

***Phtheochroa unionana* (Kennel, 1900) recognised as a dimorphic Cochylini species, with description of the hitherto unknown male genitalia (Lepidoptera, Tortricidae)**

BOYAN ZLATKOV¹, PETER HUEMER²

1 Sofia University St. Kliment Ohridski, Faculty of Biology, Dept. of Zoology and Anthropology, 8 Dragan Tsankov Blvd., 1164 Sofia, Bulgaria; bzlatkov@gmail.com

2 Tiroler Landesmuseen Betriebsgesellschaft m.b.H., Naturwissenschaftliche Sammlungen, Feldstr. 11 a, A-6020 Innsbruck, Austria; p.huemer@tiroler-landesmuseen.at

<http://zoobank.org/1F242A64-5F8B-43DB-BDAF-CE27FBD64181>

Received 29 April 2016; accepted 3 August 2016; published: 24 August 2016

Subject Editor: Jadranka Rota.

Abstract. The previously unknown male genitalia of *Phtheochroa unionana* (Kennel, 1900) are described and illustrated. The species is dimorphic: one form is white with very faint yellow scales in the fascial areas and the other is white with distinct orange fasciae. The everted vesicae of the males do not show interspecific variation but are remarkably different from those of a closely related species. Apparently, the morphology of the everted vesica is a useful tool for species recognition in this genus. The conspecificity of the two forms of *P. unionana* is further corroborated by evidence from COI barcodes.

Introduction

The genus *Phtheochroa* Stephens, 1829, comprises 107 species worldwide (Gilligan et al. 2014) and 53 species with a Palaearctic distribution (Razowski 2009). No synapomorphies are known for the genus, but some groups of species demonstrate clear morphological affinities (Razowski 1991). According to Razowski (1991), *P. unionana* (Kennel, 1900) belongs to a group of *ca.* 13 species defined by a simple valva without a free termination of the sacculus. In this group several species externally show similarity with *P. unionana*. Their genitalia are considerably simplified in comparison to other *Phtheochroa* spp. and species recognition sometimes is difficult. Further studies of the genitalia morphology combined with molecular data probably will reveal other problems and unknown facts for this group.

During an expedition to Armenia in 2014 a single male of a pure white *Phtheochroa* was collected. The genitalia of the specimen did not fit any known species, which, combined with the forewing colour, convinced us that this was a male *P. unionana*. Study of additional material also collected from Armenia revealed other *P. unionana* specimens. Unexpectedly, the genitalia of an undetermined *Phtheochroa* from the same area with orange fasciae were nearly identical to those of *P. unionana*, indicating conspecificity of the two forms, a hypothesis supported by subsequent DNA barcoding.

