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Phtheochroa carpatiana sp. nov. (Lepidoptera, Tortricidae), the Carpathian representative of the *Phtheochroa frigidana* species-group

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Abstract. *Phtheochroa carpatiana* **sp. nov.** is described from the Southern Carpathians (Romania). It is closely related to the other five members of the *P. frigidana* species-group. Adults, male and female genitalia, and the habitat of the new species are described and figured, molecular data and some details of the biology are given. The species inhabits alpine meadows above 2100 m, the highest regions of the Southern Carpathians. Adults are on the wing from the end of June to the beginning of August. *Phtheochroa carpatiana* demonstrates considerable intraspecific variation in some details of the vesica in the male genitalia.

Introduction

Phtheochroa frigidana s. lat. is a species-complex in which the true level of diversity is difficult to ascertain using traditional taxonomic methods. Initially, the group was believed to consist of only two species, the mainly western *P. frigidana* (Guenée, 1845) and the eastern *P. drenowskyi* (Rebel, 1916), both considered as widely distributed in the alpine zone of different European high mountain systems. This approach, however, led to controversial results when identifying individuals from other regions, especially from the Apennines (Trematerra 2003) and the western Balkan Peninsula (Razowski 2009).

A recent study by Zlatkov and Huemer (2017), involving material originating from the majority of the mountain ranges of Europe, resulted in major reinterpretations of the taxonomy of this group. Combining a new morphological technique, the eversion of the vesica in the male genitalia, along with DNA barcode sequencing of the mitochondrial COI gene resulted in five morphologically and genetically different geographically separated species, one from each mountain range involved in the study: *P. cantabriana* Zlatkov & Huemer, 2017 from the Cantabrian Mountains, *P. frigidana* (Guenée, 1845) from the Pyrennees, *P. alpinana* Zlatkov & Huemer, 2017 from the south-western Alps, *P. apenninana* Zlatkov & Huemer, 2017 from the Apennines and *P. schawerdae* (Rebel, 1908) (with *P. drenowskyi* Rebel, 1916 as its junior synonym) from the Dinaric, Rila and Pirin Mountains of the Balkan Peninsula. Additionally, based only on available literature sources, two other neighbouring populations from the Slovenian Alps and the Carpathian Mountains are assumed to be conspecific with *P. schawerdae*.

A subsequent re-examination of the Carpathian material originally identified as *P. drenowskyi* (Karisch and Stănescu 2003; Kovács and Kovács 2005) raised questions regarding the conspecificity of these specimens with *P. schawerdae*, as small but constant differences were found both in the external morphology and in the male and female genitalia. Further arguments for the description of a new species from the Southern Carpathians were provided by the analysis of DNA barcode sequences and examination of the everted vesica in the male genitalia, following the same procedures as in the revision by Zlatkov and Huemer (op. cit.).

Material and methods

Eighty-six specimens were examined that were collected from three of the four major mountain ranges of the Southern Carpathians: the Bucegi, Făgăraș and Parâng Mountains. The examined material was dried, pinned and set. The terminology of wing pattern follows Razowski (1970, 2009) and Zlatkov and Huemer (2017). Genitalia preparation techniques followed the methodology of Robinson (1976). Vesicae were dissected and everted under a Carl Zeiss stereomicroscope Stemi 2000c following Zlatkov (2011). The everted vesicae were drawn, photographed and measured following Zlatkov and Huemer (2017) through a Carl Zeiss Jena Amplival compound microscope equipped with a camera lucida and Canon EOS 1300D digital camera. The female genitalia were drawn in a similar manner after being placed on a cavity slide without compression. The description of the phalli follows the same publication (Zlatkov and Huemer op. cit.); terminology of the cornuti follows Anzaldo et al. (2014). Samples of the everted vesicae from all the collecting sites were studied to assess possible geographical variation. More specimens were also examined from the Făgăraș Mountains to detect potential intraspecific variability.

DNA samples from three specimens were prepared according to the prescribed standards and processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) to obtain DNA barcodes following the standard high-throughput protocol described in deWaard et al. (2008). Two of the resulting sequences (629 bp and 558 bp) and a shorter sequence (307 bp) were analysed together with 11 sequences already published by Zlatkov and Huemer (2017) and a sequence of *Phtheochroa rugosana* (Hübner, 1799) used as an outgroup. Sequences were submitted to GenBank; further details including complete voucher data and images can be accessed in the public dataset "*Phtheochroa frigidana* species-group [DS-AALARANE]" https://doi.org/10.5883/DS-AALARANE in the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007). Degrees of intra- and interspecific variation of DNA barcode fragments were calculated under the Kimura 2 parameter model of nucleotide substitution using analytical tools of BOLD systems v. 4.0. (http:// www.boldsystems.org). A Neighbor-joining tree of DNA barcode data of currently sequenced taxa was constructed using MEGA6 (Tamura et al. 2013) under the K2P model for nucleotide substitutions.

