RESEARCH ARTICLE



Two new aphid parasitoids (Hymenoptera, Braconidae, Aphidiinae) from field crops of South America

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Abstract

During two seasons of field sampling in crop fields in Argentina, a total of 2,276 Aphidiinae specimens were collected using Malaise traps positioned in maize, soybean, and wheat fields. Eight known species of aphid parasitoids were identified (*Aphidius platensis* Brethes, 1913, *A. ervi* Haliday, 1834, *A. rhopalosiphi* de Stefani-Perez, 1902, *A. uzbekistanicus* Luzhetzki, 1960, *A. matricariae* Haliday, 1834, *Diaeretiella rapae* (McIntosh, 1855), *Lysiphlebus testaceipes* (Cresson, 1880), and *Praon gallicum* Starý, 1971). Additionally, two new species of aphid parasitoids were discovered, bringing the total to ten species reported from these crops in the country. Here, we describe and provide diagnoses for *Aphidius brethes* **sp. nov.** and *Trioxys pampas* **sp. nov.**, which is the first record of winged species of the genus *Trioxys* in South America.

Keywords

A. brethes sp. nov., Argentina, Malaise trap, parasitoid wasps, T. pampas sp. nov.

Introduction

Cereal crops are among the most widespread in the world. Many different biological control programs have been used against various cereal pests, that range from fungal pathogens (Khan 2012; Newitt et al. 2019) to insect pests (Hågvar and Hofsvang 1991; Overholt et al. 1997; Starý et al. 2007; Brewer et al. 2019). Among cereal pests, aphids cause a significant decline in cereal production through direct plant damage or through the transmission and dissemination of viral pathogens (Dixon 1987; van Emden and Harrington 2017; Brewer et al. 2019). Due to their agricultural importance, aphids and their natural enemies (parasitoids and predators) are very common model systems in basic and applied research (Boivin et al. 2012). Tomanović et al. (2022) report 26 aphid parasitoid species in European cereal crops, while 12 species are known in cereals in South America (Starý 1993, 1995; Martínez-Chávez et al. 2019; Lau et al. 2023). Although there are many research efforts on the diversity and ecology of aphid parasitoids in South America (e.g. Starý 1993, 1995; Starý et al. 2007; Martínez-Chávez et al. 2019; Lau et al. 2023), in this study we additionally report, describe and diagnose two previously unknown aphid parasitoid species from crop fields in Argentina, amounting to the total number of 10 species found in these crops.

Material and methods

Systematic Aphidiinae trapping was conducted in agricultural fields within the Rolling Pampas of Argentina (Fig. 1A), a region characterized by a temperate climate (Cfa/Cfb, Köppen classification) and annual precipitation ranging from 800 to 1,200 mm. The fields followed a three-year crop rotation cycle of maize, first-crop soybean, and wheat/second-crop soybean. This study was part of a large-scale field experiment aimed at evaluating the effects of field margin management on agricultural biodiversity, including parasitoids.

Initially, the study involved 12 fields, each with ten Malaise traps systematically positioned at specific distances from the field edge (10, 50, 100, and 150 meters) (Fig. 1B). Additionally, two Malaise traps were placed directly within the field margins in a 10×100 m strip of semi natural vegetation. One of these margin strips was experimentally manipulated by sowing selected plant species and avoiding herbicide application to promote biodiversity, while the other was left as an untreated control. Due to extreme drought and crop failures, three fields were discarded, leaving nine fields included in the final analysis.

Traps were only active during periods when crops were present in the fields. They were removed immediately prior to harvest and reinstalled a few days after sowing, with no sampling conducted during fallow periods. Trapping occurred over two years (July 2022 to April 2024). During the first year (July 2022 to May 2023), traps were continuously active and emptied twice monthly. In the second year (August 2023 to April 2024), traps operated for 15 days each month. A total of 976 samples were analyzed for Aphidiinae and other agronomically relevant arthropods. Average field size was 77.25 ha (range: 40–213 ha).



Figure 1. A farm location in Argentina **B** View of a wheat field with Malaise traps' installed within soybean field.

