Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 15 (6): 1083–1091
https://doi.org/10.15560/15.6.1083

PENSOFT

# New records of aphid parasitoids (Hymenoptera) from Colombia

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**Abstract**
Aphid parasitoids have been recorded in many countries around the globe, however records in Colombia are few. Here, five primary parasitoids species, *Aphidius platensis* Brèthes, 1913, *Aphidius funebris* Mackauer, 1961, *Aphidius matricariae* Haliday, 1834, *Aphelinus varipes* (Förster, 1841), *Aphelinus paramali* Zehavi & Rosen, 1989, and two hyperparasitoids species, *Syrphophagus aphidivorus* (Mayr, 1876) and *Pachyneuron aphidis* (Bouché, 1834) are newly recorded in Colombia. Two other primary parasitoids, *Lysiphlebus testaceipes* (Cresson, 1880) and *Aphidius colemani* Viereck, 1912 are newly recorded from the department of Valle del Cauca.

**Key words**
Aphidiinae, Aphelinidae, primary parasitoids, hyperparasitoids, Neotropical Region.

**Academic editor:** Filippo Di Giovanni | Received 25 October 2018 | Accepted 10 May 2019 | Published 6 December 2019

**Citation:** Martínez-Chávez LM, Duque-Gamboa DN, Toro-Perea N (2019) New records of aphid parasitoids (Hymenoptera) from Colombia. Check List 15 (6): 1083–1091. https://doi.org/10.15560/15.6.1083

## Introduction

Members of the subfamily Aphidiinae (Hymenoptera, Braconidae) and some members of the family Aphelinidae are solitary endoparasitoids of aphids (Starý 1968). By being widespread and often quite abundant, they play an important role in aphid population control, including the reduction in number of species that affect crop plants (Godfray 1994). Contrasting this, some members of well-known families of Hymenoptera, like Encyrtidae and Pteromalidae, are hyperparasitoids of aphids and, therefore, are presumed to minimize control by primary parasitoids (Bess and Haramoto 1959, McDonald and Kok 1991), although mixed results have been described (Sullivan and Völkl 1999, Nofemela 2013). Some data on aphid parasitoids of the Neotropical region exist for Mexico, Costa Rica, Venezuela, Brazil, Chile, and Argentina, where species of *Aphidius* Nees, 1819 (Braconidae), *Lysiphlebus* Förster, 1862 (Braconidae), and some lesser-known genera (*Binodoxys* Mackauer, 1960, *Ephedrus* Haliday, 1833, *Xenostigmus* Smith, 1944, *Lipolexis* Förster 1862) have been found (Starý and Remaudiere 1982, Starý and Cermeli 1989, Starý et al. 1993, 2007, Zamoras-Mejías et al. 2010, Andorno et al. 2016). Despite the economic importance of aphids, they remain poorly studied in Colombia, and, as a consequence, studies on their parasitoids are very scarce, with only 3 species recorded in the country, *Aphidius colemani* Viereck, 1912, *Lysiphlebus testaceipes* (Cresson, 1880), and *Praon volucre* (Haliday, 1833) (Zenner de Polanía 1971, Campos 2001, Aragón et al. 2007, León et al. 1999).

Knowledge of aphid parasitoids is important because correct identification of biological control agents (parasitoids and predators) is a key element for successful biological control in agroecosystems (Pons et al. 2011).

