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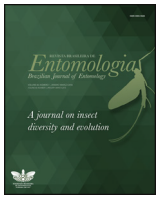

Diolcogaster choi sp. nov. from Brazil, a new gregarious microgastrine parasitoid wasp (Hymenoptera: Braconidae) reared from *Hypercompe cunigunda* (Lepidoptera: Erebidae) in Brazil

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ABSTRACT

A new species of *Diolcogaster* (Hymenoptera: Braconidae) is described and illustrated. Additionally, its position within the recently published key to New World species of the *xanthaspis* species-group (to which the described *Diolcogaster* belongs) is provided. The gregarious larval parasitoid *Diolcogaster choi* sp. nov. was collected in Maringá, Paraná State, Brazil. This natural enemy was recovered from a caterpillar of *Hypercompe cunigunda* (Stoll, 1781) (Lepidoptera: Erebidae) that was feeding on plant of passionflower, *Passiflora edulis* Sims (Passifloraceae). The fauna of the *xanthaspis* group in the New World now includes five species, including the new species from Brazil described in this paper. *Diolcogaster choi* sp. nov. differs anatomically, and is morphologically diagnosed, from all other known member of *the xanthaspis* group of the genus *Diolcogaster*, to which it belongs. The species also differs in recorded host, and its DNA barcode appears to be distinctive among described *Diolcogaster*.

Introduction

The genus *Diolcogaster* belongs to the subfamily Microgastrinae (Hymenoptera: Braconidae) which comprises 2,700+ described species (Yu et al., 2016; Whitfield et al., 2018) and many thousands of additional undescribed ones (Rodriguez et al., 2013; Fernández-Triana and Boudreault, 2016; Fernández-Triana and van Atcherberg, 2017; Moghaddam and Mokhtari, 2017; Fernández-Triana, 2018). Microgastrinae is the single most important group of parasitoid wasps attacking caterpillars (Whitfield, 1997; Avila et al., 2013; Fernández-Triana et al., 2014; Pereira et al., 2015; Fiaboe et al., 2017), with many species used or being considered as biocontrol agents against lepidopteran pests in agriculture and forestry (Yeargan and Braman, 1986; Smith et al., 2013; Pinto et al., 2014). *Diolcogaster* is currently the seventh largest genus of Microgastrinae, with 101 species described worldwide (Zeng et al., 2011; Fernández-Triana, 2015; Fernández-Triana et al., 2016; Moghaddam et al., 2019) and hundreds awaiting description. Currently this genus has 18 species recorded from the New World, 11 from the Neotropical region, and five in Brazil (Gupta and Fernández-Triana, 2014, 2015; Yu et al., 2016; Salgado-Neto et al., 2018; Whitfield et al., 2018).

This paper describes a new *Diolcogaster* species from Brazil, perhaps with potential for the biological control of *Hypercompe cunigunda* (Stoll, 1781) (Lepidoptera: Erebidae), pest of the passionflower *Passiflora edulis* Sims (Passifloraceae; Spencer and Seigler, 1983) in the state of Paraná. This plant is economically significant due to its pharmacological and medicinal properties (Patel, 2009) and food commercial value chain.

The genus *Hypercompe* occurs from the USA through the Caribbean to Argentina and Bolivia, and contains 89 species (Vincent and Laguerre, 2014). *Hypercompe cunigunda* is found from French Guiana, Suriname, Peru, Brazil, Venezuela, Ecuador, Bolivia and Guatemala

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(Silva et al., 1968; Nava et al., 2008; BoldSystems, 2019) and was described using individuals collected of samples from Paraná, Brazil. Larvae of this species have been recorded feeding on the palm *Syagrus romanzoffiana* (Cham.) Glassman (Arecaceae) (Robinson et al., 2010) as well as *Melothria pendula* L. (Cucurbitaceae) (Gernaat et al., 2016), but there are few published studies on this species, and probably few if any other *H. cunigunda* host plants have been reported in the scientific literature. Larvae of Erebidae (Arctiidae in the old classification) including *Hypercompe* spp., generally are polyphagous and feed on a variety of species and individuals plants, even during the same day (Singer and Bernays, 2009). Some of these species, such as *Hypercompe indecisa* (Walker, 1855), have been reported as a pest of leaves and ears of corn; however in recent years this species has also been reported to cause damage in the early stages of the crop, feeding at the base of the soil and causing tipping of the plant. Besides corn, they also feed on leaves of more than twenty host plants, including various vegetables, potatoes, cabbage, lettuce and tomatoes. In addition, the fruit trees avocado, persimmon, strawberry and peach have been recorded as hosts. Although *Hypercompe* was reported as a corn pest in 1968 (Silva et al., 1968) little is known about its biology, ecology and biological control (Nava et al., 2008; Gernaat et al., 2016).

