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Occurrence of *Syntomopus parisii* (Hymenoptera: Pteromalidae) parasitizing *Melanagromyza sojae* (Diptera: Agromyzidae) in Brazil and Paraguay

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ABSTRACT. Soybean stem fly (SSF), *Melanagromyza sojae* is an important pest in soybean growing regions of the Old World, and recently has been confirmed in soybean areas in Brazil, Paraguay and Bolivia. In the regions where it is endemic, the management of *M. sojae* is performed by using resistant varieties, chemical insecticides and natural control carried out by parasitoids. One of the main parasitoid species of *M. sojae*, responsible for high parasitism rates is *Syntomopus parisii* (Hymenoptera: Pteromalidae). This paper confirms the presence of *S. parisii* parasitizing pupae of SSF in Brazil and Paraguay, using morphological descriptions. In addition, we provide, for the first time, genetic information on *S. parisii* specimens, based on the fragment of Cytochrome Oxidase I gene, which indicated low genetic diversity of *S. parisii* collected in Brazil and Paraguay.

Key words: *Glycine max*; Soybean stem fly; Soybean stalk fly; Biological control

INTRODUCTION

Soybean stem fly, *Melanagromyza sojae* (Diptera: Agromyzidae) is a highly polyphagous pest, feeding on plants such as *Glycine max*, *Phaseolus vulgaris* and other members of the family Fabaceae (Dempewolf, 2004). It has been reported on five continents, and it is considered one of the main pests of soybean in parts of Indonesia (Van den Berg et al., 1995), China (Wang and Gai, 2001), India (Thapa, 2012), Australia (Brier and Charleston, 2013), Russia (Strakhova et al., 2013), Paraguay (Guedes et al., 2017) and Bolivia (Copa et al., 2018).

In Brazil, *M. sojae* has been cited as a potential pest of soybean (Hirose and Moscardi, 2012). *Melanagromyza* sp. was first reported in Brazil infesting soybean plants in the state of Rio Grande do Sul in 1983 (Gassen and Schneider, 1985), and in 2009, it was reported in five soybean production fields in the same State (Link et al., 2009). Later, specimens were collected in the Brazilian states, Rio Grande do Sul and Santa Catarina (Guedes et al., 2015) and identified as *M. sojae* (Arnemann et al., 2016). In Paraguay, *M. sojae* has been reported on Canindeyú, Alto Paraná and Itapúa Departments (Guedes et al., 2017) and recently on Bolivia soybean fields (Copa et al., 2018).

Parasitoids are frequently found parasitizing *M. sojae* larvae and pupae. The most common species in Indonesia are *Chlorocytus* sp., *Sphegigaster agromyzae* (also known as *Trigonogastra agromyzae*) (Hymenoptera: Pteromalidae), *Gronotoma* sp. (also known as Cynipoidea sp.) (Hymenoptera: Figitidae) and *Eurytoma poloni* (Hymenoptera: Eurytomidae). In addition to these species, *Bracon* sp. (Hymenoptera: Braconidae), *Eurytoma* sp., *Colotrechnus agromyzae*, *Sphegigaster* sp., *Syntomopus* sp. (Hymenoptera: Pteromalidae), *Tetrastichus* sp. and *Sympiesis* sp. (Hymenoptera: Eulophidae) have also been reported (Van der Goot, 1930; Van der Berg et al., 1995; Shepard and Barrion, 1998). Narendran (1994) described *Eurytoma melanagromyzae* from India and Huang and Xiao (2005) reported *Syntomopus thoracicus* from China. De Santis et al (1976) reared *Syntomopus parisii* from *Melanagromyza cunctanoides* in sunflower in Argentina. In addition to these records in South America, *S. parisii* and a *Leptomeraporus* species parasitizing *M. sojae* were recently reported in Brazil (Salgado-Neto et al., 2017).

Natural parasitism on larval and/or pupal stages of *M. sojae* helps to keep the population of this pest low in agricultural areas (Talekar and Chen, 1985). The percentage of parasitism varies according to the location, climate, time analyzed and sowing time; parasitism rates may reach 70% (Van den Berg et al., 1995).

In our study, suspected specimens of parasitoids were collected directly from larvae and pupae of *M. sojae* from soybean fields in Brazil and Paraguay, to identify the species using morphological characters and to sequence the partial mtDNA COI gene. We confirm the presence of *S. parisii* occurring in Paraguay and show for the first time the use of the mtDNA COI gene region to identify this important parasitoid affecting the invasive soybean pest *M. sojae*.

