

## The Distribution of *Agromyza* Species in Nagorno-Karabakh Region and Infestation Rate of Host Plants

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### Abstract

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In a survey carried out during the period 2019-2022, two species of *Agromyza* (Diptera: Agromyzidae) were identified for the first time in Nagorno-Karabakh region: *A. parca* and *A. varifrons*. This study focused on these species' description, distribution, infestation of host plants and determination of some morphometric measurements. High infestation rate (65-70%) was noted on *Alopecurus pratensis*. Morphometric measurements confirmed the presence of sexual dimorphism in both identified species, especially the male wing length of *A. parca* in Nagorno-Karabakh was shorter by 0.7-0.9 mm, and in females 0.3-0.6 mm, whereas the wing length of *A. varifrons* was shorter by 0.5-0.8 mm.

**Keywords:** Leaf miners, host-plants, morphometric measurements.

### Introduction

The study of leaf miners worldwide identified around 3,000 species, many of which are listed as quarantine species based on the degree of damage they cause. The number of *Agromyza* species in the world reached around 200, 12 of which were reported from the Neotropic region, only 6 species were found in Brazil, 99 species were found in the Palearctic Region, 72 in southwestern Europe, 34 in Spain (Boucher, 2010; Martinez & Etienne, 2002; Martinez, 2004; Ortiz, 2009). Most *Agromyza* species cause significant economic damage to the leaves of the Fabaceae and Poaceae plant families, especially to crops such as *Avena sativa*, *Hordeum vulgare*, *Oryza sativa*, *Secale cereale*, *Triticum aestivum*, *Zea mays* (Sousa & Couri, 2016; Zlobin, 2001). In Armenia, information about Agromyzidae is generally very scarce; only *A. spiraeae* of the discussed genus is known as leaf-miner of *Rubus idaeus* (Tereterian, 1976). Studies in Nagorno-Karabakh have shown that *A. parca* is a widespread and blotch leafminer of *Alopecurus pratensis* leaves, and *A. varifrons* infests trees of *Celtis caucasica*. In the USA, *A. parca* also damages the leaves of *Dichanthelium clandestinum*, *D. scoparium*, *Glyceria canadensis*, *G. striata* (Poaceae) (Eiseman *et al.*, 2021). Larvae of *A. varifrons*, common in the United States and Canada, were found not only on the leaves of *Celtis*, but also on *Ulmus* spp. (Lonsdale, 2021). Control of these pests is difficult, since the larvae are protected by the leaf epidermis. Only natural enemies, such as parasitic Hymenoptera wasps, have a certain influence on the regulation of pest numbers (Al Saffar, 2016).

### Materials and Methods

Nagorno-Karabakh is located in the southeastern part of the Lesser Caucasus, and the relief is typically mountainous. The climate is temperate and mild, with a clearly defined ascending zonality: on the plains (up to 400 m) dry

subtropical, in the foothills (400-610 m) moderately warm, in the mountains of medium altitude (700-1100 m) moderately hot and humid, in the highest areas (1300-1800 m) Alpine cold. Mined leaves, larvae and pupae were collected randomly from private and public pesticides-free areas of different regions of Nagorno-Karabakh (c. Stepanakert, 39°49'03.46"N 46°45'22.99"E; c. Shushi, 39°45'32.66"N 46°44'57.43"E; vlg. Ivanyan/Askeran, 39°54'45.99"N 46°47'28.19"E; vlg. Nor Maragha/Martakert, 39°47'41.71"N 46°53'10.86"E; vlg. Nngi/Martuni, 39°47'41.71"N 46°53'10.86"E; vlg. Skhtorashen/Martuni, 39°42'19.72"N 46°56'23.81"E), following methods generally accepted for the Agromyzidae family (Capinera, 2001; Hering, 1951).

Infected leaves were stored in glass tubes filled with wet sand (25-26°C) until pupae were formed. Leaf miner adults were reared at a temperature of 26±2°C and 70±5 % RH. The shape of mines and larval frass, the location and number of larvae and pupae spiracles, the wing venation and chaetotaxy of the head and thorax were used for species identification. In addition to morphological features, the male genitalia, in particular, the distifallus was also studied for accurate identification of the species using different identification guides (Lonsdale, 2021; Spencer & Steyskal, 1986). To enlighten the tissues, the separated abdomen of the male was boiled for 5 minutes in a 10% solution of sodium hydroxide (NaOH), or left overnight in a cold solution of NaOH (10%). After that, the abdomen was placed in a container with distilled water and a drop of 5% acetic acid was added. Then the abdomen was transferred to a 70% solution of ethyl alcohol and separated from the surrounding membranes, cuticles and muscles with the help of a dissecting needle (IPPC, 2016).

