1 **Naturalized *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera: Braconidae) complement the** 2 **resident parasitoid complex of *Tuta absoluta* (Meyrick) (Lepidopera:Gelechiidae) in Spain**

3 Carmen Denis¹, Jordi Riudavets¹, Oscar Alomar¹, Nuria Agustí¹, Helena Gonzalez-Valero², Martina
4 Cubí², Montserrat Matas³, David Rodríguez⁴, Kees van Achterberg⁵, Judit Arnó¹

5 ¹Sustainable Plant Protection Program, IRTA, Cabriels, Spain; ²Federació Selmar, Santa Susanna, Spain;
6 ³ADV Baix Maresme, Vilassar de Mar, Spain; ⁴Agrícola Maresme Segle XXI, Olèrdola, Spain; ⁵Naturalis
7 Biodiversity Center, Leiden, The Netherlands

8 9 **Abstract**

10 Our study aimed to assess the contribution of natural parasitism due to *Necremnus tutae* Ribes &
11 Bernardo (Hymenoptera: Eulophidae) to the biological control of *Tuta absoluta* (Meyrick)
12 (Lepidopera:Gelechiidae) in commercial plots where an IPM program based on the use of predatory mirid
13 bugs was implemented. During the samplings, the presence of another parasitoid was detected and,
14 therefore, a second part of our study intended to identify this species and to evaluate the importance of its
15 natural populations in the biological control of the pest. Leaflets with *T. absoluta* galleries were collected
16 during 2017–2020 from commercial tomato plots in the horticultural production area of Catalonia
17 (Northeast Spain), including greenhouses, open fields, and roof covered tunnels that lack side walls. In
18 the laboratory, *T. absoluta* larvae were classified as ectoparasitized, alive, or dead. Reared parasitoids
19 from ectoparasitized larvae were mostly morphologically identified as *Necremnus* sp. with parasitism
20 rates that peaked in summer months with values between 9 and 15%. Some of these ectoparasitized larvae
21 also yielded another parasitoid identified as *Dolichogenidea gelechiidivoris* Marsh (Hymenoptera:
22 Braconidae) by both morphological and molecular-DNA barcoding methods. In 2020, parasitism rates
23 due to *D. gelechiidivoris* that increased with season up to 22%. Our work reports for the first time in
24 Europe the presence of the neotropical species *D. gelechiidivoris* adding this biocontrol agent to the
25 resident parasitoid complex of *T. absoluta* in Spain.

26 27 **Introduction**

28 *Tuta absoluta* (Meyrick) (Lepidopera:Gelechiidae) is native to South America where has been considered
29 an important tomato pest since a long time (Larrain 1987). The first report outside its area of origin was

30 from Spain in 2006 (Urbaneja et al. 2007). From then onwards, the pest did spread over the
31 Mediterranean basin, and then quickly colonized Africa and Asia (Desneux et al. 2010; Desneux et al.
32 2011), threatening tomato production (Biondi et al. 2018).

33 Although insecticides still remain the main control tool in many world areas, many efforts have targeted
34 sustainable biological control methods (Biondi et al. 2018). In Spain, the positive role of the predatory
35 mirid bugs *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) was
36 soon acknowledged (Arnó et al. 2009; Urbaneja et al. 2009). Successful IPM programs based on both
37 predators already in use at the time of invasion did greatly contribute to the control of *T. absoluta*
38 (Urbaneja et al. 2012). These two bugs remain as cornerstones for the biological control of several pests
39 in the area (Arnó et al. 2018).

40 Surveys of parasitoids that could complement the poor predator action on *T. absoluta* larvae were soon
41 undertaken in the Mediterranean (Zappalà et al. 2013; Gabarra et al. 2014), and several larval parasitoids
42 of *T. absoluta* within the Eulophidae, Braconidae, Chalcididae, Ichneumonidae and Pteromalidae were
43 recorded (Biondi et al. 2018; Mansour et al. 2018). Out of these species, *Necremnus tutae* Ribes &
44 Bernardo (Hymenoptera: Eulophidae), first identified as *Necremnus* nr. *artynes*, was found consistently
45 parasitizing *T. absoluta* (Gebiola et al. 2015), and several studies have recognized the contribution of
46 natural populations to the biological control of the pest (Abbes et al. 2014; Crisol-Martínez and van der
47 Blom 2019; Arnó et al. in press). This parasitoid attracted the attention of the biocontrol industry, and for
48 some time it was commercially available, but the high host-killing rate was a serious drawback for
49 successful mass rearing (Calvo et al. 2016) and production has been discontinued.

