JHR 88: 115–131 (2021)
doi: 10.3897/jhr.88.75279
https://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria

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Academic editor: Elijah Talamas | Received 14 September 2021 | Accepted 19 October 2021 | Published 30 December 2021

http://zoobank.org/17C95222-AF04-49B0-A979-E18BE0EB4891

Citation: Krache F, Boualem M, Fernandez-Triana J, Bass A, Arnó J, Benourad F (2021) First record in Africa of the parasitoid *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) on tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae) from tomato fields in Algeria. Journal of Hymenoptera Research 88: 115–131. https://doi.org/10.3897/jhr.88.75279

Abstract
The Neotropical parasitoid wasp *Dolichogenidea gelechiidivoris* (Marsh, 1975) (Hymenoptera: Braconidae), one of the most important biocontrol agents of the South American tomato pinworm *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is reported for the first time from Africa, from tomato grown in open fields and greenhouses in several regions of Algeria. Color photos of specimens from Algeria, Spain and South America, as well as the holotype and one paratype are provided. Morphological and molecular details to better characterize and recognize the species are also provided. We speculate that *D. gelechiidivoris* arrived accidentally to Algeria from Spain, where it has recently been reported. The consequences for future biocontrol projects against *T. absoluta* in Africa are discussed.

Keywords
Tuta absoluta, Afrotropical region, Microgastrinae, tomato, biological control, species diagnosis, DNA barcoding, accidental introduction

Introduction

The past decade has seen Africa invaded by the tomato pinworm *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), an important pest native to South America that causes severe crop losses when not controlled. The first reported record of the pest out of its continent of origin was from Spain in 2006 (Urbaneja et al. 2007). To date, the pest insect has spread to almost the entire African continent at lightning speed (Biondi et al. 2018; Tarusikirwa et al. 2020), with devastating effects, notably, on small-scale tomato farming (Mansour et al. 2018). The first record of *T. absoluta* in Algeria was in March 2008, on tomatoes in open fields in Achaacha, Mostaganem (EPPO 2008; Guenaoui 2008). Since then, it quickly spread to all tomato-producing areas in the country. The larvae of *T. absoluta* can damage between 80% and 100% of tomato in open fields (Desneux et al. 2010). Larvae are hidden inside leaves, stems and fruits, where they feed, and are therefore protected from most insecticides (Cocco et al. 2015), resulting in repeated pesticide treatments and the development of pest resistance (Roditakis et al. 2018). Thus, integrated pest management (IPM) strategies have been developed, including augmentation and conservation biological control (Biondi et al. 2018; Mansour et al. 2018).

Biological control is often considered one of the most economical and environmentally sustainable means of managing native as well as exotic pests and crop diseases (van Lenteren et al. 2018). When compared to other biological control agents (i.e., predators and entomopathogenic organisms), insect parasitoids commonly show greater host search efficiency and host specificity, traits that are considered important in preventing non-target host attacks (e.g., Daane et al. 2016; Hardwick et al. 2016; Kenis et al. 2017; Cascone et al. 2018; Thancharoen et al. 2018; Salas Gervassio et al. 2019). Thus, biological control against *T. absoluta* using insect parasitoids is a potentially successful control tool (Han et al. 2019).

In Africa (Nigeria, Tunisia, Algeria and Morocco), the parasitoid complex of *T. absoluta* that has so far been recorded through field monitoring and surveys include the larval parasitoids *Dolichogenidea appellator* (Telenga, 1949) and several species in the genera *Apanteles* Foerster and *Bracon* Fabricius (Hymenoptera: Braconidae) as well as egg parasitoids in the genus *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) (Oke et al. 2016; Biondi et al. 2018; Mansour et al. 2018). In Algeria, the Mostaganem area showed the presence of seven parasitoids species: *Necremnus tutae* Ribes & Bernardo, 2015 (Hymenoptera: Eulophidae), which was the most abundant species, *Hyposoter didymator* (Thunberg, 1822) (Hymenoptera: Ichneumonidae), *Neochrysocharis* sp., *Sympiesis* sp., *Diglyphus isaea* (Walker, 1838) (Hymenoptera: Eulophidae), *Bracon* sp. and *Trichogramma* sp. (Boualem et al. 2012; Gebiola et al. 2015).

Among Braconidae subfamilies, Microgastrinae is a critically important group of parasitoid wasps estimated to include 17,000–50,000 species worldwide (Rodriguez et al. 2013; Fernandez-Triana et al. 2020), exclusively parasitizing larvae of Lepidoptera (caterpillars of butterflies and moths) across nearly the full spectrum of host taxa and terrestrial habitats. Microgastrinae are one of the most important groups in the biological control of agricultural and forestry lepidopterous pests worldwide (Whitfield 1997).

The microgastrine species *Dolichogenidea gelechiidivoris* (Marsh, 1975), originating from the Neotropics, is a koinobiont solitary endoparasitoid of *T. absoluta* and a few other closely related species (Marsh 1975; Yu et al. 2016). *Dolichogenidea gelechiidivoris* was originally described from specimens collected in Colombia, Chile, and Peru (Marsh 1975). Specimens were released in the USA for the control of *Phthorimaea operculella* (Zeller, 1873) (Lepidoptera: Gelechiidae) (Marsh 1975) and the species has also been considered as a biocontrol agent of *Keiferia lycopersicella* (Walshingham, 1897) (Lepidoptera: Gelechiidae) (Marsh 1975; Palacios & Cisneros 1995; Mujica & Kroschel 2013). Recently, *D. gelechiidivoris* has been reported as parasitoid of *T. absoluta* in Spain (Denis et al. 2021), where the wasp was considered as unintentionally introduced from the Neotropics along with the lepidopteran pest.

