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Molecular identification of newly recorded Lepidoptera for Cyprus and Europe (Insecta: Lepidoptera)

Peter Huemer & Özge Özden

Abstract

As part of a comprehensive molecular inventory of Lepidoptera in Northern Cyprus, 8 species have been identified as new records for the island of Cyprus through genetic comparisons of DNA barcodes with reference sequences. The following species are formally reported for the first time in Europe: *Coleophora bivittella* Staudinger, 1879 (Coleophoridae) and *Teliphasa lophotalis* (Hampson, 1900) (Pyralidae), whereas the agriculturally significant species *Spodoptera frugiperda* (Smith, 1797) (Noctuidae) was only published from the Canary Islands (Spain) and from Malta so far. The possibilities and limitations of molecular-based determinations are briefly discussed using the example of the fauna of north Cyprus.
Keywords: Insecta, Lepidoptera, DNA barcoding, *Coleophora bivittella, Anarsia acaciae*, *Teliphasa lophotalis*, *Spodoptera frugiperda*, new records, Cyprus, Europe.

Identificación molecular de Lepidoptera recién registrados para Chipre y Europa (Insecta: Lepidoptera)

Resumen

Como parte de un inventario molecular exhaustivo de Lepidoptera en el norte de Chipre, se han identificado 8 especies como nuevos registros para la isla de Chipre mediante comparaciones genéticas de códigos de barras de ADN con secuencias de referencia. Las siguientes especies se citan oficialmente por primera vez en Europa: *Coleophora bivittella* Staudinger, 1879 (Coleophoridae) y *Teliphasa lophotalis* (Hampson, 1900) (Pyralidae), mientras que la especie de importancia agrícola *Spodoptera frugiperda* (Smith, 1797) (Noctuidae) sólo se había publicado de las Islas Canarias (España) y ede Malta hasta ahora. Las posibilidades y limitaciones de las determinaciones de base molecular se discuten brevemente utilizando el ejemplo de la fauna del norte de Chipre.
Palabras clave: Insecta, Lepidoptera, ADN código de barras, *Coleophora bivittella, Anarsia acaciae*, *Teliphasa lophotalis*, *Spodoptera frugiperda*, nuevos registros, Chipre, Europa.

Introduction

The Lepidoptera fauna of Cyprus has drawn the attention of European experts since early times (Lederer, 1855; Rebel, 1939; Wiltshire, 1948). Even in recent history, the island has been visited by numerous lepidopterologists, and several comprehensive publications, especially on Microlepidoptera, have been released (Arenberger, 1994; Gozmány, 2012), continually supplemented in subsequent years (Arenberger & Wimmer, 1996, 1999, 2003; Barton, 2015, 2018). However, among the so-called Macrolepidoptera, only specific groups such as butterflies (John & Makris, 2023) or Geometridae (Fischer & Lewandowski, 2010) have been addressed in modern faunistic studies. Nevertheless, Cyprus

remains one of the few European countries without a checklist of its lepidopteran fauna. This may be due, on the one hand, to the controversial biogeographic assignment of the island to Europe and the resulting lack of consideration in major catalog works such as Karsholt & Razowski (1996) or more recently Top et al. (2023). On the other hand, it may also be attributed to the lack of local faunistic expertise. A reasonably complete online version of the Fauna Europaea with nearly 900 species is considered in need of revision (Karsholt & Nieukerken, 2013).

While the faunistic inventory of species in the southern part of the island is now largely complete, deficiencies in northern part of Cyprus are significant. A striking example of these gaps is evident in the recent revision of the family Psychidae by Weidlich (2015), with not a single recorded location in the northern part. Especially due to the island's division in 1974 and resulting restrictions, the northern part has been explored by very few lepidopterologists from Europe or Turkey (Ahola, 1998; Atay & Oğur, 2011; Can Doğanlar & Arap, 2005; Fibiger, 1997), and data on illegally collected specimens remain hidden in various collections.

