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Cotesia flavipes (Hymenoptera: Braconidae) as a biological control agent of sugarcane stem borers in Colombia's Cauca River Valley

Viviana M. Aya[1], Claudia Echeverri[1], Gloria P. Barrera[2], and German Vargas[1]*

Stem borers, *Diatraea* spp. (Lepidoptera: Crambidae), are considered among the most important pests affecting sugarcane crops in Colombia. In the Cauca River Valley, the country's main sugarcane producing area, pest damage represents a reduction of 0.83% in cane weight per each percentage unit of bored internodes, and a further 0.26% reduction of sugar yield at the sugarcane mill. Considering the mean yield in the region of 120 tons per hectare and a sucrose content of 11%, loss is estimated at approximately 144 kg of sugar per hectare with each percentage unit of internodes bored (Vargas et al. 2015). In the Western Hemisphere, Solis & Metz (2016) reported 41 species of *Diatraea*, of which at least 5 are found in Colombia: *D. busckella* Dyar & Heinrich, *D. indigenella* Dyar & Heinrich, *D. lineolata* Walker, *D. saccharalis* (F.), and *D. tabernella* Dyar.

Diatraea saccharalis and *D. indigenella* had already been reported in the Cauca River Valley, when in 2013 Vargas et al. (2013) reported a pest outbreak in the northern portion of the Valley associated with *D. tabernella*. In 2014, *D. busckella* was detected in the central region of the Cauca River Valley (Vargas unpublished), increasing the pest complex in the Cauca River Valley to 4 species. Vargas et al. (2013) also noted that the incidence of larval parasitism by *Lydella minense* (Townsend) (Diptera: Tachinidae), the main larval parasitoid used in the Cauca River Valley, was relatively low in *D. tabernella* as compared with *D. saccharalis*, suggesting the need to explore new biological control options.

One option was the larval parasitoid *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), which has been introduced in approximately 40 countries since 1950 to control stem borers in different crops (Muirhead et al. 2006). In Asia and Africa, *C. flavipes* was introduced mainly to control *Chilo* spp. (Lepidoptera: Crambidae) in sorghum (*Sorghum bicolor* [Poaceae]) and corn (*Zea mays* [Poaceae]) (Muirhead et al. 2006) and, in the Americas, its introduction targeted the sugarcane borer *D. saccharalis* (Potting et al. 1997) and was found to regulate the borer in the Caribbean, Central and South America, and southern United States (Gifford & Mann 1967; Badilla 2002; White et al. 2004).

In 1978, *C. flavipes* was introduced from Pakistan and India to Brazil, where tachinid flies were being used to manage stem borers in sugarcane without much success. The parasitoid was successfully established and now constitutes the main biological control strategy to manage stem borers in sugarcane (Botelho 1992; Postali et al. 2010). In Colombia, massive releases of *C. flavipes* were carried out between 1975 and 1982 in the Cauca River Valley and the department of Santander, in western Colombia. The parasitoid did not establish in the Cauca River Valley, but did establish in Santander, with no clear indication of why (Gaviria 1990). Efforts also were simultaneously carried out in the Cauca River Valley to introduce tachinid flies, such as *L. minense*, which established successfully on *D. saccharalis* and helped regulate this pest (Vargas et al. 2015). As a result, no further attempts were made to establish *C. flavipes* in the Cauca River Valley, explaining why there were no records of its presence in the region (Vargas et al. 2013).

However, due to recent pest outbreaks of stem borers in sugarcane crops, especially in the northern Cauca River Valley (Vargas et al. 2013; Vargas et al. 2015), the use of *C. flavipes* as a potential alternative to control the *Diatraea* complex in the region was reconsidered. Commercial releases of the parasitoid began in late 2014 and, almost immediately, there was evidence of its establishment. Parasitized stem borer larvae were detected, even in fields where no releases had been made, causing confusion as to whether the parasitism could be indeed attributed to recent releases of *C. flavipes*. *Cotesia flavipes* is a species complex formed by four species: *C. flavipes*, from the Indo-Asia region, *C. sesamiae* (Cameron) from Africa, *C. chilonis* (Matsumara) from China and Japan, and *C. nonagriae* (Oliff) from Australia and Papua New Guinea (Muirhead et al. 2012). Although morphological differentiation is possible based mainly on male genitalia, intraspecific variation can make this difficult (Muirhead et al. 2008) and, as a result, the recommendation is to compare DNA sequences.

