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New information about the third stage larva and larval habitat of *Microdon (Chymophila) bruchi* Shannon, 1927 (Diptera, Syrphidae) from Argentina

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ABSTRACT

In this paper, we provide new biological information about *Microdon* (*Chymophila*) *bruchi* Shannon, 1927. We present new records of *M. bruchi* in nests of *Camponotus mus* Roger, 1863 built inside *Vitis vinifera* L. plants from Argentina (Mendoza Province) and records of this species for Catamarca and Entre Ríos, Argentina. DNA barcodes and data on morphology and locomotion for third-stage larvae are provided. An identification key is also given to distinguish *M. bruchi* from other Neotropical species of *Chymophila*. We designate a lectotype for *Microdon bruchi* Shannon, 1927, and we consider *Microdon argentinae* Hull, 1937 a junior synonym of *M. bruchi*.

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Introduction

The hover fly subfamily Microdontinae (Diptera, Syrphidae) comprises approximately 490 described species worldwide, the majority of which live in tropical regions (Reemer 2013). The biology of the group stands out among syrphids for the close associations these species have with ants. The larvae are usually predators of immature stages of ants (Van Pelt and Van Pelt 1972; Duffield 1981; Reemer 2013), although at least one species is known to be an ectoparasitoid (Pérez-Lachaud et al. 2014). Scarce biological information is available for the majority of species, and their life cycles have not yet been studied (Akre et al. 1973; Elmes et al. 1999; Schönrogge et al. 2002; Maruyama and Hironaga 2004; Witek et al. 2012). There are few studies providing morphological descriptions of eggs, larval stages and puparia (Wheeler 1908; Dixon 1960; Garnett et al. 1990; Rotheray 1991; Rotheray and Gilbert 1999; Schmid 2004; Gammelmo and Aarvik 2007; Wolton 2011; Iwai et al. 2016; Scarparo et al. 2017). A small number of studies have addressed other

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biological aspects, such as their reproductive strategies, behaviour and locomotion (Duffield 1981; Wolton 2011; Scarparo et al. 2017).

The genus *Microdon* Meigen, 1803 currently contains 126 described species, 62 of which are known from the Neotropical Region (Reemer 2014). Until recently, the subgenus *Chymophila* Macquart, 1834 was considered to be confined to the New World, ranging from the southern USA to Argentina (Cheng and Thompson 2008). Reemer and Ståhls (2013a) included one Nearctic and 25 Neotropical species in *Chymophila*, and also recognised seven Oriental and one Eastern Palaearctic species as belonging to this group. Reemer (2014) introduced a species synonymy (*Microdon aurifex* Wiedemann, 1830 as junior synonym of *Microdon instabilis* Wiedemann, 1830); he also diagnosed a new species, *Microdon* (*Chymophila*) SUR-02, but did not formally describe it. A combined phylogenetic analysis of molecular and morphological characters by Reemer and Ståhls (2013b) recovered *Chymophila* as sister to the subgenus *Microdon sensu stricto*.

Larvae of some *Chymophila* species are known to be predators of immature stages of ants. So far, associations have been observed with ant species of the subfamilies Formicinae and Myrmicinae (Reemer 2013). The Eastern Palaearctic *Microdon* (*Chymophila*) *katsurai* Maruyama and Hironaga, 2004 has been found in nests of *Polyrhachis lamellidens* Smith, 1874 (subfamily Formicinae, tribe Camponotini) (Maruyama and Hironaga 2004; Iwai et al. 2016), and the North American *M.* (*Chymophila*) *fulgens* Wiedemann, 1830 occurs in nests of *Polyergus lucidus* Mayr, 1870, *Formica schaufussi* Mayr, 1866 (subfamily Formicinae, tribe Formicini) and *Camponotus atriceps* (Smith, 1858) (subfamily Formicinae, tribe Camponotini) (Thompson 1981). The Neotropical *M.* (*Chymophila*) *tigrinus* Curran, 1940 has been found in nests of four different *Acromyrmex* species (subfamily Myrmicinae, tribe Attini) (Forti et al. 2007; Camargo et al. 2008; Zubarán 2018), and the Neotropical *M.* (*Chymophila*) *bruchi* Shannon, 1927 has been found in nests of *Camponotus mus* Roger, 1863 (subfamily Formicinae, tribe Camponotini) (Shannon 1927). In the latter case, the species was described based on two adult females collected from a nest of *C. mus* built underneath a rock in Alta Gracia, Córdoba Province, Argentina. Male specimens of *M. bruchi* and the morphology of its larval stages and puparia are still unknown.

The host of *M. bruchi*, the ant *C. mus*, is widely distributed across Argentina, from north to south and from the western foothills to the eastern coasts, as well as in other South American countries, such as Paraguay, Uruguay and southern Brazil (Josens 2000). This species lives in humid and dry regions, as well as in forests and open places. This ant species is considered an urban pest because, in addition to its great potential as an invasive species, it causes great problems by nesting in structures and buildings (Josens et al. 2017; Werenkrut et al. 2017). On the other hand, in Argentinian crops, the carpenter ant can deteriorate wooden structures such as posts, and also use decaying wood for nesting inside of living plants, such as those in Argentinian vineyards (Chiesa Molinari 1942; Debandi personal observation). The impact of *M. bruchi* larvae on wild and vineyard nests of *C. mus* is unknown. Some authors have suggested that the impact of Microdontinae on ant colonies can be large. One *Microdon* larva may consume 125 ant larvae during its life; at an average of five or six fly larvae per nest, over 700 ant larvae would be consumed per nest (Duffield 1981; Barr 1995). In light of all the above, we deem it necessary to study the impact of this predator on wild ant nests and on agricultural crops such as grapevines.

In this study, we give some new information about *M. bruchi*. We present new records of *M. bruchi* in nests of *C. mus* built inside *Vitis vinifera* L. plants from Argentina (Mendoza Province) and the first records for Catamarca and Entre Ríos provinces. We also provide DNA barcodes (Hebert et al. 2003a, 2003b), information on morphology and locomotion of third-stage larvae, and an identification key to distinguish *M. bruchi* from other Neotropical species of *Chymophila*.