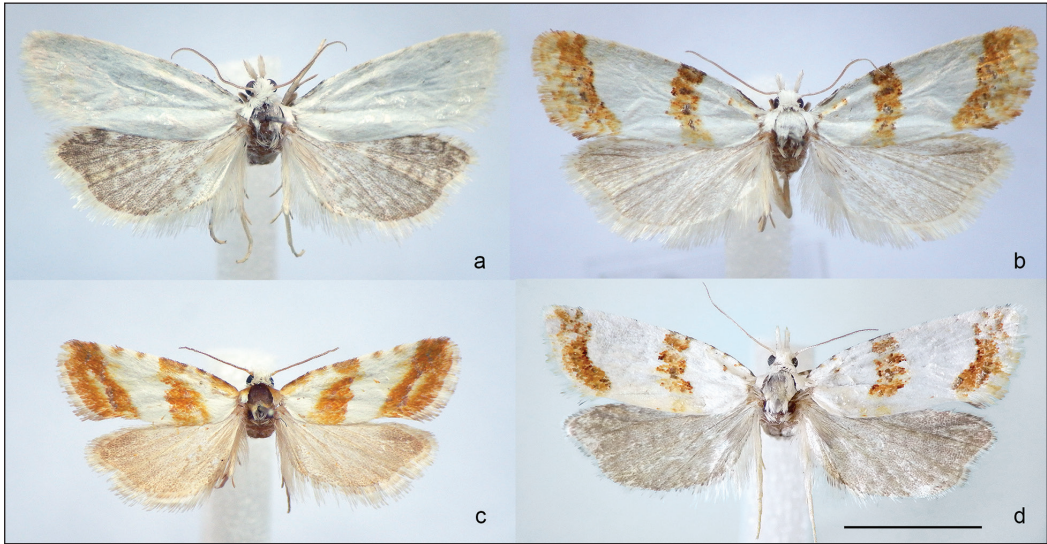


Figure 1. *Phtheochroa* adults. **a, b, d**, *P. unionana*. **a**. Male, Armenia, Dilijan, 12–14.vii.2011. **b**. Male, Armenia, Tsaghkadzor, 9–11.vii.2011. **d**. Female, Georgia, Lesser Caucasus, 28.vii.2014. **c**. Male of *P. procerana*, Bulgaria, Balchik, 21.vi.2007. All figures to the same scale, scale bar = 5 mm.

Material and methods

Material examined: *Phtheochroa unionana*: Armenia: 4 ♂♂ (2 white, 2 fasciate), Tavush region, Dilijan, N40°45', E44°51', 1340–1450 m, 12–14.vii.2011, leg. O. Karsholt, coll. ZMUC; 1 ♂ (fasciate form), Kotayk region, Tsaghkadzor, N40°32', E44°32', 1870–2350 m alt., 9–11.vii.2011, leg. O. Karsholt, coll. ZMUC; 1 ♂ (white form), Lori region, Lermontovo vill., N40°44'55", E44°39'40", 1860 m alt., 29.vii.2014, at light, leg. B. Zlatkov & D. Chobanov, coll. BFUS. Georgia: 1 ♀ (fasciate form), Lesser Caucasus, Kvemo Kartli region, Sakire, N41°14'13", E44°17'02", 1260 m alt., 28.vii.2014, leg. B. Zlatkov & D. Chobanov, coll. BFUS. *Phtheochroa procerana* (Lederer, 1863): Bulgaria: 1 ♂, Bulgaria, Black Sea coast, Balchik–Kavarna, N43°24'21", E28°12'28", 100 m alt., 21.vi.2007, leg. B. Zlatkov & S. Beshkov, coll. BFUS; 1 ♂, Bulgaria, Veliko Tarnovo region, Emen Gorge, N43°08'20", E25°21'41", 150 m alt., 16.vii.2011, leg. B. Zlatkov & O. Sivilov, coll. BFUS.

The moths were collected at a “light tower” with a 160 W MBFT lamp and blacklight fluorescent tube, and traps with blacklight tubes. The genitalia were dissected following Robinson (1976) with the exception of the phalli; they were processed following Zlatkov (2011). The description of the cornuti generally follows Anzaldo et al. (2014). The phalli with everted vesicae were submerged in Euparal essence and attached to a needle with a diameter of 0.15 mm inserted through the entering excavation of the ductus ejaculatorius into the phallus. A compound microscope with attached camera lucida was used for the line drawings.

DNA barcode sequences of the mitochondrial COI gene (cytochrome c oxidase 1) were obtained from three specimens of *P. unionana* and an other three of *P. procerana*. DNA samples from dried legs were prepared according to prescribed standards using a standard high-throughput protocol (deWaard et al. 2008). Samples were processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) to obtain DNA barcodes (Ratnasingham and Hebert 2007). DNA sequencing resulted in barcode sequence of 658 bp and a sequence

of 604 bp for *P. unionana* and two sequences of 623 bp and 627 bp for *P. procerana*; sequencing of a third specimen of each species failed. Details of successfully sequenced voucher specimens including complete voucher data and images can be accessed in the Barcode of Life Data Systems (Ratnasingham and Hebert 2007). Degrees of intra- and interspecific variation in the DNA barcode fragments were calculated using Kimura 2 parameter (K2P) model of nucleotide substitution using analytical tools in BOLD systems v3.0 (BOLD 2015). A neighbour-joining tree of DNA barcode data of European taxa was constructed using MEGA 6 (Tamura et al. 2013) under the K2P model for nucleotide substitutions.

Abbreviations

ZMUC Zoological Museum, University of Copenhagen

BFUS Zoological collection of Faculty of Biology, Sofia University

Results

Redescription of *Phtheochroa unionana*

Head (Fig. 1a, b, d). Frons, vertex, palps white. Antennae filiform, with white scales at base and inner side.

