A cosmopolitan invader – *Choreutis sexfasciella* (Lepidoptera, Choreutidae) – in Cyprus: first record, molecular characterization, and a reared parasitoid

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Abstract. The Banyan Leaf Skeletonizer moth, *Choreutis sexfasciella* (Sauber, 1902) (Lepidoptera: Choreutidae), is reported from Cyprus for the first time from *Ficus microcarpa* L.fil. and *F. benjamina* L. trees in urban areas of Larnaca, Limassol, and Paphos. *C. sexfasciella* was originally described from the Philippines and has rapidly extended its range in the last five years into several Mediterranean countries as well as into North America. DNA barcode sequencing of several specimens from Cyprus has demonstrated that they are closely related to North American specimens. The parasitoid wasp *Elasmus* cf. *cyprianus* Ferrière, 1947 (Hymenoptera: Eulophidae) has been reared from leaves infested with larvae of *C. sexfasciella*. The observed socioeconomic impacts of the non-native moth and the potential use of its parasitoid as a biological control agent are discussed.

Introduction

Ficus microcarpa L.fil., *F. benjamina* L. and other Indo-Australian representatives of the genus *Ficus* L. (Moraceae) have been introduced across the globe as ornamental plants in urban and semi-urban areas and have been followed by a large number of non-native insect species

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(Barbagallo et al. 2005; Wang et al. 2015a; Laudani et al. 2020; Laarif and Bouslama 2022; van Noort and Rasplus 2023). In Cyprus, these introduced tree species have been extensively planted in urban habitats such as parks, gardens, and roadsides, leading to the accidental introduction and establishment of numerous non-native insects. To date, more than thirty non-native species have been found to be associated with non-native *Ficus* spp. on the island, including a wide range of phytophagous species (e.g., Hemiptera and Thysanoptera) as well as chalcid wasps forming a paraphyletic group exclusively reproducing within figs, appropriately termed "fig wasps" (EPPO 2014a, b; Collins and Philippou 2016; Compton et al. 2020; Demetriou et al. 2023a; Japoshvili et al. 2023; Koutsoukos et al. 2024).

Species of the genus *Choreutis* Hübner, 1825 (Lepidoptera: Choreutidae), such as *C. aegyptiaca* (Zeller, 1867), *C. japonica* (Zeller, 1877), and *C. sexfasciella* (Sauber, 1902), are known to be associated with ornamental *Ficus* spp. (Savela 2019; Abu Ghonem et al. 2020). *Choreutis sexfasciella*, also known as the Banyan Leaf Skeletonizer (Fig. 1), was first described from the Philippines (Semper 1902) and is considered native to parts of the Eastern Palaearctic and Indomalayan biogeographic realms (Rittner 2019; Savela 2019). Nevertheless, the species has managed to escape its native range, even reaching California and Florida in North America (Beucke 2021; Hodel et al. 2021; Hayden et al. 2023; Heppner 2023). In the Mediterranean basin, it has been reported from Israel (Rittner 2019), Egypt (Abu Ghonem et al. 2020), and most recently Türkiye (Can and Koçak 2024), and it is also known from Réunion (Bippus 2020). To date, the only species of the genus known to inhabit Cyprus is *C. nemorana* (Hübner, 1799), feeding on the native *Ficus carica* L. (Karsholt and van Nieukerken 2013).

The non-native species of *Choreutis* that first appeared in California in 2021 was originally recorded under the name *Choreutis emplecta* (Turner, 1942) (Beucke 2021; Hodel et al. 2021) as its DNA barcode sequence matched the specimens of this Australian species. In the meantime, the matter was further complicated by Bippus (2020), who transferred *C. emplecta* into *Anthophila* Haworth [1811], without providing an explanation for this taxonomic act. This led to some confusion about the correct generic placement for this invasive species. In 2023, Heppner synonymized *C. emplecta* with *C. sexfasciella*. As part of our project, we tested both the synonymy hypothesis as well as the generic placement of this species with molecular data.