Statistical analysis of the data obtained was carried out using Student's t-test, and differences were considered significant at P<0.05 (Lakin, 1990). The host plants were identified and classified according to family, genus and species, by a specialist, based on Takhtajyan (2009),

Ghulyan & Balayan (2013). The extent of damage was determined by the number of damaged plants (%) per 1m<sup>2</sup>, and the intensity - by the number of damaged leaves (%) per plant. The study of the external structure of mining flies, as well as their identification by the male genitalia, was carried out using the Optika B-290 digital microscope in the laboratory of the Nagorno-Karabakh Scientific Center.

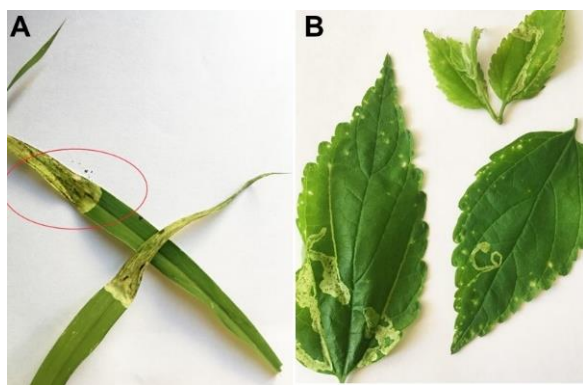
## Results and Discussion

Two species of the genus *Agromyza* were found in the fauna of Nagorno-Karabakh for the first time: *A. parca* (Spencer, 1986) and *A. varifrons* (Coquillett, 1902).

### *Agromyza parca* (Spencer, 1986)

*A. parca* leafminers are quite widespread in the Palearctic region, China, Japan, the Nearctic region (USA, Canada), they are considered oligophagous species and infest plants of the Poaceae family (Nartshuk, 2019). In Nagorno-Karabakh region *A. parca* leaf miners also damage the *Alopecurus pratensis* L. and *Elytrigia repens* (L.) Nevski plant species of the Poaceae family. *A. parca* lay eggs on the leaf margins, and 4-5 days later the larvae make narrow, short linear mines by the first and second instars, and the third instars larvae continue to feed on mesophyll tissues gradually descending from the leaf top and forming flat blotch mines (Figure 1-A). There may be 2-3 larvae on the same leaf, which feed on the entire upper palisade tissue layer. The liquid, greenish frass of the larvae spreads along the entire length of the mine, which then turns into solid, black piles. Larval development lasts 5 days at temperature of 25-26°C.

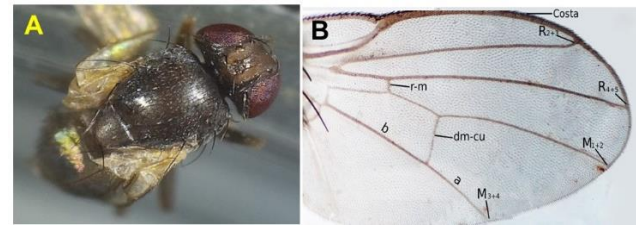
The intensity of plant infestation is 25-30%, and the extensiveness is 65-70%. Adults are dark, body length 2.0±0.02 mm (♂), 2.3±0.02 mm (♀). Frons is broad, dark or brown; they have 2 ori and 2 ors (Figure 2-A). Costa extends to M<sub>1+2</sub> vein, has a large discal cell, last section of M<sub>3+4</sub> is smaller than penultimate (Figure 2-B), wing length 1.8±0.01 mm (♂), 2.1±0.02 mm (♀).



**Figure 1.** The blotch leaf miners. (A) *Alopecurus pratensis*, and (B) *Celtis caucasica*.

Calypter is light yellow with yellow hairs and white margin. Femur and tibiae are black, tarsi light brown, and halter is white. Pupation occurs in soil, and pupae are reddish-brown (Figure 4-A), and each posterior spiracle consists of three bulbs (Figure 4-C), which are located on a short stem.

Pupal development lasts 10-15 days at 26±2°C temperature. Distiphallus consists of two fused, short, light tubes (Figure 3), sperm pump has elongated blade (Figure 3-C).



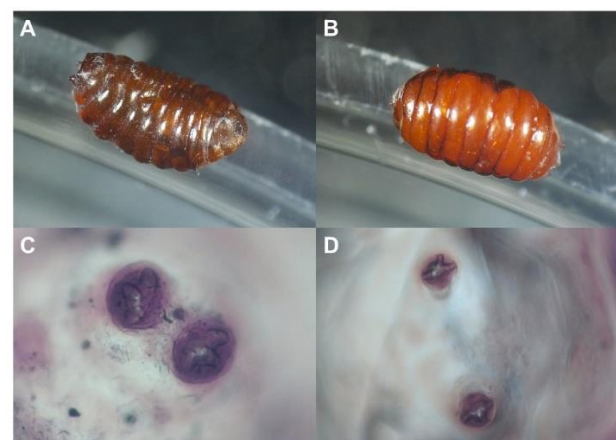
**Figure 2.** *A. parca* morphological features. (A) external view, and (B) wing veins.