The International Centre of Insect Physiology and Ecology (icipe) in Kenya, in collaboration with the International Potato Center (CIP) in Peru, also identified *D. gelechiidivoris* as an effective endemic natural enemy of *T. absoluta* in their native range (Peruvian central highlands) and imported specimens of the parasitoid wasp into Kenya from Peru in 2017 for studies of its feasibility as a candidate for classical biological control of *T. absoluta* in Africa (Aigbedion-Atalor et al. 2020). The results of these studies, under laboratory conditions, indicated the potential of *D. gelechiidivoris*. However, as far as we know, there has not been any intentional release of this parasitoid wasp in agricultural fields in Africa.

In this paper we present the first record of *D. gelechiidivoris* from Africa, recovered as a parasitoid of *T. absoluta* in tomato grown in open fields and greenhouses in Algeria. We also provide morphological and molecular information to better diagnose this parasitoid wasp species and discuss the significance of its finding for future biocontrol projects against *T. absoluta* in Africa.

Methods

In December 2020, one species of Microgastrinae was reared from larvae of *T. absoluta* collected in tomato fields of the municipality of Stidia, along the Bay of Arzew and 15 km SW from the city of Mostaganem. The locality is characterized by a mild Mediterranean climate. The same wasp species was later found in greenhouses growing tomatoes in other municipalities of Mostaganem province (Table 1 and Fig. 1), including both coastal (Hadjadj, Mazagran, Hassi Mamèche, Stidia) and interior regions (Ain Tadles, Sidi Ali).

The identification of the parasitoid wasp species was made by one of the authors (JFT) in the Canadian National Collection of Insects (CNC), Ottawa, by comparison with vouchered South American specimens of *D. gelechiidivoris* housed in the CNC, which included one paratype and other authenticated specimens from South America. The holotype (deposited in the National Museum of Natural History, Washington DC, United States) and additional samples from Catalonia (Northeast of Spain) were also examined and photographed. The identified specimens from Algeria are deposited in the CNC, Canada and the University Abdelhamid Ibn Badis of Mostaganem, Algeria.

Table 1. Collecting sites of specimens of *Dolichogenidea gelechiidivoris* parasitizing *Tuta absoluta* in Algeria.

Site	Collection dates	Field characteristics
Stidia	16/12/2020; 01/05/2021	Open fields and greenhouses
Mazaghran	21/04/2021	Greenhouses
Hassi Mameche	26/04/2021	Greenhouses
Hadjadj	26/05/2021	Greenhouses
Ain Tadles	05/06/2021	Greenhouses
Sidi Ali	28/06/2021	Greenhouses

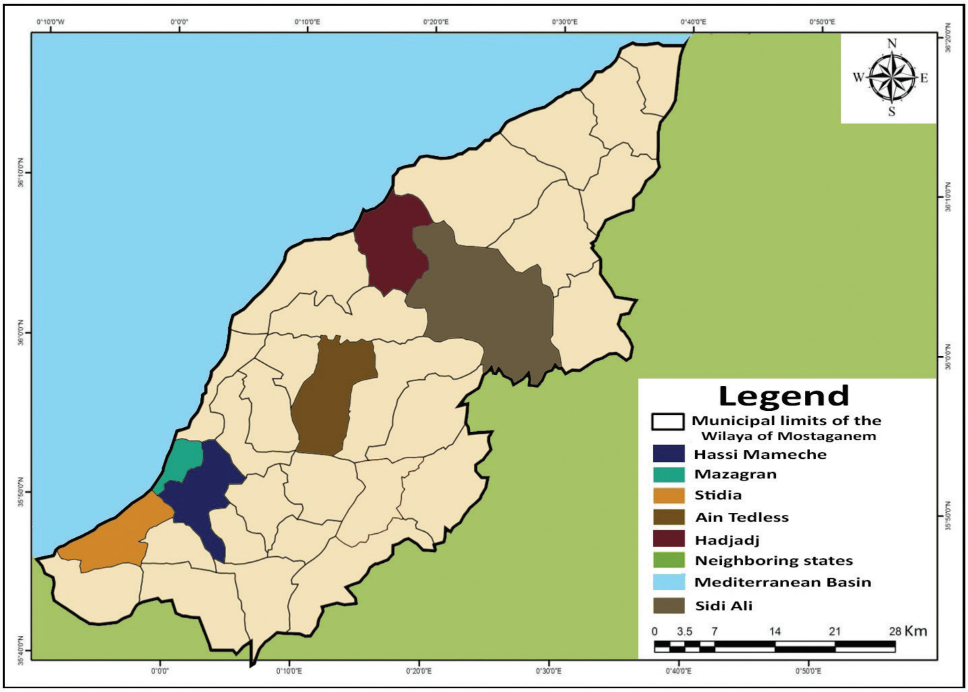

Figure 1. Map of the municipality of Stidia, along the Bay of Arzew and 15 km SW from Mostaganem, Algeria.

For morphological terms we follow several published references (see details in Fernandez-Triana et al. 2020) as well as the Hymenoptera Anatomy Ontology (HAO) website (http://portal.hymao.org/projects/32/public/ontology/).

Photographs were taken with a Keyence VHX-1000 Digital Microscope, using lenses with a range of 10–130×. Multiple images were taken of a structure through the focal plane and then combined to produce a single in-focus image using the software associated with the Keyence System. Plates were prepared using Microsoft PowerPoint 2010 and later saved as .tiff files.

