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A new species of *Dieucoila* (Hymenoptera Figitidae) associated with *Drosophila suzukii* (Diptera Drosophilidae) in Argentina

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Abstract

Drosophila suzukii (Matsumura) (Diptera Drosophilidae), spotted-wing Drosophila (SWD), has recently invaded Argentina, spreading very quickly through different fruit-growing regions. Because of the risk represented by SWD for the national berry industry, it is necessary to develop biological alternatives for its control. Determine the presence of native natural enemies associated with SWD is important to develop biological control programs against this pest. In this study, we present the results of a field survey aimed to collect and identify Hymenoptera parasitoids associated with SWD in organic raspberry crops. We found an almost irrelevant parasitization rate on SWD pupae. The few parasitoids collected were identified as a new species of *Dieucoila* Ashmead, *Dieucoila octoflagella* Reche sp. n., which is here described and characterized in comparison with other species in the genus *Dieucoila*.

Key words: Eucoilinae, organic raspberry, spotted-wing *Drosophila*, Argentina.

Introduction

Drosophila suzukii (Matsumura), commonly known as spotted-wing *Drosophila* (SWD) is known to economically important pest to affects a variety of cherry and berry crops (i.e. strawberries, raspberries, blackberries, blueberries) but can also attack other fruit crops (i.e. grapes and kiwis) (de la Vega *et al*., 2019). SWD, is native to Southeast Asia, its first record outside of this place dates from 1980 in Hawaii, but the first records from continental USA date from 2008 (Hauser, 2011; Dagatti *et al*., 2018), the same year was also reported for Canada and Europe (Walsh *et al.*, 2011; Calabria *et al.*, 2012; Cini *et al*., 2012; Asplen *et al*., 2015). In South America, the first SWD records were for Brazil in 2012 (Depra *et al*., 2014), in Argentina and Uruguay for 2014 (Santadino *et al*., 2015; Gonzalez *et al*., 2015), and the central-south of Chile for 2015-2017 (Medina-Muñoz *et al*., 2015). In Argentina, the SWD pest has spread very quickly to different fruit-growing regions. It was found for the first time in 2014, almost simultaneously in Rio Negro province on raspberry and Buenos Aires province on blueberry (Cichon *et al*., 2015; Santadino *et al*., 2015; Funes *et al*., 2018). In Entre Rios province, it was found on traps placed in orange and blackberry farms (Diaz *et al*., 2015). Subsequently, SWD is collected in the province of La Rioja on traps close to pears (Lue *et al*., 2017), in the province of Santa Fe in peach plants (Gonsebatt *et al*., 2017), and Tucumán province on semi-arid habitat plants and guava fruits (Lavagnino *et al*., 2018; Escobar *et al*., 2018a; Funes *et al*., 2018). In February 2018, Dagatti *et al*. (2018), cited to SWD for the first time for Mendoza province on blackberry and de la Vega et Corley (2019) cite to SWD on cherries and raspberry for provinces of Neuquén and Santa Cruz. However, the history of the fly invasion in our country is largely unknown. Recent studies by de la Vega *et al.* (2019) on the genetic diversity of *D. suzukii* in Argentina indicate that SWD has been introduced multiple times and that the invasion routes may have originated in areas of North America and Brazil, and did not include Asia or Europe. Because of the risk represented by SWD for the national berry industry, it is necessary to develop biological alternatives for its control.