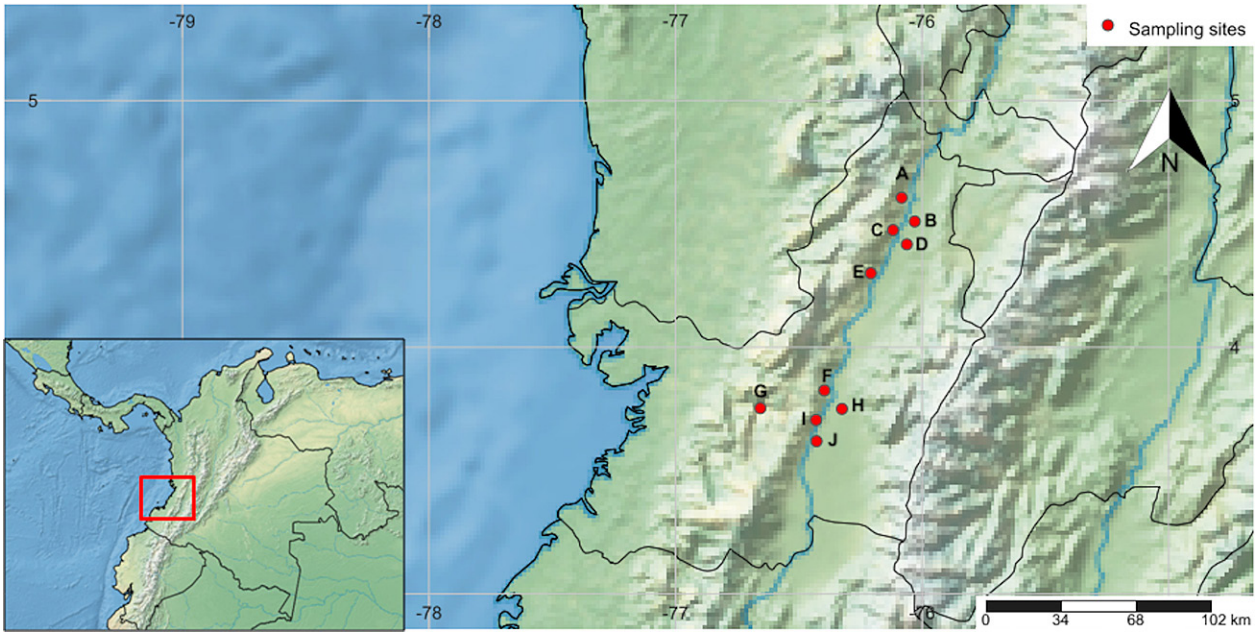



**Figure 1.** Distribution map of the sampling sites and new records localities in Colombia. A: Toro; B: La Victoria; C: Roldanillo; D: Zarzal; E:Bolivar; F: Yotoco; G: Dagua; H: Guacarí; I: Vijes; J: Palmira.

Biological control can be done by either introducing new species or using the species that are already present, but these are possible if the existing parasitoid fauna is unknown (Bortolus 2008, Raworth et al. 2008).

The lack of information and reliable identifications are the most important reasons for the incomplete knowledge of hymenopteran parasitoids in countries such as Colombia. Thus, the objective of our study is to provide an initial survey of the primary aphid parasitoids and hyperparasitoids in one of the most important crops, chili pepper (*Capsicum frutescens* L. and *Capsicum annuum* L.), in Valle del Cauca and to provide morphological and molecular information for their identification. This as a starting point for further research in the country.

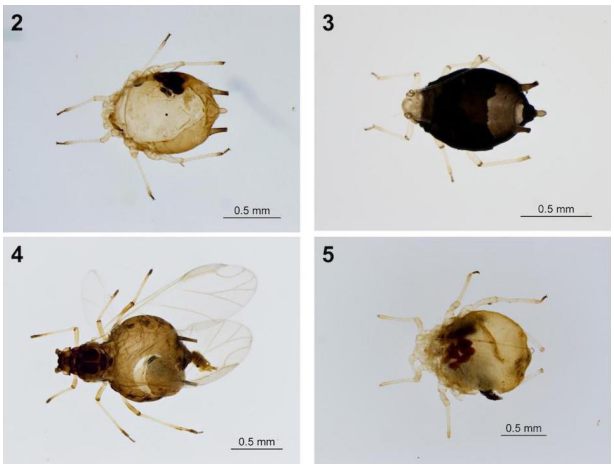
## Methods

We collected 1473 parasitized aphids (mummies) and living aphids with parasitism signs (swollen abdomen, little movement, observation of parasitoid larvae inside) from 10 sampling sites over a year-long period (2016–2017). Geographical coordinates of the sampling sites were taken with a Garmin GPS and using the WGS84 datum (Fig. 1). Rearing conditions were: 12:12 L/S, 75% RH, 25 °C, on a Sanyo MLR-351-351H environmental chamber. Emerged adult parasitoids were preserved in 96% ethanol and kept under 4 °C until morphological identification and DNA extraction process.