Parasitoid wasps of the genus *Elasmus* Westwood, 1833 (Hymenoptera: Eulophidae) are known to parasitize various Diptera, Hymenoptera, and Lepidoptera, although their hosts are largely unknown (Graham 1995; Verma et al. 2002; Yefremova and Strakhova 2012). Known cases of chalcid wasps parasitizing Choreutidae are quite rare, limited only to *E. nudus* (Nees, 1834), which has been catalogued as a larval and pupal parasitoid of *Tebenna bjerkandrella* (Thunberg, 1784), and *Conura side* Walker, 1843, a parasitoid of *T. silphiella* (Grote, 1881) (Labeyrie 1962; Noyes 2019). In Cyprus, five species of *Elasmus* have been identified, namely: *E. bicolor* (Fonscolombe, 1840), *E. platyedrae* Ferrière, 1935, *E. cyprianus* Ferrière, 1947, *E. phthorimaeae* Ferrière, 1947, and *E. steffani* Viggiani, 1967 (Ferrière 1947; Graham 1995). These species are known parasitoids of moths belonging to numerous lepidopteran families, such as Elachistidae Bruand, 1851, Gelechiidae Stainton, 1854, Erebidae: Lymantriinae Hampson, 1893, Plutellidae Guenée, 1845, Psychidae Boisduval, 1828, Tortricidae Latreille, 1803, and Yponomeutidae Stephens, 1829, which include notorious pests of agricultural plants e.g. *Pectinophora gossypiella* (Saunders, 1844), *Phthorimaea operculella* (Zeller, 1873) (Graham 1995), and *Prays oleae* Bernard, 1788, affecting cotton, potato, and olive production, respectively.

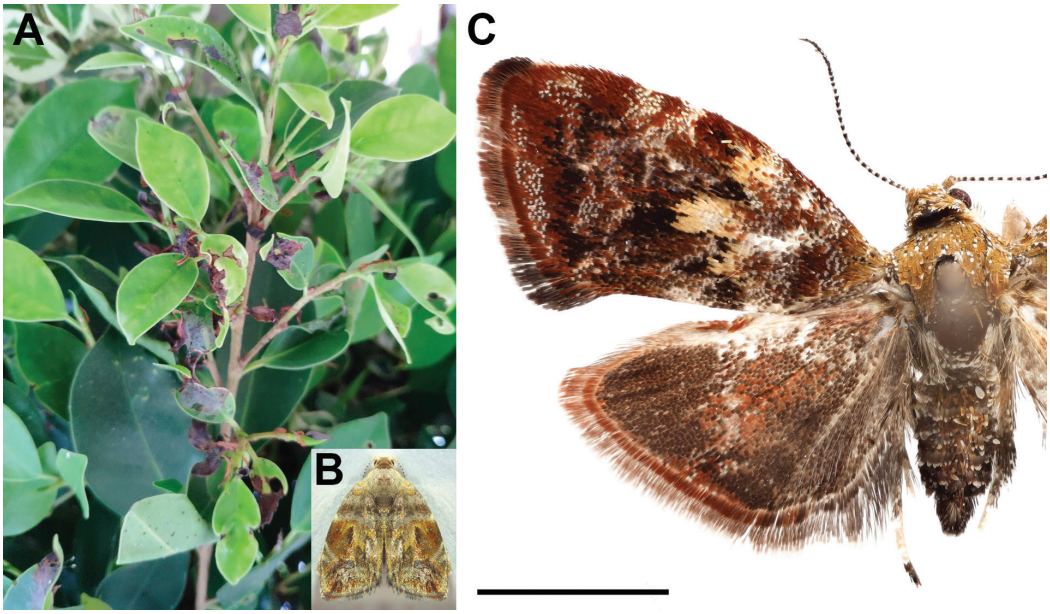

Figure 1. Observed signs of infestation by *Choreutis sexfasciella* (Sauber, 1902) on *Ficus microcarpa* L. (**A**); imago collected from Paphos, Chloraka, Melanos (**B**); pinned imago (♀) deposited at Lund University, MZLU-00208361 (**C**).