Photographs of the adults were taken using a Canon EOS–6D camera with a Sigma 105 mm lens and focus stacking method. Photos of the habitats and adults in nature were taken using Sony DSC–H2 and DSC–W830 digital cameras.

Institutional abbreviations

NMNHS National Museum of Natural History, Sofia, Bulgaria;
TLMF Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria.

Results

Phtheochroa carpatiana Kovács, Kovács, Zlatkov & Huemer, sp. nov.

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Figs 1–6, 12

Type material. ***Holotype.*** Romania; • ♂; Carpaţii Meridionali, Munţii Făgăraş, Căldarea Bâlea; [45°36’12”N, 24°37’18”E]; 2100–2200 m; 17 Jul. 2019; S. & Z. Kovács legit & coll. (Miercurea Ciuc) (Fig. 1).

Paratypes. 78 ♂♂, 7 ♀♀, all collected by S. & Z. Kovács and if not otherwise mentioned are deposited in the collection of S. & Z. Kovács (Figs 2–3).

Romania; • ♀; Carpaţii Meridionali, M[un]ţii Făgăraş, Bâlea; 2100 m; 9 Aug. 1992; genit. prep. no. 468/♀/ Kovács (1998); • 3 ♂♂; Carpaţii Meridionali, Munţii Făgăraş, Vârful Lăiţel; 2300 m; 21 Jul. 2015; • 40 ♂♂, 3 ♀♀; Carpaţii Meridionali, Munţii Făgăraş, Căldarea Bâlea; 2100–2200 m; 14 Jul. 2016; genit. prep. no. 1909/♂/ and 1910/♀/ Kovács (2017), genit. prep. no. 1/3.2.2020/♂ and 3/20.1.2020/♀/ Zlatkov; [Barcode identification number] TLMF Lep 27414, TLMF Lep 27444; 1 ♂, 1 ♀ coll. TLMF, 1 ♀ coll. NMNHS; • 24 ♂♂; same data as for preceding; 17 Jul. 2019; genit. prep. no. 2262/♂/ Kovács (2019), genit. prep. no. 1/20.1.2020/♂, 2/3.2.2020/♂ and 3/3.2.2020/♂/ Zlatkov; 1 ♂ coll. NMNHS; • 3 ♂♂, 2 ♀♀; same data as for preceding; 18 Jul. 2019; • ♂; Carpaţii Meridionali, Munţii Făgăraş, Iezerul Caprei; 2350 m; 17 Jul. 2019; • 2 ♂♂, 1 ♀; Carpaţii Meridionali, Munţii Bucegi, V[âr]f[ul]. Caraiman; 2380 m; 27 Jun. 2003; genit. prep. no. 1486/♂/ Kovács (2017); • 2 ♂♂; same data as for preceding; 9 Jul. 2013; genit. prep. no. 1/4.2.2020/♂/ Zlatkov; [Barcode identification number] TLMF Lep 27445; 1 ♂ coll. NMNHS, 1 ♂ coll. TLMF; • ♂; same data as for preceding; 2300 m; 22 Jul. 2006; • 2 ♂♂; Carpaţii Meridionali, Munţii Parâng, Mohorul; 2100–2200 m; 13 Jul. 2016; genit. prep. no. 2/20.1.2020/♂/ Zlatkov; 1 ♂ coll. NMNHS.

Diagnosis. *Phtheochroa carpatiana* male is characterized by the yellowish grey colour of the forewing with indistinct reddish brown markings and the female by its yellow coloured forewing with distinct markings. The male genitalia are also distinct: the apex of the ventral phallic process is curved to the right at about 45 degrees in ventral view and slightly sinuous in both ventral and lateral view, and the diverticula of the vesica are in dorso- and ventrolateral positions. The female genitalia are characterized by the short ductus bursae, wide ventral diverticulum of the ductus bursae, and ellipsoidal corpus bursae.

Description. Male (Figs 1, 2). Head. Frons and vertex covered with yellow scales, reddish brown around eye and scapus. Labial palpus about 2.5 times length of diameter of eye; first segment short; second segment long and wider; third segment slightly shorter than the second, all with long grey scales densely covered with pale yellow scales on the medial surface and reddish brown scales on the lateral surface. Antennae filiform, reaching 1/2 of the forewing’s length, dark brown, scapus and first two flagellar segments covered with reddish brown scales, the rest of the flagellum covered with yellow scales on the dorsal surface.

