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Trichogramma canariensis (Insecta: Hymenoptera: Trichogrammatidae) a parasitoid of eggs of the twinspot moth *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) in the Canary Islands

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Abstract

A new species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) parasitizing eggs of the golden twin-spot moth (or tomato looper) *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) on banana crops in the Canary Islands, Spain, is described as *Trichogramma canariensis* del Pino & Polaszek, sp.n. The new species is closely related to *T. brassicae* Bezdenko. Limited aspects of morphology, coupled with ITS2 and COI sequences and reproductive data are presented to distinguish *T. canariensis* sp.n. from *T. brassicae*.

Key words

Chalcidoidea, egg parasitoid, taxonomy, ITS2, COI, biocontrol.

1. Introduction

Banana (*Musa acuminata* Colla) is the most important economic crop in the Canary Islands (Spain), with a production area of close to 9100 ha in 2010, and a total production of 400 thousand metric tons (MARM 2011). In recent years, one of the most harmful pests of banana greenhouse crops in this region has been the golden twin-spot moth, or tomato looper, *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) (Perera & Molina 2007; del Pino et al. 2011). This species is highly polyphagous, feeding on many fruit, vegetable and ornamental crops and weeds in many plant families and countries (CABI 2007). In the Canary Islands, *C. chalcites* occurs throughout the entire growing cycle of bananas, and larvae feeding on leaves and fruits can cause losses of up to 30% of total production under greenhouse conditions (del Pino et al. 2011). Traditionally, the control of *C. chalcites* in banana crops has been with the application of pesticides (Perera & Molina 2007). However, chemical based control measures require multiple applications that can increase the risk of pest resistance, increase production costs, and can hamper the commercialization of products that contain pesticide residues (Broza & Sneh 1994; Perera & Molina 2007). At present, the management of different banana pests has shifted from application of chemical insecticides to integrated pest management (IPM) that includes cultural practices, pheromone trapping and biological control (Cabrera-Cabrera et al. 2010).

In the Palaearctic region, several parasitoids and predators of *C. chalcites* have been recorded (Alcázar et al. 2002; Cabello 1989; Garzia et al. 2003; Linden 1996; Vilardebo & Guerout 1964) and evaluated as biological control agents (Bell et al. 2000; Bolckmans & Teteeroo 2002; de Clercq et al. 1998; Messelink 2002; Pizzol et al. 1997; Vacante et al. 1996; Zimmermann 2004), although they are not yet commercially available (Cabello 2009). However, surveys conducted in banana crops in the Canary Islands have shown the presence of different parasitoid and predator species (del Pino et al. 2009) as well as entomopathogens (Bernal et al. 2013). High levels of egg parasitism have been detected during these surveys with a significant impact on the pest populations (del Pino et al. 2011). Prospecting for potential natural enemies of the South American tomato pinworm *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) and *C. chalcites*, Polaszek et al. (2012) and del Pino et al. (2013) reported the discovery of five species of *Trichogramma* on the Canary Islands archipelago: four relatively widespread, *T. achaeae* Nagaraja & Nagarkatti, *T. bourarachae* Pintureau & Babault, *T. euproctidis* (Girault) and *T. evanescens* Westwood; and, a fifth species close to *T. brassicae* Bezdenko that was probably new to science.

Worldwide, egg parasitoids of the genus *Trichogramma* Westwood, 1833 (Hymenoptera: Trichogrammatidae) are commonly employed as biological control agents of lepidopteran pests in both agricultural and natural environments (Smith 1996; Mills 2010). Traditionally, identification of *Trichogramma* species was based mainly on morphological characteristics of the male genitalia and the male antennae (Nagarkatti & Nagaraja 1971, 1977; Pinto 1999). However, the taxonomy of *Trichogramma* is very complicated due to their small size (<1 mm) and the absence of clear morphological differences between closely related species (Nagarkatti & Nagaraja 1977), and morphological identification to species typically requires expert knowledge of the genus (Pinto & Stouthamer 1994; Ciociola et al. 2001). Consequently, the development of alternative identification methods such as the use of reproductive compatibility studies (Pinto et al. 1991) and, more recently, the incorporation of molecular methods (Stouthamer et al. 1999) as tools for identification, have continued to refine *Trichogramma* taxonomy. Currently, around 210 species of *Trichogramma* are formally recognized worldwide (Pinto 2006), but this figure may change considerably with increasing use of molecular methods. The vast majority of *Trichogramma* species are known to be polyphagous, attacking a wide range of lepidopterans as well as insects belonging to other orders (Smith 1996).

The purpose of this paper is to incorporate morphological characters, ITS2 and COI sequence differences, and data from cross-breeding experiments, to describe *Trichogramma canariensis* sp.n., a species that was previously reported from the Canary Islands as "*T.* sp. nr. *brassicae*" in Polaszek et al. (2012) and del Pino et al. (2013), and to distinguish this new species from *T. brassicae*.