50 To further assess the contribution of *Necremnus tutae* to the biological control of *T. absoluta*, our first
51 goal was to estimate the natural parasitism in commercial plots where the IPM program based on the use
52 of predatory mirid bugs was implemented. However, since we observed the presence of another
53 parasitoid, the second part of our study aimed to identify this species and to evaluate the importance of its
54 natural populations in the biological control of *T. absoluta*.

55

56 **Materials and Methods**

57 Samples were collected during 2017–2020 from commercial tomato plots in the horticultural production
58 area of Catalonia (Northeast Spain), including greenhouses, open fields, and roof covered tunnels that

59 lack side walls (Table 1). Each plot was sampled up to 21 times depending on crop duration and
60 *T. absoluta* infestation levels. Plots were sampled by randomly walking in the plot and collecting leaflets
61 that had galleries big enough to host a second to third instar larva of *T. absoluta*. Sampling terminated
62 after 20 minutes or a maximum of 25 leaflets, whichever was reached first, in order to minimize sampling
63 time and costs, particularly when infestation levels were low (Naranjo 2008).

64 Leaflets were taken to the laboratory and inspected under a stereomicroscope. *Tuta absoluta* larvae were
65 classified as “ectoparasitized” (with pupae, larvae or eggs of a parasitoid on the *T. absoluta* larva), “alive”
66 (not ectoparasitized and able to crawl when touched with a fine brush), or “dead” (not ectoparasitized and
67 unable to crawl when touched with a fine brush), and the number of each category was recorded. As our
68 initial interest was on the contribution of *N.tutae* to the control of *T. absoluta*, during 2017, 2018 and
69 2019, only the ectoparasitized larvae were placed inside Petri dishes (maximum of 3 larvae per dish), and
70 kept at room temperature for at least 2-3 weeks.

71 During 2019, the recurrent presence in the samples, albeit at a low rate, of a parasitoid belonging to the
72 Braconidae was observed. Therefore, additional samples were collected from nine tomato fields in
73 September. From these samples, alive, dead and ectoparasitized larvae were individualized in Petri dishes
74 and kept in the climatic chamber (25°C) for a maximum of 42 days until the emergence of *T. absoluta* or
75 of adult parasitoids.

76 From the samples collected in 2020, the number of alive, dead, and ectoparasitized *T. absoluta* larvae was
77 also recorded as before, but only larvae that were alive, with no clearly visible ectoparasitoids, were kept
78 in order to determine the parasitization rate by endoparasitoids. Larvae were placed in a Petri dish
79 (maximum of 3 larvae per dish) together with the leaflet and stored at room temperature for a maximum
80 of 3 weeks until the pupation of *T. absoluta* or the emergence of a parasitoid. Emerged parasitoids from
81 the four years samples were collected and stored in 70% alcohol for further identification.

82 Due to the high irregularity of infestation levels in the plots, the number of leaflets collected from each
83 plot and date (a sample) was very variable, and furthermore, not all the galleries had a *T. absoluta* larvae
84 inside. Consequently, the total number of larvae (alive, dead or ectoparasitized) collected in each plot was
85 very variable, and sometimes very low. Leaflets with only empty galleries and no *T. absoluta* larvae were
86 discarded. To summarize the levels of parasitism, samples were pooled for each month and year. The
87 percentage of monthly ectoparasitism was calculated by dividing the number of ectoparasitized larvae by

the total number of larvae recorded each month. Additionally, for the 2020 samples of alive *T. absoluta* larvae, monthly parasitism due to endoparasitoids was calculated dividing the number of emerged parasitoids by the total number of larvae recorded each month.

Adult parasitoids were first identified to family and sub-family level using the keys by [Grissell and Schauff \(1990\)](#) and [Hanson and Gauld \(2006\)](#). Eulophidae were further identified to genus using the keys by [Askew \(1968\)](#) and [Gebiola et al. \(2015\)](#). Microgastrinae (Hymenoptera: Braconidae) were first identified to genus following the descriptions of [Fernandez-Triana et al. \(2020\)](#), and then as *Dolichogenidea gelechiidivoris* Marsh (= *Apanteles gelechiidivoris*) using the description of [Marsh \(1975\)](#). Additional material examined for morphological identification were 10 ♂♂ and 10 ♀♀, from a laboratory rearing started with adults that emerged from *T. absoluta* larvae collected in 2019 from several tomato fields in El Maresme county (31TDF49 to 31TDG92, Catalonia, Spain). These voucher specimens were prepared and deposited in Naturalis Biodiversity Center (Leiden, The Netherlands).