The region experiences distinct seasonal variations, with summer temperatures often exceeding 30 °C and winter temperatures dropping below freezing. The diurnal temperature range is particularly significant in summer, with average annual temperatures between 13 °C and 17 °C. Most precipitation occurs during summer months (Soriano et al. 1991; Bianchi and Cravero 2010).

Specimens were preserved in 70% alcohol and later identified to the species level. External morphology was examined using a ZEISS Discovery V8 stereomicroscope, with several specimens dissected and slide-mounted in Berlese medium. Morphological terminology follows Sharkey and Wharton (1997). All specimens are housed in the collections of the Bernardino Rivadavia Natural Sciences Argentine Museum (Buenos Aires, Argentina; MACN-En), Basel Natural History Museum (Basel, Switzerland; NMB), and the Institute of Zoology, Faculty of Biology, University of Belgrade (FBUB).

Results

In total we collected 2276 aphid parasitoid specimens, from eight species: *Aphidius platensis* Brethes, 1913 [866 specimens ($842 \ 243$); 38.05%], *A. ervi* Haliday, 1834 [731 ($588 \ 1433$) (32.12%)], *A. rhopalosiphi* de Stefani-Perez, 1902 [2 (22); below 1%], *A. uzbekistanicus* Luzhetzki, 1960 [7 (5233); below 1%], *A. matricariae* Haliday, 1834 [5 (52); below 1%], *Diaeretiella rapae* (McIntosh, 1855) [621 (5842373); 27.28%], *Lysiphlebus testaceipes* (Cresson, 1880) [30 (26243); 1.32%], and *Praon gallicum* Starý, 1971 [6 (5213); below 1%]. All collected parasitoid species are associated with pest aphids from investigated crops.

Additionally, we collected two unknown species: six specimens $(5 \ 1 \ 3)$ of *Aphidius* (*Lysaphidus*) sp. and two individuals of *Trioxys* sp. $(2 \ 2)$. After their examination, we found that they are new to science. Unfortunately, DNA extraction was unsuccessful, probably due to bad preservation of specimens.

Taxonomic part

Order Hymenoptera L., 1758 Family Braconidae Nees, 1811 Subfamily Aphidiinae Haliday, 1833

Aphidius brethes Tomanović & Kocić, sp. nov. https://zoobank.org/22360475-0BA2-4300-9818-5E64447AA0EE Figs 2, 3A–H, 4A–H

Type material. *Holotype:* • 1 \bigcirc , Argentina, sample 85APHI, 34°27'35"S, 61°48'46"W (Leandro N. Alem County), 29.XI.2022, collected by Malaise traps in field margin of first-crop soybean, leg. M. Devoto & collabs. Holotype deposited in coll. Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Argentina (MACN-En).

Paratypes: • 1 ♀, sample 540APHI, Argentina, 33°56'16"S, 59°37'04"W (Baradero County), 28.IV.2023, collected by Malaise traps in field margin of first-crop soybean, leg. M. Devoto & collabs. (MACN-En); • 1 ♀, sample 609APHI, Argentina, 34°29'22"S, 61°44'47"W (Exaltación de la Cruz County), 04.VIII.2023, collected by Malaise traps in margin of wheat field, leg. M. Devoto & collabs. (MACN-En); • 1 ♀, sample 519APHI, 34°28'56"S, 61°47'18"W (Leandro N. Alem County), Argentina, 04.V.2023, collected by Malaise traps in margin of second-crop soybean field, leg. M. Devoto & collabs. (FBUB); • 1 ♀, sample 580APHI, 33°56'16"S, 59°37'04"W (Baradero County), Argentina, 04.V.2023, collected by Malaise traps in wheat field, leg. M. Devoto & collabs. (NMB); • 1 ♂, 549APHI, 33°55'16"S, 59°37'42"W (Baradero County), Argentina, 04.VIII.2023, collected by Malaise traps in wheat field, leg. M. Devoto & collabs. (MACN-En).