The new *Diolcogaster* parasitoid wasp species, described in this paper, should be considered for future studies about its possible potential for biological control of *Hypercompe cunigunda* caterpillars that cause crop damage on cultivated passion fruits.

Materials and methods

One last instar caterpillar of *H. cunigunda* was observed feeding on leaves of passion fruit, *Passiflora edulis,* on 28 December 2018, in the garden of one of the authors (Í. M. Medri), in Maringá, (Fig. 1), Paraná, Brazil (23º 24' 22"S 51º 55' 53"W; 553 m above sea level).

The caterpillar was collected with a fine-tipped brush, placed in a 500 mL plastic container lined with filter paper with the lid aerated, and with leaves provided *ad libitum* of the respective host plant as food and substrate for pupation (Tavares et al., 2012). On the next day, 39 cocoons were observed on the caterpillar's exterior after it had died. After 12 days, in 9 January 2019, 39 adult wasps emerged from the cocoons, 14 individuals were released and 25 wasps were sacrificed using ethyl ether and preserved by immersion in 70% ethanol. The caterpillar, *H. cunigunda*, and 5 open cocoons also were preserved by immersion in 70% ethanol and were sent to Geraldo Salgado-Neto of "Coleção de Parasitóides do Laboratório de Biologia Evolutiva da Universidade Federal de Santa Maria – UFSM", with registration *Hypercompe cunigunda* Erebidae 2019 and *Diolcogaster* Ísis 2019, respectively.

A total of 25 wasps collected (22 females and 3 males) had the following destination: a) 2 females and 1 male were sent to Alvaro Doria dos Santos of "Museu de Zoologia da Universidade de São Paulo – MZUSP"; b) 5 females and 1 male were sent to José L. Fernández-Triana of Canadian National Collection (CNC) of Insects, Arachnids, and Nematodes; c) 5 females and 1 male were sent to James Bryan Whitfield of Illinois Natural History Survey – INHS Insect Collection; d) 5 females were sent to Geraldo Salgado-Neto of "Coleção de Parasitóides do Laboratório de Biologia Evolutiva da Universidade Federal de Santa Maria – UFSM"; e) 4 females were sent to Ricardo Harakava of "Instituto Biológico" for analyses of the COI barcode; f) 1 female stayed with the collector Ísis Meri Medri for provide part of pictures for article illustration.

Photographs of the wasps were taken using DSLR camera with a 100mm macro lens, in Brazil, and with digital camera photography attached to stereoscopic microscopes, at the University of Illinois. Morphological terms and measurements of structures are mostly those used by Fernández-Triana et al. (2014).

To check the molecular-specific characterization of the new species, the mitochondrial gene Cytochrome Oxidase I (COI) was analyzed. For the amplification of a fragment of approximately 460 bp of this gene, we used the following primer pair: COI-F (5'-GATTTTTTGGKCAYCCMGAAG-3') and COI-R (5'CRAATACRGCTCCTATWGATAAWAC-3') (Gusmão et al., 2010). DNA extraction of one specimen was performed with the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich®) and followed the manufacturer's protocol. The product was amplified via PCR according to the following schedule: 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 40 seconds and 72°C for 4 minutes. Then the PCR product was purified using polyethylene glycol precipitation (PEG; Schmitz and Riesner, 2006). These samples were sequenced using the Big Dye 3.1 reagent (Life Technologies®) and 3500 xL automatic sequencer (Life Technologies®).

Molecular identification

The molecular characterization of *D. choi* was carried out by sequencing the mtDNA COI gene of two specimens from Brazil. Consensus sequences from Brazil showed one SNP (single nucleotide polymorphism) located 280 bp into the alignment; it was identified as a pyrimidine substitution (T/C). The NCBI/Genbank deposit generated the accession number MK952186 for Brazil. The cladogram (Fig. 2) was reconstructed based on analyses of the COI region performed by the General Time Reversible nucleotide substitution model with Gamma distributed with invariant sites (GTR + I + gamma); parameters for partial exemption (95%) were estimated as the best substitution model using MEGA 5.0 software (Tamura et al., 2011). The largest possible

Figure 1 Geographical location of the Brazilian municipality of Maringá in the Paraná State.

number of accessioned comparative sequences already deposited in NCBI was included to perform the phylogenetic analyses of barcodes, which was inferred with the maximum likelihood method as in Tamura and Nei (1993).

