DNA BARCODES REVEAL A NEW HOST RECORD FOR CARCELIA ATRICOSTA HERTING (DIPTERA TACHINIDAE) IN ITALY

INTRODUCTION

Tachinids are one of the largest and most diverse families of Diptera worldwide (STIREMAN et al., 2006). The adults usually feed on nectar and pollen, while the larvae are parasitoids of arthropods, mostly exophytic caterpillars and other herbivorous insects. Compared to hymenopteran parasitoids, tachinids are generally considered to have a broad host range (STIREMAN et al., 2006). However, recent studies show that their host range is extremely variable, with both polyphagous and specialized species (STIREMAN, 2016). Understanding the real extent of tachinid host ranges is extremely difficult because the family is taxonomically challenging, there are many undescribed and cryptic species, and rearing tachinids from all possible hosts poses practical problems (STIREMAN, 2005).

**Orgyia antiqua** (Linnaeus) (Lepidoptera: Lymantriidae), the rusty tussock moth, is a polyphagous moth native to Europe, but now introduced and widespread throughout Asia and North America. As with many other lymantriids, it can reach high population densities, becoming an important defoliator and pest of trees and cultivated plants. In Italy, **O. antiqua** is known to be parasitized by the tachinids **Compsilura concinnata** (Meigen) and **Exorista larvarum** (Linnaeus) (CERRETTI & TSCHORSNIG, 2010); in the Palearctic Region as a whole, it is parasitized by an additional 15 species of Tachinidae (TSCHORSNIG, 2017).

As part of a sampling study aimed at understanding the parasitoid community of phytophagous insects in a managed forest near Arzergrande (Padua, Veneto Region, Northern Italy), a single caterpillar of **O. antiqua** was collected in Northern Italy (Arzergrande, Padua, Veneto Region) in June 2015. The specimen was reared and died after a few days. A tachinid larva emerged and pupariated immediately after the caterpillar died. After one year of rearing, an adult tachinid had not eclosed, and the puparium was thus placed in ethanol for preservation. DNA barcoding was used to identify the parasitoid.

**MATERIALS AND METHODS**

**LABORATORY ANALYSIS**

Genomic DNA was extracted and isolated by cutting out a section of the tachinid pupa using standard proteinase K–phenol/chloroform method with ethanol precipitation. The pupa has died and dried inside the puparium. Remains of the puparium and pupa have been deposited in the Museum of Zoology, Sapienza University of Rome, in Rome, Italy. The mitochondrial DNA fragment Cytochrome c Oxidase subunit I (COI) was amplified using two primer pairs: M13F-LCO (5'-TGTTATCAAAACGCTAGCCGCTGACACACCATATAATGATATTGG-3') and M13R-HCO (5'-CACGAAAACGCTATGCACAACTCAACTACGGGTAACAAAATCAT-3'). Amplification was carried out in 25 µl reaction volume containing 50mM of MgCl2, 10mM of dNTP, 25 pM of each primer, 0.75 U Taq of Polymerase (Bioline), 1X NH4 reaction Buffer and 50ng of DNA. Cycling parameters were as follows: initial denaturation (94°C, 5m), 35 cycles (94°C, 30s; 50°C, 30s; 72°C, 30s) and final extension (72°C, 10m). PCR products were purified using Exosap-IT (USB Corporation) and sequenced by Macrogen Inc.

**PHYLOGENETIC RECONSTRUCTION**

Consensus sequences were generated using Geneious R7.0.6 (Biomatters Inc.). Alignment was carried out using the ClustalW program in Geneious R7.0.6 with 26 sequences (Table 1). The Neighbor-joining clustering method was run with MEGA v.6 (KUMAR et al., 2008), using the default parameters.

**RESULTS**

The COI DNA sequence of the tachinid pupa was deposited in GenBank (NCBI) and is available under accession number MF539618. It was compared with all the sequences of species belonging to the Carcelia genus.
Carcelia atricosta is scattered distributed throughout Europe from the Mediterranean to Norway (PAPE et al., 2015). Only two specimens have been previously collected in Italy, both captured in Malaise traps in Central Italy (Collolongo site- Selva Piana (AQ), Abruzzo Region; 3-17 August 2004; lat. 41.8930°, long. 13.5968°; 1500 m; legit. M. Romano; collection P. Cerretti, Museum of Zoology, Sapienza University, Rome, Italy) (CERRETTI, 2010). This represents the first record for Northern Italy (Arzergrande site (PD), Veneto Region; 26 June 2015; lat. 45.2565°, long. 12.0551°, 8 m; legit. D. Corcos).

**DISCUSSION**

The association between Orgyia antiqua and Carcelia atricosta had already been reported for the Czech Republic, the Netherlands and the United Kingdom (TSCHORSNIG, 2017 and literature therein), but is here recorded for the first time for Italy. Other known lepidopteran hosts of C. atricosta are: Orgyia recens Hübner (Lymyntriidae), Malacosoma neustria Linnaeus (Lasiocampidae) and Acronicta psi Linnaeus (Noctuidae) (TSCHORSNIG, 2017).

The importance of tachinids as natural enemies of phytophagous pest insects is well documented. However, tachinid-host associations are still poorly understood (STIREMAN, 2016), in part because of the difficulties in reproducing the optimal conditions for rearing specimens in the laboratory. The identification of tachinid larvae or puparia based on morphological characters is seldom possible. As an alternative to morphological identifications of these lesser known life stages, the increasing number of COI sequences of tachinids in molecular libraries are creating a growing inventory of data that allows for the rapid and affordable identification of taxa (POIKHOSMAKI et al., 2016). The use of molecular tools, as well as the availability of DNA sequences online, can dramatically improve our knowledge of parasitoid-host associations, especially in the case of rare or poorly-known species. Investigating the degree of tachinid host specificity and how widespread species conserve or change their host species in different regions, may help us to better understand the forces driving the diversification and evolution of these parasites (STIREMAN 2005). Also, because of their importance as enemies of pest insects, improving our knowledge of tachinid-host associations may be particularly useful in planning successful biological control programs (STIREMAN et al., 2006).

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