

First rearing record and DNA sequence of *Chromatomyia aizoon* (Hering, 1932) (Diptera: Agromyzidae) associated with *Saxifraga paniculata* Miller from the Czech Republic

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Abstract: A male of *Chromatomyia aizoon* (Hering, 1932) (Diptera: Agromyzidae) was reared from a leaf mine at *Saxifraga paniculata* collected in Štramberk (Northern Moravia, Czech Republic). Partial sequence of the mitochondrial cytochrome oxidase subunit I (COI barcode region, 658 bp) is also provided.

Key words: Diptera, Agromyzidae, *Chromatomyia aizoon*, Czech Republic, biology, faunistics, DNA barcoding

Introduction

Chromatomyia aizoon (Hering, 1932) is a rare species recorded so far only from Austria, Czech Republic, Poland, and Switzerland (Martinez 2013). However, only the records from Austria and Switzerland are based on specimens reared from its host plant *Saxifraga paniculata*. For this reason the species has not been included in the Checklist of Diptera of the Czech Republic and Slovakia (Černý & Vála 2009), pending a recent finding based on rearing. The species-richness of the agromyzid fauna in the Czech Republic is increased to 495 species in this paper.

Material and methods

A field survey of relict populations of the plant *Saxifraga paniculata* occurring on the limestone outcrops on Zámecký vrch hill and in an old inactive limestone quarry called Dolní Kamenárka (both localities are located in the town of Štramberk, Northern Moravia, Czech Republic) was held by the second author in order to check the plants for the presence of *Chromatomyia aizoon* mines. This agromyzid species was reported from Kotouč hill by Zavřel (1953) – our current survey was inspired by this literary record. Zámecký vrch hill was surveyed on 31.viii.2020 and 9.ix.2020, Dolní Kamenárka quarry on 9.ix.2020. Kotouč hill was not surveyed since the second author did not notice the host plants there during his earlier floristic inventory of the area (cf. Kocián 2020).

A male of *Chromatomyia* was reared from a single leaf of *Saxifraga paniculata* by the third author. The specimen is preserved in 75 % ethanol and deposited in the private collection of the first author who carried out a morphological examination of the specimen. The other leaves with mines of this agromyzid species have been left at the locality in order to maintain and protect the local population of the species. The identification and nomenclature used were based on Hering (1932), Griffiths (1972, 1974), Martinez (2013) and Spencer (1990). Nomenclature of vascular plants follows Danihelka *et al.* (2012).

The method of DNA extraction and amplification used here is principally described by Ševčík *et al.* (2016). PCR product was sequenced by Eurofins Genomics.

Abbreviations used in the text: det. = determined, distr. = district, leg. = legit (collected), m a.s.l. = meters above sea level, PP = Natural Monument.

Results and Discussion

FAMILY: AGROMYZIDAE

Subfamily: Phytomyzinae

Chromatomyia aizoon (Hering, 1932) (Fig. 1a)

Phytomyza aizoon Hering, 1932: 162.

Material examined: CZECH REPUBLIC, Northern Moravia: Nový Jičín distr., Štramberk, PP Štramberk, 49°35'32.0"N, 18°06'53.8"E, 415 m a.s.l., leaf mine with puparium collected 31.viii.2020 by J. Kocián, 1 ♂ reared 10.ix.2020 by J. Ševčík, det. J. Kocián (host plant) and M. Černý (the adult fly).

Biology: Larva forms a mine on the upper side of the leaf of *Saxifraga paniculata* (= *S. aizoon* Jacq.). The mine is initially narrow and inconspicuous but later becomes broader, finally forming a large area covering almost the entire surface of the leaf (Figs 1b-c). Puparium is whitish and 2.3 mm long (Fig. 1d), its anterior spiracles are knob-shaped with 9-10 bulbs, posterior spiracles are small knob-shaped with 9-12 bulbs (Hering 1932, Griffiths 1972). Puparium remains in mine, with its ventral surface adjacent to surface of leaf, and its anterior spiracles projecting ventrally through epidermis.

A single plant of *Saxifraga paniculata* with agromyzid mines in leaves (Fig. 1b) was found on the western slope of Zámecký vrch hill (Fig. 2b) within the Štramberk Natural Monument. The habitat includes partly shaded limestone outcrops covered by semixerothermic vegetation containing several relict, phytogeographically remarkable and rare species such as *Saxifraga paniculata*, *Scabiosa lucida* subsp. *calicicola*, *Libanotis pyrenaica*, *Allium senescens* subsp. *montanum* and *Festuca pallens* (Fig. 2a). The locality is a part of Štramberk karst, a small limestone area well known for its biological richness. Limestone rocks, rock steppes and dry grasslands are the most valuable habitats of this area providing ecological niches for many endangered organisms. These habitats were best developed, both in quality and quantity, on Kotouč hill. After their destruction there (see below) the best preserved ones remained on Zámecký vrch hill.