The external morphology of each parasitoid was observed and described using a Leica Z24 stereomicroscope and photographs were taken using a Nikon SMZ-1500 stereomicroscope. Species were identified with morphological characteristics using the available keys (Menke and Evenhuis 1991, Huang and Polaszek 2007, Starý et al. 2007, 2010, Rakhshani et al. 2012, 2015, Ferrer-Suay et al. 2013, 2014, Tomanović et al. 2014, Ullah et al. 2015, Kavallieratos et al. 2016, Zamora-Mejías and Hanson 2017). Additionally, comments on identifications were made by Robert Kula (United States Department of Agriculture, USA) and Marcus Sampaio (Universidade Feredal de Uberlândia, Brazil). Voucher specimens were deposited on the Entomological Museum of the Universidad del Valle (MUSENUV), Cali, Colombia, with accession numbers 28960 to 28976.

Available keys (Blackman and Eastop 2000) were used for morphological identification of aphid hosts as *Aphis gossypii* Glover, 1877 or *Myzus persicae* Sulzer, 1776 (Figs 2–5).

A 700-base pair (bp) fragment of the mitochondrial cytochrome-Oxidase subunit I (COI) gene was sequenced for confirmation of taxonomic identification. Polymerase chain reactions (PCR) were carried out using




**Figures 2–5.** Aphid mummies. **2.** *Aphis gossypii* (apterous) attacked by Aphidiinae. **3.** *A. gossypii* (apterous) attacked by Aphelinidae. **4.** *A. gossypii* (alate). **5.** *Myzus persicae* (apterous).



**Figures 6, 7.** Habitus of *Lysiphlebus testaceipes*. **6.** Female. **7.** Male.

the universal primers LCO1490 and HCO2198 (Folmer et al. 1994) in a 25 uL amplification cocktail containing: 2.5 uL Buffer Taq + KCL – $MgCl_2$ at 1X, 2 uL $MgCl_2$ at 1.5 mM, 0.125 uL dNTPs at 0.2 mM, 0.625 uL of each primer at 0.5 mM, 0.2 uL Taq polymerase at 5U/uL, 2 uL extracted DNA at 5 ng/uL and 16.925 uL of MiliQ water. PCRs were performed with an initial denaturation temperature of 95 °C for 5 min, followed by 35 cycles (30 s denaturation at 95 °C, 30 s annealing at 50 °C and extension for 60s at 72 °C) and a final extension at 72 °C for 10 min. Finally, 20 uL of the PCR products were purified and bidirectional sequencing was performed using a specialized provider.

Sequences were edited with Sequencher (4.1) (Gene Codes Corporation 2010), and alignments were generated with MUSCLE using MEGA 7.0.21 (Kumar et al. 2016), with sequences segregated by family (information provided by morphological identification). Unique sequences were deposited in GenBank (Benson et al. 2013) under accession numbers MF807198, MF807199, MF807200, MF807201, MF807202, MF807203, MF807204, MF807205 and MF807206.

An exploratory clustering using Neighbor Joining method and a pair wise matrix of Kimura 2-parameter (K2P) was performed in MEGA 7.0.21 to compare the genetic distances within and among morphologically identified species. Sequences from each species, were compared with the GenBank database with nBLAST tool for molecular confirmation.

## Results

### Primary parasitoids

### Braconidae

#### *Lysiphlebus testaceipes* (Cresson, 1880)

Figures 6, 7

**New records.** Colombia, Valle del Cauca, La Victoria, 17.vi.2017, 04°30′36″N, 076°01′48″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 9 females (MUSENUV: 28960 / GenBank accession number: MF807198). Guacarí, 04.iii.2017, 03°45′01″N, 076°19′30″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 6 males (MUSENUV 28961).

