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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 Boulétreau, 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

Table 1
Ant species, collection code and locality, geographic positions and *Wolbachia* strains.

Species	Collection code and locality	Geographic positions	Wolbachia strains
S. geminata	E1820 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1825 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1818 Manaus, AM	S03°06′25″ W60°01′34″	H17
S. geminata	E1823 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1830 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1826 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1827 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1832 Manaus, AM	S03°06′25″ W60°01′34″	–
S. geminata	E1822 Manaus, AM	S03°06′25″ W60°01′34″	H26
S. invicta	E1710(2) Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H32, H40
S. invicta	E1709(1) Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H10, H26, H44
S. invicta	E1739 Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H44
S. invicta	E1741 Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H35, H36
S. invicta	E1628 Campo Grande, MS	S20°27′59″ W54°35′33″	–
S. invicta	Colômbia, SP	S20°10′40″ W48°41′38″	H45
S. invicta	E1652 Miranda, MS	S20°14′29″ W56°22′43″	–
S. invicta	E1786 Bento Gonçalves, RS	S29°0948″ W51°31′54″	H30, H41
S. invicta	E1680 Bento Gonçalves, RS	S29°07′22″ W51°20′58″	–
S. invicta	E1683 Ubatuba, SP(1)	S23°30′21″ W45°07′55″	–
S. invicta	E1684 Ubatuba, SP(2)	S23°30′21″ W45°07′55″	–
S. invicta	E1685 Ubatuba, SP(2)B	S23°30′21″ W45°07′55″	–
S. invicta	E1744 Ubatuba, SP	S23°30′21″ W45°07′55″	–
S. invicta	E1748 Ubatuba, SP	S23°30′21″ W45°07′55″	–
S. invicta	E1749 Ubatuba, SP	S23°30′21″ W45°07′55″	H1
S. invicta	E1752 Ubatuba, SP	S23°30′21″ W45°07′55″	–
S. invicta	E1686 Picinguaba, SP	S23°19′02″ W44°54′04″	H46
S. invicta	E1704 Corumbá, MS	S19°30′31″ W57°20′05″	–
S. invicta	E1705 Corumbá, MS	S18°45′11″ W57°07′09″	–
S. invicta	E1706 Corumbá, MS	S18°50′00″ W57°18′55″	–
S. invicta	E1707 Corumbá, MS	S18°50′00″ W57°18′55″	–
S. invicta	E1722 Corumbá, MS	S19°00′23″ W57°39′10″	–
S. invicta	E1721 Corumbá, MS	S19°00′23″ W57°39′10″	–
S. invicta	E1720 Paraná	S25°25′46″ W49°16′18″	–
S. invicta	E1723 Porto Esperança, MT	S14°09′40″ W56°04′38″	–
S. invicta	E1724 Porto Esperança, MT	S14°09′40″ W56°04′38″	–
S. invicta	E1725 Porto Alegre, RS	S29°59′14″ W51°09′580″	H1
S. invicta	E1726 Porto Alegre, RS	S29°59′14″ W51°09′580″	H1
S. invicta	E1727 Porto Alegre, RS	S29°59′14″ W51°09′580″	H1
S. invicta	E1737 Rio Claro, SP	S22°23′34″ W47°33′21″	H1
S. invicta	E1754 São Luiz do Paraitinga, SP	S23°12′22″ W45°20′43″	–
S. invicta	E1768 Anastácio, MS	S20°28′46″ W55°48′08″	–
S. invicta	E1770 Aquidauana, MS	S20°28′42″ W55°47′03″	–
S. invicta	E1771 Aquidauana, MS	S20°28′42″ W55°47′03″	–
S. invicta	E1780 Registro, SP	S24°31′46″ W47°51′24″	H1, H7, H39
S. invicta	E1781 Registro, SP	S24°31′46″ W47°51′24″	H1, H2, H6
S. invicta	E1783 Alfredo Wagner, SC	S27°41′42″ W49°19′53″	H1, H22, H27, H29
S. invicta	E1787 Pinto Bandeira, RS	S29°07′21″ W51°26′56″	H26, H37, H44
S. invicta	E1788 Pinto Bandeira, RS	S29°07′21″ W51°26′56″	H1, H28
S. invicta	E1789 Pinto Bandeira, RS	S29°07′21″ W51°26′56″	H1
S. invicta	E1794 Curitiba, PR	S25°25′42″ W49°16′25″	H25, H26, H38
S. invicta	E1808 Corrientes, Argentina	S27°18′39″ W58°33′44″	H23
S. invicta	E1807 Corrientes, Argentina	S27°18′39″ W58°33′44″	H26
S. invicta	E1805 Corrientes, Argentina	S27°18′39″ W58°33′44″	H26
S. invicta	E1801 Corrientes, Argentina	S27°18′39″ W58°33′44″	H26
S. invicta	E1803 Corrientes, Argentina	S27°18′39″ W58°33′44″	H2
S. invicta	E1802 Corrientes, Argentina	S27°18′39″ W58°33′44″	H26
S. invicta	E1806 Corrientes, Argentina	S27°18′39″ W58°33′44″	H26
S. invicta	E1810 Corrientes, Argentina	S27°18′39″ W58°33′44″	H31
S. invicta	E1798 Recife, PE	S08°07′49″ W34°54′09″	–
S. invicta	E1799 Maringá, PR	S23°25′35″ W51°56′46″	–
S. invicta	E1800 Maringá, PR	S23°25′35″ W51°56′46″	–
S. invicta	E1784 Lages, SC	S27°48′57″ W50°22′17″	H1
S. invicta	E1790 Capão Alto, RS	S28°00′23″ W50°32′26″	H1, H9
S. invicta	E1815 Pelotas, RS	S31°46′33″ W52°20′33″	H8
S. invicta	E1816 Pelotas, RS	S31°46′33″ W52°20′33″	H8
S. invicta	E1646 Caçador, SC	S26°46′32″ W51°00′56″	H44
S. invicta	E1645 Caçador, SC	S26°46′32″ W51°00′56″	H44
S. invicta	E1648 Caçador, SC	S26°47′06″ W50°59′27″	–
S. megergates	E1782 São Francisco, SC	S26°33′53″ W48°43′10″	H44
S. megergates	E1793 Areia Branca, PR	S25°51′45″ W19°21′45″	H24, H42
S. megergates	E1644 Caçador, SC	S26°46′32″ W51°00′56″	H44
S. megergates	E1643 Caçador, SC	S26°46′32″ W51°00′56″	H44
S. pusillignis	E1657 Ladário, MS	S19°01′05″ W57°33′04″	–
S. pusillignis	E1708 Hotel, Ladário, MS	S19°01′03″ W57°34′11″	–

