



## RESEARCH ARTICLE

## Trophic networks associated with the corn leaf aphid, *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) in a cornfield, Manabí, Ecuador

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### Abstract

The corn leaf aphid is considered an important pest associated with maize. This study aimed to discover the trophic associations around *Rhopalosiphum maidis* in Manabí, Ecuador. Maize leaves were sampled to determine the numbers of parasitized aphids, and the identities of predators and parasitoids. Nine taxa of natural enemies were detected: the primary parasitoid was *Lysiphlebus testaceipes* Cresson, 1880 (Hymenoptera: Braconidae); the hyperparasitoid *Syrphophagus aphidivorus* (Mayr, 1876) (Hymenoptera: Encyrtidae); the predatory hoverfly *Ocyrtamus dimidiatus* (Fabricius, 1781) (Diptera: Syrphidae), four species of coccinellids, *Cheilomenes sexmaculata* (Fabricius, 1781), *Cycloneda sanguinea* (Linnaeus, 1763), *Hippodamia convergens* Guerin-Meneville, 1842 and *Paraneda pallidula guticollis* (Coleoptera: Coccinellidae) and an assassin bug, *Zelus* sp. (Hemiptera: Reduviidae). A parasitoid, *Pachyneuron formosum* Walker, 1833 (Hymenoptera: Pteromalidae) emerged from hoverfly pupae. This study reports the presence of the parasitoids *S. aphidivorus* and *P. formosum* in Ecuador for the first time. These results increase the knowledge of a four-trophic level relationship (host plant – pest – parasitoids, predators – hyperparasitoids) in a maize agroecosystem as a fundamental basis for biological control programs.

**Keywords:** biological control; predation; hyperparasitism; maize; parasitoids; biocontrol.

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## 1. Introduction

Maize, *Zea mays* L. (Poaceae), is a crop widely sown in the world since it can adapt to various agroecological conditions (Suganya et al., 2020). In Ecuador, 1,572,943 tons of maize are produced annually, from 328,214 ha harvested, and it is the second most important crop after cacao (MAG, 2020). Throughout its phenological cycle, maize can be attacked by approximately 250 species of insects and mites worldwide (Singh & Singh, 2018), of which in Ecuador the following are considered of economic importance, the fall armyworm, *Spodoptera* spp., the corn earworm, *Heliothis zea* (Boddie, 1850) (Lepidoptera: Noctuidae), the stem borer, *Diatraea* spp. (Lepidoptera: Crambidae), thrips (*Frankliniella* spp.) (Thysanoptera: Thripidae), the leafhopper, *Dalbulus maidis* (DeLong, 1923) (Hemiptera: Cicadellidae) and the corn leaf aphid, *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) that can severely limit production (Caviedes et al., 2022; Quito-Avila et al., 2016).

The corn leaf aphid, *R. maidis* stands out as a pest because it can cause damage in the different growth stages of corn, but mainly in the vegetative phase, in which the nymphs and adults feed on all parts of the plant. In the reproductive phase, this aphid interferes with pollination and introduces fungi to the cob (Alam et al., 2020). In addition, to the direct damage caused by sucking photoassimilates from the plant, the aphid is also a vector of some viruses that negatively impact production, namely, *Maize yellow dwarf virus* (MDMV), *Barley yellow dwarf virus* (BYDV), *Sugarcane mosaic virus* (SCMV), and *Cucumber mosaic virus* (CMV) (Chen et al., 2019; Quito-Avila et al., 2016).

As a fundamental basis for the management of phytosanitary problems of maize, it is necessary to review its ecological bases as an agroecosystem. Consumers turn to plant crops (first trophic level) as synthesizers of organic matter to obtain vital resources that they cannot synthesize, generating consumption chains. The second

trophic level is made up of herbivores or phytophagous organisms (some with pest status), the third by primary carnivores (parasitoids and predators), which are interconnected by sharing functions in one of their links (e.g., a predator consuming in two or more food chains), thus forming complex networks, structurally and functionally. Additionally, hyperparasitism is involved in the fourth trophic level as a highly evolved relationship that exists between entomophages (Kos et al., 2012).