**Identification.** Female: 1.5–1.6 mm. Filiform antennae with 13–15 segments and 3–5 longitudinal placodes. Maxillary palpi 3-segmented and labial palpi 2-segmented. Petiole trapezoidal and smooth. Forewing venation incomplete. Stigma widely triangular and distinctly longer than R1; r&Rs shorter than stigma and R1; M&m-cu incomplete, just visible near r&Rs. Transversal vein between M&m-cu and r&Rs incomplete.

Male: similar to female, but antennae with 14 or 15 segments.

#### *Aphidius colemani* Viereck, 1912

Figure 8

**New records.** Colombia, Valle del Cauca, Bolivar, 22.xi. 2016, 04°17′60″N, 076°12′29″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 124 females (MUSENUV: 28962 / GenBank accession number: MF 807199), 92 males (MUSENUV 28963).

**Identification.** Female: 1.5–1.6 mm. Filiform antennae with 15 or 16 segments and 0–2 longitudinal placodes. Maxillary palpi 4-segmented and labial palpi 2-segmented. Petiole trapezoidal with costate anterolateral area. Forewing venation incomplete, with R1 longer than stigma length; r&Rs same length than stigma but shorter than R1; M&m-cu complete, ending before r&Rs; transversal vein between M & m-cu and r&Rs incomplete. Stigma with elongate triangular shape.

Male: similar to female but filiform antennae with 16 segments.

#### *Aphidius funebris* Mackauer, 1961

Figure 9

**New record.** Colombia, Valle del Cauca, Roldanillo, 09.iv.2017, 04°28′30″N, 076°07′05″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 20 females


**Figures 8–11.** Habitus of *Aphidius*. **8.** *A. colemani*. **9.** *A. funebris*. **10.** *A. matricariae*. **11.** *A. platensis*. All are females.

(MUSENUV: 28964 / GenBank accession number: MF807200), 1 male (MUSENUV 28965).

**Identification.** Female: 2.1–2.2 mm. Filiform antennae with 18 or 19 segments and 0–3 longitudinal placodes. Maxillary palpi 4-segmented and labial palpi 3-segmented. Petiole trapezoidal with finely costulate anterolateral area. Forewing venation incomplete, with R1 with similar length than stigma; r&Rs same length than stigma and R1; M&m-cu complete, ending before r&Rs; transversal vein between M & m-cu and r&Rs almost complete. Stigma elongately triangular.

Male: 1.6–1.7 mm. Similar to female but filiform antennae with 16–18 segments.

### *Aphidius matricariae* Haliday, 1834

Figure 10

**New record.** Colombia, Valle del Cauca, Palmira, 05.v.2017, 03°37′11″N, 076°23′23″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 3 females. (MUSENUV: 28966 (female) / GenBank accession number: MF807201).

**Identification.** Female: 1.8 mm. Filiform antennae with 15 segments and 0–2 longitudinal placodes. Maxillary palpi 3- to 4-segmented and labial palpi 2-segmented. Petiole trapezoidal with costulate anterolateral area. Forewing venation incomplete, with R1 slightly longer than stigma length; r&Rs same length than stigma but shorter than R1; M&m-cu complete, ending before r&Rs; transversal vein between M & m-cu and r&Rs complete. Stigma elongately triangular.

No males found.

### *Aphidius platensis* Brèthes, 1913

Figure 11

**New records.** Colombia, Valle del Cauca, Yotoco, 11.vii. 2017, 03°49′31″N, 076°23′49″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 13 females (MUSENUV: 28967 / GenBank accession number: MF 807202), 2 males (MUSENUV 28968).

**Identification.** Female: 1.75–1.8 mm. Filiform antennae with 14 or 15 segments and 0–2 longitudinal placodes. Maxillary palpi 4-segmented and labial palpi 2-segmented. Petiole almost parallel-sided with costate anterolateral area. Forewing venation incomplete, with R1 approximately ⅓ shorter than the stigma length; r&Rs same length than stigma but a bit longer than R1; M&m-cu complete, ending before r&Rs; transversal vein between M&m-cu and r&Rs incomplete. Stigma elongately triangular.

