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AKADÉMIAI KIADÓ

First record of *Coleophora gazella* Toll, 1952 in Central Europe with notes on its biology

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RESEARCH ARTICLE

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ABSTRACT

During the past years, we found a new *Coleophora* taxon in Hungary that proved to be unknown for the fauna of the country. We identified the unknown specimens using combined information from morphological examination including the female genitalia and genetic analyses. We sequenced the Cytochrome Oxidase "COI" gene of the mitochondrial DNA, and used the resultant base-pair sequence for species identification, using the BOLD platform, and for preparing a phylogenetic tree with the most closely related taxa involved. Our results show that larval and adult morphology, genitalia structure, and the sequence of the COI region of the unknown specimens are most similar to those of *Coleophora gazella* Toll, 1952. The habitats of the species in Hungary are strongly isolated from the nearest population in Turkey (Anatolia). We also studied habitat characteristics and life history of the species, which are briefly presented in this paper. The article contains four figures and two tables.

KEYWORDS

Lepidoptera, distribution, habitat, Carpathian basin, Artemisia

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INTRODUCTION

Several new *Coleophora* species have been recorded in recent years for the European and Hungarian fauna (Pastorális & Buschmann 2018, Tabell & Kosorín 2020, Szabóky & Takács 2021, Baldizzone et al. 2022, Tabell & Baldizzone 2023). One of them is *Coleophora gazella* Toll, 1952, a leaf miner on *Artemisia* species, which is distributed primarily in Central-Asia and enters Europe only in the extreme east of European Russia. Until recently, this species has been thought to inhabit only desert and semidesert areas, where its primary food plant, *Artemisia turanica* Krasch abounds (Falkovich 1988).

In the autumn of 1996, the second author found and collected 11 cases of an unknown *Coleophora* species on *Artemisia santonicum* L. near Csikóspuszta in the township of Királyhegyes, Hungary. Because rearing was not successful, the cases had been put away and then were forgotten. Seventeen years later, the cases were found accidentally, which prompted us to investigate further. We were particularly interested in the identity and life-history of the moth that prepared the cases and the characteristics of the habitat where the species is typically found. To obtain larvae and imagines of the unknown species, we decided to search for fresh cases in spring at the location where the first cases were found. However, the conditions of the only known site had changed substantially since 1996. The site has been heavily grazed lately, resulting in a dramatic change in the distribution and density of the host plant. As a consequence, no cases were found at this location despite our repeated attempts. Clearly, we needed first to find new areas of suitable habitat, with ungrazed stands of large woody *Artemisia* plants, to launch a systematic search for the unknown species.

MATERIAL AND METHODS

Field work, record and rearing

To locate suitable habitats we turned to Tibor Danyik, a local expert, who guided us to the vicinity of Csanádalberti (Fig. 1a. red dot). Another site was located by Adrienn Patalenszki in the township of Tiszafüred near the Hortobágy (Fig. 1a. green dot).

Collection of cases and adults took place on five occasions, four times at the Csanádalberti location from 2021 to 2023 and once at the Tiszafüred site in 2022. Adults were collected at Csanádalberti in 2023 using artificial light. The collected cases were transferred to the experimental garden of the first author with cultivated *Artemisia santonicum* plants to rear imagines. To keep the larvae on the plants, we secured mesh bags on the plants with cases, so the larvae were free to move around and feed, but could not leave the plant. The bag also protected them from parasitoids. After pupation, the cases were placed in a plastic container where they were kept until emergence.

The specimens are deposited in the private collections of Cs. Szabóky and A. Takács, and I. Richter.