Aphids constitute one of the most important groups of pests in agriculture, causing economic damage to many crops worldwide (Van Emden & Harrington, 2007). Aphidophagous braconids (Hymenoptera: Braconidae) play an important role in population regulation and can attack more than one aphid-host species (Kos et al., 2012). On the other hand, hyperparasitoids have a great impact on the dynamics of arthropod communities, and their effect on biological control programs remains uncertain (Kos et al., 2012; Sullivan & Völkl, 1999). Likewise, predators represent natural biological control agents of aphids, among them hoverflies (Diptera: Syrphidae) and coccinellids (Coleoptera: Coccinellidae) (Amorós-Jiménez & Marcos-García, 2020; Bouvet et al., 2021). Despite the importance of *R. maidis* as a corn pest in Ecuador, little is known about the multitrophic associations that are entangled around this pest. Given the relevance of natural enemies in the design of integrated pest management (IPM) strategies, as well as for the establishment of applied biological control programs, this study aimed to determine the insect species that make up the food webs

associated with the corn leaf aphid, *R. maidis* in a cornfield in the province of Manabí, Ecuador.

## 2. Materials and methods

This study was carried out during the year 2021 in the town of Charapotó, Manabí province, Ecuador (00°49'15"S, 80°30'18"W, 30 m a.s.l., Figure 1). The climatic conditions correspond to a tropical dry forest: average annual temperature: 25 °C (range: 24.74 °C to 30.9 °C), relative humidity: 72%, average annual rainfall: 550 mm per year (INAMHI, 2021). A 1000 m<sup>2</sup> corn plot of the INIAP 583® variety was established, planted at a distance of 0.20 m between plants and 0.80 m between rows. Within the lot, 60 rows which were not treated with chemical insecticides were sampled weekly, from which 36 leaves on 18 plants were randomly selected and taken to the Entomology Laboratory, Faculty of Agricultural Engineering, Technical University of Manabí for examination.

The samples were observed under a Carl-Zeiss® stereoscope (magnification: 18–40X) and the numbers of live and parasitized aphids were counted. Parasitized aphids were recognized by their inflated integument and brown coloration (mummies). Mummies were placed individually in transparent gelatin capsules to the emergence of adult parasitoids and allow later identification.

Larvae of hoverflies (Diptera: Syrphidae) preying on *R. maidis* were observed on the leaves and were reared to adults for identification. Small wasps emerged from hoverfly pupae, which were identified using dichotomous keys of Narendran et al. (2007) and Xiao et al. (2009).