As part of a collaboration initiated in September 2023 between Near East University (Nicosia, north Cyprus) and the Tyrolean State Museums (Innsbruck, Austria), the significant faunistic research deficit is intended to be addressed through intensive sampling and the use of molecular identification methods (DNA barcoding). The ultimate goal is to establish a molecular reference database and based on that, create an annotated checklist of the fauna of Cyprus. New findings from an initial survey phase are presented here.

Material and Methods

Material was collected with ultraviolet light (15W/12V tubes) illuminating a gauze tower, and portable light traps or other devices in a two-week survey period lasting from 6th to 21st of September 2023. All samples were immediately pinned after euthanasia with ethyl acetate, spread, and dried to ensure high DNA quality. After full labelling of samples and morphology based macroscopic identification of undisputed species, object data were digitized using BioOffice2 software. A single specimen at minimum of all presumed morphospecies was selected for further molecular analysis, covering altogether 344 specimens. Tissue samples of these specimens (a single leg) were finally transferred to well plates containing 99% ethanol, with each plate holding 95 samples, and sent to the Canadian Centre for DNA Barcoding (CCDB, University of Guelph, Ontario, Canada). DNA isolation, PCR amplification, and subsequent DNA sequencing of the mitochondrial Cytochrome C Oxidase I (COI, Barcode fragment 5') were carried out at CCDB following the standard protocols described by deWaard et al. (2008). For all samples, after successful sequencing, DNA barcodes were compared with reference sequences in BOLD (Barcode of Life Data Systems) (Ratnasingham & Hebert, 2007). In further steps, potential new records for Cyprus were identified from the aforementioned literature sources and Lepiforum e.V. (2008-2021) and Karsholt & Nieukerken (2013). Subsequently, 8 noteworthy new records for Cyprus were selected and further analyzed. All collection data of the analyzed 78 specimens, including images, and their genetic sequences can be found in the public dataset DS-NEWLECYP "New records of Lepidoptera for Cyprus" on BOLD. Voucher material is preserved in the Natural History Collections of the Tyrolean State Museum Ferdinandeum (Austria).

For each new record a Neighbor Joining Tree was constructed based on the Kimura-2-Parameter model (K2P) and using the MEGA11 software (Tamura et al. 2021). Focal species were compared with the most similar 2-3 congeneric species in BOLD, usually based on Barcode Index numbers (BINs) (Ratnasingham & Hebert, 2013).

Pinned voucher specimens were photographed using a Canon 750D camera in combination with a Canon MP-E-65 mm lens. For each photograph, sets of 50-60 images were taken at different focal planes and focus-stacked using Helicon Focus 6. The final images were edited in Adobe Photoshop.

Results

Overall, 339 sequences were determined from a total of 344 specimens. These sequences are

attached to 188 taxa identifiable at the species level and approximately 60 barcode clusters that currently cannot be assigned to any known species.

Below, 8 selected taxa of particular faunistic significance are presented. The discussions of these species include specific collection data, DNA barcode IDs (specimen ID), and, where applicable, taxonomic and/or faunistic comments. Only specimens with DNA barcodes are taken into consideration.

Cedestis civitatensis Nel & Varenne, 2015 (Yponomeutidae) (Figure 1)

Records: Cyprus, Kyrenia/Girne, Lapta, Selvili tepe, 890 m, 35.316ºN, 33.168ºE, 14-IX-2023, leg. Huemer, DNA Barcode IDs TLMF_Lep_39180, TLMF_Lep_39181.

Remarks: Two barcoded specimens from Spain, one incorrectly identified as *Elachista* sp. (DNA Barcode ID MM19987, BIN: BOLD:AAZ9164), cluster next to *C. civitatensis* at a p-distance of 3.25% and likely belong to a possibly undescribed species of *Cedestis*. *Cedestis civitatensis* was hitherto only known from two type specimens colected in France nearby *Pinus halepensis* (Nel & Varenne, 2015), a tree also well present at Selvili tepe in north Cyprus and indicating a much more widespread distibution pattern. **New record for Cyprus**.

