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SCIENTIFIC NOTES

First Record of *Telenomus dilophonotae* (Hymenoptera, Scelionidae), Parasitizing Eggs of *Erinnyis ello* (Lepidoptera, Sphingidae) in Western Paraná, Brazil, with Molecular Characterization and Records of Occurrences

Ana Paula G. S. Wengrat[1] · Leidiane Coelho Carvalho[2] · Vanda Pietrowski[2] · Karine Schoeninger[3] · Valmir Antonio Costa[3] · Norman F. Johnson[4]

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Abstract
There are few records for *Telenomus dilophonotae* Cameron, 1913 (Hymenoptera, Scelionidae) from South America. In Brazil, the first occurrence was reported in Bahia in rubber crops, *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. - Arg., there parasitizing eggs of *Erinnyis ello* Linnaeus, 1758 (Lepidoptera, Sphingidae). It was also found parasitizing the same host in cassava, *Manihot esculenta* Crantz (Euphorbiaceae). This is the first record of occurrence of *T. dilophonotae* in the state of Paraná, parasitizing eggs of *E. ello* in areas of cassava production in the western region of Paraná, this being the southernmost record of the species. Here, photographs, the first sequence of DNA barcode of this species of parasitoid wasp, and a distribution map are provided.

Keywords Egg parasitoids · *Manihot esculenta* · Neotropical region · Telenominae

Introduction

Telenomus Haliday, 1833 (Platygastroidea, Scelionidae) comprise egg parasitoids of a diversity of insects in the orders Hemiptera, Diptera, Neuroptera, and especially Lepidoptera (Austin et al. 2005; Wengrat et al. 2021). In Brazil, the occurrence of *Telenomus* has been formally registered in several states, with twenty species recorded (Wengrat 2023).

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✉ Ana Paula G. S. Wengrat
anawengrat@usp.br

1 Depto de Entomologia e Acarologia, Escola Superior de Agricultura "Luiz de Queiroz" (USP-ESALQ), Univ de São Paulo, Piracicaba, SP, Brazil

2 Depto de Produção Vegetal, Univ Estadual do Oeste do Paraná (UNIOESTE), Rua Pernambuco, Marechal Cândido Rondon, PR, Brazil

3 Centro Avançado de Pesquisa em Proteção de Plantas e Saúde Animal, Instituto Biológico, Campinas, SP, Brazil

4 Dept of Evolution, Ecology, and Organismal Biology, The Ohio State Univ, Columbus, OH, USA

These parasitoid wasps parasitize mainly eggs of Pentatomidae (Corrêa-Ferreira and Moscardi 1995) and lepidopteran pests of the families Noctuidae (Polaszek and Foerster 1997; Wengrat et al. 2021) and Sphingidae (Johnson 1990; Noronha et al. 2020) from several cultures.

Around the world, three species of *Telenomus* parasitizing eggs of Sphingidae (Lepidoptera) have been recorded: *Telenomus connectans* Ashmead, 1895, recorded from the USA, Dominican Republic, Grenada, Costa Rica, and Brazil; *Telenomus monilicornis* Ashmead, 1894, from the Dominican Republic and Costa Rica; and *Telenomus dilophonotae* Cameron, 1913, in Costa Rica, Guyana, Guadeloupe, and Brazil (Johnson 1990) and Colombia (Johnson and various contributors: Triplehorn Insect Collection, Ohio State University, USA, 2017).

There are few reports of the natural occurrence of *T. dilophonotae* parasitizing eggs of *Erinnyis ello* Linnaeus, 1758 (Lepidoptera, Sphingidae). The first recorded parasitism of this pest occurred in southern Bahia in the 1980s, where more than 90% of the collected eggs were parasitized (Freire 1985). In this work, two *Telenomus* haplotypes were found, but they were not identified at the species level. The second record, also in the same decade and state, was made for *E. ello* in rubber crop - *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. - Arg. - (Abreu and Sgrillo 1989). Noronha et al. (2020) reported the presence of *T. dilophonotae* from the state of Pará in cassava crops parasitizing *E. ello* eggs. This is the most recent occurrence record for this species. The same authors also made important observations of its morphological plasticity.