The population of *Saxifraga paniculata* on Zámecký vrch hill consists of a few hundred rosettes growing in several clusters dispersed on the western slope of the hill. Since the population of the host plant is small and the mines were found only on a single plant, *Chromatomyia aizoon* population is arguably small too. Although the insect species was not found on the second surveyed site – in Dolní Kamenárka quarry – the population of the host plant is much larger there and further survey could bring a positive result.

DNA barcode sequence: Partial sequence of the mitochondrial cytochrome oxidase subunit I (COI) gene (barcode region, 658 bp) is provided below. GenBank accession number is MW458947.

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AACTTTATATTTTATTTTTGGAGCTTGAGCCGGAATAGTAGGAACTTCTTTAAGTATT  
TTAATTCGAGCAGAATTAGGTCATCCTGGAGCCTTAATTGGGGATGATCAAATTTAT  
AATGTAATTGTTACAGCTCATGCAATTTATTATAATTTTCTTCATAGTTATACCAATTAT  
AATTGGAGGATTTGGAAATTGACTAGTACCCTTAATACTAGGAGCCCCAGATATAGC  
CTTTCCTCGTATAAATAATATAAGCTTTTGATTACTACCCCCAGCTTTAACCCTTTTA  
CTAATAAGTAGTATAGTAGAAAACGGAGCTGGTACAGGGTGAAGTGTATACCCCC  
TTTATCCTCAATTATTGCTCATGGAGGAGCTTCTGTGATCTTGCAATTTTTTCTCTT  
CACTTAGCTGGAATTTTCATCTATTTTAGGGGCTGTAAATTTTATTACAACAATTATTA  
ATATACGATCAACTGGAATTTCTTTTGATCGAATACCTTTATTTGTTTGATCAGTTTT  
AATTACTGCAGTATTACTTTTACTATCCCTTCCTGTTTTAGCTGGGGCTATTACTATAC  
TTTAAACAGATCGAAATTTTAATACTTCATTTTTTGATCCTGCTGGTGGAGGAGACC  
CTATTTTATACCAACACTTATTT
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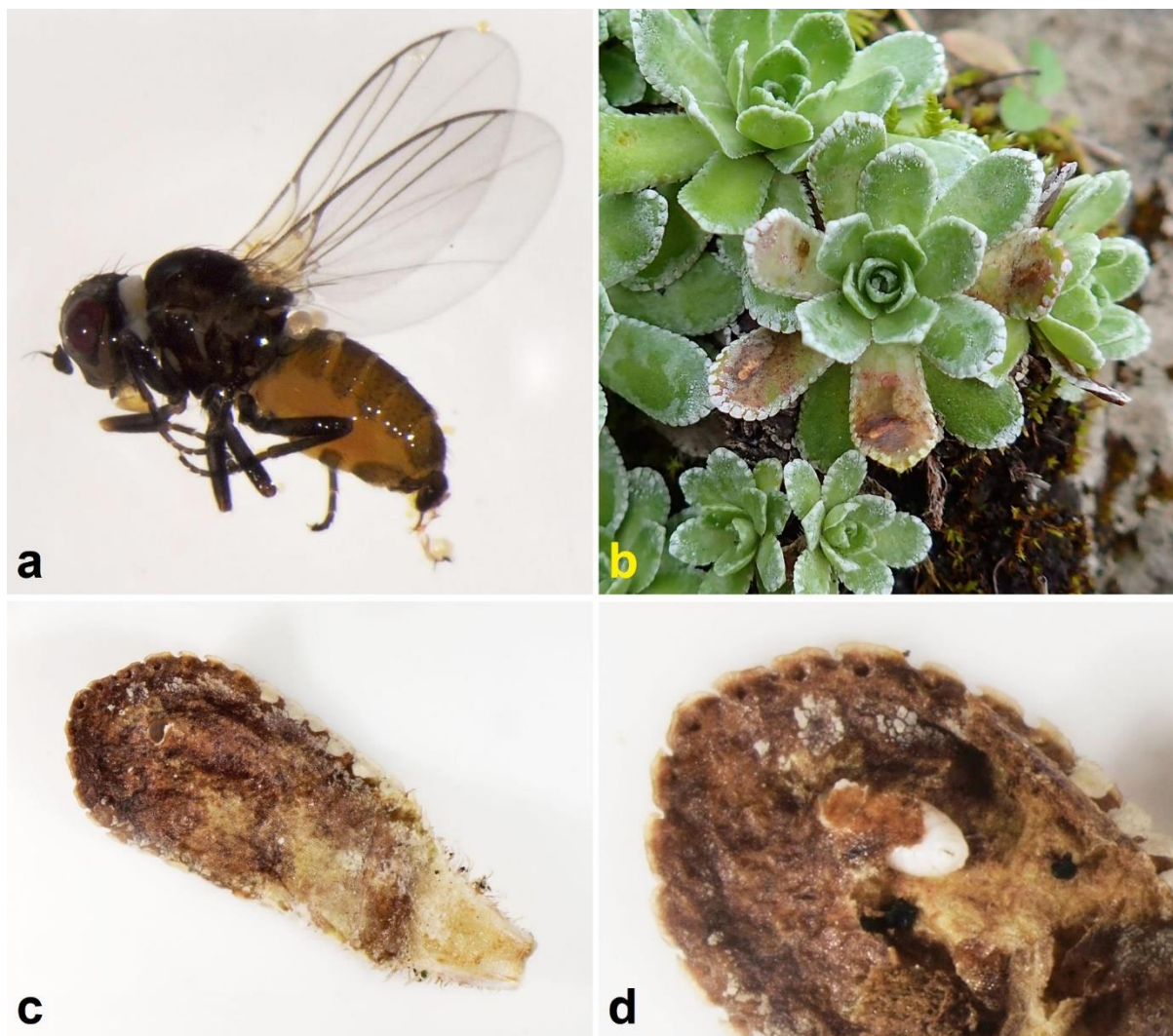