Results and discussion

Diolcogaster choi sp. nov. belongs to the *xanthaspis* species-group, as characterized and keyed for the New World by Salgado-Neto et al. (2018).

Accounting for the new species from Brazil being described in this paper, the fauna of the *xanthaspis* group in the New World now includes five species (Table 1). The new species will key out in the key of Salgado-Neto et al. (2018) to *D. flammeus* Salgado-Neto and Fernandez Triana, 2018, but differs from that species in a number of features listed in the differential diagnosis.

Species description

***Diolcogaster choi* Whitfield & Salgado-Neto sp. nov.**

Holotype. 1 female deposited at the "Museu de Zoologia da Universidade de São Paulo" – MZUSP 62277, Maringá, Paraná State, Brazil, 23º 24' 22"S 51º 55' 53"W, 553 m above sea level, 09.i.2019, coll. Ísis Meri Medri, ex. larva *Hypercompe cunigunda*. **Paratypes.** 1 female and 1 male deposited at the "Museu de Zoologia da Universidade de São Paulo" – MZUSP 62278 and MZUSP 62279 respectively. 4 females and 1 male deposited at the Canadian National Collection (CNC) of Insects, Ottawa, Ontario – CNC 649484, CNC649485, CNC 649486, CNC 649487 and CNC 649488 respectively. 5 females and 1 male deposited at the Illinois Natural History Survey (INHS Insect Collection) – INHS 834744, INHS 834745, INHS 834746, INHS 834747, INHS 834748 and INHS 834749 respectively. 3 females deposited at the "Coleção de Parasitóides do Laboratório de Biologia Evolutiva