MATERIAL AND METHODS

During the 2016 cropping season, in Ibirubá, RS State, Brazil and Bella Vista, Amambay Department, Paraguay, *M. sojae* pupae were collected from soybean plants that demonstrated feeding damage by *M. sojae*, as described by Van den Berg et al. (1998). The

soybean plants were collected randomized, and analyzed in a laboratory in Brazil. The soybean stems were opened with a longitudinal cut in their main and secondary stems and the pupae that were found were collected. The pupae were reared in growth chambers (25±1°C and 12:12h light: dark photoperiod) until adult parasitoids were obtained. The parasitoids were collected and individually stored in 1.5-mL Eppendorf containing 99.9% ethanol, for morphological and molecular characterization.

Specimens were identified according to De Santis et al (1976) and Heydon (1993). Three *S. parisii* specimens from Brazil and three from Paraguay were used for molecular characterization of part of the mtDNA COI gene region; the remaining specimens were deposited in the collection Insetos Entomófagos Oscar Monte (Instituto Biológico, Campinas, São Paulo, Brazil).

Total genomic DNA from *S. parisii* specimens was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The mitochondrial gene fragment of COI (mitochondrial Cytochrome Oxidase I) (Hebert et al., 2003) was amplified by PCR using the primers LCO 1490 (5´-GGTCAACAAATCATAAAGATATTGG-3´) and HCO 2198 (5´-TAAACTTCAGGGTGACCAAAAAATCA-3´) (Folmer et al., 1994). Each PCR mixture contained 0.25µL AccuTaq™ LA DNA Polymerase (5U/µL), 2.5 µL of AccuTaq LA 10 Buffer, 1.25 µL dNTP mixture (10mM of each), 2 µL of each primer (10pM), 3 µL of DNA template and 15 µL distilled water, with a final volume of 26 µL. PCR reactions were performed using an initial denaturation at 94°C for 5 min, followed by 34 cycles at 94°C for 30s, 48°C for 30s, and 72°C for 1.5 min, and a final extension at 72°C for 5 min. The amplified products were resolved on 1.0% agarose electrophoresis gels, pre-stained with Nancy-520 DNA gel stain (Sigma-Aldrich, St. Louis, MO, USA) and visualized with a gel documentation system. Sequencing was performed by Helixxa genomics service provider. The software Pregap and Gap4 within the Staden package (Staden et al., 1998) were used for editing and analyzing the DNA sequences and to generate a sequence consensus. For identification of collected specimens, sequences were aligned by BioEdit software, version 7.2.5 (Hall, 1999).

Consensus sequences were deposited in the GenBank and a comparative search by means of Blastn at NCBI (network services using National Center for Biotechnology Information, USA database) was performed. The phylogenetic relationship of the sequences by country was reconstructed based on analyses of the COI region in MEGA 5.0 software (Tamura et al 2011), with the analysis of Maximum Likelihood (ML) followed by 1,000 bootstrap replications to estimate node confidence. Owing to the low number of SNPs, a BULK DNA sequence sampling analysis was used.

RESULTS

Morphological description

This is the first record of *S. parisii* parasitizing *M. sojae* in Paraguay. *Syntomopus parisii* (Fig. 1) is a Pteromalinae species with a large pronotum, with the collar less than four times as broad as median length, and with rectangular shoulders; the clypeal margin is symmetrical, with three angular teeth, the median tooth longer than the other two, and the mesosoma is usually clearly flattened (Bouček, 1988; Heydon, 1993). *Syntomopus parisii* is

a black colored species with a greenish metallic sheen on the head, mesosoma, and the base of the gaster; there are also some purple reflections on the mesosoma; scape and tibiae are yellowish. Head and mesosoma are reticulately sculptured; the gaster is smooth and shiny.

Figure 1. *Syntomopus parisii* (Hymenoptera, Pteromalidae) male (left) and female (right).

Among the *Syntomopus* species known to occur in the New World, *S. parisii* is closer to *Syntomopus arpedes*, differing from the latter species because of its yellowish scape and the median tooth on the clypeus being only slightly longer than the lateral teeth; in *S. arpedes*, the scape is yellowish brown, with strong metallic green reflections, and the median tooth on the clypeus is much longer than the lateral ones (Heydon, 1993).

Molecular identification

Molecular characterization of *S. parisii* was carried out by sequencing mtDNA COI gene of eight specimens from Brazil and Paraguay. The sequences were combined by country to obtain the consensus sequences. The consensus sequences from Brazil and Paraguay showed one SNP (single nucleotide polymorphic) located 280 bp from the alignment between cladogram data sets; it was identified as a pyrimidine substitution (T/C). The NCBI/Genbank deposit generated the accession numbers NCBI KY468264 and KY468263 for Brazil and Paraguay, respectively. The cladogram (Fig. 2) was reconstructed based on analyses of the COI region performed by the General Time Reversible nucleotide substitution model with Gamma distributed with invariant sites; parameters for partial exemption (95%) were estimated as the best substitution model using MEGA 5.0 software (Tamura et al., 2011). Thereby, the largest possible number of comparative accessed already deposited in NCBI was included to perform the cladogram analyses. According to morphological and molecular data sets from our study, the cladogram suggests a close relationship between the *Syntomopus* specimens from Brazil and Paraguay and a sharp distinction between these accessions compared to other sequences retrieved from the GenBank database.