Diagnosis. By wing venation pattern (M+m-cu vein partly developed under r-m vein) (Fig. 3H) Aphidius brethes sp. nov. belongs to subgenus Lysaphidus. Aphidius brethes sp. nov. belongs to Aphidius species group with costulate anterolateral area of petiole like all known Aphidius species in South America (e.g. A. matricariae Hal., A. rhopalosiphi Luzhetzki, A. uzbekistanicus De Stefani-Perez), except for A. ervi Hal. that possesses rugose and A. avenae Hal., A. platensis Brèthes and A. colemani Viereck that have costate anterolateral area of petiole. New species differs from the mostly known Lysaphidus species by having 16-17-segmented antennae (Fig. 3B), four maxillar palpomeres and two labial palpomeres, while other species have 12-14 segmented antennae and usually three maxillar and two or one labial palpomeres (e.g. Aphidius arvensis (Starý), A. erysimi (Starý), A. adelocarinus Smith, A. rosaphidis Smith). Aphidius brethes sp. nov. by 16–17-segmented antennae and four maxillar and two labial palpomeres morphologically resembles A. viaticus (Sedlag) (15, 16-segmented antennae) and A. ramithyrus Smith (15, 16-segmented antennae). Moreover, it differs from A. viaticus on having the proportion between length and width of petiole at spiracles level $2.3-2.4 \times$ (Fig. 3F), while A. viaticus possesses 3.0-3.5 petiole proportion. The new species differs from A. ramithyrus by shorter flagellomere 1 (proportion between length and width of F1 is $2.6-2.8 \times$ (Fig. 3C), while in *A. ramithyrus* it is $3.0-3.2 \times$) and longer petiole (2.3-2.4



Figure 2. Aphidius brethes sp. nov., habitus of the paratype female (sample ID 519APHI).

× as long as width at spiracles level (Fig. 3F), while in *A. ramithyrus* 2.0–2.2). Furthermore, *Aphidius brethes* sp. nov. is mostly yellow colored (Fig. 2), while *A. ramithyrus* and *A. viaticus* are darker. *Aphidius viaticus* parasitizes *Pleotrichophorus glandulosus* (Kalt.) aphid associated with *Artemisia* plants, while *Aphidius ramithyrus* parasitize *Capitophorus* spp. aphids. We assume that *A. brethes* sp. nov. parasitizes cereal or soybean aphids.

Description. Female (holotype). *Length.* Body 1.5–1.7 mm; fore wing 1.6 mm; hind wing 1.2 mm.

Head (Fig. 3A). Transverse, wider than mesosoma at tegulae. Eyes medium sized, oval, laterally prominent. Face moderately setose. Tentorial index (tentoriocular line/ intertentorial line) 0.60–0.65. Clypeus with 8–12 long setae. Labrum distinct, with 7–8 short setae on outer margin. Malar space equal to 0.33–0.38 of longitudinal eye diameter. Mandible bidentate, with 9–10 setae on outer surface. Maxillar palpi with 4 palpomeres, labial palpi with 2 palpomeres. Antenna 16, 17-segmented, moderately thickened (Fig. 3B). Pedicel subsphaerical (Fig. 3C). First flagellomere (F_1) subequal or slightly shorter than second flagellomere (F_2) and 2.6–2.8 × as long as its maximum width (Fig. 3C). F_1 and F_2 bearing 0–1 and 2–3 longitudinal placodes, respectively (Fig. 3C). Flagellomeres covered uniformly with semi-erect setae subequal to half of segments diameter.

Mesosoma. Mesoscutum (Fig. 3D) smooth, covering pronotum from above. Notaulices not present. Mesoscutum sparsely setose with one row of setae laterally (Fig. 3D). Scutellum oval, bearing 6–7 long setae. Propodeum areolated with wide central areola, but sometimes missing upper carina (Fig. 3E). Upper and lower areolae of propodeum with 1–2 and 1–3 long setae on each side. Fore wing (Fig. 3H) densely pubescent, marginal setae longer than those on surface; pterostigma, 2.9–3.1 × as long as its width, 1.9–2.4 × as long as R1 vein (=metacarpus).