Male: 2.0 mm. Similar to female but filiform antennae with 14 segments.

**Table 1.** Genetic distances between COI sequences of Aphidiinae (*Lysiphelebus* and *Aphelinus*). Conducted using the K2P model. Genetic distances in % are shown below the diagonal, SE in % above the diagonal.

| | *Lysiphelebus testaceipes* | *Aphelinus colemani* | *Aphelinus funebris* | *Aphelinus matricariae* | *Aphelinus platensis* |
|---|---|---|---|---|---|
| ***Lysiphelebus testaceipes*** | | 1.7 | 1.5 | 1.5 | 1.6 |
| ***Aphelinus colemani*** | 12.2 | | 1.6 | 1.6 | 0.8 |
| ***Aphelinus funebris*** | 10.7 | 10.4 | | 0.9 | 1.5 |
| ***Aphelinus matricariae*** | 10.2 | 11.1 | 4.2 | | 1.5 |
| ***Aphelinus platensis*** | 10.5 | 3.5 | 10.1 | 9.9 | |

**Braconidae molecular confirmation.** Levels of interspecific variation between morphological identified species varied from 3.5 to 12.4% while levels of intraspecific variation ranged from 0 to 0.2% (Table 1). Morphological identifications were congruent with molecular information, since the sequences, once blasted, recovered 99–100% similarity with the ones already present in NCBI.

Aphelinidae

***Aphelinus varipes* (Förster, 1841)**
Figure 12

**New records.** Colombia, Valle del Cauca, Zarzal, 07.viii.2017, 04°25′02″N, 076°03′44″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 3 specimens (MUSENUV: 28969 / GenBank accession number: MF 807203).

**Identification.** 0.6–0.7 mm. Head and thorax black, abdomen almost brown with the last 2 terguites darker. Legs yellow, with infuscate coxae, hind tibia and last tarsal segment. Costal cell of forewings with 2 or more complete row of hairs. Antennae light-yellow, with third funicular segment subquadrate and club 1-segmented. Mouth parts yellow.

***Aphelinus paramali* Zehavi & Rosen, 1989**
Figure 13

**New record.** Colombia, Valle del Cauca, Vijes, 22.iii.2017, 03°42′18″N, 076°25′48″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 1 specimen (MUSENUV: 28970 / GenBank accession number: MF 807204).

**Identification.** 0.75–0.95 mm. Head and thorax black, abdomen black with only the first terguite dark yellow. Legs yellow, with dark coxae and hind tibia. Costal cell of forewings with only 1 row of hairs. Antennae dark yellow, with first and second funicular segments subquadrate, third funicular segment slightly longer than wide and club 1-segmented. Mouth parts black.




**Figures 12–14.** Habitus of Aphelinidae. **12.** *Aphelinus varipes*. **13.** *Aphelinus paramali*. **14.** *Encarsia* sp.

***Encarsia* sp.**
Figure 14

**New record.** Colombia, Valle del Cauca, Vijes, 22.iii. 2017, 03°42′18″N, 076°25′48″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 1 specimen (MUSENUV: 28971 / GenBank accession number: MF 807205).

**Identification.** 0.4–0.5 mm. Body entirely yellow, with the first and the last abdominal segment darker. Antennae light yellow, with 3 funicular segments and a 3-segmented club. Legs light yellow, middle leg with 5 tarsal




**Figures 15–17.** Habitus of hyperparasitoids. **15.** *Syrphophagus aphidivorus*. **16.** *Pachyneuron aphidis* **17.** *Cerapteroceroides* sp.

segments. Forewing hyaline, infuscated below marginal vein and with an asetose area around stigmal vein. Mouth parts yellow.