There are few studies of parasitism by *Telenomus* in Sphingidae of Brazil. Knowledge of the diversity and distribution of parasitoids in the territory of Brazil is important, both from the ecological point of view and for pest management of crops. DNA barcode data of *Telenomus* from Lepidoptera of the Brazilian fauna are even more scarce, with only the single sequence for *Telenomus remus* Nixon, 1937 (Wengrat et al. 2021).

Therefore, it would be helpful to develop studies in Brazil to verify the genetic diversity of *Telenomus* species and to build a database of these parasitoids that occur on pests of Brazilian agroecosystems. These wasps are of paramount importance in natural and applied biological control, and their use is currently one of the main control methods within integrated pest management (IPM) programs. This work aims to make the first record of occurrence of *T. dilophonotae* parasitizing eggs of *E. ello* in cassava crop areas in western Paraná, to make available the sequence of DNA barcode for species, as well as to expand our understanding of its distribution.

Material and methods

Specimens

Parasitism by *T. dilophonotae* was detected from egg collections of *E. ello* carried out in the 2017/18 and 2021 harvests in areas of commercial production of cassava in the municipality of Marechal Cândido Rondon (24°50′96,92″S, 54°30′80,31″W) and Entre Rios do Oeste, in the western region of Paraná (Fig. 1).

Sampling was performed every 2 weeks in Marechal Cândido Rondon from 15 Oct 2017 to 28 Mar 2018, by zigzag walking sampling 30 equidistant points, and inspecting 6 plants/point. In the 2021 harvest, the collections were sporadic and carried out in Entre Rios do Oeste. The eggs collected were transferred to the Laboratory of Biological Control of the Universidade Estadual do Oeste do Paraná (UNIOESTE), where they were identified, placed in gelatin capsules, and kept in BOD type climate chambers at 25 ± 2°C and a photophase of 14 h. The parasitoids that emerged were placed in 1.5-mL microtubes containing 70% ethanol. In addition, 16 specimens were obtained that were laboratory-reared in *E. ello* eggs (10♀, 6♂).