(*continued on next page*)

Table 1 (*continued*)

Species	Collection code and locality	Geographic positions	Wolbachia strains
S. saevissima	E1608 Eirunepé, AM	S6°38′55″ W69°52′32″	–
S. saevissima	E1615 Rio Claro, SP	S22°23′34″ W47°33′44″	H4
S. saevissima	E1712 Rio Claro, SP	S22°23′47″ W47°32′51″	–
S. saevissima	E1631 Ipeúna, SP	S22°26′11″ W47°43′10″	H4
S. saevissima	E1640 Belém, PA	S01°23′28″ W48°28′43″	–
S. saevissima	E1650 Juiz de Fora, MG	S21°45′51″ W43°20′56″	H26, H43
S. saevissima	E1662 Macapá, AP UFAP	S00°00′23″ W51°05′06″	–
S. saevissima	E1666 Macapá, AP IEPA	S00°02′19″ W51°05′39″	–
S. saevissima	E1671 Macapá, AP	S00°02′19″ W51°05′39″	––
S. saevissima	E1682 Bento Gonçalves, RS	S29°04′31″ W51°14′13″	H4
S. saevissima	E1713 Buritizeiro, MG	S17°25′20″ W44°56′54″	H26
S. saevissima	E1714 Buritizeiro, MG	S17°25′20″ W44°56′54″	H26
S. saevissima	E1738 Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H26
S. saevissima	E1740 Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H26
S. saevissima	E1738 Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H26
S. saevissima	E1740 Rio de Janeiro, RJ	S22°58′51″ W43°16′75″	H26
S. saevissima	E1742 São Paulo, SP	S23°32′53″ W46°38′11″	H4
S. saevissima	E1743 Ubatuba, SP	S23°30′21″ W45°07′55″	H4
S. saevissima	E1746 Ubatuba, SP	S23°30′21″ W45°07′55″	H26
S. saevissima	E1747 Ubatuba, SP	S23°30′21″ W45°07′55″	–
S. saevissima	E1750 Ubatuba, SP	S23°30′21″ W45°07′55″	–
S. saevissima	E1751 Ubatuba, SP	S23°30′21″ W45°07′55″	H4
S. saevissima	E1753 Ubatuba, SP	S23°30′21″ W45°07′55″	H26
S. saevissima	E1769 Três Lagoas, MS	S20°47′37″ W51°37′59″	–
S. saevissima	E1791 São Cristóvão do Sul, SC	S27°15′32″ W50°26′50″	H3, H5
S. saevissima	E1792 São Cristóvão do Sul, SC	S27°15′32″ W50°26′50″	H4
S. saevissima	E1716 Porto Nacional, TO	S10°42′37″ W48°24′34″	–
S. saevissima	E1717 Taquaruçu, TO	S10°19′07″ W48°09′22″	–
S. saevissima	E1718 Palmas, TO	S10°19′07″ W48°09′22″	–
S. saevissima	E1719 Palmas, TO	S10°19′07″ W48°09′22″	–
S. saevissima	E1718 Palmas, TO	S10°12′46″ W48°21′37″	–
S. saevissima	E1819 Manaus, AM	S03°06′25″ W60°01′34″	–
S. saevissima	E1821 Manaus, AM	S03°06′25″ W60°01′34″	H26
S. saevissima	E1824 Manaus, AM	S03°06′25″ W60°01′34″	–
S. saevissima	E1829 Manaus, AM	S03°06′25″ W60°01′34″	–
S. saevissima	E1831 Manaus, AM	S03°06′25″ W60°01′34″	–
S. saevissima	E1828 Manaus, AM	S03°06′25″ W60°01′34″	–
S. saevissima	E1833 Pindoretama, CE	S04°01′33″ W38°18′24″	
S. saevissima	E1785 Lages, SC	S27°48′57″ W50°22′17″	H26, H31, H34
S. saevissima	E1795 Recife, PE	S08°07′44″ W34°54′13″	–