To confirm the morphological identification of *D. gelechiidivoris*, 11 specimens were sequenced for DNA barcoding identification. The specimens emerged from samples collected in different locations from eastern Catalonia along a transect of 100 Km (from 31TDF17 to 31TDG84 and 31TEG03, in the municipalities of Viladecans (2018), Mataró (2019, 2020), Argentona (2018), 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. in press](#)) collected in 2016 in Malgrat de Mar was also DNA barcoded.

Total genomic DNA was extracted from whole insects by using SpeedTools Tissue DNA Extraction Kit (Biotools, Madrid, Spain) following the manufacturer protocol and eluted in 100 µl of BBE buffer provided by the manufacturer and stored at -20°C. A 658-bp region of the CO1 gene was amplified with the following protocol using primers LepF1 5-ATTCAACCAATCATAAAGATATTGG-3 and LepR1 5-TAAACTTCTGGATGTCCAAAAAATCA-3 ([Smith et al. 2006](#)). PCR reaction volumes (20 µl) contained 2 µl of resuspended DNA, 10 µl of Master Mix (Biotools, Madrid, Spain) and 0.4 µl of each primer [10 µM]. Samples were amplified in a 2720 thermal cycler (Applied Biosystems, CA, USA) using the thermocycling profile of one cycle of 2 min at 94°C; five cycles of 40 sec at 94°C, 40 sec at 45°C, and 1 min at 72°C; followed by 35 cycles of 40 sec at 94°C, 40 sec at 51°C, and 1 min at 72°C, with a final step of 5 min at 72°C ([Smith et al. 2006](#)). PCR products were analysed by electrophoresis in 2.4% agarose gels stained with GelRed® (Biotium, Hayward, CA) and visualized under UV light. They were purified with QIAquick PCR Purification kit (Qiagen) and bidirectionally sequenced by using BIGDYE

118 3.1 on an ABI 3730 DNA Analyzer (Applied Biosystems) at the Genomics Unit of the Barcelona Science
119 Park (University of Barcelona). Obtained sequences were compared against the reference database
120 *Barcode of Life Data System* (BOLD, <http://www.boldsystems.org/>), to find the matching species.

121

122 **Results**

123 DNA barcoding successfully confirmed the initial identification based on morphological characters of the
124 11 analyzed specimens as *D. gelechiidivoris*, regardless of location and year of collection. Obtained
125 similarity percentages ranged from 100 to 99,48% compared with the 13 *D. gelechiidivoris* available
126 sequences at the time of the analysis (February 2021) in the GenBank database (Accession codes:
127 KX443088, HQ558975-HQ558977, JN282071-JN282078 and JQ849955). . Obtained sequences were
128 also deposited to the GenBank database (Accession codes: MZ298974-MZ298984).

129 Monthly levels of ectoparasitized larvae over the four sampling years are displayed in Table 2. They were
130 recorded from April to November, with levels ranging from 0.1% (May 2017) to 35.7% (November
131 2020). Apart from this exceptionally high value, each year the parasitism peaked during the summer
132 months of August and September with values between 9 and 15%.

133 Most parasitoids that emerged from ectoparasitized larvae from 2017 to 2019 were Eulophids (87%) and
134 a smaller percentage (11%) belonged to Braconidae (Table 3). Among the Eulophidae, eight genera were
135 recorded: *Necremnus* (162 individuals), *Pnigalio* (7), *Neochrysocharis* (5), *Diglyphus* (4), *Stenomesus* (4),
136 *Aprostocetus* (3), *Cirrospilus* (2), and *Sympiesis* (1). Out of the 25 individuals belonging to Braconidae
137 that emerged from larvae that had an ectoparasitoid on them (Table 3), 23 were morphologically
138 identified as *D. gelechiidivoris* same as the four individuals collected in 2016.

139 The additional samples collected in September 2019 yielded 170 *T. absoluta* larvae. From 21
140 ectoparasitized *T. absoluta* larvae emerged 11 *Necremnus* sp., 2 *Diglyphus* sp., 1 *Neochrysocharis* sp.,
141 and 1 unidentified Eulophid. From 114 dead *T. absoluta* larvae emerged 6 *Necremnus* sp., and from the
142 35 alive larvae emerged 13 *D. gelechiidivoris*.