DNA barcodes were obtained from specimens collected in Algeria and Spain. DNA was extracted destructively from the hind leg of 4 specimens with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). Modifications to the manufacturer's protocol from Moreau (2014) and Cruaud et al. (2019) were incorporated into the extraction. The 658 base pais (bp) barcoding region of the mitochondrial COI gene was amplified on an Eppendorf MasterCycler Pro S in reactions containing 2 µl 2.5 mM dNTPs, 2 µl 25 mM $MgCl_2$, 2.5 µl 10 X Taq Buffer, 1 µl of each 10 µM universal primer LCO1490 and HCO2198 (Folmer et al. 1994), 0.2 µl of ExTaq HS DNA polymerase (Takara Bio USA, Madison, WI), 1–4 µl of DNA template, and ddH_20 to 25 µl. The amplification program was as follows: 95 °C for 1 minute, 35 cycles of denaturation (95 °C for 15 s), annealing (49 °C for 15 s), extension (72 °C for 45 s), and a final extension at 72 °C for 4 minutes. Amplification success was confirmed by visualization on a 1% gel. Samples that successfully amplified were cleaned with ExoSAP-IT (PE Applied Biosystems, Foster City, CA, USA). Cycle sequencing was completed with the BigDye Terminator v3.1 Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA) in 10 µl reactions. Samples were purified and sequenced at the Agriculture & Agri-Food Canada Eastern Cereal and Oilseed Research Centre Core Sequencing Facility (Ottawa, ON, Canada) on a 3500xl DNA Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). Chromatograms were trimmed, aligned, and assembled with Geneious Prime v2020.0.4. Assembled sequences were compared to available sequences for similarity.

Sequences were deposited in the Barcode of Life Data Systems (BOLD; http://www.boldsystems.org). A dataset containing all available sequences of *D. gelechiidivoris* (ours and others previously obtained from specimens in South America) was created in BOLD and assigned a doi for future reference (dx.doi.org/10.5883/DS-DOLIGELE). A Neighbour joining (NJ) tree with the 16 sequences of the species over 600 base pairs (bp) was built using BOLD standard parameters.

Results and discussion

The parasitoid wasp specimens reared in Algeria from *T. absoluta* on tomato were identified as *D. gelechidiivoris*, based on the original description (Marsh, 1975) and comparison with the species holotype, one paratype and other material deposited in the CNC (Figs 2–6). The Algerian specimens have slightly darker hind legs (Figs 6, 7) but otherwise match the rest of the material studied.

Additional corroboration of the species identity came from Algerian specimens from which DNA barcodes were obtained and were a perfect match to specimens from South America and Spain (Fig. 8).

A total of 25 species of *Dolichogenidea* have been so far recorded from Africa (Fernandez-Triana et al. 2020), but many more remain undescribed. There has never been a taxonomic review of the genus in the continent, although some species, at that time under the genus *Apanteles*, were treated by Wilkinson (1932) and Nixon (1965), and the faunas of the Arabian Peninsula and Réunion Island have been partially studied recently (Rousse & Gupta 2013; Fernandez-Triana & van Achterberg 2017).

At present, there are no keys to separate African species of *Dolichogenidea*, but the following combination of morphological characters should suffice to characterize and