In this study, *C. sexfasciella* is recorded for the first time in Cyprus, where it is damaging ornamental *F. microcarpa* foliage in urban habitats of Larnaca, Limassol, and Paphos districts. A parasitoid, *Elasmus* cf. *cyprianus* (Fig. 2), was reared from *C. sexfasciella* larvae, constituting the first known parasitoid for the species. We sequenced one mitochondrial (COI-5P – the DNA barcode) and one nuclear gene from several specimens and combined our sequences with the publicly available data for a phylogenetic analysis. We briefly discuss the relationship of the Cyprus population of *C. sexfasciella* to the other sequenced populations of this species, test the validity of its synonymy with *C. emplecta*, and elucidate the taxonomic placement of *C. sexfasciella*. The impacts of this non-native moth on *Ficus* spp. in Cyprus, as well as its biological control are also discussed.

Materials and methods

Material examined

Choreutis sexfasciella (Sauber, 1902)

2 ex. Pafos (Paphos), Chloraka, Melanos (34.7915°N, 32.4070°E), 15.vii.2021, alt. 74 m, coll. J. Demetriou, reared from larvae collected on *F. microcarpa*, in house garden; 9 ex. Pafos (Paphos), Chloraka, Melanos (34.7915°N, 32.4070°E), 12.ix.2021, alt. 74 m, coll. J. Demetriou, collected on *F. microcarpa* foliage, in house garden; 2 ex. Pafos (Paphos), Chloraka (34.8048°N, 32.4027°E), 12.ix.2021, alt. 60 m, coll. J. Demetriou, collected on kumquat and basil foliage, in house garden; 1 ex. Pafos (Paphos), Chloraka, Melanos (34.7915°N, 32.4070°E), 02.x.2021, alt. 74 m, coll. J. Demetriou, collected on *F. microcarpa*, in house garden (stored in 96% EtOH); 3 ex. Pafos (Paphos), Panagias Theoskepastis Gymnasium environs

(34.7762°N, 32.4102°E), 26.ix.2022, alt. 25 m, coll. J. Demetriou, larvae collected feeding on ornamental *F. benjamina* foliage near roadsides. Specimens reared to adults; 3 ex. Lemesos (Limassol), Kato Polemidia (34.6830°N, 33.0060°E), 21.vii.2021, alt. 40 m, coll. J. Demetriou, reared from larvae collected on *F. microcarpa*, in house gardens; 4 ex. Lemesos (Limassol), Kato Polemidia (34.6830°N, 33.0060°E), 06.x.2021, alt. 40 m, coll. J. Demetriou, collected on *F. microcarpa*, in house gardens (two stored in 96% EtOH); 2 ex. Larnaka (Larnaca), Alethriko (34.8653°N, 33.4939°E), 03.x.2021. alt. 125 m, leg. M. Aristophanous, in garden, at light; 1 ex. Larnaka (Larnaca), Alethriko (34.8653°N, 33.4939°E), 14.x.2022. alt. 125 m, leg. M. Aristophanous, in garden, at light; 3 ex. Pafos (Paphos), Pafos, close to Tombs of the Kings (34.7761°N, 32.4100°E), 11.xii.2022, alt. 25 m, coll. J. Demetriou, reared from larvae collected on *F. benjamina*, in house garden.

Eleven of these specimens from various localities were sequenced; voucher information is presented in Suppl. materials 1, 2, and in the BOLD database as DS_CHSEX dataset at 10.5883/DS-CHSEX. These 11 specimens are deposited in the Entomology Collection, Biological Museum, Lund University, Lund, Sweden (MZLU).

Elasmus cf. *cyprianus* Ferriere, 1947

2 ♀. Lemesos (Limassol), Kato Polemidia (34.683°N, 33.006°E), 21.vii.2021, alt. 40 m, coll. J. Demetriou, det. R. R. Askew and E. Koutsoukos, reared from *C. sexfasciella* larvae collected on *F. microcarpa*, in house gardens.

Species mapping

A map depicting the distribution of *C. sexfasciella* in the Mediterranean basin and its collection sites in Cyprus was created in QGIS (Fig. 3).