MORPHOLOGICAL EXAMINATIONS

Photography and genitalia preparation – The cases, adults, and feeding signs of the larvae were photographed and described in detail. Images were made with a Canon 450 D camera applied to

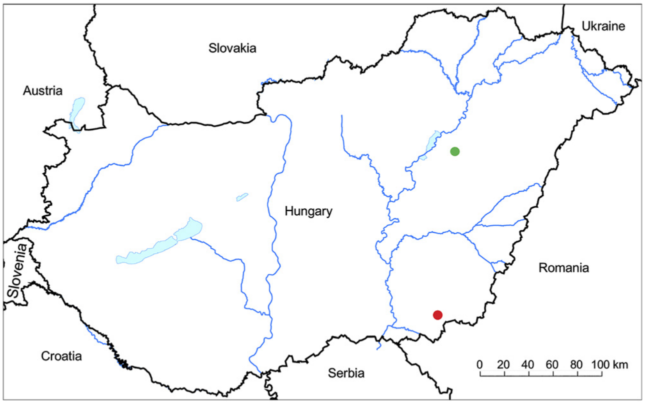

Fig. 1. Collection localities of *Coleophora gazella* used in this study. Red dot: Csanádalberti, green dot: Tiszafüred, Kosárhát. Map by Kristóf Antal (Budapest)

a Carl Zeiss Stemi-2000 binocular stereomicroscope and were edited with the Adobe Photosop CS6 software. The habitat image was taken with a Sony A7R2 camera.

One captured imago was used for making a genitalia preparation that was also photographed. Preparation of the genitalia followed the method of van Nieukerken et al. (2012, 2018).

Molecular methods analysis – For molecular analyses, we obtained DNA from the legs of three adult specimens collected at Csanádalberti and Kosárhát near Tiszafüred (Table 1). The specimens were killed and stored in 70% alcohol and DNA was isolated with E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek, Inc.) according to the recommended protocol of the manufacturer.

Amplification of the gene encoding the cytochrome c oxidase I subunit (COI) was performed with the primers LCO-1490 and HCO-2198 (Folmer et al. 1994). The PCR products were purified using the USB ExoSAP-IT® PCR Product Clean-Up reagent (Affymetrix) and amplicons were sequenced bidirectionally (BaseClear B.V., the Netherlands). The new sequences were assembled with Staden Package 2.0.0b9 (Staden et al. 1998).

To identify the specimens with the examined COI sequences, we performed a holistic search for the most similar sequences using pairwise comparisons and carried out a phylogenetic analysis with the most similar sequences included.

To identify the most similar sequences, we used the BOLD ID search engine (boldsystems.org/index.php/IDS_OpenIdEngine) available on the BOLD version 4.0 platform (accessed on 06/10/2023).

For phylogenetic reconstruction, multiple sequence alignment was carried out by ClustalW (Larkin et al. 2007), with default parameters with the downloaded gene sequences. The most appropriate model of nucleotide substitution was determined with MEGA7 (Kumar et al. 2016) under the Bayesian Information Criterion (BIC). The Tamura's 3-parameter model with Gamma Distribution (T92+G) (Tamura & Nei 1993) was selected for our phylogenetic reconstruction and distance analysis. The phylogenetic tree was constructed using the Maximum-Likelihood (ML) Method implemented in Mega 7 with the default initial rearrangement settings. To obtain an estimate of the support for each node, a bootstrap analysis using 1,000 replicates was performed. Bootstrap support is indicated on the appropriate branches in the ML tree.

Table 1. Coleophora specimens from Hungary used for molecular analyses

Species	Host plant	Locality	GPS coordinates	Date of collection	Collector	Stage	BOLD Accession number	Location of specimens
C. gazella	*Artemisia santonicum* L.	Tiszafüred, Kosárhát	47°31′33.3″N 20°55′08.8″E	30.vi.2021	Adrienn Patalenszki	ex larva, imago	CLGAH001-23	Private collection of Csaba Szabóky
C. gazella		Csanádalberti	46°19′27.1″N 20°41′23.4″E	10.vii.2023	Attila Takács, Cs. Szabóky, C. Plant	imago	CLGAH002-23	Private collection of Attila Takács
C. gazella	*Artemisia santonicum* L.	Csanádalberti	46°19′27.1″N 20°41′23.4″E	09.vii.2023	Attila Takács	ex larva imago	CLGAH003-23	Private collection of Attila Takács

RESULTS

We collected six cases and three adults in the field during the study period. We obtained only two adults reared from the cases. Both were females, one from Tiszafüred and one from Csanádalberti. We collected three additional females near Csanádalberti using artificial light.