Thorax. Dorsally covered with yellow scales, similar to tegulae and the head, the lateral edge of tegulae reddish brown. Forewing length of holotype 10 mm and that of the paratypes between 7.5 and 10.5 mm. Forewing long and trapezoidal, narrower at base and wider terminally, termen slightly concave, apex pointed; ground colour yellowish grey; faint reddish brown markings consisting of: a small diffuse spot at base of wing extending along the subcostal vein to 1/5 of the forewing’s length; a dorsal patch extending from dorsum to mid-cell; a narrow, apically curved subterminal fascia extending to M_1 vein; fringe light yellow with grey basal line. Hindwing dark grey, with a faint reddish brown longitudinal line along the median cell and M_2 vein; fringe light yellow with a

Figure 1. *Phtheochroa carpatiana* sp. nov., male, holotype, wingspan: 21 mm, Făgăraş Mountains, Căldarea Bâlea, 2100–2200 m, 17.vii.2019, photograph Z. Csata.

Figure 2. *Phtheochroa carpatiana* sp. nov., male, paratype, wingspan: 19 mm, same data as for holotype, photograph Z. Csata.

Figure 3. *Phtheochroa carpatiana* sp. nov., female, paratype, wingspan: 19 mm, same data as for holotype, but 18.vii.2019, photograph Z. Csata.

broad grey basal line. Underside of thorax dark grey, the similarly coloured forewing pale yellow at termen and apex, hindwing dark grey at base and the subcostal area, light yellow along M_2 vein and yellowish grey on the external parts of the wing. Legs yellowish grey similar to the forewing.

Abdomen covered with dark grey scales, last segment reddish brown dorsally.

Male genitalia (Figs 4, 5A, B, 6). Uncus tapered apically, strongly sclerotized. Tegumen rounded. Socii rounded. Valva broad, curved dorsally, valva width uniform. Transtilla dorsally spinulous, apically with median indentation. Phallus broad, slightly curved ventrally, ventral phallic process moderately long, slightly sinuous in both ventral and lateral view, in ventral view moderately wide and with apex curved to the right at about 45 degrees (Fig. 6A, C). Vesica with large main part protruded to the right, and two large diverticula each terminating with aciculate, capitate, robust cornutus. Small diverticulum with uneven surface emerges ventrally from main part of vesica. Gonopore located dorsally, surrounded by sclerotized wrinkled plate. Main part of vesica dorsally constricted by median furrow into which gonopore is sunk. Portion located distally of furrow large, protruding dorsally. Large diverticula emerge from right portion of main part of vesica in opposite directions. Right diverticulum directed dorsolaterally terminating with larger cornutus (435–510 μm, mean 469; n = 6), left diverticulum pointed ventrolaterally bearing smaller cornutus (385–448 μm, mean 408; n = 6). Smaller cornutus ca 0.5× the length of phallus and 0.8–0.9× the length of longer cornutus. Axis of right diverticulum and sclerotized phallus form angle of 55–90° (mean 76; n = 6). Barely discernible acanthae cover left portion laterally, right portion dorsally and apical third of large diverticula (Figs 4B, C, 5A, B).

Female (Fig. 3). Forewing length 6.5–9 mm. Forewing parallel sided, apex more pointed compared with male, ground colour yellow, markings distinct. Abdomen grey, densely covered with dispersed yellow scales, last segment reddish brown.