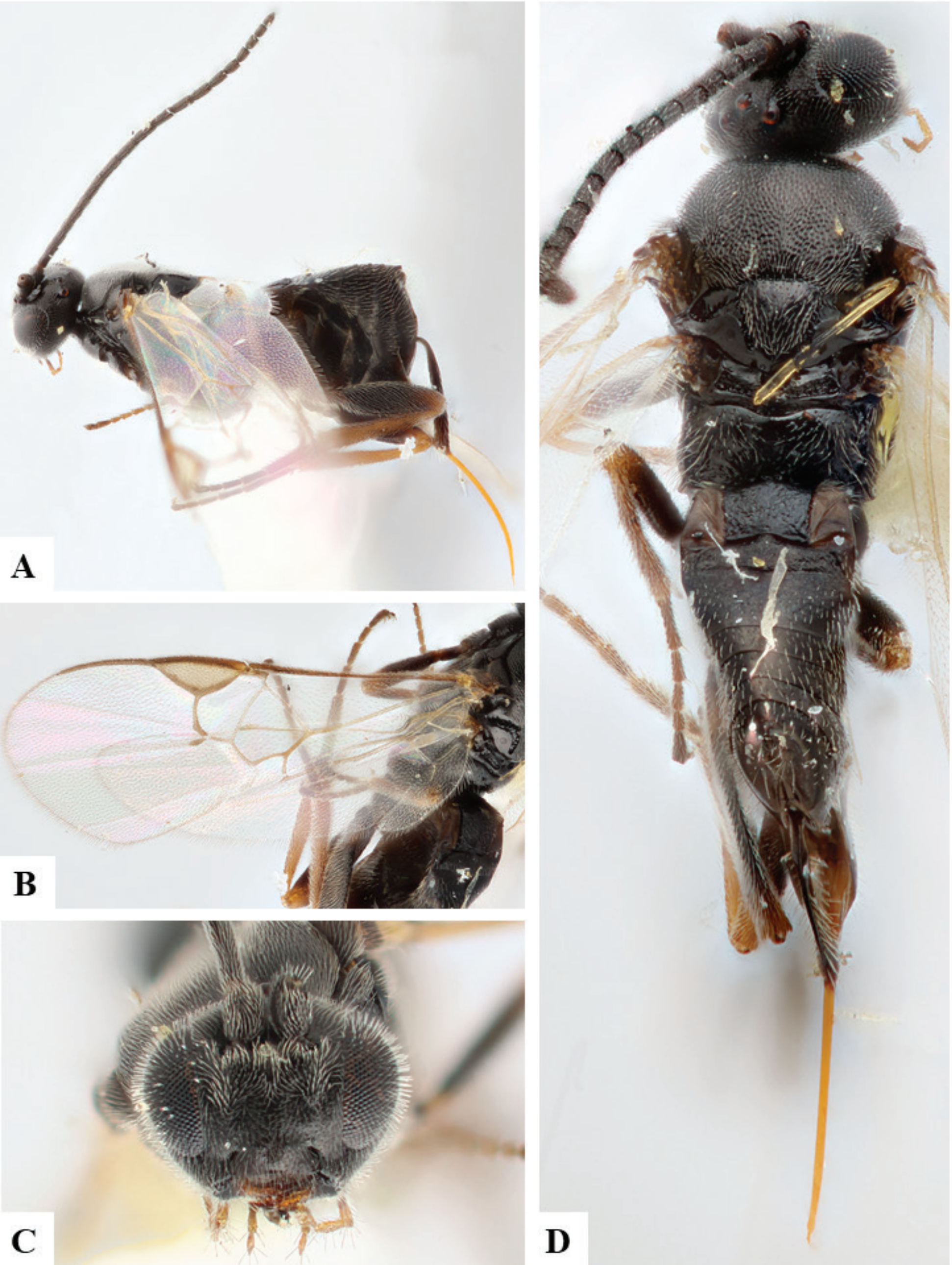

Figure 2. Female holotype of *Dolichogenidea gelechiidivoris*, Colombia, voucher code USNMENT00831760.

unequivocally recognize *D. gelechiidivoris* among all other described species of the genus in Africa: 1) antenna shorter than body; 2) anteromesoscutum mostly shiny, covered with white setae and with relatively sparse but evenly distributed and well-marked

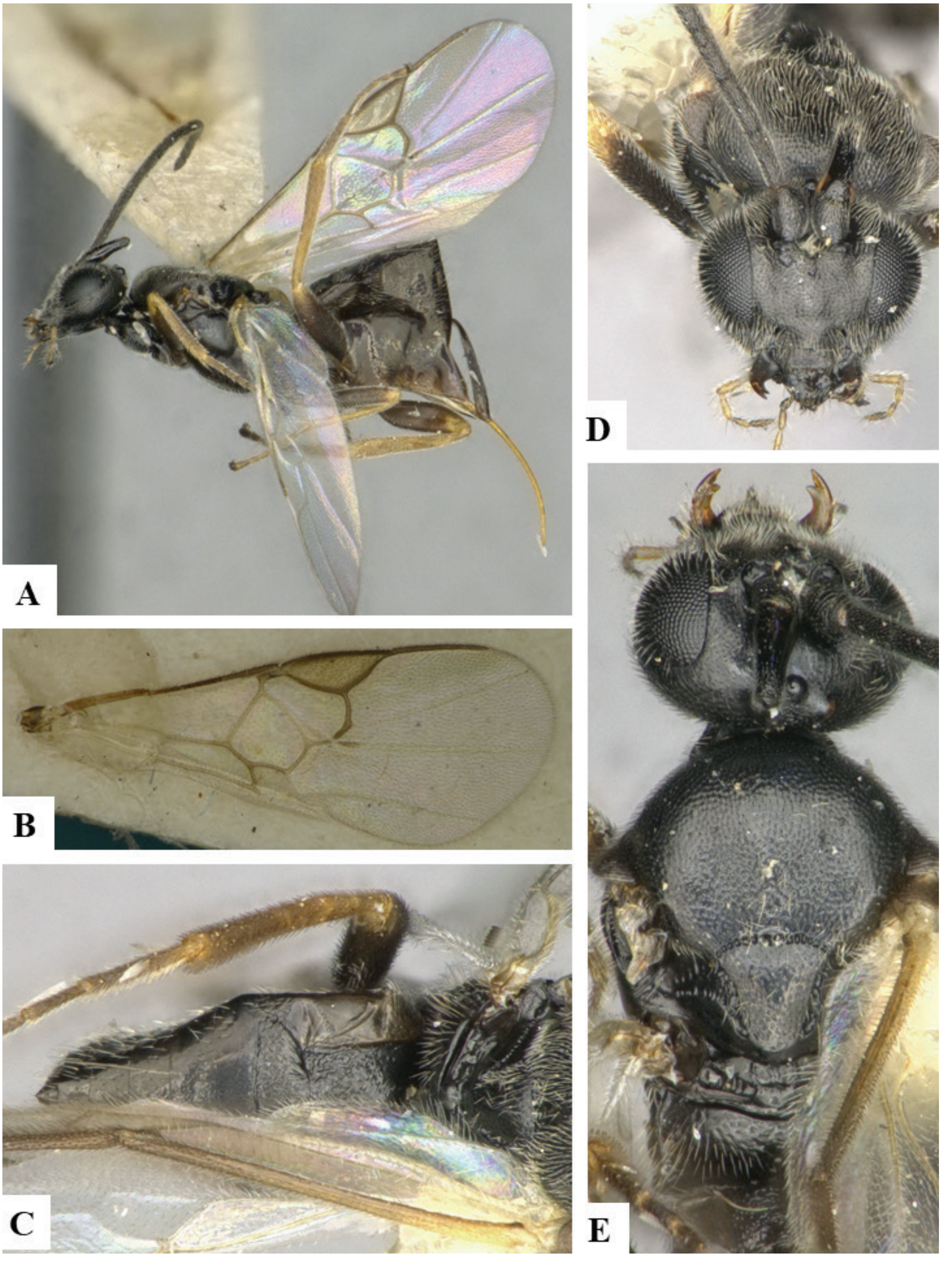

Figure 3. Female paratype of *Dolichogenidea gelechiidivoris*, Colombia, voucher code CNC678061.