MATERIAL EXAMINED

Coleophora gazella Toll, 1952

Fig. 2a–d

Adults: 1 ♀, Hungary: Hajdú-Bihar county, Tiszafüred, Kosárhát, ex. larva on *Artemisia santonicum*, 30.vi. 2021. leg. A. Patalenszki.

1 ♀, Hungary: Csongrád-Csanád county, Csanádalberti, ex. larva on *Artemisia santonicum*, 09.vii. 2023. leg. A. Takács. 3 ♀ Csongrád-Csanád county, Csanádalberti on light, 10.vii. 2023. leg. A. Takács, Cs. Szabóky, C. W. Plant (Fig. 4).

Cases: 11 cases, Békés county, Királyhegyes 09.x.1996, on *Artemisia santonicum*, leg. Cs. Szabóky. 2 cases, Csongrád-Csanád county, Csanádalberti, 15.v.2021, on *Artemisia santonicum*. 2 cases, Csongrád-Csanád county, Csanádalberti 19.v.2022. leg. A. Takács.

Habitat characteristics – The habitats at Királyhegyes-Csikóspuszta, Csanádalberti and Tiszafüred-Kosárhát are very similar. All three are dominated with salt-affected, mostly solonetzic, soils on which various plant communities have developed in response to the variable salt and moisture content. The dominant community at both sites is shortgrass wormwood-steppe dominated with *Artemisia santonicum* and *Festuca valesiaca*. It is interspersed with patches of annual halophytic communities, saline meadows in depressions, and species-rich steppe-like dry meadows on the highest ground.

MORPHOLOGY AND RECORDED HOST PLANTS

Adults – Head and thorax yellowish white. The base of the wings light ochre; antennae segmented, ochre coloured from the base to 1/4 of their length. Palps whitish. Forewings light ochre with alternating darker yellowish-brown lengthwise stripes. Fringes brown. The most prominent stripe below the frontal (leading) edge very narrow at the base gradually widening to the wingtip. The narrow stripe along the sc vein runs from the base to only the 1/5 of the length of the wing. The two stripes are separated by a gradually widening ochreous area grading into white towards wingtip. Below the prominent stripe less clearly defined brown stripes separated by whitish area. Outer margin of wing is bordered by broad ochre area. On the wingtip, there is an ovoid white patch below prominent brown stripe extending onto the fringes. Hindwings light whitish ochre with scattered brown scales that become denser towards wingtip. Abdomen ochre with short abdominal lines. Wingspan 20 mm.

Female genitalia – Anal papillae short and broad with rather dense, long hairs. Posterior apophyses twice as long as anterior ones. Sterigma broad and rounded, wider than its length with caudolateral corners rounded. Inner side of sterigma hairy. Ductus bursae strongly sclerotized. Colliculum broad and long. Signum in corpus bursae well visible and sclerotized.