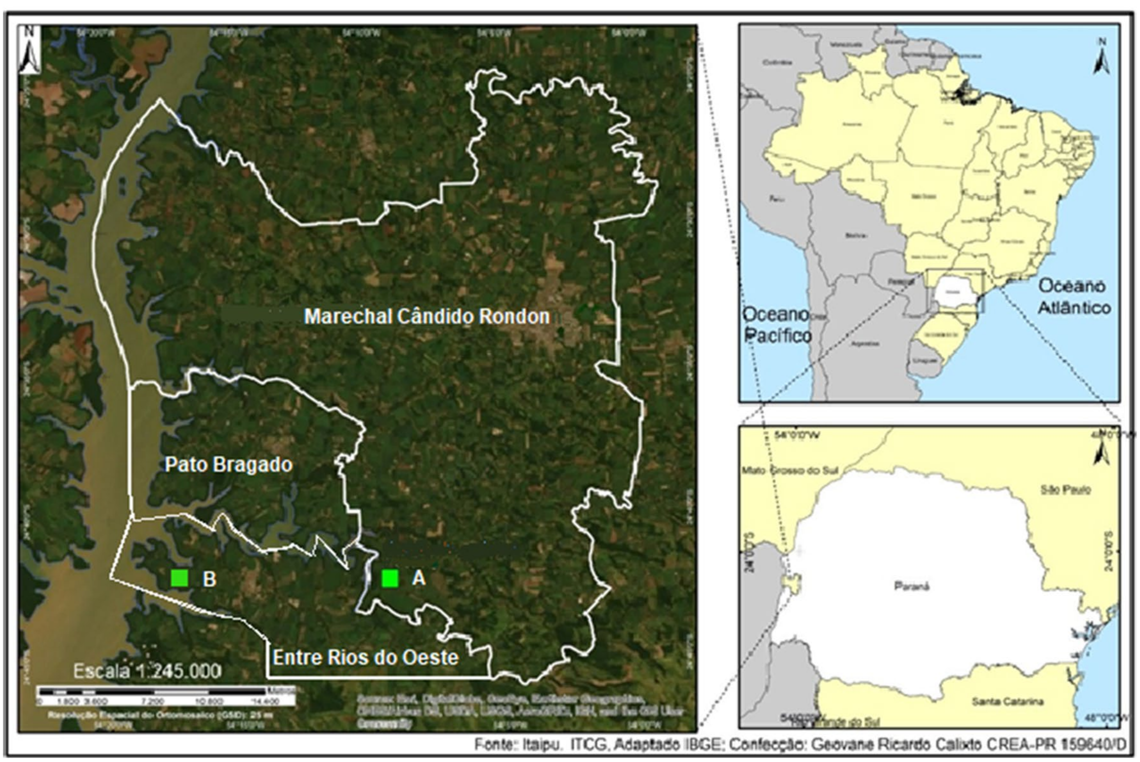

Fig. 1 Location of the egg collection areas of *Erinnyis ello* (Lepidoptera: Sphingidae), in commercial cultivation of cassava (*Manihot esculenta*), area A: Marechal Candido Rondon and area B: Entre Rios do Oeste, Paraná, Brazil