Thorax. White, legs grey-brown. Forewing with small costal fold (*ca.* 1.5 mm), forewing length 7.9–9.5 mm. Uppervide pattern dimorphic: fasciate or white with some yellow scales. Fasciate form: white ground colour and ochreous-orange fasciae. Median fascia equal in width for its entire length, subterminal and terminal fascia not separated but the latter paler, with reticulate pattern. Fasciae with small groups of raised rust brown reflective scales. Cilia pale orange with alternating darker areas. White form: white, with more or less pronounced groups of yellow scales in the fasciate areas. Raised reflective scales correspond to those of the fasciate form but are pearly white. Underside in both forms dark grey brown, costa white with dark grey spots, cilia white. Hindwing upperside in males of both forms grey with pale anal area and reticulate pattern of darker and paler areas and white cilia, in females more uniform, with less pronounced reticulate pattern. Underside of both forms whitish with more or less prominent grey scattered spots, especially in the costal area, cilia white.

Abdomen. Grey-brown.

Male genitalia (Figs 2, 3). Uncus slender and long, slightly widened at the middle, with small setae on the apical area. Socii large, pendant, emerging close to the base of uncus, rounded apically, with external surface setose. Valva relatively narrow, with costa and sacculus nearly parallel and apex rounded, densely covered with setae. Sacculus strongly sclerotized and extending for more than 1/3 of the lower margin of valva. Transtilla slightly trapezoidal, dorsally spinulose. Central area of juxta ovoid. Phallus large, almost length of valva, ventrally bent, with short straight ventral process and two unequal cornuti the larger of which is *ca.* 0.5 the length of phallus, coecum wide. Vesica voluminous, strongly asymmetrical; median area mainly membranous bearing narrow, conical, intensively stainable diverticulum on the ventral side that posteriorly forms a sclerotized plate; left portion large and covered with minute spines (acanthae); right portion considerably larger, dorsally widened, with acanthae and extended posteriorly with two long cylindrical diverticula,

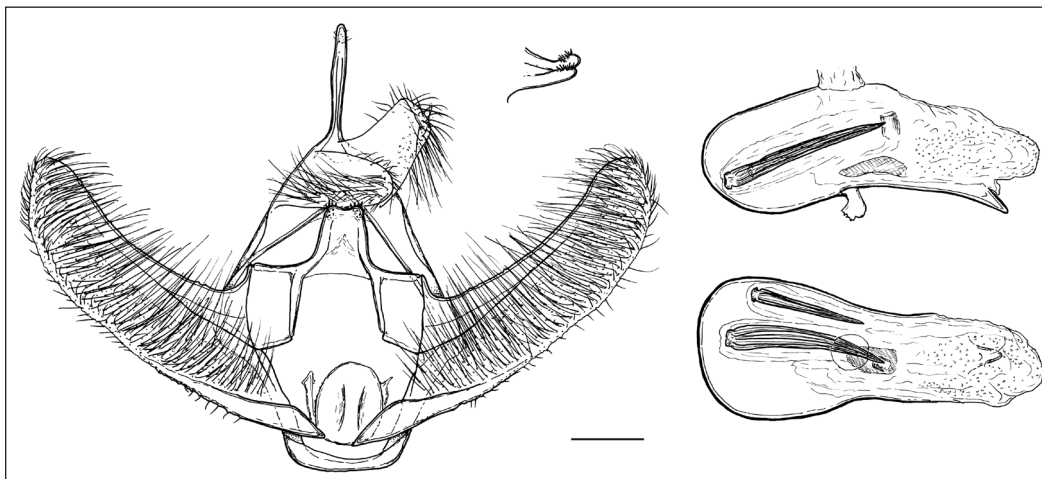


Figure 2. Male genitalia of *Phtheochroa unionana*. Inset: transtilla in dorso-lateral view; phallus with inverted vesica, lateral (above) and dorsal (below) view. All drawings to the same scale, scale bar = 250 μ m.

pointing left and right, each of which ending with a strong cornutus; right diverticulum and associated cornutus smaller than the left ones; cornuti acicular, non-deciduous, slightly curved, strongly sclerotized, longitudinally striated, with large sockets (capitate); gonopore located dorsally on the middle area of vesica, surrounded by a spinose, sclerotized semi-cylinder.