Material and methods

Collecting sites and field sampling

Field sampling was conducted in a vineyard located in San Martín, Mendoza, Argentina (32.985°S, 68.360°W), in the winter season of 2016. During the end of July and the beginning of August, a total of 17 third-stage larvae and 10 eggs were collected from six different nests built inside *Vitis vinifera* plants. The procedure to access ant nests consisted of boring a hole and splitting open the trunk of the grapevine in which the presence of ants was observed (Figure 1). Only six of 13 examined nests contained third-stage larvae of *Microdon*. Eight third-stage larvae were found inside two nests built inside a grapevine, four per trunk in this case. Another six larvae were found inside two trunks (three per trunk), plus another trunk contained two larvae and the last had one larva. These were transferred to the Laboratorio de Entomología, Instituto Nacional de

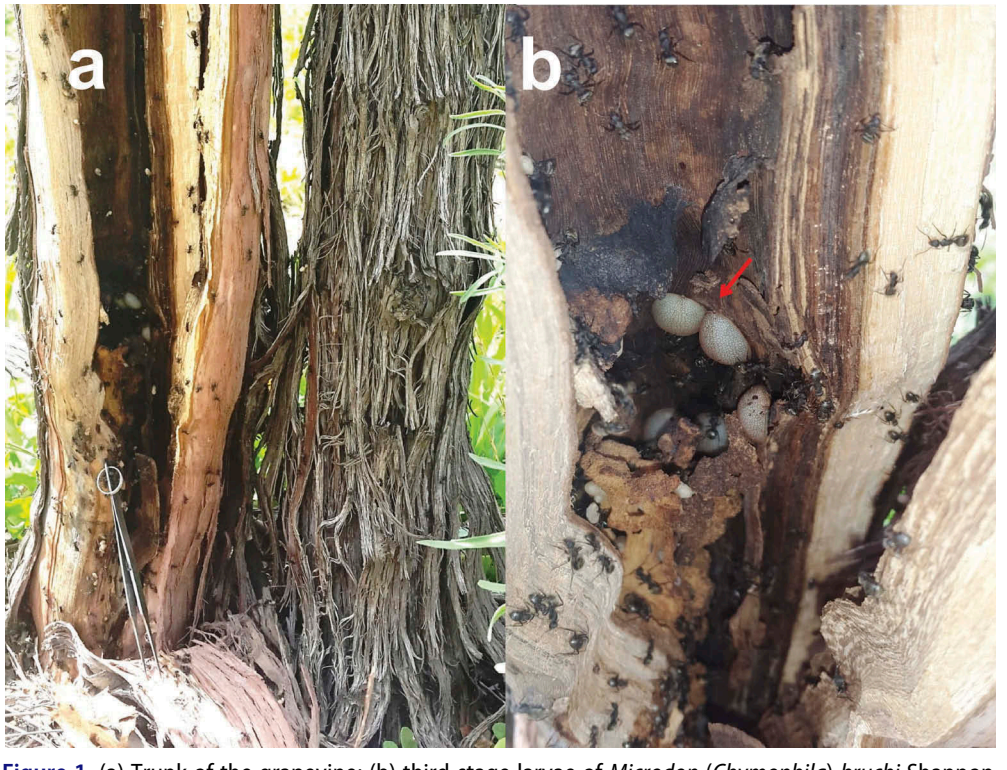

Figure 1. (a) Trunk of the grapevine; (b) third-stage larvae of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927 in ant nests inside *Vitis vinifera* L. plant.

Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria (EEA) Junín, Mendoza, Argentina, in covered plastic containers. Nine third-stage larvae were preserved in absolute ethanol for subsequent DNA barcoding analysis and eight were kept in 200-mL plastic containers at room temperature (24–27°C) and a relative humidity of 40–50%, until the adults emerged (September). The top of each container was sealed with 0.5-mm mesh screen. In this case, for obtained adults, it is important to highlight that larvae were not fed; pupation started just after transferring them into the laboratory. The obtained *Microdon* specimens (nine third-stage larvae and eight adults) were sent to the Zoologisches Forschungsmuseum Alexander Koenig (ZFMK) in Bonn, Germany, for molecular studies, and later to the Naturalis Biodiversity Centre (RMNH) in Leiden, Netherlands, for taxonomic studies. Three larvae were preserved in 90% ethanol for later morphological studies with scanning electron microscopy (SEM) at the Laboratorio de Entomología, Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA) Centro Científico Tecnológico (CCT) - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Mendoza, Argentina.

Adult terminology, measurements and photography

The morphological terminology used for adults follows Thompson (1999) and Cumming and Wood (2009). The abbreviations used for collections follow the standard of the *Systema Dipterorum* (Thompson 2013), and their equivalents are given below:

CSCA: California State Collection of Arthropods, Sacramento, USA.

MACN: Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos, Argentina.

MCZ: Museum of Comparative Zoology, Harvard University, Cambridge, USA.

RMNH: Naturalis Biodiversity Centre, Leiden, the Netherlands.

USNM: United States National Museum, Smithsonian Institution, Washington DC, USA.

ZFMK: Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany.

In the description of type labels, the contents of each label are enclosed by quotation marks (‘ ’), italics denote handwriting, and the individual lines of data are separated by a double forward slash (//). At the end of each record, the holding institution and the unique identifier or number are given.

All measurements are in millimetres and were taken using a reticule in a Leica® M165 C microscope. Body length was measured from the anterior oral margin to the posterior end of the abdomen, in lateral view. Wing length was measured from the wing tip to the basicosta. Photographs were composed using the software Zerene Stacker® 1.04 (Richland, Washington, USA), based on images of pinned specimens taken with a Canon EOS 7D® camera mounted on a P–51 Cam-Lift (Dun Inc., VA, USA) and with the help of Adobe Lightroom® (version 5.6). Simple-Mappr (Shorthouse 2010) was used to create Figure 2.

