

First record and DNA barcode of the clearwing moth *Tinthia tineiformis* (Esper, 1789) from Malta, central Mediterranean

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Abstract

A sesiid species, *Tinthia tineiformis* (Esper, 1789), is reported for the first time from the Maltese Islands, central Mediterranean. This new record represents the first species belonging to the subfamily Tinthiinae in Malta. The specimen was identified through morphological and genetic analyses. Observations of the live specimen revealed the use of jumping strategies by this species.

Keywords

Biodiversity conservation, Lepidoptera, Maltese Archipelago, Sesiidae.

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Introduction

Clearwing moths, Sesiidae, exhibit Batesian mimicry and usually mimic hymenopterans in their morphology and in some cases also in their behavior (Skowron et al. 2015; Skowron Volponi et al. 2018). Sesiid moths are morphologically readily distinguished from other moth families by the presence of transparent areas on their wings due to the lack of scales and the presence of a hair tuft at the tip of the abdomen (Laštůvka and Laštůvka 2001). Sesiid moths have been classified into two subfamilies, Sesiinae and Tinthiinae (Naumann 1971; Laštůvka and Laštůvka 2001), although some authors recognise a third subfamily, Paranthreninae (Duckworth and Eichlin 1974; Heppner and Duckworth 1981; McKern et al. 2008). The subfamilies Tinthiinae and Sesiinae are distinguished by the morphology of the antennae. Tinthiinae do not possess clavate antennae and possess either filiform or setiform antennae (Duckworth and Eichlin 1974; Laštůvka and Laštůvka 2001; Paolucci 2016).

The larvae of clearwing moths are generally monophagous or oligophagous being associated with a specific host plant species or a plant family and are rarely polyphagous (Špatenka et al. 1999; Laštůvka and Laštůvka 2001; Bella et al. 2017). The endophagous larvae are usually univoltine and require one year for development inside stems or roots of herbaceous or woody plants (Duckworth and Eichlin 1974; Karimpour et al. 2007; Gorbunov and Efetov 2018). Rhizophagous species in contrast to xylophagous species are generally more restricted to a few locations as they do not disperse far from their host plant (Garrevoet et al. 2007; Bąkowski and Hołowiński 2010; Bąkowski et al. 2010). Sesiid moths have also been described as colonial, where colonies are usually located upon a few individual host plants (Garrevoet et al. 2005; Paolucci 2016). These habitual characteristics make it generally difficult to observe and capture sesiid moths without the use of species-specific pheromone lures (Naka et al. 2008; Levi-Zada et al. 2011).

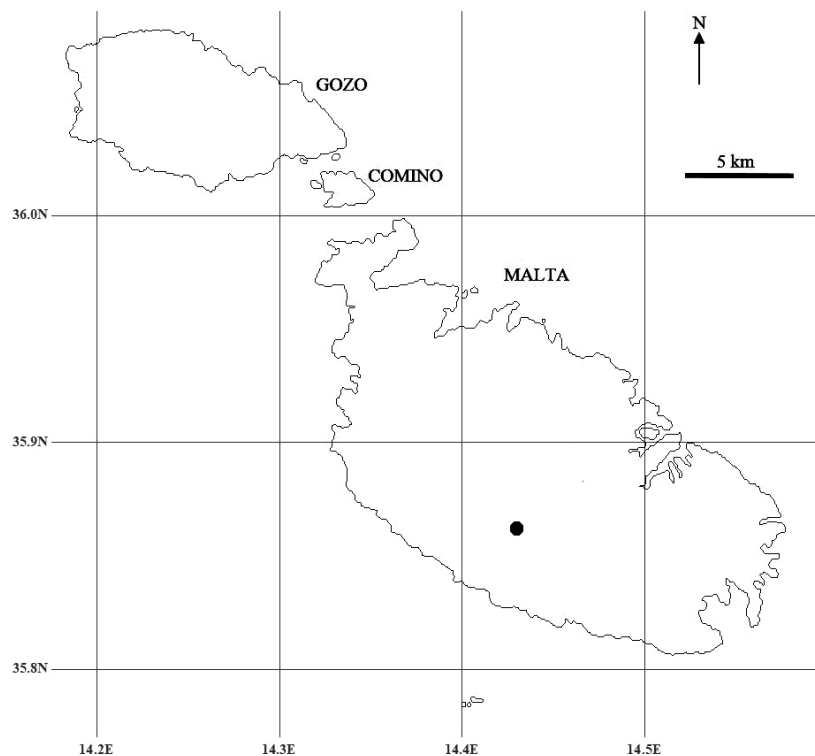


Figure 1. Map of the Maltese Islands, central Mediterranean and the geographical location of the collection site (black dot) for the first record of *Tinithia tineiformis* from Siġġiewi, Malta.