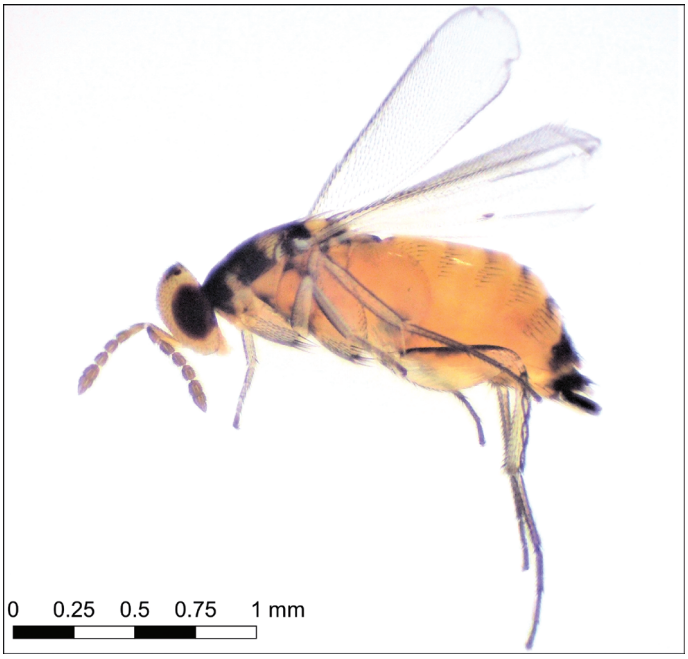

Figure 2. *Elasmus* cf. *cyprianus* Ferrière, 1947, adult female reared in Limassol, Kato Polemidia from pupae of *Choreutis sexfasciella* (Sauber, 1902).

Figure 3. Distribution of *Choreutis sexfasciella* (Sauber, 1902) in the Mediterranean Basin. Literature records and examined material are shown in points. Inset: localities in Cyprus where specimens were collected and reared.

Laboratory work and phylogenetic analyses

The molecular phylogeny dataset included two outgroups from the choreutid genus *Brenthia* Clemens, 1860, representatives of several genera in addition to *Choreutis* from the subfamily Choreutinae (*Anthophila* Haworth, [1811], *Caloreas* Heppner, 1977, *Niveas* Rota, 2013, *Prochoreutis* Heppner, 1981, *Tebenna* Billberg, 1820), two additional species of *Choreutis* [C. *nemorana* and *C. pariana* (Clerck, 1759)] and 24 specimens of *C. sexfasciella*. Eleven of these were from Cyprus and were newly sequenced for this project, while the rest are from BOLD (Ratnasingham and Hebert 2007), from the following countries: Australia (8 specimens), Papua New Guinea (2), and the USA (3) (Suppl. material 1). DNA extraction was done from one or two legs or from abdomens. We used the DNA, NucleoSpin® Tissue Kit (MACHEREY-NAGEL, Düren, Germany) for DNA extraction. We followed the laboratory protocols described by Wahlberg and Wheat (2008) for sequencing cytochrome oxidase I (COI, mitochondrial; Simon et al. 1994) and wingless (nuclear gene; Brower and DeSalle, 1998). We visualized the PCR products on an agarose gel with electrophoresis and selected the PCR products showing a single band for cleaning with Exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (ThermoFisher Scientific, Waltham, MA, USA). We used Macrogen Europe (Amsterdam, Netherlands) for Sanger sequencing. Taking into account the genetic codes and reading frames, alignments for each gene were checked by eye against a reference sequence from another *Choreutis* in the VoSeq database (Peña and Malm 2012). The remaining sequences are from earlier choreutid phylogenies or other studies (Mutanen et al. 2010; Rota 2011; Rota and Wahlberg

2012; Regier et al. 2013; Rota and Miller 2013; Rota et al. 2016; Falck et al. 2020) or from public databases, such as GenBank and BOLD. The final dataset consisted of the following eight gene fragments: COI (mitochondrial; Simon et al. 1994), CAD (Wiegmann et al. 2000), EF1-alpha (Cho et al. 1995), GAPDH, IDH, MDH, RpS5 (Wahlberg & Wheat, 2008), and wingless (Brower & DeSalle, 1998) (all seven nuclear). All accession numbers are listed in Suppl. material 1. The final alignment was 6401 base pairs long. We used the VoSeq application (Peña and Malm 2012) for storage of sequences and creation of datasets. The datasets can be accessed at 10.5281/zenodo.14264419. For the newly sequenced *Choreutis sexfasciella* specimens from Cyprus, we created a BOLD dataset DS-CHSEX that is also publicly accessible at 10.5883/DS-CHSEX.