Female genitalia (Fig. 4). Apophyses anteriores *ca.* 1.8 \times longer than apophyses posteriores. Sterigma nearly twice as broad as ductus bursae, the latter weakly sclerotized posteriorly and membranous anteriorly. Corpus bursae ovoid; the wall sclerotized into three large plates. An elongated lateral sclerite on the left side connects the ductus bursae with the dorsal side; a second –the largest– sclerite covering most of the ventral side, extends ventrolaterally and then dorsally and forms folds near the middle area of corpus bursae; a third relatively small sclerite located lateroposteriorly on the left side. A densely folded membranous area present at the right side anterolaterally. Ductus seminalis emerges medioventrally from corpus bursae. No sclerotized spines visible under a stereomicroscope, but observation at high magnification under the microscope (e.g., 200 \times) reveals small unsclerotized spiniform structures at the left side of the emerging area of ductus seminalis.

Diagnosis. The wing pattern of the fasciate form of *P. unionana* is similar to *P. chalcantha* (Meyrick, 1912), *P. durbonana* (Lhomme, 1937), *P. purissima* (Osthelder, 1938), *P. procerana*, *P. aureopunctana* (Ragonot, 1894), and *P. purana* (Guenée, 1845). The white form is easily distinguished from all other *Phtheochroa*. The male genitalia of *P. unionana* are also similar to the aforementioned species. The uncus is relatively long and slender and the transtilla bears a broad, rectangular, median process as in *P. chalcantha*, *P. durbonana*, *P. procerana* and *P. purana*, but the cornuti in *P. procerana* and *P. durbonana* are of nearly equal size; however, the size of cornuti is not absolutely constant (e.g., Fig. 3a–d). The cornuti of *P. chalcantha* are similarly unequal but look much larger compared with the length of the phallus. The uncus in *P. purana* is widened at the apex, and the cornuti are more curved. The shape of the valva should be used with caution because it varies slightly depending on the pressure applied on the coverslip, at least in *P. unionana*. The female genitalia superficially resemble those of the discussed species but details in the shape of

