

Redescription of *Mononeuron duguetiae* Fischer (Braconidae, Doryctinae), a gall associated species on *Duguetia furfuracea* (St. Hil.) (Annonaceae)

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Abstract

We redescribe the poorly known, gall associated doryctine wasp *Mononeuron duguetiae* Fischer (Braconidae) based on museum and recently collected material. This species shares various morphological features with the members of the also gall associated *Allorhogas* Gahan, suggesting their close relationship. A Bayesian phylogenetic analysis using mitochondrial (COI) and nuclear (28S) markers recovered *M. duguetiae* within a clade with another five gall associated doryctine genera + *Heterospilus* Haliday. Surprisingly, *M. duguetiae* was recovered, though with low support, as the sister taxon of a *Monitoriella* Hedqvist + *Labania* Hedqvist clade, and not closely related to *Allorhogas*. Information is provided about the galls on *Duguetia furfuracea* (St. Hil.) (Annonaceae) where the specimens of *M. duguetiae* were reared.

Keywords

redescription, Braconidae, Doryctinae, gall associated, *Mononeuron*

Introduction

The braconid subfamily Doryctinae is a cosmopolitan, highly diverse group of parasitoid wasps containing more than 1,300 described species (Yu et al. 2005), although this probably only represents half of its actual species diversity (Jones et al. 2009).

Species of this subfamily are grouped into about 200 recognized genera, some of which are considerably species rich with wide geographical distributions (e.g. *Heterospilus* Haliday, *Spathius* Nees, *Notiospathius* Matthews and Marsh, *Allorhogas* Gahan).

The doryctine genus *Mononeuron* Fischer was erected by Fischer (1981) to contain a single species, *M. duguetiae* Fischer, reared from galls on *Duguetia furfuracea* (St. Hil.) (Annonaceae) at a locality in the state of São Paulo, Brazil. In this work, Fischer distinguished *Mononeuron* from other doryctine genera by its reduced hind wing vein cu-a, first metasomal tergite without wing-like projections, and remaining metasomal tergites without grooves. These features, however, are also present in other doryctine genera. *Mononeuron duguetiae* instead appears to be morphologically very similar to the species of the mainly New World *Allorhogas* Gahan, which are also known to be gall associated.

Here, we redescribe *M. duguetiae* based on museum (including the holotype specimen) and recently collected material, and provide information about the galls where the species was reared. We also employed one mitochondrial (mt) [cytochrome oxidase I (COI)] and one nuclear [28S ribosomal (r) RNA gene] marker to elucidate the phylogenetic affinities of this taxon.

Methods

All the specimens examined in this study were collected in the type locality of the species, the vegetation of which consists of the remains of Brazilian savannah ('cerrado') forest owned by the Universidade Federal de São Carlos, in São Carlos, São Paulo, Brazil. Some of the specimens were reared from galls on *D. furfuracea* in February of 2011. The specimens were preserved in 100% ethanol and kept at -20°C until they were mounted or processed for DNA sequencing. The examined material is deposited in the Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, São Carlos, SP, Brazil (DCBU), the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México (CNIN IB-UNAM), and the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN).

The terminology employed follows Sharkey and Wharton (1997), except for the surface sculpture, which follows Harris (1979). Colour digital photographs were taken and edited with a Leica® Z16 APO-A stereoscopic microscope, a Leica® DFC295/DFC290 HD camera, and the Leica Application Suite® program. Digital SEM photographs were taken with a FEI Quanta™ 250 SEM in low vacuum mode.

Phylogenetic affinities of *Mononeuron* based on DNA sequence data

The phylogenetic placement of *Mononeuron* was assessed using DNA sequences of one fast evolving (629 bp of the COI mtDNA gene) and one slow evolving [-650 bp of

the second and third domain regions of the 28S rRNA gene] gene marker. Two COI and one 28S sequence were generated from specimens of *M. duguetiae* (COI: GenBank accession nos JQ320372-3; 28S: GenBank accession no. JQ320371). Protocols for genomic DNA extraction of the samples (using a non-destructive technique) and amplification of the PCR products were the same as those employed by Ceccarelli et al. (2012). Amplification of the COI and 28S products employed the LepF1/LepRI (Hebert et al. 2004) and the 28Sfwd/28Srev primers (Belshaw and Quicke 1997; Mardulyn and Whitfield 1999), respectively. PCR products were sent directly to the High-Throughput Genomics Unit at the University of Washington (<http://www.ht-seq.org/index.html>) for sequencing. All sequences were edited using Sequencher 4.1.4 (Gene Codes Corp.).