an absence of the bacteria rather than low quality (low yield of PCR product), a high concentration of genomic DNA or an error associated with the PCR setup (Shoemaker et al., 2000). However, in the absence of the EF1α fragment and of the *wsp* gene fragment, it is not possible to conclude the absence of the endobacteria. In this case, the genomic DNA was diluted and the PCR protocol repeated.

The amplifications were carried out with final volume of 25 μL, with 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 according to Braig et al. (1998) and Zhou et al. (1998). The confirmation of the amplification was visualized in 2% agarose gel.

2.4. Cloning

The presence of noise in the electropherogram of the sample of the sequenced *wsp* gene required cloning of the sample to separate the strains.

PCR products were cloned 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. For each cloned sample it was sequenced at least 10 clones to track all possible strains present in the sample.

2.5. DNA sequencing

DNA was sequenced with the BigDye Terminator Kit (Applied Biosystem Inc). Both DNA chains of each sample were sequenced separately with the corresponding primers, the mitochondrial DNA for ants, and the *wsp* gene of endobacteria, using an automatic sequencer ABI Prism 377 (Applied Biosystem Inc.). DNA sequencing was carried out according to standard protocols. The final volume was 10 μL. The extension products were precipitated with 75% isopropanol.

2.6. Phylogenetic 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. A second and more refined alignment was performed with the software MUSCLE3.6 (Edgar, 2004). The resulting alignment was used for the construction of the network of strains and for the analysis of the phylogenetic signal.

Based on the *wsp* gene, protein sequences were obtained by conceptual translation, and sequences were reconstructed and aligned with the software BioEdit. The nucleotide sequences were aligned manually by comparing the alignment of proteins. This alignment was used in the phylogenetic analysis.

The construction of a network of *Wolbachia* strains was carried out with the software DnaSP4.90 (Rozas et al., 2003) and Network4.5 (fluxus-engineering.com) using the median-joining method (Bandelt et al., 1999).

After the alignment, the data set of the *wsp* gene was analyzed with the software DAMBE (Xia and Xie, 2001).