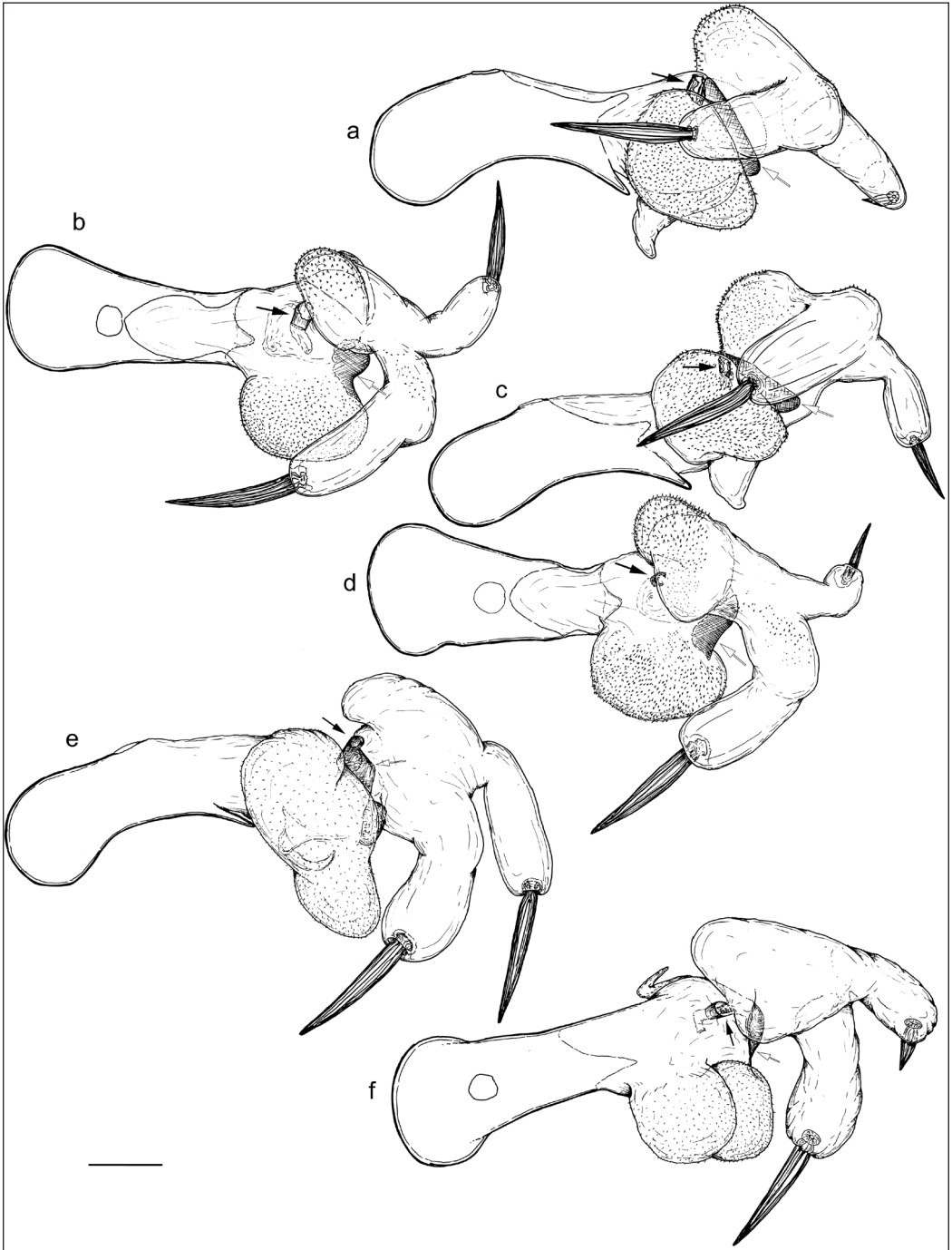


Figure 3. Phallus with vesica everted of *Phtheochroa* spp. **a–d**, *P. unionana*; **a, b**, white form, Armenia, Lori region, 29.vii.2014; **c, d**, fasciate form, Armenia, Tsaghkadzor, 9–11.vii.2011. **e–f**, *P. procerana*, Bulgaria, Emen Gorge, 16.vii.2011; **a, c, e**, left; **b, d, f**, dorsal. The black arrows shows semicylindrical sclerotisation around the gonopore, the white arrows – posterior sclerotisation of the median part of vesica. Scale bar = 250 μ m.



Figure 4. Female genitalia of *Phtheochroa unionana*, striated form, Georgia, Lesser Caucasus, 28.vii.2014. Scale bar = 500 μm .

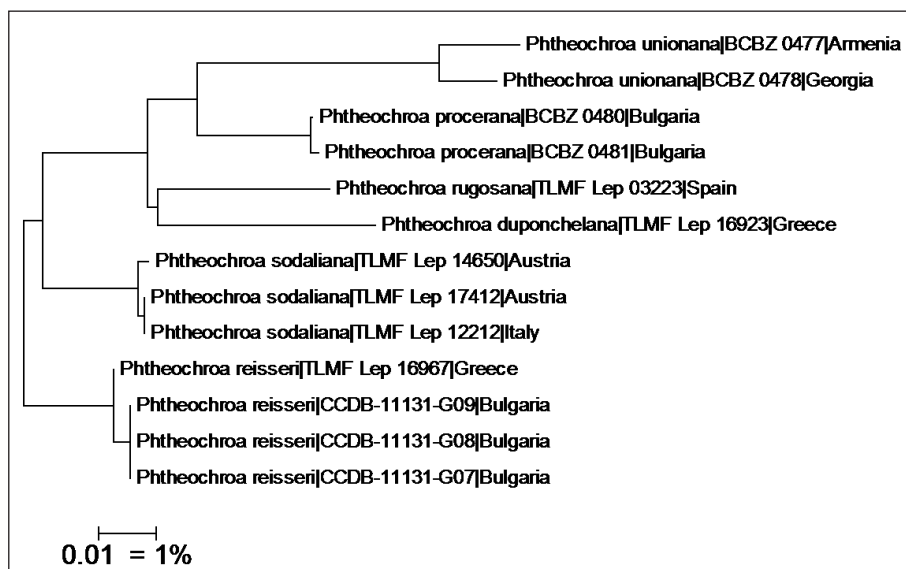


Figure 5. Neighbour-joining tree with a subset of *Phtheochroa* spp. (Kimura 2 parameter, built with MEGA 6; cf. Tamura et al. 2013). Source: DNA Barcode data from BOLD (Barcode of Life Database, cf. Ratnasingham and Hebert 2007).