To confirm the identity of the parasitoids found in the study region, 26 samples were collected from sugarcane fields of 7 different sugarcane mills located throughout the Cauca River Valley region. One sample also was collected in the municipality of Suaita in Santander. Samples also were taken from 3 commercial insectaries located in the Cauca River Valley (Biocol, Bioagro, and Corpoama). The DNA was extracted using complete bodies of 5 *Cotesia* specimens per sample. Bodies were macerated using two 3 mm diameter steel balls, generating a mechanical force of 30 Hz per s for 3 m (Retsch® MM400, Haan, Germany), and then suspended in 500 µl buffer Tris/HCl (25 mM Tris, 100 mM NaCl, 2 mM EDTA, pH 8.0) and again macerated for 3 m. Next, 20 µl SDS (10% w per v in water) were added, steel balls discarded, and 50 µl proteinase K (10 mg per ml) added, followed by incubation for 1 h at 56 °C and centrifugation at 8,000 rpm for 5 m. The pellet was discarded after centrifugation, and the supernatant was treated with 1 volume phenol-chloroform and centrifuged at 14,000 rpm for 5 m. The resulting supernatant was recovered and extracted again with 1 volume chloroform-isoamyl alcohol (24:1) and centrifuged at 14,000 rpm for 10 m. The DNA was precipitated adding 2 µl sodium acetate

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Fig. 1. Neighbor-joining tree generated under the Kimura 2-parameter (K2P) nucleotide substitution model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches. Abbreviations for sugarcane mills in Colombia's Cauca River Valley are as follows: Manuelita (MN), Mayagüez (MY), Pichichí (PC), Providencia (PV), Riopaila (RP), Risaralda (RS), Sancarlos (SC). GeneBank *C. flavipes* accessions: Uganda - JQ396735.1, Brazil - DQ232320.1, India - DQ232336.1, Kenya - DQ232317, Thailand - DQ232340.1, USA - DQ232330.1, South Pakistan - JQ396714.1, Jamaica - DQ232321.1, Pakistan - DQ232335.1, Sri Lanka - DQ232327.1, Indonesia - DQ232337.1, Mauritius - DQ232319.1, Reunion - DQ232329.1, Papua New Guinea - DQ232316.1.