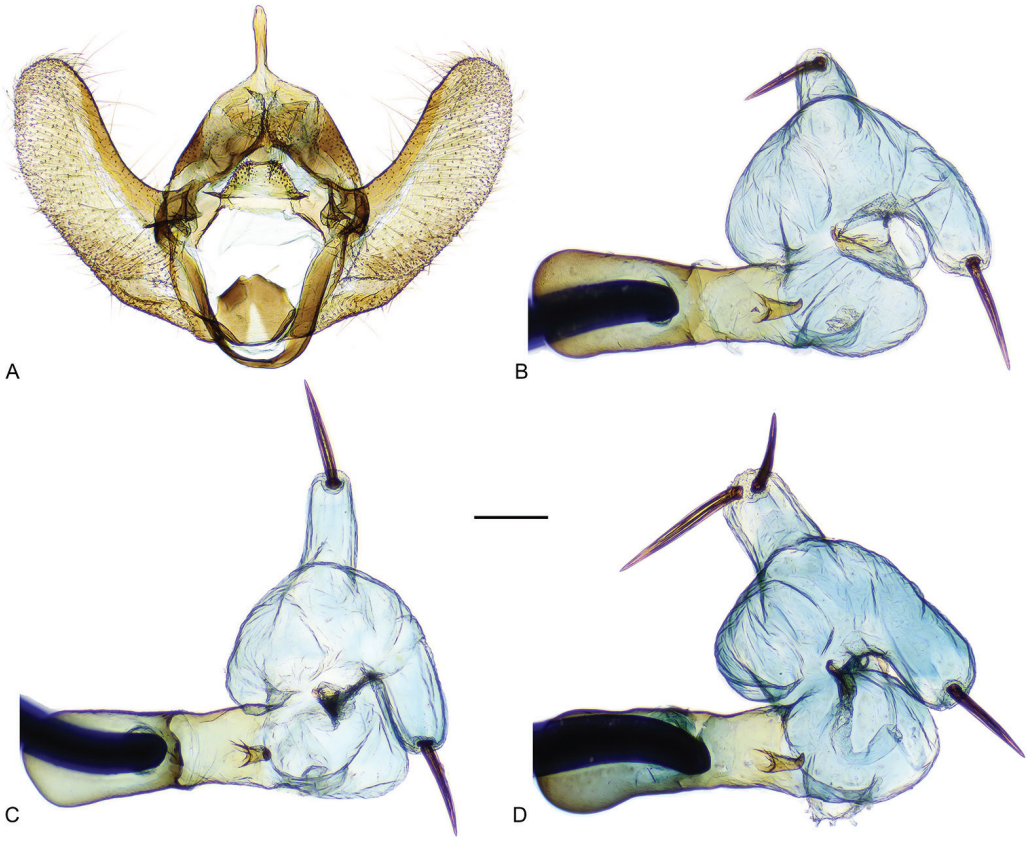

Figure 4. Male genitalia of *Phtheochroa carpatiana* sp. nov., paratypes. **A.** genitalia without phallus, Făgăraș Mountains, genitalia slide 1/20.1.2020; **B–D** variation of the phallus with vesica everted in dorsal view: **B.** Făgăraș Mountains, genitalia slide 1/3.2.2020; **C.** Bucegi Mountains, genitalia slide 1/4.2.2020; **D.** specimen with two cornuti on the right diverticulum, Făgăraș Mountains, genitalia slide 3/3.2.2020. The black object in the proximal part of the phallus is the tip of a minute needle used to fix the structure in position. Scale bar: 250 μm, all to scale.

Female genitalia (Fig. 5C). Papillae anales large, apophyses anteriores slightly shorter than apophyses posteriores. Tergum 8 medially membranous, sterigma with two spatulate protuberances. Antrum trapezoidal, with a shallow posterior incision. Ductus bursae short, with a wide subtriangular diverticulum on the membranous ventral wall, and a sclerite extending from the corpus bursae ending on its dorsal wall. Corpus bursae ellipsoidal, with a large, moderately sclerotized ribbon-like sclerite. Sclerite starting on the right ventral side of the corpus bursae with a few (ca 5) longitudinal folds, from there expanding anteromedially, then bending posterodorsally continuing on left side of corpus bursae and ending on dorsal wall of ductus bursae.

Variability. The dorsal patch and subterminal fascia are not distinct in some male specimens (Fig. 2). One female has a deep yellow forewing without markings. In worn specimens the yellow and reddish brown colour become almost indistinguishable and so these moths look more or less uniformly grey. The male genitalia (n = 12) show small variations: the ventral phallic process is not sinuous in one