Determine the presence of native natural enemies associated with SWD is important to develop biological control programs against this pest. Multiple parasitoid species target larvae and pupae, all they have potential like biological control agents. Among them, we find pupal parasitoids *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera Diapriidae). In South Korea, China, Japan, North America and, Europe, the most commonly collected larval-pupal parasitoids associated with spotted-wing drosophila are *Asobara japonica* Belokobylskij (Hymenoptera Braconidae), *Ganaspis brasiliensis* (Ihering) and, *Leptopilina japonica japonica* Novkovic et Kimura (both Hymenoptera Figitidae) (Lee e*t al.*, 2019). In Argentina, the figitids *Leptopilina clavipes* (Hartig), *Ganaspis hookeri* Crawford (Lue *et al*., 2017), *Leptopilina boulardi* (Barbotin, Carton et Kelner-Pillaut) (Garrido *et al*., 2018), were cited in association with SWD. In this study we present the results

of a field survey aimed to collect and identify Hymenoptera parasitoids associated with SWD in organic raspberry crops. We found an almost irrelevant parasitization rate on SWD pupae. The few parasitoids collected were identified as a new species of *Dieucoila* Ashmead, *Dieucoila octoflagella* Reche sp. n., which is here described and characterized in comparison with other species in the genus *Dieucoila*.

Materials and methods

Raspberry fruit (*Rubus idaeus* L. cv. "Heritage") still on the plants were randomly collected from a one-hectare organic farm located in Tafí del Valle (26°50'47"S 65°42'32"W), Tucumán province, northwestern Argentina. Fruit samples (table 1) were taken to the INTA-EEA Famaillá entomological laboratory, Tucumán. Each fruit sample was placed in 20 × 30 cm plastic trays with a sand floor as pupation substrate and voile cloth cover to allow air exchange and prevent insects from escaping. The trays were maintained at ≈25 °C and 60-70% RH for 15-20 days until the emergence of adult flies and parasitoids. All adult flies that emerged from the *Drosophila* pupae recovered from raspberry samples were identifies as *D. suzukii*. SWD specimens were morphologically identified by C. Funes (INTA-EEA Famaillá) following the taxonomic key by Takamori *et al*. (2006) and the morphological description by Hauser (2011). Only a single adult parasitoid emerged from SWD pupae. Two more adult parasitoids were collected as they walked on ripe fruits during fruit sampling by using an entomological aspirator. The parasitoid specimens were individually placed in 2 ml Eppendorf tubes with 70% alcohol, and sent to the División Entomología, Museo de La Plata (MLP), Universidad Nacional de La Plata, Buenos Aires, Argentina, for morphological identification. The specimen was provisionally identified as *Dieucoila* sp. and compared with other very similar specimens deposited in the MLP. These specimens were collected from different Argentinean regions, two of them were of the same parasitoid species, one of Horco Molle (Tucumán Province) and other of Bonpland (Misiones Province, Northeastern Argentina). The five eucoiline specimens were identified by using taxonomic keys by Weld (1952) and original descriptions of Eucoilinae species by Kieffer (1907; 1909), Weld (1921), Loiacono and Diaz (1977), and Diaz (1978), and to define the new species, were compared with photographs of the specimens types of *Dieucoila brasiliana* (Kieffer 1909), *Dieucoila brasiliensis* (Kieffer 1909), *Dieucoila rufopetiolata* (Kieffer 1909), *Dieucoila striatifrons* (Kieffer 1908), *Dieucoila variicornis* (Kieffer 1909), *Dieucoila nigriceps* (Kieffer 1907), *Dieucoila rufa* (Kieffer 1907) and *Dieucoila scutellaris* (Kieffer 1907), all of them deposited at California Academy of Sciences (CAS), USA (images available in http://www.calacademy.org/scientists/entomology). Morphological terminology follows Gallardo *et al.* (2017) for taxonomic characters and Harris (1979) for surface sculpturing. Morphological terms used matched to the Hymenoptera Anatomy Ontology (HAO, Yoder *et al*., 2010). Biogeographical regions follow to scheme of Arana *et al*. (2017). The photographs were taken with a Leica digital camera model DFC295 adapted to a Leica steromicroscope (S8APO) and a scanning electron microscope (SEM FEI Quanta200) at the Laboratorio Investigaciones de Metalurgia Física (LIMF) (UNLP). The examined specimens were deposited in the División Entomología (MLP).