143 In 2020, 1,872 alive larvae were collected from 228 samples from 32 plots. From these larvae, we
144 obtained a total of 264 parasitoids that emerged from 92 different samples collected from 20 plots. A
145 subsample of 165 were morphologically identified as *D. gelechiidivoris*, together with one
146 *Neochrysocharis* sp., and one from the subfamily Alysiinae. As can be observed in Figure 1, the

percentage of endoparasitism steadily increased from May until October. No endoparasitoids were recorded in March, April and November.

Discussion

Results of our four-years field survey confirmed the relevance of Eulophidae as larval parasitoids of *T. absoluta* in the Mediterranean basin. All genera except *Aprostocetus* had been previously reported (Biondi et al. 2018). We reared three individuals of this genus from a single sample collected in September 2017. *Aprostocetus* are parasitic or hyperparasitic in Lepidoptera (Sakaltaş and Tüzün 2014), and Mirchev et al. (2001) refer to an *Aprostocetus* sp. as an hyperparasitoid of a Braconid parasitizing a Gelechiid.

Among Eulophidae, our results corroborate the importance of *Necremnus* sp. as the most widespread ectoparasitoid of *T. absoluta* larvae in the Mediterranean, as reported before (Ferracini et al. 2012; Zappalà et al. 2013; Gabarra et al. 2014; Gebiola et al. 2015; Biondi et al. 2018). In fact, *N. tutae*, which is by far the predominant *Necremnus* species in the area of our survey (Arnó et al. in press), was considered a promising parasitoid to be released for *T. absoluta* control (Calvo et al. 2013; Chailleux et al. 2014; Bodino et al. 2016; Calvo et al. 2016), although currently it is not commercially available. Rates of ectoparasitized larvae found in the present study, mostly due to *Necremnus* sp., were similar to those recorded in other field studies in the Mediterranean basin. In the same area of the present study, parasitism rates by *N. tutae* in sentinel plants were close to 20% (Arnó et al. in press), and in Tunisia it was of 26% in sentinel plants, and between 11 and 15% when sampling the crop (Abbes et al. 2014). Much higher rates, up to 73%, were recorded in tomato greenhouses in the southeast of Spain (Crisol-Martínez and van der Blom 2019).

Contribution of *N. tutae* to the control of *T. absoluta* goes further than only parasitization. As many Eulophidae, it kills more larvae than parasitizes in order to obtain nutrients that have a strong positive effect on its reproduction (Ferracini et al. 2012; Balzan and Wäckers 2013; Calvo et al. 2013; Chailleux et al. 2014; Calvo et al. 2016; Bodino et al. 2019). In the present study, although the number of dead larvae was recorded, we could not determine the exact causes of mortality because even all sampled plots were managed according to an IPM program based on predatory mirids, insecticide applications were also occasionally required.

176 An important outcome of our study was the detection of *D. gelechiidivoris* in field collected samples from
 177 2017 to 2020, and also in one sample collected in 2016. *Dolichogenidea gelechiidivoris* is native of South
 178 America, where it is considered as an important agent of biological control (Larrain 1987, Agudelo and
 179 Kaimowitz 1997; Vallejo 1999; Salas-Gervasio et al. 2019). In Colombia, mass rearing protocols were
 180 developed to release this parasitoid for *T. absoluta* control (Morales et al. 2013). Furthermore, in 2017 it
 181 was imported to Kenia from Peru to contribute to the control of *T. absoluta* in Africa (Aigbedion-Atalor
 182 et al. 2020). Another species, *Dolichogenidea appellator* (Telenga) (= *Dolichogenidea litae* (Nixon)), was
 183 occasionally found parasitizing *T. absoluta* in the same area as the present study (Gabarra et al., 2014),
 184 and was found also associated with this pest in Sudan (Mansour et al. 2018).

185 To our knowledge, this is the first report of *D. gelechiidivoris* naturally occurring outside its area of
 186 origin. Since there is no record of intentional introduction of *D. gelechiidivoris* into Europe, and the
 187 importation to Africa took place in 2017, one year after our first detection of this species in 2016, the
 188 results of our survey suggest that this parasitoid was unintentionally introduced from the Neotropics
 189 together with the pest. Accidental introductions of natural enemies in new territories are not strange. Roy
 190 et al (2011) stated that most of the alien arthropod predator and parasite species in Europe arrived
 191 accidentally, as part of worldwide movement of invasive pests that is facilitated by global trade. Trade of
 192 infested fruits has been pointed out as the most probable cause of the arrival of *T. absoluta* to Spain from
 193 South America and the quick spread of the pest (Desneux et al. 2010).