**Aphelinidae molecular confirmation.** Levels of interspecific variation between morphological identified species varied from 7.9 to 17.3% (Table 2), and we found intraspecific variation for *Aphelinus varipes* but none for *Aphelinus paramali*. Morphological identifications were congruent with molecular information, since the sequences, once blasted, recovered 99-100% similarity with the ones already present in NCBI. In the particular case of *Encarsia* sp. we could not found sequences with more than 89% of identity, morphological identification was more informative in this case.

**Table 2.** Genetic distances between COI sequences of Aphelinidae. Conducted using the K2P model. Genetic distances in % are shown below the diagonal, SE in % above the diagonal.

| | *Encarsia* sp. | *Aphelinus varipes* | *Aphelinus paramali* |
|---|---|---|---|
| ***Encarsia* sp.** | | 1.7 | 1.8 |
| ***Aphelinus varipes*** | 14.7 | | 1.1 |
| ***Aphelinus paramali*** | 17.3 | 7.9 | |

Hyperparasitoids

Encyrtidae

***Syrphophagus aphidivorus* (Mayr, 1876)**
Figure 15

**New records.** Colombia, Valle del Cauca, Dagua, 27. ii.2017, 03°45′11″N, 076°39′22″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 1 specimen (MUSENUV 28973).

**Identification.** 1.1–1.5 mm. Body with a metallic green color. Mouth parts black. Antennae yellow, with 6 funicular segments and 3-segmented club. Forewing longer than body, with a very long submarginal vein, marginal vein enlarged but short and an enlarged stigmal vein. Stigma and post-marginal vein short. Second leg with long, thick tibial spur.

***Cerapteroceroides* sp.**
Figure 16

**New record.** Colombia, Valle del Cauca, Vijes, 22.iii.2017, 03°42′18″N, 076°25′48″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 1 specimen (MUSENUV 28974).

**Identification.** 0.5–0.6 mm. Body entirely metallic blue. Mouth parts yellow. Antennae black and segments very close, giving the appearance like a single unit; scape the longest segment; funicular segments 6, very close to each other; club 3-segmented. Legs with coxa, femur and anterior part of tibia brownish. Wings typically infuscate.

Pteromalidae

***Pachyneuron aphidis* (Bouché, 1834)**
Figure 17

**New records.** Colombia, Valle del Cauca, Palmira, 05. v.2017, 03°37′11″N, 076°23′23″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 3 specimens. (MUSENUV 28972 / GenBank accession number MF 807206).

**Identification.** 0.8–1.5 mm. Body entirely metallic blue. Mouth parts yellow. Antennae black, anulli 3, funicular



**Figures 18, 19.** Habitus of hyperparasitoids. **18.** *Alloxysta* sp. **19.** *Aprostocetus* sp.

segments 5, and club 3-segmented; all segments densely covered with sensilia. Forewing with a long submarginal vein; marginal vein enlarged but short; post marginal vein long; stigma enlarged. Foreleg with a curved tibial spur.

Figitidae

***Alloxysta* sp.**
Figure 18

**New record.** Colombia, Valle del Cauca, Toro, 08.xi.2016, 04°36′22″N, 076°04′59″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 1 specimen. (MUSENUV: 28975).

**Identification.** 1.4–1.6 mm. Body entirely dark brown. Mouth parts brown. Filiform antennae with 15 segments. Mesoscutum rounded in dorsal view, with a few setae. Forewing as long as body, covered with pubescence. Radial cell trapezoidal and closed. Marginal setae absent.

Eulophidae

***Aprostocetus* sp.**
Figure 19

**New record.** Colombia, Valle del Cauca, Vijes, 22.iii.2017, 03°42′18″N, 076°25′48″W, collected by hand, L.M. Martinez, D.N. Duque, E. Aguirre, 4 specimens. (MUSENUV: 28976).

**Identification.** 1.8–2.0 mm. Body entirely metallic green. Mouth parts yellow. Antennae dark yellow, with 3 anulli, 3 funicular segments, and a 3-segmented club. Forewing as long as body. Submarginal vein shorter than a very long marginal vein. Post marginal vein short. Stigma and stigmal vein thin.