sclerites distinguish *P. unionana* from the other related species, particularly the sclerite connecting ductus bursae with corpus bursae is diagnostic.

Phallus and vesica of *Phtheochroa procerana* (Fig. 3e, f)

The phallus of *P. procerana* is similarly shaped as in *P. unionana*, with relatively wider coecum. The asymmetrical vesica comprises all components found in the previous species. The median part is sclerotized posteriorly and bears a small curved diverticulum ventrally; a semi-cylindrical sclerotized spiny plate is located dorsally, around the gonopore. The right part is larger than in the previous species, with two unequal diverticula pointed ventrally. The right diverticulum is smaller than the left one, but the cornuti are of equal size. Acanthae are seen only on the right portion of vesica and are comparatively smaller than in *P. unionana*.

Molecular data (Fig. 5)

The intraspecific divergence is considerable with 2.38% (n=2) in *P. unionana* but low with only 0.16% (n=2) in *P. procerana*. The variation in the former is also reflected by two different BINs: BOLD:ACZ3163, BOLD:ACZ3164. Based on the two BINs the distance to the Nearest Neighbour in BOLD of *P. unionana* is *P. procerana* with 7.4% and the Nearctic *P. aegrana* (Walsingham, 1879) with 7.9% divergence whereas the distance of *P. procerana* to its Nearest Neighbour *P. rugosana* (Hübner, 1796) is 5.42%.

Discussion

P. unionana was described from two male specimens from the Caucasus (without details of the locality), both with white forewings with barely discernible yellow fasciae (Kennel 1900). The specimens were later lost and the male genitalia remained unstudied (Razowski 1970). Female

specimens collected subsequently were assigned to this taxon based entirely on the appearance of the forewing, but no further males were reported prior to the present study. Thus for the first time we are able to describe the genitalia morphology of the male. Razowski (1970, 2009) placed *P. unionana* near *P. procerana* based on the external appearance and female genitalia. With regards to the male genitalia, this position seems correct. The everted vesicae of both species demonstrate similarity though there are certain differences in the details: the same components are present in both species but their position and shape is different. It should be emphasised that the shape of the vesica, even the relative position of the diverticula and cornuti, are relatively constant at specific level though some small differences can be detected (Fig. 3a–d). A remarkable character is present in both species: a sclerotized semi-cylindrical plate around the gonopore. Though its function is not known, it suspends eversion of the ductus ejaculatorius. The peculiar ventral “diverticulum” with a conical shape and uneven surface does not correspond to any structure of the tortricid vesica known to us. It may be even non-eversible in the living moth. The female genitalia agree well with the illustrations by Razowski (1970, 2009) but the latter probably were drawn from the dorsal side instead of the ventral. This assumption is supported by the genitalia of the related species: the lateral sclerite connecting the corpus bursae with the ductus bursae is located at the left side in *P. procerana* (specimens studied by us), *P. durbonana* and *P. purissima* (judging from illustrations in literature).

Though a special case, comparison of the vesicae of two related species proves the taxonomical significance of this structure in the Cochylini, at least in *Phtheochroa*. Detailed comparison is achievable only after complete inflation of the vesicae, then numerous characters became visible and can be used for morphological analysis. The taxonomic significance of this character was tested by comparison with the closely related species *P. procerana* from which fresh material was available (Fig. 1c). As well as the morphology, the barcode divergence of 7.4% between *P. unionana* and *P. procerana* also clearly supports two separate species.

