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INSECTS ASSOCIATED WITH THE EUROPEAN MISTLETOE (*VISCUM ALBUM*) IN WESTERN PART OF UKRAINE: A PILOT STUDY

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Insects Associated with the European Mistletoe (*Viscum album*) in Western Ukraine: a Pilot Study. Zamoroka, A. M., Shparyk, V. Yu., Dovhanyuk, I. Ya. & Varga, O. O. — The first insect-targeted study of the European mistletoe, *Viscum album* L., was conducted in Ukraine. In total, 35 species of insects reared from mistletoe were identified to belong to 5 orders, 20 families and 34 genera. Twenty-seven identified species were reared from mistletoe for the first time, including 17 species of Hymenoptera, 8 species of Coleoptera and 2 species of Diptera. Our results revealed significant gaps in knowledge about insects associated with mistletoe. This is appeared in the fact that more than 77 % of the insects we reared have never been detected on mistletoe before. It is obviously that 70 % to 90 % of the insects associated with mistletoe remain unknown. We also found a highly probable tripartite link between the European mistletoe, parasitic fungus *Sphaeropsis visci* and the saproxylic and mycetophagous Coleoptera. The interaction of Coleoptera with *S. visci* is facultatively mutualistic, and beetles act as vectors to spread spores and mycelium among mistletoes. In addition, we found the mass breading of invasive species *Leiopus femoratus* on *V. album*. Hence, *V. album* might play a crucial role in the mass and rapid invasion of *L. femoratus* in Europe.
Key words: *Viscum album*, insects, saproxylic beetles, parasitoids, aphidophagous Diptera, invasive species, insect-plant interaction, parasitism, mutualism.

Introduction

The European mistletoe (*Viscum album* L.) is a hemiparasitic plant of arboreal species that alters host physiology affecting the ecosystem services (Watson, 2001; Mathiasen et al., 2008). Mistletoe attracts attention of wide range of experts in biology and ecology, including entomologists. However, arthropods associated with mistletoe remained scarcely studied.

The interaction between white or European mistletoe and its host plants is fairly well studied, but mistletoe as an arthropod host is still poorly understood (Krasylenko et al., 2020). Since the beginning of the 20th century, the number of known species of herbivorous insects associated with mistletoe has gradually increased. Schumacher (1918) presented the first data on insects feeding and living on *V. album* and listed 21 insect species, 6 of which he proposed as mistletoe specialists. The remaining 15 species were recognised as secondary migrants. Schumacher's data were duplicated in Tubeuf (1923) monograph on mistletoe.

Throughout the 20th century, there were no specific studies of insect groups on mistletoe, and very little information on the development of insects on mistletoe in the publications of the time. Horion (1935) reported the rearing of the beetle *Stenostola ferrea* (Schrank, 1776) (Cerambycidae) on mistletoe. Duffy (1953) listed European mistletoe as a food plant for cerambycid species in Great Britain, including *Pogonocherus hispidus* (Linnaeus, 1758) and *Pogonocherus hispidulus* (Piller & Mitterpacher, 1783). Gutowski (1995) has repeated the same data.

At the beginning of the 21st century, mistletoe and associated insects again attracted the attention of scientists. Hellrigl (2006) published a paper on insects living on mistletoe in South Tirol. He summarized data from different sources and his own study presenting the list of 21 species of insects. He suggested 8 species as mistletoe specialists and 13 as secondary migrants. Later, Varga et al. (2012) presented very similar data from Hungary. Lazaro-Gonzalez et al. (2017) conducted a comparative study of insect communities on European mistletoe and its host plants, black pine (*Pinus nigra*) and Scots pine (*P. sylvestris*). Although their research is important for understanding the ecological heterogeneity of ecosystems and biodiversity, unfortunately most of the insects they collected on European mistletoe were only identified by order and family. The only exception was the well-known specialists on *V. album*, in particular *Cacopsylla visci*, *Pinalitus viscicola*, *Anthocoris visci*. Several partial studies of the recent decades (Hansen & Hodkinson, 2006; Struwe et al., 2009; Krasylenko et al., 2023), that non-targeted on the viscum insect communities (hereinafter — non-target studies), provided the same data as the authors mentioned above.

In this insect-focused study, we present the most comprehensive data on mistletoe-associated insects from Ukraine, significantly expanding knowledge on the subject. We reared and identified 35 insect species from *V. album*, 27 of them for the first time. *Viscum album* was identified as a new food plant for four insect species.

Material and Methods

The whole clumps of mistletoe (n = 15) were sampled within the Central Ciscarpathia (Ivano-Frankivsk Region, Ukraine) in four localities namely: Ivano-Frankivsk (48.911879, 24.684231), Sokil (49.097852, 24.625574), Maydan (49.007488, 24.578528), Tsenzhiv (48.995938, 24.599763). Each locality represents four types of ecosystems with different degree of anthropogenic transformation and mistletoe spreading. The first type was a park orchard with various tree species in the city of Ivano-Frankivsk (257 m a. s. l.). The second type was an artificial lime hedgerow with various shrubs along H10 highway in the Maydan locality (329 m a. s. l.). The third type was an apple and pear orchard in the Sokil locality (236 m a. s. l.). The fourth type was the natural oak-hornbeam forest in the Tsenzhiv locality (377 m a. s. l.). Samples were obtained from four host tree species namely small-leaved lime (*Tilia cordata* Mill.), apple (*Malus domestica* Borkh.), sycamore maple (*Acer pseudoplatanus* L.) and Canadian poplar (*Populus×canadensis* Moench). Twelve of fifteen sampled *V. album* were infected by parasitic fungus *Sphaeropsis visci*.