Results

Parasitoids collected and identified

The number of SWD flies and adult parasitoids recovered per fruit sample are summarized in table 1. The apparent parasitization rate was negligible (one adult parasitoid emerged against 1,438 SWD flies). Overall, five specimens were identified: one adult emerged from SWD pupae infesting ripe berries, two adults collected in the field while walking on ripe berries, and two specimens from MLP's entomological collection. All of them were assigned to a new species in the genus *Dieucoila*.

Table 1. Sampling dates, number of raspberry fruit per sample, number of SWD males, females and total individuals recovered from each fruit sample, incidence of SWD per fruit and number of parasitoids recovered from an organic farm in Tafí del Valle, Tucumán province, northwestern Argentina (2016 and 2017).

Sampling date	No. of fruit/ sample	Total No. of SWD individuals			Incidence (No. of SWD per fruit)	No. of parasitoids	
		Females	Males	Total		emerged from SWD puparia	collected on infested fruit
Feb 5, 2016	16	2	28	30	1.9		1
Feb 19, 2016	50	118	146	264	5.3	1	
Mar 15, 2016	50	218	237	455	9.1		
Mar 22, 2016	50	55	145	200	4.0		
Jan 3, 2017	40	4	16	20	0.5		
Jan 18, 2017	48	2	4	6	0.1		
Feb 15, 2017	50	128	107	235	4.7		
Mar 3, 2017	50	38	25	63	1.3		
Mar 15, 2017	50	70	95	165	3.3		1
Total	404	635	803	1.438	3.6	1	2