2. Materials and methods

2.1. Origin of parasitoids

Trichogramma canariensis sp.n. adults were first collected from parasitized eggs of *C. chalcites* found in banana groves at Arucas (28º07′58″N 15º30′54″W), Gran Canaria, Canary Islands, Spain between May 2009 and May 2010. Parasitized eggs were returned to the laboratory and individually stored in glass tubes in a climatic chamber (25 ± 1ºC, 65 ± 10% relative humidity and 16 : 8 h [L : D] photoperiod) until emergence of the adult wasps. Emergent wasps were either collected directly into 96% ethanol and stored at –20˚C, or used to initiate a lab colony by allowing them to mate and confining them with fresh eggs of an alternative host, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). After being given the opportunity to mate and oviposit, these individuals were also collected into 96% ethanol. *T. brassicae* adults used in the crossing experiments were supplied by Biotop (France) and previously reared on eggs of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae).

2.2. *Trichogramma* colonies

Colonies of both *Trichogramma* species were reared and maintained on UV-sterilized eggs (<24 h old) of *E. kuehniella* at 25 ± 1ºC, with a 65 ± 10% relative humidity and 16 : 8 h [L : D] photoperiod in a climatic chamber, according to the methodology described by Cabello (1985). Host eggs were obtained from a culture of *E. kuehniella* routinely reared following the procedure described by Cerutti et al. (1992). Wasps were reared in glass vials (95 × 15 mm) with an egg card providing approximately 500 host eggs, covered with cotton and supplied with water-honey (1 : 1) drops as a food source for adult wasps.

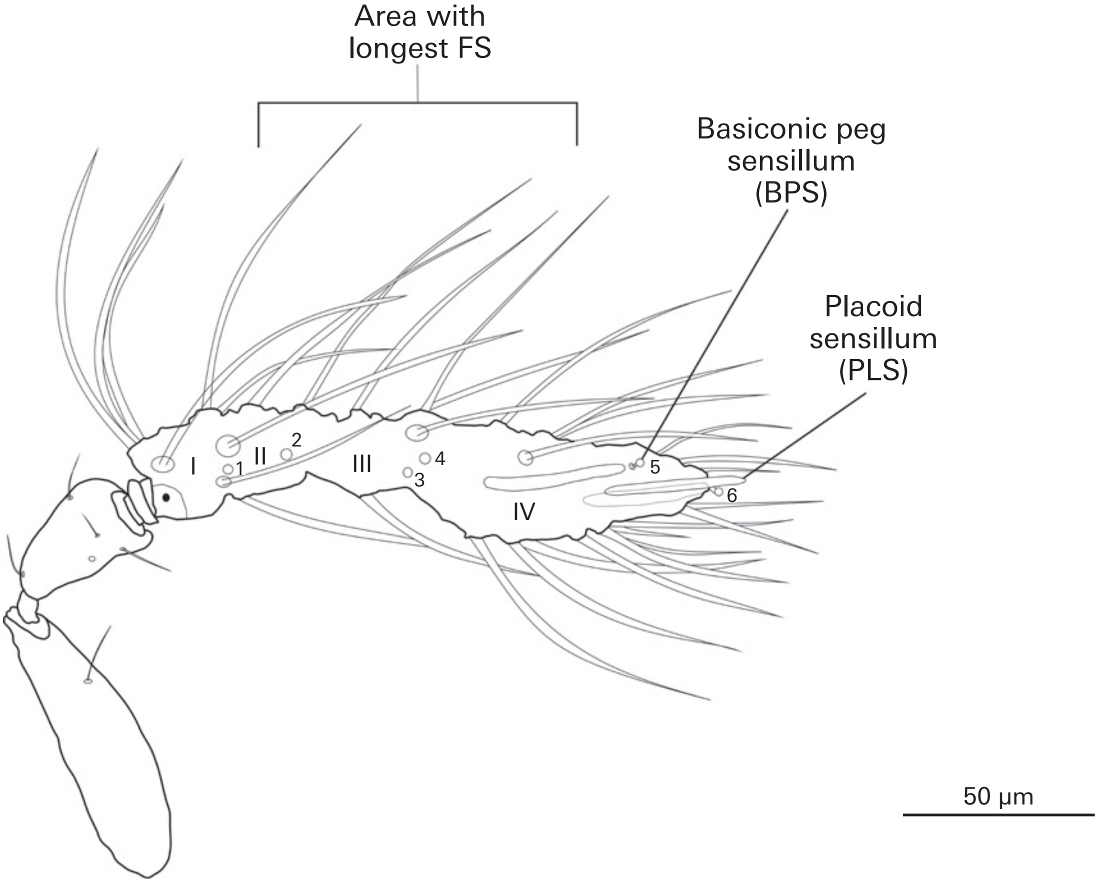

Fig. 1. Morphological terminology for antennal male characters of *Trichogramma canariensis* sp.n. used in this study (after Pinto 1999). Arabic numerals on flagellum refer to "positions 1–6" that carry basiconic peg sensilla (BPS); Roman numerals I–IV refer to "sections" of the flagellum.