COI genetic distances between the two sequenced specimens of *M. duguetiae* and among these and previously published sequences of species belonging to *Heterospathius*, *Heterospilus* and to the gall associated doryctine genera *Allorhogas* Gahan, *Labania* Hedqvist, *Monitoriella* Hedqvist, *Percnobracon* Kieffer & Jörgensen and *Psenobolus* Reinhard were calculated using the K2P distance model (Kimura 1980) with PAUP version 4.0b10 (Swofford 2002). GenBank accession numbers for the above taxa are found in Zaldívar-Riverón et al. (2008). *Heterospilus* was recovered in previous studies as the sister group of a clade with the species of the above gall associated doryctine genera (Zaldívar-Riverón et al. 2007, 2008). The phylogenetic affinities of *M. duguetiae* were assessed by including the 28S and COI sequences of one specimen of *M. duguetiae* in a previously published COI+28S matrix (Zaldívar-Riverón et al. 2008) composed of 94 species belonging to 64 different doryctine genera. This matrix excluded the regions of ambiguous alignment detected for the 28S marker from the analyses performed.

A Bayesian MCMC partitioned analysis was run with MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003) through the University of Oslo bioportal (<http://www.bioportal.uio.no/>). The analysis employed two simultaneous runs of 10 million generations each, using four chains, default priors, and four partitions, one for 28S and three for COI according to its first, second and third codon positions. The evolutionary model employed for each partition was obtained with MrModeltest version 2.3 (Nylander 2004) and PAUP version 4.0b10 (Swofford 2002) using the Akaike information criterion. The first five million sampled trees of each run were deleted and the remaining trees were pooled to build a majority rule consensus tree, considering posterior probability (PP) of clades ≥ 0.95 as significantly supported (Huelsenbeck and Ronquist 2001).

We used a Bayesian approach (Buckley et al. 2002; Reeder 2003) to test for an alternative topology not present in the 50% majority rule consensus trees derived from the two simultaneous analyses. This alternative topology places the three included specimens of *Allorhogas* and *M. duguetiae* as monophyletic. Details about this approach are found in Zaldívar-Riverón et al. (2008).

Results

Phylogenetic relationships

Corrected COI genetic distances between the two specimens of *M. duguetiae* and among the latter specimens and the species belonging to other gall associated genera were of 0.5% (3 bp) and of 13.5 to 16.5% (71 to 86 bp), respectively. Corrected COI distances among species of the above gall associated taxa and species of *Heterospilus* varied from 14.4 to 21.1 % (79 to 111 bp). Our Bayesian phylogram recovered the same major doryctine clades obtained in Zaldívar-Riverón's (2008) phylogenetic study: a South American, an Australian, a Holcobraconini, and an African-Holarctic-Madagascan clade. Within the South American clade, *M. duguetiae* is nested within a significantly supported clade (PP= 1.0) composed of members belonging to the five gall associated doryctine genera and the included species of *Heterospilus* Haliday (Fig. 1). This latter clade is composed of two subclades, one (PP = 0.92) with *Perconobracon* at the base of a monophyletic *Allorhogas* (PP = 0.96) that are sister to a cluster containing *M. duguetiae* and *Monitoriella* + *Labania* (PP = 0.94), and a second one with a *Heterospilus* + *Heterospathius* cluster that is sister to *Psenobolus* (PP = 0.95).