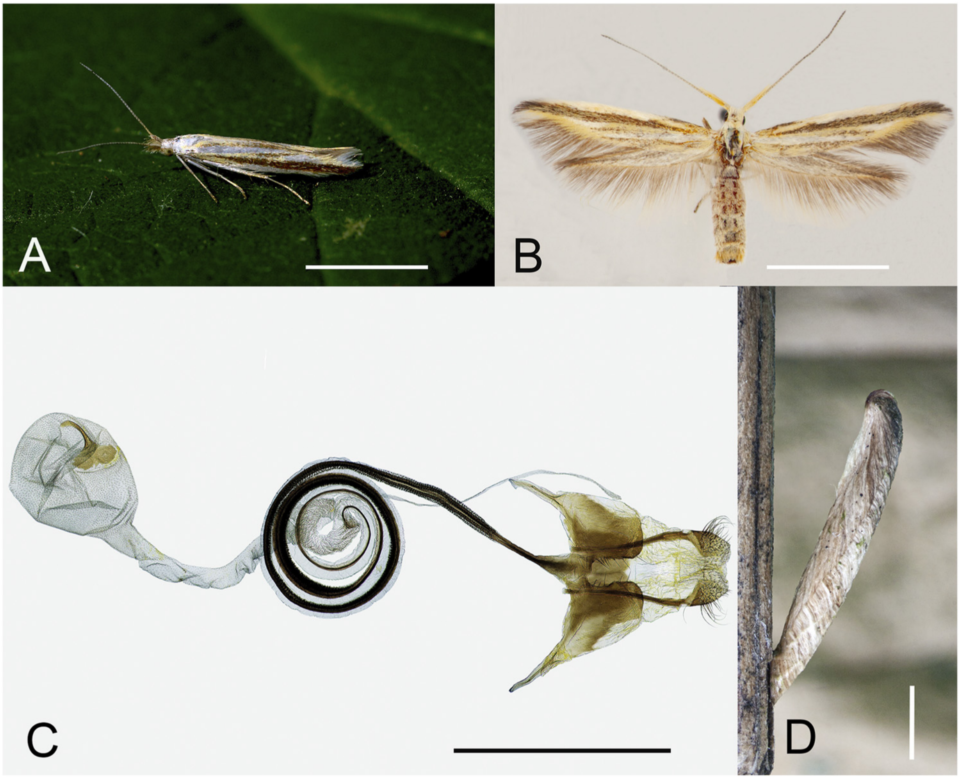

Fig. 2. Coleophora gazella.
a. adult female in resting position. Hajdú-Bihar county, Tiszafüred, Kosárhát, ex. larva on *Artemisia santonicum*, 30.vi. 2021. leg. A. Patalenszki. b. Mounted female. Csongrád-Csanád county, Csanádalberti, ex. larva on *Artemisia santonicum*, 09.vii. 2023. leg. A. Takács. c. Female genitalia. Csongrád-Csanád county, Csanádalberti on light, 10.vii. 2023. leg. A. Takács, Cs. Szabóky, C. W. Plant. Preparation by I. Richter. d. Sheath case of larva in pupation position. Csongrád-Csanád county, Csanádalberti, 01.vi. 2023. leg. A. Takács. Scale bars: Fig a: 2.5 mm Fig b: 4.5 mm. Fig c: 0.16.mm. Fig d: 2.5 mm. Photos: Cs. Szabóky (a), A. Takács (b–d), I. Richter (c)

Cases And Larval Behaviour– The case of the young larva is unknown. The final larval instar has a sheath case, which is 12.0–13.5 mm long with somewhat wrinkled surface. The posterior end rounded, two-valved. The colour of the case is pale chocolate brown with a dark brown patch tinged with pinkish ochre at the posterior end.

The dorsal part is patterned by numerous short, dark and light stripes arranged in several broad and somewhat twisted longitudinal bands. Ventral part chocolate brown turning into conspicuous light brown near the posterior end, where it is flattened. The two parts separated by a noticeable suture. The mouth of the case is at a 30–35° angle with the axis. The case is attached upside down onto the woody parts of the host plants.

The larvae seem to prefer the old and rather tall *Artemisia santonicum* plants with somewhat woody stem and dense foliage. They feed in the interior of the foliage where they mine the leaves.

Based on the morphology of the cases and one adult female reared from larva collected at Tiszafüred, Kosárhát on 30 June 2021 by A. Patalenszki, the unknown taxon was misidentified and published as *C. solenella* (Szabóky & Takács 2021). Later, with more specimens at hand and consulting the available literature on Central Asian Coleophoridae (Falkovich 1988) we concluded that the unknown taxon was more likely to be *Coleophora gazella*.

