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First record of *Compsilura concinnata* (Meigen, 1824) (Diptera: Tachinidae) attacking *Orgyia trigotephras* (Boisduval, 1829) in Tunisia

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Abstract

Background: The polyphagous tachinid *Compsilura concinnata* is an endoparasitoid fly recorded as attacking larvae of Lepidoptera and Hymenoptera. Larvae of *Orgyia trigotephras* [Lepidoptera: Erebidae] were collected from northern Tunisia and reared in the laboratory.

Results: Adult flies that emerged from these larvae were identified on the basis of morphological description and DNA analysis (PCR) as *Compsilura concinnata*.

Conclusion: *Compsilura concinnata* is recorded here for the first time from *Orgyia trigotephras* in Tunisia. This species could be of great interest as a potential biological control agent of pests in Tunisia and neighboring countries.

Keywords: Endoparasitoid, Larvae. *Orgyia trigotephras*, DNA analysis, Tunisia

Background

The Tachinidae are the second largest family of the order Diptera and the most important group of entomophagous parasitoids Mellini (1990). Most tachinid species are endoparasitoids of many insect orders' host orders English-Loeb et al. (1990), predominantly Lepidopteran larvae, Stireman et al. (2006), and the majority are ovoviviparous, Evenhuis et al. (2008). Among this family, the genus *Compsilura* Bouché, 1834, is the smallest one of the tribe Blondeliini (Tachinidae: Exoristinae) including three species, namely *Compsilura solitaria* Curran., *C. samoaensis* Malloch. and *C. concinnata* Meigen (Crosskey 1973; 1976; O'Hara and Cerretti 2016). The species *C. concinnata* was described for the first time by Meigen (1824) from Germany. It is an endoparasitoid of endoparasitoid of larvae of the Lepidoptera, Hymenoptera and Coleoptera (Herting 1960; Arnaud 1978; Tschorsnig 2017).

In this paper, we combined morphological and molecular data to diagnose and confirm the parasitoid species *Compsilura concinnata* (Meigen 1824) attacking larvae of the Erebidae, *Orgyia trigotephras* [Lepidoptera: Erebidae], and to report it for the first time in Tunisia.

Methods

Investigations were conducted in northern Tunisia from 2013 to 2018. A total of 1060 larvae of *Orgyia trigotephras* were collected (with 926 larvae from Jebel Abderrahmane (Cap-Bon, alt. 432 m; 36°52′N, 10°48′E) and 134 larvae from Dam Ziatine (Sejnane, alt. 48 m; 37°11′N, 9°11′E). Each larva was kept individually in a plastic box (30 × 70 ml) at 25 ± 2 °C and reared on fresh leaves of its host plant, *Quercus coccifera* (Fagaceae). A periodic check of rearing boxes was carried out, and we noticed the emergence of parasitoids from all larval instars. Adult flies were stored in ethanol (95%) until identification. The morphological characters of adult flies were described

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using keys of Shima (1997); Tschorsnig and Herting (1994); Tschorsnig and Richter (1998).

For specific final recognition, molecular analysis of 1–3 legs of the 8 specimens of flies was carried out by amplification of 650pb fragment of the mitochondrial cytochrome c oxidase gene subunit 1 (COX1) using the universal primers LCO1490/HCO2198 (Folmer et al. 1994). DNA extraction was carried out using the Syngen DNA Mini Kit (Syngen, Poland) according to the manufacturer's instructions. Polymerase chain reaction (PCR) amplification mix was prepared in 25 µl contained 1 × PCR Buffer (Taq PCR Core Kit, QIAGEN), 1.5 mM MgCl2, 0.4 mM of each dNTP, 0.2 µM of each primer, 1 U of Taq polymerase and 10–20 ng of template DNA. Thermal cycling was executed on a T gradient thermal cycler (Bio-Rad) with an initial denaturation at 96 °C for 3 min, followed by 40 cycles of denaturation at 96 °C for 30 s, annealing at 48 °C for 56 s, extension at 72 °C for 1 min and 20 s, and a final extension at 72 °C for 10 min. Amplicons were analyzed by electrophoresis, visualized in a 1% agarose gel stained with the GelRed® dye (Biotium, USA) and purified using the CleanUp Kit (A&A Biotechnology, Poland). Sequencing of the purified amplification products was performed at Genomed Company (Warsaw, Poland). The obtained sequences were analyzed using the Basic Local Alignment Search Tool Nucleotide (BLASTN) searches at GenBank (National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/) in order to determine the closest matches and confirm the morphological identification. To perform phylogenetic analysis, the generated sequences were supplemented with additional sequences of *Compsilura concinnata* specimens obtained from GenBank and other species from Tachindae family used as outgroups. The phylogenetic tree was carried out by using the Maximum Likelihood Method based on the Kimura 2-parameter model (Kimura 1980). The analysis involved 18 nucleotide sequences and was conducted in MEGA v.6.