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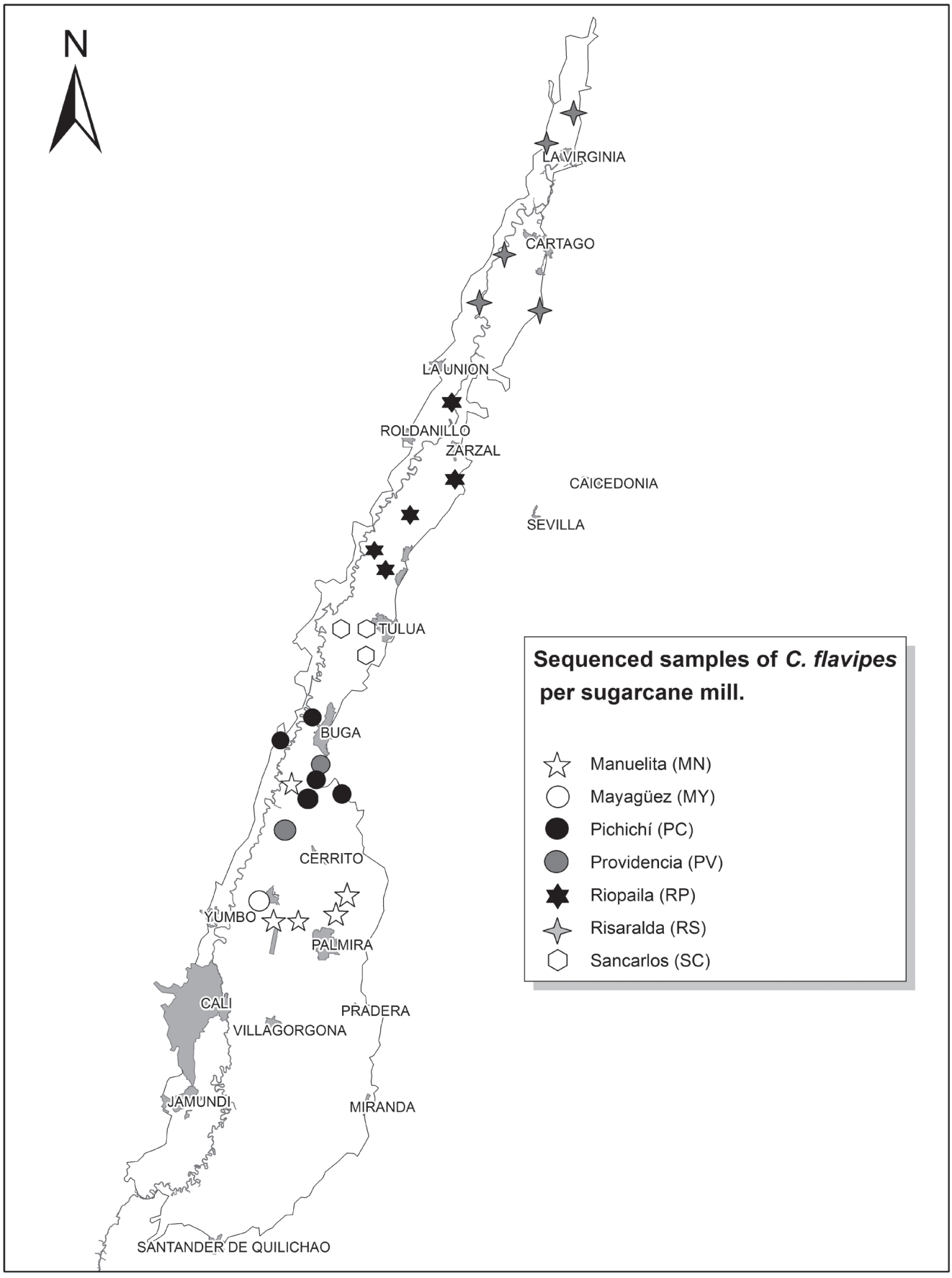

Fig. 2. Distribution of *Cotesia flavipes* in different sugarcane mills of Colombia's Cauca River Valley.

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(3M, pH 5.2) and 500 µl absolute alcohol. The resulting pellet was suspended in 50 µl ultrapure water, treated with 2 µl RNAse (10 mg per ml), and incubated at 37 °C for 1 h. In vitro amplifications of a fragment of the cytochrome oxidase subunit I (*COI*) mitochondrial gene were carried out using the universal primers previously described by Folmer et al. (1994): LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3').

The polymerase chain reaction (PCR) was performed under standard conditions using Go Taq® Master Mix (Promega, Madison, Wisconsin, USA) and a BIO-RAD S1000™ Thermal Cycler. The PCR products were sequenced using amplification primers (Macrogen, Rockville, Maryland, USA). Thermal cycling was performed as follows: 1 cycle at 95 °C for 5 m; 35 cycles at 95 °C for 45 s; 50 °C for 45 s; and 72 °C for 30 s; and one cycle at 72 °C for 3 m. Sequences were manually curated and used to infer phylogeny and evolutionary distances by the neighbor-joining method MEGA version 7 (Kumar et al. 2016), with the following set of parameters: bootstrap with 1,000 replicates; model = Kimura 2-parameters; substitutions to include = D: transitions + transversions; rates among sites = uniform; patterns among sites = same (homogeneous); and gaps per missing data = pairwise deletion. Genbank sequences of *Cotesia glomerata* accession JQ396719.1 and *Cotesia koebelei* accession AY333888.1 were included as outgroups.

