



UDC 632.76

Hymenopteran Parasitoids of Leaf Miners (*Diptera: Agomyzidae*)

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ARTICLE INFO

Keywords:

Hymenoptera,
Agromyzidae,
parasitoid,
wasp,
infestation

ABSTRACT

The current article discusses the taxonomic composition of parasitoids and the infestation degree of leaf miners with parasitoids.

The species composition of hymenopteran parasitoids of the Braconidae, Eulophidae and Pteromalidae families is studied and presented for the first time in the Artsakh Republic. Eight parasitoid wasps were reared from the 10 leaf-mining flies which attacked 19 plant species.

Introduction

Many species of leaf miners (Diptera, Agromyzidae) are serious pests for the cultivated, medicinal and ornamental plant species necessary for humans, and even such species as vegetable leaf miner (*L. sativae*), tomato leaf miner (*L. bryoniae*), South American or serpentine leaf miner (*L. huidabrensis*) and others are considered quarantine species (Spencer, 1989, 1990, Lonsdale, 2011, CABI, 2013, Grigoryan, et al., 2020). As a result of climate changes observed in recent years, as well as the import of new types of cultivated and ornamental plants in open and covered soils, the habitats of leaf miners and the intensity of infection are noticeably increasing. Based on the degree of damage caused by mining flies, various pesticides are often used against plant pests in many countries, which generally do not have a selective effect.

Repeated application of pesticides also destroys useful entomofauna, including the natural enemies of leaf miners, which play an important role in influencing the number of pests. In addition, leaf miners gain a high tolerance through the multiple use of chemicals (Keil, et al., 1985).

The most effective method of controlling leaf miners is the use of natural enemies, such as pathogenic bacteria, parasitoids and insect predators, which are also harmless to the environment (Shojaey, et al., 2020). Based on the undesirable consequences of chemical control in recent years, in many countries, including Artsakh, much attention has been paid to the research of the natural enemies of leaf miners. Therefore, our main purpose was to study the parasitoids' species diversity of mining flies in Artsakh. The species diversity of the family Eulophidae (Hymenoptera) and the peculiarities of their parasitism

were studied by Askew and Shaw in England (Askew and Shaw, 1974). Recently, the species composition of subfamilies Eulophinae, Entedoninae and Tetrastichinae has been studied in Iran (47 species) (Yefremova, et al., 2007), 17 species of ecto- and endoparasitoids of chalcidoid parasites (Hymenoptera, Eulophidae) have been explored in the Middle Volga River Basin (Strakhova, et al., 2013), while 15 species of hymenopterous parasitoids were found in northwestern Iran (Iranian Azerbaijan) in 2015, 3 of which belong to the Braconidae family, 10 - to the Eulophidae family, 2 - to the Pteromalidae (Lotfalizadeh, et al., 2015). As a result of research in 2020, the entomofauna of Iran was added with new parasitic species of the family Pteromalidae (Shojaey, et al., 2020).

It has been confirmed that parasitoids play an essential role in the regulation of leaf miners' number in nature. Hymenoptera is considered one of the largest orders of insects with huge species diversity. More than two dozen families of the order, such as Ichneumonidae, Braconidae, Sphecidae, Pteromalidae and others, parasitize insects and pests reducing their numbers in nature. Larvae of hymenopteran parasitoids having developed through the early instar larvae of host insects gradually destroy them. This is one of the important features that distinguish parasitoids from parasites (Askew, 1971), which do not kill their hosts. Parasitoid larvae can live inside host organism (endoparasitoid) or outside the host organism (ectoparasitoid).

Noyes J. (2004) recorded 300 species of parasitoids, more than 80 of which parasitize leaf miners of the genus *Liriomyza* (Diptera: *Agromyzidae*). The complexity of the study of leaf miners' parasitofauna occurs during pupation when their larvae come out of the mines, taking with them the chalcid parasitoids (Askew and Shaw, 1974).

Our task was to detect the leaf miner pupae infested with parasitoids and rear adult parasitic species, to conduct structural research for precise differentiation, to study the biological and ecological features of the parasitoids' development as well as the character and rate of infestation.

Materials and methods

The research material was leaves infected with leaf miners and collected from different regions of Artsakh Republic for the periods of April 2018 to August 2020. The infected leaves were stored in glass tubes, filled with wet sand (23-25°C) until pupae formation. Usually the development of the larva lasts 4-5 days (23-25°C), pupal development takes 7-14 days (25-28°C). Prolonged pupal development is associated with the formation of mold or

with the development of natural enemies inside leaf miner pupa, which usually lasts 10 - 20 days. We separated the infested pupae, moved them to another vial (25-28°C, 70 ± 5 % RH) and constantly followed the development process. After 24 hours the emerged adult parasitoids were stored in 75 % ethyl-alcohol for future work. Species were classified according to the accepted taxonomic classification (Boucek, 1988), and various identification guides were used to differentiate morphological features (Hansson, 1985, Graham, 1959, LaSalle and Parella, 1991, Achterberg, 1993, Shaw, 1991). Morphological studies of parasitoids were carried out in the Biological Research Laboratory of Artsakh State University using XSZ-0800 and ADSM302 digital microscopes.