Coleophora bivittella Staudinger, 1879 (Coleophoridae) (Figure 2)

Record: Cyprus, Iskele, Bafra/Vokolida, Thalassa Beach, 6 m, 35.33ºN, 34.065ºE, 6-IX-2023, leg. Huemer, DNA Barcode ID TLMF_Lep_38989.

Remarks: Originally described from southern Turkey, furthermore, known from Iran (Baldizzone, 1989). **New record for Europe**.

Scrobipalpa geomicta (Meyrick, 1921) (Gelechiidae) (Figure 3)

Records: Cyprus, Iskele, Bafra/Vokolida, Thalassa Beach, 4 m, 35.331ºN, 34.068ºE, 8-IX-2023, leg. Huemer, DNA Barcode IDs TLMF_Lep_39040, TLMF_Lep_39058, TLMF_Lep_39061.

Remarks: In Europe so far only known from Spain and Greece (Crete) (Huemer & Karsholt, 2010; Karsholt & Huemer, 2017). **New record for Cyprus**.

Ephysteris iberica Povolný, 1977 (Gelechiidae) (Figure 4)

Records: Cyprus, Iskele, Bafra/Vokolida, Thalassa Beach, 4 m, 35.331ºN, 34.068ºE, 8-IX-2023, leg. Huemer, DNA Barcode IDs TLMF_Lep_39057.

Remarks: The nearest neighbor, *Ephysteris promptella* (Staudinger, 1859), shows a considerable intraspecific variation of the DNA barcode which may be due to cryptic diversity. Sproadic distribution in the Mediterranean (Huemer & Karsholt, 2010). **New record for Cyprus**.

Anarsia acaciae Walsingham, 1896 (Gelechiidae) (Figure 5)

Records: Cyprus, Iskele, Bafra/Vokolida, Thalassa Beach, 6 m, 35.33ºN, 34.065ºE, 6-IX-2023, leg. Huemer, DNA Barcode ID TLMF_Lep_38961; Cyprus, Iskele, Bafra/Vokolida, Thalassa Beach, 4 m, 35.331ºN, 34.068ºE, 8-IX-2023, leg. Huemer, DNA Barcode ID TLMF_Lep_39056.

Remarks: The identification is based on a barcoded specimen from Morocco, identified by O. Karsholt. It was already collected in Cyprus and identified as *Anarsia* nr. *acaciae* by I. Barton (BOLD). Furthermore, long series of DNA barcoded specimens of a yet unidentified *Anarsia* sp. from Kenya and Tanzania in BOLD fully correspond with specimens from Cyprus. According to Bildzilya et al. (2019) unknown from Europe but published for the Continent by Huemer & Karsholt (2020) without any details **New record for Cyprus**.

Stenoptilia aridus (Zeller, 1847) (Pterophoridae) (Figure 6)

Records: Cyprus, Kyrenia/Girne, Yilgaz E, 280 m, 35.322ºN, 33.227ºE, 11-IX-2023, leg. Huemer, DNA Barcode ID TLMF_Lep_39161.

Remarks: The species is absent from large parts of the Eastern Mediterranean but known from Turkey and Israel (Lepiforum e.V., 2008-2021). **New record for Cyprus**.

Teliphasa lophotalis (Hampson, 1900) (Pyralidae) (Figure 7)

Record: Cyprus, Kyrenia/Girne, Yilgaz, 225 m, 35.322ºN, 33.227ºE, 12-IX-2023, leg. Huemer, DNA Barcode ID TLMF_Lep_39075.

Remarks: The species was described from Turkey but is more widespread in the Middle East with records from Iran, Israel, and Lebanon (Koçak, 1987). Unpublished records are available from Greece (Samos) (Lepiforum e.V., 2008-2021) and a reference sequence from this country based on material collected by. D. Fritsch is used in the NJ tree. A small series of the species was collected in north Cyprus, clearly indicating a residential status. **New record for Europe**.