The mistletoe samples were cut with part of the host branch and placed in a plastic zip-lock bag for transport to the laboratory, where they were placed in closed plastic containers. The samples collected in October were kept at 4 °C for 14 days and at 24 °C for the following 50 days. The first adult parasitoid wasps were reared on the 60th day, and saproxylic beetles appeared on the 78th day after collecting mistletoe in the field. The mistletoe collected in February was kept at 24 °C for 14 days before the first insects were reared.

The reared insects were identified at least to genus level and stored in 96 % ethyl alcohol in labelled plastic tubes. All material was collected by the authors and deposited in the collection of the Department of Biology and Ecology, Vasyl Stefanyk Precarpathian National University (PUIF).

Additionally, to reveal the nature of the xylobiont insects galleries within the plants tissues, we manually made longitudinal sections of the mistletoe stems at the point of its connection with the branch of the host plant. The photographs were taken using Nikon D90 camera. Images were then aligned and stacked in theDLT-CamViewer x86, 3.7.7892 software package and additionally.

Results

Resultantly, we identified 36 species of insects reared from mistletoe, which belong to 5 orders (Coleoptera — 12 species, Hemiptera — 2 species, Hymenoptera — 18 species, Lepidoptera — 1 species, Diptera — 3 species), 20 families and 34 genera. Families and genera are presented in alphabetic order in the annotated list below.

Coleoptera
Anthribidae
Rhaphitropis marchica (Herbst, 1797)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 269 specimens, ex larva; Maydan, 28.02.2014, 10 specimens, ex larva; Ivano-Frankivsk, 16.02.2014, 262 specimens, ex larva (A. Zamoroka) (PUIF).

Biology. Polyphagous on Sordariomycetes (Ascomycota) fungi associated with dead wood of deciduous trees (Yunakov et al., 2018). We reared numerous specimens of *R. marchica* from *V. album* heavily infected with the fungus *Sphaeropsis visci* (Sollm.) Sacc. (Dothideomycetes).

Cerambycidae
Exocentrus lusitanus (Linnaeus, 1767)

Reared material. Ukraine, Ivano-Frankivsk Region: Maydan, 28.02.2014, 7 specimens, ex larva; Tsenzhiv, 28.02.2014, 8 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are polyphagous on deciduous trees with a preference for *Tilia* spp.; *V. album* is a new and previously unknown food plant for *E. lusitanus*. Adults feed on thin twigs and probably fruit bodies of saproxylic fungi (Zamoroka & Kapeliukh, 2016).

Leiopus femoratus Fairmaire, 1859

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 100 specimens, ex larva, Maydan, 28.02.2014, 29 specimens, ex larva; Ivano-Frankivsk, 16.02.2014, 262 specimens, ex larva; Tsenzhiv, 28.02.2014, 14 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. An invasive Mediterranean species that is expanding its range northwards (Zamoroka & Kapeliukh, 2012; Zamoroka, 2022). The larvae are polyphagous on deciduous trees. *Viscum album* is a new and previously unknown food plant for *L. femoratus*. Adults probably feed on phloem of twigs and thin branches (Zamoroka & Kapeliukh, 2016).

Mesosa curculionoides (Linnaeus, 1761)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 2 specimens, ex larva; Maydan, 28.02.2014, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are polyphagous on deciduous trees. There are known observations of *M. curculinoides* adults feeding on fruit bodies of the saproxylic fungus *Schizophyllum commune* Fr. (Zamoroka & Kapeliukh, 2016).

Oplosia cinerea (Mulsant, 1839)

Reared material. Ukraine, Ivano-Frankivsk Region: Maydan, 28.02.2014, 1 specimen, ex larva; Ivano-Frankivsk, 16.02.2014, 2 specimens, ex larva (A. Zamoroka) (PUIF).

Biology. Larvae are polyphagous on deciduous trees; *V. album* is a new food plant for *O. cinerea*, which was unknown previously.

Pogonocherus hispidus (Linnaeus, 1758)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 17 specimens, ex larva; Maydan, 28.02.2014, 1 specimen, ex larva; Ivano-Frankivsk, 16.02.2014, 14 specimens, ex larva; Tsenzhiv, 28.02.2014, 2 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are polyphagous on deciduous trees, especially on *Tilia*. The feeding of adults on fruit bodies of the saproxylic fungus *Nectria* sp. is known (Zamoroka & Kapeliukh, 2016).

Stenostola ferrea (Schrank, 1776)

Reared material. Ukraine, Ivano-Frankivsk Region: Maydan, 28.02.2014, 3 specimens, ex larva; Tsenzhiv, 28.02.2014, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are polyphagous on deciduous trees with preferences on *Tilia* spp. (Zamoroka & Kapeliukh, 2016).

Chrysomelidae

***Longitarsus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 1 specimen, ex larva (A. Zamoroka) (PUIF).

Biology. Larvae develop mainly on herbaceous plants. Our record of *Longitarsus* sp. on *V. album* is probably occasional.

Ciidae

Orthocis alni (Gyllenhal, 1813)

Reared material. Ukraine, Ivano-Frankivsk Region: Tsenzhiv, 28.02.2014, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae and adults of *O. alni* are associated with the fruiting bodies of a wide range of saproxylic fungi, mostly Polyporaceae but also Corticiaceae and others (Alexander, 2002). The species has not previously been seen on *V. album*. We reared the only specimen on a twig of *V. album* infected by *S. visci*.

