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***Chrysoclista karsholti*, sp. n., from Turkey, and a new record of *C. germanica* from central Europe (Lepidoptera: Elachistidae: Parametriotinae)**

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Abstract

Chrysoclista karsholti Šumpich, sp. n., is described from a single male collected in Turkey. This species most resembles *C. germanica* Šumpich & Huemer, 2016, but differs in the colouration of the dorsum of the forewing and in the shape of the valva in the male genitalia. Differences in the DNA barcode region between these two species are rather low compared to differences between other species of the genus. *Chrysoclista germanica*, previously known only from the holotype, is recorded from the Czech Republic for the first time. An updated checklist of western Palaearctic *Chrysoclista* Stainton, 1854 is provided.

Key words: Taxonomy, new species, western Palaearctic, barcoding, cytochrome oxidase I, genetic distances

Introduction

The genus *Chrysoclista* Stainton, 1854 was recently reviewed by Šumpich & Huemer (2016), who recognized 11 species and described *C. germanica* Šumpich & Huemer, 2016. They also commented on the rarity of most species of *Chrysoclista*; i.e., five species are known only from males, four of which are known only from the holotype (Šumpich & Huemer 2016). Hence, it is not surprising that a recently described species, i.e., *C. soniae* Corley, 2017 from Portugal, is known only from the holotype (Corley 2017).

During the study of the Lepidoptera collections in the Naturhistorisches Museum, Wien, a very small specimen of *Chrysoclista* was found in the F. Kasy collection. At first glance it resembled *C. gabretica* Šumpich, 2012 in its habitus, but dissection of its genitalia confirmed its close morphological relationship to *C. germanica*. Morphological differences between this small specimen and *C. germanica* are enough to confirm their distinction, and the species is described here as new.

Material and methods

The specimen of *Chrysoclista germanica* discussed herein was captured at a portable light trap with 8-Watt ultraviolet tube. The specimen of *Chrysoclista karsholti*, sp. n., was discovered in the collection of NHMW. Male genitalia were dissected and prepared using standard methods (e.g., Huemer and Karsholt 2010) and studied in glycerol. Terms for genitalia structures follows Koster & Sinev (2003). Photographs of genitalia were taken using a Canon EOS 1100D digital camera attached to an Olympus BX41 stereomicroscope. Pinned specimens were photographed with a Canon 750D + MP-E 65mm f/2,8 1-5x macro lens. Final pictures were edited in Adobe Photoshop®.

One of the goals of this work was to confirm the species status of *C. karsholti*, sp. n., using the 658-basepair fragment of cytochrome oxidase 1 (COI) known as the barcode region. Holotypes of *C. karsholti* and *C. germanica*

were barcoded at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using the Next-Generation Sequencing Technology (NGS protocol) described by Prosser *et al.* (2016). The two samples yeilded 457-basepair sequences. The Czech specimen of *C. germanica* was processed in the molecular laboratory of the National Museum (Czech Republic, Prague). For details regarding sequencing procotols see Vondráček *et al.* (2018). Sequencing of this sample was done in Macrogen Europe (the Netherlands), and a full barcode sequence was obtained (658 basepairs). Sequence data are available on the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007) under their process ID – DEEUR1078-17 (*C. karsholti*), DEEUR1101-17 and DNMPMC301-18 (*C. germanica*). For a more complete genetic assesment, we used all other known public barcodes of the genus *Chrysoclista* available in the public dataset “Lepidoptera of Europe – Chrysoclista” dx.doi.org/10.5883/DS-LEEUCHRY in the BOLD.

Sequences were edited in Geneious software (Kearse *et al.* 2012, <http://www.geneious.com>) and aligned using the Geneious inbuilt algorithm. The best-fit models of molecular evolution for each codon position in the aligned dataset were chosen using PartitionFinder v2.1.1 (Guindon *et al.* 2010, Lanfear *et al.* 2016) with Bayesian information criterion and the “greedy” algorithm (Lanfear *et al.* 2012). A phylogenetic tree was constructed under Bayesian inference with MrBayes 3.2.6 (Ronquist *et al.* 2012), using eight chains (in two runs) of 15×10^6 generations, sampling the chains every 500 generations. Stationarity in MCM chains was determined using Tracer 1.7 (Rambaut *et al.* 2018), and burn-in was set accordingly. Genetic distances were calculated using Kimura 2-parameter model (Kimura 1980) as implemented in MEGA-X software (Kumar *et al.* 2018)

Examined material is deposited in the following collections:

NHMW Naturhistorisches Museum Wien, Austria
RCJJ Research collection of Josef Jaroš, České Budějovice, Czech Republic

Results

Updated checklist of western Palaearctic *Chrysoclista*

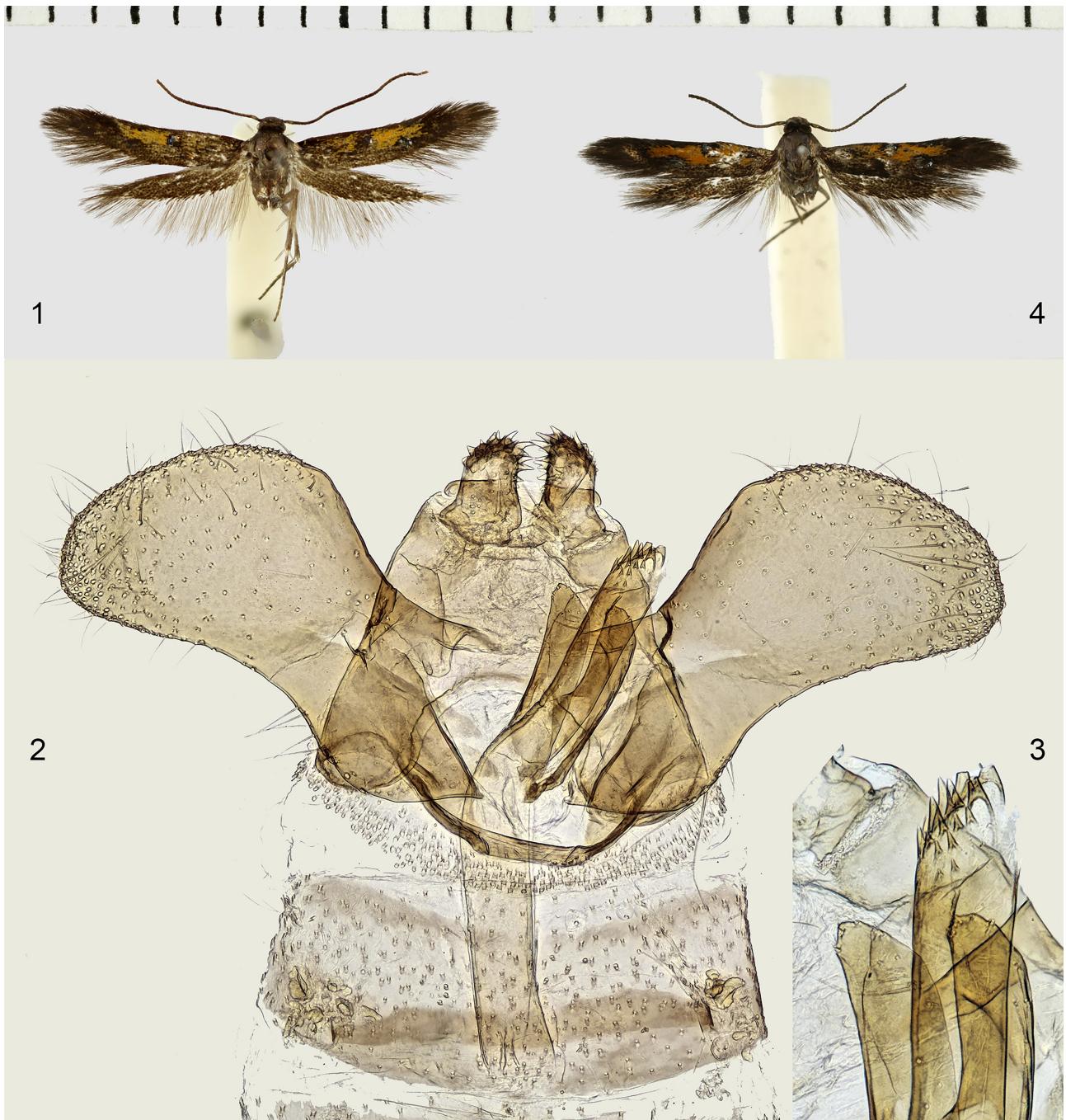
abchasica (Sinev, 1986)
ankaraensis Seven, 2014
gabretica Šumpich, 2012
germanica Šumpich & Huemer, 2016
karsholti Šumpich, sp. n.
lathamella Fletcher, 1936
linneella (Clerck, 1759)
soniae Corley, 2017
splendida Karsholt, 1997
zagulajevi Sinev, 1979

Chrysoclista karsholti Šumpich, sp. n.