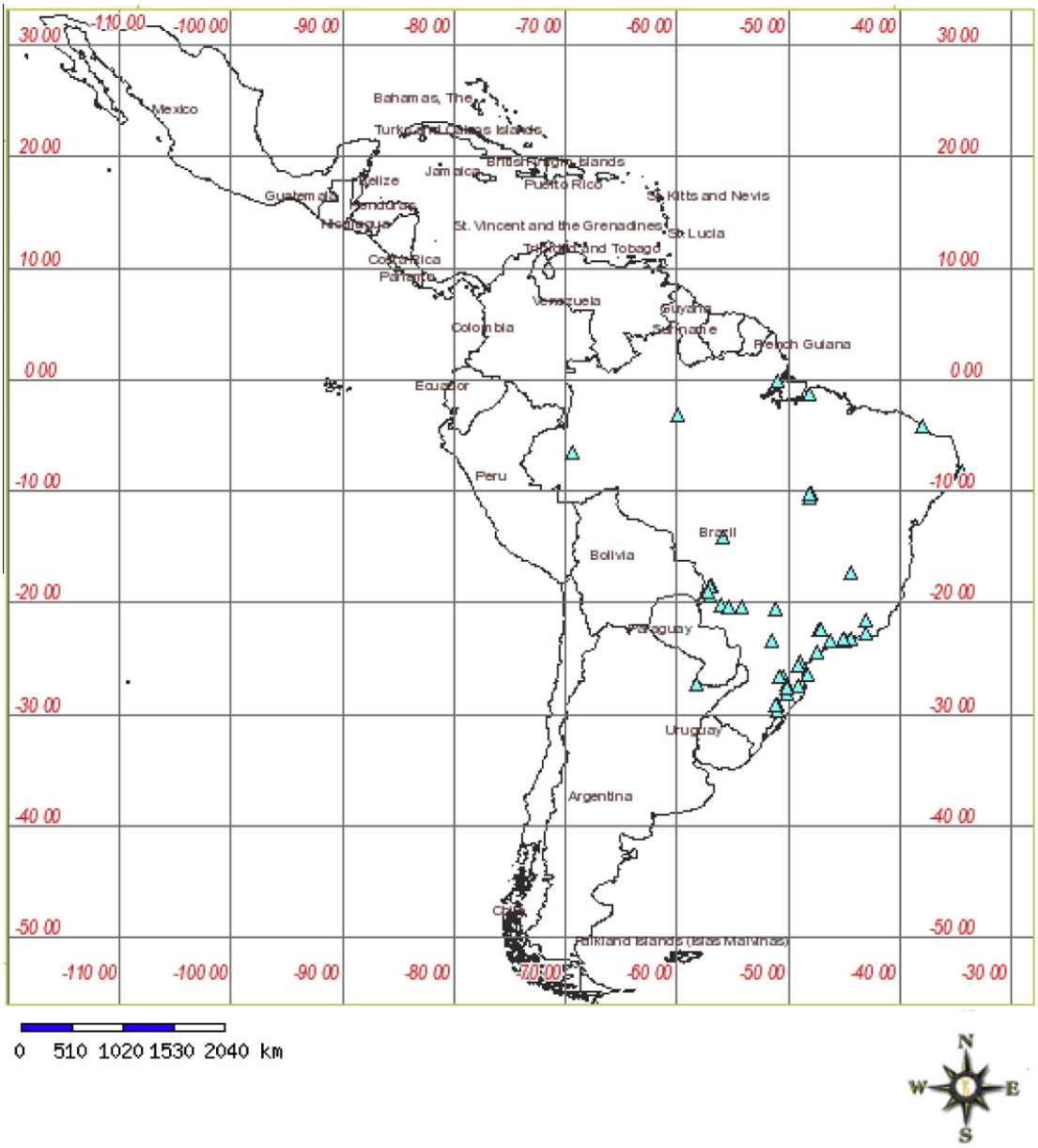

Fig. 1. Collection places from studied populations. Table 1 show localities. Map: speciesMapper (http://splink.cria.org.br/mapper).

After all sequences were aligned with the sequences retrieved from the GenBank (Table 4), some bases at the end of the fragment were excluded due to unsatisfactory alignment. The resulting matrix consisted of approximately 480 bp.

The reconstruction of the phylogeny based on maximum parsimony analysis was conducted using the software PAUP 4.0 (Swofford, 2003). The data set were analyzed using the settings 1 for gap and 3 for substitutions. One thousand replicates were used to generate bootstrap values.

Before carrying out the Bayesian analyzes, appropriate model of sequence evolution were chosen via the Akaike Information Criterion using Modeltest v 3.06 (Posada and Crandall, 1998) and the model selected was GTR + G. The reconstruction of the phylogeny based on the Bayesian analysis was carried out using the software MrBayes (Huelsenbeck and Ronquist, 2001). A Markov chain was run for 1,000,000 generations and sampled at each 100 generations. To summarize the parametric values and the trees generated, the first 10% of the trees were excluded as burnin

Table 2
Wolbachia strains diversity (A or B) of each analyzed species ant. Only the strain type for each species is shown here ignoring repetition of occurrence.

Species	Strain A	Strain B	Total
S. geminata	–	H17, H26	2
S. invicta	H1, H2, H6, H7, H8, H9, H10	H22, H23, H25, H26, H27, H28, H29, H30, H31, H32, H35, H36, H37, H38, H39, H40, H41, H44, H45, H46	27
S. megergates	–	H24, H42, H44	3
S. pusillignis	–	–	–
S. saevissima	H3, H4, H5	H26, H31, H34	6

Table 3
Strain diversity (A or B) of *Wolbachia* for each analyzed region.

Occurence region	Strain A	Strain B	Total
North	–	H17, H26	2
Northwest	–	–	–
South	H1, H3, H4, H5, H8, H9	H22, H24, H25, H26, H27, H28, H29, H30, H31, H34, H37, H38, H41, H42, H44	21
Southeastern	H1, H2, H4, H6, H7, H10	H26, H32, H35, H36, H39, H40, H43, H44, H45, H46	16
Central-western	–	–	–
Argentina	H2	H23, H26, H31	4

Table 4
Genera and lineage of endobacteria or host species and respective GenBank access numbers.