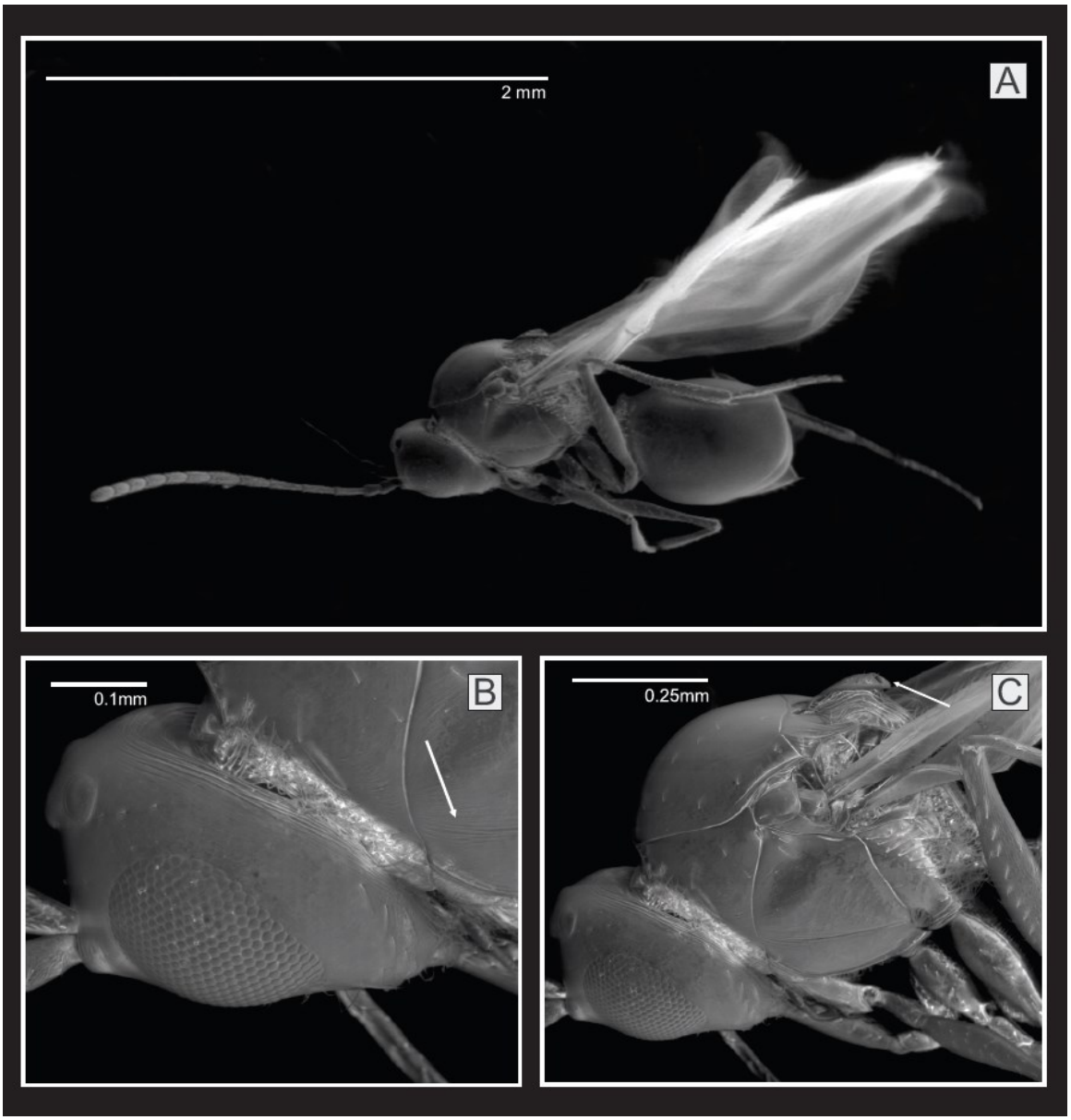

Figure 1. *Dieucoila octoflagella* Reche sp. n., female. **1A**) Habitus; **1B**) Head and mesosoma in lateral view, arrow indicated mesopleuron aciculate; **1C**) Mesoscutum in lateral view, arrow indicated scutellar plate which in lateral view has a snake's open mouth shape.

Taxonomic description

Dieucoila octoflagella Reche sp. n.
Female (Holotype):

Body length 1.60 mm (figure 1A) scarcely setose. Head and mesosoma slightly aciculate (figure 1B, 1C). Head and mesosoma dark brown almost black. Metasoma reddish brown. Antenna brown yellowish. Mandibles, wing venation and legs yellowish.

Head - (figure 2A) in anterior view subcircular, broader than mesosoma, with striations between malar sulcus and torulus (arrow in figure 2A). Central area of face smooth, scattered setose. Antenna (figure 2B) filiform, with 13 segments; F1-F3 subequal in length, slender and longer than the following; antennal club with eight flagellomeres, all with rhinaria. Malar sulcus inconspicuous. Without genal carinae. Compound eyes glabrous. Occiput and vertex striated (figure 1B).

Pronotum - pronotal plate aciculate, medial bridge broad, with lateral foveae open, deep and small. Dorsal margin slightly emarginated. Sides of pronotum aciculate and highly setose behind genae (figure 1B).

Mesoscutum - in lateral view (figure 1C), as long as wide. Aciculate, scarcely setose. Notauli absent. Parapsidal hair line present. Parascutal impression conspicuous.

Mesopectus - mesopleuron aciculate (arrow in figure 1B) and slightly striated with mesopleural carina and precoxal carina inconspicuous. Subalar pit small. Mesopleural triangle undefined.

Mesoscutellum - scutellar fovea large, deep, wider than long. Lateral bars of scutellum (figure 1C) striated with a fovea under them. Dorsal surface of scutellum coarsely areolate, laterally striated, posterior margin round. Scutellar plate suboval, covering ¼ of the scutellum in