punctures; 3) mesoscutellar disc shiny with punctures that are comparatively shallower and more sparse than in anteromesoscutum; 4) polished area (lunules) of the lateral side of the axillar complex about half the height of lateral side; 5) propodeum mostly

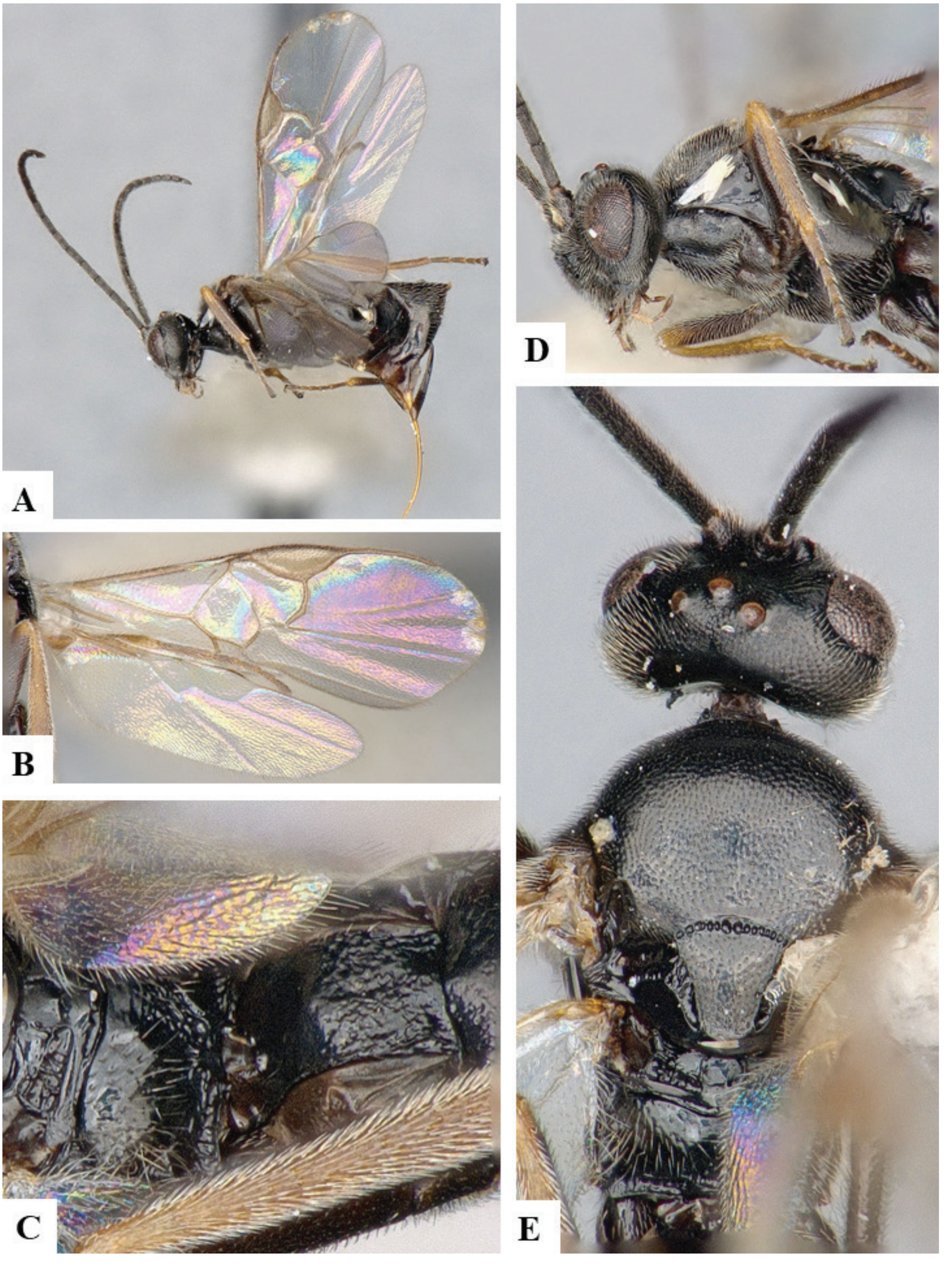

Figure 4. Female paratype of *Dolichogenidea gelechiidivoris*, Colombia, voucher code CNCHYM 01043.

smooth and shiny on anterior half, posterior half rugose medially, with rugae near nucha and forming lower part of an areola; 6) metasoma relatively short and slightly compressed laterally; 7) first mediotergite (T1) mostly parallel-sided, but slightly nar-