The conspecificity of two strikingly different forms in *P. unionana* is less supported by molecular data because the intraspecific distance between the forms is considerable at 2.38% but this may be due to geographic variation as one sequenced specimen originated from Armenia and the other from Georgia. Such divergence rates have been attributed either to intraspecific variation or interspecific divergence, varying from case to case (Huemer et al. 2014 and references within). However, the full conformity of genitalia morphology in both forms and their co-occurrence support a single species hypothesis. Male genitalia structures including the everted vesicae of the fasciate and white syntopic specimens appeared identical. Furthermore, the genitalia of a female specimen with fasciate forewings appeared identical to the available illustrations of the female genitalia of *P. unionana*. As a result we conclude that *P. unionana* has two forms that differ by forewing pattern. Extreme variation in the wing pattern is common in the subfamily Tortricinae, especially in the genus *Acleris*, and in many Cochylini, e.g. *Cochylimorpha* and *Aethes*.

Acknowledgements

We are most grateful to Paul Hebert and his team at the Canadian Centre for DNA Barcoding (Guelph, Canada) whose sequencing work was enabled by funding from the Government of Canada to Genome Canada through the Ontario Genomics Institute. We are also grateful to the Ontario Ministry of Research and Innovation and to

NSERC for their support of the BOLD informatics platform. Special thanks go to Ole Karsholt (Copenhagen, Denmark), who provided most of the *P. unionana* specimens. Robert J. Heckford (Plymouth, U.K.) improved an earlier draft of the paper linguistically and provided valuable comments. Thanks to Joaquín Baixeras (Valencia, Spain) and John Brown (Washington DC, USA) for critical review of the manuscript.

References

- Anzaldo SS, Dombroskie J, Brown JW (2014) Morphological Variation, Taxonomic Distribution, and Phylogenetic Significance of Cornuti in Tortricinae (Lepidoptera: Tortricidae). *Proceedings of the Entomological Society of Washington* 116(1): 1–31. doi: 10.4289/0013-8797.116.1.1
- BOLD (2015) The Barcode of Life Data System (www.barcodinglife.org). <http://www.boldsystems.org>
- deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA Barcodes: Analytical Protocols. In: Cristofre M (Ed.) *Methods in Molecular Biology: Environmental Genetics*. Humana Press Inc., Totowa, USA, 275–293. doi: 10.1007/978-1-59745-548-0_15
- Gilligan TM, Baixeras J, Brown JW, Tuck KR (2014) T@RTS: Online world catalogue of the Tortricidae (Ver. 3.0) <http://www.tortricid.net/catalogue.asp> [accessed 26.iii.2015]
- Huemer P, Mutanen M, Sefc KM, Hebert PDN (2014) Testing DNA Barcode Performance in 1000 Species of European Lepidoptera: Large Geographic Distances Have Small Genetic Impacts. *PLoS ONE* 9(12): e115774. doi: 10.1371/journal.pone.0115774
- Kennel J (1900) Neue paläarktische Tortriciden, nebst Bemerkungen über einige bereits beschriebene Arten. *Deutsche entomologische Zeitschrift. Lepidopterologische Hefte* 13: 124–159. <http://www.biodiversitylibrary.org/item/45903#page/9/mode/1up>
- Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology Notes* 7: 355–364. doi: 10.1111/j.1471-8286.2007.01678.x
- Razowski J (1970) Cochylidae. In: Amsel HG, Gregor F, Reisser H (Eds) *Microlepidoptera Palaearctica* 3. Georg Fromme, Wien, iv + 528 pp., 161 pls.
- Razowski J (1991) Notes on cochyline genus *Phtheochroa* Stephens (Lepidoptera: Tortricidae) with descriptions of new American species. *Acta zoologica cracoviensia* 34(1): 163–187.
- Razowski J (2009) Tortricidae of the Palaearctic Region. Volume 2. Cochylini. František Slamka, Bratislava, 195 pp.
- Robinson G (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. *Entomologist's Gazette* 27: 127–132. http://idtools.org/id/leps/tortai/Robinson_1976.pdf
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729. doi: 10.1093/molbev/mst197
- Zlatkov B (2011) A preliminary study of everted vesicae of several leafrollers (Tortricidae). *Nota Lepidopterologica* 33(2): 285–300. <http://www.biodiversitylibrary.org/page/46832594#page/111/mode/1up>