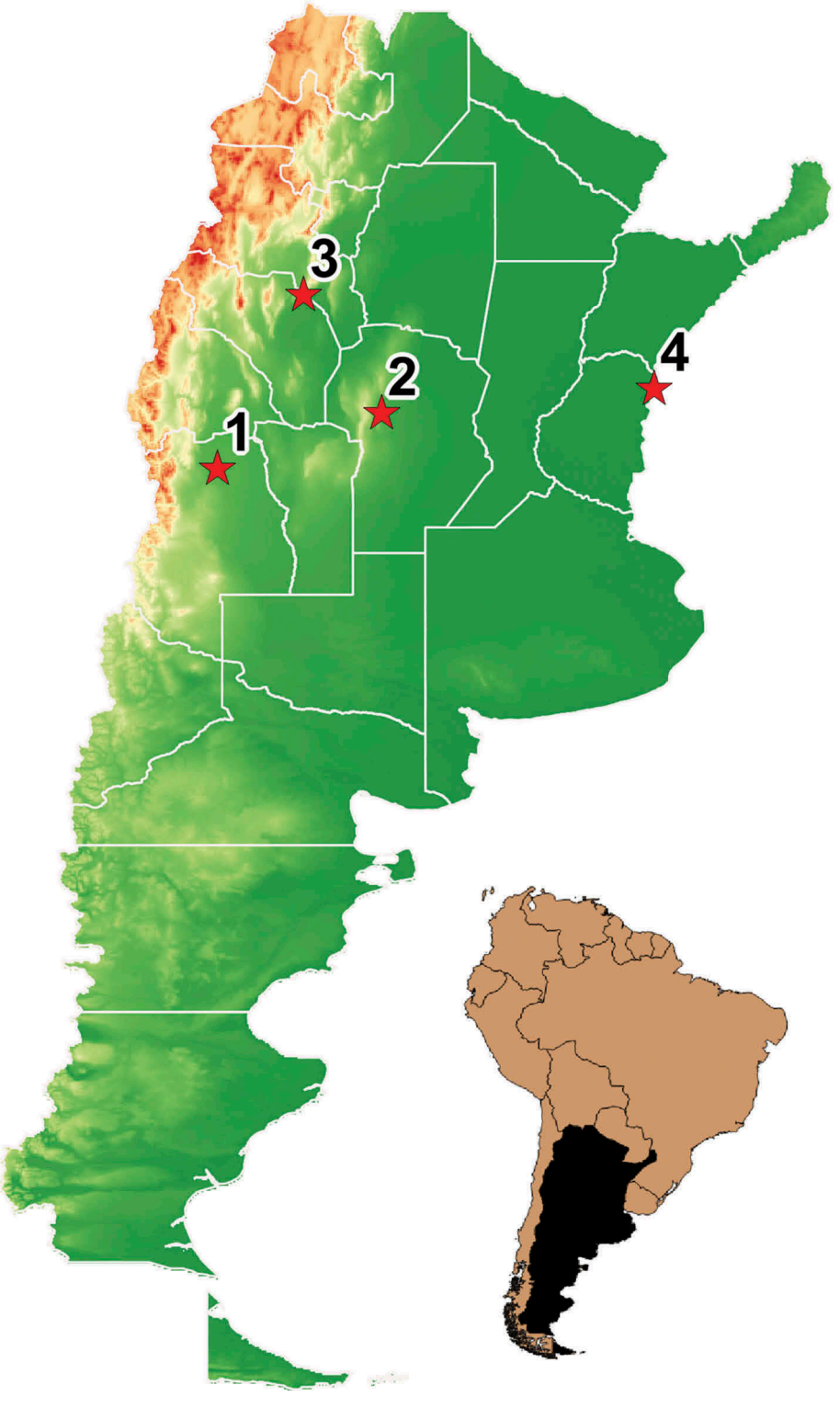

Figure 2. Records of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927 in Argentina. (1) vineyard located in San Martín, Mendoza, 32.985°S, 68.360°W; (2) Alta Gracia, Córdoba, 31.652°S, 64.428°W; (3) Catamarca, 28.816°S, 66.300°W; (4) Chaviyú Forest Reserve, Federación, Entre Ríos, 31.091°S, 57.930°W.

Adult identification

The publications of Thompson (1999) and Reemer and Ståhls (2013a) were used to identify the genus of the Argentinian specimens collected during the field work. Subsequently, we studied the original descriptions of all 25 Neotropical species listed under this subgenus by Reemer and Ståhls (2013a), plus the species diagnosed by Reemer (2014); notes, photographs of type specimens and material deposited in collections also were used to identify the specimens to species level.

Molecular studies (DNA barcoding)

One or two legs from dry pinned adult specimens, or a small section of ethanol-preserved larva, were used for DNA extraction. Extractions were carried out using the NucleoSpin Tissue DNA Extraction kit (Machery-Nagel, Düren, Germany) following the manufacturer's instructions; samples were resuspended in 100 µL of ultra-pure water. Remnants of specimens were preserved, labelled as DNA voucher specimens and deposited at the Zoological Museum Alexander Koenig [ZFMK], as listed in the *Material examined*. DNA primers and Polymerase Chain Reaction (PCR) amplification protocols for sequencing the 5′ end of the mitochondrial gene of the cytochrome c oxidase subunit I (COI) were the same as described in Mengual et al. (2008, 2012) and Rozo-Lopez and Mengual (2015). Amplified DNA was electrophoresed on 1.5% agarose gels for visual inspection of amplified products. PCR products were cleaned using the commercially available QIAquick PCR Purification Kit (QIAgen®). Bi-directional sequencing reactions were carried out by Macrogen© Inc. Chromatograms were edited for base-calling errors and assembled using Geneious 7.1.3 (Biomatters© Ltd).

Morphological studies on third-stage larvae

The morphological terminology used in the description of *Microdon* larvae follows Rotheray (2019). Third-stage larvae were examined using SEM. High-resolution images were obtained with a JSM-6610 LV microscope (JEOL, www.jeol.com) at the Laboratorio de Microscopía Electrónica de Barrido y Microanálisis (MEByM), CONICET-Mendoza, Argentina.

Three third-stage larvae were prepared for morphological study. The methodology and sample preparation were as follows: larvae were immersed in 25% acetone (C_3H_6O) and then gradually dehydrated by placing them in higher acetone concentrations (50%, 75% and up to 100%), at 15-min intervals. Then, larvae were critical-point dried using a Denton DCP-1 dryer, mounted on standard stubs, and finally gold sputtered using a Denton Vacuum Desk IV coater. The following morphological structures were analysed: cuticle surface area, posterior spiracle, marginal band (dorsal surface) and ventral surface.

Locomotion

In order to observe the locomotion movements, a third-stage larva of *M. bruchi* was kept in a Petri dish (9 cm diameter) and was filmed through an ®S6D Leica microscope equipped with an ®ES3 Leica camera.

Results

We identified the collected Argentinian specimens as *Microdon* (*Chymophila*) *bruchi* Shannon, 1927 based on the adult morphology (for details see Redescription and diagnosis). *Microdon argentinae* Hull, 1937 is here considered to be a junior synonym of *M. bruchi* (for details see below).

Microdon* (*Chymophila*) *bruchi Shannon, 1927
(Figures 3(a–l), 4(a–h), 5(a–g), 6)

Microdon bruchi Shannon, 1927: 38. (Lectotype: ♀, USNM; here designated. Type locality: Argentina, Córdoba, Alta Gracia).

Microdon argentinae Hull, 1937: 18. (Holotype: ♂, MCZ; by monotypy. Type locality: Argentina, Córdoba). New synonym.

***Microdon* (*Chymophila*) sp.** of Zubarán (2018).

Material examined

Lectotype of *Microdon bruchi* Shannon, 1927. ARGENTINA: 1♀; *Alta Gracia // Cord. 20.1.27 // R.C. Shannon' 'Laying eggs in // trail of ants' 'Microdon // bruchi // Shannon' 'Cotype //* No. *40822* // U.S.N.M' 'USNMENT 01295601' [barcode]; USNM coll. USNM type database: http://n2t.net/ark:/65665/395cc169c-24d2-42a0-a81c-cca5df2904f5.

Holotype of *Microdon argentinae* Hull, 1937. ARGENTINA: 1♂; Cordova. // Argent. Davis. Davis; *Microdon // argentina // FMH Hull*; 'M.C.Z. Type // *22218*'; MCZ coll.; MCZ type database: https://mczbase.mcz.harvard.edu/MediaSearch.cfm?action=search&media_id=115547,115548,115549,115550,115551.