We analysed two datasets, one with COI sequences with the focus on the populations of *Choreutis sexfasciella* only (*C. sexfasciella* dataset) and one with the addition of the so-called legacy genes (Wahlberg and Wheat 2008) to test the placement of the species within the genus *Choreutis* (Choreutinae dataset) as well as the designation of *C. emplecta* as the junior synonym of *C. sexfasciella* (Suppl. material 1). The *C. sexfasciella* dataset included 11 specimens of *C. sexfasciella* from Cyprus, eight from Australia, two from Papua New Guinea, and three from the USA. The Choreutinae dataset included the type species for genera *Anthophila* and *Choreutis*. We analysed both datasets with IQ-Tree 2.2.0 (Nguyen et al. 2015) implemented on a local server, using ultrafast bootstrap (UFB) approximation to examine branch support (Minh et al. 2013), as well as SH-like approximate likelihood ratio test (SH) (Guindon et al. 2010), both with 1000 replicates. We followed the recommendation in the IQ-Tree tutorial (Minh et al. 2022) and considered a clade well-supported with SH ≥ 80 and UFB ≥ 95. We partitioned the data by gene and codon position for the Choreutinae dataset and we used IQ-Tree (-mTEST) to find the best-fit model for each partition using the ModelFinder (Kalyaanamoorthy et al. 2017). We chose to allow different rates for partitions (--p), choosing the option TESTNEWMERGE to merge similar partitions thereby reducing over-parameterization using the greedy algorithm of the PartitionFinder (Lanfear et al. 2012). Trees were visualised using FigTree v1.4.4 (Rambaut 2010) and Adobe Illustrator. The *C. sexfasciella* dataset was rooted with *Alasea*, one of the choreutine genera closely related to *Choreutis*, and the Choreutinae dataset was rooted with *Brenthia*, a genus belonging to the choreutid subfamily Brenthiinae.

Results

During sampling for material in urban areas of Larnaca, Limassol, and Paphos districts in July and August 2021, damaged and withered leaves of *F. microcarpa* and *F. benjamina* were observed being covered in small, dark-coloured excrement and silk-web (Fig. 1A). *Choreutis sexfasciella* larvae were observed resting under silk-webs on the adaxial leaf lamina. Withered leaves with excrement and web evidenced the presence of the larvae, while thick silken cocoons located on the midrib indicated the presence of developing pupae (Abu Ghonem et al. 2020). Adult individuals were sparsely observed on the foliage during the evening hours before nightfall. Infested and healthy fresh leaves were collected and stored in plastic containers until the emergence of imagines (Fig. 1B, C). Imagines were preliminarily identified based on photographic material and species diagnoses in Rittner (2019) and Abu Ghonem et al. (2020), with their identity being molecularly confirmed. Two specimens of *Elasmus* sp. were also reared from pupae in Limassol district and identified as *E.* cf. *cyprianus* (Graham 1995) (Fig. 2).

We sequenced the mitochondrial COI and the wingless gene from all 11 specimens. Based on the analysis of the DNA barcode sequences from these 11 specimens and the additional 13 specimens from BOLD, the specimens of *C. sexfasciella* from Cyprus are closely related to the specimens from the USA (Fig. 4). All of the haplotypes from Cyprus are grouped together, suggesting that there was likely a single introduction event, and they are not showing any population structure with respect to the different regions of Cyprus. The specimens from Australia, which were previously considered as *C. emplecta*, show less than 2% divergence from the rest of the specimens from different parts of the world. In the analysis of the Choreutinae dataset, *C. sexfasciella* is deeply nested within *Choreutis*, with full branch support from both SH and UFB (Fig. 5).