Spodoptera frugiperda (Smith, 1797) (Noctuidae) (Figure 8)

Records: Cyprus, Iskele, Bafra/Vokolida, Thalassa Beach, 6 m, 35.33ºN, 34.065ºE, 6-IX-2023, leg. Huemer, DNA Barcode IDs TLMF_Lep_39219, TLMF_Lep_39220.

Remarks: The highly rapid spread of the fall armyworm, *Spodoptera frugiperda*, a species originally described in North America and introduced to West Africa in 2016, and now established in parts of Asia and Australia, is extensively documented by Lepiforum e.V. (2008-2021). In addition to a note of the discovery of the species in the Canary Islands (Vives Moreno & Gastón, 2019), and from Malteses islands (Seguna et al. 2024), three adult specimens from December 2022 in Cyprus (Souni Zanatzia, Limassol) are illustrated. Furthermore, EPPO (2023) reports on three additional specimens collected by a private collector in January 2023, also from the vicinity of Limassol (Pissouri village). Additional reports and measures are described as follows: "Official surveys were conducted, and the NPPO of Cyprus reported that a larva has been found in a maize (*Zea mays*) crop in Larnaca district in June 2023, and multiple larvae in maize crops for animal feed in Nicosia district in July 2023. An infested area has been demarcated, as well as a buffer zone. Official measures are taken to eradicate the outbreaks. The pest status of *Spodoptera frugiperda* in Cyprus is officially declared as: Present, at low prevalence, only in some parts of the Member State concerned, under eradication." However, our additional findings from Northern Cyprus suggest a significantly expanded colonisation, casting doubt on the success of measures already implemented. Confirmed record for Cyprus.

Discussion

For the first time ever, a significant number of species of Lepidoptera have been sampled in Northern Cyprus, comprising over 250 species. Equally unique is the consistent application of molecular identification methods, specifically DNA barcoding.

The success rate of approximately three-quarters of taxa identifiable at the species level may initially seem disappointing. However, it aligns with the extent of previous efforts for the complete sequencing of European Lepidoptera. Particularly from Mediterranean regions, many so-called "gap species" are known, lacking reference sequences in large databases like BOLD (Huemer & Mutanen, 2022). Nevertheless, it is already assured that the well over 60 currently unidentified barcode clusters from Northern Cyprus include a multitude of taxonomic challenges and further faunistic discoveries for Cyprus and even Europe. Even in morphologically identified samples, remarkable findings exist, although they are here not considered due to the absence of comparative sequences. Examples include *Choreutis sexfasciella* (Sauber, 1902) which is a previously unpublished species in Europe.

The faunistic examples already secured and discussed here include a notable case of recent introduction with *Spodoptera frugiperda*, a highly invasive and agriculturally significant neozoon originated in the Neotropics. This species was first introduced to Africa in 2016 and has since spread extensively. Thanks to the molecular identification of samples from Northern Cyprus, its presence on the Island and in Europe is confirmed. In combination with few individual sightings in Cyprus from 2023, further spread on the island is already anticipated.

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*Peter Huemer
Naturwissenschaftliche Sammlungen
Tiroler Landesmuseen
Betriebsgesellschaft m. b. H.
Krajnc-Strasse, 1
A-6060 Hall
AUSTRIA / *AUSTRIA*
E-mail: p.huemer@tiroler-landesmuseen.at
https://orcid.org/0000-0002-0630-545X

Özge Özden
Near East University
Faculty of Agriculture
Mersin, 10
99138 Nicosia
CHIPRE DEL NORTE / *CYPRUS NORTH*
E-mail: ozge.ozden@neu.edu.tr
https://orcid.org/0000-0001-9479-3862