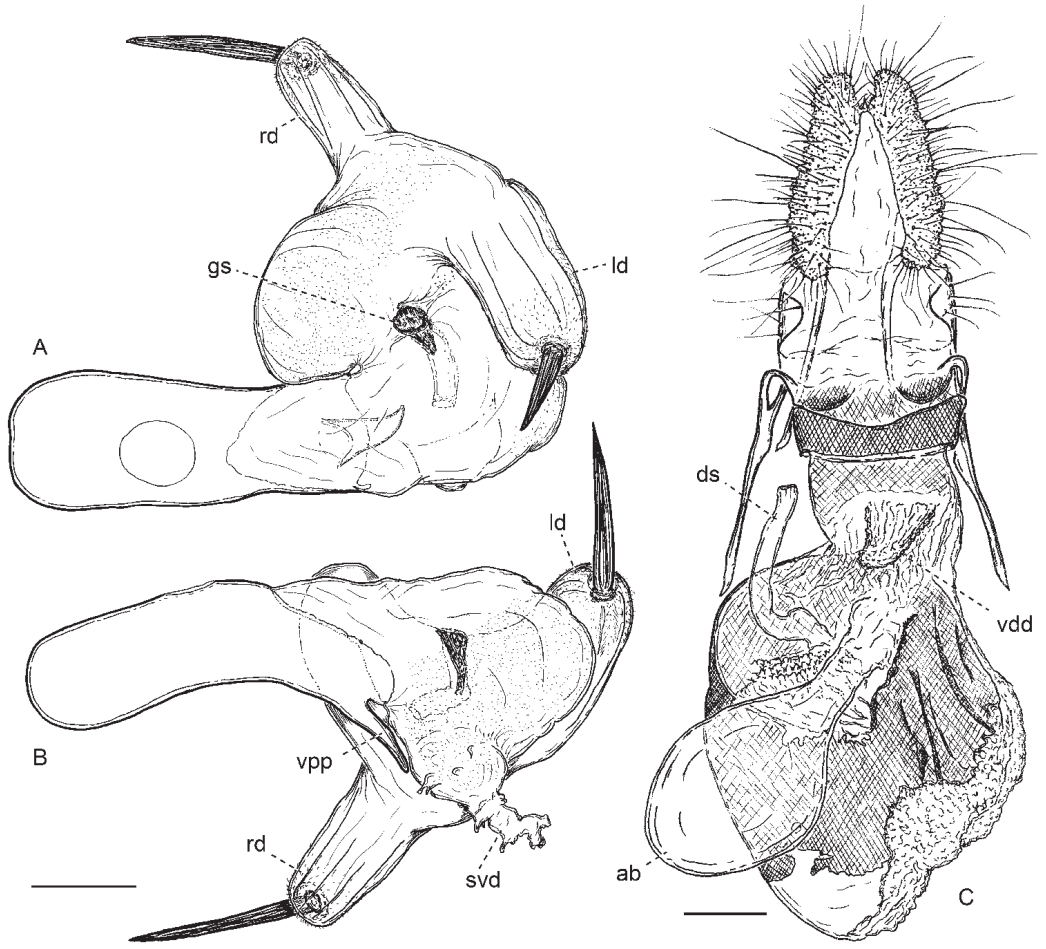

Figure 5. Male (phallus with vesica everted) and female genitalia of *Phtheochroa carpatiana* sp. nov. **A.** phallus in dorsal view, Făgăraș Mountains, genitalia slide 1/20.1.2020; **B.** phallus in left view, the same specimen; **C.** female genitalia, Făgăraș Mountains, genitalia slide 3/20.1.2020. **ab** – accessory bursa; **ds** – ductus seminalis; **gs** – gonopore sclerotization; **ld** – left diverticulum; **rd** – right diverticulum; **svd** – small ventral diverticulum; **vdd** – ventral diverticulum of ductus bursae; **vpp** – ventral phallic process. Scale bars: 250 µm, **A** and **B** to scale.

specimen (Fig. 6B); the right diverticulum may be oriented at a different angle towards the sclerotized phallic tube; the orientation of the large diverticula varies from nearly dorsoventral to almost lateral; in some specimens the cornuti are slightly curved (Fig. 4B, C); one specimen had two cornuti of normal size on the right diverticulum and diverticula with larger diameters (Fig. 4D). The female genitalia (n = 3) show small variations in the shape of the antrum as it is nearly rectangular in one specimen.

Molecular data. BIN URI: BOLD:AEA2346. No intraspecific variation in the barcode region was observed (n = 3). The minimum distance to the Nearest Neighbor, *P. schawerdae* from the Dinaric Mountains, is 3.14 % (Fig. 7).

Systematic position. Because of its external morphology and genitalia structure, *Phtheochroa carpatiana* should be placed between *P. alpinana* and *P. apenninana*.