Species-level diagnoses can be routinely obtained by *COI* analysis of *Cotesia* species (Muirhead et al. 2006; Muirhead et al. 2012; Kaiser et al. 2015). A total of 684 base pairs (bp) of *COI* mtDNA genes were obtained for the 30 samples analyzed in this study. Sequence comparisons carried out with 11 species of *Cotesia* yielded an identity percentage of 98% of the samples tested with those of *C. flavipes* registered in the GenBank (Fig. 1). An estimate of genetic divergence between the sequences showed no differences among analyzed samples (field samples collected in the Cauca River Valley, field sample from Santander, and samples from commercial insectaries), with genetic distances ranging from 0.00 to 0.03. The resulting phylogenetic tree shows a large branch containing the 30 analyzed samples, together with *C. flavipes* from India, Brazil, Jamaica, Kenya, and Pakistan (Fig. 1), thus corroborating not only the origin of the introductions of *C. flavipes* to the Americas from India and Pakistan through the Caribbean islands and then to countries such as the United States, Brazil, and Colombia (Potting et al. 1997), but also the great proximity between *C. flavipes* and *C. nonagriae*, considered by Muirhead et al. (2008) as sister species within the complex.

Vargas et al. (2013) had recommended the release of *C. flavipes* in the Cauca River Valley, and commercial releases of the parasitoid began in northern Cauca River Valley in late 2014. This study revealed that *C. flavipes* was attacking *Diatraea* larvae from the northern part of the region (municipality of La Virginia, department of Risaralda) southward to central Valle del Cauca in the municipality of Palmira, covering a straight line distance of 152 km between each another (Fig. 2) and evidencing a rapid, extensive, and progressive dispersion of the parasitoid from northern Cauca River Valley. According to Sallam et al. (2001), the dispersal of *C. flavipes* could be as far as 64 m per generation depending on wind flow. The rapid dispersal could have been enhanced by the anthropogenic movement of vegetative material with parasitized larvae in harvested stalks and seedlings between several points. Of the 26 parasitoid samples collected in Cauca River Valley fields, 5 came from a non-identified species of *Diatraea*, 3 from *D. saccharalis*, 9 from *D. busckella*, and 9 from *D. tabernella*, whereas the sample from Suaita came from *D. busckella*. These results suggest that the parasitoid distribution in the Cauca River Valley may be associated with that of the two new *Diatraea* species in the region, *D. busckella* and *D. tabernella*. Further research is needed to assess the potential use of this parasitoid to control other stem borer species attacking sugarcane in Colombia.

Summary

Releases of the parasitoid *Cotesia flavipes* have been carried out since late 2014 in Colombia's Cauca River Valley to complement other biological control on sugarcane stem borers (*Diatraea* spp.). To confirm the identity of the species being released as well as those recovered in the field, including samples from the department of Santander in western Colombia, specimens were examined using a fragment of cytocrome oxydase I (*COI*). Results confirmed that the genetic identity of the specimens sequenced corresponded to *C. flavipes*, with a genetic divergence of 0.00 to 0.03 as compared with GenBank registers. In the Cauca River Valley, parasitoid distribution currently extends from La Virginia (Risaralda) to Palmira (Valle del Cauca), and is mainly associated to the geographical distribution of the new *Diatraea* species reported in the region, *D. busckella* and *D. tabernella*.

Key Words: *Diatraea tabernella*; *Diatraea busckella*; *Diatraea saccharalis*; *Diatraea indigenella*; *Lydella minense*; Cytochrome oxidase I

Sumario

Desde finales del año 2014 se ha venido liberando al parasitoide *Cotesia flavipes* en el valle del río Cauca de Colombia para complementar otros esfuerzos de control biológico de los barrenadores del tallo de la caña de azúcar (*Diatraea* spp.). Para confirmar la identidad tanto de los parasitoides que se han venido liberando como de aquellos recuperados en campo, incluso de aquellos provenientes del departamento de Santander al oeste de Colombia, se analizaron especímenes utilizando un fragmento de la citocromo oxidasa I (*COI*). Se confirmó que la identidad genética de los especímenes secuenciados corresponde a *C. flavipes*, con un estimativo de divergencia genética de 0.00 y 0.03 en comparación con los registros Genbank. En el valle del río Cauca la distribución del parasitoide se extiende actualmente entre La Virginia (Risaralda) y Palmira (Valle del Cauca), y es asociada principalmente a la distribución de las nuevas especies de *Diatraea* reportadas en la región, *D. busckella* y *D. tabernella*.

Palabras Clave: *Diatraea tabernella*; *Diatraea busckella*; *Diatraea saccharalis*; *Diatraea indigenella*; *Lydella minense*; Citocromo oxidasa I

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