From the Palaearctic region, 347 sesiid species have been listed in the most recent global checklist (Kallies and Pühringer 2004). The Maltese Islands are known to host four sesiid species (Karsholt and Nieukerken 2013), i.e. *Synanthedon myopaeformis* (Borkhausen, 1789), *Bembecia tunetana* (Le Cerf, 1920), *Chamaesphexia aerifrons* (Zeller, 1847), and *C. anthraciformis* (Rambur, 1832), all belonging to the subfamily Sesiinae, tribe Synanthedonini. In this paper, we report an additional sesiid species to the Maltese entomofauna, *Tinithia tineiformis* (Esper, 1789), which we identified using morphological features and confirmed by means of the DNA barcoding approach. Our discovery represents the first record of the subfamily Tinithiinae in Malta. *Tinithia tineiformis* is a Holo-Mediterranean species that occurs in southern Europe including the Iberian, Apennine, and Balkan peninsulas, France, major Mediterranean islands including Sicily, Sardinia, Corsica, and Crete; it is also recorded from Asia Minor, the Middle East, and northern Africa (Laštůvka 1985; Špatenka et al. 1999; Laštůvka and Laštůvka 2001; Karsholt and Nieukerken 2013; Bella et al. 2017).

Methods

Ongoing biodiversity field research and monitoring undertaken by us in the Maltese Islands has allowed both the collection of dead and live insects. The individual specimen described here was collected from a limestone rubble wall surface in Siġġiewi, Malta, central Mediterranean (Fig. 1). The geographical coordinates were

obtained using Google Earth® v. 7.3.2 (geodetic datum: WGS84; map data: 2017-9-17, Google, DigitalGlobe). The live specimen was kept in a large enclosure indoors for behavioral observations, after which it was pinned for detailed morphological study and photography (Fig. 2). Morphological identification of the specimen was based on Duckworth and Eichlin (1974), Heppner and Duckworth (1981), Laštůvka (1985), Laštůvka and Laštůvka (2001), and Paolucci (2016). The pinned voucher specimen is deposited in the wild species collection of the Conservation Biology Research Group, University of Malta (CBRG-UM), Msida, Malta. Two legs were stored in absolute ethanol at -20°C as tissue vouchers for molecular analyses at CBRG-UM.

A total DNA was extracted from a single leg using GF-1 Tissue DNA Extraction Kit (Vivantis, Malaysia). The 658 bp barcode region of the mitochondrial cytochrome *c* oxidase subunit I (COI) was amplified using standard LepF1/LepR1 primers (Hebert et al. 2004). Three PCR reaction replicates were carried out in 25 μL reaction volume containing approximately 10ng DNA template, 1X FIREPOL® Master Mix containing 2.5 mM Mg^{2+} , 200 μM of each dNTP and 1 U FIREPOL® DNA polymerase (Solis BioDyne, Estonia), and 0.5 μM of each primer. PCRs were carried out on a Nexus Gradient Mastercycler® (Eppendorf, Germany) using the following temperature profile: 95 $^{\circ}\text{C}$ for 5 min; followed by 6 cycles of 95 $^{\circ}\text{C}$ for 45 s, 45 $^{\circ}\text{C}$ for 30 s, 72 $^{\circ}\text{C}$ for 1 min and 36 cycles of 95 $^{\circ}\text{C}$ for 45 s, 50 $^{\circ}\text{C}$ for 30 s, 72 $^{\circ}\text{C}$ for 1 min, and a final extension at 72 $^{\circ}\text{C}$ for 15 min. From each PCR reaction, 2 μL of the PCR product was visualised



Figure 2. Dorsal photograph of *Tinthia tineiformis* (voucher ID SESDM001) collected from Malta.

on a 1.5% agarose gel stained with ethidium bromide, together with a 100 bp DNA ladder (Solis BioDyne, Estonia). Each of the remaining PCR product was subsequently purified and sequenced in both directions with an ABI3730XL sequencer. Quality check, editing, and assembly of complementary barcode sequences was conducted in Geneious® 11.1.2 (<https://www.geneious.com>; Kearse et al. 2012). The final COI sequence of 619 bp was searched against NCBI GenBank® database (GenBank, <https://www.ncbi.nlm.nih.gov/genbank>; Benson et al. 2012) nucleotide collection (nr/nt) using BLASTn v. 2.9.0 (Zhang et al. 2000; Morgulis et al. 2008). The 619 bp sequence was also searched against the species level barcode records available at the Barcode of Life Data System (BOLD, <http://www.boldsystems.org>; Ratnasingham and Hebert 2007). The COI sequence data of the specimen was deposited to GenBank (MK803278).

Results

Tinthia tineiformis (Esper, 1789)

New records. Malta: Siġġiewi (35°51'4"N, 014°25'31"E; 140 m a.s.l.), D. Magro leg., June 18, 2018 (1 female, CBRG-UM, voucher ID SESDM001).