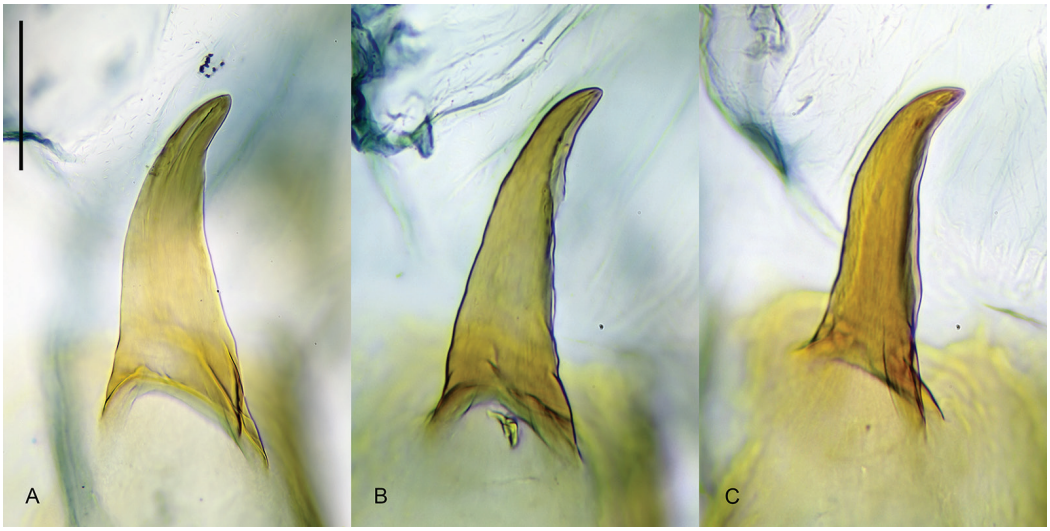

Figure 6. Variation of the ventral phallic process in *Phtheochroa carpatiana* sp. nov., all specimens originate from Făgăraș Mountains. **A.** genitalia slide 1/20.1.2020; **B.** genitalia slide 1/3.2.2020; **C.** genitalia slide 2/3.2.2020. Scale bar: 100 µm.

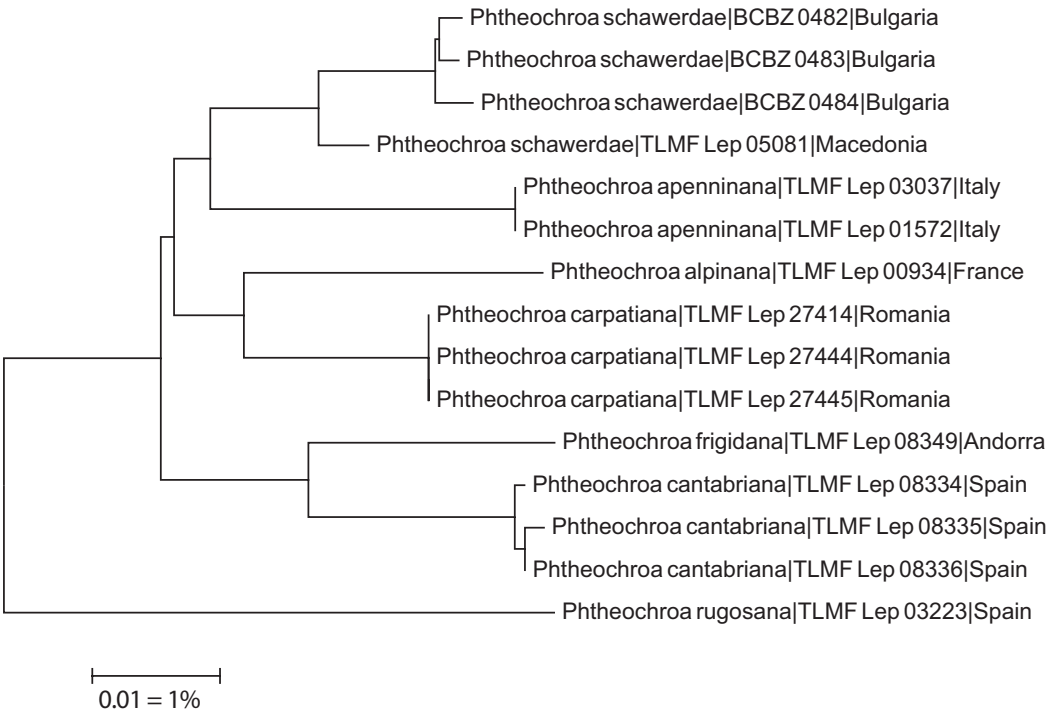

Figure 7. Neighbor-joining tree (Kimura 2 parameter) of members of the *Phtheochroa frigidana* species group with *P. rugosana* as outgroup.

Figure 8. The habitat of *Phtheochroa carpatiana* sp. nov., Făgăraş Mountains, general view, 2100–2500 m, westwards of Iezerul Caprei Peak.

Habitat. *Phtheochroa carpatiana* inhabits the highest mountains in the Romanian Carpathians. The moths can be found in the alpine zone from 2100 to 2380 m above sea level. The substrate is silicate in Făgăraş (Fig. 8) and the Parâng Mountains (Fig. 9), and conglomerate in the Bucegi (Fig. 10). This species prefers sunny meadows, thickly covered with grasses (Fig. 11). In most of its collecting sites it is found with other Carpathian endemic species such as *Dichrorampha carpatalpina* Kovács & Kovács, 2019 (Tortricidae) and *Catoptria orientellus* (Herrich-Schäffer, [1855]) (Crambidae).

Phenology. Univoltine, adults are on the wing from the end of June to the beginning of August, being highly dependent on general weather conditions.