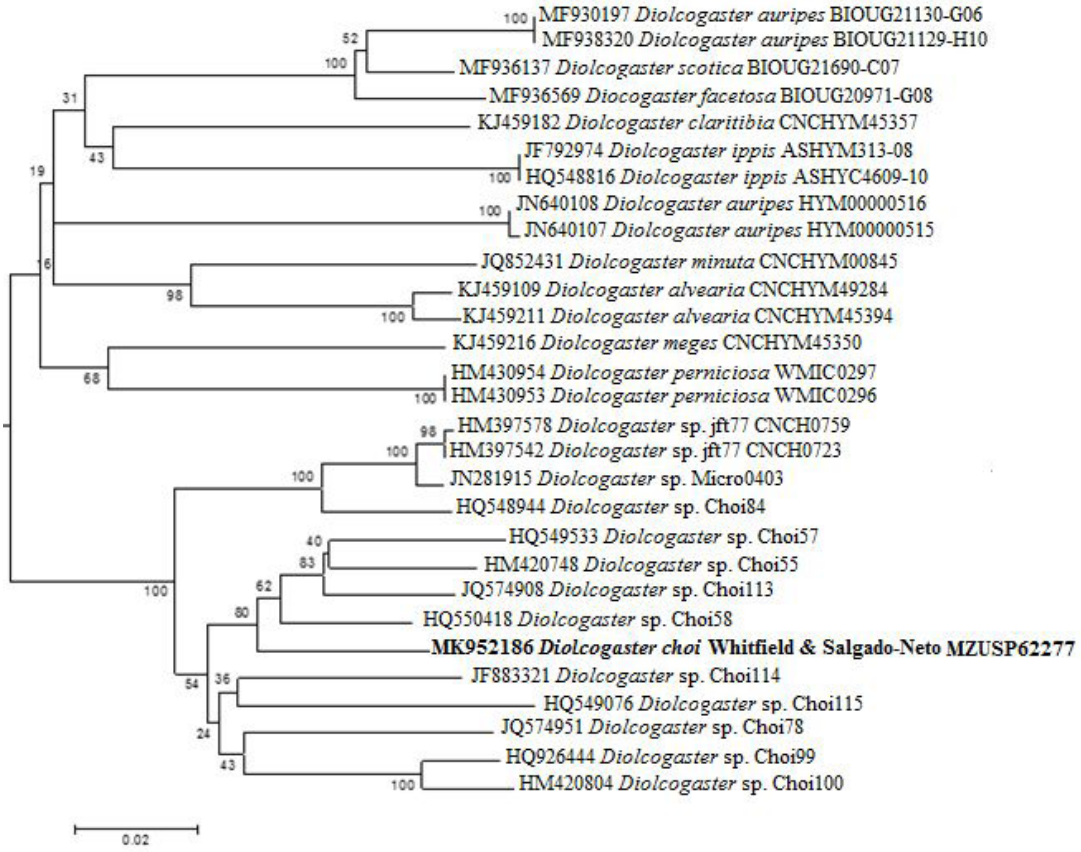

Figure 2 Phylogenetic tree of *Diolcogaster choi* (Hymenoptera: Braconidae) and close relatives inferred from partial mtDNA COI gene region (barcode) sequences, using maximum likelihood (ML) analysis. The numbers preceding the scientific names are the respective COI GenBank access codes.

Table 1.
Species of *Diolcogaster* (Hymenoptera: Braconidae) in the New World and respective hosts (Lepidoptera), geographical distribution, caterpillar hostplants and references.

Diolcogaster spp.	*Geographical Distribution*	*Host Caterpillar*	*Caterpillar Hostplants*	*Reference*
D. choi Whitfield & Salgado-Neto, 2019	Maringá, State of Paraná, Brazil	*Hypercompe cunigunda* (Stoll, 1781); Erebidae	*Passiflora edulis* Sims; Passiflorae	Present Study
D. flammeus Salgado-Neto & Fernández-Triana (2018)	Mexico to Brazil	*Agaraea minuta* (Schaus, 1892); Erebidae	*Costus spicatus* (Jacq.) Sw. and *C. spiralis* (Jacq.) *Roscoe var. spiralis;* Costaceae	Salgado-Neto et al. (2018)
D. iridescens (Cresson, 1865)	Cuba and Southern USA (Florida)	*Asciodes gordialis* Guenée, 1854; Crambidae	Species, of *Bougainvillea*; Nyctaginaceae	Patton (1958)
D. bakeri (Muesebeck, 1922)	Canada (Ontario, Quebec and Saskatchewan), USA (Arkansas, Florida, Georgia, Illinois, Iowa, Kansas, Louisiana, and Texas)	*Ponometia candefacta* (Hübner, 1831) and *P. erastrioides* (Guenée, 1852); Noctuidae	Asteraceae including species of *Aster* and *Ambrosia*	Pogue (2010); Stojanović et al. (2011)
D. xanthaspis (Ashmead, 1900)	Saint Vincent and the Grenadines	Unknown	Unknown	Ashmead (1900)

da Universidade Federal de Santa Maria" – UFSM, with registration *Diolcogaster* Ísis 2019. Same data of locality, data, host and collector as holotype for all paratypes.

Description. Female (Fig. 3 and 4). Body color entirely yellow to orange yellow, except for dark brown to black on flagellomeres, parts of the scape, and most of the interocellar area, black ovipositor sheaths, darkened hind tibial apices and hind tarsi, and occasionally dorsum of extreme posterior metasomal segments partly brown. Fore wing with pterostigma and veins very dark brown to black. Head mostly with striate sculpture on face, frons, gena and vertex; occiput strongly concave and smooth. Mesosoma with anteromesoscutum and scutellar disc coarsely punctate, scutellar disc posteriorly with a slight keel; propodeum with strong and complete median carina, and additional transverse rugosity on posterior 0.6 of propodeum. Metasoma with T1 slightly widening towards posterior margin and with strong and deep median sulcus running throughout the anterior ¾ of the tergite; T2 subtriangular and posterior about same width as medial length, with relatively strong sulcus delimiting curved lateral margins; T3+ smooth; ovipositor sheaths weakly decurved and polished with a few scattered setae. Metacoxa relatively large, extending to end of T2. Measurements (all in mm). Body length: 3.0-3.2. Fore wing length: 3.1-3.3.

Male (Fig. 5). Similar to female except medial groove of T1 reaching nearly to posterior end of tergite.

Molecular data. COI barcode deposited in GenBank (Accession MK952186).

Biology. A gregarious parasitoid, reared from *Hypercompe cunigunda* (Lepidoptera: Erebidae), that has potential for biological control of this caterpillar species.

Distribution. So far it has only been found in Maringá, Paraná State, Brazil; the municipality is inland, 350 km northwest of Curitiba city and west of the coast by 470 km.

Etymology. The specific epithet, *"choi"* is in honor of the researcher Won-Young Choi, formerly of the National Institute of Biological Researches, Gyeongseo-Dong, South Korea. Choi produced a monograph on neotropical *Diolcogaster* as his Ph.D. dissertation at the University of Illinois, under orientation of J. B. Whitfield (Choi, 2005). Unfortunately, he tragically died in 2016 before publishing this work.