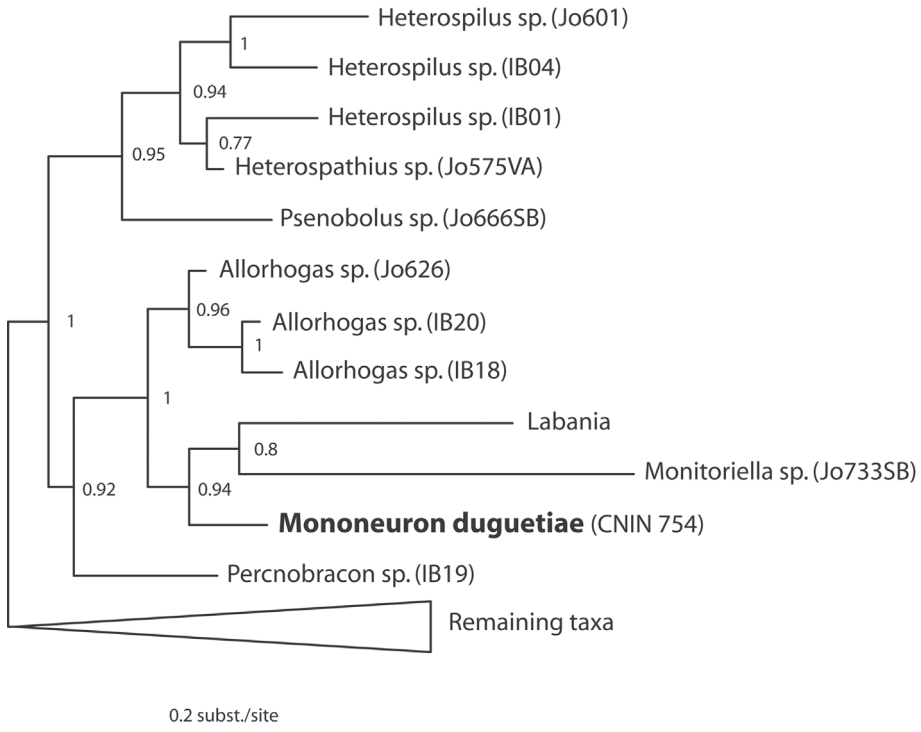


Figure 1. Part of the Bayesian phylogram obtained in this study showing the phylogenetic affinities of *M. duguetiae* Fischer with respect to other gall associated doryctine genera (see results for details of the complete reconstructed topology). Numbers near clades represent posterior probabilities.

The Bayesian test showed that the alternative topology with the three included specimens of *Allorhogas* and *M. duguetiae* as monophyletic is present in some of our 95% credible set of trees sampled from Bayesian analysis. We therefore cannot statistically reject the paraphyly of *Allorhogas* with respect to *M. duguetiae*.

Taxonomy

Mononeuron Fischer

<http://species-id.net/wiki/Mononeuron>

Mononeuron Fischer, 1981: 47–51 (original description); Marsh 1997: 214 (Key); Yu et al. 2005 (catalogue).

Type species. *Mononeuron duguetiae* Fischer

Diagnosis. *Mononeuron* may be distinguished from the remaining doryctine genera by having the following combination of features: frons excavated; occipital carina meeting hypostomal carina; propodeum with semicircular or subtriangular shaped basal median areas (Fig. 2C); fore wing vein r-m present (Fig. 3C); first subdiscal cell of fore wing open at apex (Fig. 3C); hind wing vein SC+R absent (Fig. 3D); hind wing vein cu-a absent or spectral (Fig. 3D); hind wing vein 1A incomplete (Fig. 3D); hind wing vein M+CU equal to or slightly greater than vein 1M (Fig. 3D); hind wing vein m-cu slightly curved toward wing apex or straight to wing margin but never angled toward wing base (Fig. 3D); hind coxa with a distinct baso-ventral tubercle; basal sternal plate of first metasomal tergum less than 0,25 length of tergum; metasoma sculptured on first three terga (Fig. 2C); ovipositor apex strongly sclerotized and with a single nodus (Fig. 2D).