Biology. The early stages and the host-plant are unknown. Adults fly during the day in sunshine or they rest on the upper part of grasses. In cloudy weather they immediately hide deep in the vegetation. The flight of the males is not fast, and only when disturbed do they fly fast and hide deep within the vegetation. Females call on the vegetation. They were much more rarely collected than males: when we observed freshly emerged females we counted 40 males and only 4 females. One pair in copula was also observed on low vegetation (Fig. 12). Following the end of the copulation with the first male, a second attracted male immediately started to mate with the female. *Phtheochroa carpatiana* does not seem to be active by night as none were collected by light traps installed at sites where specimens were observed during the same day.

Figure 9. The habitat of *Phtheochroa carpatiana* sp. nov., Parâng Mountains, general view, 2100–2500 m, westwards of Mohorul Peak.

Distribution. *Phtheochroa carpatiana* seems to be widespread and locally common in the three main mountain ranges in the Southern Carpathians: Bucegi, Făgăraş and Parâng (Fig. 13). It may also occur in the other parts of the Southern Carpathians, at least in the very similar Retezat Mountains, where we made only one unsuccessful attempt to find it. *Phtheochroa carpatiana* replaces *P. drenowskyi* in the checklist of the Romanian Lepidoptera (Rákosy and Goia 2007).

Etymology. The specific name is a feminine adjective derived from the name of the Carpathians, the mountain range where the new *Phtheochroa* species was discovered.

Discussion

The members of the *Phtheochroa frigidana* species-group are geographically separated and their collecting sites are a guide to their identification. Externally the species are similar, and only the genitalia provide reliable diagnostic characters in this group. In ventral view of the male genitalia the ventral phallic process is straight in *P. frigidana*, curved to the right at almost 90° in *P. schawerdae* and almost similarly curved (to around 45°) in all other species: it is widest in *P. apenninana*, slenderer in *P. cantabriana*, evenly narrowing in *P. alpinana*, and slightly sinuous in *P. carpatiana*. The everted vesica has a single dorsal diverticulum in *P. frigidana*, but in all other species has two distinct diverticula which differ in position: in *P. alpinana* dorsal and ventral, in *P. carpatiana*

Figure 10. The habitat of *Phtheochroa carpatiana* sp. nov., Bucegi Mountains, Caraiman Peak, 2380 m, west-facing slope.

dorso- and ventrolateral, in *P. apenninana* lateral, and distal and lateral with the angle between the axes of the right diverticulum and the sclerotized phallus smaller (100–120°) in *P. schawerdae* and larger (120–140°) in *P. cantabriana*.

The female genitalia are unknown in *P. apenninana* and *P. cantabriana*; the ductus bursae is longer in *P. frigidana*, *P. alpinana* and *P. schawerdae* and shorter in *P. carpatiana*. The small membranous diverticulum of the ductus bursae is long and slender in *P. schawerdae*, shorter and wider in *P. alpinana* (Zlatkov and Huemer op. cit.), widest in *P. carpatiana* and has not been figured in *P. frigidana*. Female genitalia figures referring to the *P. frigidana* species-group in the literature before Zlatkov and Huemer (op. cit.) are incomplete, as they do not depict the membranous diverticulum on the ventral wall of the ductus bursae (Razowski 1961, 1970, 2002, 2009; Karisch and Stănescu 2003; Kovács and Kovács 2005).

The paucity of available material of most species is the major hindrance to a thorough study of the group. *Phtheochroa carpatiana* is the only species with a larger type series allowing a study of variability, which appears to be much higher than expected. The variation of the size of *P. carpatiana* is the same as in all other related species of the *P. frigidana* species-group. The variable shape of the transtilla in specimens of the same population has already been mentioned in *P. schawerdae* by Zlatkov and Huemer (op. cit.) and observed in the case of the

Figure 11. The habitat of *Phtheochroa carpatiana* sp. nov., Făgăraș Mountains, Căldarea Bâlea, 2100–2200 m, west-facing slope.

vesica and antrum of *P. carpatiana* during the present study. The presence of two cornuti of normal size and development on the right diverticulum instead of one, and diverticula having larger diameters in a specimen from the Făgăraș Mountains is remarkable. Such a condition has never been found either in any species of the *P. frigidana* species-group or in other Cochylini groups with two cornuti, each attached to its own diverticulum (*P. reisseri* (Razowski, 1970), *P. unionana* (Kennel, 1900), *P. procerana* (Lederer, 1863), *Eugnosta magnificana* (Rebel, 1914), *E. lathoniana* (Hübner, [1800])). In general, *P. carpatiana* demonstrates variation in some details of the vesica. The lack of detected variation in other species of the *P. frigidana* species-group may be due to the small number of available males (e.g., only two males are known of *P. alpinana*). It must be emphasized that the observed variation in *P. carpatiana* is not geographically related. Another representative in the tribe Cochylini, *Eugnosta magnificana*, also demonstrates remarkable variation of the vesica, mainly in its size (Zlatkov 2018). Since there are no particular studies on variation of the phallus and especially the vesica in the Cochylini or other Tortricidae groups, any further conclusions on the variation of the vesica in *P. carpatiana* would be speculative.