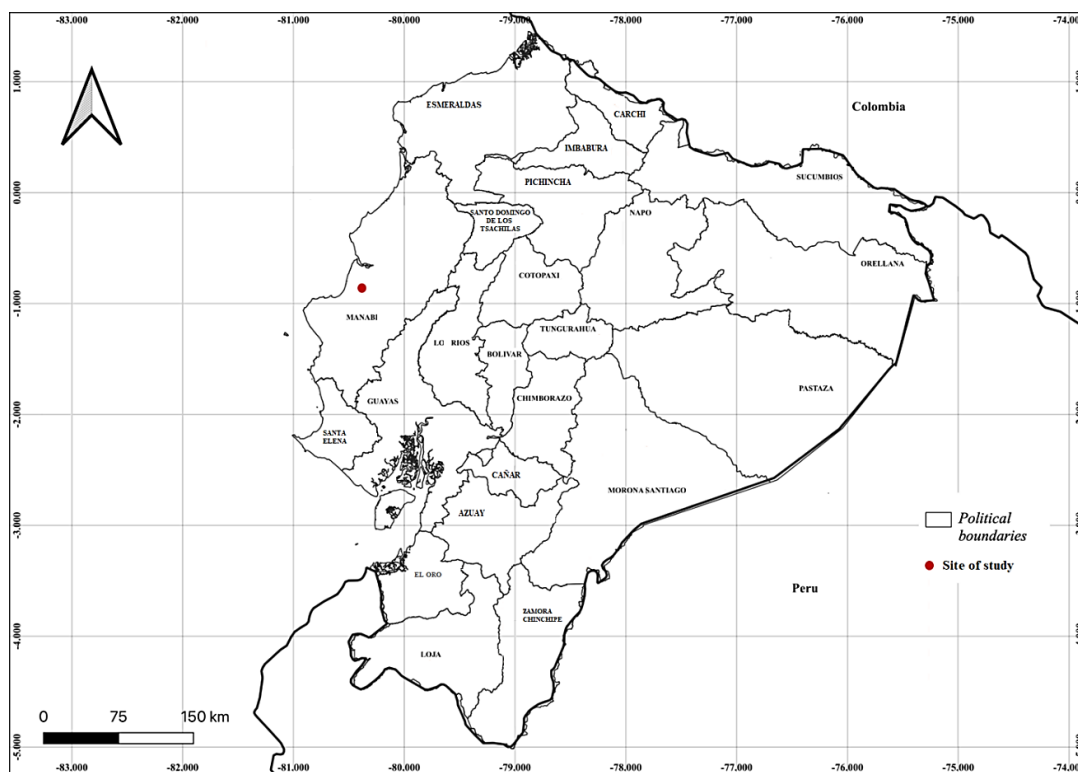


Figure 1. Map of Ecuador illustrating the area of study (red dot).

Individuals of lady beetles (Coleoptera: Coccinellidae) preying on *R. maidis* were observed in the field, and were collected into flasks containing 70% ethyl alcohol and the species were identified by comparison with voucher specimens collected in previous studies carried out in the same province (Bailon et al., 2022).

Hoverfly adults were identified with the keys of Marinoni et al. (2007) and Mengual et al. (2018). The hymenopteran parasitoids emerged from the parasitized aphids were identified using the key provided by Zamora-Mejías and Hanson (2017). Trophic interactions between detected taxa were also plotted. All identifications were made in the Entomology Laboratory, Faculty of Agricultural Engineering, Technical University of Manabí, where 180 voucher specimens (phytophagous and natural enemies) were deposited.

### 3. Results and discussion

#### Trophic networks

Nine taxa of natural enemies were detected associated with *R. maidis* and their interconnections are shown in Figure 2. From the 775 parasitized aphids emerged the primary parasitoid, *Lysiphlebus testaceipes* Cresson, 1880 (Hymenoptera: Braconidae) and the hyperparasitoid, *Syrphophagus aphidivorus* (Mayr, 1876) (Hymenoptera: Encyrtidae) (Figure 3).

The larvae of a hoverfly species preying on *R. maidis* was identified as *Ocyptamus dimidiatus* (Fabricius, 1781) (Diptera: Syrphidae) and the parasitic wasps that emerged from the pupae of *O. dimidiatus* were identified as

*Pachyneuron formosum* Walker, 1833 (Hymenoptera: Pteromalidae) (Figure 3). An undetermined species of assassin bug, *Zelus* sp. (Hemiptera: Reduviidae) was found preying on *R. maidis*. Furthermore, the following lady beetles (Coleoptera: Coccinellidae) were also found feeding on *R. maidis*: *Cheilomenes sexmaculata* (Fabricius, 1781), *Cycloneda sanguinea* (Linnaeus, 1763), *Hippodamia convergens* Guerin-Meneville, 1842, and *Paraneda pallidula guticollis* (Mulsant, 1850). Four trophic levels around *R. maidis* are entangled (Figure 2), with maize as the host plant at the first trophic level and the pest at the second trophic level. The third trophic level showed the highest number of taxa, one primary parasitoid and six predators. Interestingly, the assassin bug, *Zelus* sp., in addition to preying on *R. maidis* attacked also coccinellids, thus consuming at two levels. Finally, in the fourth trophic level, hyperparasitoids associated with the primary parasitoid of *R. maidis*, as well as the parasitoid of the predatory hoverfly were detected.