Identification. The genus of the specimen was identified as *Tinthia* Walker, 1865 and distinguished from a closely related genus, *Pennisetia* Dehne, 1850, due to the filiform antennae, the lack of transparent areas on the fore wings, and the hind wing vein pattern with Cu_1 arising before crossvein (Fig. 2) (Laštůvka and Laštůvka 2001; Paolucci 2016). The species identity of the specimen was classified

as *T. tineiformis* and was distinguished from other *Tinthia* species by absence of distinct yellow bands on abdomen, brown coloration of the fore wings and abdomen, the lack of orange coloration of the hind tibia, coloration of the first abdominal segment, and the lack of yellowish-white spots in the distal part of the fore wings (Fig. 2) (Laštůvka 1985; Laštůvka and Laštůvka 2001). The specimen was identified as a female based on the shape of abdomen and anal tuft, following Laštůvka (1985).

BLAST hit results of the 619 bp COI sequence from the specimen examined resulted in the highest pairwise identity of 99.8% to two sequences with the accession numbers AJ864349 and AJ864350 assigned to *T. tineiformis* in GenBank. The identification tool in BOLD assigned the sequence from our specimen to the BIN URI: BOLD: AAD9679 which corresponds to the species *T. tineiformis*. The assigned BIN intraspecific *p*-distance ranged from 0% to 0.78%.

Observations. The specimen was found at 11 a.m. on a sunny day in an open area dominated by agricultural land and herbaceous vegetation along rural roadsides. The specimen was found resting on a sunlit stone wall, which had been cleared of vegetation prior to the discovery of the specimen. The plant remains from the location where the specimen was found were identified as *Convolvulus* spp. and *Hedera helix* L. Within the enclosure, the live specimen was observed using two different jumping strategies. One strategy was jumping and landing without opening the wings and a second strategy involved jumping for take-off and then transitioning into powered flight.

For the newly recorded Maltese sesiid species, *T. tineiformis*, we propose the Maltese name “Baħrija Żunžan tas-Sejġieħ”. In Maltese “Sejġieħ” refers to the stone used to construct rubble walls, which is the habitat where this species was first discovered in Malta.

Discussion

This first record of *T. tineiformis* from Malta at one location, despite the ongoing sampling efforts done by the authors, may suggest that this species has a restricted local distribution, a common characteristic among other sesiid species (Garrevoet et al. 2005, 2015). In Sicily, *T. tineiformis* was recorded from multiple locations (Bella et al. 2017); therefore, further research may elucidate the distribution of *T. tineiformis* in Malta and be able to confirm whether the species is localized and to assess the conservation status of this taxon in the Maltese Islands. *Tinthia brosisiformis* (Hubner, 1813), a species closely related to *T. tineiformis*, is a widespread species in Turkey and both related taxa depend on the same host plants, *Convolvulus* spp. (Garrevoet et al. 2005). *Convolvulus* spp. are widespread in the Maltese Islands (Weber and Kendzior 2006) and may therefore serve as indicators of suitable habitat for *T. tineiformis*.

The discovery of an individual of *T. tineiformis* occurred after the wall where it was found had been cleared of roadside vegetation which included bindweeds (*Convolvulus* spp.) and the Common Ivy (*Hedera helix*). The larvae of *Tinthia* spp. are known to live inside roots of *Convolvulus* spp. (Bertaccini and Fiumi 2002; Garrevoet et al. 2005), and our results might support this observation. The habitat was modified by roadworks before the specimen was found and only plant remains were recovered from the location. Therefore, the host-plant association could not be ascertained. Due to the colonial behavior documented for many sesiid species (Garrevoet et al. 2005), the removal of vegetation from roadsides, especially in rural areas, could have a substantial detrimental impact on the population of this species in Malta.

Observations of the specimen within an enclosure revealed that *T. tineiformis* uses two different jumping strategies. These behavioral adaptations might allow faster escape from predators and provide the necessary propulsive force for take-off (Burrows and Dorosenko 2015). Such behavior could partially explain why this species was never recorded in Malta before now. We are not aware of previous published data suggesting this behavior for *T. tineiformis*. However, the two jumping strategies observed and described are common for other small species of Lepidoptera (Burrows and Dorosenko 2015).

The DNA barcoding approach assists in the taxonomy of sesiid species (Hansen et al. 2012; Garrevoet et al. 2013) and contributes towards the formulation of their conservation and management plans (Lait and Hebert 2018). Such a molecular technique is highly valuable

if included as a routine process when documenting the presence of taxa across their geographical range. Despite the small area of the Maltese Islands, new insect records are still made frequently, which indicates that the knowledge on insect biodiversity is still progressing. Ongoing field research accompanied by DNA barcoding of tissues from insect specimens sampled by us is helping to fill these knowledge gaps in order to provide an essential baseline for biodiversity conservation.

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Authors' Contributions

AV and CMM assessed the location and habitat features associated with the specimen under investigation, undertook the morphological and genetic data analyses and prepared the manuscript; DM observed and captured the live specimen and pinned the specimen in preparation for morphological analyses after taking records of its behavioral characteristics.

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