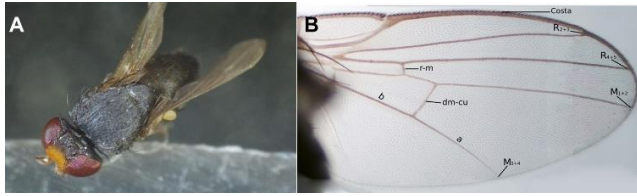
**Figure 3.** Male genitalia of *A. parca*. (A) aedeagus, ventral view, (B) aedeagus, lateral view, and (C) sperm pump.

### *Agromyza varifrons* (Coquillett, 1902)

In Nagorno-Karabakh region, leaf miner *A. varifrons* damages especially young lower-level leaves of *Celtis caucasica* Willd. The first and second instars larvae form irregularly narrow, linear mines that grow into blotch mines by the third instar larvae (Figure 1-B). The frass of the larvae looks like dots and piles. Larval development at 25-26°C temperature lasts 4-5 days. Pupation takes place in the soil, and the pupae first have first a slight yellow tint, and then turn to reddish-orange (Figure 4-B). Each posterior spiracle consists of 3 pores (Figure 4-D). Pupal development lasts 10-15 days depending on the air temperature and soil relative humidity. The intensity of the plant infestation is 35-40%, and the extensiveness is 45-50%.



**Figure 4.** Pupa and posterior spiracles. (A, C) *A. parca*, (B, D) *A. varifrons*



**Figure 5.** *A. varifrons* morphological features. (A) external view, and (B) wing structure

Body length of *A. varifrons* is  $1.7 \pm 0.04$  mm (♂),  $2.0 \pm 0.01$  mm (♀). The lower frons is yellow or orange with 2 ors, 2 ori, the upper part is brown or black, and the frons slightly projects above the eye in profile. Thorax is dark covered with a gray coating, mesonotum with 3+1 dc (Figure 5-A). Costa extends to the  $M_{1+2}$  vein, has a large discal cell, the last section of  $M_{3+4}$  is small or almost equal to penultimate (Figure 5-B). The wing length is  $1.5 \pm 0.03$  mm (♂),  $1.8 \pm 0.03$  mm (♀). Legs are black, knees and tarsi are brown, halteres are yellow.

Mesophallus looks like a long narrow tube (Figure 6-A), distiphallus consists of two petals fused at the bottom (Figure 6-B), the sperm pump is small (Figure 6-C). Cerci have short, strong setae lying at the lower corner of the inner surface.

Analyzing the morphometric parameters of *A. parca*, it was found that the males' wing length is 0.7-0.9 mm, and in females 0.3-0.6 mm, which is less than what has been reported in the literature, where the males' wing length varied from 2.5 to 2.7 mm, and in females from 2.4 to 2.7 mm

(Lonsdale, 2021). Based on previous reports (Spencer & Steyskal, 1986), wing length of the species *A. varifrons* reached 2.3 mm (2 mm by Malloch, 1913), whereas the wing length of *A. varifrons* in Nagorno-Karabakh region was shorter by 0.5-0.8 mm.



**Figure 6.** Male genitalia of *A. varifrons*. (A) aedeagus, ventral view, (B) aedeagus, lateral view, and (C) sperm pump.

It can be concluded from this study that this is the first report of *Agromyza* miners (*A. parca*, *A. varifrons*) in Nagorno-Karabakh. *A. parca* is found at altitudes of 400-1100 m, and *A. varifrons* at 700-1100 m. High rates of plant infestation were observed in *Alopecurus pratensis* (extensiveness was 65-70%). *A. varifrons* was less distributed species and caused damage to the young leaves of *Celtis caucasica* (extensiveness was 45-50%). The results of morphometric measurements showed that the 2 species of the genus *Agromyza* have pronounced sexual dimorphism, which is expressed in the prevalence of body and wing length in females ( $P < 0.001$ ).

## الملخص

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أمكن من خلال مسح أجري خلال الفترة 2019-2022، تحديد نوعين من حافرات الأوراق من الجنس *Agromyza* (Diptera: Agromyzidae) لأول مرة في منطقة ناغورنو كاراباخ، وهما: *A. parca* و *A. varifrons*. ركزت هذه الدراسة على وصف هذين النوعين، انتشارهما، إصابة نباتات العائل وتحديد بعض القياسات الجسمية. سُجّل معدل إصابة مرتفع (65-70%) على نبات ذيل الثعلب (*Alopecurus pratensis*). أكدت القياسات الجسمية وجود الشكل الجنسي المزدوج في كلا النوعين المحددين، لا سيما أن طول جناح ذكور النوع *A. parca* في ناغورنو كاراباخ كان أقصر بمقدار 0.7-0.9 مم، وفي الإناث بمقدار 0.3-0.6 مم، بينما كان طول جناح النوع *A. varifrons* أقصر بمقدار 0.5-0.8 مم.

كلمات مفتاحية: حافرات أوراق، نبات عائل، قياسات جسمية

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