Mononeuron is morphologically similar to *Allorhogas*, but it can be distinguished from members of this genus by having the vertex at least partially striate (Fig. 2B) (coriaceous in all described species of *Allorhogas*); hind wing vein SC+R absent (present in all described species of *Allorhogas* except *A. ingavera* Marsh); hind wing vein cu-a absent or spectral (sclerotized or nebulous in most described species of *Allorhogas*, only spectral in *A. argentinus* Brèthes and *A. shawi* Marsh); hind wing vein 1A incomplete (always reaching vein cu-a in other described species of *Allorhogas*); ovipositor long, about 1.5 times length of metasoma (Fig. 3B) (generally shorter than metasoma in most of the described species of *Allorhogas*, longer than metasoma in *A. gallicola* Gahan).

Mononeuron duguetiae Fischer, 1981: 47–51.

http://species-id.net/wiki/Mononeuron_duguetiae

Description. Female. *Colour.* Body and first three flagellomeres honey yellow, remaining flagellomeres, fifth tarsomeres and ovipositor sheath light brown (Fig. 3B). *Body*

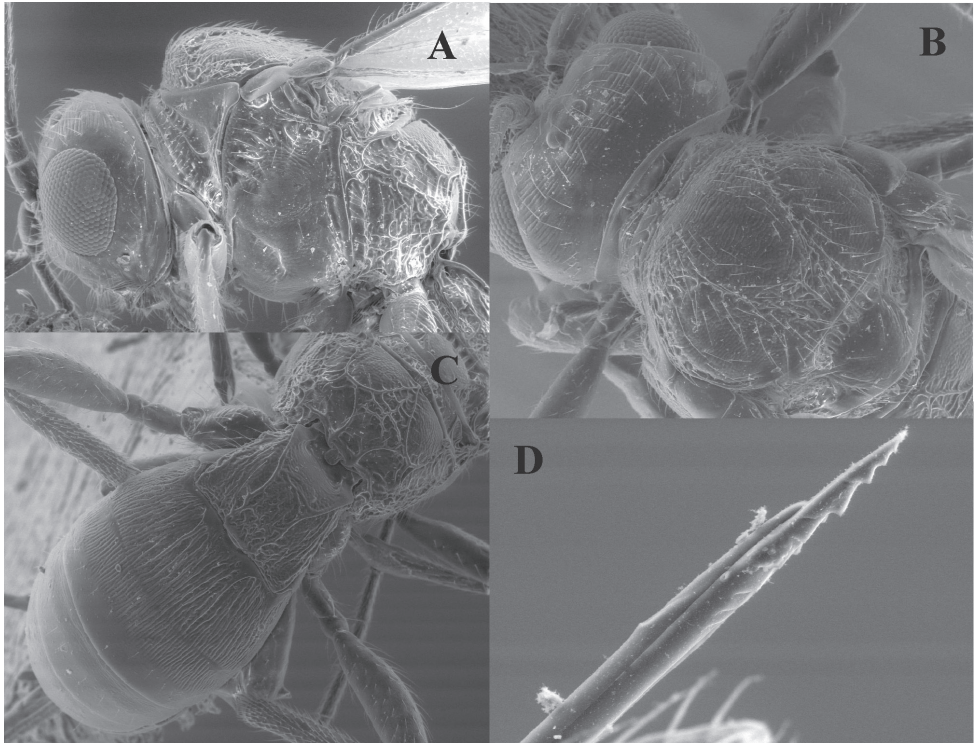


Figure 2. *Mononeuron duguetiae* Fischer (holotype, female) **A** head and mesosoma, lateral view **B** head and mesoscutum, dorsal view **C** metasoma, dorsal view **D** ovipositor.