194 As all Microgastrinae, *D. gelechiidivoris* is a koinobiont solitary larval endoparasitoid (Fernandez-Triana
 195 et al. 2020), and the host remains alive until the end of the parasitoid development. Of the 2017–2019
 196 samples of *T. absoluta* larvae, we only kept those that had an ectoparasitoid egg or larva on them, but
 197 11.5% of the emerged parasitoids were *D. gelechiidivoris* (Table 3). This suggests that there is no clear
 198 recognition of previous parasitism between ectoparasitoids (mainly *Necremnus* sp.), and *D.*
 199 *gelechiidivoris*.

200 When we maintained all alive larvae to record parasitoid emergence (additional 2019 samples, and all
 201 2020 samples), the endoparasitism rate was 7.6% in September 2019 and increased up to 22% from May
 202 to October 2020 (Fig. 1). However, the real rate of parasitism by *D. gelechiidivoris* in 2020 was probably
 203 underestimated since about 11% of ectoparasitized larvae collected between 2017 and 2019 yielded *D.*
 204 *gelechiidivoris*, and ectoparasitized larvae of 2020 had been discarded.

205 In this scenario of coexistence of several natural enemies, the interactions between *D. gelechiidivoris* and
 206 *Necremnus* sp. will be of great importance. For example, young females of the ectoparasitoid
 207 *Dineulophus phthorimaeae* De Santis (Hymenoptera: Eulophidae) avoided multiparasitism on the
 208 microgastrinid endoparasitoid *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae), but older
 209 females did not discriminate heterospecific parasitized *T. absoluta* larvae, and joint action of both
 210 parasitoids exerted an important control of *T. absoluta* (up to 80% of host larvae mortality) (Salas-
 211 Gervasio et al., 2019). The outcome of competition between parasitoids attacking the same host depends
 212 on many factors that may explain the dominance of one parasitoid over another, e.g. where the venom of
 213 idiobiont ectoparasitoids has little or no effect on the development of endoparasitic koinobionts (Harvey
 214 2013), although in multiple parasitisms between an ectoparasitoid and an endoparasitoid, the former
 215 normally wins (Mitsunaga and Yano 2004). Furthermore, the interaction between *D. gelechiidivoris* and
 216 predatory mirids will be also of interest. These predators prefer to prey on eggs but may also feed on
 217 young *T. absoluta* larvae (Arnó et al. 2009; Urbaneja et al. 2009), which are the preferred host instar of
 218 the parasitoid (Aigbedion-Atalor et al. 2020). *Nesidiocoris tenuis* did not prey on nor did reduce the
 219 oviposition by *D. gelechiidivoris*, and the efficacy of both natural enemies together on *T. absoluta* was
 220 significantly higher than either natural enemy alone (Aigbedion-Atalor 2020). The outcome of the
 221 interactions among these biocontrol agents will be determinant for a more successful control of *T.*
 222 *absoluta* (Tarusikirwa et al. 2020).

223

224 Acknowledgements

225 The present research was supported by the Spanish Ministry of Economy and Competitiveness
 226 (AGL2016-77373-C2-1-R) and the Ministry of Agriculture, Livestock, Fisheries and Food of the
 227 Generalitat de Catalunya. Authors from IRTA were supported by the CERCA Programme/Generalitat de
 228 Catalunya. Carmen Denis was supported by a PhD grant of BECAL-PY. We are in debt to Dr. Valmir
 229 Antonio Costa (Instituto Biológico of Campinas, Brazil) and Dr. José Fernandez-Triana (Canadian
 230 National Collection of Insects, Ottawa, Canada) for their advice in the identification of parasitoids.