Other studied material. ARGENTINA: 1♀; Catamarca Province, Trampasacha, 8 km W Chumbicha; 28.816°S, 66.300°W; alt. 650 m.; 24 October 2003; F.D. Parker and M.E. Irwin leg.; CSCA coll.; hand netted in damp wash. 1♀; Mendoza Province, San Martín, Estación Experimental INTA Junín; 32.985°S, 68.360°W; 19 September 2016; G. Debandi leg.; ZFMK coll.; ZFMK-DIP-00015973, ZFMK DNA voucher D291. 1♀; same locality as previous; 20 September 2016; ZFMK coll.; ZFMK-DIP-00015974, ZFMK DNA voucher D293. 1♂; same locality as previous; 17 September 2016; ZFMK coll.; ZFMK-DIP-00015975, ZFMK DNA voucher D296. 1♂; same locality as previous; 15 September 2016; ZFMK coll.; ZFMK-DIP-00015976, ZFMK DNA voucher D298. 1♂; same locality as previous; 5 November 2016; on *Camponotus mus* colony; RMNH coll.; ZFMK-DIP-00046334, ZFMK DNA voucher D369. 2♀; same locality as previous; 15 October 2016; ZFMK coll.; ZFMK-DIP-00046223, ZFMK DNA voucher D371; RMNH coll.; ZFMK-DIP-00046225, ZFMK DNA voucher D373.

Diagnosis

Body length: male 11–12 mm, female 12–14 mm. *Microdon bruchi* is assigned to the subgenus *Chymophila* based on the characteristic shape of wing vein M1, which has an outward angle and is anteriorly recurrent (Figures 4(a), 5(a) and 5(b)). The morphology of the male genitalia is also distinctive for the subgenus, with both phallic processes very

long and slender (Figure 6). *Microdon bruchi* belongs to the group of Neotropical non- (or only faintly) metallic species without conspicuous stripes of pale pile on the abdomen. Among these species, *M. bruchi* can be recognised by the combination of the following characters: metatibia with pile shorter than width of tibia; scutellum with calcars shorter than 1/3 of scutellar length; tergite 3 largely brown to black pilose with anterolateral patches of whitish pile; anterior fascia of whitish pile on mesoscutum absent or covering at most 1/3 of mesoscutum; scutellum at least partly pale pilose.

Redescription (based on lectotype)

(Figure 4(a–h)). Adult female. Body size: 12 mm. **Head**. Face occupying 0.44 of head width in frontal view; blackish brown; white pilose. Gena blackish brown; white pilose. Lateral oral margin weakly produced; blackish brown; white pilose. Frons and vertex blackish brown; white pilose, except vertex black pilose at level of ocellar triangle. Occiput blackish brown; white pilose. Eye bare. Antennal fossa about as wide as high. Antenna brown. Ratio of scape: basoflagellomere approximately 1:1.3. Basoflagellomere parallel sided with somewhat acute apex. Arista slender, about 2/3 length of basoflagellomere **Thorax**. Mesoscutum shining blackish brown; semi-erect black pilose, except for narrow uninterrupted fasciae of semi-erect white pile along anterior and posterior margins (width of anterior fascia is approx. 1/6 length of scutum, width of posterior fascia approx. 1/8). Postpronotum blackish brown; white pilose. Postalar callus blackish brown; white pilose. Scutellum blackish brown; white pilose; with two apical calcars of about 1/10 length of scutellum, with mutual distance approximately 1.4 length of scutellum. Pleura blackish brown. Anterior and posterior part of anepisternum separated by deep sulcus; white pilose anteriorly and posteriorly, with wide bare area in between. Anepimeron entirely white pilose. Katepisternum white pilose dorsally, bare ventrally. Other pleurae bare (except for microtrichiae). Calypter grey with black fringe, halter brown with blackish knob. **Wing** hyaline, brownish in antero-apical cells; veins around cell br and vena spuria yellow. Wing microtrichose, except cell br only microtrichose along vena spuria and at apical 1/8; cell bm widely bare along anterior and posterior margins, leaving wedge-shaped field of microtrichia with narrowest part at base of cell; cell cup bare on anterobasal 2/5; alula bare at basomedial 1/2. Legs blackish, pale brown pilose. **Abdomen** broadly oval, wider than thorax, widest at base of tergite 3. Tergites blackish brown with weak metallic sheen. Tergites 1 brown pilose. Tergites 2 brown pilose on lateral 1/4, white pilose medially. Tergites 3–5 brown pilose except white pilose at anterolateral corners and lateral margins. Sternites 1–5 brown; brown pilose.

Variation in additionally studied females

The additionally studied females (see *Material examined*) differ from the type of *M. bruchi* most notably in the colouration of the pile. The occiput is black pilose dorsally in the recently collected specimens, and all pile on the mesoscutum and pleura is black. The scutellum is white pilose, except black pilose on anterior 1/3 and along posterolateral margins. The legs are entirely black pilose. The tergites are black pilose on parts where holotype is brown pilose.

Male (based on the recently collected material from Argentina; see Material examined)

As for the female, except for the following differences: Occiput black pilose dorsally. Ratio of scape: basoflagellomere approximately 1:1.2. Width of anterior fascia of yellowish white pile on mesoscutum varies from approx. 1/8 to 1/3 of length of scutum; also, with yellowish white pile along transverse suture; white pile along posterior margin of mesoscutum absent to covering approx. 1/8 of length of scutum. Apical tarsomeres reddish brown to black. Tergite 3 with lateral margin reddish brown. Tergite 4 reddish brown, clearly getting paler laterally and posteriorly, but to variable degree among examined specimens; yellowish pilose laterally and on posterior half. Sternites 3 and 4 reddish brown. Sternites yellow pilose. Genitalia as in Figure 6.

Colour variation and synonymy

Colouration of body pile is quite variable among the studied specimens. Most notable is the variation in width of the fascia of pale pile along the anterior margin of the scutum. In males, its width ranges from approximately 1/8 to 1/3 of the length of the scutum. In females, the anterior fascia of pale pile is entirely absent in the studied specimens from Catamarca and Mendoza provinces, whereas in the lectotype specimen of *M. bruchi* its width is about 1/4 the length of the scutum. In the female specimen in the photos of Zubarán (2018, figs 16 and 17), an intermediate width is found of about 1/6 the length of the mesoscutum. As the studied specimens are highly similar in many other characters, we choose to consider them conspecific. Moreover, all specimens have the same host ant species, and the COI variability among the Mendoza specimens is less than 1%. Variation in pile colouration has previously been recorded in a number of other *Microdon* species (Akre et al. 1973; Thompson 1981, 2007; Wolton 2017). The male holotype of *Microdon argentinae* shares the diagnostic characters of *M. bruchi* with the holotype of the latter taxon: abdomen non-metallic without conspicuous stripes of pale pile, metatibia with very short pile, scutellum with small (shorter than 1/3 of scutellar length) calcars, tergite 3 largely black or brown pilose with anterolateral patches of pale pile, and mesoscutum with less than anterior half pale pilose. Consequently, *M. argentinae* is here considered to be a junior synonym of *M. bruchi* (Figure 5(a–g)).

The original descriptions of *M. bruchi* by Shannon (1927) and of *M. argentinae* by Hull (1937) are not entirely accurate with regard to the colour of the pile on the mesoscutum. Shannon (1927: 39) wrote '[Thorax …] clothed with short yellow hairs', but he did not mention the black pile clearly visible in the holotype. Hull (1937: 18) wrote 'Pile of thorax short, pale, appressed, with a few scattered darker hairs', whereas in the holotype much of the posterior half of the mesoscutum is covered with black pile. Shannon (1927: 39) observed a male *Microdon* at the site where he had collected the female. He noted that 'It was smaller in size and the abdomen was of a distinct brown colour'. This concurs with the reddish-brown colour we observed in the recently collected Argentinean males, as well as in the male holotype of *Microdon argentinae*.