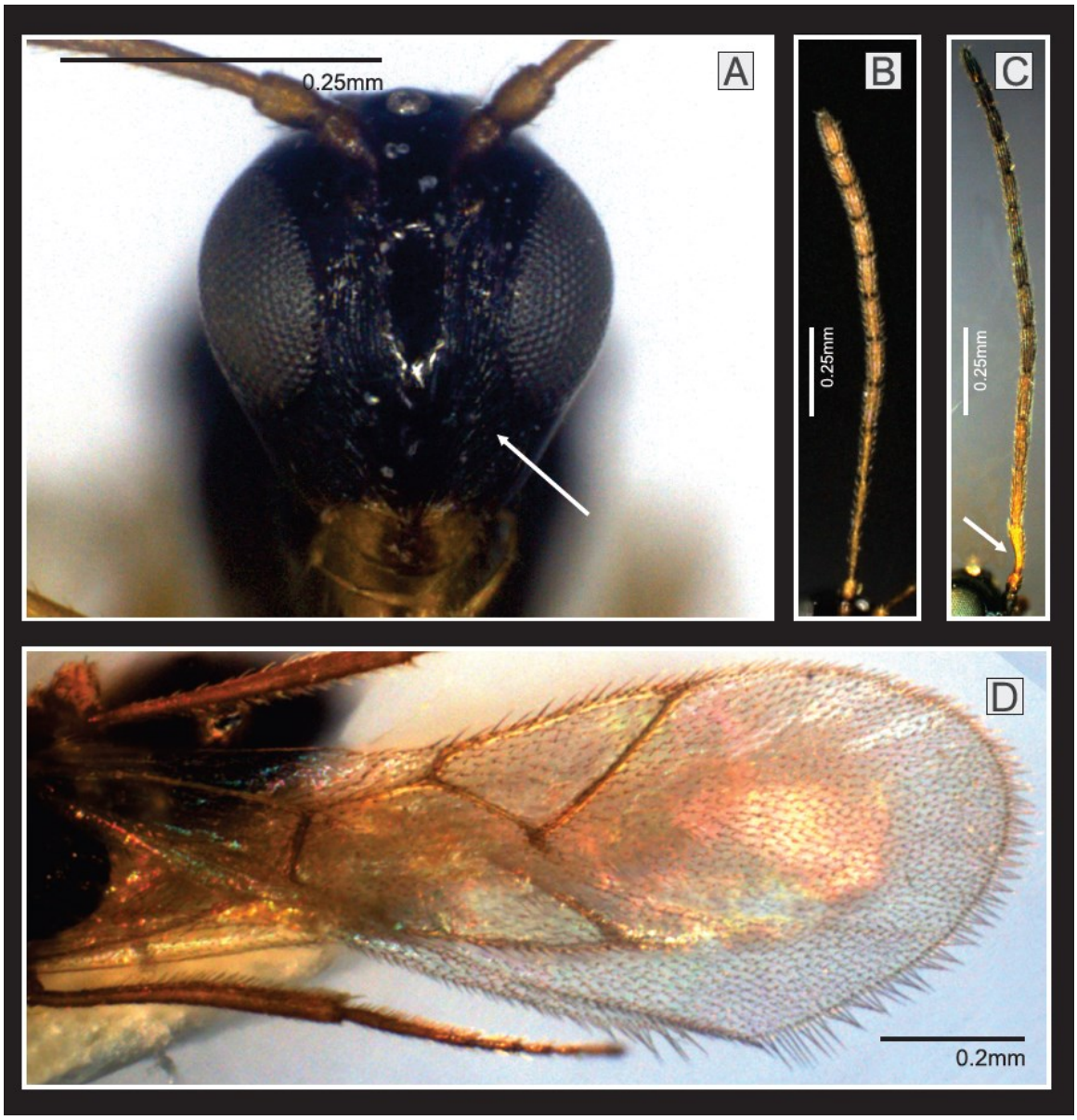

Figure 2. *Dieucoila octoflagella* Reche sp. n. **2A**) Anterior view of female head, arrow indicated striations between malar sulcus and torulus; **2B**) Antenna of female; **2C**) Antenna of male, arrow indicated F1 curved outwardly and brown pale; **2D**) Forewing of female.

dorsal view, elevated above the surface of scutellum in lateral view, at its posterior end there is a small midpit, covered by a small tooth, which in lateral view has a snake's open mouth shape (arrow in figure 1C).