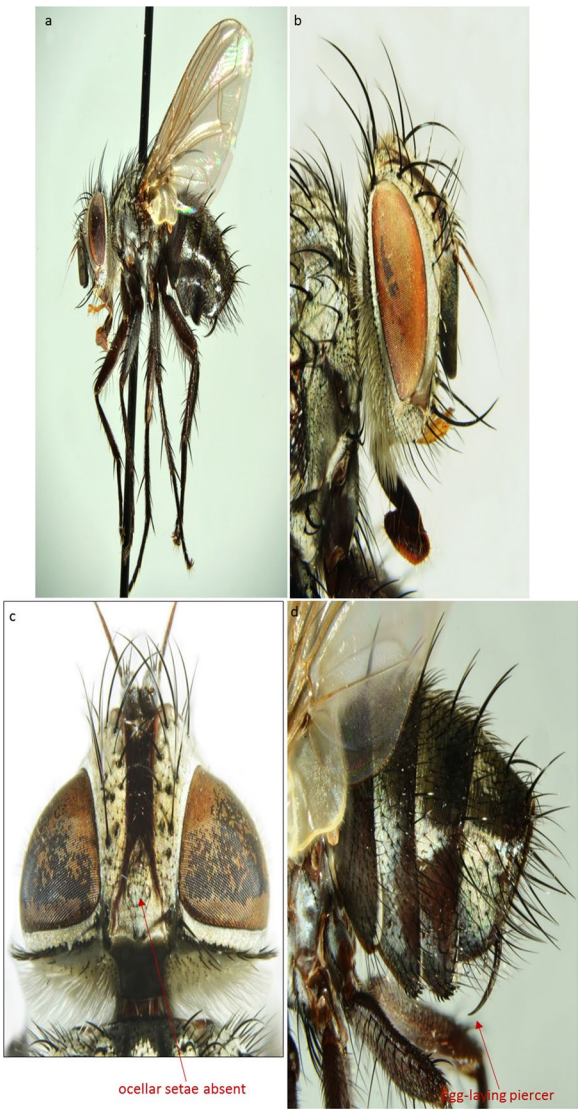

Fig. 1 *Compsilura concinnata;* **a** Adult; **b** head; **c** Ocellar setae; **d** abdomen

Results

The morphological description showed that the flies have eyes densely covered with long hairs (Fig. 1a–c). Above the vibrissa, strong upright bristles reach further than the middle of the facial ridges (Fig. 1b). Ocellar are setae absent (Fig. 1c). The third antennal segment on its base did not noticeably pull forward. Scutum with four pairs of post-sutural dorso-central bristles behind the suture. The third and fourth female tergites are ventrally compressed, and the 7th abdominal sternite is modified into a piercer (Fig. 1d).

PCR amplification of the partial sequence of the barcoding region of the cytochrome oxidase subunit I gene (COI) resulted in a 623 bp fragment for each specimen. The sequencing of these fragments showed that the 8 specimens shared 100% identity at all sequenced sites. BLAST searches in GenBank revealed that the eight generated sequences had 100% homology with sequences of *Compsilura concinnata* (LC516575 and LC516576) originating from Spain.

The phylogenetic tree with the highest log likelihood (−1767.5128) is shown in Fig. 2. The analyzed specimens clustered unambiguously with *Compsilura concinnata* specimens (LC516575, LC516576, LC516571, and LC516564) with 99% of support in the ML and formed a cluster clearly separate from outgroups (Fig. 2). Accordingly, this confirms the identity of the eight specimens