Cucujidae

Lathropus sepicola (P. W. J. Müller, 1821)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae of *Lathropus sepicola* feed on saproxylic fungi (Uliana, 2003). The species was first reared from *V. album* heavily infected by *S. visci*.

Curculionidae
Ernoporus tiliae (Panzer, 1793)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 134 specimens, ex larva, (A. Zamoroka & V. Shparyk) (PUIF); Ukraine, Ivano-Frankivsk Region: Maydan, 28.02.2014, 6 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae feed under the bark of *Tilia* spp. (Alexander, 2002). It was also reported in association with lime wood infected by the parasitic fungus *Geosmithia* sp. (Kubátová et al., 2004). Our records of *E. tiliae* on *V. album* infected by *S. visci* were the first evidence of the association of these three species.

Trogossitidae
Nemozoma elongatum (Linnaeus, 1761)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 4 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae and adults are predators and feed on the eggs of bark beetles as well as on larvae of wood-boring beetles and other insects (Miłkowski et al., 2019). We first spotted *N. elongatum* on *V. album* inhabited by *E. tiliae*, *Rh. marchica*, *L. femoratus* and others.

Hemiptera
Aphididae
Aphis fabae Scopoli, 1763

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 4 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Polyphagous on various angiosperms (Wieczorek et al., 2019).

Psyllidae
Cacopsylla visci (Curtis, 1835)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 62 specimens, ex larva; Maydan, 28.02.2014, 24 specimens, ex larva; Ivano-Frankivsk, 16.02.2014, 131 specimens, ex larva; Tsenzhiv, 28.02.2014, 10 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Monophagous specialist on *V. album* (Schumacher, 1918; Hellrigl, 2006).

Hymenoptera
Braconidae
***Eubazus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are the primary koinobiont endoparasitoids of Curculionidae eggs and larvae (Alauzet, 1987). *Rhaphitropis marchica* is likely a possible host of *Eubazus* sp. reared during this study. The association of *Eubazus* sp. with insect communities on *V. album* was previously unknown.

***Cyanopterus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae of wasps of the genus *Cyanopterus* Haliday, 1835 are primary ectoparasitoids of Cerambycidae larvae (Tobias, 1978). The association of *Cyanopterus* sp. with insect communities on *V. album* was previously unknown.

***Spathius* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 1 specimen, ex larva (A. Zamoroka) (PUIF).

Biology. Larvae of *Spathius* sp. are primary parasitoids of Cerambycidae and Curculionidae larvae, including *E. tiliae*. The association of *Spathius* sp. with insect communities of *V. album* was previously unknown.

Cynipidae
***Phanacis* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 1 specimen, ex larva (A. Zamoroka) (PUIF).

Biology. Neither *Phanacis* sp. nor Aylacini were previously known to be trophically associated with the mistletoe. Here, we report the association of cynipids with *V. album* for the first time.

Colletidae
Hylaeus confusus Nylander, 1852

Reared material. Ukraine, Ivano-Frankivsk Region: Tsenzhiv, 28.02.2014, 4 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. *Hylaeus confusus* is a solitary bee species nesting in the xylophagous beetles' galleries. Here, we firstly reported nesting of *H. confusus* on *V. album*.

Eulophidae
***Aprostocetus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 5 specimens, ex larva; Maydan, 28.02.2014, 2 specimens, ex larva; Ivano-Frankivsk, 16.02.2014, 8 specimens, ex larva (A. Zamoroka) (PUIF).

Biology. The species of the genus are ectoparasitoids parasitising eggs of a large of the variety of such insects as Hemiptera, Lepidoptera, Orthoptera, Odonata, and Coleoptera (Graham, 1987; Hérard et al., 2005). The association of *Aprostocetus* sp. with insect communities on *V. album* was previously unknown.

Sympiesis gordius (Walker, 1839)

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 2 specimens, ex larva (A. Zamoroka) (PUIF).

Biology. Larvae are ectoparasitoids of leafrollers larvae (Lepidoptera) (Yefremova, 1997). It is likely that *S. s gordius* parasitises *C. woodiana* larvae, the *V. album* monophagous specialist. The association of *S. gordius* with insect communities on *V. album* is described for the first time.

Eupelmidae
Eupelmus pini Taylor, 1927

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 1 specimen, ex larva (A. Zamoroka) (PUIF).

Biology. *Eupelmus pini* is a primary parasitoid on wide range of xylophagous Coleoptera larvae (e. g., Scolitinae, Curculionidae, Cerambycidae) (Fusu, 2009; Gibson & Fusu, 2016). It is also suggested as hyperparasitoid through Braconidae (Gibson & Fusu, 2016). The association of *E. pini* with insect communities on *V. album* was previously unknown.

Eusandalum inerme (Ratzeburg, 1848)

Reared material. Ukraine, Ivano-Frankivsk Region: Tsenzhiv, 28.02.2014, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. *Eusandalum inerme* is a primary parasitoid on wide range of xylophagous Coleoptera larvae (e. g., Anobiidae, Bostrichidae, Buprestidae, Cerambycidae, Lucanidae, Lyctidae, Scolytinae) (Bouček, 1967; Noyes, 2018). The association of *E. inerme* with insect communities on *V. album* was previously unknown.