Lectotype designation and additional remarks

In the original description of *M. bruchi*, Shannon (1927) studied two females on which the description was based, but he did not mention the holding institutions of these syntypes. Thompson et al. (1976) stated that one syntype is in the USNM collection and the other is

likely to be (question mark added) in the MACN collection. In our survey, only the female specimen deposited in the USNM was studied, and it is here designated as the lectotype to fix and ensure the universal and consistent interpretation of the name.

After the preparation of the identification key and the survey of the published literature and type material for Neotropical species, we could identify as *M. bruchi* the female specimen named as *Microdon* (*Chymophila*) sp. by Zubarán (2018, figs 16 and 17), photographed laying eggs in a decayed tree with a nest of *C. mus*. This female specimen represents the first record of *M. bruchi* in the Entre Ríos Province, Argentina.

DNA barcoding

We were able to successfully amplify and sequence the DNA barcodes (COI, 658 bp) for three males [D296, D298 and D396], four females [D291, D293, D371 and D373], eggs [D299], and two third-stage larvae [D301 and D303] (Table 1). The uncorrected pairwise distances among *M. bruchi* specimens ranged from 0% to 0.91%. The obtained variability among sequenced specimens suggests the presence of only one taxon, and confirms the identification of third-instar larvae and eggs collected in the field.

In BOLD (www.boldsystems.org/) there is no barcode of *M. bruchi*, but there are DNA barcodes of other *Chymophila* species such as *M. fulgens* Wiedemann, 1830. The closest taxon to our barcodes has 91.2% similarity, and it is not identified to the species level (listed as *Chymophila* sp.); the second closest has 89% similarity (*M. fulgens* specimen CNC DIPTERA 106171; Sequence ID: CNCDB3539-11 = GenBank accession number JN992012).

New information on third larval stage of Microdon bruchi

Mean body length = 11.2 mm, mean body width = 8.12 mm (N = 3). Body hemispheric, convex dorsally and greenish-blue in colour (Figure 3(a)).

Dorsal cuticle surface area

Dorsal tegument surface with reticulate pattern formed by star-shaped processes (Figure 3(b)). Each single star-shaped process showing two or sometimes three spines (Figure 3(c)).

Table 1. Specimens of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927 sequenced in the present study, with GenBank accession numbers for the COI sequences.

Unique identifier	Lab code	Stage and sex	GenBank accession number
ZFMK-DIP-00015973	D291	Adult, female	MK751121
ZFMK-DIP-00015974	D293	Adult, female	MK751122
ZFMK-DIP-00015975	D296	Adult, male	MK751123
ZFMK-DIP-00015976	D298	Adult, male	MK751124
	D299	Eggs (N = 7)	MK751125
ZFMK-DIP-00015977	D301	Third-instar larva	MK751126
ZFMK-DIP-00015978	D303	Third-instar larva	MK751127
ZFMK-DIP-00046334	D369	Adult, male	MK751128
ZFMK-DIP-00046223	D371	Adult, female	MK751129
ZFMK-DIP-00046225	D373	Adult, female	MK751130

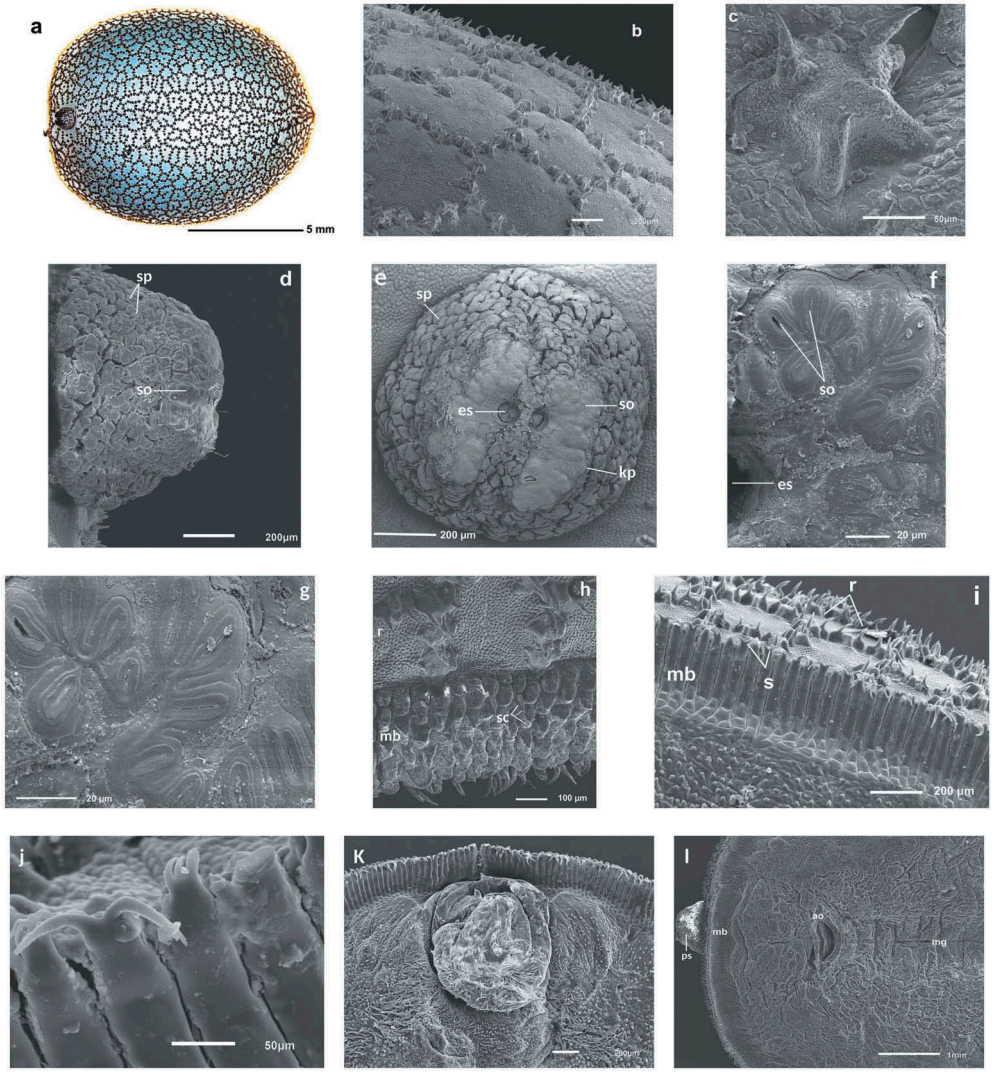

Figure 3. (a). General habitus of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927. Note the greenish-blue body colour. Image taken with a Leica S6D stereomicroscope equipped with a Leica ES3 camera. (b). Dorsal tegument surface, with reticulate pattern formed by star-shaped processes, of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927. (c). Single star-shaped process, showing spines, of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927. (d). Posterior spiracle of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, lateral view. (sp) scale-shaped plates, (so) spiracular opening. (e). Posterior spiracle of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, dorsal view. (sp) scale-shaped plates, (es) ecdysial scars, (kp) kidney-shaped plates. (f). Spiracular opening, forming ramifications, of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927. (es) ecdysial scars, (so) spiracular opening. (g). Detail of ramification and spiracular opening of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927. (h). Marginal band of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, lateral view. (r) reticulate pattern, (mb) marginal band, (sc) scale-like plates. (i). Edge of marginal band with spinules of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, ventral view. (r) reticulate pattern, (mb) marginal band, (s) spinules. (j). Detail of bifid and trifid spinules on marginal band edge of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, ventral view. (k). Anterior region of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, ventral view. (l). Posterior region of third-stage larva of *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, ventral view. (mg) medial groove, (ao) anal opening, (ps) posterior spiracle.