Results and discussions

As a result of our research, in Artsakh Republic we found 8 parasitoid species belonging to 8 genera, 6 subfamilies and 3 families: Braconidae (2 species), Eulophidae (4 species) and Pteromalidae (2 species) (Table 1). If the development of uninfested pupae at a temperature of 25-28°C takes 7-14 days, then the development of infested pupae takes longer, about 10-20 days.

Most species of Braconidae family (Hymenoptera: Ichneumonoidea) are endo- and ectoparasitoids of eggs and larvae. A number of parasitoids of this family are isolated from the host organism at the end of the development cycle to complete development beyond it (Shaw and Huddleston, 1991). Family Braconidae is represented by two subfamilies: *Opiinae*, Blanchard, 1845 and *Alysiinae*, Leah, 1815, which are endoparasitoids. The development of leaf miners' larvae infested with parasitoids is not interrupted, their larvae continue to feed and mature, and even pupate. The development of parasitoids ends inside host larvae and pupae, and the adult braconid emerges from the pupa (Li, et al., 2013).

Species of the family Eulophidae (Hymenoptera: Chalcidoidea) are widely distributed and are considered ectoparasitoids of larvae and pupae. The family includes 290 genera and 4300 species (Noyes, 2001).

Family Pteromalidae (Hymenoptera: Chalcidoidea) is one of the largest families of the Chalcidoidea superfamily, numbering 3 500 species and 600 genera (Noyes, 2002, 2003). Parasitic pteromalids lay eggs inside hosts' eggs, larvae and pupae. It should be noted that at the embryonic stage, the infested leaf miners' development is not interrupted and continues until the formation of larva, then pupa, where the development of leaf miners is ceased and the adult parasitic wasp emerges from pupa.

Table 1 shows the species composition of mining flies, which attack various plant species, and their parasitoids.

The following table (Table 2) shows the number of parasitoids developing in different species of mining flies on one plant.

Table 1. Taxonomic composition of the studied parasitic wasps*

Super-family	Family	Subfamily	Genus	Species	Host species of mining flies	Infected plant species
Ichneumonoidea Haliday, 1838	Braconidae Nees, 1812	Opiinae Blanchard, 1845	Opius Wesmael, 1835	Opius sp.	Phytomyza horticola (Goureau, 1851)	Cardaria draba (L.) Desv. (Brassicaceae)
					Liriomyza bryoniae (Kaltenbach, 1858)	Capsicum annum L. (Solanaceae)
					Liriomyza sativae (Blanchard, 1938)	Phaseolus vulgaris L. (Fabaceae)
						Cucurbita pepo L. (Cucurbitaceae)
					Calycomyza humeralis (von Roser, 1840)	Erigeron canadensis L. (=Conyza canadensis (L.))
					Phytomyza lappae (Goureau, 1851)	Arctium lappa L. (Asteraceae)
		Alysiinae Leah, 1815	Chaenusa seminervata van Achterberg, 2012	Chaenusa seminervata sp.	Amauromyza leonuri (Spencer, 1971)	Ballota nigra L. (Lamiaceae)
						Lamium album L. (Lamiaceae)
					Liriomyza congesta (Becker, 1903)	Trifolium repens L. (Fabaceae)
					Liriomyza endiviae (Hering, 1955)	Lactuca sativa L. (Asteraceae)
Chalcidoidea	Eulophidae Westwood, 1829	Eulophinae Westwood, 1829	Diglyphus Walker, 1844	Diglyphus isaea (Walker, 1838)	Liriomyza endiviae (Hering, 1955)	Lactuca sativa L. (Asteraceae)
		Entedoninae Forster, 1856	Pediobius Walker, 1846	Pediobius metallicus (Nees, 1834)	Phytomyza horticola (Goureau, 1851)	Dahlia pinnata Cav. (Asteraceae)
						Sisymbrium loeselii L. (Brassicaceae)
			Neochrysocharis Kurdjumov, 1912	Neochrysocharis sp.	Phytomyza horticola (Goureau, 1851)	Galinsoga parviflora Cav. (Asteraceae)
			Apleurotropis Girault, 1913	Apleurotropis sp.	Phytomyza horticola (Goureau, 1851)	Leucanthemum maximum Ramond (DC.) (Asteraceae)
		Pteromalinae Dalman, 1820	Nasonia vitripennis (Walker, 1836)	Nasonia sp.	Amauromyza flavifrons (Meigen, 1830)	Alliaria petiolata (Bieb.) Cavara et Grande (Brassicaceae)
		Miscogastrinae Walker, 1833	Halticoptera Spinola, 1811	Halticoptera sp.	Liriomyza bryoniae (Kaltenbach, 1858)	Saponaria officinalis L. (Asteraceae)
						Beta vulgaris L. (Amaranthaceae)

*Composed by the authors.