#### Species detected for the first time in Ecuador

*Syrphophagus aphidivorus* (Mayr, 1876) (Hymenoptera: Encyrtidae) (Adapted from lemma et al. (2017) and Zamora-Mejías & Hanson (2017)).

**Diagnosis. Female:** Length 1.0 – 1.5 mm, bluish-green, bronze body (Figure 3a). Geniculate antennae with the scape longer than other segments (Figure 3b). Forewing with small pterostigma, very short marginal vein and dark brown venation (Figure 3c), axillae almost touching in the middle region of the mesoscutellum. Median tibia with a large spur at the apex (Figure 3d).

#### Trophic Levels

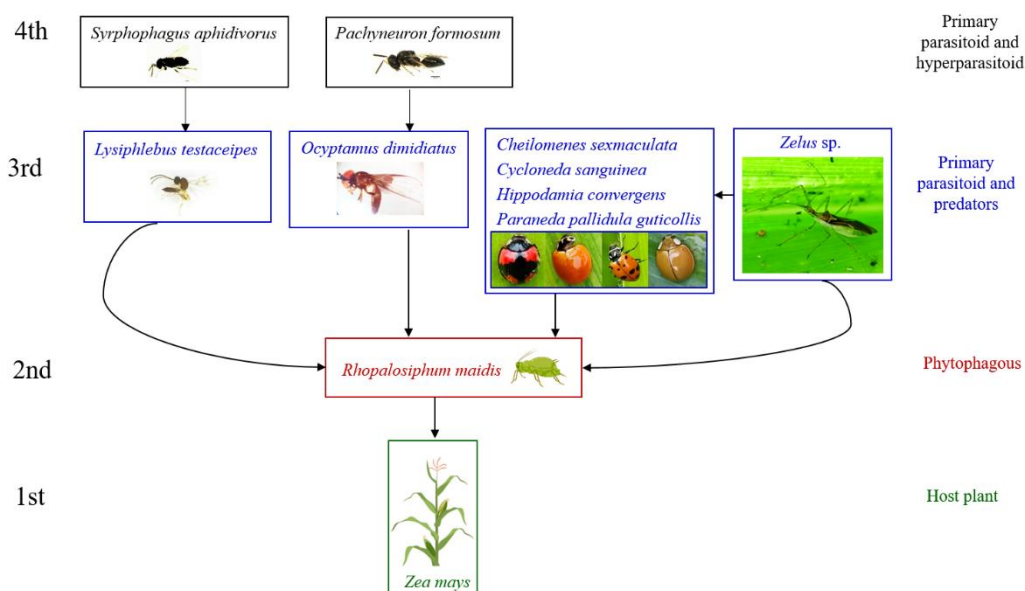
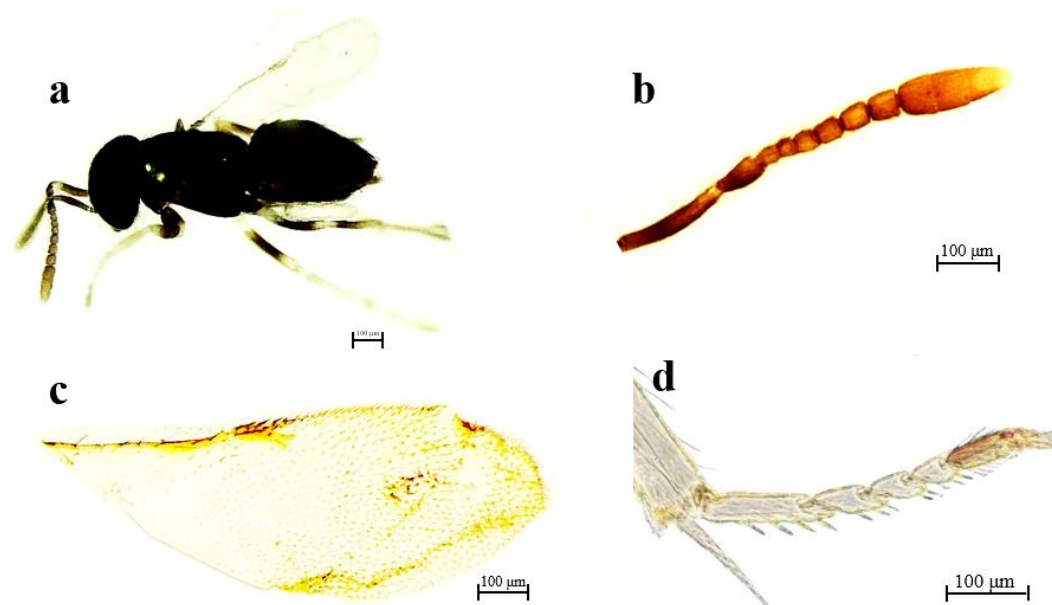