Eurytomidae
Eurytoma tilicola Hedquist, 1966

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 10 specimens, ex larva; Maydan, 28.02.2014, 1 specimen, ex larva; Ivano-Frankivsk, 16.02.2014, 11 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are the primary ectoparasitoids of *S. ferrea* larvae (Zerova, 2010). The association of *E. tilicola* with insect communities on *V. album* was previously unknown.

Ichneumonidae
Dolichomitus agnoscendus (Roman, 1939)

Reared material. Ukraine, Ivano-Frankivsk Region: Tsenzhiv, 28.02.2014, 17 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are the primary idiobiont ectoparasitoids of wood-boring beetles, mainly on Cerambycidae (Varga, 2012, 2014). The association of *D. agnoscendus* with insect communities on *V. album* was previously unknown.

Platygastridae
***Synopeas* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Tsenzhiv, 28.02.2014, 6 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are solitary koinobiont endoparasitoids ovipositing inside the eggs or newly hatched larvae of gall midges (Diptera, Cecidomyiidae) (Abram et al., 2012). We reared several specimens of Cecidomyiid from the species *A. viscicola* the monophagous specialist of *V. album*. The association of *Synopeas* sp. with insect communities on *Viscum album* was previously unknown.

Pteromalidae

***Aggelma* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Unknown. *Aggelma* sp. might be a primary parasitoid of *E. tiliae*. The association of *Aggelma* sp. with insect communities on *V. album* was previously unknown.

Platygerrhus affinis (Walker, 1836)

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 26 specimens, ex larva (A. Zamoroka) (PUIF).

Biology. Larvae are primary parasitoids of wood-boring Coleoptera, in particular *S. ferrea* and *E. tiliae* (Mitroiu, 2005). The association of *P. affinis* with insect communities on *V. album* was previously unknown.

***Platygerrhus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Ivano-Frankivsk, 16.02.2014, 9 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are primary parasitoids of wood-boring Coleoptera (Mitroiu, 2005). The association of *Platygerrhus* sp. with insect communities on *V.album* was previously unknown.

***Anisopteromalus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 1 specimen, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. *Anisopteromalus* sp. is a primary parasitoid on beetles' larvae in stored products (e. g., cereals, etc.). Its development on *S. paniceum* (Linnaeus, 1758) larvae was reported by Baur et al. (2014). The association of *Anisopteromalus* sp. with xylophagous insects in general and insects developing on *V. album*, in particular, was documented for the first time.

Trigonoderus princeps Westwood, 1832

Reared material. Ukraine, Ivano-Frankivsk Region: Tsenzhiv, 28.02.2014, 2 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are solitary ectoparasitoids of Cerambycidae and Curculionidae (*Scolytus ratzeburgi* Janson, 1856) larvae (Hérard et al., 2005). The association of *T. princeps* with insect communities on *V. album* was previously unknown.

***Trigonoderus* sp.**

Reared material. Ukraine, Ivano-Frankivsk Region: Maydan, 28.02.2014, 2 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are solitary ectoparasitoids of Cerambycidae and Curculionidae. The association of *Trigonoderus* sp with insect communities on *V. album* was previously unknown.

Lepidoptera
Tortricidae
Celypha woodiana (Barrett, 1882)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 2 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Monophagous specialist on *V. album* (Schumacher, 1918; Hellrigl, 2006).

Diptera
Cecidomyiidae
Asynapta viscicola Skuhravá, 2007

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 7 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Monophagous specialist on *V. album* (Schumacher, 1918; Hellrigl, 2006).

Syrphidae
Scaeva pyrastri (Linnaeus, 1758)

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 2 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are typically aphidophagous predators. The association of *S. pyrastri* with aphidids feeding on *V. album* was observed for the first time.

Syrphus vitripennis Meigen, 1822

Reared material. Ukraine, Ivano-Frankivsk Region: Sokil, 13.10.2013, 2 specimens, ex larva (A. Zamoroka & V. Shparyk) (PUIF).

Biology. Larvae are typically aphidophagous predators. The association of *S. vitripennis* with aphidids feeding on *V. album* was recorded for the first time

Discussion

Our results confirmed the high diversity of insects associated with mistletoe and added to the list of known species. We significantly increased the number of known species of such insects from 49 to 77 species (13 new from Hymenoptera, 8 from Coleoptera and 2 from Diptera). A comparison of our results with those of previous researchers is shown in table 1.

Table 1. The data on mistletoe-associated insects (species number) from published and current studies

Taxa	Schumacher, 1918	Hellrigl, 2006	Varga et al., 2012	Current study	Non-target studies*	General diversity
Coleoptera	8	10	5	12	2	**27**
Hemiptera	11	5	9	2	5	**19**
Hymenoptera	0	3	4	17	1	**24**
Lepidoptera	2	2	2	1	0	**3**
Diptera	0	1	0	3	1	**4**
Total	**21**	**21**	**20**	**35**	**9**	**77**

*Hansen & Hodkinson, 2006 (1 species); Struwe et al., 2009 (2 species); Krasylenko et al., 2023 (9 species)

It is noteworthy that our results differ significantly from those of previous studies (table 2). In particular, our list of species reared from mistletoe only agrees by 7.2 % with the list published by Schumacher (1918), by 7.7 % with Varga et al. (2012) and by 13.1 % with Helrigl (2006). The non-target studies were not systematic in nature and are extremely difficult to compare. This is due to the very small number of insect species (1–9) included in these studies (Hansen & Hodkinson, 2006; Struwe et al., 2009; Krasylenko et al., 2023). Nevertheless, these studies are an important source for understanding the distribution of certain species, mostly mistletoe monophages, in Europe (Hansen & Hodkinson, 2006; Struwe et al., 2009). However, our analysis shows that the similarity of our results to non-target studies does not exceed 4.8 %. It should be noted that such a result is not statistically significant.