231

232 References

- 233 Abbes K, Biondi A, Zappalà L, Chermiti B (2014) Fortuitous parasitoids of the invasive tomato leafminer
234 *Tuta absoluta* in Tunisia. *Phytoparasitica* 42:85–92. <https://doi.org/10.1007/s12600-013-0341-x>
- 235 Agudelo LA, Kaimowitz D (1997) Tecnología agrícola sostenible: retos institucionales y metodológicos.
236 Dos estudios de caso en Colombia. Instituto interamericano de Cooperación para la Agricultura
237 (IICA) / Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH. San José, Costa
238 Rica.
- 239 Aigbedion-Atalor PO (2020) Elucidating *Tuta absoluta* (Meyrick) invasion and enhancing its
240 management in Eastern Africa: spread, socio-ecological impacts, and potential of a newly
241 imported larval parasitoid for classical biological control Eastern in Africa. PhD dissertation.
242 Rhodes University.
- 243 Aigbedion-Atalor PO, Mohamed SA, Hill MP, Zalucki, MP, Azrag AGA, Srinivasan R, Ekesi S (2020)
244 Host stage preference and performance of *Dolichogenidea gelechiidivoris* (Hymenoptera:
245 Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biol Control*
246 144:104215. <https://doi.org/10.1016/j.biocontrol.2020.104215>
- 247 Arnó J, Castañé C, Alomar O, Riudavets J, Agustí N, Gabarra R, Albajes R (2018) Forty years of
248 biological control in Mediterranean tomato greenhouses: The story of success. *Isr J Entomol*
249 48:209–226. <http://doi.org/10.5281/zenodo.1486574>
- 250 Arnó J, Molina P, Aparicio Y, Denis C, Gabarra R, Riudavets J (in press) Natural enemies associated
251 with *Tuta absoluta* and functional biodiversity in vegetable crops. *BioControl*
- 252 Arnó J, Sorribas R, Prat M, Matas M, Pozo C, Rodríguez D, Garreta A, Gómez A, Gabarra R (2009) *Tuta*
253 *absoluta*, a new pest in IPM tomatoes in the northeast of Spain. *IOBC/WPRS Bull* 9:203–208.
- 254 Askew RR (1968) Handbooks for the identification of British insects. Vol. VIII. Hymenoptera 2.
255 Chalcidoidea section (b). Royal Entomological Society of London.
- 256 Balzan MV, Wäckers FL (2013) Flowers to selectively enhance the fitness of a hostfeeding parasitoid:
257 Adult feeding by *Tuta absoluta* and its parasitoid *Necremnus artynes*. *Biol Control* 67:21–31.
258 <https://doi.org/10.1016/j.biocontrol.2013.06.006>
- 259 Biondi A, Guedes RNC, Wan FH, Desneux N (2018) Ecology, worldwide spread, and management of the
260 invasive south american tomato pinworm, *Tuta absoluta*: Past, present, and future. *Ann Rev*
261 *Entomol* 63:239–258. <https://doi.org/10.1146/annurev-ento-031616-034933>

262 Bodino N, Ferracini C, Tavella L (2016) Is host selection influenced by natal and adult experience in the
 263 parasitoid *Necremnus tutae* (Hymenoptera: Eulophidae)? *Anim Behav* 112:221–228.
 264 <https://doi.org/10.1016/j.anbehav.2015.12.011>

265 Calvo FJ, Soriano JD, Bolckmans K, Belda JE (2013) Host instar suitability and life-history parameters
 266 under different temperature regimes of *Necremnus artynes* on *Tuta absoluta*. *Biocontrol Sci*
 267 *Technol* 23:803–815. <https://doi.org/10.1080/09583157.2013.795930>

268 Calvo FJ, Soriano JD, Stansly PA, Belda JE (2016) Can the parasitoid *Necremnus tutae* (Hymenoptera:
 269 Eulophidae) improve existing biological control of the tomato leafminer *Tuta absoluta*
 270 (Lepidoptera: Gelechiidae)? *Bull Entomol Res* 106:502–511.
 271 <https://doi.org/10.1017/S0007485316000183>

272 Chailleux A, Desneux N, Arnó J, Gabarra R (2014) Biology of two key Palaearctic larval ectoparasitoids
 273 when parasitizing the invasive pest *Tuta absoluta*. *J Pest Sci* 87:441–448.
 274 <https://doi.org/10.1007/s10340-014-0557-7>

275 Crisol-Martínez E, van der Blom J (2019) *Necremnus tutae* (Hymenoptera, Eulophidae) is widespread
 276 and efficiently controls *Tuta absoluta* in tomato greenhouses in SE Spain. *IOBC/WPRS Bull*
 277 147:22–29.

278 Desneux N, Luna MG, Guillemaud T, Urbaneja A. (2011) The invasive South American tomato
 279 pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: The new threat to
 280 tomato world production. *J Pest Sci* 84:403–408. <https://doi.org/10.1007/s10340-011-0398-6>

281 Desneux N, Wajnberg E, Wyckhuys K, Burgio G, Arpaia S, NarváezVasquez C, González-Cabrera J,
 282 Catalán-Ruescas D, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T, Urbaneja A. (2010)
 283 Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion
 284 and prospects for biological control. *J Pest Sci* 83:197–215. [https://doi.org/10.1007/s10340-010-](https://doi.org/10.1007/s10340-010-0321-6)
 285 [0321-6](https://doi.org/10.1007/s10340-010-0321-6)