Figure 2. Natural enemies associated with *Rhopalosiphum maidis* on maize. The black arrows show the interconnections in the agroecosystem. Herbivore (aphid pest) in red, primary carnivores in blue and secondary carnivores in black. The pictures of maize plant and aphid were created in Biorender.



**Figure 3.** *Syrphophagus aphidivorus* (Mayr, 1876): (a) Habitus of female, lateral view; (b) Antenna; (c) Wing; (d) Mesothoracic tibia showing a long thick spur.

*Pachyneuron formosum* Walker, 1833 (Hymenoptera: Pteromalidae) (Adapted from Xiao et al. (2009)).

**Diagnosis. Female:** Length ca. 2.0 mm, body green with metallic reflections; antenna brown except scape and pedicel yellowish brown; leg yellow or brown except coxa which is similar to body color; gaster brown (Figure 4a and 4b). Antennal formula 1-1-2-6-3 (Figure 4c); scape reaching anterior ocellus; funicular segments 1.5X as long as wide (Figure 4c). Head broader than thorax dorsally (Figure 4b). Marginal vein 4X times longer than maximum width, marginal vein longer than stigma (1.2X). Gaster wider than thorax, 1.2X as long as wide, with reticulate petiole longer than wide.

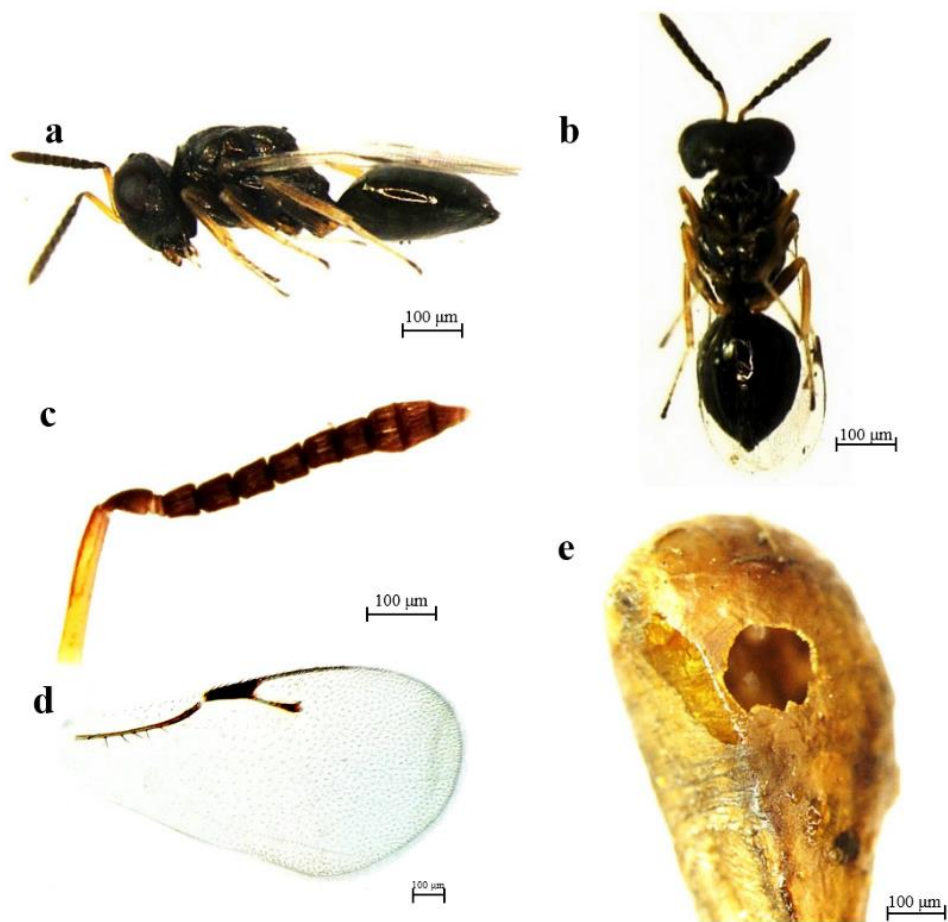
The natural enemies observed constitute important biological control agents associated with the corn leaf aphid. *Lysiphlebus testaceipes* belongs to the subfamily Aphidiinae (Hymenoptera: Braconidae) that includes solitary endoparasitoids, koinobionts, exclusive to adult and immature aphids (Zamora-Mejías & Hanson 2017). This parasitoid in Ecuador had not been reported associated with *R. maidis* in maize. In fact, hitherto, *L. testaceipes* had been reported in Ecuador as a parasitoid associated with the species *Aphis gossypii* Glover, 1877 and *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) in cotton, melon, pepper and tobacco crops (Chirinos et al., 2021).

From a high percentage (38.2%) of the reared aphid mummies, the hyperparasitoid *S. aphidivorus* emerged, which translates to an adult emerging from each mummy. This is the first record of the species for Ecuador. lemma et al. (2017) reported that *S. aphidivorus* is a secondary endoparasitoid of other hymenopteran parasitoids, in which hyperparasitoid females have two oviposition strategies: ovipositing in live parasitized aphids, as well as in mummified aphids. Both strategies aim to attack the larvae of primary parasitoids (lemma et al., 2017).

