Naturalized *Dolichogenidea gelechiidivoris* complement the resident parasitoid complex of *Tuta absoluta* in North-eastern Spain

Short title: *Dolichogenidea gelechiidivoris* in North-eastern Spain

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Abstract

Our work reports on the establishment of the neotropical parasitoid *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) for the first time in Europe. This larval parasitoid has been recorded in samples collected in commercial tomato crops in Catalonia (North-eastern Spain) from 2016 to the present. *Dolichogenidea gelechiidivoris* is considered to be a new biocontrol agent among the resident parasitoid complex of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae).

Key words: Conservation biological control, introduced natural enemies, tomato, parasitoids, Invasive pests

Introduction

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is an important pest of tomato and other Solanaceous plants worldwide. It is native to South America and was recorded in Spain in 2006; since then, it has rapidly spread from the Mediterranean basin to Africa and Asia (Desneux et al., 2010, 2011). To date, considerable efforts have been taken targeting the sustainable management of *T. absoluta* (Biondi et al,. 2018; Desneux et al. 2022). In Spain, existing integrated pest management (IPM) programs based on predatory mirid bugs have significantly contributed to controlling *T. absoluta* soon after its invasion (Urbaneja et al,. 2012; Arnó et al., 2018). Moreover, many naturally occurring parasitoid species within several Hymenopteran families have been identified attacking *T. absoluta* across the Mediterranean (Biondi et al., 2018). The conducted surveys mostly recognised the key role of the ectoparasitoid *Necremnus tutae* Ribes & Bernardo (Hymenoptera: Eulophidae), first identified as *Necremnus* nr. *artynes*, for the biocontrol of *T. absoluta* (Gebiola et al., 2015). To estimate the contribution of the natural parasitism of *T. absoluta* by *N. tutae* in commercial tomato plots, pest control advisors in Catalonia (North-eastern Spain) conducted extensive sampling from 2017 to 2019. As a result, we recorded, sporadically and recurrently, the presence of a non-eulophid parasitoid that emerged from the ectoparasitized *T. absoluta* larvae (with eggs, larvae or pupae, of a parasitoid on them). Therefore, in 2019 and 2020 we conducted specific samplings to identify this parasitoid and evaluate its prevalence in the area.

Materials and Methods

In 2019 and in 2020, leaflets with galleries large enough to host a second to third larva of *T. absoluta* were collected by random walks in commercial tomato plots (including open field and protected crops). Sampling terminated after 20 minutes or after collection of a maximum of 25 leaflets, whichever was reached first; this method is useful to minimise sampling time and costs, particularly at low infestation levels (Naranjo, 2008). Under a stereomicroscope, *T. absoluta* larvae were classified as “ectoparasitized” (paralyzed and with pupae, larvae or eggs of a parasitoid on them), “alive” (not ectoparasitized and still able to crawl) or “dead” (unable to crawl and symptoms of ectoparasitism not detected). Table 1 summarizes the number of plots, farms and municipalities sampled each year covering an area of approximately 1600 ha of horticultural crops.

In September 2019, all the larvae were individualised with the leaflet in Petri dishes and stored at room temperature for up to 27 days until the emergence of either *T. absoluta* or adult parasitoids, which were then stored in 70% alcohol. In 2020, samples were taken from March to November. Each plot was sampled up to 18 times, depending on the crop cycle and *T. absoluta* infestation levels, and only the alive larvae were retained to evaluate the presence of endoparasitoids. Additionally, from 2017 to 2019, pest advisors had collected 23 adult parasitoids from field samples, which, even if they came from ectoparasitized *T. absoluta* larvae, did not morphologically match with Eulophidae.

Adult parasitoids were identified to family and sub-family using available keys (Grissell & Schauff, 1990; Hanson & Gauld, 2006). All Microgastrinae (Hymenoptera: Braconidae) were first identified to genus (Fernandez-Triana et al., 2020) and then to species using the description found in Marsh (1975). Additional specimens (10 ♂♂ and 10 ♀♀) from a laboratory rearing were also morphologically identified. This rearing was initiated with adults that emerged from *T. absoluta* larvae collected in 2019 in El Maresme county (31TDF49 to 31TDG92, Catalonia), and is kept in a climatic chamber (25ºC, 70% RH and 16:8 L:D photoperiod) on *T. absoluta* larvae infesting tomato plants..