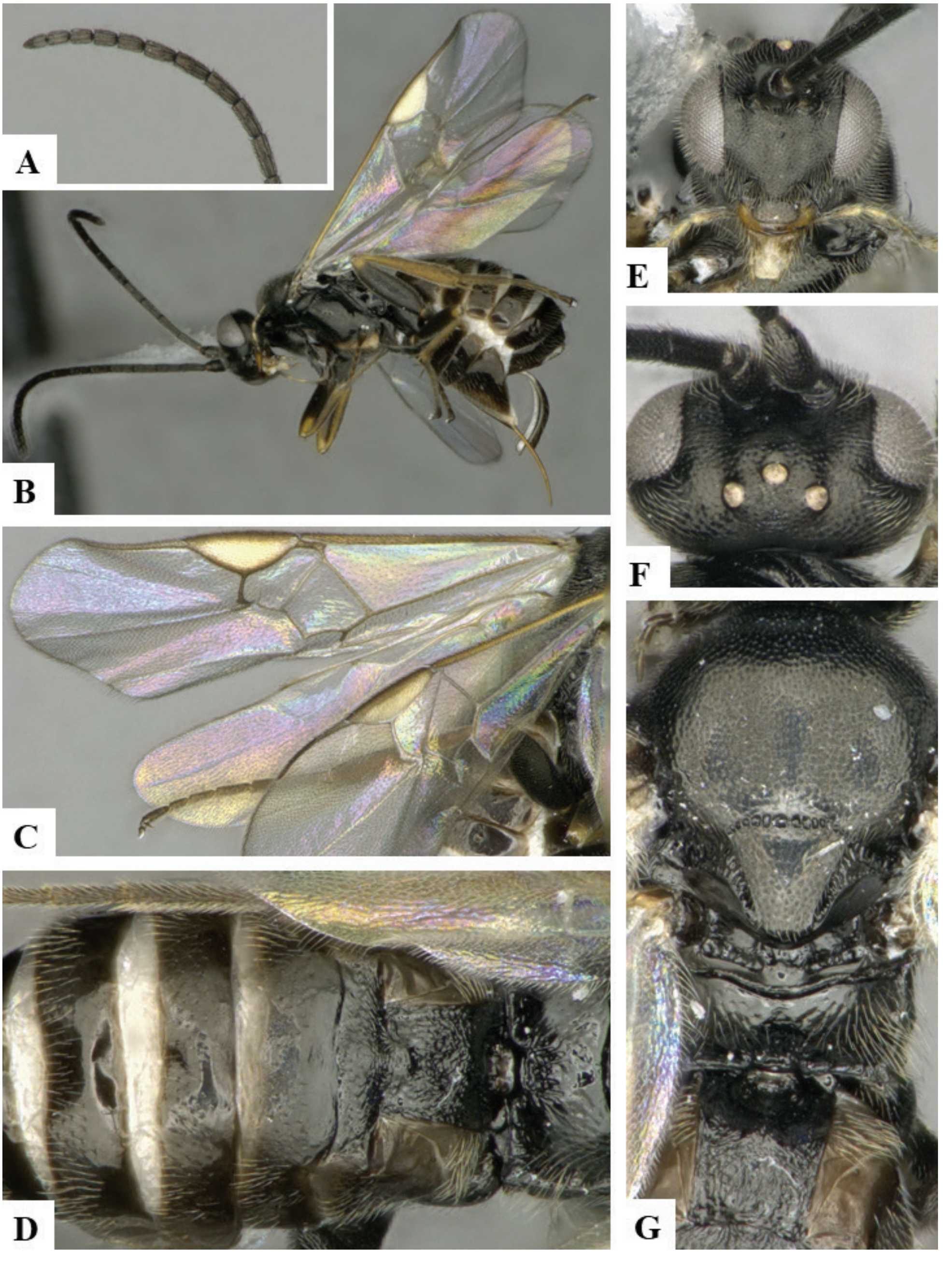

Figure 5. Female specimen of *Dolichogenidea gelechiidivoris*, Spain, voucher code CNC1196542.

rowing near posterior margin; 8) T1 smooth on anterior half, rugose on posterior half; 9) second mediotergite (T2) relatively transverse, its width at posterior margin more than 3.0× its medial length, with lateral sides oblique and posterior margin sinuate