Genera and lineage of endobacteria or host species	GenBank access numbers	wsp Haplotype
Wolbachia sp. wInv-B1	AF217722	H18
Wolbachia sp. S. invicta	DQ842483	H33
Wolbachia sp. Sdag-B4	AY878106	H26
Wolbachia sp. SinvictaB	AF243436	H26
Wolbachia sp. SdagB1	AY878102	H19
Wolbachia sp. SdagB3	AY878101	H20
Wolbachia sp. SdagB2	AY878107	H21
Wolbachia sp. SdagA5	AY878104	H15
Wolbachia sp. SdagA4	AY878099	H16
Wolbachia sp. SdagA3	AY878105	H13
Wolbachia sp. SdagA2	AY878100	H14
Wolbachia sp. SdaqA1	AY878103	H11
Wolbachia sp. S. richteri	AY755414	H12
Wolbachia sp. WrichteriA	AF243437	H8
Wolbachia sp. wSinvictaA	AF243435	H1
Wolbachia sp. S. saevissima	EU251432	H4
Wolbachia sp. SS1A	EU251431	H4

and the probability values were then calculated with the remaining trees.

In the absence of a suitable outgroup for rooting the inferred Trees (see Lo et al., 2002), the evolutionary rate was assumed to be approximately uniform to all branches. Based on this premise trees were midpoint rooted.

3. Results

Of the 114 analyzed colonies of *Solenopsis* (59 of *S. invicta*, 40 of *S. saevissima*, 9 of *S. geminata*, 4 of *S. megergates*, 2 of *S. pusillignis*) from the southern, southeastern, northern, northeastern, and west-central Brazil, 58 (51%) were infected with the endosymbiont *Wolbachia*, and 13% had multiple infections. All *wsp* sequences generated in this study have been deposited in the GenBank database under access numbers HM747138 to HM747161.

Table 1 presents the species identified by COI, the collecting sites, the presence/absence of *Wolbachia* infection, and the *Wolbachia* strains found.

The sequences H10, H17, H28, and H38 were not included in the analysis, as they generated proteins that were not similar to those of other sequences and therefore could be represent errors in the sequencing.

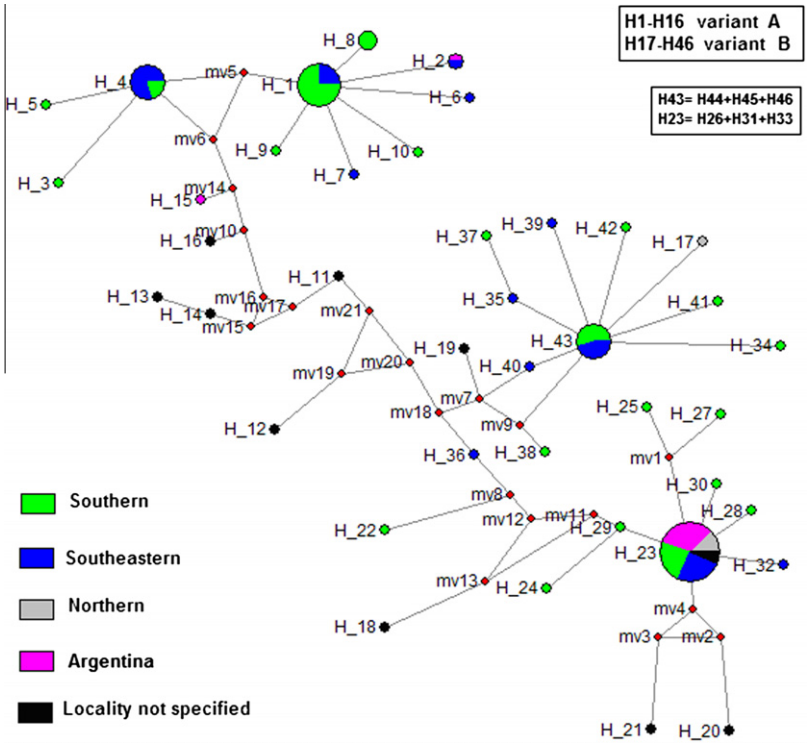

Fig. 2. Network of strains from *wsp* gene constructed with software NETWORK4.5 (fluxus-enginnering.com). H1–H16 strains from supergroup A, H17–H46 stains from supergroup B. The red points indicate hypothetical ancestors. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

