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

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Corcos D., Centorame M., Cerretti P. – DNA barcodes reveal a new host record for *Carcelia atricosta* Herting (Diptera Tachinidae) in Italy

The parasitoid-host association between *Orgyia antiqua* (Linnaeus) (Lepidoptera: Lymantriidae) and *Carcelia atricosta* Herting (Diptera: Tachinidae) is recorded here for the first time in Italy. A single caterpillar of *O. antiqua* was collected in Northern Italy (Arzergrande, Padua, Veneto Region) in June 2015. After the specimen died, a single tachinid larva emerged and pupariated. The emerged parasitoid was identified using DNA barcoding, with DNA extracted from the tachinid pupa. This is the first distributional record of *C. atricosta* in Northern Italy and the second for Italy, only two other specimens having being recorded previously (Abruzzo Region, Central Italy).

KEY WORDS: Parasitoid, parasitoid-host association, host range, Lepidoptera, *Orgyia antiqua*, pupa.

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 Palaearctic 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 on a blackberry shrub (*Rubus* sp.; Rosaceae) 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'-TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG-3') and M13R-HCO (5'-CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA-3') (FOLMER *et al*., 1994, modified). 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

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Table 1 – Sequences used to run the analysis which results are showed in Fig. I. Our sequence was compared with all COI sequences of the species belonging to the genus *Carcelia* Robineau-Desvoidy available in GenBank (NCBI), excluding sequences without species identification. A minimum of three sequences for haplotype have been retained for each species.

ID in tree	Species	Acc. Num.
MF539618	*C. atricosta*	MF539618
GU142048.1	*C. flavirostris*	GU142048.1
GU142049.1	*C. flavirostris*	GU142049.1
KM960164.1	*C. reclinata*	KM960164.1
KP049202.1	*C. reclinata*	KP049202.1
KP189254.1	*C. formosa*	KP189254.1
KR390013.1	*C. atricosta*	KR390013.1
KR428353.1	*C. reclinata*	KR428353.1
KR435682.1	*C. reclinata*	KR435682.1
KX843832.1	*C. puberula*	KX843832.1
KX843855.1	*C. puberula*	KX843855.1
KX843862.1	*C. lucorum*	KX843862.1
KX843955.1	*C. lucorum*	KX843955.1
KX844011.1	*C. gnava*	KX844011.1
KX844177.1	*C. laxifrons*	KX844177.1
KX844236.1	*C. atricosta*	KX844236.1
KX844238.1	*C. tibialis*	KX844238.1
KX844339.1	*C. lucorum*	KX844339.1
KX844462.1	*C. atricosta*	KX844462.1
KX844500.1	*C. tibialis*	KX844500.1
KX844513.1	*C. tibialis*	KX844513.1
KX844522.1	*C. bombylans*	KX844522.1
KX844525.1	*C. bombylans*	KX844525.1
KX844543.1	*C. laxifrons*	KX844543.1
KX844474.1	*C. rasa*	KX844474.1
HQ548469.1	*Blepharipa* sp.	HQ548469.1

available in GenBank [July 2017], revealing a similarity of 100.0 % with *Carcelia atricosta* Herting (Diptera: Tachinidae) (Fig. I). Other than *C. atricosta*, eight additional species belonging to the same genus have been recorded in Italy: *Carcelia alpestris* Herting, *Carcelia bombylans* Robineau-Desvoidy, *Carcelia dubia* (Brauer & Bergenstamm), *Carcelia gnava* (Meigen), *Carcelia laxifrons* Villeneuve, *Carcelia lucorum* (Meigen), *Carcelia rasa* (Macquart), and *Carcelia rasella* Baranov. Among them, *C. gnava* and *C. rasa* are known as parasitoids of *O. antiqua* in the Palaearctic Region (TSCHORSNIG, 2017). Both species were included in the analysis.

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 (Collelongo 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 (Lymantriidae), *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 (Pohjoismaki *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 parasitoids (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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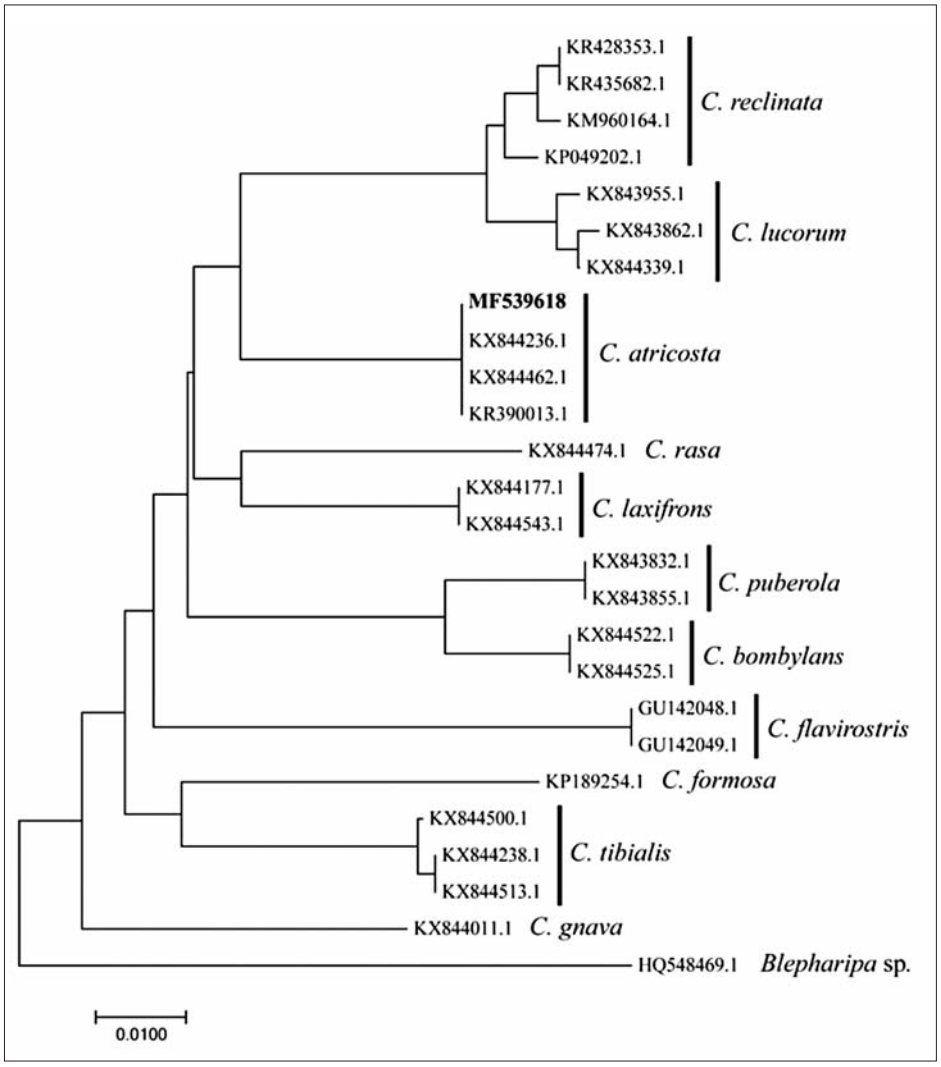

Fig. I – Phylogenetic tree based on 543bp of the COI gene built with the Neighbor Joining method. Our sequence (MF539618) clusters with available sequences for *C. atricosta* sharing the same haplotype.

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