Parasitoids – No parasitoid specimens have hatched from the collected cases used for rearing adults to date.

MOLECULAR ANALYSES AND SPECIES IDENTIFICATION

The aligned CO1 sequence from the examined females numbered 515 nucleotide positions in the final data set. The sequences of the three Hungarian specimens were identical in the studied region. The search engine did not recover an exact match to this sequence in the BOLD database, but the studied sequence was most similar to that of a *C. gazella* specimen from Russia (ELACA918-11). The two sequences were 99.42% similar and differed only in three positions. The next most similar sequence was coded as *Coleophora* TAB26 with no further information on its taxonomic identity in the BOLD database. For all other species compared, the similarity was below 94% (Table 2).

These results were supported by our phylogenetic reconstruction. The sequence of the studied specimens from Hungary formed a monophyletic clade with the *C. gazella* and *Coleophora TAB26* sequences in the reconstructed ML tree (Fig. 3a). Morphological examination of the female genitalia and its comparison with published illustrations (Toll 1952) also supported the identification of the studied specimen as *C. gazella*. Ignác Richter also identified the specimen as *C. gazella* based solely on genitalia structure using reference material in his possession from Russia and Armenia. We conclude therefore that the unknown *Coleophora* taxon we found in Hungary is most likely to be *C. gazella*. As our results indicate here, our prior identification of one of the females as *C. solenella* was erroneous. *C. solenella* has not been recorded in Hungary to date.

DISCUSSION

The type locality of *C. gazella* is found in Anatolia, Turkey (Akschehir), where the type specimens were collected by Wagner between 10 and 20 August 1928 (Glaser 1971). In addition to

Table 2. Top matches to the unknown Hungarian *Coleophora* specimens in the BOLD System database found by the identification engine

Genus	Species	Similarity	Remarks
Coleophora	*Gazella*	99.42	Published
Coleophora	*TAB26*	97.66	Private
Coleophora	*atromarginata*	93.76	Early-Release
Coleophora	*tiliaefoliella*	93.57	Published
Coleophora	*caelebipennella*	93.57	Published
Coleophora	*albovanescens*	93.18	Private

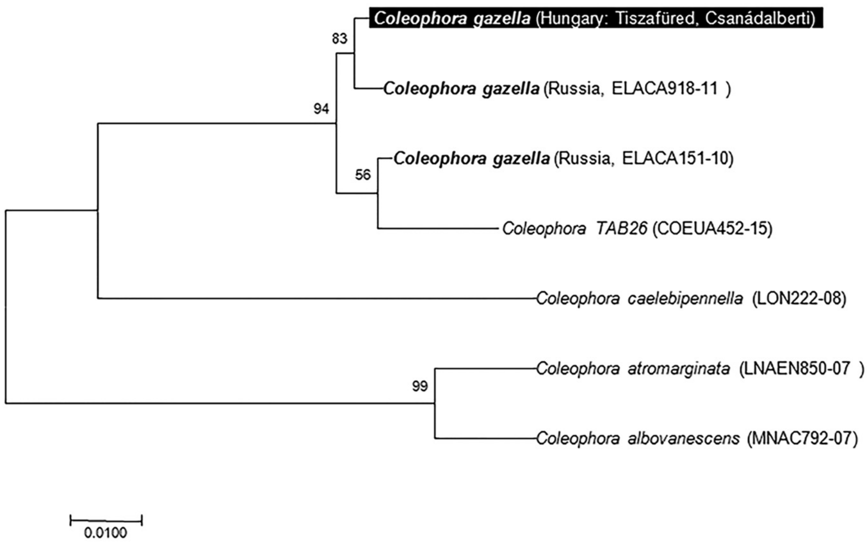

Fig. 3. Maximum-likelihood tree of the sequence from the Hungarian specimens and the six most similar sequences of *Coleophora* taxa. Scale bar indicates 2% T92+G divergence of nucleotide substitution

Fig. 4. Collection localities of Coleophora gazella used in this study in Csanádalberti, picture by Gusztáv Boldog (Békéscsaba)

Turkey, this species also occurs in Afghanistan, Armenia, Iraq, Iran, Russia (Southern Ural), Turkmenistan, Uzbekistan (Kyzil-kum) (Toll 1952, Falkovich 1988, Baldizzone 1994, Baldizzone et al. 2006), and now in Hungary.