(Figs. 1–3)

Type material. Holotype ♂: ‘Anatolien | Kizilcahamam | leg. Pinker | 25.vii.1970’, ‘Photo / | Gen. prep. | J. Šumpich | 18/419’, ‘HOLOTYPE ♂ | Chrysoclista karsholti | Šumpich, 2018’ [red label], coll. NHMW.

Description. Adult (Fig. 1). Wingspan 11 mm. Labial palpus narrow, short, slightly curved, covered with bronze scales on ventral side, and with silvery-grey on dorsal side, tip of third segment brown. Head and frons bronze with metallic shine. Forewing costa, dorsum, basal, apical and subapical areas brown, interspace in middle of wing light orange. Three dark spots with several silver scales in postbasal, tornal, and discal areas. Fringe and hindwing uniform chocolate brown, same shade as brown parts of forewing.



FIGURES 1–4. *Chrysoclista* species. 1. Holotype ♂, of *Chrysoclista karsholti*, Turkey. 2. Male genitalia of *Chrysoclista karsholti*. 3. Detail of the terminal part of phallus of *Chrysoclista karsholti*. 4. *Chrysoclista germanica*, ♂, Czech Republic.

Male genitalia (Figs. 2–3). Valva broad, slightly curved and elongated ventrally, rounded terminally. Phallus slightly bent, without cornuti, and with group of approximately 15 smaller spines in terminal part.

Female genitalia. Unknown.

Molecular data. BIN URI not stated previously. Process ID in BOLD: DEEUR1078-17. According to the phylogenetic tree, *C. karsholti* is sister to the genetically very close *C. germanica* with only 1,10 % difference between them (see Fig. 5 and Table 1). These two species are sister to *C. gabretica* with genetic distances of 7.86 and 7.96 % respectively.

TABLE 1. Mean average genetic distances between main phylogenetic clades defined in Fig. 5 which are corresponding with morphospecies in the genus *Chrysoclista*.

	<i>C. linneella</i>	<i>C. lathamella</i>	<i>C. gabretica</i>	<i>C. karsholti</i> sp. n.
<i>C. lathamella</i>	8,94 %			
<i>C. gabretica</i>	8,19 %	9,31 %		
<i>C. karsholti</i> sp. n.	11,47 %	11,34 %	7,96 %	
<i>C. germanica</i>	11,86 %	11,56 %	7,86 %	1,10 %