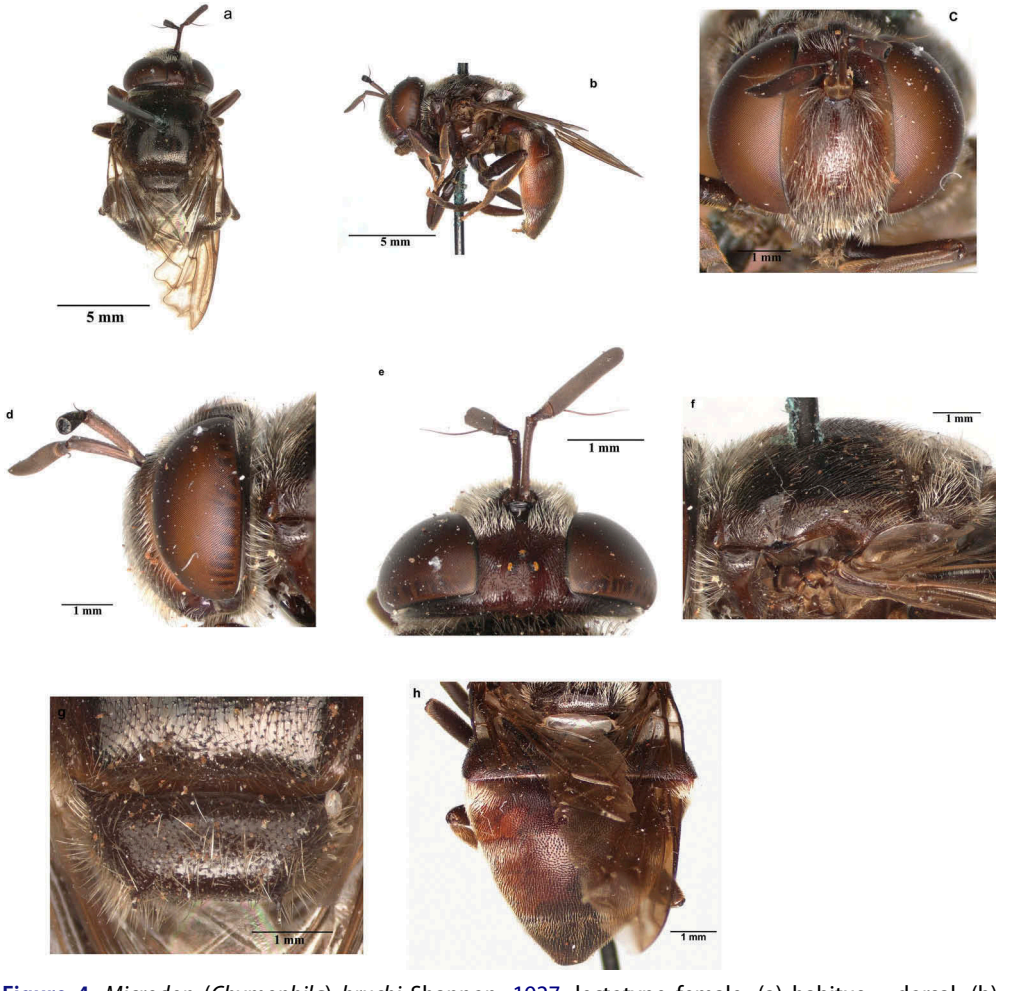

Figure 4. *Microdon* (*Chymophila*) *bruchi* Shannon, 1927, lectotype female. (a) habitus – dorsal; (b) habitus – lateral; (c) head – frontal; (d) head – lateral; (e) head – dorsal; (f) mesoscutum – lateral; (g) scutellum; (h) abdomen – dorsal.

Posterior spiracle

Posterior spiracle strongly sclerotised on dorsal region with truncated cone shape in lateral view, base covered laterally with scale-shaped plates (Figure 3(d)). Dorsal surface of posterior spiracle with scale-shaped plates, ecdysial scars and respiratory opening forming two kidney-shaped plates (Figure 3(e)). The respiratory opening forms ramifications (Figure 3(f,g)).

Marginal band

Marginal band processes appear as a continuous horizontal fringe on dorsolateral region of body, only absent on the small V-shaped anterior part, near the cephalic region. The marginal band is composed of scale-like plates (Figure 3(h)) and its edge has bifid and trifid spinules (Figure 3(i,j)).