In general, the low similarity between our and published insect faunal lists can be explained by methodological differences in their collection. In particular, we reared insects from mistletoe during the winter diapause, while other studies mainly used the less effective method of shaking mistletoe to collect insects (Varga et al., 2012; Krasylenko et al., 2023), and partly the method of rearing insects from mistletoe clumps collected in summer (Varga et al., 2012). Our study shows that collecting mistletoe during the winter diapause of insects and their larvae gives better results for rearing parasitic wasps and saproxylic beetles. On the other hand, sampling mistletoe in summer gives better results for the collection of Hemiptera.

Our insect list (35 species) has only 8 species in common with all previously published data (42 species). This is 11.6 %. At the same time, we found a maximum similarity of 24.2 % between the insect lists of Varga et al. (2012) and Hellrigl (2006). We believe that such significant differences are due to an insufficient study of insects associated with mistletoe. Based on these data, we estimate that between 70 % and 90 % of the insect diversity associated with European mistletoe remains unknown. Further research will certainly reveal the considerable diversity and complexity of insect communities on European mistletoe.

Table 2. The percentage (%) variation of similarity between published and current studies

Study	Schumacher, 1918	Hellrigl, 2006	Varga et al., 2012	Current study
Schumacher, 1918	100	17.1	17.7	7.2
Hellrigl, 2006	17.1	100	24.2	13.1
Varga et al., 2012	17.7	24.2	100	7.7
Current study	7.2	13.1	7.7	100

We found only three insect species that are considered to be specialised monophages on *V. album*, namely *Celypha woodiana* (Lepidoptera), *Asynapta viscicola* (Diptera) and *Cacopsylla visci* (Hemiptera). The species we discovered, *Aphis fabae*, is polyphagous on a wide range of angiosperms. The species listed above were also discovered by previous researchers in Europe (Schumacher, 1918; Hellrigl, 2006; Hansen & Hodkinson, 2006; Struwe et al., 2009; Varga et al., 2012; Krasylenko et al., 2023). Of greatest interest, however, are species whose association with mistletoe was not previously known. In our study, these are two large groups of species representing the orders Coleoptera and Hymenoptera.

Among the 12 species of beetles reared from *V. album*, no oligophagous specialists were found, on the contrary, all of them are polyphagous. The rearing of three mycetophagous beetles such as *Lathropus sepicola*, *Orthocis alni* and *Rhaphitropis marchica* from mistletoe was unexpected. These beetles inhabit the fruiting bodies of saproxylic macromycetes (Ulyana, 2003; Yunakov et al., 2018), which were not found on *V. album*. The other seven species reared on European mistletoe were the primary xylophages. They include *Ernoporus tiliae*, *Exocentrus lusitanus*, *Leiopus femoratus*, *Mesosa curculionoides*, *Oplosia cinerea*, *Pogonocherus hispidus* and *Stenostola ferrea*. It is noteworthy that the larvae of these species are also partially related to wood-inhabiting fungi. Moreover, most of them cannot develop without the presence of fungal hyphae in the tissues of the host plant. Fungi play a very important role in the larval development and feeding of adults of many saproxylic insect species, and even in the evolution of their ability to decompose cellulose (Sasakura et al., 2022; Shin & Pauchet, 2023). In our study, the only fungal species we found was the parasitic fungus *S. visci*, of which numerous pycnidia were found on *V. album* specimens. In our opinion, it is *S. visci* that ensures the development of fungal-associated mycetophages and saproxylic beetles on European mistletoe. This suggests a link between the parasitic fungus *S. visci* and the beetles reared on *V. album* in this study.

Varga et al. (2012) discussed that the mechanism of infection of *V. album* by the parasitic fungus *S. visci* is not fully understood. They suggested that abiotic factors such as temperature and relative humidity play a significant role in this process. However, our observations indicate that the most likely mechanism of *V. album* infection is the transfer of spores and mycelia of *S. visci* by the vectors, which are the longhorn beetles (Cerambycidae), fungus weevils (Anthribidae), true weevils (Curculionidae) etc. The most of these beetles have special internal and external organs for the accumulation, transfer and distribution of spores and mycelium, called mycangia (Bartnik et al., 2001; Grebennikov & Leschen, 2010; Sasakura, 2022; Kishigami et al., 2023). Females in typical cases bring fungi culture during oviposition, which contributes to the formation of both the environment for the development of larvae and the dispersing of fungi (Kishigami et al., 2023). It is clear that *S. visci* spreads by this mechanism.