Hymenopteran Parasitoids	Leaf-mining flies (n*)										
	Amauromyza leonuri (9)	Amauromyza flavifrons (5)	Calycomyza humeralis (6)	Liriomyza congesta (6)	Liriomyza endiviae (4)	Liriomyza sativae (29)	Liriomyza bryoniae (8)	Phytomyza horticola (29)	Phytomyza plantaginis (14)	Phytomyza lappae (4)	Total
Opius sp.	9		2			9	2	4		3	29
Chaenusa seminervata sp.				5	2			2	6		15
Diglyphus isaea					2						2
Pediobius metallicus								6			6
Neochrysocharis sp.								3			3
Apleurotropis sp.								2			2
Nasonia sp.		4									4
Halticoptera sp.							2				2
Total	9	4	2	5	4	9	4	17	6	3	63
Degree of infestation (%)	100	80	33.3	83.3	100	31	50	59	43	75	

**Composed by the authors.

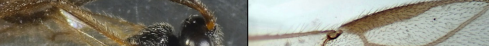
A composite image of a wasp. The main image on the left shows the whole wasp, which has a black head and thorax, a brown and black abdomen, and yellow legs. Two inset images on the right provide details: the top inset shows a close-up of a wing with its venation, and the bottom inset shows a close-up of the segmented antennae.

Figure 2. Family Braconidae Nees, 1812.

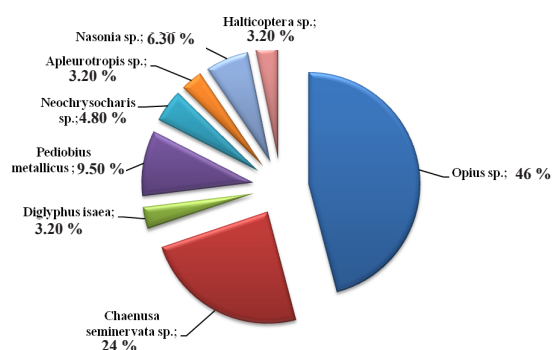
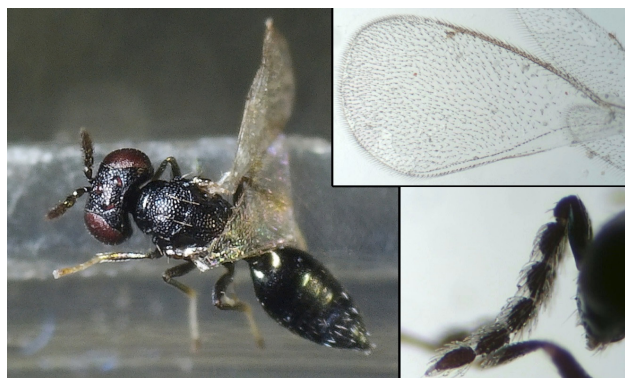
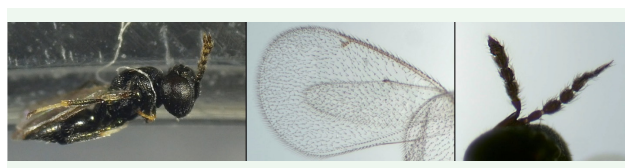


Figure 1. Quantitative ratio of parasitoids (*composed by the authors*).

Genus *Pediobius* Walker, 1846 *Pediobius metallicus* (Nees, 1834)Genus *Apleurotropis* Girault, 1913 *Apleurotropis* sp.Genus *Diglyphus* Walker, 1844 *Diglyphus isaea* (Walker, 1838)Genus *Neochrysocharis* Kurdjumov, 1912 *Neochrysocharis* sp.**Figure 3.** Family Eulophidae Westwood, 1829.Genus *Nasonia* vitripennis, Walker, 1836 *Nasonia* sp.Genus *Halticoptera* Spinola, 1811 *Halticoptera* sp.**Figure 4.** Family Pteromalidae Dalman, 1820.

The infestation rate of *L. congesta* parasitized by *Chaenusa seminervata* sp. is 83.3 %, and the infestation rate of *Ph. plantaginis* is 43 %.

It can be noted that specialized parasitoids are more effective in regulating the number of leaf miners than non-specialized species with a density of less than 10 %.

We have also presented the reared parasitic species of the Braconidae (Figure 2), Eulophidae (Figure 3) and Pteromalidae families (Figure 4).

Conclusion

During the research, we found 8 species of parasitoids belonging to the Braconidae, Eulophidae and Pteromalidae

families, which parasitize the species of the families Amauromyza, Calycomyza, Liriomyza and Phytomyza. Species of Braconidae family (*Opius* sp. - 46 %, *Chaenusa seminervata* sp. - 24 %) have a high occurrence rate, but the Eulophidae family stands out with its species diversity. 5 parasitic species were reared from the polyphagous *Ph. horticola* species, which is considered as a pest of cucurbits and ornamental plants. Considering the high rate of parasitism, we can assume that parasitic species play an important role in regulating the number of mining flies. As above-mentioned, when we use pesticides against leaf-mining flies, it is necessary to take into account that in order to preserve important fauna, we should also know the infestation period and development features of parasitic wasps.

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Accepted on 28.10.2021

Reviewed on 09.11.2021