2.3. Morphology and species identification

We follow the anatomical terminology, morphological characters, measurements and ratios as used by Pinto (1999) and previously described in Polaszek et al. (2012), see Figs. 1–2. Male specimens were mounted in Canada Balsam using the method described by Noyes (1982) with some modifications (see Polaszek et al. 2012). The specialized terminology of the male genitalia is given in Fig. 2, modified from Pinto (1999). Linear measurements were reported as a mean ± standard deviation (SD), measured to the nearest 0.01 mm. Species were initially identified based on morphology, using a combination of the following published taxonomic accounts: Lin (1994), Nagaraja & Nagarkatti (1970), Nagarkatti & Nagaraja (1971), Pinto (1999), Pintureau & Babault (1988) and Pintureau (2008).

2.4. Cross-breeding experiments

Cross-breeding experiments were conducted to determine the reproductive compatibility of *Trichogramma canariensis* sp.n. and *T. brassicae*. The crossing experiment consisted of all possible crosses between males and females of the two species, resulting in four combinations. It followed the general procedures outlined by Pinto et al. (1991) and Stouthamer et al. (2000) and 20–25 crosses for each combination were set up. To ensure that the females used in crosses were unmated and arrhenotokous, we also set up a total of 20 replicates of virgins of each species. Adults used in all crosses were less than 24 h old. Wasps were obtained by isolating parasitized eggs of *E. kuehniella* from the laboratory cultures. This was performed by removing them with a wet fine brush (no. 0). Individual eggs were isolated in glass vials (5 × 1 cm) covered with cotton and supplied with a water-honey (1 : 1) drop as a food source and subsequently reared to adulthood. Upon emergence, their sex was determined and couples (1 male + 1 female) and virgin females (1 female alone) were separated and used for the tests. After mating, the male and the female were kept together in the vial and an egg card (2 × 2.5 cm) with approximately 100 host eggs and a water-honey drop as a food source were supplied daily until the death of the female. Three days after the first offspring emerged, the vials were placed in a freezer to kill the wasps. The number of parasitized eggs and number and sex of the offspring were then determined. Differences in the numbers of off-