Sphaeropsis visci is certainly a source of fungal protein for the larvae, and probably also for the adults of mycetophagous and saproxylic beetles that we found. Thus, we consider *S. visci* to be a new, previously unknown food resource for mycetophagous and saproxylic beetles. For many saproxylic beetles, additional nutrition with the fruiting bodies of various fungi has been previously recorded (Adlbauer, 2004; Kubátová et al., 2004; Zamoroka & Kapeliukh, 2016). In particular, *Ernoporus tiliae* often inhabits linden wood affected by the parasitic fungus *Geosmithia* sp. (Kubátová et al., 2004). Adlbauer (2004) reported an additional feeding of *Exocentrus lusitanus* on the fruiting bodies of unidentified species of saproxylic fungi and *Mesosa curculionoides* on *Schizophyllum commune* and *Pogonocherus hispidus* on *Nectria* sp. We reared 405 imagines of *Leiopus femoratus* from mistletoe, whose additional feeding on fungi remains unknown. However, it was documented feeding of the imago of the closely related species of *Leiopus nebulosus* Linnaeus, 1758 on the saproxylic fungus *Diatrype bullata* (Hoffm.: Fr.) Tul. (Michalcewicz, 2002). Therefore, we assume that *Leiopus femoratus* can also additionally feed on fungi fruiting bodies. It should be emphasized that feeding of mentioned Cerambycidae on *S. visci* fruit bodies parasitising the European mistletoe has never been directly observed. However, we hypothesize that mistletoe infected by *S. visci* attracts adult female Cerambycidae and leads them to oviposit on both, infected and healthy plants. At the same time, Cerambycidae act as vectors for the spread of spores and mycelium of *S. visci*, actively infecting new *V. album* clumps. It is likely that a facultative mutualistic relationship exists between Cerambycidae and *S. visci*. Thus, Cerambycidae mediate the infection of new mistletoe clumps with the fungus. We reared three species of mycetophagous beetles, 2 of which had not been observed on *V. album* before. These include *Lathropus sepicola* and *Orthocis alni*. A third species, *Rhaphitropis marchica*, was recorded on mistletoe in the study of Schumacher (1918). We reared 333 specimens of *Rhaphitropis marchica* from mistletoe samples infected by the fungus *Sphaeropsis visci*. Larvae and adults of *Rhaphitropis marchica* usually feed on saproxylic fungi from the class Sordariomycetes (Ascomycota) (Yunakov et al., 2018). The association of *Rhaphitropis marchica* with *S. visci* has been never described before. We reared only one specimen each of *Lathropus sepicola* and *Orthocis alni* from mistletoe. *Lathropus sepicola* feeds on a very wide range of saproxylic fungi (Ulyana, 2003). Similarly, *Orthocis alni* is known from many species of Polyporaceae and Corticiaceae (Ulyana, 2003). However, none of these species have been previously recorded on *Sphaeropsis visci*.

Among *Viscum*-associated insects, a predatory beetle *Nemozoma elongatum* was found, which both in the larval and imaginal stages, actively preys on the larvae and eggs of a wide range of saproxylic beetles (Miłkowski et al., 2019).

Our finding of *Longitarsus* sp. on mistletoe remains unclear since the larvae develop in the roots of various grasses, while their pupation occurs in the soil. Probably, the last-stage larva might be transferred from the soil to the mistletoe by bird vectors. It is noteworthy Lazaro-Gonzalez et al. (2017) observed nonidentified Alticinae on the European mistletoe in Spain.

Since all the saproxylic beetles we reared from European mistletoe during the study are polyphagous on deciduous trees, it is natural to ask whether the larvae of these insects migrated secondarily from the tissues of the host plant to the mistletoe stems or whether they developed exclusively in the mistletoe stems. In order to clarify this question, we have made a series of longitudinal sections of the mistletoe stems (fig. 1, D–D1) and of the branches of the host plants at their junctions (fig. 1, A–C, A1–C1). As can be seen from the sections, mistletoe stems contain mainly small-diameter galleries (fig. 1, D–D1) of small species (e. g. *Rhaphitropis marchica*, *Ernoporus tiliae*, *Exocentrus lusitanus*, *Leiopus femoratus*, *Pogonocherus hispidus*). At the same time, large-diameter galleries extend from the mistletoe stems into the tumour on the branch of the host plant (fig. 1, B–B1, C–C1). Such galleries are found in relatively large species such as *Mesosa curculionoides*, *Oplosia cinerea*, *Stenostola ferrea*. Thus, many species can develop exclusively in the stems of *V. album* without any contact with the tissues of the host plant. Such a result is very important in view of the current problem in the ecological literature of the spread of broadleaf-associated saproxylic beetles into coniferous forests (Hellrigl, 2006; Lazaro-Gonzalez et al., 2017). In this study, we did not use samples of *V. album* parasitising on coniferous trees. However, our results provide a starting point for understanding how to solve this ecological problem.

The complex of hymenopteran parasitoids associated with *V. album* includes several families of Chalcidoidea, Ichneumonoidea and Platygastroidea. On the basis of their life strategy, parasitoids can usually be divided into two broad categories — idiobionts, which