The species *L. testaceipes* and *S. aphidivorus* have been found in other field studies in different geographical

regions. Studies carried out by Villa-Ayala et al. (2020) detected *L. testaceipes* as a primary parasitoid of the yellow aphid, *Melanaphis sacchari* (Zehntner, 1897), on sorghum crop in the state of Morelos, Mexico, and among the hyperparasitoids they observed *S. aphidivorus*. Research carried out by lemma et al. (2017) on alfalfa crop in the state of São Paulo, Brazil, reported *S. aphidivorus*, as a secondary parasitoid of *Aphidius ervi* (Haliday, 1834) (Hymenoptera: Braconidae). Zamora-Mejías & Hanson (2017) sampled plants infested with aphids in various regions of Costa Rica and detected nine species of primary parasitoids, including *L. testaceipes* and hyperparasitoids, including *S. aphidivorus*.

The species of predatory coccinellids and syrphids found in this study had been previously reported for Ecuador. Bailon et al. (2022) in an inventory carried out in three geographical areas of the province of Manabí detected 13 taxa of coccinellids associated with aphids in corn, with *Ch. sexmaculata* being the most abundant species followed by *C. sanguinea* and *H. convergens*. The hoverfly, *O. dimidiatus* was listed by Marín-Armijos et al. (2017) as part of the syrphid fauna of Ecuador. On the other hand, Fortoul-Díaz et al. (2020) carried out a field study on sorghum in the state of Puebla, Mexico and identified among the predators associated with *M. sacchari*, the coccinellids *H. convergens* and *C. sanguinea*, followed in abundance by various species the hoverflies. Maza et al. (2022) recorded for the first time three species of hoverflies, *O. dimidiatus*, *Ocyrtamus gastrostactus* (Wiedemann, 1830) and *Toxomerus watsoni* (Curran, 1930), whose larvae were found feeding on whiteflies and aphids on pepper in Tucumán, Argentina. Fidelis et al. (2018) found that the mortality of *M. persicae* was a consequence of the predation exerted by larvae of various species of coccinellids, including *C. sanguinea*, together with the action of hoverflies species.



