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A SURVEY FOR *SOLENOPSIS INVICTA* VIRUSES (SINVS) IN BRAZIL

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Abstract *Solenopsis invicta* viruses (SINVs) were surveyed in samples collected in municipalities of São Paulo and Minas Gerais States, Brazil, using a multiplex RT-PCR method. Species identification was performed by mitochondrial COI gene sequencing. All three SINVs described in the US were detected in Brazil but with low prevalence. Detection of a highly divergent SINV-3 haplotype indicates that other variants might exist in Brazil and may have passed undetected by the primers employed. Predominance of *S. saevissima* in samples collected in the countryside contrasts with the predominance of *S. invicta* in São Paulo city.
Key Words Multiplex RT-PCR, mtDNA, positive strand RNA virus

INTRODUCTION

The fire ant *Solenopsis invicta* is native to Brazil and is an invasive species in the southeastern US since around 1940 and more recently in California, Caribbean, Australia, New Zealand, Taiwan, Hong Kong, Macao, and China (Ascunce et al., 2011; Valles et al., 2009). Given its importance many aspects of the biology of this species has been elucidated including genetics and genomics. Expressed sequence tags and genome projects have already been completed (Valles et al., 2008; Wang et al., 2007; Wurm et al., 2011). Viral sequences were found among the EST clones that provided a start point for the characterization of the complete genome of the viruses SINV-1, SINV-2, and SINV-3, and part of the genome of SINV-1A. Experimental transmission of SINV-1, -2, and -3 have been demonstrated and increased mortality was associated with the presence of SINV1 and SINV3. (Valles and Hashimoto, 2009; Valles and Strong, 2005; Valles et al., 2004; Valles et al., 2007a, Valles et al., 2007b). These characteristics confer *S. invicta* viruses a potential use as biological control agents. Valles et al. (2009) developed a set of primers for multiplex RT-PCR detection of SINV -1, -2, and -3. These primers were employed in the present work for a preliminary survey of *Solenopsis* spp viruses in Brazil.

MATERIALS AND METHODS

Solenopsis spp workers were collected in 42 nests in different locations in São Paulo and Minas Gerais States and kept in 92 % ethanol until processing in the lab. Before nucleic acids extraction, ants were dried in a centrifuge evaporator. Ten workers for each DNA or RNA extraction were ground under liquid nitrogen in microtubes with a plastic pestle. DNA was extracted using DNeasy Tissue and Blood kit (QIAGEN), according to manufacturer's instructions. RNA was extracted using Trizol reagent (Life Technologies). For species identification, a 420 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified using primers COI-F (5' - GATTTTTTGGKCAYCCMGAAG - 3') and COI-R (5' - CRAATACRGCTCCTATWGATAAWAC- 3') (Gusmao et al, 2010). For detection of SINVs, the primers described by Valles et al. (2009) were employed. Primers p524: (5' - ATTGCATTTTCACAATTAATCTTAGTGCTCTC - 3') and p244 (5' - CATCTGGCAATCCTGCAACCAC - 3') amplified a 481 bp product of SINV-1; p548 (5' - TGCATACTCGTTGTAAACAATCTGCTCATCT - 3') and p555 (5' - TGCCGTGACAATCCTGAATATCGTCAGATGTA - 3') a 717 bp product of SINV-2; p812 (5' - AATATCAGCATATTGATGATCCAAAATGCCAA - 3') and p813 (5' - AAGAGAACGTATGCCTACTCCATCAGAAGCAT - 3') a 259 bp product of SINV-3. Amplification products were purified and submitted to DNA sequencing using the reagent Big Dye 3.1 (Applied Biosystems) and an ABI 377 DNA sequencer (Applied Biosystems). Obtained sequences were compared with those deposited in the GenBank using BLAST. Sequences alignment and analysis were made using ClustalW and BioEdit.

RESULTS

Forty two nests were sampled and identified through COI gene sequencing resulting in 27 nests of *S. saevissima* (6 haplotypes) and 15 of *S. invicta* (1 haplotype) (Table 1). Only five nests were positive for SINV. Interestingly, the nest of São Paulo municipality, located in a garden lawn inside Instituto Biológico, which was sampled twice, resulted positive for SINV3 in winter and for SINV-1 in summer. SINV-1 (two haplotypes) were detected in two

Table 1. Location, species identification and virus detection of *Solenopsis* spp nests sampled in São Paulo and Minas Gerais States, Brazil. Species identification was done through COI gene sequencing and virus detection through multiplex RT-PCR using specific primers (Valles et al., 2009).