Coleophora gazella is one of a number of *Coleophora* species (*Coleophora impalella* Toll, 1961, *Coleophora nigridorsella* Amsel, 1935, *Coleophora klimeschiella* Toll, 1952, *Coleophora eurasiatica* Baldizzone, 1989, *Coleophora remizella* Baldizzone, 1983, *Coleophora nomgona*

Falkovitsh, 1975, *Coleophora santonici* Baldizzone & Takács, 2022 (Baldizzone & Tokár 2008, Buschmann et al. 2014, Buschmann & Richter 2015, Baldizzone et al. 2022) that reach the western boundary of their geographical range in Hungary. It seems to be a habitat specialist in Hungary living only in alkaline wormwood steppe community where it exclusively utilizes *Artemisia santonicum* as its host.

We have collected this species only at two distant locations so far despite the widespread occurrence of its host plant in Hungary. This might be an indication that the species has a disjunct distribution in the country.

Considering its overall distribution pattern and habitat characteristics, we speculate that *C. gazella* could have colonized the Carpathian Basin as early as the end of the Pleniglacial period and survived there in areas where the habitats retained certain characteristics of the Pleniglacial environment.

According to current knowledge, *C. gazella* is distributed in the zones of the dry steppes and wormwood steppes throughout much of its geographical range. The host plants of *C. gazella*, *Artemisia santonicum* in Hungary and *A. turanica* in Central Asia, and their relatives in the subgenus *Seriphidium* (*A. austriaca* Jacq., *A. lercheana* Weber ex Stechm., *A. pauciflora* Weber ex Stechm., *A. sublessingiana* Krasch. ex Poljakov, *A. taurica* Willd.) are the main components of present-day wormwood steppes (Lavrenko et al. 1991, Karamysheva 2003). Currently, these steppes form a continuous vegetation belt only from the Caspian Lowland in Southern Russia to Central Asia and Mongolia between the belts of the dry fescue-feathergrass steppes in the north and the true deserts in the south (Lavrenko et al. 1991, Karamysheva 2003). Related communities with similar structure and species composition also occur in isolated, climatically extrazonal outposts on exposed ridges and solonetz soils along the shores of the Black Sea (Karamysheva 2003) as far west as the Carpathian Basin (Borhidi et al. 2012).

The periglacial steppe also could have provided suitable habitats for *C. gazella*, like the dry steppes and wormwood steppes today. The periglacial steppe shows striking similarities to present-day dry steppes and wormwood steppes in terms of composition. The composition and abundance relations of its pollen profiles (Tarasov et al. 2000) correspond well to the abundance relations at the generic and family level, especially the high abundance of Poaceae, Chenopodiaceae, Asteraceae and particularly the genus *Artemisia* in the dry and wormwood steppes (see Lavrenko et al. 1991, Korolyuk & Lakhtionov 2021).

There is increasing evidence that the periglacial steppe during and shortly after the last glacial maximum formed a nearly continuous belt from western Mongolia to the Carpathians (Tarasov et al. 2000) and even to Hungary (Magyari 2011) providing an almost barrier-free colonization route for the species. In addition, analyses of paleosoils also suggest that suitable habitats might have persisted in the Danube basin throughout the Late Glacial up to the present (Sümegi 2005, Barczi et al. 2006).

To ascertain that *C. gazella* is indeed a relict in Hungary, more detailed knowledge is needed on its current distribution pattern and habitat preference as well as on the ecological characteristics of the periglacial steppe.

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