Diagnosis. *Diolcogaster choi* sp. nov. differs, along with *D. flammeus* Salgado-Neto and Fernandez-Triana, from other described species in the *Diologaster xanthaspis*-group New World species in being predominantly yellow/orange in body color. From *D. flammeus*, it differs in the following combination of characters: abdominal terga almost completely orange-yellow throughout except sometimes at posterior tip of metasoma (*D. flammeus* has much of T2-T4 darkened dorsally), percurrent medial longitudinal groove of T1 extending only 0.75 or less of distance to posterior end of tergite (in *D. flammeus*, essentially 0.95 or more), T2 posteriorly approximately as broad as medially length

Figure 3 Female holotype of *Diolcogaster choi*, new species. A – dorsal shot of live female; B – dorsal habitus; C – lateral habitus; D – ventral habitus. Photos by Ísis Meri Medri.

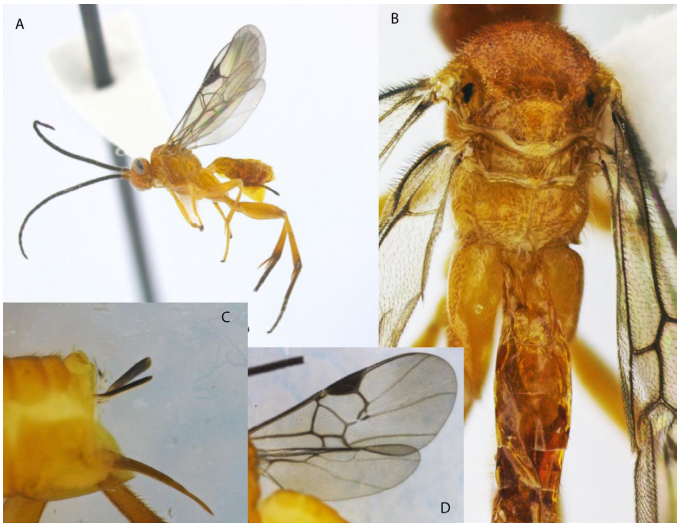

Figure 4 *Diolcogaster choi*, new species. A – lateral habitus of point-mounted female; B –dorsum of mesosoma and anterior metasomal tergites; C – lateral view of tip of metasoma showing ovipositor and sheaths; D – wings venations. Photos by James Bryan Whitfield.

Figure 5 Male paratype of *Diolcogaster choi*, new species, dorsal view. Photo by Ísis Meri Medri.

of tergite (in *D. flammeus* close to 1.5X as broad as long); ovipositor sheaths black throughout (in *D. flammeus* much paler proximally). The species also differs in recorded host, and its DNA barcode appears to be distinctive among described *Diolcogaster*.

Janzen does not use 2% as a cut-off for species boundaries, but instead uses extrapolation from clustering of clear within-species % differences versus clear between-species distances. There is typically a strong difference between the magnitudes of within-species versus between-species divergences. D. choi is more or less as distinct as many other recognized species, and is definitely distinct morphologically from any of the described ones as well as very distinct in terms of barcodes from any previously described species with barcodes (top half of tree). The species status is actually based on integration of information from the morphological description, host and ecological data, and DNA barcodes, rather than simply any one of these lines of evidence.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

Geraldo Salgado-Neto: Lead author, new species descriptor, review and organization of the main article. Ísis Meri Medri: Species collector and article writer. José L. Fernández-Triana: Assistance in the details of the description of the new species. James Bryan Whitfield: Principal descriptor of the new species, organizer of morphometric photos and finalization of the English review. Principal financier of publication costs

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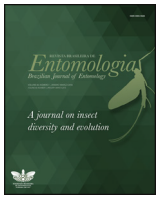

Erratum

In the article "*Diolcogaster choi* sp. nov. from Brazil, a new gregarious microgastrine parasitoid wasp (Hymenoptera: Braconidae) reared from *Hypercompe cunigunda* (Lepidoptera: Erebidae) in Brazil" with DOI number https://doi.org/10.1590/1806-9665-rbent-2019-82 published at Revista Brasileira de Entomologia v64n1:e201982 in page 5-6.

Where it was written:
Ísis Meri Medri: Species collector and English review.

Should read:
Ísis Meri Medri: Species collector and article writer.

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