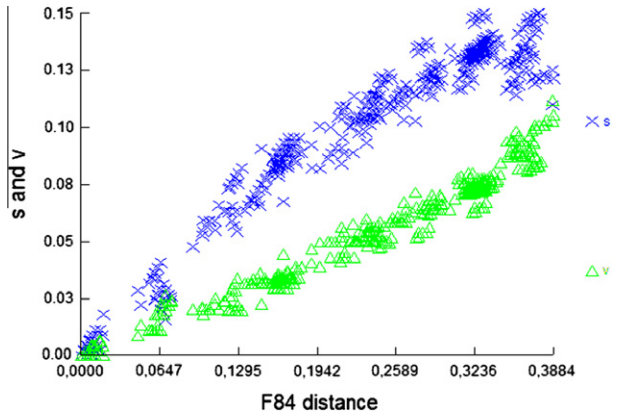

Fig. 3. Analysis of the phylogenetic signal of the *wsp* gene sequences from *Wolbachia* endosymbiont of the samples studied in this paper. The x axis indicates a divergence around 39% between the sequences. The blue dots represent transitions and the green dots represent transversions. The graph shows a satisfactory phylogenetic signal where the transitions appear more often than transversions in most of the graph. The regions where the dots overlap is probably due to one of the four HVR (high variable region) occurring in *wsp* (Baldo et al., 2005). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.1. Distribution of Wolbachia in the genus Solenopsis

Wolbachia infections were found in four of five species of *Solenopsis* examined (*S. invicta*, *S. saevissima*, *S. geminata*, and *S. megergates*). The frequency of *Wolbachia* infections were highest in *S. invicta*, with 33 infected colonies (22%), while in *S. saevissima*, *S. megergates*, and *S. geminata*, 19 (47%), 4 (100%), 2 (22%), colonies were infected, respectively.

Tables 2 and 3 present the type of *Wolbachia* supergroup (A or B) in each ant species examined, and by region, respectively. Supergroup B was more commonly found in *S. invicta*, with 27 strains (Table 2). The number of variants found in the remaining species was low. In Table 3, the highest incidence was observed in populations from southern (with 21 strains) and southeastern (16 strains) Brazil. The supergroup B was the most frequent, with 15 strains found in southern areas and 10 strains in southeastern Brazil. The infection rate was lower in the remaining regions. Low infection rates were found in the northern region, while in central-western and northwestern Brazil, no nests were found to be infected with *Wolbachia*.

3.2. Network of strains of Wolbachia

Ninety-one sequences of the *wsp* gene were generated and analyzed along with sequences of strains retrieved from GenBank (presented in Table 4) using the software NETWORK4.5 to generate a network of strains (Fig. 2). The resulting network revealed the existence of 46 variants of the *wsp* gene in the populations examined. From these 46 variants, 35 were present in the populations surveyed. Some strains were very abundant in the samples and were named H1 and H4 (supergroup A), H23/H26 and H43 (supergroup B).

3.3. Analysis of the phylogenetic signal

After alignment, the strength of the phylogenetic signal was measured using the software DAMBE (Xia and Xie, 2001). The results indicated a strong phylogenetic signal, with transitions exceeding transversions (Fig. 3).

