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Eucosma subvittana (Staudinger 1892) stat. rev., a Mediterranean species resurrected by DNA barcodes and morphology (Lepidoptera, Tortricidae)

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Abstract

Eucosma subvittana is resurrected from synonymy with *E. cana* (Haworth, 1811) and redescribed from extensive material collected in Greece (Crete) and Tunisia. It is distinguished from the similar *E. cana* by constant differences in wing pattern and the female genitalia, whereas the male genitalia are inseparable. A lectotype of *E. subvittana* is designated in order to fix the identity of this species. Adults and genitalia of both species are figured extensively. Furthermore, the DNA barcodes (cytochrome c-oxidase subunit 1) of both species are clearly divergent. In comparison, several cryptic taxa of related species-groups are genetically inseparable.

Key words: Olethreutinae, DNA barcode, morphology, redescription, revised status

Introduction

Eucosma Hübner, 1823 is a diverse genus of the Tortricidae, recently redefined based on a unique sterigma-sternum 7 structure (Gilligan & Wright 2013a). The first phylogenetically based analysis covered 231 valid species (including 96 synonyms) and introduced a considerable number of taxonomic changes, mainly affecting the Nearctic fauna (Gilligan *et al.* 2012). The European fauna covers 57 species (Aarvik 2011), with 46 species already analysed and figured by Razowski (2003) who also added four unplaced species. Only relatively few taxa have been described in recent years from Europe, namely *E. apocryphoides* Budashkin, 2009, *E. halophilana* Budashkin, 2009, *E. ukrainica* Budashkin, 2009, *E. gustavelsneri* Šumpich, 2011, *E. bimaculatana* Labonne, Nel & Varenne, 2017, as well as *E. paulorosea* Tsvetkov, 2020 and *E. fulvana suncretana* Tsvektov, 2020 (Budashkin 2009, Šumpich 2011, Labonne *et al*. 2017, Tsvetkov 2020). The latter taxon, however, is a subspecies of the disputed *E. fulvana* (Stephens, 1834) belonging to the species group of *E. hohenwartiana* (Denis & Schiffermüller, 1775) and allies (Agassiz & Langmaid 2004, Haslberger & Segerer 2016, Huemer 2013).

Despite a relevant identification guide (Razowski 2003) the morphological identification of several taxa is difficult. In particular, the genitalia structures are similar in several species with only subtle differences. DNA barcode-based identification as a potential alternative suffers from many obviously mismatched sequences in the Barcode of Life Database BOLD (Ratnasingham & Hebert 2007, BOLD 2023). Furthermore, DNA barcode sharing seems to be relevant for several closely related or taxonomically uncertain species complexes of *Eucosma* as well (Mutanen et al. 2016). In consequence a correct identification is often mainly supported on external characters such as wing pattern.

One of the externally inconspicuous and therefore relatively easy to confuse species in Europe is *E. cana*, which is widespread in large parts of the continent. Male and female genitalia of this species are also similar to many other taxa and differ only slightly. In contrast, however, the DNA barcode of this species is apparently unique with a single BIN (Barcode Index Number) (Ratnasingham & Hebert 2013). The species demonstrates considerable variability,

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which resulted in description and subsequent synonymisation of several taxa with *E. cana.* Specimens from Crete that differed in appearance and in the DNA barcode were therefore already assessed as worthy of verification by Huemer (2016). Further field surveys and subsequent genetic and morphological investigations revealed that these specimens belong to the taxon *Grapholitha carduana* var. *subvittana* Staudinger, 1892, which was hitherto considered a synonym of *E. cana* (Gilligan et al. 2012). Our findings demonstrate that the taxon should be treated as a bona species and we propose a revised status: *E. subvittana* (Staudinger, 1892) **stat. rev.**

The taxon *Grapholitha carduana* var. *subvittana* was described as a variety of *Catoptria carduana* Guenée, 1845. Later Rebel (1901) considered it a synonym of *Epiblema cana* (Haworth, 1811) and therefore, a variation of the latter taxon: *E. cana* var. *subvittana*. Notably, Obraztsov (1968) preserved the separate status of the taxon *Eucosma cana* f. *subvittana* Stgr., though with a question mark, but further catalogues simply treated it as a synonym of *E. cana*.