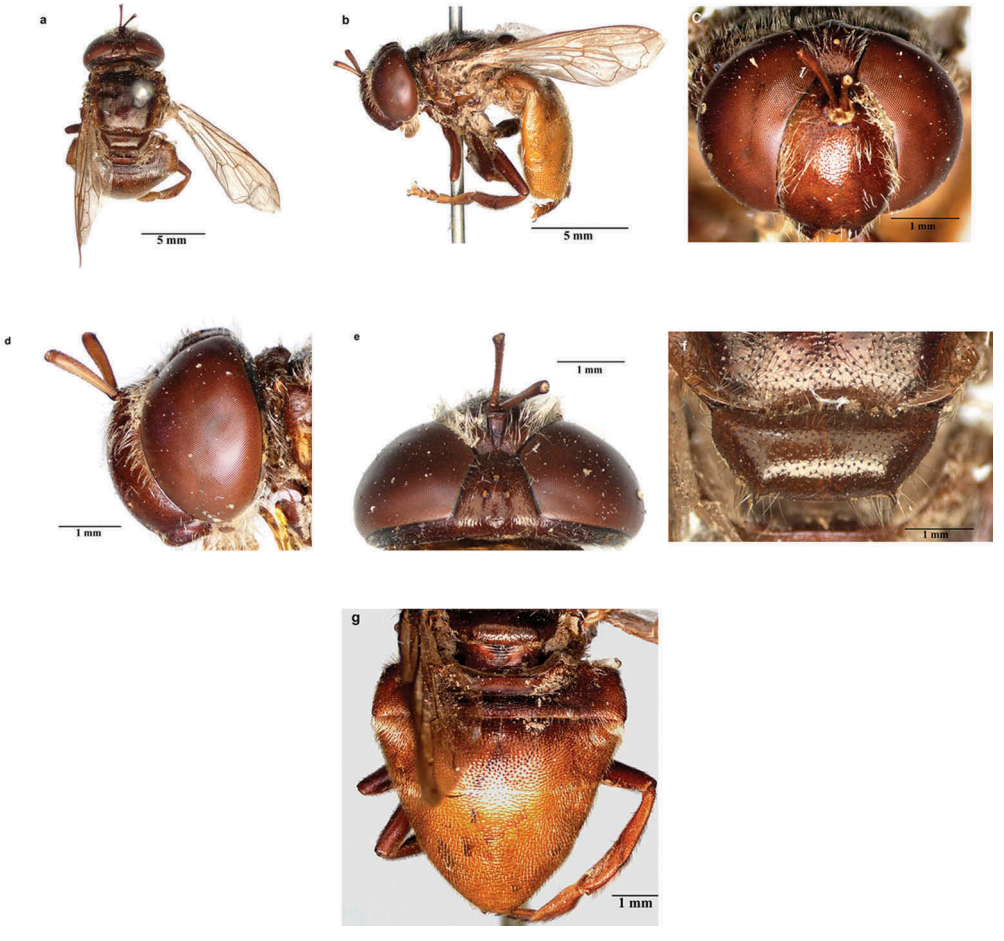

Figure 5. *Microdon* (*Chymophila*) *argentinae* Hull, 1937, holotype male. (a) habitus – dorsal; (b) habitus – lateral; (c) head – frontal; (d) head – lateral; (e) head – dorsal; (f) scutellum; (g) abdomen – dorsal.

Ventral surface

Anterior ventral region with cephalic segment retracted, mouthparts reduced and internal (pseudocephalon) with many sensillae producing high pilosity (Figure 3(k)). Posterior ventral region with anal opening and mid ventral surface containing a medial groove (Figure 3(l)).

Locomotion of third-stage larvae

Locomotion movements of ventral muscular plate (muscular foot) consist in repeated contraction and relaxation of muscles (peristaltic movement). When larva is moving forward, the peristaltic movements begin on the posterior region of the ventral plate (see supplemental material Video 1, third-stage larva of *M. bruchi* moving, ventral view). Conversely, the peristaltic movements start on the anterior region of the plate when the larva is moving backward; moreover, this larva can move sideways (see supplemental material Videos 2 and 3, third-stage larva of *M. bruchi* moving, ventral view). The head of the larva remains

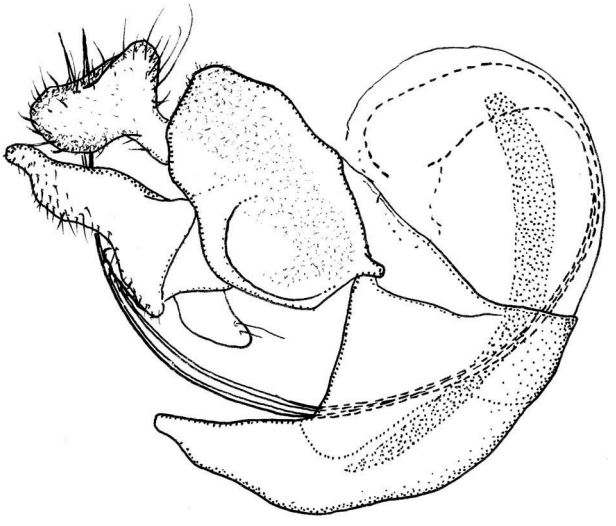

Figure 6. *Microdon* (*Chymophila*) *bruchi*, ZFMK-DIP-00046334, male genitalia, lateral view.

completely protracted during locomotion and is used to explore the environment (see supplemental material Video 4, third-stage larva of *M. bruchi* moving, ventral view).

Identification key for Neotropical species of Microdon (Chymophila)

The purpose of this key is to distinguish *Microdon* (*Chymophila*) *bruchi* from other Neotropical species of the subgenus *Chymophila*. The characters of the other species are only based on the type specimens, so their variability is unknown. Therefore, the utility of this key for distinguishing the other included species is probably very limited. The subgenus is in need of revision, but this is not the aim of the present study. Species of the subgenus *Chymophila* differ from other groups of *Microdon* in the characteristic shape of apical crossvein M1, which is recurrent in its anterior part (Figure 7). Reemer and Ståhls (2013a) may be used to key out the *Chymophila* species to subgenus level.

1. Abdomen with conspicuous pattern of pale pilose stripes (Figure 7) . . .. **striped-abdomen species group**

   *This group includes the following species: *Microdon flavoluna* Hull, 1943, *M. histrio* Wiedemann, 1830, *M. shannoni* Curran, 1940, *M. stramineus* Hull, 1943, *M. superbus* Wiedemann, 1830 and *M. tigrinus* Curran, 1940.
- Abdomen without conspicuous stripes of pale pile 2

2. Brightly metallic species, clearly metallic on both thorax and abdomen (Figure 8)..... **metallic species group**

   *This group includes the following species: *Microdon barbiellini* Curran, 1936, *M. dives* Rondani, 1848 (listed as a junior synonym of *M. instabilis* Wiedemann by Reemer and Ståhls 2013a, based on Thompson et al. 1976, but this needs confirmation), *M. emeralda*

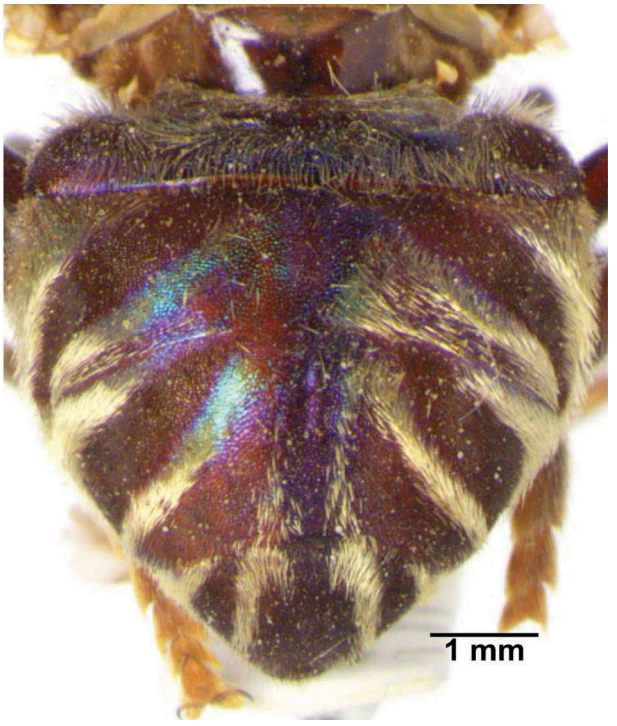

Figure 7. *Microdon* (*Chymophila*) *histrio* Wiedemann, holotype female, abdomen; representative of the 'striped-abdomen species group'.