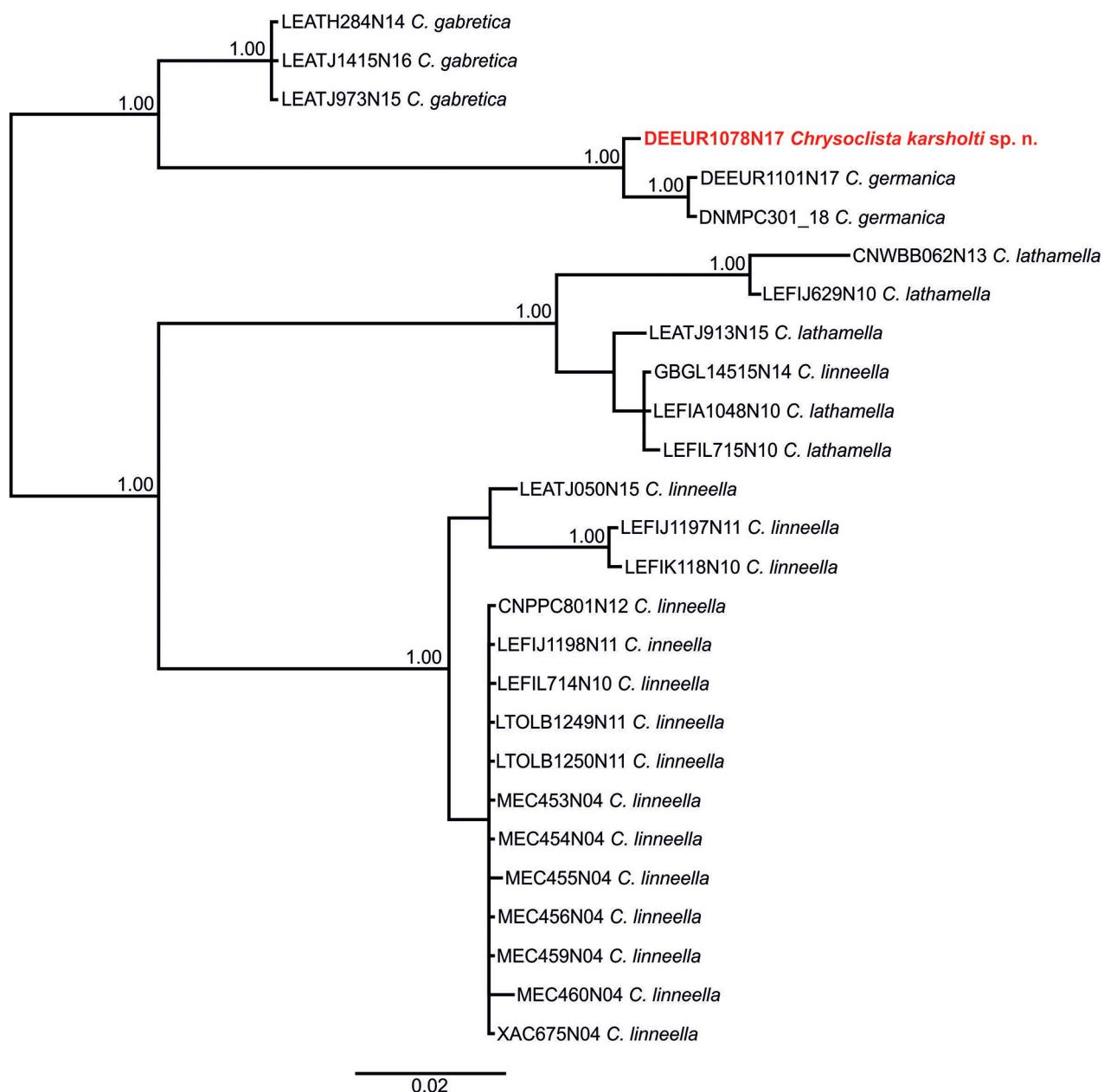


FIGURE 5. Majority-rule consensus tree from Bayesian analysis based on the barcode fragment of all *Chrysoclista* samples. Posterior probabilities are provided only for splits indicating 0.95 or higher probability. Tip labels include Process IDs from BOLD and species names.

Diagnosis. *Chrysoclista karsholti* resembles *C. gabretica*; the orange scales of the forewing of the latter are of the same shade as those of *C. karsholti*, but in *C. gabretica* the orange area reaches the dorsal margin (terminating before the margin in *C. karsholti*). Also, there is a conspicuous basal dark longitudinal streak in *C. gabretica* that is

indistinct in *C. karsholti*. The genitalia of these two species are very similar but the valvae are rounded in *C. gabretica*, whereas they are distinctly elongated dorsoventrally in *C. karsholti*. The shape of the anellus lobes are distinct in both species. *Chrysoclista karsholti* is also similar to *C. germanica*, but in the latter species the orange area is red-orange, and the brown colour is darker. In the male genitalia, the main differences are the shapes of the valvae (much more elongated in *C. germanica*), and the size and number of spines in the terminal part of the phallus (smaller and fewer in *C. karsholti*). *Chrysoclista karsholti* is closely related to *C. germanica* based on the DNA barcode sequences. We cannot exclude the presence of cryptic diversity in this genus as was indicated by Šumpich & Huemer (2016) in which a similar dataset was interpreted in detail. For a clarification of this problem, additional material for genetic and morphological analysis is needed. Because the description of the new species is based on a single specimen, it is possible that *C. karsholti* merely represents variation with *C. germanica*. However, evidence from forewing pattern and genital morphology suggest otherwise.

Distribution. The species is known only from Turkey.

Bionomics. The early stages are unknown. The adult was collected in July. The exact collecting locality of the holotype is unknown - Kizilcahamam is located in an area with altitudes between 1000 m and 1400 m.

Etymology. The name of the new species is a patronym for Ole Karsholt, who has contributed significantly to the research of gelechioid moths, including *Chrysoclista* species.

Chrysoclista germanica Šumpich & Huemer, 2016

(Fig. 4)

Material studied. ♂, Czech Republic, southern Bohemia, Třísov env. (1200 m SE), Vltava valley, 480 m a.s.l., 20.vi.2013, J. Jaroš leg. (RCJJ).

This species was recently described from Germany based only on the holotype. The Czech specimen (9 mm) is smaller than the holotype (12 mm), but its genitalia are nearly identical to those of *C. germanica*. Molecular data of both specimens are available (Process ID in BOLD: DEEUR1101-17 [holotype], DNMPG301-18), and the intraspecific variability in the barcode region is 0.0 %. **New record for the Czech Republic.**

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