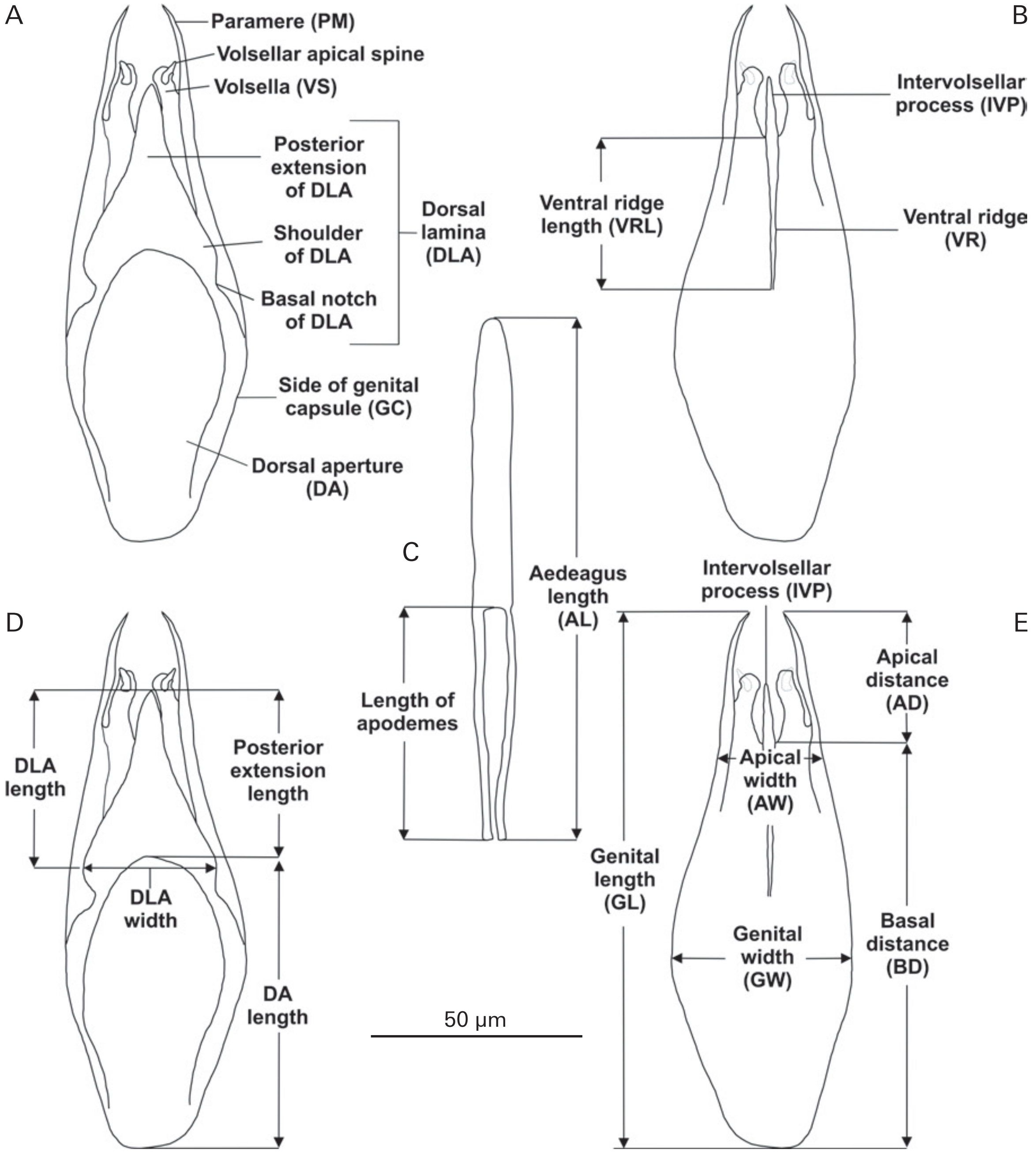

Fig. 2. Morphological terminology for male genitalia characters of *Trichogramma canariensis* sp.n. used in this study (after Pinto 1999). **A**: Dorsal structures of genital capsule. **B**: Ventral structures of genital capsule. **C**: Aedeagus. **D**: Dorsal measurements of genital capsule. **E**: Ventral measurements of genital capsule.

spring produced by females of each species, under the different mating regimes (virgin, conspecific mating, and heterospecific mating) were investigated using simple ANOVA and Tukey's pairwise comparisons in Minitab ® 15.1.30.0 (Minitab Inc.).

2.5. Molecular identification

Two females of *T. canariensis* were selected from the original samples for DNA extraction. Genomic DNA was extracted separately from each individual using Chelex 100 ® resin (Bio-Rad, Hucules, CA) (see Stouthamer et al. 1999; Sumer et al. 2009). The polymerase chain reaction (PCR) was used to amplify the nuclear ITS2 region of each specimen using primers and conditions described in Stouthamer et al. (1999). In addition, the mitochondrial COI of one of these females of *T. canariensis* was amplified using the primers and conditions given in Rugman-Jones et al. (2012). Amplicons were directly sequenced in both directions at the Institute for Integrative Genome Biology, University of California, Riverside, USA. The sequences were aligned manually using BioEdit version 7.0.9.0 (Hall 1999), and compared with the ITS2 and COI sequences of *T. brassicae* (Sumer et al. 2009) and other known *Trichogramma* spp. (Kumar et al. 2009; Silva et al. 1999; Sumer et al. 2009; Thomson et al. 2003). Complete newly acquired ITS2 and COI sequences were deposited in GenBank (accession numbers: ITS2 JF415947, JF415948; COI KF275668) to facilitate molecular characterization of future samples.

3. Results

3.1. Taxonomic description

Trichogramma canariensis **del Pino & Polaszek, sp.n.**

Figs. 1–3

Species-group/section placement. Following the morphological characteristics established by Pintureau (2008), it is considered that *T. canariensis* belongs to the *evanescens*-group defined by the following character set: long flagellar setae (Fig. 3A,B); genital capsule (GC, Fig. 3C–E) not elongated and not retracted; dorsal lamina (DLA, Fig. 3D) with relatively developed basal notches, located behind the basal end of the chelate structures (CS) (= parameres (PM) according to Pinto's terminology) and behind the basal end of the intervolsellar process (IVP); distal ends of the gonoforceps (GF) (= volsellae (VS) according to Pinto's terminology) very advanced beyond the distal ends of the chelate structures (CS) (= PM) and the distal end of the intervolsellar process (IVP).

The *evanescens*-group is a part of the *minutum*-group *sensu* Nagarkatti & Nagaraja (1977) renamed by Pintureau & Babault (1980) and incorporated into the Exiguum-section by Pinto (1999). This group includes three species formally cited in Europe (Pintureau 2008, 2012): *T. brassicae* Bezdenko (= *maidis* Pintureau & Voegelé), *T. euproctidis* (Girault) (= *meyeri* Sorokina, = *voegelei* Pintureau), and *T. evanescens* Westwood (= *barathrae* Skriptshinskij, = *carpocapsae* Schreiner, = *latipennis* Haliday, = *niveiscapus* Morley, = *rhenana* Voegelé & Russo, = *vitripennis* Walker); *T. canariensis* sp.n. is added herein.

Species of the *evanescens*-group have a wide natural host range that extends across the orders Coleoptera (Chrysomelidae incl. Bruchinae, Curculionidae, Dermestidae, Rhynchitidae, Tenebrionidae), Diptera (Anthomyiidae, Tachinidae, Stratiomyiidae, Syrphidae, Tabanidae), Hemiptera (Cimicidae), Hymenoptera (Pamphiliidae, Tenthredinidae), Megaloptera (Sialidae) and Neuroptera (Chrysopidae) (Polaszek 2010). However, the majority of egg hosts are in Lepidoptera, belonging to a wide range of families: Arctiidae, Bombycidae, Danaidae, Gelechiidae, Geometridae, Glyphipterygidae, Hesperiidae, Lasiocampidae, Leptidae, Lymantriidae, Noctuidae, Notodontidae, Notodontidae, Nymphalidae, Oecophoridae, Papilionidae, Pieridae, Pyralidae, Saturniidae, Sphingidae, Tortricidae, Yponomeutidae and Zygaenidae (Cabello 1986; Nikolskaya & Tryapitsyn 1988; Pintureau 2008; Polaszek 2010).