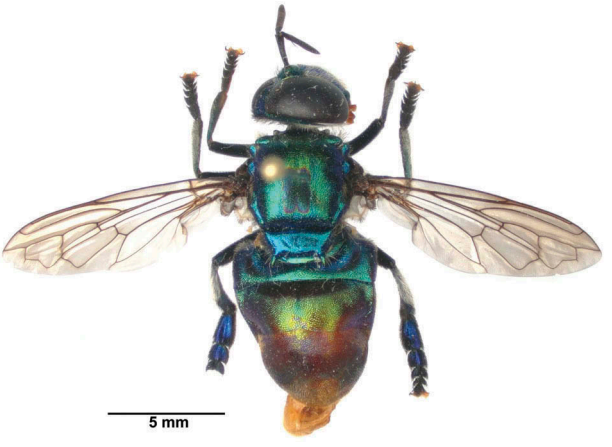

Figure 8. *Microdon* (*Chymophila*) SUR-02 of Reemer (2014), male, habitus; representative of the 'metallic species group' (Suriname, Peperpot, 24 February 2006, leg. M. Reemer, coll. RMNH).

Hull, 1943, *M. inaequalis* Loew, 1866, *M. instabilis* Wiedemann, 1830 (= *M. aurifex* Wiedemann, 1830; see Reemer 2014), *M. marceli* Curran, 1936, *M. opulentus* Bigot, 1883, *M. pulcher* Williston, 1887, *M. splendens* Wiedemann, 1830 and *M.* SUR-02 of Reemer (2014).

- Non-metallic species or only weakly metallic on abdomen 3

3. Metatibia with long, brush-like pile (stingless bee mimic), longest pile longer than width of tibia (Figure 9)..... ***angulatus*** Hull, 1943
- Metatibia with shorter pile, none longer than width of tibia 4

4. Scutellum without calcars..... ***willistoni*** Mik, 1899
- Scutellum with calcars 5

5. Scutellar calcars large, about 1/3 of scutellum length or longer (Figure 10)..... ***cyaneiventris*** (Macquart, 1846) and ***nero*** Curran, 1936 (differences between these taxa not studied)
- Scutellar calcars small, shorter than 1/3 of scutellar length (Figures 4(g) and 5(f))..... 6

6. Tergite 3 largely pale pilose or with large patches of pale pile on posterior half (Figure 11) ..... ***limbatus*** Wiedemann, 1830
- Tergite 3 largely black or brown pilose with anterolateral patches of pale pile (Figures 4(h) and 5(g))..... 7

7. Mesoscutum pale pilose on anterior half. Scutellum entirely black pilose (Figure 12) ..... ***aurifacius*** Hull, 1937 and ***cyaneus*** Perty, 1833..... ..... (differences between these taxa not studied)
- Mesoscutum with only anterior margin pale pilose (less than anterior half) or entirely black pilose. Scutellum with only anterior margin black pilose or entirely pale pilose..... ***bruchi*** Shannon, 1927

Discussion

The richest and most diverse fauna of Microdontinae is found in the Neotropical Region (Reemer 2013, 2014). As pointed out by Thompson (2007), although many species await description, the priority is to resolve the nomenclature after two centuries with little

Figure 9. *Microdon* (*Chymophila*) *angulatus* Hull, 1943, holotype male, hind leg.

Figure 10. *Microdon* (*Chymophila*) *cyaneiventris* (Macquart, 1846), syntype female, scutellum.

Figure 11. *Microdon* (*Chymophila*) *limbatus* Wiedemann, 1830, holotype male, habitus.

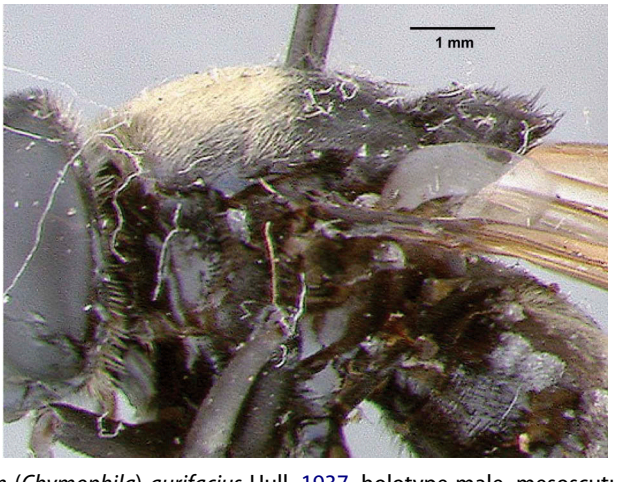

Figure 12. *Microdon* (*Chymophila*) *aurifacius* Hull, 1937, holotype male, mesoscutum, lateral view.

synthetic, monograph work – a common problem in the study of the Neotropical Syrphidae. The challenges are to understand the species and their characters, variation and distribution, then re-examine types of old names where available or original descriptions where types are lost, and finally work out synonymies if any, especially important for the Microdontinae. The present study helps with the systematics of the subgenus *Microdon* (*Chymophila*), improving the current knowledge of this subgenus in the Neotropics with a lectotype designation, a synonym and an identification key. Moreover, DNA barcodes corroborated the identity of eggs and larvae of *M. bruchi*, and they can be used in future studies for precise identification using molecular characters.

Following the above argument, the information on the third-stage larva of *M. bruchi* fills a gap in the morphological information for this species and will help in the morphological comparison with other Neotropical *Chymophila* specimens. SEM images revealed the morphology of the distinctive star-like structures present on the dorsal tegument of the third-stage larva of *M. bruchi*, a characteristic reticulate pattern (Figure 3(b,c)), as well as the particular morphology of the posterior spiracle (Figure 3(d–g)) and marginal band (Figure 3(h–j)) specific to this species. This information will allow the differentiation of *M. bruchi* from other third-stage larvae of Microdontinae (Wheeler 1908; Dixon 1960; Garnett et al. 1990; Rotheray 1991; Schmid 2004; Gammelmo and Aarvik 2007; Wolton 2011; Iwai et al. 2016; Scarparo et al. 2017).

The association of the syrphid fly *Microdon bruchi* with *Camponotus mus* has been known since its original description by Shannon (1927). Zubarán (2018) also recorded this association based on observations of ovipositing females. We corroborate this association based on our own findings and his figures (Zubarán 2018) and report a new record of *M. bruchi* inside *Vitis vinifera* plants from Mendoza, Argentina (Figure 1). To the best of our knowledge, this is the first record of a microdontine fly associated with crops, and these are new records of *M. bruchi* for Catamarca and Mendoza provinces, together with the records for Entre Ríos Province based on Zubarán (2018). This is particularly important considering that the host of *M. bruchi*, the 'carpenter ant' *C. mus*, can deteriorate wooden structures (Josens et al. 2017; Werenkrut et al. 2017), such as posts, and also uses decaying wood for nesting inside of living plants (Chiesa Molinari 1942), like those in Argentinian vineyards (Debandi personal observations). From an agronomic perspective, it would be beneficial to closely explore the impact of *M. bruchi* on the populations of *C. mus* in vineyard nests, as well as in wild nests, to assess its potential role as a biocontrol agent of the carpenter ant.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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