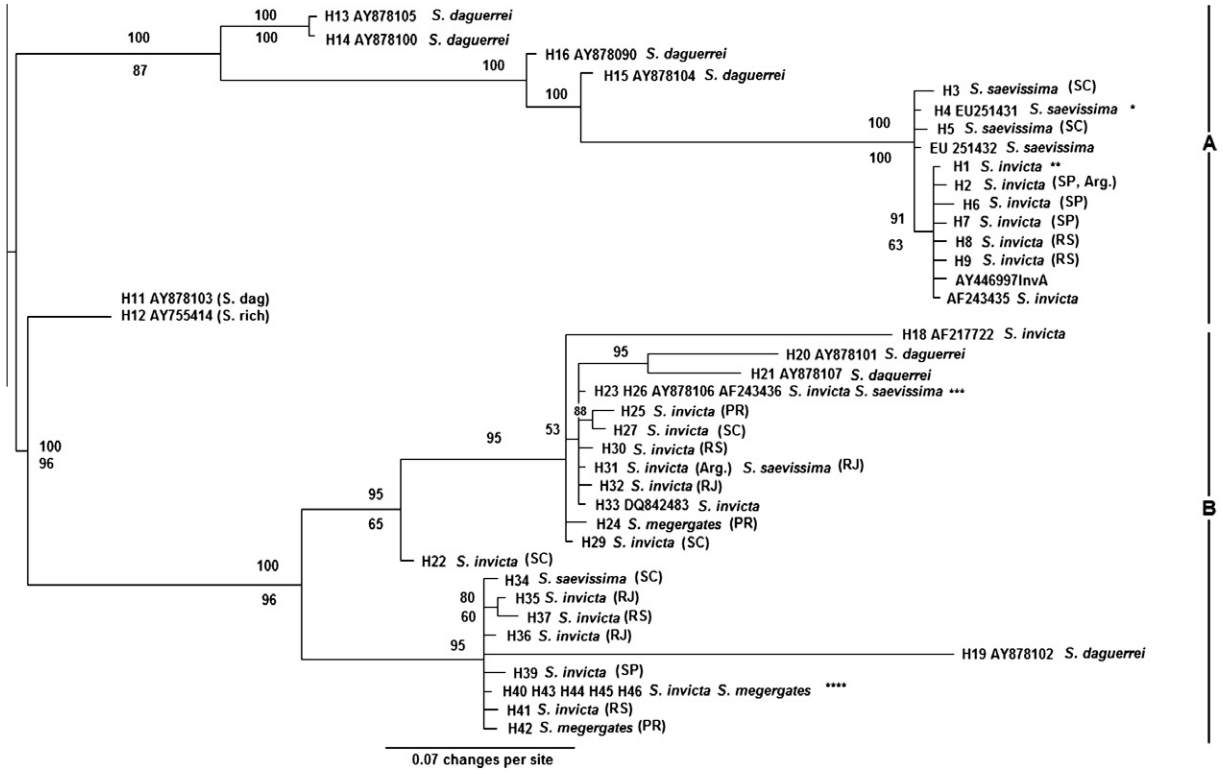

Fig. 4. Phylogenetic tree based on *wsp* sequence gene from ants of Brazilian genera *Solenopsis*. The maximum parsimony and Bayesian analyses recovered virtually identical trees in topology. The illustrated tree was generated by software MrBayes. Numbers above branches indicate posterior probability values from Bayesian analyses. Numbers below branches are bootstrap values recovered from the maximum parsimony analyses. Bootstrap values below 60 are not indicated. *H4: *S. saevissima* SP, RS, SC. **H1: *S. invicta* RS, SP, SC, ***H23 *S. invicta* Argentina; H26 *S. invicta* RS, Argentina; *S. saevissima* RJ, AM, SC, MG; *S. geminata* AM. ****H40, H43, H44, H45, H46: *S invicta*: RJ, RS, SP, SC; *S. saevissima*: MG.

3.4. Phylogeny of Wolbachia on the populations surveyed

The result of the phylogenetic analysis of *Wolbachia* strains based on the *wsp* gene is summarized in Fig. 4. A total of 483 characters were used in the maximum parsimony analysis, 267 were constant and 182 were parsimony-informative characters. Three equally parsimonious trees were found.

Both maximum parsimony analysis and Bayesian inference were congruent and only the Bayesian phylogenetic tree is presented with posterior probability and MP bootstrap values. The resulting tree was midpoint rooted, based on sequences of *wsp* from *S. invicta*, *S. saevissima*, *S. geminata*, and *S. megergates* (Table 1) as well as on sequences from *Wolbachia* strains from other hosts of the genus *Solenopsis* retrieved from the GenBank (Table 4).

Six *Wolbachia* strains of the supergroup A were found in *S. invicta* and three in *S. saevissima*. Two strains (AF243435 and AY446997) found in *S. invicta* retrieved from the GenBank were grouped in the branch of *Wolbachia* strains of this ant, forming a derived polytomy. At the base of this clade, a group of *Wolbachia* strains forms a polytomy with strains from *S. saevissima* retrieved from the GenBank (EU251431 and EU251432).

Within supergroup B, fifteen strains were found in *S. invicta*, three in *S. saevissima*, and two in *S. megergates*. Three strains, termed H23 and H26; and H31 were also found in *S. invicta* and *S. saevissima*, respectively. Supergroup B was separated in two groups. One of them exhibited a unresolved node (polytomy) formed by a *Wolbachia* sequence found in *S. daguerrei* retrieved from GenBank (AY878102), along with *Wolbachia* strains from *S. invicta* and *S. megergates*. The second group was a sister group of the first group, formed by *Wolbachia* strains found in *S. invicta* (H22) at the base, followed by a branch from strains found in *S. invicta* retrieved from GenBank (AF217722), and a strain found in *S. megergates* and another in *S. invicta*. A derived group in relation to the previous ones was comprised by strains found in *S. daguerrei* (AY878101, AY878107), followed by a group of strains found in *S. invicta*, forming a polytomy with strains found in *S. invicta* and S. *daguerrei* retrieved from GenBank (AF243436, DQ842483, and AY878106).

4. Discussion

The analysis of *Wolbachia* sequences of different species of *Solenopsis* indicates a higher frequency of supergroup B rather than A, unlike the observed by Ahrens and Shoemaker (2005) in *S. invicta.* These authors reported a similar occurrence of the two supergroups in some South-American populations. In the distribution of these supergroups in the network generated and in the reconstructed phylogeny, there is a complete separation of supergroups, in agreement with the described by Zhou et al. (1998) and Ahrens and Shoemaker (2005), the variants H1–H16 (Fig. 2) correspond to strains of the group A and H17–H46 correspond to strains of the group B.