Fig 1: *Chromatomyia aizoon* (Hering) and its host plant (a-d): **a** – *Ch. aizoon*, male, laterally (10.ix.2020); **b** - Host plant *Saxifraga paniculata* with leaf mines (31.viii.2020); **c** – Leaf of *Saxifraga paniculata* with the mine after the emergence of the adult (10.ix.2020); **d** – Leaf of *Saxifraga paniculata* with the mine and puparium (10.ix.2020). Photos a, c, d by J. Ševčík, photo b by J. Kocián.

Comments

According to the Fauna Europaea Database, *Chromatomyia aizoon* is known only from Austria, Czech Republic, Poland and Switzerland (Martinez 2013) and adult specimens were reared only in Austria and Switzerland. Hering (1932) described the species based on adult specimens reared from mines found on the host plant *Saxifraga paniculata* in Mauthen (Carinthia, Austria) and Zerneß (Canton Graubünden, Switzerland).

In the Czech Republic, Zavřel (1953) found the rare relict plant *Saxifraga paniculata* with the mines of *Phytomyza aizoon* Hering, 1932 at the western slope of the Kotouč Hill on 12.ix.1952 (see Griffiths 1972) but he did not rear any adult specimens. However, the locality does not exist anymore, due to extensive limestone quarrying at Kotouč hill during the 20th century. The first adult male of *Phytomyza aizoon* reared from its host plant *Saxifraga paniculata* is thus herewith recorded from a nearby locality at Zámecký vrch hill in Štramberk. The new locality is about 1.2 km northeast of the former one. The new finding represents the first definitive record of *Chromatomyia aizoon* (Hering, 1932) from the Czech Republic.



Fig 2: Habitat of *Chromatomyia aizoon* (Hering): **a** – vegetation on the site of *Ch. aizoon* occurrence (31.viii.2020); **b** – part of the western slope of Zámecký vrch hill in Štramberk (26.x.2020). Photos by J. Kocián.

In Slovenia, Maček (1975, 1999) reported leaf mines of *Phytomyza aizoon* on *Saxifraga rotundifolia* from Vintgar near Bled (October 1974) and Velika planina (Tiha dolina Valley, July 1974). Unfortunately these records were not supported by rearing of adults, so this species cannot be considered as reliably recorded from Slovenia and also *S. rotundifolia* should not be considered a confirmed host plant of *Ch. aizoon*. Beiger (1979, 1981) recorded a leaf mine on *Saxifraga paniculata* from a locality near Krakow (4.ix.1961) but again without a confirmation by the rearing of an adult. This indicates that *Chromatomyia aizoon* is undoubtedly more widely distributed in suitable habitats in Europe and any well documented record of an adult male reared from its host plant is valuable and worth publishing.

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