Discussion

Choreutis sexfasciella is reported for the first time from Cyprus, supplementing monitoring efforts on the non-native insects associated with ornamental *Ficus* spp. on the island, as well as adding a second representative of the family Choreutidae to the entomofauna of Cyprus. By the end of 2024, a total of 30 non-native species of Lepidoptera had been reported from Cyprus [excluding *Dichelia cedricola* (Diakonoff, 1974), a species of questionable status] (Demetriou et al. 2023b). Since then, two more species have been added to this list, including the presented record of *C. sexfasciella* as well as the fall armyworm, *Spodoptera frugiperda* (Smith, 1797), a species included in the EPPO A2 list (EPPO 2023a, b). Rittner (2019) hypothesised that *C. sexfasciella* may have been introduced to Israel through the import of Asian *Ficus* spp. The species has probably been introduced to Cyprus as a contaminant of nursery material and/or as contaminant on infested alien fig trees (*Ficus* spp.) [see introduction pathways of alien species UNEP/CBD/SBSTTA/18/9/Add.1, 26 June 2014, as modified according to Pergl et al. (2020)], from neighbouring Egypt, Israel or straight from the species native range.

A molecular analysis of the Cyprus populations of *C. sexfasciella*, together with the American, Australian, and Papua New Guinean populations, has shed some light on the invasion history of the species, demonstrating that populations in Europe and North America are closely related. However, questions remain. For example, was the introduction of *C. sexfasciella* into the Mediterranean a single event that has led to a rapid spread of this species since its hosts are widely grown in the region as ornamentals, or were there a number of separate introductions into different areas through trade in ornamental plants? A far greater sequencing effort, covering the full range, is needed to try to answer these questions. Also, so far, no specimens of *C. sexfasciella* have been sequenced from the Philippines – the species' type locality.

Our results support Heppner's (2023) decision to synonymize *C. emplecta* with *C. sexfasciella*. The divergence in the COI sequences is below 2% and there is no clear separation between the Australian specimens and those from the other regions. Likewise, *C. sexfasciella* is firmly placed within the genus *Choreutis* based on the mitochondrial and nuclear genes, as its morphology clearly suggests.

Larval stages of *C. sexfasciella* were responsible for damaging stands of ornamental plants (Fig. 1A) undermining the aesthetics of nature (Kueffer and Kull 2017) and thus indicating some potential socioeconomic impacts of the species. Nevertheless, the extent of the observed damage to the host-plant did not seem to significantly affect the survival of the plants. Despite being commonly used as an ornamental plant, *F. microcarpa* has escaped urban areas, reaching natural habitats in the Mediterranean basin (Wang et al. 2015b). In invaded habitats, it has also been associated with