length: 2.5 mm; ovipositor 2.5 mm. *Head:* Clypeus smooth, face and frons smooth, frons slightly excavated, vertex slightly striate laterally and anteriorly, smooth posteriorly (Fig. 2B), temple and gena smooth (Fig. 2A); eye 1.45 times higher than wide (lateral view); malar space 0.45 times eye height (lateral view); temple 0.46 times eye width (dorsal view); ocular-ocellar distance 3.3 times diameter of lateral ocellus; length of scape 1.4 times its width (frontal view); antenna with 20 flagellomeres. *Mesosoma:* Length of mesosoma 1.5 its maximum height; pronotum smooth laterally, pronotal collar short (Fig. 2A); pronotal groove weakly scrobiculate, propleuron smooth; mesoscutal lobes coriaceous; notauli shallow but distinct, obscured before scutellum at middle of mesoscutum in a costate-rugose area (Fig. 2B); scutellar disc slightly coriaceous; median length of scutellar sulcus 0.33 times median length of scutellar disc, with six carinae (Fig. 2B); mesopleuron coriaceous, weakly coriaceous medially (Fig. 2A); subalar groove scrobiculate; precoxal sulcus wide, smooth, 0.5 length of mesopleuron; venter of mesosoma slightly coriaceous; metapleuron strongly rugose; propodeum with a pentagonal areola diverging at the base of propodeum, areolar area rugose, basal median areas coriaceous (Fig. 2C). *Wings:* Fore wing length 2.75 times its maximum width, length of pterostigma 3.2 times its maximum width, vein r about 0.5 times length of vein 3RSa, vein 3RSb reaching the wing margin as a tubular vein; vein m-cu

interstitial to vein 2RS, vein 1cu-a distinctly postfurcal with vein 1M (Fig. 3C); hind wing vein SC+R absent; vein cu-a absent (Fig. 3D); vein m-cu nebulous (Fig. 3D); vein 1A reduced, only present basally; vein M+CU about equal length of vein 1M (Fig. 3D). *Legs*: Fore tibia with a row of 12 spines; hind femur weakly coriaceous, length 3.86 times its maximum width; hind coxa weakly striate dorsally, coriaceous laterally, with a distinct basal tubercle. *Metasoma*: Length of first metasomal tergum 0.9 times its apical width, median area slightly coriaceous basally, costate-rugose apically, costate-rugose laterally, with complete longitudinal lateral carinae; dorsope present, acrosternite 0.2 times length of first metasomal median tergite; second metasomal median tergite longitudinally costate (Fig. 2C); third metasomal median tergite costate on basal half, smooth on apical half (Fig. 2C); remaining metasomal median tergites smooth; ovipositor 1.8 times length of metasoma. Ovipositor strongly sclerotized apically, with a single nodus (Fig. 2D).

Male. Slightly smaller than female. Body length 2.0–2.5 mm; hind femur swollen, length 2.35 times its maximum width.

Variation. Females: Antenna with 16–21 flagellomeres; scutellar sulcus with 5–6 carinae.

Biology. We collected 20 leaves of *D. furfuracea* infested with galls at the type locality of *M. duguetiae* in February of 2011. All leaves were placed in a plastic bag and maintained at room temperature. Each of the collected leaves had between three to 10 spheroidal galls with a maximum diameter of approximately 1 cm (Fig. 3A). Twenty five specimens of *M. duguetiae* emerged from the galls a few days after being collected, and two or more individuals were observed to emerge from each gall. Moreover, two specimens of an unidentified cecydomiid species and the following wasp taxa emerged after most of the specimens of *M. duguetiae* had emerged: Torymidae (six specimens belonging to an unidentified species), Eurytomidae (13 specimens of an unidentified species assigned to *Sycophila* Walker, and eight specimens of an unidentified species), Eulophidae (21 individuals of an unidentified species), and Diapriidae (one unidentified specimen). Members of some of these wasp families are usually parasitoids (Torymidae, Eulophidae), whereas others are generally phytophagous (e.g. Eurytomidae). We could not confirm that *M. duguetiae* is the species that induces the above galls, though its early emergence suggests that it might be cecidogenic.

Comments. The genus *Mononeuron* has been mainly characterised by the absence of hind wing vein cu-a. This feature was employed to distinguish the genus in the key to New World doryctine genera provided by Marsh (1997). We found that some of the examined specimens of *M. duguetiae* have hind wing vein cu-a spectral (*sensu* Mason 1986) but present. Some described species of *Allorhogas* also have a nebulous or even spectral hind wing vein cu-a (e.g. *A. argentinus*, *A. shawi*; Marsh 2002).