Metapectal-propodeal complex - metapleuron aciculate. Upper part with stout and long hairs, lower part glabrous. Anteroventral cavity conspicuous, suboval, setose. Propodeum elongate, highly setose on the carinae and between them. Lateral propodeal carinae subparallel, separated in middle portion.

Wings - forewings (figure 2D) hyaline, apical marginal with hair fringe, with membrane prubescence, rounded. Marginal cell closed, elongate, twice longer than wide.

Key to species of *Dieucoila* in Argentina

1 - Head and mesosoma strongly aciculate (figure 3B). Antennal club with six flagellomeres (figure 3C). Central area of face striated. Scutellar plate with a small midpit placed close to posterior margin of plate, covered by a large tooth (figure 3B, arrow) . . . . . *Dieucoila subopaca* Ashmead 1903 (figure 3A)

-- - Head and mesosoma slightly aciculate (figure 1B). Antennal club with eight flagellomeres (figure 2B). Central area of face smooth. Scutellar plate with a small midpit placed close to posterior margin of plate, covered by a small tooth (figure 1C, arrow) . . . . . *Dieucoila octoflagella* Reche sp. n. (figure 1A)

Figure 3. *Dieucoila subopaca* Ashmead, female. **3A**) Habitus; **3B**) Head and mesosoma in lateral view, arrow indicated midpit of scutellar plate covered by a large tooth; **3C**) Antenna.

Legs - patch of hairs dorsolaterally on midcoxa and row of hairs posterodorsally on hindcoxa present.

Metasoma - smooth shining, not aciculate. Hairy ring at base of syntergum completed dorsally, as wooly; distally with micropunctures.

Variation - body length 1.60-1.70 mm.

Male:

Similar to female, except for the following features: Body length 1.60-1.80 mm. Antenna (figure 2C) with last nine flagellomeres dark brown, with 15 segments, filiform; F1 curved outwardly and brown pale (arrow in figure 2C), subequal to following flagellomeres; F2-F5 straight, subequal in length; F6-F13 become progressively shorter and dark brown; all with rhinaria.

Type material

Holotype - ♀, on pin card, Argentina, Tucumán, Tafí del Valle 26°53'10"S 65°41'28"W, 19.ii.2016, reared from *Drosophila suzukii* obtained from raspberry fruit (*Rubus idaeus* L. cv. "Heritage") collected in an organic farm, Escobar and Funes colls. (MLP).

Paratypes - on pin card: 1♀, Argentina, Tucumán, Tafí del Valle 26°53'10"S 65°41'28"W, 5.ii.2016, recovered from the surface of infested raspberry ripe fruits (*Rubus idaeus* L. cv. "Heritage") in an organic farm, Escobar and Funes colls. (MLP); 1♂, Argentina, Tucumán, Tafí del Valle 26°53'10"S 65°41'28"W, 15.iii.2017, recovered from the surface of infested raspberry ripe fruits (*Rubus idaeus* L. cv. "Heritage") in an organic farm, Escobar and Funes colls. (MLP); 1♂, Horco Molle 26°55'00"S 65°05'00"W, without data (MLP); 1♀, Misiones, Bonpland 27°29'6.66"S 55°28'139.61"W, 27.xi.1986, Fidalgo coll. (MLP).

Etymology

The specific name refers to the number of flagellomeres that form the female antennal club.

Distribution

Argentina, Tucumán and Misiones, this distribution belongs to the biogeographic Chacoan province and Parana Forest province, respectively (Chacoan subregion).

Biology

Specimens from Tafí del Valle (Tucumán) were collected in raspberry (*Rubus idaeus* L. cv. "Heritage") organic farm, the holotype was reared from *D. suzukii*.