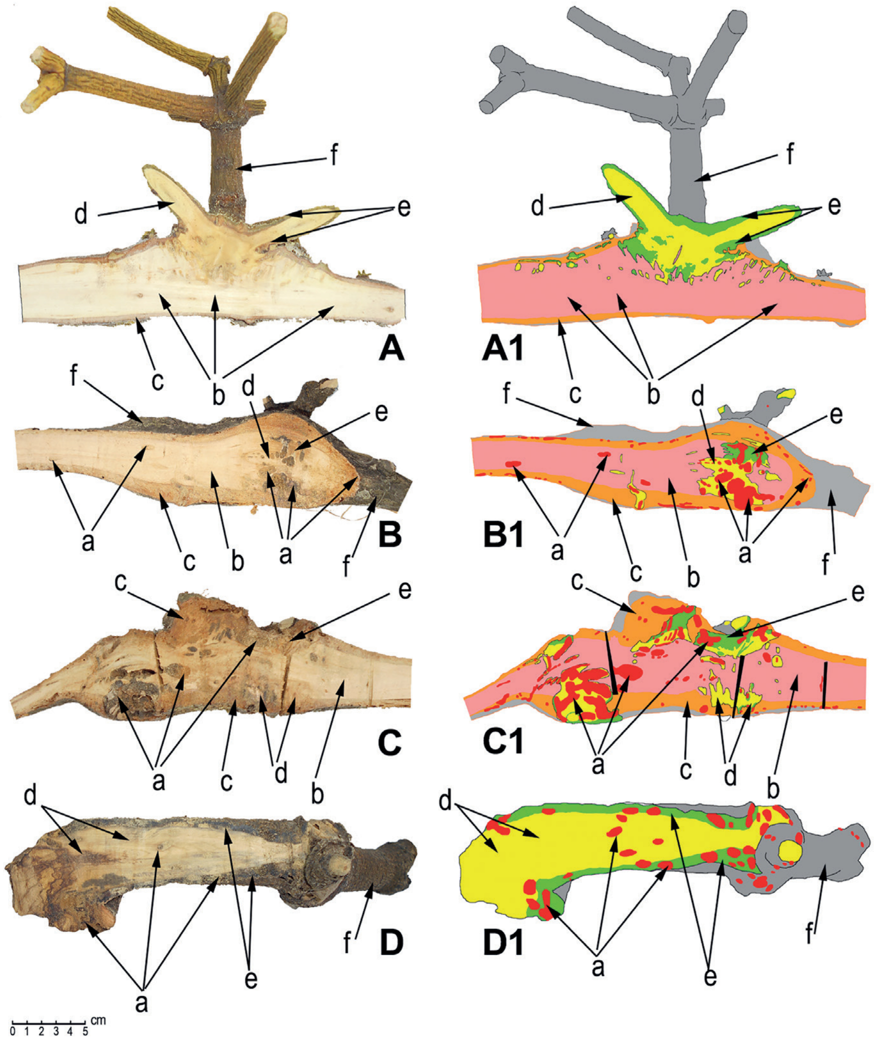

Fig. 1. Longitudinal section through the tissues of the host tree (*Tilia cordata*) and European mistletoe (*Viscum album*): A–A1 — section through intact tissues of mistletoe and host tree; B–B1, CC1 — section through infested by xylophagous insects' tissues of mistletoe and host tree; D–D1 — section through infested by xylophagous insects' mistletoe tissue. A–D — sections in the original colors, A1–D1 — sections in the false colors. Labels: a (red) — xylophagous insects' galleries; b (pink) — wood of the host tree; c (orange) — bark and phloem of the host tree; d (yellow) — mistletoe wood; e (green) — bark and phloem of mistletoe; f (grey) — the external surface of plants.

kill or permanently paralyse their hosts at the time of oviposition, and koinobionts, which allow their hosts to continue developing after the attack (Askew & Shaw, 1986). Parasitoid wasp species differ in which host life stage they attack — eggs, larvae, pupae or adults. They mainly follow one of two main strategies of parasitism: either they are endoparasitic, developing inside the host, or ectoparasitic, developing outside the host. The hymenopteran parasitoid complex associated with *Viscum album* can be formally divided into two groups: 1) wood borer parasitoids and 2) parasitoids of other insects.

Parasitoids of wood-borers. The species of Braconidae, Eurytomidae, Eupelmidae, Ichneumonidae and Pteromalidae are included in this group. The ichneumonoid wasp families Braconidae and Ichneumonidae include numerous genera of parasitoids of saproxylic beetles, four of which have been associated with *Viscum* logs. *Dolichomitus agnoscendus* belongs to the ichneumonid subfamily Pimplinae. The species of the genus are idiobiont ectoparasitoids of wood-boring beetles, mainly Cerambycidae (Varga, 2012; 2014). *Dolichomitus agnoscendus* is reported as a parasitoid of *Pogonocherus hispidulus* (Aubert, 1969). Nevertheless, the other reared large-bodied beetle species, *Exocentrus lusitanus*, *Leiopus femoratus*, *Mesosa curculionides*, *Oplosia cinerea*, *Stenostola ferrea*, are believed to be hosts of the current *Dolichomitus* species as well. The hymenopteran complex of parasitoids of the above cerambycid species includes also Braconidae species of the subfamilies Braconinae and Doryctinae. The Braconinae species of the genus *Cyanopterus*, reported as ectoparasitoid of cerambycid larvae (Tobias, 1978) were reared from *V. album* stems during this study. Similarly, most of the Doryctinae genera contain mainly idiobiont ectoparasitoids of different saproxylic beetles' larvae, especially subcortical species. Thus, the member of the unidentified Doryctinae genus and *Spathius* species are believed to parasitise some of the cerambycid larvae found during the study as well as curculionoid species, *Ernoporus tiliae*. *Ernoporus tiliae* larvae are probably hosts also for Eupelmidae species. *Eupelmus pini* is reported as idiobiont ectoparasitoid of bark beetles' larvae (Gary & Gibson, 2011), while *Eusandalum inerme* — as parasitoid of *Ernobius* spp. (Bouček, 1967). Among recorded pteromalid wasps there is at least one species, *Platygerrhus affinis*, which is known as the primary parasitoid of wood-boring Coleoptera, mainly *Stenostola ferrea* and *Ernoporus tiliae* (Mitroiu, 2005). The biology of *Aggelma* species is unknown, but it was reared from *Pinus nigra* twigs infested by *Magdalis* spp. (Curculionidae), *Anthaxia quadripunctata* (Buprestidae), *Pityophtorus glabratus*, *Pityogenes* sp. (Scolytidae) and *Ernobius mollis* (Anobiidae) in Romania by Mitroiu et al. (2007). Thus, we suppose that our specimens might be parasitoids of *Ernobius tiliae* reared from *V. album*. Another pteromalid parasitoid species, *Trigonoderus princeps*, is known as a solitary ectoparasitoid of different cerambycid larvae as well as birch bark beetle, *Scolytus ratzeburgi* (Hérard et al., 2005). The chalcidoid species of the family Eurytomidae, *Eurytoma tilicola*, is known as the ectoparasitoid of *Stenostola ferrea* larvae (Zerova, 2009).