Interspecific DNA barcode divergences to the Nearest Neighbor in the *P. frigidana* species-group roughly range from 3 % to 4 % (Table 1) though based on low sampling. Where such high levels of barcode variation are found within a series believed to be from a single species, it often reflects

Figure 12. *Phtheochroa carpatiana* sp. nov., pair in copula, Făgăraş Mountains, Căldarea Bâlea, 2100–2200 m, 14.vii.2016.

Table 1. Barcode gap analysis in the *Phtheochroa frigidana* species-group. For each species the number of barcoded specimens are given (n), the mean and maximum intra-specific values are compared to the Nearest Neighbor (NN) distance (in %). For singletons N/A is displayed for intra-specific values.

Species	n	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Distance to NN
Phtheochroa apenninana	2	0	0	*Phtheochroa schawerdae*	3.93
Phtheochroa cantabriana	3	0.2	0.31	*Phtheochroa frigidana*	3.62
Phtheochroa alpinana	1	N/A	0	*Phtheochroa carpatiana*	3.47
Phtheochroa frigidana	1	N/A	0	*Phtheochroa cantabriana*	3.62
Phtheochroa schawerdae	4	0.98	1.61	*Phtheochroa carpatiana*	3.14
Phtheochroa carpatiana	3	0	0	*Phtheochroa schawerdae*	3.14

overlooked species or at least requires further integrative analysis (Hausmann et al. 2013), but there is no fixed level of divergence that indicates species status (Kekkonen et al. 2015).

It is notable that the flight period for *P. frigidana* mentioned by Razowski (1970, 2002, 2009) is from April to July, which is not reflected by the material in recent studies, as the collecting dates of all six currently known species in the *P. frigidana* species-group are between the end of June and beginning of August. Further studies are necessary to clarify which one of the species of the *P. frigidana* species-group has an earlier flying period.

The status of two specimens from the Slovenian Alps, one mentioned as *P. frigidana* by Lesar et al. (2009) and another deposited in the J. Klimesch collection (Lepiforum), thought to be *P. schawerdae* by Zlatkov and Huemer (op. cit.) is uncertain.

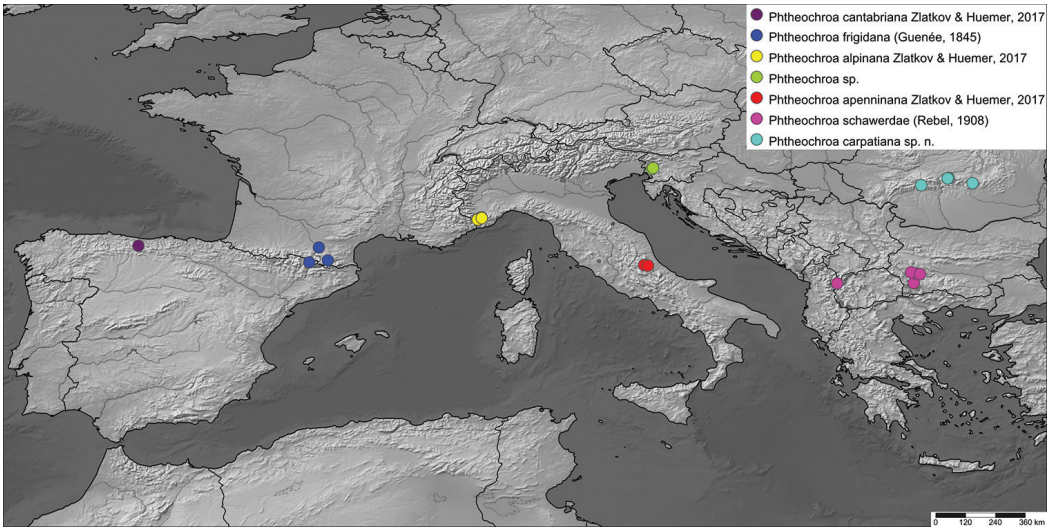

Figure 13. Distribution map of the *Phtheochroa frigidana* species-group based on examined material (after Zlatkov and Huemer 2017, modified) and literature sources for *Phtheochroa* sp. from the Slovenian Alps. Map created with SimpleMappr (http://www.simplemappr.net).

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