**Figure 4.** *Pachyneuron formosum* Walker, 1833: a and b, Habitus of female, lateral and ventral view; c, Antenna; d, Wing; e, Pupa of *Ocyptamus dimidiatus* (Fabricius, 1781) showing exit hole perforated by *P. formosum*.

In our study, *P. formosum* was detected for the first-time parasitizing *O. dimidiatus* pupae, with approximately five adults emerging from each pupa. *Pachyneuron* is a cosmopolitan genus as they can be found as hyperparasitoids of Hemiptera (Aphidoidea, Coccoidea and Psylloidea) through Hymenoptera (Ichneumonoidea: Braconidae, Aphidiinae; Chalcidoidea: Encyrtidae, Aphelinidae), or primary and secondary parasitoids of predators of various orders, i.e., Diptera, Coleoptera (Coccinellidae), and Neuroptera (Chrysopidae) (Dzhanokmen, 2009). Previous studies have reported *P. formosum* as a parasitoid of hoverflies in other regions. In Turkey, Tek & Okyar (2018) found individuals of *P. formosum* and individuals of *Euneura lachni* (Ashmead, 1887) (Hymenoptera: Pteromalidae), emerging together from an unidentified hoverfly puparium. Santo et al. (2018) observed and identified parasitized pupae of two syrphid species *Episyrphus balteatus* (De Geer, 1776) and *Sphaerophoria scripta* (Linnaeus, 1758), from which hymenopterans of the Ichneumonidae (Diplazontinae) and Pteromalidae families emerged.

The combined action of parasitoids and predators could contribute to the reduction of population densities of *R. maidis* in maize. The biological control of aphids is exerted by numerous parasitoids belonging to the families

Braconidae, Encyrtidae and other Chalcidoidea (Bañol et al., 2017; Zamora-Mejías & Hanson, 2017) as well as predators of the families Coccinellidae and Syrphidae are present in most ecosystems, both agricultural and urban (Amorós-Jiménez & Marcos-García, 2020; Fortoul-Díaz et al., 2020). Hyperparasitoids have been pointed out for causing adverse effects in classical biological control programs, but hyperparasitoids cannot be eliminated from food webs (Tougeron & Tena, 2019). Consequently, studies on their ecology in different environmental conditions are necessary to estimate the interactions between species and their function in trophic networks (Tougeron & Tena, 2019). This research constitutes a significant contribution to the knowledge of the occurrence of natural enemies in an ecological system surrounding maize crop. The information on the trophic associations around *R. maidis* will contribute to the planning of IPM strategies.

#### 4. Conclusions

Nine taxa of natural enemies associated with *R. maidis* were found in this study, and we record for the first time for Ecuador the species *S. aphidivorus* (Hymenoptera: Encyrtidae) attacking *L. testaceipes* (Hymenoptera:



Braconidae) and *P. formosum* (Hymenoptera: Pteromalidae) parasitizing pupae of the aphid-feeding hoverfly, *O. dimidiatus* (Diptera: Syrphidae). In terms of biological control, these results increase the knowledge of four-trophic interactions between maize plants, aphids, aphidophagous parasitoids, hyperparasitoids, predators consuming in more than one network, and predators and parasitoids interacting within an agroecosystem. Future studies should be conducted to elucidate how tertiary consumers (fourth trophic level), i.e., hyperparasitoids and predators (e.g., *Zelus* sp.), affect the efficacy of secondary consumers (third trophic level), i.e., primary parasitoids and predators.

#### Conflict of interest

The authors express no conflict of interest associated with this work.

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