base of these clades, some GenBank retrieved were grouped showing a certain divergence from the *S. invicta* and *S. saevissima Wolbachia* strains found in our study. Within supergroup B we can clearly see two major groups: one of them forming an unresolved node (polytomy) formed by *Wolbachia* sequence derived from *S. daguerrei* (AY878102) along with *Wolbachia* strains from *S. megergates*, *S invicta* and *S. saevissima*. The second group was a sister group from the first group formed by *Wolbachia* strains found in *S. invicta*, *S. saevissima* and *S. megergates*. A derived group from the previous one was comprised by strains found in *S. daguerrei* (AY878101, AY878107). The analysis of *Wolbachia* sequences indicates a higher frequency of supergroup B rather than A, unlike the observed by Ahrens and Shoemaker (2005) in *S. invicta*.

Relatively high incidence of *Wolbachia* infection in ants, as reported in previous studies, was also found in the genus *Solenopsis* in Brazil. This high incidence might be due to the more favorable conditions of invasion and maintenance of the *Wolbachia* infection in haplodiploid social hosts when compared with solitary hosts (Wenseleers et al., 1998). In addition, the occurrence of multiple infections found in some nests can influence reproductive conflicts and combined with other reproductive barriers, it might accelerate speciation (Werren, 1997). The number of strains was very high and was not associated with the number of *Solenopsis* species examined (*S. invicta*, *S. saevissima* and *S. megergates*), which might be a strong indication of horizontal transmission within the genus *Solenopsis*, as suggested by Ahrens and Shoemaker (2005). Similarly, Souza et al. (2009) reported horizontal transmission in Brazilian populations of *Solenopsis saevissima*.

A strong evidence of horizontal transmission in the species examined is the grouping of *Wolbachia* strains from the social parasite *S. daguerrei* with strains of supergroup A and B, forming an unresolved node (polytomy) in supergroup B. If a parasite plays a role in the transmission of *Wolbachia*, both the social parasite and the host are expected to have identical or almost identical *Wolbachia* strains (Dedeine et al., 2005). Therefore, horizontal transmission is the most likely explanation for this result, as the intimate interaction between the social parasite and its host (such as trophallaxis and egg carrying, Hölldobler and Wilson, 1990) may provide enough opportunities for the transmission of *Wolbachia* from the host to the social parasite and possibly from the social parasite to the host.

CONCLUSIONS

High incidence of *Wolbachia* infection was found in ants of *Solenopsis* genus corroborating several previous studies. This high incidence might be due to a more favorable conditions and maintenance in haplodiploid hosts

compared with solitary ones (Wensellers et al., 1998). In addition, multiple occurrences of infections might be accelerating speciation by influencing reproductive conflicts (Werren, 1997).

Furthermore, our findings are important as an ecological recovery of which strains are present in *Solenopsis* species in its natural distribution in Brazil, as there is an interest of *Wolbachia* in biological control of insect pests such as some species of *Solenopsis* genus. But this only will be possible if more information about the distribution and evolutionary history of *Wolbachia* strains are recovered in a more detailed way. Bourtzis (2007) discussed that analysis via comparative genomics and post genomics can help the development of a genetic transformation system and also helping elucidation of the molecular mechanisms of some alterations induced by *Wolbachia*.

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