*Autor para la correspondencia / *Corresponding author*

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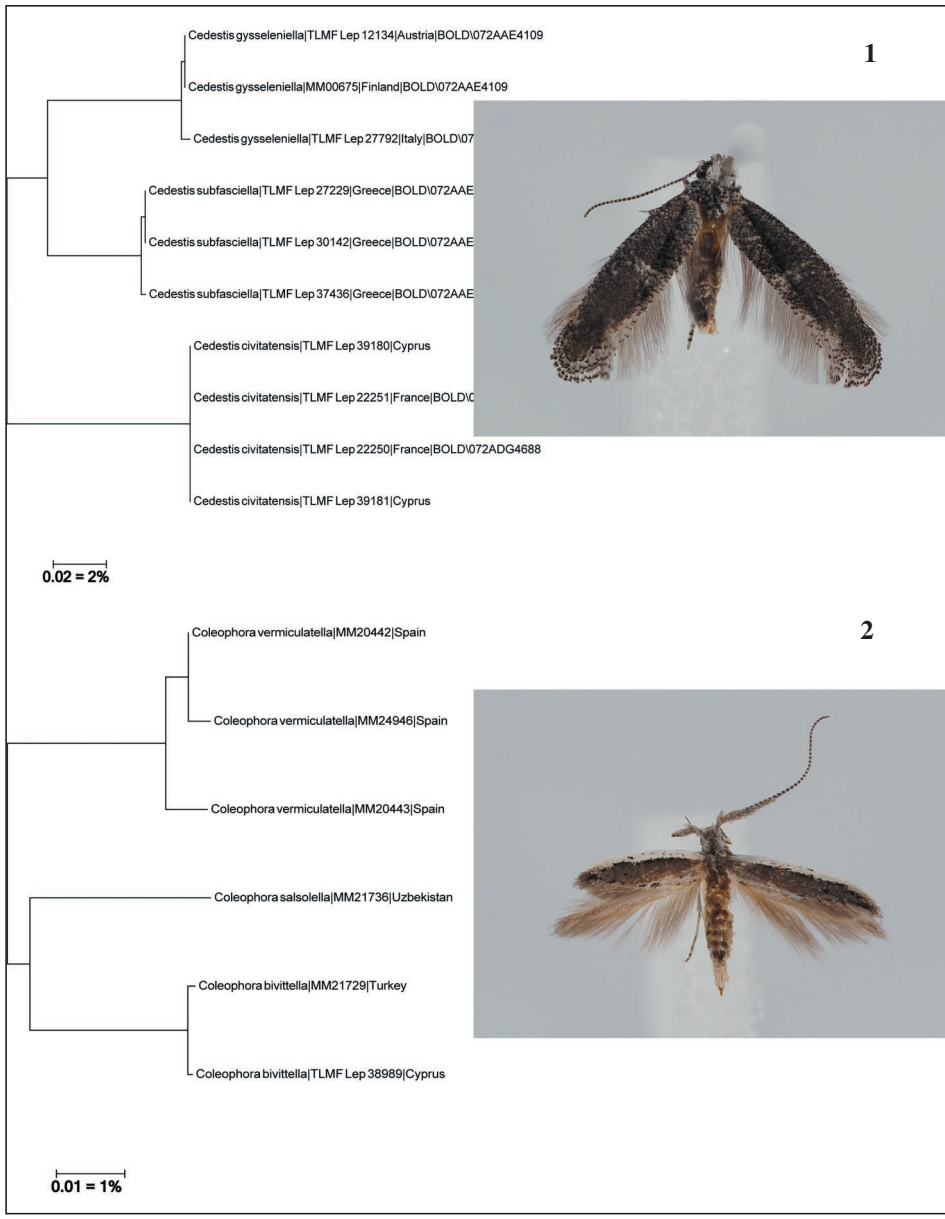