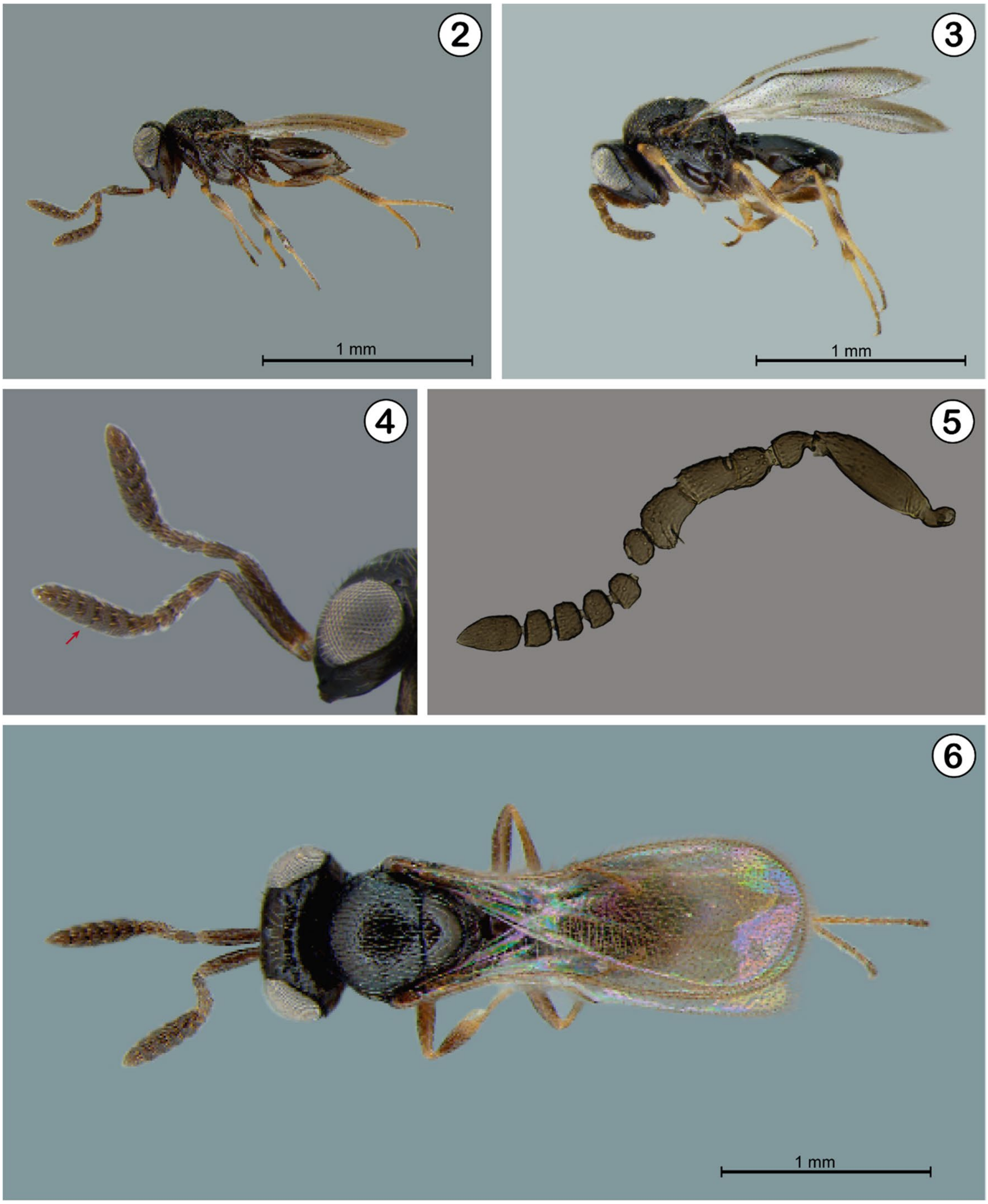

Fig. 2-6 *Telenomus dilophonotae* Cameron, 1913 (Platygastroidea, Scelionidae). **2**, lateral view, female; **3**, lateral view, male; **4**, clava female, arrow indicating; **5**, antenna, male; **6**, dorsal view, female

Generic identification was performed following Masner (1976), and at the species level according to Johnson (1990) and Noronha et al. (2020). Color images were taken with a Leica MC 190HD digital camera attached to the Leica stereomicroscope M205A, and the specimens illuminated with a Leica LED5000 HDI high-light diffused dome (Kerr et al. 2008). The final extended focusing image was combined with the software *Leica LAS X*. The distribution map was generated using *SimpleMappr* (Shorthouse 2010). Geographic coordinates of the species records, if not present in labels, were derived from *geoLoc* (*species*-Link, CRIA).

The specimens of *T. dilophonotae* are deposited in the Coleção de Insetos Entomófagos "Oscar Monte"

Table 1 Country of occurrence, Lepidoptera host, and host plant of *Telenomus dilophonotae* (Platygastroidea: Scelionidae)

Country	Host	Host plant
Brazil	*Erinnyis ello* (Linnaeus)	*Manihot esculenta* Crantz
Colombia	*E. ello*	Unidentified
Costa Rica	*Perigonia stulta* Herrich-Schäffer and unidentified sphingid	Unidentified
Guadeloupe	*Erinnyis* sp.	Unidentified
Guyana	*Phryxus caicus* Cramer; *E. ello*	Unidentified

(IB-CBE), in Unidade Laboratorial de Referência em Controle Biológico do Instituto Biológico, in Campinas, São Paulo, Brazil, under the unique identifier numbers IB-CBE-003116 to IB-CBE-003132.

Molecular data

To obtain the DNA barcodes, we used 3 females of *T. dilophonotae*. The extraction of total genomic DNA was performed by non-destructive methodology, following work by Wengrat et al. (2021). For amplification of the mitochondrial gene, the Cytochrome C Oxidase Subunit I (COI) primers SCEL-F1 (5′- GCAATAATTCGAATAGAA TTAAGAGT-3′) (Gariepy et al. 2014) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) were used.