Note. Given the morphological similarity between *T. canariensis*, *T. brassicae* and other species within the *evanescens*-group, a minimal morphological description is provided here, with emphasis on diagnostic differences. Proportions of various parts of the male genitalia, traditionally used in *Trichogramma* taxonomy, were found to vary greatly, depending on the exact way in which the genitalia were mounted, in particular the degree of dorso-ventral flattening. Morphological characters, relative measurements and ratios are based largely on characters used by Pinto (1999, see Figs. 1, 2).

Description, male (n = 8; measurements represent means, followed by ranges)**.** ***Colouration***: Unremarkable, largely brown with the legs, scutellum and posterior mesoscutum pale, the antennal flagellum and pedicel noticeably darker than the pale scape. Pronotal plates dark. Wings basally infuscate. ***Morphology***: length antennal flagellum (Figs. 1, 3A,B) / length of scape: 2.08 (1.93–2.27); length antennal flagellum / width antennal flagellum (not including setae): 6.04 (5.64–6.59); maximum length flagellar seta / maximum width basal flagellum: 3.0 (2.80–3.40). Terminal placoid sensilla extending beyond the end of the flagellum. Basiconic peg sensilla formula (BSPS formula): 1-2-2-2. Genital capsule (GC, Figs. 2, 3C,D) length 2.7× maximum width (n = 4; range 2.20–2.90), sides narrowed at level of intervolsellar process (IVP). IVP (Figs. 2, 3C) large, prominent. Dorsal lamina (DLA, Figs. 2, 3D) originating at middle of GC, triangular and rounded at apex. Shoulders present at base of DLA. Aedeagus as in Fig. 3E. Length of the basal distance (BD) / length of the ventral ridge (VR): 2.88 (2.71–3.23). Wing comparatively broad, length 2× width (Fig. 3F). Most males are macropterous, i.e. having normally developed wings, but both brachypterous and completely apterous males also occur in this species, and one specimen of each is included in the paratype series.

Description, female (n = 5)**.** ***Colouration***: As in male. ***Morphology***: Without distinguishing morphological characters. Length mid tibia / length ovipositor: 1.04 (1.01–1.08).

Distribution. Europe: Spain, Canary Islands, Gran Canaria, Arucas (village). Known so far only from type locality.

Hosts. LEPIDOPTERA: Noctuidae: *Chrysodeixis chalcites* (Esper); Pyralidae: *Ephestia kuehniella* Zeller (alternative host).