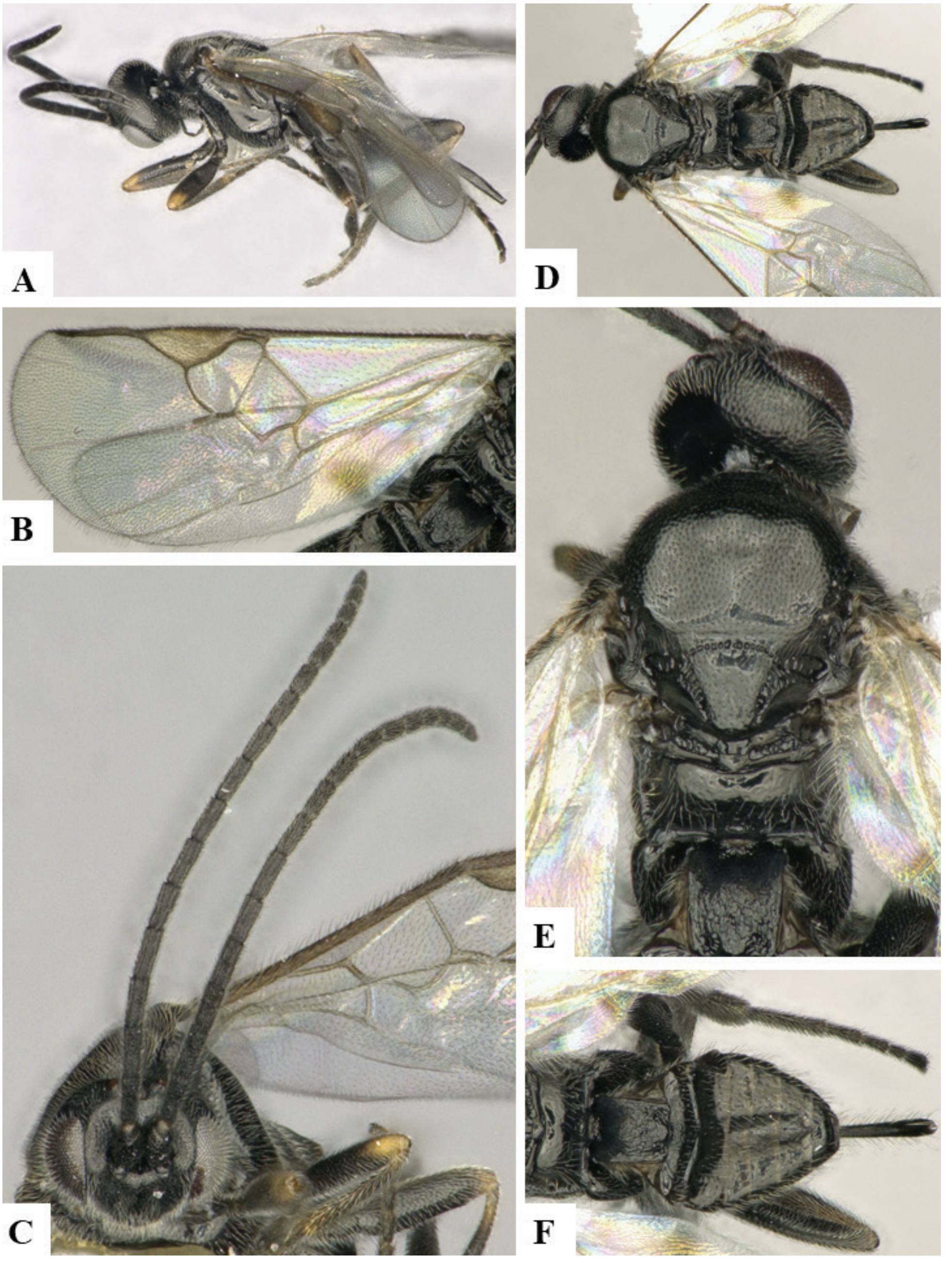

Figure 6. Female specimen of *Dolichogenidea gelechiidivoris*, Algeria, voucher code CNC1196948.

(shape of T2 varies slightly and it is sometimes less transverse in some specimens from all geographical areas examined); 10) T2 mostly sculptured, with small area smooth along posterior margin; 11) ovipositor sheaths about same length (or slightly shorter)

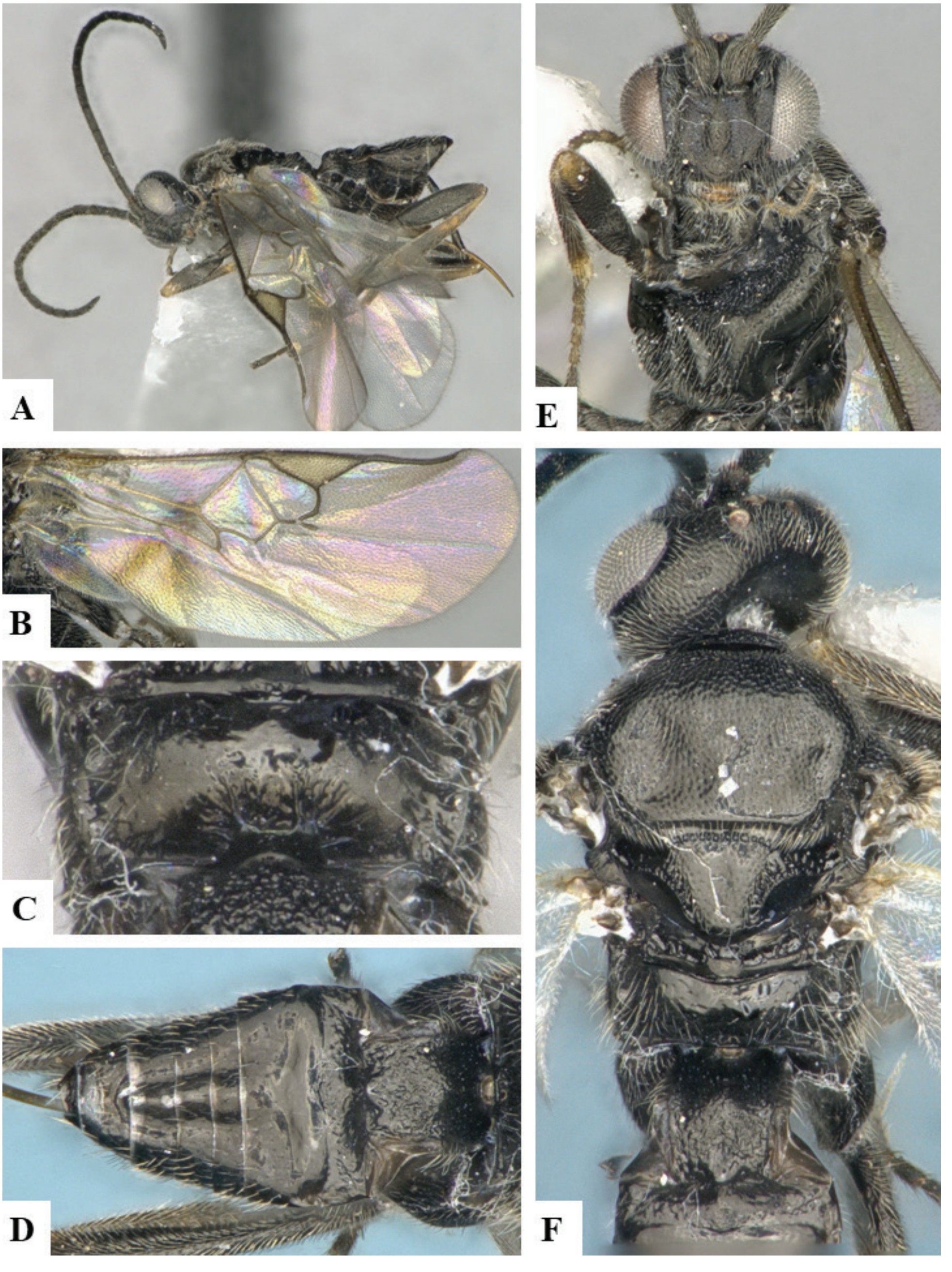

Figure 7. Female specimen of *Dolichogenidea gelechiidivoris*, Algeria, voucher code CNC1180035.