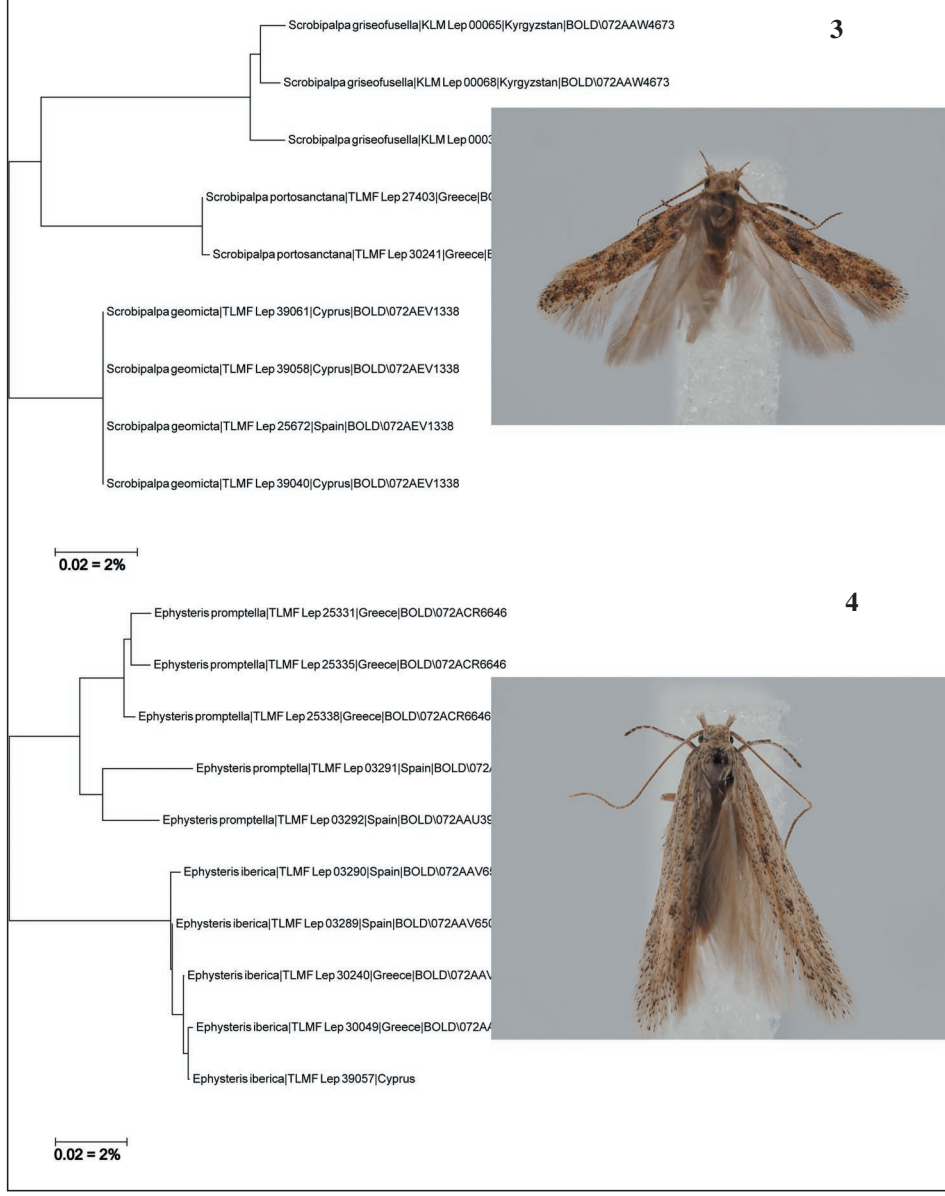
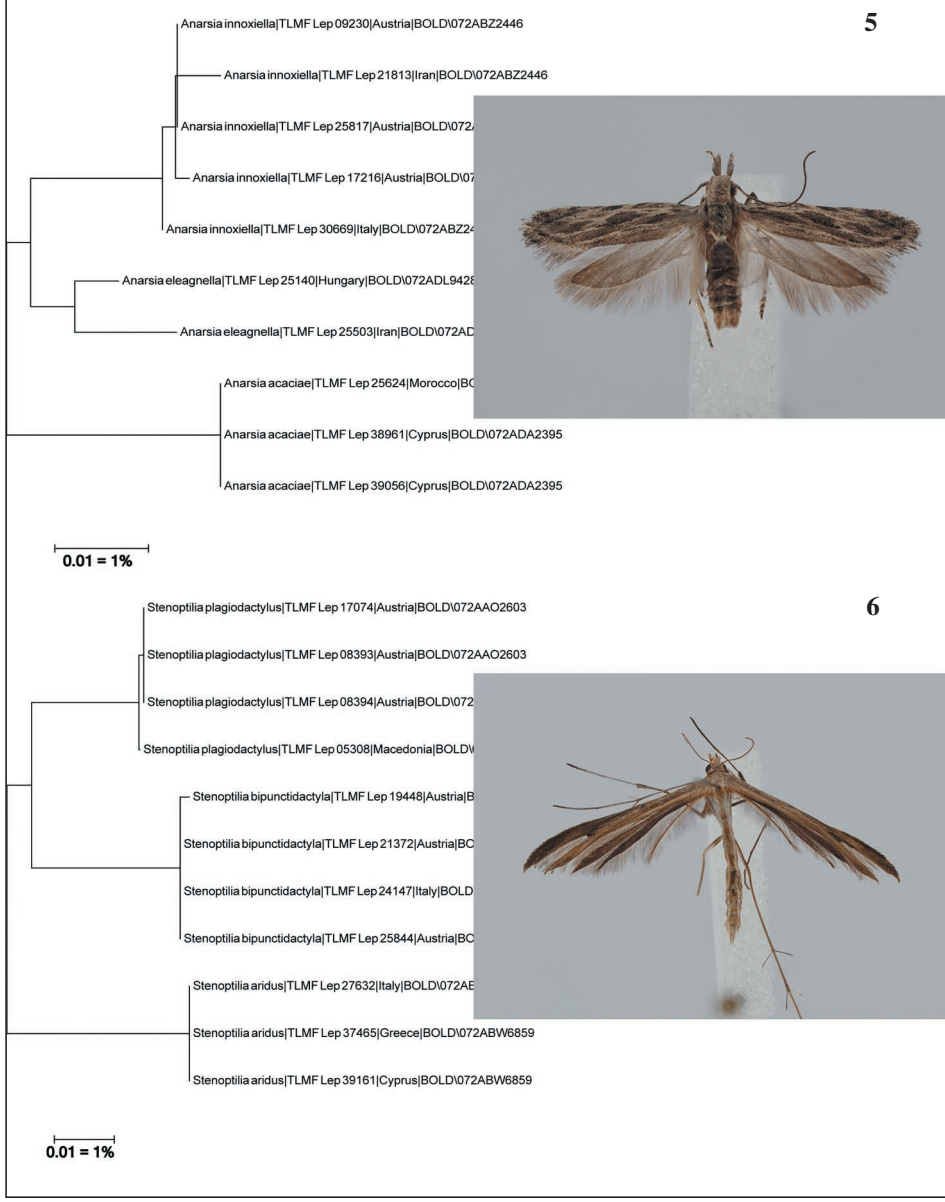


