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Presence and distribution of the endosymbiont *Wolbachia* among *Solenopsis* spp. (Hymenoptera: Formicidae) from Brazil and its evolutionary history

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ABSTRACT

Wolbachia are intracellular bacteria that commonly infect arthropods. Its prevalence among ants of the genus *Solenopsis* is high. In the present study, the presence and distribution of these endosymbionts was examined among populations of *Solenopsis* spp. from Brazil. A phylogenetic analysis based on the *wsp* gene was conducted to infer the evolutionary history of *Wolbachia* infections within the populations surveyed. A high frequency of *Wolbachia* bacteria was observed among the genus *Solenopsis*, 51% of the colonies examined were infected. Incidence was higher in populations from southern Brazil. However, little genetic variability was found among different *Wolbachia* strains within supergroups A and B. Our findings also suggest that horizontal transmission events can occur through the social parasite *S. daguerrei*.

1. 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* (*S. invicta* and *S. 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). Currently, millions of dollars have been spent in the attempt to control them, but despite these efforts, they continue to spread to new areas. *Solenopsis invicta* invasions have also been reported in several countries such as Puerto 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 (Dedeine et al., 2005; Hilgenboecker et al., 2008; Shoemaker et al., 2003a,b).

Wolbachia variants found in New World ants are more closely related, and differ from other strains found in other insect groups, suggesting they may have become specialized in ants (Tsutsui et al., 2003). These bacteria can cause reproductive alterations in their hosts to increase transmission to subsequent generations (Bandi et al., 1998; O'Neill et al., 1992; 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; Bourtzis, 2008; Girin and Bouletreau, 1995; Stouthamer, 1993).

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).

Some aspects of *Wolbachia* are well known. It was clear by Werren et al. (1995) that in arthropods there were two mains groups (A and B). Zhou et al. (1998) went further indicating that those two clades had at least eight potential groups within A and

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four within B. Recently, A and B were termed "supergroups" (Lo et al., 2007) and other supergroups have also been described, including on *Wolbachia* infecting nematoids (C and D supergroups) (Bandi et al., 1998), supergroup E in Collembola (Czarnetzki and Tebbe, 2004; Vandekerckhove et al., 1999), F in arthropods and nematoids (Casiraghi et al., 2005), G in spiders (Rowley et al., 2004) and H in termites (Bordenstein and Rosengaus, 2005).

Wolbachia transmission within host species occurs maternally through the egg cytoplasm (Stouthamer et al., 1999; Werren, 1997). However, several independent studies have shown that *Wolbachia* can be transmitted horizontally, within as well as between host species (Ahrens and Shoemaker, 2005; Dedeine et al., 2005; O'Neill et al., 1992; Vavre et al., 1999).

Studies conducted in ant populations of several species of the genus *Solenopsis* in areas where they were introduced and native ranges indicated the presence of the two *Wolbachia* supergroups (A and B), and reported that the frequency of infection varies dramatically between different regions (Shoemaker et al., 2000). In addition, there is a strong association between the *Wolbachia* variant and the host mitochondrial DNA, as also reported by Shoemaker et al. (2003a,b).

Ahrens and Shoemaker (2005) suggested that the evolutionary history of *Wolbachia* in *S. invicta* is more complex and involve multiple invasions or horizontal transmission events of the bacteria into this species. These authors also suggest that *Wolbachia* infections might have been lost secondarily within different lineages and that the effects of *Wolbachia* on the mitochondrial genome of the host are less severe than originally predicted.

While some parasites are successful inside their hosts, others benefit from the ant nest as a super-organism and are successful as social parasites. Originally described as *Labauchena daguerrei*, *Solenopsis daguerrei* is a workerless parasitic ant. Its hosts are restricted to *Solenopsis* species of the group *saevissima* (*S. richteri*, *S. invicta*, *S. saevissima*, *S. quinquecuspis*, and *S. macdonaghi*) (Tschinkel, 2006). Queens of the parasitic ant attract workers of the host nest so that they tend preferentially the brood of the parasite and neglect the host queen and as the parasitic ant produce eggs, the colony tends the sexual brood of the parasite (Tschinkel, 2006).

Parasitism rates are low (Calcaterra et al., 1999) and the populations of parasites are small and localized (Tschinkel, 2006). The strongest effect of *S. daguerrei* is the collapse of the parasitized colony, but typically the detrimental effects are not extreme (Tschinkel, 2006).