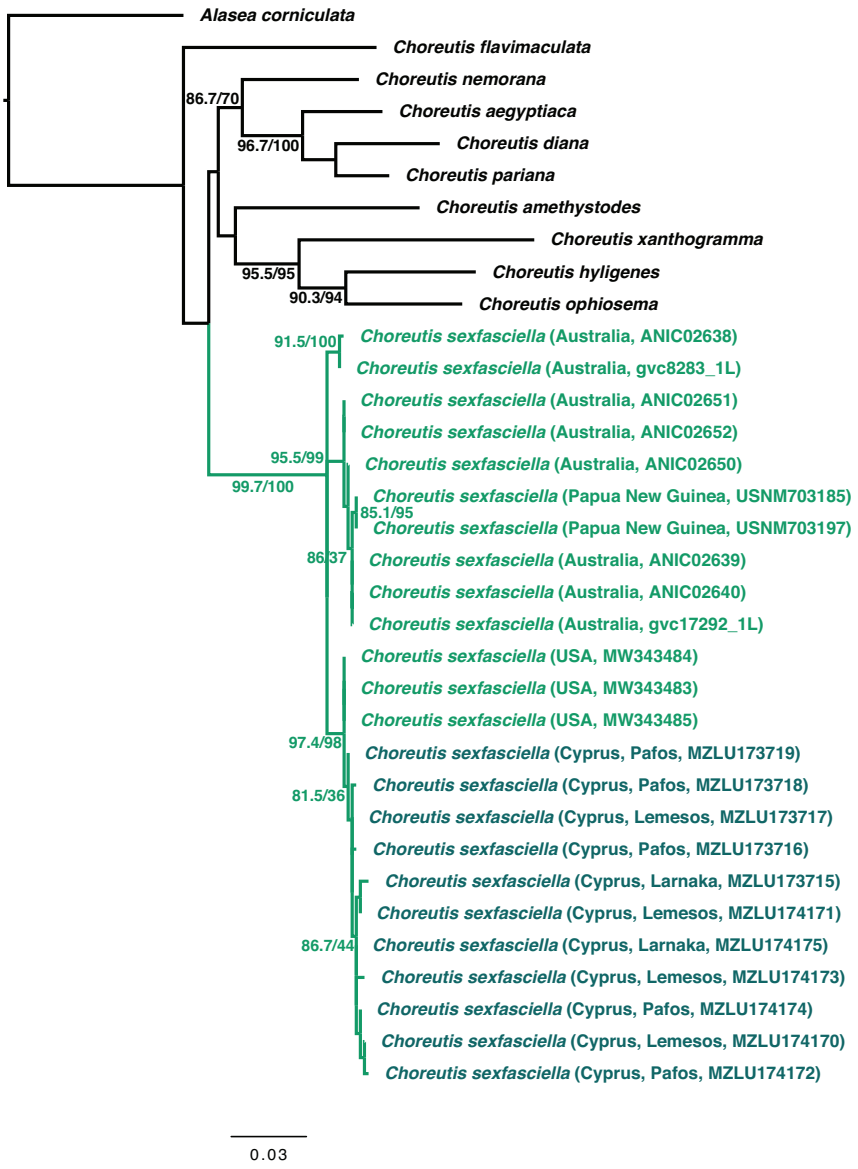

Figure 4. Maximum likelihood tree from the *Choreutis sexfasciella* (Sauber, 1902) dataset based on the COI sequences. The numbers next to the branches represent branch support as SH-like approximate likelihood ratio test / ultrafast bootstrap. When branch support by both measures is below the level of statistical significance, it is not shown.

the destruction of infrastructure and replacement of native flora (Ramírez and Montero 1988; Starr et al. 2003; Wang et al. 2015a), although such outcomes have not been reported from Mediterranean countries. Tsintides et al. (2002) and Demetriou et al. (2023a), document socioeconomic and human-health risks linked to *F. microcarpa* in Cyprus, including slippery sidewalks and road crossings due to fallen figs as well as damage to cars and pavements.