Figure 3. *Aphidius brethes* sp. nov., holotype female (sample ID 85APHI) **A** head **B** antenna **C** scape, pedicel, anellus, F1 and F2 flagellar segments **D** mesonotum **E** propodeum **F** petiole **G** ovipositor sheath **H** forewing.



Figure 4. *Aphidius brethes* sp. nov., paratype male (sample ID 549APHI) **A** head **B** antenna **C** scape, pedicel, anellus, F1 and F2 flagellar segments **D** mesonotum **E** propodeum **F** petiole **G** aedeagus **H** forewing.

Metasoma. Petiole (Fig. 3F) elongate, dorsally striated at the middle, slightly convex dorsally; its length $2.3-2.4 \times$ its width at spiracles, $1.8-2.0 \times$ its width at the base (Fig. 3F); 10-12 setae positioned on dorsal posterior part; one long seta posterior to spiracles. Anterolateral area of the petiole with 10-12 costulae. Ovipositor sheath (Fig. 3G) moderately elongated, wide at base, dorsally slightly convex, narrowed toward tip, apically truncated, bearing two long setae at dorsal margin. Length of ovipositor sheath $1.6-2.0 \times$ its maximum width at base, $2.7-3.0 \times$ its minimum width at tip.

Coloration. General body color yellow to light brown (Fig. 2). Scapus and pedicel yellow to light brown, annellus yellow, yellow ring at the base of F1, remaining part of antennae brown. Head light brown, eyes dark. Mouthparts except tips of mandible yellow. Pronotum, mesoscutum and mesopleuron light brown. Propodeum yellow. Legs with yellow coxae and trochanters, remaining parts of legs light brown. Wings hyaline, venation yellowish brown. Petiole yellow, other metasomal terga yellow to light brown, dorsally darker. Ovipositor sheath dark brown.

Male (Figs 4A–H). Antenna 17-segmented (Fig. 4B). Maxillar palpi with 4 palpomeres, labial palpi with 2 palpomeres. Flagellomeres 1 and 2 shorter than in female, and both 1.9 × as long as wide, each bearing 7–8 longitudinal placodes (Fig. 4C). Tentorial index approx. 0.43, malar index approx. 0.63. Fore wing venation as in female (Fig. 4H). Fore wing marginal setae distinctly longer than those on surface; pterostigma widely triangular, 2.8 × as long as its width and 1.9 × as long as R1 vein (=metacarpus) (Fig. 4H). Petiole (Fig. 3F) 2.9 × its width at spiracles. Aedeagus oval (Fig. 4G) with 10–15 setae laterally. Body slightly darker than in female, head and thorax light brown, antenna brown, mouthparts yellow. Wings slightly transluscent. Legs brown with light yellow patches at ventral and tip of segments. Petiole and propodeum yellow. Coxae and trochanters yellow, remaining parts of legs light brown to brown. Metasoma light brown to brown. Body length: 1.5 mm.

Distribution. *Aphidius brethes* sp. nov. was collected in Argentina and currently it is its only known distribution.

Aphid host. Unknown, collected by Malaise traps within field crops.

Etymology. The new species takes its name by Juan Brèthes, an Argentine scientist, naturalist, entomologist who has described several aphid parasitoid species.

Trioxys pampas Tomanović & Kocić, sp. nov.

https://zoobank.org/ABFB846A-FC16-4966-9EAC-CC5BBC8E8471 Figs 5A–H

Type material. *Holotype*: • 1 Q, Argentina, sample 122APHI, 34°29'30"S, 61°44'39"W (Leandro N. Alem County), Argentina, 31.X.2022, collected by Malaise traps in margin of maize field, leg. M. Devoto & collabs. Holotype deposited in coll. Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Argentina (MACN-En).

Paratypes: • 1 \bigcirc , 8APHI, 34°28'56"S, 61°47'18"W (Leandro N. Alem County), Argentina, 11.XI.2022, collected by Malaise traps in wheat field, leg. M. Devoto & collabs. (NMB).