Comments

Dieucoila is characterized by the following combination of features: Head and mesosoma aciculate, metasoma smooth, shiny, not aciculate. Face in frontal view with striations between malar sulcus and torulus. Central area of faces striated or smooth, scattered setose. Antenna subfiliform in both sexes. Dorsal surface of scutellum areolate. Scutellar plate narrow, elevated above the disk, at its posterior end with a small midpit covered by a tooth, which in lateral view has a snake's open mouth shape. Marginal cell closed or partially closed never fully open. The common drosophilid parasitoids have a very similar overall appearance (Buffington and Forshage, 2016). Among them, the dorsal surface of scutellum coarsely areolate and the scutellar plate lack of midpit covered by a tooth (resulting in has a snake's open mouth shape in lateral view), are particularly useful to separate *Dieucoila* from *Ganaspis* Forster and *Leptopilina* Forster, two genera commonly encountered among *Drosophila* hosts. The species of *Ganaspis* have a body smooth and a large scutellar plate; in *Dieucoila*, the body is aciculate and has a small scutellar plate. The species of *Dieucoila* can be separated from all *Leptopilina* by having a hairy ring at the base of the syntergum complete dorsally (typically interrupted and incomplete dorsally in *Leptopilina*), the metapleuron is aciculate (smooth in *Leptopilina*) and the male antenna with the first flagellomere distinctly modified and curving outward (the second flagellomere is modified in *Leptopilina*).

Dieucoila is distributed in the Neotropical region: Belize, Brazil, Cuba, and Nicaragua (Ashmead, 1903; Kieffer, 1907; 1908; 1909; Weld, 1921). The host of this genus was unknown until Escobar *et al.* (2018b) mentioned the association with Drosophilidae. In Argentina, only *Dieucoila subopaca* Ashmead was recorded from Misiones province (Loiácono and Díaz, 1977). We report *D. octoflagella* sp. n. from Tucumán and Misiones provinces.

The new species differs from *D. subopaca* and *D. brasiliensis* by the following characteristics: color dark brown almost black (reddish brown in *D. subopaca* and *D. brasiliensis*); central area of face smooth (striated in *D. subopaca* and *D. brasiliensis*); head and mesosoma slightly aciculate (strongly aciculate in *D. subopaca*, *D. nigriceps*, *D. rufopetiolata* and *D. variicornis*); antennal club with eight flagellomeres (six flagellomeres in *D. subopaca*); medial bridge of pronotal plate broad (narrow in *D. subopaca* and *D. brasiliensis*); lateral bars of scutellum smooth (striated in *D. subopaca*); scutellar plate narrow with a small midpit placed close to posterior margin of plate, covered by a small tooth (large tooth in *D. subopaca*). Marginal cell closed twice longer than wide (three times longer than wide in *D. subopaca*). *D. octoflagella* can be distinguished from *D. nigriceps* by the following features: colour dark brown almost black (reddish brown in *D. nigriceps*); central area of face smooth (striated in *D. nigriceps*); antennal club with eight flagellomeres (five flagellomeres in *D. nigriceps*); medial bridge of pronotal plate broad (narrow in *D. nigriceps*); scutellar plate narrow with a small midpit placed close to posterior margin of plate, covered by a small tooth (large tooth in *D. nigriceps*). Marginal cell closed (partially closed in *D. nigriceps*). *D. octoflagella* differs from *D. variicornis* by having the antennal club with eight flagellomeres light brown (six flagellomeres of which F6-F8 white, the others brown in *D. variicornis*). The new species can be distinguished from *D. striatifrons* and *D. rufopetiolata* by the following features: dorsal margin of scutellar plate slightly emarginated (deeply emarginated in *D. striatifrons* and *D. rufopetiolata*); antenna of male with F1 equal to F2 (F1 longer than F2 in *D. rufopetiolata*). *D. octoflagella* differs from

D. rufa and *D. scutellaris* by having antennal club with eight flagellomeres (seven in *D. rufa*) and antenna of male with F1 equal to F2 (F1 longer than F2 in *D. scutellaris*). The new species can be distinguished from *D. brasiliana* by the following features: head and mesosoma aciculate (smooth in *D. brasiliana*); scutellar plate with a tooth (scutellar plate without tooth in *D. brasiliana*); antenna of male with F1 subequal to F2 (F1 longer than F2 in *D. brasiliana*).

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