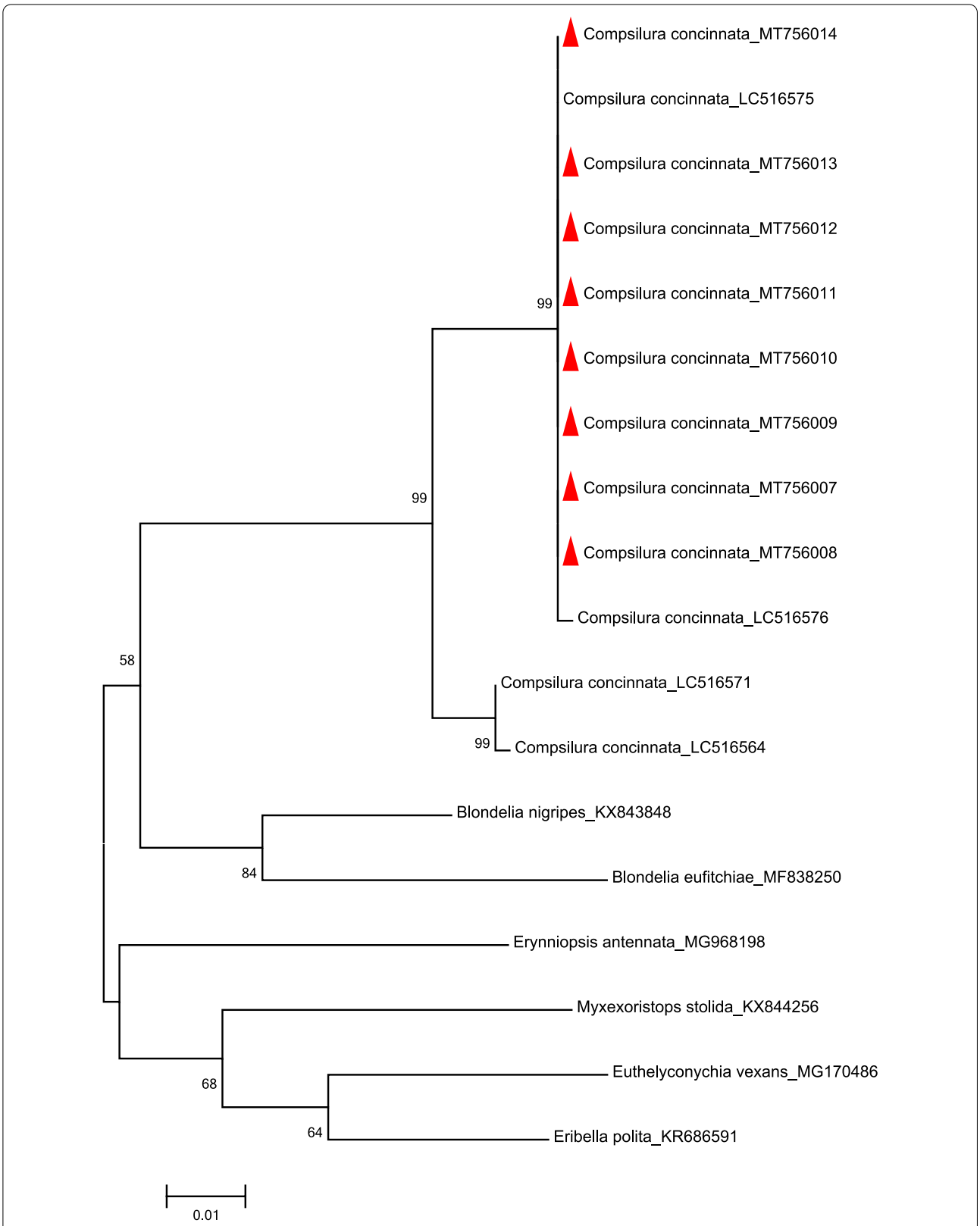

Fig. 2 Molecular phylogenetic analysis of *Compsilura concinnata* by maximum likelihood method using the Kimura 2-parameter model (Kimura 1980)

as *Compsilura concinnata*. The generated sequences were deposited in GenBank under the following accession numbers: MT756007, MT756008, MT756009, MT756010, MT756011, MT756012, MT756013 and MT756014 (Fig. 2).

Discussion

In Tunisia, the diversity of tachinid has been less studied for a long time but many updates to the host list were provided afterward (Tschorsnig 2017). Most tachinid species are able to parasitize only one or a few closely related host species of insects. From 1906 to 1911, *Compsilura concinnata* was one of the first parasite enemies of the gypsy moth (*Lymantria dispar*) and the brown-tail moth (*Euproctis chrysorrhoea*) to be obtained from Europe (Burgess 1929). It was introduced in the northeastern United States to control the populations of gypsy moth, and brown-tailed moth, (Sabrosky and Reardon 1976). This species was recorded on about 275 host species (Herting 1960; Arnaud 1978; Tschorsnig 2017) distributed in the Palaearctic region, particularly in Europe and North Africa to Japan. *Compsilura concinnata* was introduced in the northeastern United States to control the populations of gypsy moth, *Lymantria dispar* and brown tail moth, *Euproctis chrysorrhoea* (Sabrosky and Reardon 1976). In Tunisia, the species was observed for the first time in 2013 as a parasitoid on larvae of *Orgyia trigotephras.*

Conclusions

For the first time, the tachinid fly, *Compsilura concinnata* is recorded here attacking the erebid caterpillar, *Orgyia trigotephras* in Tunisia. This species could be of great interest as a potential biological control agent of pests in Tunisia and neighboring countries.

Abbreviations

DNA: Deoxyribonucleic acid; PCR: Polymerase chain reaction; COX1: The mitochondrial cytochrome c oxidase gene subunit 1; COI: Cytochrome c oxidase 1; MEGA V.6: The molecular evolutionary genetics analysis version 6.

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Authors' contributions

SH designed the project, performed the field and laboratory work and wrote the paper. IY performed the analysis of molecular data and reviewed the paper. OE planned and designed the research experiments, performed data analysis and reviewed the paper. CB and KS performed the morphological identification of Diptera parasitoids and reviewed the paper. MLB supervised and provided funding acquisition, administration and validation. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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