**Hyperparasitoids molecular confirmation.** Molecular data were only available for *Pachyneuron aphidis*.

Comparison with public databases showed that the sequence found in our study is identical to sequences of *Pachyneuron* sp. in GenBank, which were originally identified as *P. siphonophorae* Ashmead, 1886. *Pachyneuron siphonophorae* was synonymized with *P. aphidis* (Bouché, 1834) on the basis of morphological characteristics in 1988 (Zuparko and Dahlsten 1993). However, our sequence has only 90% of sequence identity with *P. aphidis* sequences available on GenBank. Our specimens cannot be identified as *P. siphonophorae* because it is not an accepted species but neither can they be identified as *P. aphidis* because of the dissimilarity with the available sequences. Considering this inconsistency, molecular confirmation to species was not achievable.

## Discussion

In this work we expand the distributions of 5 species of primary parasitoids and 2 hyperparasitoids in Colombia. All of these species are newly recorded from the country. *Aphidius funebris* and *A. paramali* have not been previously recorded in either Colombia or South America (Godfrey and McGuire 2004) until now, while *A. platensis*, *A. matricariae*, and *A. varipes* have already been found in Chile (Japoshvili and Abrantes 2006, Tomanović et al. 2014).For the hyperparasitoids, *S. aphidivorus* and *P. aphidis,* their closest previously known records are in Costa Rica (Zamora-Mejías and Hanson 2017). The genera *Cerapteroceroides* and *Aprostocetus* have not been previously recorded in Colombia. Our findings considerably expand the distribution of these species and genera.

The genus *Encarsia* has been used in Colombia as control agents for whiteflies (Hemiptera, Aleyrodidae) (Pérez et al. 2011), but the unknown species that we found was different from *Encarsia formosa* Gahan, 1924, the only *Encarsia* species recorded in the country; our unidentified species differs morphologically from *E. formosa*, and there was the genetic distance was between COI sequences was more than 8%.

Several species of *Alloxysta* have been recorded from other departments in Colombia (Ferrer-Suay et al. 2013) but none previously from Valle del Cauca, so our record represents the first for this genus from this department. The identification of our specimen to

species requires further study.

The distribution of *Lysiphlebus testaceipes* and *Aphidius colemani* is extended to the Valle del Cauca department and the Pacific region. *Lysiphlebus testaceipes*, which occurs throughout Central America and most of South America, was previously recorded in Colombia near the capital city of Bogotá and in Meta, in an eastern department (Zenner de Polanía 1971, León et al. 1999). *Aphidius colemani* occurs in warmer regions around the world and is common in South America. It is sold for biological control in Colombia (Monguí et al. 1986). Both species have been frequently described as cosmopolitan with a great capacity of adaptation to diverse climatic conditions (Starý 1975, Kavallieratos et al. 2001, Andorno et al. 2016) and therefore widely distributed around the world.

Our study records more than 3 times the number of species of aphid parasitoids previously found in Colombia. We show that it is still not rare to expand the distributions for primary parasitoids and hyperparasitoids, as aphid parasitoids are still poorly studied in the Neotropical region. Knowledge of aphid parasitoids in Colombia is still scarce, and more effort is needed on other crops and natural habitats, and in regions, to truly understand the parasitoid diversity present in Colombia.

## Acknowledgements

We thank the Center of Innovation in Bioinformatics and Photonics for financial support as well as Hugo Restrepo and Cia for allowing us to conduct this study on their chili pepper crops. We are grateful for M. Sampaio and R. Kula for their comments on the identifications. Finally, we thank the Postgraduate Program in Science-Biology of the Universidad del Valle for the use of the Nikon SMZ-1500 stereomicroscope.

## Authors' Contributions

LMMC, DNDG and NTP planed and designed the study. Host sampling was done by LMMC and DND. LMMC reared the parasitoids, made the morphological identifications, performed the molecular processes (extraction, amplification), conducted data analyses, and wrote the manuscript. DNDG and NTP made corrections to the manuscript.

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