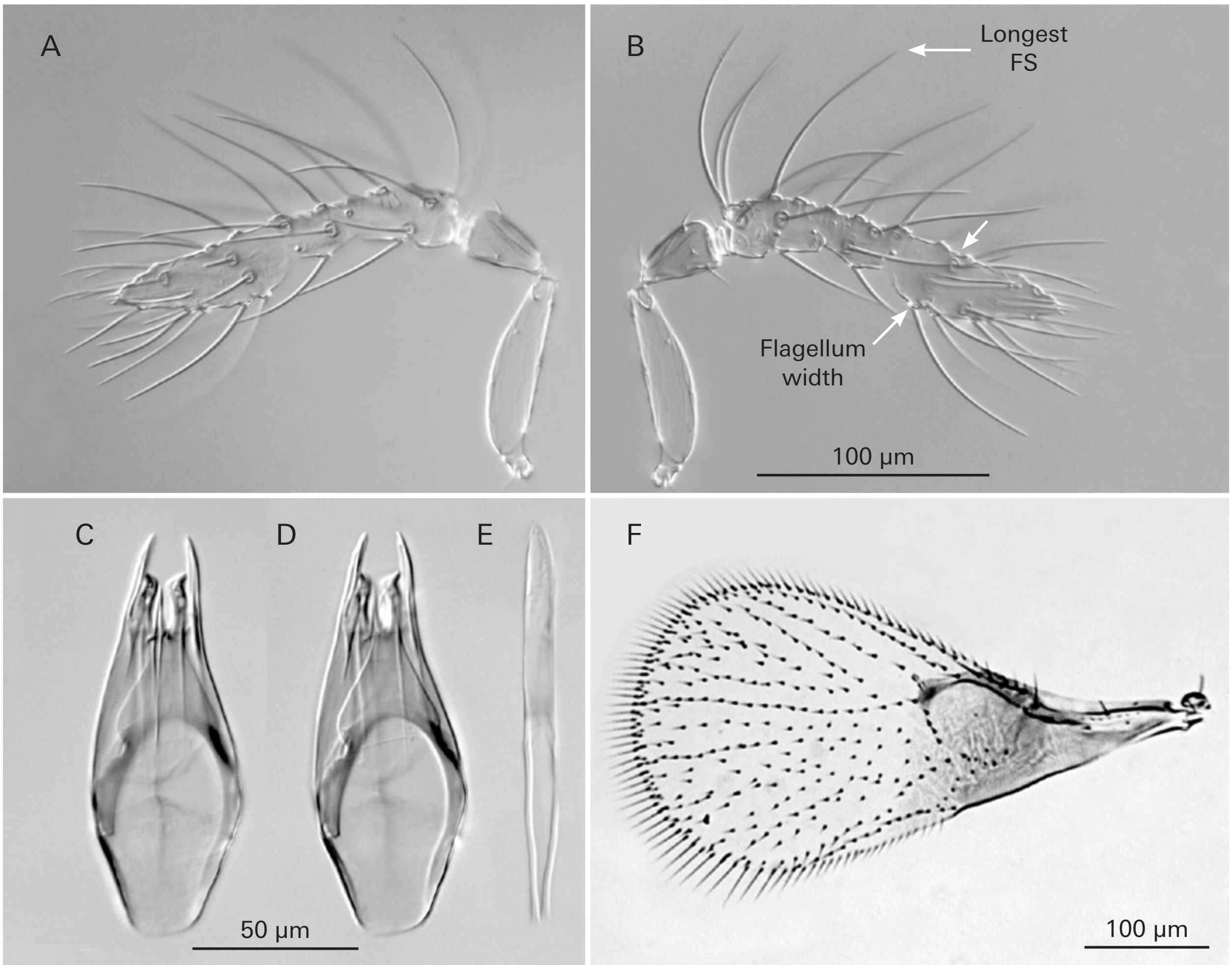

Fig. 3. Morphology of *Trichogramma canariensis* sp.n. **A**, **B**: Male antennae, inner (A) and outer (B) aspects; arrows in 3B indicate longest basal antennal seta (LBAS) and point of measurement of flagellum width; see Fig. 1 for morphological terms. **C–E**: Genital capsule (GC) and aedeagus: Genital capsule (GC; aedeagus removed) with focus on parameres, volsellae, and intervolsellar process (C) and with focus on dorsal lamina (D); isolated aedeagus (E); see Fig. 2 for morphological terms. **F**: Fore wing.

Material examined (confirmed by sequencing ITS2)**.** Holotype ♂ SPAIN: Canary Islands, Gran Canaria, Arucas, 28º07′58″N 15º30′54″W, 11.v.2009, *Musa acuminata* GC07/09-17 ex egg *Chrysodeixis chalcites*. – Paratypes: 1 ♂, same data as holotype, except GC07/09-50; 17 ♂♂ 16 ♀♀ *Solanum lycopersicum* ex egg *Chrysodeixis chalcites*. All specimens from the morphological study are deposited in Department of Life Sciences, Natural History Museum, London, UK (NHM).

Remarks. Morphometric comparisons were made between males of *T. canariensis* (n = 8) and *T. brassicae* (n = 8). Genitalia measurements and ratios vary greatly and consequently almost complete overlap was found in these parameters (see Figs. 3C–E, 4C,D). However, one ratio stood out consistently as separating the two species, the ratio between the length of the longest basal seta on the male antenna (measured as a curved line) and the maximum width of the basal antennal flagellum (see Figs. 1, 3B, 4B). In *T. canariensis,* the mean ratio was exactly 3.0 (range 2.8–3.4), in *T. brassicae* it was 4.2 (range 3.6–4.4). This appears to be an extremely useful and easily-measured character for separating males of these two species.

3.2. Crossing compatibility of *T. canariensis* and *T. brassicae*

Conspecific crosses involving *T. canariensis* and *T. brassicae* resulted in the production of female and male offspring, with sex ratios biased towards females (Table 1). However, when crossed to a heterospecific male, females of both species produced only male offspring (Table 1). Similarly, virgin females of each species produced exclusively male broods, as expected under arrhenotoky. In *T. canariensis*, virgin females produced as many total emergent offspring (males only) as those mated to a conspecific male (males and females). However, crossing with a *T. brassicae* male resulted in a reduction in the total number of emergent offspring (male only) ($F_{2,56} = 20.76$, $p < 0.001$; Table 1), indicating post-zygotic isolation – i.e. eggs are at least partly fertilized by heterospecific sperm, but problems early on result in a failure to develop. In contrast, in case of *T. brassicae* females heterospecifc crossing did not affect offspring emergence, but there was a marginally significant re-