As evidenced by Dedeine et al. (2005) the intimate relationship (trophallaxis and egg carrying) between workers of the infected nest and the social parasite creates enough opportunities for horizontal transmission of microorganisms, such as *Wolbachia*, from the host to the social parasite and, possibly from the social parasite to the host. Dedeine et al. (2005) found two *Wolbachia* variants infecting *S. daguerrei* identical to known variants infection other *Solenopsis* species (*S. invicta* and *S. richteri*) and suggested that possible transfer of *Wolbachia* between *S. daguerrei* and their hosts have occurred.

This study was aimed for investigating the presence and distribution of the endobacteria *Wolbachia* in populations of *S. invicta*, *S. saevissima*, *S. megergates*, *S. geminata*, and *S. pusillignis* in Brazil, using the hypervariable region of the *wsp* gene.

2. Material and methods

2.1. Collection, identification and preservation of ants

We analyzed specimens of 114 colonies of five species of the genus *Solenopsis* from south, southeast, north, northeast, and west-central Brazil (Table 1 and Fig. 1).

Ant workers of several sizes were collected directly from nests and frozen in 80% ethanol to avoid DNA degradation.

The material was identified using mitochondrial DNA, more specifically the cytochrome oxidase I (COI), for the identification of the species. The visual differentiation between different species of *Solenopsis* is hampered due to poor definition of morphological characteristics (Pitts et al., 2005). In this sense, molecular data can clarify the doubts created by morphological identifications and may even be the main tool used to differentiate species by allowing for the creation of a DNA barcode (Hebert et al., 2003a,b; 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). The identification was considered positive when there was a strong similarity between compared sequences with high scores and *E*-values equal to 0 or very close to those deposited in the database.

2.2. DNA extraction

Total DNA was extracted out using a non-phenolic method. Five whole ant workers (pool) were used. Samples were homogenized in lysis buffer consisted of 100 mM Tris, pH 9.1, 100 mM NaCl, 50 mM EDTA, 0.5% SDS. The homogenized samples were incubated at 55 °C, for 3 h; protein residues were precipitated with 5 M NaCl. DNA precipitation was carried out with 100% ethanol alcohol, followed by 70% ethanol. DNA elution was conducted with TE buffer (10 mM Tris, 1 mM EDTA, pH 8).

2.3. PCR amplification

2.3.1. Mitochondrial DNA

Mitochondrial DNA fragments of approximately 920 bp were amplified by PCR. These fragments are part of the cytochrome oxidase I gene (approximately 780 bp), leucine transfer RNA (70 bp), and part of the cytochrome oxidase II (approximately 60 bp). The amplifications were carried out with a final volume of 25 μL, containing 250–500 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 as proposed by Ross and Shoemaker (1997): 1 min at 94 °C (initial denaturation) and 35 cycles at 94 °C for 1 min, annealing temperature of 48 °C for 1 min, and extension temperature of 68 °C for 2 min, followed by a final extension step at 72 °C for 5 min.

The primers used were: C1-J-2195 (COI-RLR) (5′-TTGATTTTTT GGTCATCCAGAAGT-3′) and DDS-COII-4 (5′-TAAGATGGTTAATGAA-GAGTAG-3′) (Ahrens et al., 2005; Ross and Shoemaker, 1997). When the combination of primers did not amplify the desired fragment, the second primer was used instead of DDS-COII-4, named JerryGarcia-CI (5′-GGGAATTAGAATTTTGAAGAG-3′) (Shoemaker et al., 2006), which produces fragments of approximately 780 bp that includes only the gene cytochrome oxidadese I (COI).

2.3.2. Wolbachia gene isolation

Two pairs of primers were used to examine the presence of *Wolbachia* in ants. The first pair was the control: EF1α-532F (5′-AGG-CAAATGTCTTATTGAAG-3′) and EF1α-610R (5′-GCGGGTGCGAAGG TAACAAC-3′) (Shoemaker et al., 2000) that amplify a fragment of 400 bp of the nuclear gene EF1α (elongation factor). The second pair amplifies the variable fragment of a gene that decodes a surface protein of the bacteria of approximately 600 bp, named *wsp*81F (5′-TGGTCCATTAAGTGATGAAGAAAC-3′) and *wsp*691R (5′-AAAAATTAAACGCTACTCCA-3′) (Braig et al., 1998; Zhou et al., 1998).

The presence of the control primer (EF1α) fragment and the absence of the *Wolbachia*-specific fragment (*wsp*) most likely reflects