286 Fernandez-Triana J, Shaw MR, Boudreault C, Beaudin M, Broad GR. (2020) Annotated and illustrated
 287 world checklist of Microgastrinae parasitoid wasps (Hymenoptera, Braconidae). *ZooKeys*
 288 920:1–1089. <https://doi.org/10.3897/zookeys.920.39128>

289 Ferracini C, Ingegno BL, Navone P, Ferrari E, Mosti M, Tavella L, Alma A (2012) Adaptation of
290 indigenous larval parasitoids to *Tuta absoluta* (Lepidoptera: Gelechiidae) in Italy. J Econ
291 Entomol 105:1311–1319. <https://doi.org/10.1603/ec11394>

292 Gabarra R, Arnó J, Lara L, Verdú MJ, Ribes A, Beitia F, Urbaneja A Téllez MM, Mollá O, Riudavets J
293 (2014) Native parasitoids associated with *Tuta absoluta* in the tomato production areas of the
294 Spanish Mediterranean Coast. BioControl 59:45–54. <https://doi.org/10.1007/s10526-013-9545-8>

295 Gebiola M, Bernardo U, Ribes A, Gibson GAP (2015) An integrative study of *Necremnus* Thomson
296 (Hymenoptera: Eulophidae) associated with invasive pests in Europe and North America:
297 Taxonomic and ecological implications. Zool J Linn Soc-Lond 173:352–423.
298 <https://doi.org/10.1111/zoi.12210>

299 Grissell EE, Schauff ME (1990). A handbook of the families of Nearctic Chalcidoidea (Hymenoptera).
300 Entomol Soc Wash, Washington, DC. Handbook 1: 1 - 85.

301 Hanson PE, Gauld ID (eds) (2006). Hymenoptera de la Región Neotropical. Memoirs of the American
302 Entomological Institute 77, pp. 1–994. The American Entomological Institute. Gainesville.

303 Harvey JA, Poelman EH, Tanaka T (2013) Intrinsic inter- and intraspecific competition in parasitoid
304 wasps. Annu Rev Entomol 58:333–351. <https://doi.org/10.1146/annurev-ento-120811-153622>

305 Larrain, P (1987) Plagas del tomate. I parte: descripcion, fluctuacion poblacional, daño, plantas
306 hospederas, enemigos naturales de las plagas principales. IPA La Platina 39:30–35.

307 Mansour R, Brévault T, Chailleux A, Cherif A, Grissa-Lebdi K, Haddi K, Mohamed SA, Nofemela RS,
308 Oke A, Sylla S, Tonnang HE (2018) Occurrence, biology, natural enemies and management of
309 *Tuta absoluta* in Africa. Entomol Gen 38:83–112. <https://10.1127/entomologia/2018/0749>

310 Marsh PM (1975). A new species of *Apanteles* from South America being introduced into California. The
311 Pan-Pacific Entomol 51:143–146.

312 Mirchev P, Georgiev G, Tsankov G (2001) Studies on the parasitoids of *Gelechia senticetella* (Stgr.)
313 (Lepidoptera: Gelechiidae) in Bulgaria. J Pest Science 74:94–96. <https://doi.org/10.1046/j.1439-0280.2001.01017.x>

314

315 Mitsunaga T, Yano E (2004) The effect of multiple parasitism by an endoparasitoid on several life history
316 traits of leafminer ectoparasitoids. Appl Entomol Zool 39: 315–320.
317 <https://doi.org/10.1303/aez.2004.315>

318 Morales J, Rodríguez D, Cantor F (2013). Estandarización de la cría masiva de *Apanteles gelechiivoris*
319 Marsh (Hymenoptera: Braconidae) para el control de *Tuta absoluta* Meyrick (Lepidoptera:
320 Gelechiidae). Revista Facultad de Ciencias Básicas 9:20–37.

321 Naranjo SE (2008) Sampling arthropods. In: Capinera J.L. (eds) Encyclopedia of Entomology. Springer,
322 Dordrecht. https://doi.org/10.1007/978-1-4020-6359-6_4014

323 Sakaltaş E, Tüzün A (2014) Contribution to the knowledge of the *Aprostocetus* Westwood, 1833
324 (Hymenoptera: Tetrastichinae) from Kırıkkale and Çankırı (Turkey) with some new records.
325 Türk Entomol Derg 38:415–426.