The braconid species of the subfamily Brachistinae, chiefly of the genus *Eubazus*, are koinobiont endoparasitoids of predominantly Curculionidae larvae. At least part of the species lay their eggs in eggs of the host or very young larvae and develop internally when the larva is full-grown and has a final ectoparasitic phase (ovo-larval parasitoid) (Alauzet, 1987). It is possible that *Eubazus* species attack hosts from the other Curculionoidea families with similar biology, e.g., those living in a dead wood. It is likely that *Rhaphitropis marchica* is a possible host of *Eubazus* parasitoids reared during this study. The species of the pteromalid genus *Anisopteromalus* are known as parasitoids of various stored-product pests, e. g., *Stegobium paniceum* (Baur et al., 2014). The latter beetle species can also destroy wooden furniture. Thus, it might be assumed that the reared *Anosopteromalus* species infested small beetles with similar biology, e. g., *Ernoporus tiliae* in our study.

Parasitoids of other insects. Most of Eulophidae species of the genus *Aprostocetus* are parasitic, though phytophagy is known in some cases. Species of the genus are external egg parasitoids of Hemiptera, Lepidoptera, Orthoptera, Odonata, and Coleoptera (Graham, 1987). Nevertheless, at least one species, *Aprostocetus anoplophorae* was found to be a gregarious egg parasitoid of some cerambycids (Hérard et al., 2005). Thus, it is difficult to state clearly, are our reared specimens associated with hosts on leaves or saproxylic ones. The Eulophidae genus *Sympiesis* contains ectoparasitoids of leafrollers larvae (Lepidoptera) (Yefremova, 1997). Similarly, the microlepidopterans are also hosts for ichneumonid species of the tribe Phaeogenini, which are endoparasitoids (Siytan, 1977). Most *Synopeas* species (Platygastridae) are solitary koinobiont endoparasitoids ovipositing inside the eggs or newly hatched larvae of gall midges (Diptera, Cecidomyiidae) (Abram et al., 2012). We recorded one ichneumonid species from the subfamily Cryptinae. Unfortunately, the identification even to genera level, especially males (in our case) is difficult and almost impossible even for Ichneumonidae experts without experience in studying the subfamily. Thus, it is difficult to suppose the host association for our specimen. Cryptines are mostly ectoparasitoids of different insect pupae (Jonaitis, 1981).

The rearing of the gall wasp from the cynipoid genus *Phanacis* was one of the most unexpected findings during the study. All species of this relatively small Palaearctic genus, with a few exceptions, are associated mainly with Asteraceae, forming small cell-like galls hidden in the plant without any visible external deformation of its stem (Melika, 2006). We have reared only one specimen of *Phanacis* and it remains entirely unknown whether it is actually associated with mistletoe or whether our finding is occasional.

Next, our finding of two aphidophagous Syrphidae (Diptera) species — *Scaeva pyrastri* and *Syrphus vitripennis* — on *V. album* is novel, but not unexpected. Mistletoe is a food plant for several species of aphids (Schumacher, 1918; Hellrigl, 2006; Varga et al., 2012), which are a food source for the mentioned Syrphidae. It should be noted that *S. pyrastri* and *S. vitripennis* are considered to be inhabitants of trees, bushes and shrubs (Speight, 2020). Hypothetically, there are two ways of getting hoverflies larvae on mistletoe: 1) Syrphidae larvae feed and develop on the host plant and accidentally migrate to pupate on mistletoe; 2) syrphids indeed associated with the trophic web of mistletoe. However, it is obvious that their stratigraphic distribution in ecosystems remains underestimated. We assume that other aphidophages might also inhabit mistletoe infested with aphid colonies, and their diversity should be much higher.

Finally, our unexpected find was a species of a solitary bee *Hylaeus confusus* (Hymenoptera Colletidae), reared from *V. album*. The feeding of *H. confusus* on mistletoe is unknown, and it might be a pollinator and/or feed on the nectar from mistletoe flowers. In our study, *Hylaeus confusus* might use cerambycids galleries for nest building and breeding and the interaction between *H. confusus* and *V. album* is probably only topical.

In conclusion, our study shows a significant underestimation of the insect diversity associated with European mistletoe. This is due to a number of key factors. Firstly, the limited number of studies focusing on insects. Second, the different methods used in the studies. Thirdly, the difficulty in identifying separate groups of insects. In our study, we showed that over 77 % of the identified insects associated with European mistletoe were previously unknown. In addition, all the mycetophagous and saproxylic beetles we identified are broadly polyphagous, so there is a very high probability that other species from these ecological groups will be recorded in future studies. Next, the diversity of mycetophagous and saproxylic beetles determines the diversity of their associated parasitic wasps. An important part of future studies will be to compare insect communities associated with European mistletoe from different biomes in Europe and from different host plants, especially conifers. Of particular interest is the comparison of insect communities associated with European mistletoe and other parasitic Santalales species of Europe, especially yellow-berry mistletoe (*Loranthus europeus* Jacq.).

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