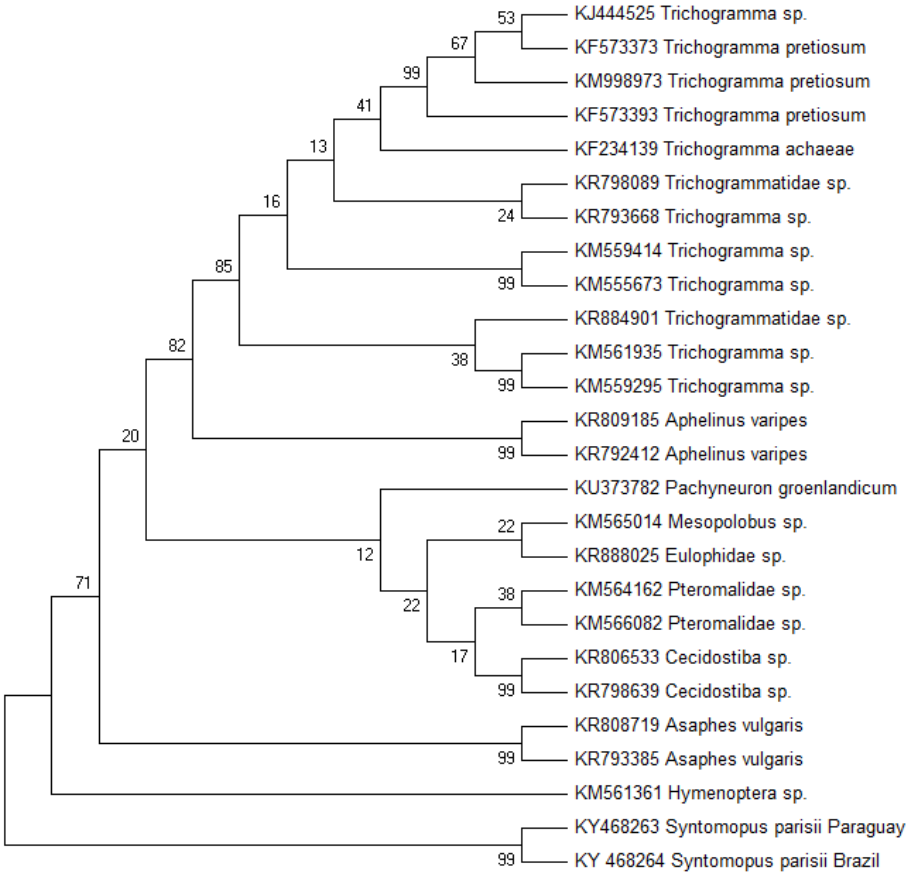

Figure 2. Cladogram of the *Syntomopus parisii* (Hymenoptera, Pteromalidae) inferred from partial mtDNA COI gene region sequences, using Maximum Likelihood analysis.

DISCUSSION

The identification of parasitoids and natural enemies using morphological and molecular tools is possible and essential to understand which species are associated with *M. sojae* in the New World. Analysis of part of the COI gene is an efficient and accurate method to identify *S. parisii* parasitizing *M. sojae* and is an alternative tool that could help to overcome some of the difficulties associated with its morphological identification. Furthermore, knowing parasitism rates and whether seed coat insecticides affect the parasitism of *M. sojae* by *S. parisii* is important to make management decisions to control the soybean stem fly in South American soybean fields.

DNA barcoding is useful and informative to identify *S. parisii* specimens. The mitochondrial COI gene has been used to discriminate similar species in almost all animal phyla, being a useful marker for a DNA barcoding identification of parasitoids, e.g. *Encarsia* sp. (Hymenoptera: Aphelinidae, Monti et al., 2005) and pests e.g. *Anastrepha* sp. (Lopes et al., 2013; Bomfim et al., 2011). Further studies that include more samples of *S.*

parisii are necessary to better understand its genetic diversity and routes of dispersion in Brazil and Paraguay.

Outbreaks of *M. sojae* have been observed in some soybean areas of southern Brazil, Paraguay and recently in Bolivia. Nevertheless, the large number of parasitized pupae, associated with the low adult emergence rate found in the plants examined in this study, indicate that biological control has collaborated to avoid widespread outbreaks of *M. sojae* in soybean fields.

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