Figure 5. *Trioxys pampas* sp. nov., holotype female (sample ID 122APHI) **A** head **B** antenna **C** scape, pedicel, anellus, F1 and F2 flagellar segments **D** mesonotum **E** propodeum **F** petiole **G** ovipositor sheath with accessory prongs **H** forewing.

Diagnosis. The new species is distinguishable from its congeners by combination of characters: apical clawlike bristle and two short setae at the tip of prongs (Fig. 5G), propodeum carinated with irregular carinae, without central areola (Fig. 5E) and one to

two and two to three longitudinal placodes on flagellomere 1 and 2, respectively (Fig. 5C). We assume that *T. pampas* sp. nov. parasitizes cereal aphids. It differs from *T. sunnysidensis* Fulbright & Pike, 2007, which attack cereal aphids in North America, Europe and New Zealand, by irregular propodeal areola (regular pentagonal in *T. sunnysidensis*) and by an apical clawlike bristle and two short setae at the tip of prongs (two simple bristles in *T. sunnysidensis*) (Fulbright et al. 2007). *Trioxys pampas* sp. nov. shares irregular propodeal areola and clawlike bristle on the top prongs with *T. californicus* Starý & Zuparko, 1995. However, it easy differentiated from *T. californicus* by 11-segmented antennae (*T. californicus* has 13-segmented antennae) and almost straight prongs (*T. californicus* has more curved prongs) (Fulbright et al. 2007). Additionally, *T. californicus* parasitizes *Eucallipterus tiliae* (L.) on *Tilia cordata* (Starý and Zuparko 1995).

Description. Female (holotype). *Length.* Body 1.3 mm; fore wing 1.2 mm; hind wing 1.0 mm.

Head. Eye large (Fig. 5A), parallel sided, only slightly converging toward clypeus; interocular line $0.3 \times \text{of}$ the head width; malar space approx. 0.1 of longitudinal eye diameter. Genae densely setose, remaining parts of head sparsely setose (Fig. 5A). Clypeus narrow, with 6–8 long setae. Tentorial index (tentorio-ocular line/ intertentorial line) 0.21. Malar index 0.12. Maxillary palp with 4 palpomeres, labial palp with 2 palpomeres (Fig. 5A). Mandible monodentate, bearing 6–7 long setae at outer surface. Labrum with two rows of setae with 6 setae at outer margin and three setae at inner part. Antennae (Fig. 5B) 11-segmented, moderately thickened, prevailingly with semi-erect setae which are subequal to the half of the segments diameter; flagellomere 1 (F₁) 3.10–3.43 × as long as its median width, and with 1–2 longitudinal placodes (Fig. 5C); flagellomere 2 (F₂) 2.8–3.1 × as long as its median width, with 2–3 longitudinal placodes; F₁ equal to F₂ (Fig. 5C).

Mesosoma. Mesoscutum with short notauli distinct anteriorly with 1–2 rows of sparse long setae at each side (Fig. 5D). Scutellum (Fig. 5D) sub-triangular, with 4 long setae at each side. Forewing: Pterostigma (Fig. 5H) triangular, $2.5-2.9 \times$ as long as its width and $1.5-2.0 \times$ as long as R1 vein; r & RS vein reaching to the middle of R1 vein in maximum length. Forewing surface densely covered with medium size setae and with long marginal setae (Fig. 5H). Propodeum (Fig. 5E) carinated with irregular carinae, without central areola.

Metasoma. Petiole (Fig. 5F) short, $1.6-1.7 \times as$ long as wide at spiracles, with a pair of setae on postero-dorsal aspect, and four long lateral setae along the side. Ovipositor sheath (Fig. 5G) elongate, $3.0 \times as$ long as its maximal width (at base); with a single long and 3–4 short setae on lateral and dorsal aspects. Prongs (Fig. 5G) almost straight and long with 3 long perpendicular dorsal setae, followed by an apical clawlike bristle and two short setae at the tip.