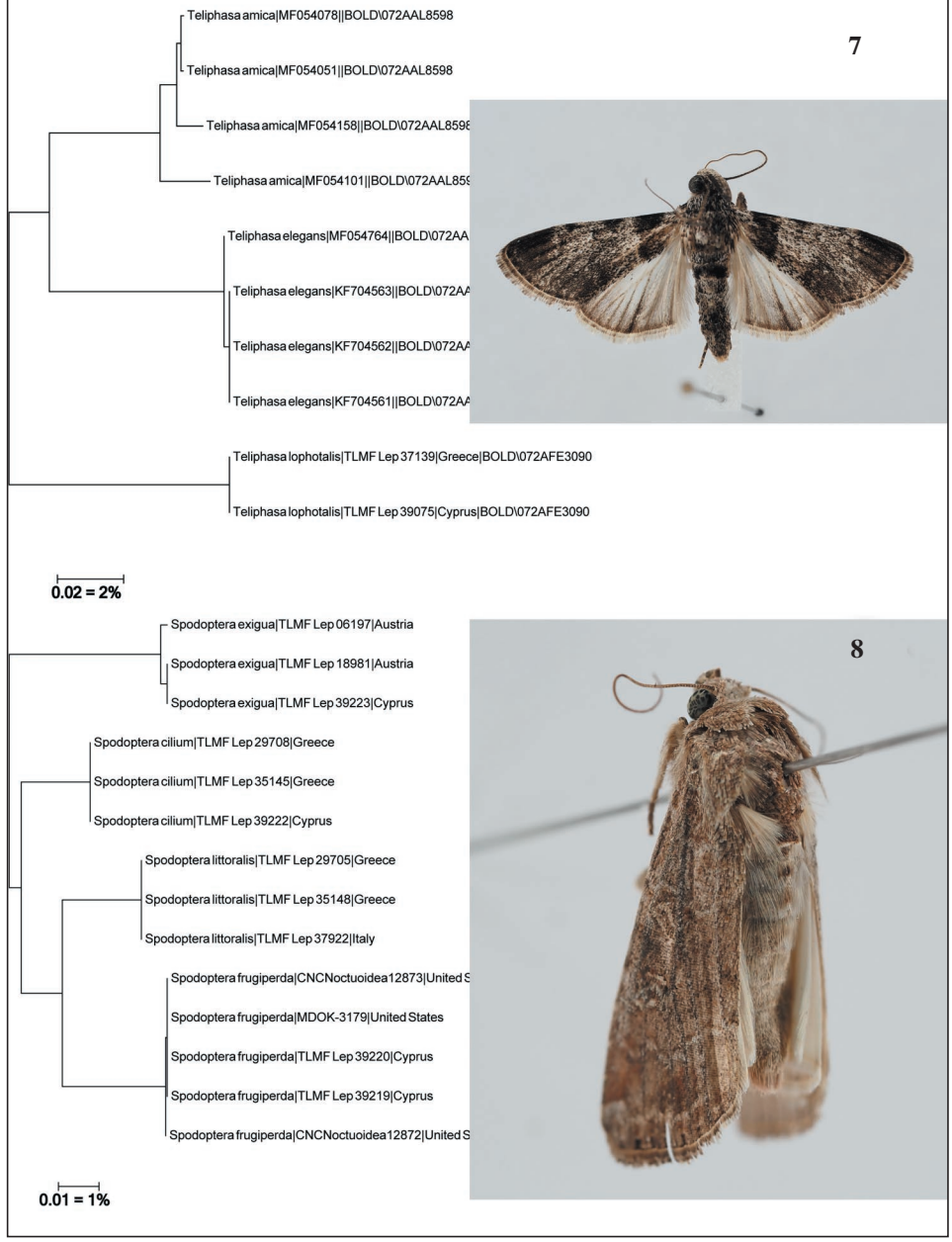
Figs 1-2. Neighbour-Joining tree and adult. **1.** *Cedestis civitatensis* Nel & Varenne. **2.** *Coleophora bivittella* Staudinger.



Figures 3-4. Neighbour-Joining tree and adult. **3.** *Scrobipalpa geomicta* (Meyrick). **4.** *Ephysteris iberica* Povoln .



Figures 5-6. Neighbour-Joining tree and adult. **5.** *Anarsia acaciae* Walsingham. **6.** *Stenoptilia aridus* (Zeller



Figures 7-8. Neighbour-Joining tree and adult. **7.** *Teliphasa lophotalis* (Hampson). **8.** *Spodoptera frugiperda* (Smith).