The concentration and reagents of the Polymerase Chain Reaction (PCR), as well as the thermocycling conditions for the SCEL-F1/HCO-2198 primers, followed the protocol of Gariepy et al. (2014). The amplicons were observed after electrophoresis under ultraviolet light on a 1.5% agarose gel stained with SYBR Safe (LIFE TECHNOLOGIES). The subsequent purification process for 10 μL of the final PCR product was performed using 1 μL (20 U μL^{-1}) of Exonuclease I (Thermo Fisher Scientific™) and 2 μL (1 U μL^{-1}) of thermosensitive alkaline phosphatase FastAP™ (Thermo Fisher Scientific) per 10 μL of the final PCR product. The purification conditions were 37°C for 30 min, followed by 80°C for 15 min. Bidirectional Sanger sequencing was performed at the Animal Biotechnology of ESALQ.

After sequencing, the material was manually aligned and edited, when necessary, for 562 base pairs (bp) for the SCEL-F1/HCO-2198 primer set. The chromatograms of each of the individual sequences were checked, edited, and aligned to produce the consensus sequence in Geneious Prime 2022.1 (https://www.geneious.com). The presence of NUNTs (nuclear parallels of mitochondrial origin) was observed in MEGA X, following the steps described in Corrêa et al. (2017). Sequences were submitted to NCBI.

Results

We recorded *T. dilophonotae* for the first time from the state of Paraná (Fig. 3). Three sequences of COI were obtained for *T. dilophonotae*, one with 553 bp and two with 562 bp. These are the first sequences that have been generated and deposited in the NCBI database for this parasitoid species OQ720987, OQ720988, and OQ720989.

According to Johnson (1990), *T. dilophonotae* (Figs. 2, 3, 4, 5, and 6) has the following morphological characters: legs and antennae dark brown (Figs. 2, 3, 4, and 5); occipital

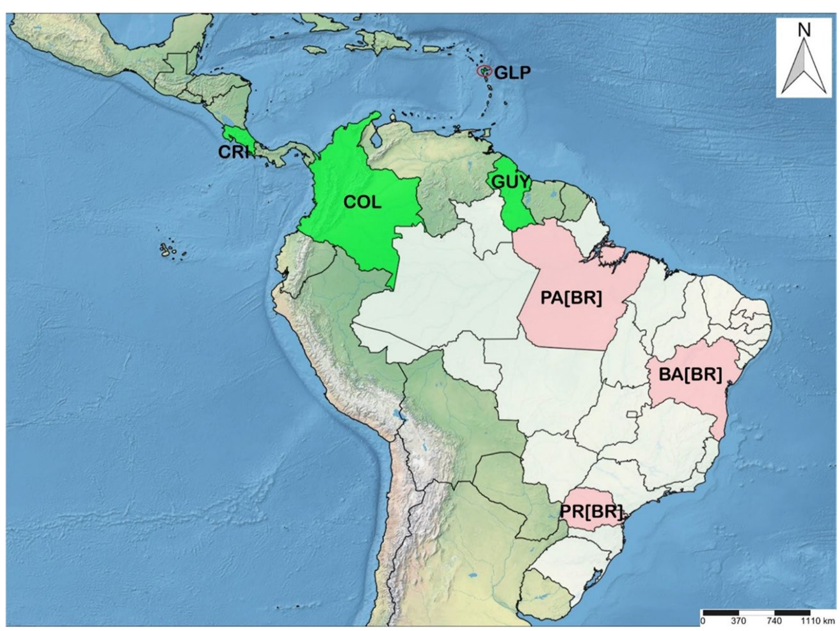

Fig. 7 Worldwide distribution of *Telenomus dilophonotae* Cameron, 1913 (Platygastroidea: Scelionidae). COL, Colombia; CRI, Costa Rica; GLP, Guadeloupe; GUY, Guyana; BA [BR], Bahia state, Brazil; PA [BR], Para state, Brazil; PR[BR], Paraná state, Brazil

carina complete medially; female clava with 6 clavomeres (see Fig. 4) and claval formula (A11-A7/1,2,2,2); T1 with 1 pair of sublateral setae; genitalia of the male with four small teeth per digitus, usually weakly melanized (see Fig. 1 in Johnson 1990). According to Noronha et al. (2020), the male (Fig. 3) antennae has eleven antennomeres (Fig. 5) and a preocellar pit is present (see images 3a, b in Noronha et al. 2020).