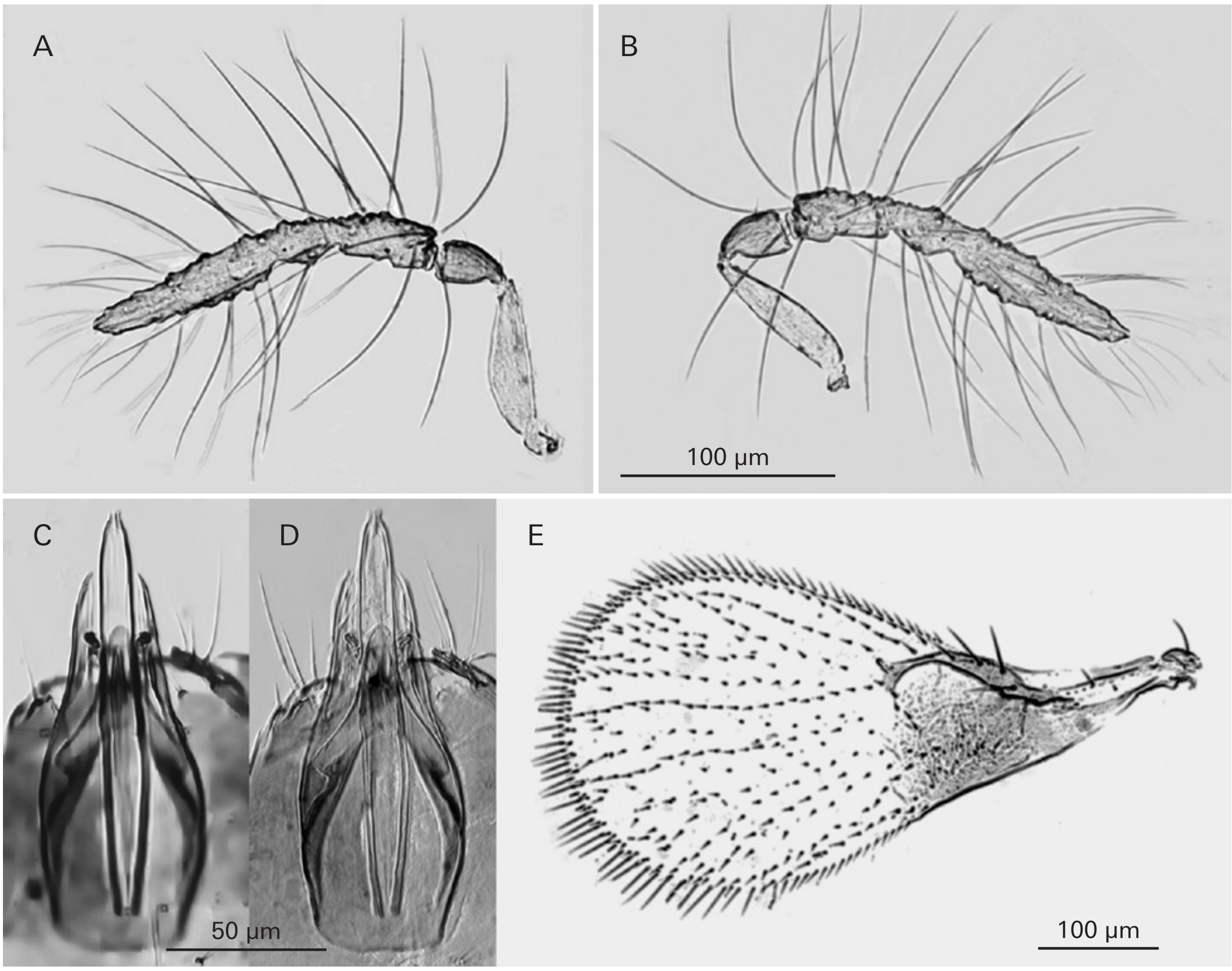

Fig. 4. Morphology of *Trichogramma brassicae*. **A**, **B**: Male antennae, inner (A) and outer (B) aspects. **C**, **D**: Genital capsule (GC) including aedeagus with focus on dorsal structures (C) and ventral structures (D). **E**: Fore wing.

duction in the number of emergent offspring produced by virgin females ($F_{2,51} = 3.81$, $p = 0.029$; Table 1), indicating pre-zygotic isolation – i.e. mating does not occur and/or sperm is not successfully transferred or stored.

3.3. Molecular identification

Trichogramma canariensis can be distinguished by its ITS2 sequence. Sequences taken from the two individuals collected from *C. chalcites* eggs were 409 bp in length (Genbank accessions JF415947 and JF415948), and when compared to the standard *T. brassicae* ITS2 sequence (JF415949) of a specimen from Turkey showed clear differences, with several indels and substituions (Table 2). In addition, *T. canariensis* COI sequences differed by about 6% from COI sequences of both *T. brassicae* (FM210196–FM210198) and *T. evanescens* (GQ367960) (Table 3).

4. Discussion

Reproductive compatibility, or cross-breeding, studies have frequently been used to complement morphology in solving taxonomic problems in *Trichogramma* (Pinto et al. 1991; Stouthamer et al. 2000). In some cases, as with *T. deion* Pinto & Oatman and *T. pretiosum* Riley, reproductive data have helped verify species originally indicated by minor morphological differences (Pinto et al. 1983). The most common means of breeding in *Trichogramma* is arrhenotokous parthenogenesis, the dominant mode of sex determination in the Hymenoptera, where an unfertilized egg develops into a haploid male and a fertilized egg into a diploid female (Heimpel & de Boer 2008). In the present case, not a single female was produced in interspecific crosses between *T. canariensis* and *T. brassicae*, which indicates reproductive isolation existing between these species.

Several studies have also demonstrated the usefulness of techniques such as RAPD, RFLP, micro-satellites

Table 1. Mean number of male and female descendants and total number of offspring (mean ± SE) obtained in crosses between *Trichogramma canariensis* sp.n. and *T. brassicae* at 25ºC under laboratory conditions (60–80% relative humidity and 16 : 8 hours L : D).