326 Salas Gervassio NG, Aquino D, Vallina C, Biondi A, Luna MG (2019) A re-examination of *Tuta*
327 *absoluta* parasitoids in South America for optimized biological control. J Pest Sci 92:1343–1357.
328 <https://doi.org/10.1007/s10340-018-01078-1>

329 Tarusikirwa VL, Machekano H, Mutamiswa R, Chidawanyika F, Nyamukondiwa C (2020). *Tuta*
330 *absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on the “Offensive” in Africa: Prospects for
331 Integrated Management Initiatives. Insects 11, 764. <https://doi.org/10.3390/insects11110764>

332 Urbaneja A, González-Cabrera J, Arnó J, Gabarra R (2012) Prospects for the biological control of *Tuta*
333 *absoluta* in tomatoes of the Mediterranean basin. Pest Manag Sci 68:1215–1222.
334 <https://doi.org/10.1002/ps.3344>

335 Urbaneja A, Montón H, Mollá O (2009) Suitability of the tomato borer *Tuta absoluta* as prey for
336 *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. J Appl Entomol 133:292–296.
337 <https://doi.org/10.1111/j.1439-0418.2008.01319.x>

338 Urbaneja A, Vercher R, Navarro V, García-Marí F, Porcuna JL (2007) La polilla del tomate, *Tuta*
339 *absoluta*. Phytoma España 194:16–23.

340 Vallejo FA (1999) Mejoramiento genético y producción de tomate en Colombia. Universidad Nacional de
341 Colombia. pp. 216.

342 Zappalà L, Biondi A, Alma A, Al-Jboory IJ, Arnó J, Bayram A, Chailleux A, El-Arnaouty A, Gerling D,
343 Guenaoui Y, Shaltiel-Harpaz L, Siscaro G, Stavrinides M, Tavella L, Vercher-Aznar R,
344 Urbaneja A, Desneux N (2013) Natural enemies of the South American moth, *Tuta absoluta*, in
345 Europe, North Africa and Middle East, and their potential use in pest control strategies. J Pest
346 Sci 86:635–647. <https://doi.org/10.1007/s10340-013-0531-9>

Table 1: Number of plots surveyed, number of samples that had leaflets with at least one *T. absoluta* larva, and week of the year when first and last samples were taken. Each sample corresponds to a maximum of 25 leaflets during up to 20 minutes from one crop on one date.

Year	Number of		Week of the year	
	Plots	Samples	First	Last
2017	32	235	17	41
2018	61	296	11	40
2019	37	186	12	39
2020	32	228	12	46

351

352

353

Table 2. Percentages of ectoparasitized *T. absoluta* larvae recorded from tomato leaflets collected in commercial tomato plots. Percentages were calculated as the number of ectoparasitized larvae over the total number of larvae collected per month. In brackets are the number of samples that had leaflets with at least one *T. absoluta* larvae.

Month	% ectoparasitized larvae				<i>T. absoluta</i> larvae (num. samples)			
	2017	2018	2019	2020	2017	2018	2019	2020
Mar	-	0	0	0	-	480 (25)	113 (12)	11 (3)
Apr	0	0.8	1.0	0	32 (5)	612 (47)	308 (22)	14 (6)
May	0.1	3.0	5.0	0	714 (50)	833 (52)	360 (25)	148 (19)
Jun	0.6	7.3	6.2	0.3	519 (52)	575 (43)	389 (27)	398 (48)
Jul	4.9	5.2	4.3	0.6	409 (53)	515 (55)	531 (45)	530 (43)
Aug	10.6	5.5	13.5	1.8	256 (31)	455 (48)	319 (36)	610 (47)
Sep	11.6	14.6	7.6	9.2	242 (27)	213 (21)	197 (19)	370 (40)
Oct	9.1	3.1	-	7.4	186 (16)	65 (5)	-	202 (17)
Nov	-	-	-	35.7	-	-	-	14 (5)

358

359

360 **Table 3:** Number of adult parasitoids of different Hymenoptera families reared from ectoparasitized *T.*

361 *absoluta* larvae in samples collected from commercial plots in the different years of sampling.

Hymenoptera family	Year of sampling		
	2017	2018	2019
Eulophidae	23	68	98
Braconidae	5	8	12
Torymidae	1	0	0
Platygastridae	1	0	0
Diapriidae	0	1	0
Aphelinidae	0	0	1

362

363

364

365

366 **Figure 1:** Monthly percentage of endoparasitized *T. absoluta* larvae in commercial tomato plots in 2020.

367 Percentages were calculated as the number of emerged parasitoids over the total number of larvae

368 recorded per month. Total number of *T. absoluta* larvae and number of samples are displayed in Table 2.

369