In our Bayesian analysis, *M. duguetiae* was more closely related to *Monitoriella* and *Labania* than to the species of *Allorhogas*, although with marginally non-significant support. This contrasts with our morphological examinations, which show that there are various external morphological features in *M. duguetiae* shared with species of *Allorhogas*. The latter genus is a mainly Neotropical and Nearctic group with 33

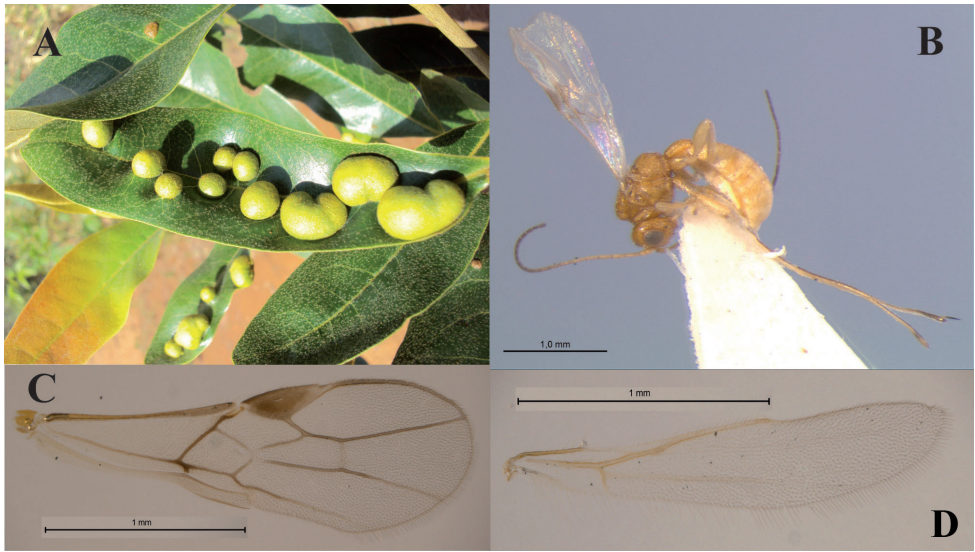


Figure 3. **A** Galls on *Duguetia furfuracea*. **B–D** *Mononeuron duguetiae* Fischer (holotype, female). **B** Habitus, lateral view **C** fore wing **D** hind wing.

described and an undetermined number of undescribed species. Among the features shared by *M. duguetiae* and members of *Allorhogas* are an excavated frons, hind wing vein r-m present, pronotal collar very short or absent, propodeum usually with semicircular or subtriangular shaped basal median areas, basal sternal plate of first metasomal tergum less than 0.25 length of tergum; metasoma sculptured on first three terga, hind coxa with small but distinct basal tubercle, fore wing vein r-m present, first subdiscal cell open at apex, and hind wing vein m-cu usually curved toward wing apex, often straight to wing margin but never angled toward wing base. Further molecular phylogenetic studies employing additional markers and more taxa, especially for the species of *Allorhogas*, will confirm the relationships of *M. duguetiae* with respect to other gall associated doryctine genera.

We also examined various specimens assigned to *M. duguetiae* deposited in the DCBU collection that show some morphological differences, and which might represent an undescribed species. These specimens were collected in the type locality of *M. duguetiae* and all of them were reared from the same type of galls on *D. furfuracea*. They differ from our concept of *M. duguetiae* by having the vertex, mesopleuron and basal areas of propodeum mostly smooth, and the first metasomal median tergite smooth and with brown colour. Further molecular data will allow us to confirm whether this represents an undescribed species of *Mononeuron* or intraspecific variation within *M. duguetiae*.

Examined material. Holotype (DCBU).—Female. Brazil, São Carlos, São Paulo, geschlüpft von Gallen von *Duguetia furfuracea* ST. HILL. (Annonaceae). Other examined material: 31 specimens, 12 males, 19 females. Twelve specimens: Brazil, SP, São Carlos, UFSCar, Cerrado forest, 21°58'7.96"S, 47°53'9.68"W, ex. *Duguetia furfuracea*

galls, 26 January 2011, A. Zaldívar-Riverón and J. Nunes coll. Nineteen specimens: same locality as above, 18 March to 11 April 2008, A. M. Penteado-Dias, coll.

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