Cross	N	No. of females	No. of males	Total offspring
♀ *T. canariensis* x ♂ *T. canariensis*	14	38,9 ± 12,5	21,4 ± 9,60	62,1 ± 10,9 a
♀ *T. canariensis* x ♂ *T. brassicae*	20	0	34,0 ± 21,0	34,0 ± 21,0 b
♀ *T. canariensis* virginal	25	0	64,5 ± 14,2	64,5 ± 14,2 a
♀ *T. brassicae* x ♂ *T. brassicae*	19	42,2 ± 24,5	27,4 ± 14,8	69,6 ± 26,3 ab
♀ *T. brassicae* x ♂ *T. canariensis*	18	0	71,4 ± 22,5	71,4 ± 22,5 b
♀ *T. brassicae* virginal	17	0	50,2 ± 26,5	50,2 ± 26,5 a

Table 2. Aligned ITS2 sequences of *Trichogramma canariensis* (JF415947) and *T. brassicae* (JF415949). Dots (.) indicate identical nucleotide in the T *brassicae* sequence to the *T. canariensis* sequence. Dash (–) indicates gap in the alignment and when a nucleotide is shown the *T. brassicae* sequence differs from the *T. canariensis* sequence at that position.

T. canariensis *T. brassicae*	GTTTATAAAAACGAACCCGACTGCTCTCTCGCAAGAGAGAGCGTTGATCTGGGCGCTCGT	**60**
T. canariensis *T. brassicae*	CTCTATCTC--TTACTCTTCTTCGAAGTGTAT-AGCAGTGTGATACGTCGCCTCAAACGA .........TC................C.C.GG...........................	**120**
T. canariensis *T. brassicae*	AACGCAAGAAAAAAGATGAATTCGTTCGTCTAGCTGGCGCGCGCTTACCGCTTGGAGA	**180**
T. canariensis *T. brassicae*	GTACTCGCTCGCGCGAGTACTTCCGATCGTTCTGCGTCGAGTCCCGGAGCTTTCTCGACT	**240**
T. canariensis *T. brassicae*	CGTCGAGCAGCGGACCGACGTCTAGCACACGATCAGGCTCGTCCATGCATCGGTCATTGA	**300**
T. canariensis *T. brassicae*	ACGCGCGCGTGCAA-----TTTTAAACACACACACACACACACAACACTCGTTGTGTGCG .........C..GCACTTT........G...........CGTGTGTG--....-------	**360**
T. canariensis *T. brassicae*	GTGCTTGTTTATAAAACAGGCTAGCTCGAATTTTTGCTGAACGAGTCTTTTTT-CTCGAT -----.A..A.G....AT...................................T......	**420**

Table 3. Difference of COI sequence of *Trichogramma canariensis* with the related species *T. brassicae* and *T. evanescens*. GenBank accession numbers: *T. canariensis* KF275668; *T. brassicae* 1–3 FM210196–8; *T. evanescens* GQ367960.

	T. canariensis	*T. brassicae* 1	*T. brassicae* 2	*T. brassicae* 3
T. brassicae 1	6.5%			
T. brassicae 2	6.6%	0.6%		
T. brassicae 3	6.4%	0.4%	0.2%	
T. evanescens	5.5%	4.9%	5.2%	5.0%

and sequencing of ITS1 and ITS2 in the identification of species of *Trichogramma* (Gariepy et al. 2007). In this sense, Stouthamer et al. (1999) demonstrated the utility of the ITS2 (internal transcribed spacer 2) region of rDNA as a new technique in the species identification of *Trichogramma*. In our study, the morphological and reproductive data suggesting two separate species are also supported by sequences of the nuclear ribosomal ITS2 and the mitochondrial COI gene regions. *Trichogramma* in general show little intraspecific divergence in ITS2 sequences. In addition, the sequences of some cryptic species actually show such a high degree of similarity that they cannot be reliably used to distinguish between them (Stouthamer et al. 1999, 2000). Thus, the differences found in the ITS2 sequences in our study (Table 2) fall well outside the range of any intraspecific differences noted before in *Trichogramma* (Stouthamer et al. 1999), supporting *T. canariensis* and *T. brassicae* as two different species. Similarly, COI sequence differences between *T. canariensis* and the related species from the *evanescens*-group (Pintureau 2008) show that *T. canariensis* differs by at least 5% from both *T. brassicae* and *T. evanescens* (Table 3). A sequence divergence of more than 3% in COI has been suggested as a reason to question the conspecific status of individuals from which the sequences originated (Hebert et al. 2003).

According to the literature, *T. brassicae* is a polyphagous parasitoid; it has been found on a wide range of lepidopterous hosts (Orr et al. 2000) and has been described as a common parasitoid of *C. chalcites* eggs in Eurasia (Polaszek 2010). This species originated from Moldavia (Black Sea region) and was introduced into several countries in Central Europe in order to control the European corn borer, *Ostrinia nubilalis* (Hubner), in maize fields (Parra et al. 2010). However, *T. brassicae* has not been identified on the Canary Islands (Arechavaleta et al. 2010; Polaszek et al. 2012; del Pino et al. 2013) and information about its previous introductions to the archipelago is absent (Jacas et al. 2006; Rasplus et al. 2010; Roy et al. 2011).

In conclusion, results obtained with the combination of morphological, molecular and breeding procedures used in the present study have allowed the successful discovery and description of *T. canariensis* as a new species parasitizing eggs of *C. chalcites*. Consequently, this species could be considered as an appropriate candidate as a biological control agent against the twin-spot moth.

5. Acknowledgements

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