The number of strains was very high and was not associated with the number of *Solenopsis* species examined (*S. invicta*, *S. saevissima*, *S. megergates*; *S. geminata*, and *S. pusillignis*), which might be indicative of horizontal transmission within the genus *Solenopsis*, as suggested by Ahrens and Shoemaker (2005). Similarly, Souza et al. (2009) suggested horizontal transmission in Brazilian populations of *S. saevissima*.

Zhou et al. (1998) reported that the divergence in the sequence obtained from different insect species in supergroup A was 14% and in supergroup B, 22%. A low divergence was found in both supergroups from *Solenopsis*, as indicated by polytomies in the consensus tree.

An 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 (Dedeine et al., 2005).

Solenopsis invicta and *S. saevissima* were the most frequent species collected. The former had the highest frequency of colonies infected with *Wolbachia*, as well as the highest diversity of strains. The highest frequency of colonies with multiple infections was also found in *S. invicta* colonies, mainly from southern Brazil.

Although samples were collected in disturbed sites, similar results regarding *Wolbachia* infections would be expected where *Solenopsis* was introduced. However, no individuals from populations of introduced *Solenopsis* were found to be infected with *Wolbachia* by Shoemaker et al. (2000).

On the other hand, the bacterial surface protein *wsp* shows homology with antigenic proteins of pathogens, with a heterogeneous variation characterized by hypervariable regions (HVRs) flanked by highly conserved regions (CRs) (Braig et al., 1998). This protein might be under strong positive selection, affecting its hypervariable region. In addition, evidences indicate the existence of recombination in this sequence (Jiggins, 2002; Reuter and Keller, 2003; Werren and Bartos, 2001). These factors can alter the function of this protein in host-*Wolbachia* interactions (Baldo et al., 2005).

In our study, *Wolbachia* infection was not uniform, confirming the results obtained by Ahrens and Shoemaker (2005).

The low *Wolbachia* infection rate found in populations from Manaus, Amazonas state, Brazil, was characterized by less intense bands of the *wsp* gene. Several dilutions and repeated amplification of the *wsp* gene where made in order to have more intense bands and sequence this samples but no improvement where done on amplification final concentration. As a result sequencing of these samples was not possible. This low concentration of amplified *wsp* gene and consequently possible low infection rates in those samples could be explained by several hypothesis but based on recent publications (Arthofer et al., 2009; Wolfgang et al., 2009) about the low-titre infections not traceable by conventional PCR techniques (i.e. low copy numbers of *Wolbachia* in the infected individuals) we infer that this could be the case of those populations. A possible strategy to confirm the low infection rates on those populations could be to perform a high sensitive nested PCR technique, such as that on Wolfgang et al. (2009), an interesting subject of study in future and further investigation in those Brazilian ants.

A positive relationship has also been found between *Wolbachia* infections and latitudinal distribution. Northern, central-western, and northeastern populations have low or no *Wolbachia* infection rates, indicating that incidence is apparently lower in regions with long dry seasons or high daily average temperatures. This has been observed in the beetle *Chelymorpha alternans* and in ants of the genus *Solenopsis* (Ahrens and Shoemaker, 2005; Keller et al., 2004). The distribution of *Wolbachia* in *S. invicta* can be influenced by differences in environmental conditions, with higher *Wolbachia* prevalence occurring in more southerly temperate populations (Ahrens and Shoemaker, 2005).

The higher frequency of some *Wolbachia* strains in colonies from southern and southeastern regions might be due to infection by a strain in several local populations, or even a strain in many populations of two or more species. The polytomies found in the phylogenic analysis support this hypothesis. The high frequency

of a few strains might also be a consequence of the original foundresses infected (founder effect) with *Wolbachia* and their expansion in these regions. The "satellite" strains (Fig. 2), which are linked to more frequent variants, might result from few differences in gene sequence due to mutations, as described by Ahrens and Shoemaker (2005) or recombination of the most frequent one.

All ant populations from Corrientes, Argentina were infected with *Wolbachia*, with only three variants. Two of them belong to supergroup B, one was found in most colonies sampled, H26, and another one from supergroup A. The strains of group B are very closely related, and are part of the polytomy revealed in the phylogenic tree (Fig. 4). These data corroborates the results found in populations from southern Brazil, where *Wolbachia* infections were more successful and are more abundant.

5. Conclusion

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 in some nests can influence reproductive conflicts and combined with other reproductive barriers, it might accelerate speciation (Werren, 1997).

In general, our observations support the results obtained by Ahrens and Shoemaker (2005) regarding the possibility of several introductions of *Wolbachia* in *S. invicta* and the diversification of the bacteria within the genus. In the ant species examined, several horizontal transmission events might have occurred, followed by a possible founder effect and expansion of some strains in some regions.

The grouping of the *Wolbachia* strain from the parasite *S. daguerrei* with strains from supergroups A and B, suggests its participation in horizontal transmission.

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