To confirm the morphological identification of the species, one specimen from this lab rearing and nine specimens from the previous field samplings were also identified by DNA barcoding. Those specimens were collected in seven locations along a transect of 100 km (from 31TDF17 to 31TDG84 and 31TEG03), in the municipalities of Viladecans (2018), Argentona (2018), Mataró (2020), Santa Susana (2017, 2019, 2020), Blanes (2017), Fornells de la Selva (2019) and Calonge (2018). An additional specimen from a previous study (Arnó et al., 2021) collected in 2016 in Malgrat de Mar was also barcoded. For this, total genomic DNA was extracted from each insect by using the SpeedTools Tissue DNA Extraction Kit (Bio Tools, Madrid, Spain) following the manufacturer's protocol and resuspended in 100 µl of elution buffer. A 658-bp region of the CO1 gene was amplified using primers LepF1 and LepR1 (Smith et al., 2006). The polymerase chain reaction (PCR) reaction volumes (20 µl) contained 2 µl of resuspended DNA, 10 µl of Master Mix (BioTools) and 0.4 µl of each primer [10 µM]. The samples were amplified in a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA) using the thermocycling profile described in Smith et al. (2006). The products were visualized on 2.4% agarose gels stained with GelRed® (Biotium, San Francisco, CA, USA) under ultraviolet light, purified with a QIAquick PCR Purification kit (Qiagen, Hilden, Germany) and bidirectionally sequenced using BIGDYE 3.1 on an ABI 3730 DNA Analyzer (Applied Biosystems) at the Genomics Unit of the CCiTUB (University of Barcelona). The obtained sequences were compared against the Barcode of Life Data (BOLD) reference database (http://www.boldsystems.org/) to find the matching species.

Results

Table 1 summarizes the number of *T. absoluta* larvae ectoparasitized, dead and alive obtained in 2019 and 2020 samplings. In 2019, only 21 eulophids emerged from ectoparasitized and dead larvae, being 81% *Necremnus* sp., and 13 *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) emerged from 13 alive larvae. In 2020, 264 parasitoids emerged from the alive larvae (from 92 samples, 20 plots and 10 farms). Of them, 262 were classified as Braconidae, of which a sample of 163 adults were morphologically identified and all found to be *D. gelechiidivoris*. This year, the endoparasitism levels (number of adult parasitoids over number of larvae collected each month) steadily increased from May (2.7%) to October (21.8%). No endoparasitoids were recorded in March, April and November. All the adult parasitoids (23) from the field ectoparasitized samples collected in 2017–2019 by farm advisors from 12 farms located in nine municipalities were also morphologically identified as *D. gelechiidivoris*.

DNA barcoding confirmed the initial morphological identification of the 11 analysed specimens as *D. gelechiidivoris*, regardless of location and year. The obtained sequences were deposited in the GenBank database (Accession codes: MZ298974-MZ298984). Their similarity percentages ranged from 100% to 99.48% when compared with the 13 available sequences in the GenBank database in February 2021 (Accession codes: KX443088, HQ558975-HQ558977, JN282071-JN282078 and JQ849955).

Discussion

Our study allowed for the detection of *D. gelechiidivoris* in field collected *T. absoluta* larvae samples samples from 2016 to 2020 in an area spanning more than 100 km in Catalonia. This species is a parasitoid native of South America, where it is considered to be an important control agent of *T. absoluta* (Salas-Gervassio et al., 2019). It was imported to Kenya from Peru in 2017 (Aigbedion-Atalor et al., 2020) and it has been recently found in Algerian tomato crops (Krache et al., 2021). Because there is no record of the intentional introduction of this parasitoid into Europe, its wide establishment in the area of our study suggests that it was probably unintentionally introduced from the Neotropics some years ago as a consequence of global trade, as has been reported for other natural enemies (Roy et al., 2011). This was also the most likely cause of the arrival of *T. absoluta* in Spain and its rapid spread (Desneux et al., 2010).

Under IPM programs based on conservation and/or augmentation of predators and parasitoids (Arnó et al., 2018), the outcome of the interactions between parasitoids, and parasitoids and mirids will be decisive for successful control of *T. absoluta*. As all Microgastrinae, *D. gelechiidivoris* is a koinobiont solitary larval endoparasitoid (Fernandez-Triana et al., 2020) that maintains the host alive until just before the parasitoid pupates.. However, *D. gelechiidivoris* also emerged from 11.5% of the ectoparasitized larvae collected in 2017–2019 (authors' unpublished data), suggesting that there is no clear detection of previous parasitism between *Necremnus* sp. and *D. gelechiidivvoris*. On the other hand, Aigbedion-Atalor et al. (2021) reported no effect of *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) on oviposition and progeny of *D. gelechiidivoris*. Further studies are needed to understand the impact of conservation and/or augmentation of *D. gelechiidivoris* in tomato IPM programs.

Conflicts of interest: The authors declare no conflicts of interest

Author contribution: JA and JR designed the study. HGV, MC, MM and DR did the sampling. CD identified the parasitoids. KVA and NA confirmed Dg identity. CD wrote the manuscript with input from JA, JR, OA and NA. All the authors approved the manuscript.

Data availability Statement: https://dataverse.csuc.cat/privateurl.xhtml?token=29fb30d3-b80c-4d25-a85d-8163217ff747. Voucher specimens of *Dolichogenidea gelechiidivoris* were deposited at the Naturalis Biodiversity Center (Leiden, The Netherlands).

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Table 1. Number of plots, farms and municipalities surveyed in 2019 and 2020 and number of ectoparasitized, dead and alive *T. absoluta* larvae registered under the stereomicroscope.

Year	No. of plots	No. of farms	No. of municipalities	Number of larvae		
				Ectoparasitized	Dead	Alive
2019	9	7	4	21	114	35
2020	31	13	5	69	356	1872