Municipality/State	Fire ant species	SINV
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. invicta* haplotype 1	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Andradas/Minas Gerais	*S. saevissima* haplotype 5	
Caieiras/São Paulo	*S. invicta* haplotype 1	
Caieiras/São Paulo	*S. saevissima* haplotype 4	
Campinas/São Paulo	*S. invicta* haplotype 1	SINV-1b
Campinas/São Paulo	*S. saevissima* haplotype 4	
Campinas/São Paulo	*S. invicta* haplotype 1	
Campinas/São Paulo	*S. invicta* haplotype 1	
Campinas/São Paulo	*S. invicta* haplotype 1	
Campinas/São Paulo	*S. invicta* haplotype 1	
Campinas/São Paulo	*S. invicta* haplotype 1	
Cunha/São Paulo	*S. invicta* haplotype 1	
Cunha/São Paulo	*S. saevissima* haplotype 1	
Cunha/São Paulo	*S. saevissima* haplotype 2	
Cunha/São Paulo	*S. saevissima* haplotype 2	
Cunha/São Paulo	*S. saevissima* haplotype 3	SINV-3b
Cunha/São Paulo	*S. saevissima* haplotype 3	
Cunha/São Paulo	*S. saevissima* haplotype 2	
Cunha/São Paulo	*S. saevissima* haplotype 2	
Cunha/São Paulo	*S. saevissima* haplotype 2	
Espirito Santo do Pinhal/São Paulo	*S. invicta* haplotype 1	
Espirito Santo do Pinhal/São Paulo	*S. invicta* haplotype 1	
Piracicaba/São Paulo	*S. invicta* haplotype 1	
Piracicaba/São Paulo	*S. saevissima* haplotype 4	
Piracicaba/São Paulo	*S. saevissima* haplotype 4	
Ribeirão Preto/São Paulo	*S. saevissima* haplotype 6	
Ribeirão Preto/São Paulo	*S. saevissima* haplotype 6	
Ribeirão Preto/São Paulo	*S. saevissima* haplotype 6	SINV-2
São Paulo/São Paulo	*S. invicta* haplotype 1	SINV-1a and SINV-3a
Santa Bárbara d'Oeste/São Paulo	*S. invicta* haplotype 1	
Santa Bárbara d'Oeste/São Paulo	*S. saevissima* haplotype 1	
Uberaba/Minas Gerais	*S. saevissima* haplotype 6	SINV-2
Uberlândia/Minas Gerais	*S. invicta* haplotype 1	

S. invicta nests, SINV-2 (1 haplotype) in two *S. saevissima* nests, and SINV-3 (two haplotypes) in two *S. saevissima* nests (Table 1). SINV-1a and SINV-1b haplotypes were 95.0% identical at the nucleotide level between each other and 96.0% and 96.6% identical, respectively, to the completely sequenced SINV-1 (GenBank AY634314). SINV-2 haplotype was 90.4% identical to the completely sequenced SINV-2 (GenBank EF428566). SINV-3a and SINV-3b haplotypes were 76.9% identical between each other and 96.7% and 75.5% identical, respectively, to the completely sequenced SINV-3 DM strain (GenBank NC012531).

DISCUSSION

In this work we report for the first time the occurrence of *Solenopsis invicta* viruses in Brazil. All three SINVs reported in the USA were detected separately in samples collected in the States of São Paulo and Minas Gerais. We also report for the first time the occurrence of SINV viruses in *S. saevissima*. The nucleotide similarities of the Brazilian strains to the North American strains were above 90% for all SINVs except for one haplotype of SINV-3 which was only 75.5% identical. The prevalence of each SINV species was 4.8% and can be considered very low compared to the prevalence observed in the US (SINV-1 – as high as 50%, SINV-2 – 40%, SINV-3 – 20%) and in Argentina (SINV-1 – 28.9%, SINV-2 – 16.6%, SINV-3 – 7.9%) (Valles et al., 2009). It is possible that virus prevalence in *Solenopsis* spp in Brazil is much higher than the values observed in the current work because the specific primers employed in the multiplex RT-PCR may have hindered the detection of divergent SINV haplotypes. The amplification of a highly divergent SINV-3 haplotype was a fortuitous result given that the primers employed were very long and not degenerated. Also, the SINV-1A variant that is prevalent in many locations in the US was not probed by the method employed.

Species identification of the surveyed nests showed predominance of *S. saevissima* over *S. invicta* (27 and 15 nests, respectively). Municipalities located in the countryside of São Paulo and Minas Gerais States prevailed in our sampling and might explain the divergence with the results of Gusmao et al. (2010) whom observed predominance of *S. invicta* in nests collected in 7 parks in São Paulo city. These results suggest that *S. invicta* is better adapted to more disturbed environments. The observation of several *S. invicta* nests in Campinas, the third largest city in São Paulo State located 90 km north of São Paulo city, also supports this assumption. Also, the observed genetic diversity for *S. saevissima* was higher than for *S. invicta*, with 6 haplotypes detected for the former and only one for the latter.

CONCLUSIONS

Solenopsis invicta viruses -1, -2, and -3 occur in Brazil and infect *S. invicta* and *S. saevissima*. *S. invicta* predominates in more disturbed areas while *S. saevissima* predominates in less disturbed areas.

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