Coloration. Head brown, gena, face, clypeus, mouthparts light brown. Pedicel and scapus light brown to brown, annellus yellowish, flagellomere 1 and flagellomere 2 light brown, remaining part of antennae brown. Mesonotum and propodeum light brown, the rest of mesosoma yellow. Legs light brown with yellow trochanters. Petiole yellow to light brown, the rest of metasoma light brown to brown. Ovipositor sheaths and prongs light brown.

Male. Unknown.

Distribution. *Trioxys pampas* sp. nov. was collected in Argentina and it is only known species distribution at the moment.

Aphid host. Unknown, collected by Malaise traps within maize field and wheat fields margin.

Etymology. The new species takes its name by Rolling Pampas of Argentina where was collected.

Discussion

Species composition and diversity of aphid parasitoids as important natural enemies of aphids in cereal and soybean crops are well known. The recent study on parasitoids parasitizing aphids from cereal crops in Europe reported 26 parasitoid species (Tomanović et al. 2022). Many of these species are distributed in the Palaearctic (Starý 1981; Rakhshani et al. 2008), Africa (Laamari et al. 2012), Australia (Ward et al. 2021), North America (Pike and Starý 1995; Pike et al. 2000) and South America (Starý 1993, 1995; Lau et al. 2023). The North American fauna of aphid parasitoids in cereal crops includes six species of European or Palaearctic origin (see Pike et al. 2000), but also several species that are probably native to North America: Aphidius avenaphis (Fitch, 1861), Ephedrus californicus Baker, 1909, Lysiphlebus testaceipes (Cresson, 1880), Monoctonus washingtonensis Pike & Starý, 1995, Praon occidentale Baker, 1909, P. unicum Smith, 1944 and P. yakimanum Pike & Starý, 1995. In South America, however, of the 12 known species of aphid parasitoids from cereal crops (Starý 1993, 1995; Martínez-Chávez et al. 2019; Lau et al. 2023), except for A. platensis (see Tomanović et al. 2014), all other species are introduced and not native to that region. Here, we described and diagnosed two new aphid parasitoid species from field crops in Argentina. Although we did not rear them from aphid hosts, but sampled them with the Malaise trap, we assume that both species parasitize aphids from cereals (wheat or corn) or soybean. We collected them inside field crops. We suspect that A. brethes sp. nov. and T. pampas sp. nov. originate from South America, because they have never been sampled before from any crops or non crop habitats outside this region. Aphid parasitoids of the genus Trioxys are not common in cereal crops. Starý (1981) reported T. auctus (Haliday) on the bird cherry oat aphid, Rhopalosiphum padi (L.), in Europe. Fulbright and Pike (2007) described T. sunnysidensis Fulbright & Pike from the Pacific Northwest (Washington) on the same aphid host. Later, Čkrkić et al. (2019) reported T. sunnysidensis in Europe and New Zealand. Trioxys pampas sp. nov. can be easy differentiated from North American congenerics (Fulbright et all. 2007) while A. brethes belongs to subgenus Lysaphidus, that was reviewed by Tomanović et al. (2007). Ragsdale et al. (2011) reviewed 8 North American braconid parasitoids on soybean aphids and among them no Trioxys species were found. Starý and Delfino (1986) and Starý (1995) reviewed aphid parasitoids from Tucuman Province (Argentina) and Chile, respectively and described two new *Binodoxys* species (originally described within genus

Trioxys) – *B. tucumanus* Starý, 1986 and *B. chilensis* Starý, 1995. Subsequently, two additional *Binodoxys* species were reported from central Argentina, newly described *B. achalensis* Starý, 2004 (Delfino & Starý, 2004) and *B. brevicornis* (Zummofen et al. 2013). Surprisingly, *T. pampas* sp. nov. is the first recorded species of the genus *Trioxys* in South America (Yu et al. 2016) and only winged *Trioxys* species from crop dominated landscape in South America. Gärdenfors (1990) described *T. apterus* from Napo Province in Ecuador, and it was the only known *Trioxys* species in South America prior to our research. The distribution and biology of both newly described species in South America should be evaluated by further research, as well as their host range pattern.

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