than metatibia length, ovipositor evenly down curved; 12) fore wing with pterostigma relatively thick, light coloured (pale yellow-brown) but with thin brown margins; 13) fore wing vein R1 comparatively short, its length about the same than pterostigma length, but less than 2.5× the distance between its end and end of vein 3RS; 14) most veins in wings either light brown to yellow-brown or transparent; 15) legs mostly dark brown to black but with posterior 0.1–0.2 of femora and most of tibiae yellow to yellow brown (specimens from Algeria and some specimens from Spain we have examined have darker tibiae, which can be mostly dark brown to black); 16) body color mostly black, including tegula and humeral complex.

Especially, the following characters are useful to recognize the species: the sculpture of propodeum; sculpture and shape of T1 and T2; color and shape of pterostigma; short vein R1; relative short antenna and metasoma relatively compressed laterally. Among the specimens included in the following study, the morphological variation was relatively minor (with some specimens having slightly darker legs and slightly less transverse T2 shape), thus the species is fairly recognizable and uniform.

Dolichogenidea appellator (Telenga, 1949) is the only other species of *Dolichogenidea* from Africa which has been recorded parasitizing *Tuta absoluta* in the continent (Idriss et al. 2018; Mansour et al. 2018). It can be clearly distinguished from *D. gelechiidivoris* because of different sculpture of first two metasomal segments (T1 and T2 are entirely smooth); fore wing pterostigma mostly light brown with a white or transparent spot on anterior 0.2–0.3; tegula (entirely) and humeral complex (partially) yellow; profemur (entirely) and mesofemur (anterior half) pale (yellow to light brown-yellow); and longer antenna, as long as body length.

From a molecular perspective, *D. gelechiidivoris* is also clearly distinctive and diagnosable. The specimens with available data in BOLD come from six different countries, Algeria, Chile, Colombia, Peru, Spain and Venezuela. They comprise 19 sequences, with 16 of them over 600 bp (15 representing full barcodes). Out of the three short sequences (107–164 bp) two are especially important as they were retrieved from paratype specimens housed in the CNC (voucher codes: CNCHYM 01042 and CNCHYM 01043, see details in dataset dx.doi.org/10.5883/DS-DOLIGELE); those paratype sequences match very well with the full barcodes. There were no differences between specimens from South America, Spain or Algeria (Fig. 8); the only exception was a specimen from Chile (voucher code WAM 0495) which had one base pair different (0.16%) compared to all other sequences. The Barcode Index Number (BIN, see more details on the BIN concept in Ratnasingham and Hebert 2013) assigned to *D. gelechiidivoris* is BOLD:AAM4042. That BIN has 31 bp (4.72%) of difference with the closest available BIN in BOLD (BOLD:AAZ9580) which comprises a single, unidentified specimen of *Dolichogenidea* specimen from California, USA; therefore making the identification of *D. gelechiidivoris* using DNA barcoding fairly easy and very reliable (http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAM4042).

Although South American specimens of *D. gelechiidivoris*, from Peruvian central highlands, were brought to Kenya in 2017 by researchers at icipe, they have only been studied in laboratory conditions for potential classical biological control of *T. absoluta* (Aigbedion-Atalor et al. 2020). Based on available reports and scientific publications, *D. gelechiidivoris* has not yet been released into field conditions in Africa. Thus, our finding of the parasitoid wasp species in open tomato fields and greenhouses in Algeria

Figure 8. Neighbour joining (NJ) tree with all available sequences of *Dolichogenidea gelechiidivoris* over 600 base pairs. For every specimen details of the voucher code, country of origin and total of base pairs is shown in the corresponding branch.

is the first report of individuals of this species naturally occurring in Africa. Its presence is unlikely to be related to the efforts being undertaken in Kenya; rather, it is more likely that the species arrived in northern Africa from Spain, where it had been recently found (Denis et al. 2021). In any case, the introduction seems to be accidental, probably following its host (*T. absoluta*), as it is supposed to have been the case in Spain.

The documentation in this paper of *D. gelechiidivoris* already established in Africa (at least in northern areas of the continent) is very relevant for the future of biological control of *T. absoluta* in the continent. Since this wasp species occurred spontaneously in tomato crops but autonomous spreading to isolated fields seems limited, it is possible to recommend its mass rearing and augmentation release for its rapid establishment in other areas, and to include its management in combination with other biocontrol agents in the IPM programs of *T. absoluta* in the region.

Acknowledgments

We are grateful for the excellent comments and suggestions provided by the reviewers Kaoru Maeto, Ilgoo Kang and Sergey Belokoblylskij as well as the editor Elijah Talamas, which considerably improve the final version of this paper. The work of JFT and AB was supported by Project J-002276 "Systematics of beneficial arthropods in support of resilient agroecosystems", from Agriculture and Agri-Food Canada. JA was supported by Horta.Net and the CERCA Programmes of the Generalitat de Catalunya.

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