We also observed that throughout the sampling period, *T. dilophonotae* was collected in only three parasitized eggs of *E. ello*, demonstrating a low parasitism index, 0.2%. In the 2017/2018 harvest, 1 egg of *E. ello* parasitized by *T. dilophonotae* was collected, from which six (4♀, 2♂) parasitoids emerged. *Telenomus dilophonotae* is one of the few species in this very large genus that is a gregarious parasitoid. In the 2021 harvest, 2 eggs of *E. ello* parasitized by *T. dilophonotae* were collected, from which two females emerging from one and 5 females and 1 male from the other.

Telenomus dilophonotae occurs in Central and South America parasitizing eggs of Lepidoptera (Table 1). Records have been made from Guadeloupe, Colombia, and Guyana (Johnson and various contributors: Triplehorn Insect Collection, Ohio State University - USA, 2017), all of which from a single site each, in Costa Rica (Johnson 1990), and from two sites in the Brazilian states of Bahia (Freire 1985; Abreu and Sgrillo 1989) and Pará (Noronha et al. 2020). Paraná is now the third state record for Brazil (Fig. 7).

Discussion

This is the first record of *T. dilophonotae* (Fig. 2) in *E. ello* in cassava crop areas in the state of Paraná and is the third record of this parasitoid for Brazil. Until now, the known occurrence of *T. dilophonotae* was restricted to the states of Bahia and Pará (Noronha et al. 2020). The new data are the southernmost records for the species. Considering that the distance between the records of occurrence of *T. dilophonotae* in Brazil is approximately 2000 km, this species probably has a much wider distribution than has so far been formally recorded.

Furthermore, Brazil is a continental country with different biomes and edaphoclimatic conditions, as well as having a high diversity of Sphingidae (Lepidoptera) species, with about 200 species registered for the country (Orlandin et al. 2024). These abiotic and biotic factors can influence the adaptation of this parasitoid in different agroecological zones. In northern Brazil, the parasitism rate of *T. dilophonotae* in *E. ello* eggs was 11% (Noronha et al. 2020), while in this study it was 0.02%, indicating that abiotic factors may influence the abundance of this species of parasitoid.

Species of the genus *Telenomus* have been studied and used in the management of insect pests of economic importance in Brazil, mainly for *Spodoptera frugiperda* (Smith 1797) (Lepidoptera: Noctuidae) in maize, *Telenomus remus* (Wengrat et al. 2021), and *Telenomus podisi* for *Euschistus heros* (Fabricius, 1798) (Hemiptera: Pentatomidae) in soybean (Corrêa-Ferreira and Moscardi 1995).

Although the parasitism of this genus in *E. ello* eggs was low in the present study, it is important to know the species and establish their distributions in Brazil. *Telenomus dilophonotae* had so far been recorded parasitizing *E. ello* only in rubber crop in Bahia and cassava in the state of Pará, where it had a natural parasitism of approximately 5% (Noronha et al. 2020), a higher index in comparison to the other genera found in the study, such as *Trichogramm*a Westwood, 1833 (Hymenoptera: Trichogrammatidae), *Chrysonotomyia* Ashmead, 1904 (Hymenoptera: Eulophidae), and *Ooencyrtus* Ashmead, 1900 (Hymenoptera: Encyrtidae). For the state of Paraná, the predominant egg parasitoids in *E. ello* are the species of *Trichogramma*. The dynamics between the different species of *Trichogramma* and *Telenomus* is not understood, nor are the factors that interfere in the abundance of their populations in different Brazilian regions. With improved capabilities to make confident identifications, the future studies needed to address these important questions are possible (Wengrat et al. 2021).

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Declarations

Conflict of interest The authors declare no competing interests.

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