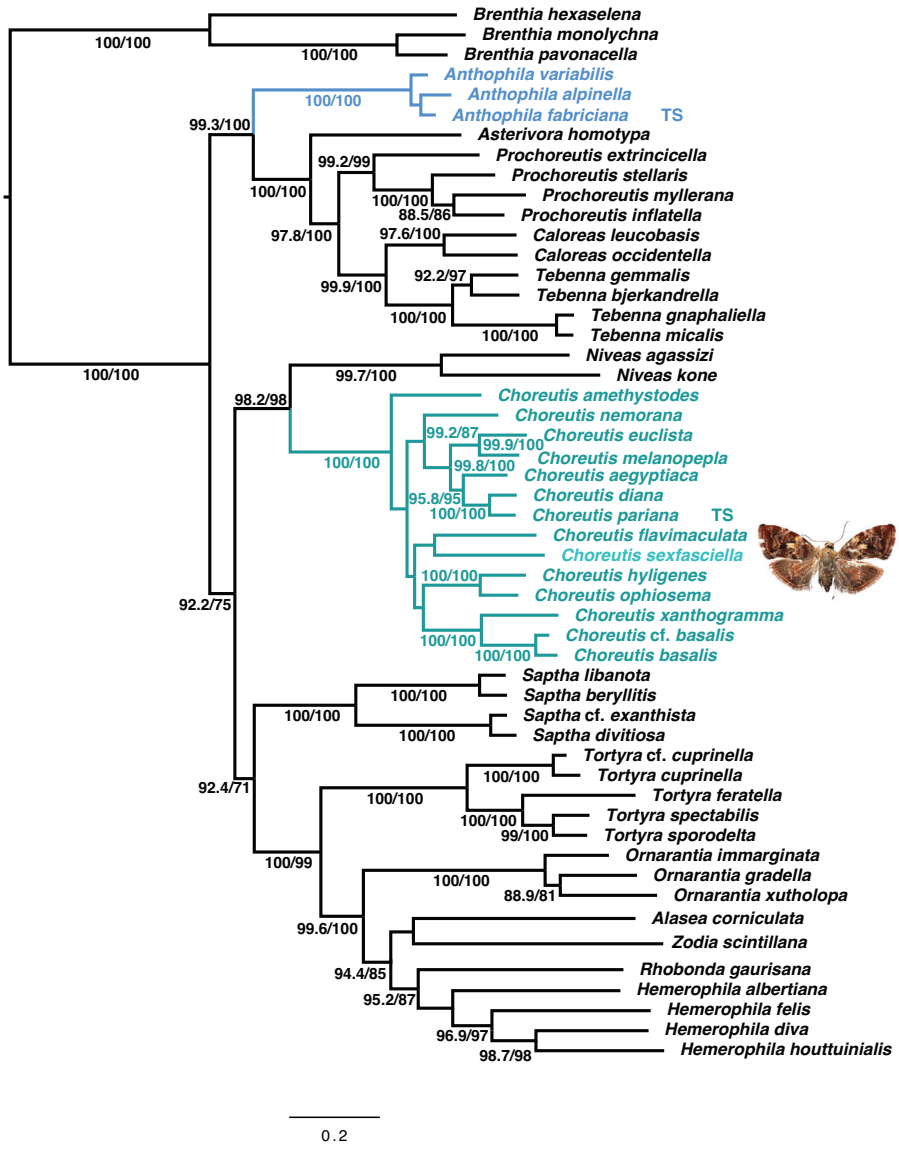

Figure 5. Maximum likelihood tree from the Choreutinae dataset based on DNA sequences from COI and seven nuclear genes (Suppl. material 1). The numbers next to the branches represent branch support as SH-like approximate likelihood ratio test / ultrafast bootstrap. When branch support by both measures is below the level of statistical significance, it is not shown. Type species for genera *Anthophila* and *Choreutis* are marked with TS.

Elasmus cyprianus was first described from the island by Ferrière (1947), "breeding in young green carob pods" (Graham 1995). Its host species are unknown and presumably belong to some lepidopteran developing in pods of *Ceratonia siliqua* L. (Graham 1995). Additionally, male individuals of the species have not been described (Ferrière 1947; Graham 1995). The identification of reared samples was performed using the identification key of Graham (1995) and compared with

the re-description of morphologically similar species *E. phthorimaeae* (Strakhova and Yefremova 2010). The reared specimens resemble those of *E. cyprianus*, although comparison with type material is required (R.R. Askew, pers. comm.). Additional examination of the material using keys for Asian *Elasmus* provided no results (R.R. Askew, pers. comm.). As such, our samples are identified as *E.* cf. *cyprianus* (pedicel longer than F1; F3 approx. 1.3 times as long as broad) unveiling a new, non-native host for the European *Elasmus* species.

Future research on *C. sexfasciella* in Cyprus should be carried out in order to map the extent of its distribution, determine other possible host-plants and identify any further adverse impacts. The potential use of *E.* cf. *cyprianus* for the biological control of *C. sexfasciella* could also be assessed by measuring its parasitism rate and seasonal dynamics. Rearing of specimens may provide us with male samples, which are currently undescribed, while the comparison of reared specimens with type material will be conducted once additional individuals are collected.

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Supplementary material 1

Voucher data and accession numbers for sequenced specimens

Authors: Jakovos Demetriou, Marios Aristophanous, Evangelos Koutsoukos, Eddie John, Helen E. Roy, Angeliki F. Martinou, Jadranka Rota

Data type: xlsx

Explanatory note: The accession numbers are either from GenBank or are BOLD ProcessID numbers.

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Link: https://doi.org/10.3897/nl.48.137778.suppl1

Supplementary material 2

Voucher data for the specimens deposited at the Biological Museum, Lund University, including the 11 sequenced specimens

Authors: Jakovos Demetriou, Marios Aristophanous, Evangelos Koutsoukos, Eddie John, Helen E. Roy, Angeliki F. Martinou, Jadranka Rota

Data type: xlsx

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