Material and methods

Morphological analysis: the study is based on c. 80 specimens of the *Eucosma cana* complex. Material was traditionally set or spread and dried. Genitalia preparations followed standard techniques in microlepidoptera (Robinson 1976). The habitus and wings of the specimens were photographed under a stereomicroscope Stemi 2000-c (Zeiss) with a digital camera EOS 1300D (Canon) on a grey background with consistent parameters of the light source and the camera, apart of the specimens from Museum für Naturkunde der Humboldt Universität, Berlin, Germany. The wings were set perpendicular to the optical axis, regardless of the setting of the specimens. The genitalia were photographed and drawn under a compound microscope Amplival (Carl Zeiss Jena) equipped with a Canon EOS 2000D digital camera and camera lucida. All images were edited with Photoshop (Adobe).

Molecular analysis: DNA samples (dried hindlegs) to obtain the 658 base-pair long barcode segment of the mitochondrial COI gene (cytochrome c oxidase I) of 38 samples of the *E. cana* complex were prepared according to the prescribed standards and processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using the standard high-throughput protocol described in deWaard *et al*. (2008). In addition, we analysed five public sequences of *E. fulvana* from the University of Oulu (Finland) on BOLD. Only complete DNA barcode sequences of 658 bp were considered for analysis, with the exception of a single shorter sequence of 524 bp belonging to *E. subvittana*. Details including complete voucher data and images of analysed material can be accessed in the public dataset "Overlooked species of *Eucosma* [DS-Eucosma]" in the Barcode of Life Data Systems (BOLD 2023). Sequences were finally submitted to GenBank. All sequences were assigned to the Barcode Index Numbers (BIN), algorithm-based operational taxonomic units that provide an accurate proxy for the true species. BINs were automatically calculated for records in BOLD that comply with the DNA Barcode standard (Ratnasingham & Hebert 2013). 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 from the dataset was constructed using MEGA7 (Kumar *et al*. 2016) under the Kimura 2 parameter model for nucleotide substitutions.

Abbreviations
IBER = Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria.
MNHN = Muséum national d´Histoire naturelle, Paris, France.
MNHU = Museum für Naturkunde der Humboldt Universität, Berlin, Germany.
TLMF = Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria.

Results

Taxonomy

***Eucosma subvittana* (Staudinger, 1892) stat. rev.**
(Figs 1A–H, 2A–G, 3A–C)

Grapholitha carduana var. *subvittana* Staudinger 1892: 299.

Type material examined

Lectotype ♂, hereby designated: Tunisia, with five labels: "Origin." [printed on red paper], "Tunis / 91. Vaulog.[er]" [handwritten], "v. subvittana / Stgr." [handwritten], "Lectotype / *Eucosma subvittana* / (Staudinger, 1892) / Zlatkov & Huemer des. 2023", [URI QR-code label] (MNHU). Paralectotypes: 1 ♂ Italy, with four labels: "Origin." [printed on red paper], "Sardinia / m." [handwritten], "Paralectotype / *Eucosma subvittana* / (Staudinger, 1892) / Zlatkov & Huemer des. 2023", [URI QR-code label] (MNHU); 1 ♀ Spain, with four labels: "Origin." [printed on red paper], "Barcelona" [handwritten], "Paralectotype / *Eucosma subvittana* / (Staudinger, 1892) / Zlatkov & Huemer des. 2023", [URI QR-code label] (MNHU).

Other material examined

Greece: 6 ♂, 4 ♀ Greece, Crete, Agia Galini, 2.5 km NE of, 35°06'42''N, 24°41'56''E, 100–110 m, 25.iv.2022, leg. P. Huemer; , DNA Barcode TLMF Lep 32066, TLMF Lep 32067, TLMF Lep 32065, genitalia slides No. 3/31.1.2023, 3/16.1.2023, 6/16.1.2023, 7/16.1.2023, 5/16.1.2023 (TLMF); 1 ♂ ditto, genitalia slide No. 2/8.3.2023 (IBER); 6 ♂, 6 ♀ Crete, Georgioupoli, 2 km E of, 35°21'16''E, 24°17'04''E, 3 m, 4.v.2022, leg. P. Huemer, DNA Barcode TLMF Lep 32091, genitalia slides No. 8/16.1.2023, 1/24.1.2023, 1/8.3.2023 (TLMF); 1 ♀ ditto, genitalia slide No. 4/16.1.2023 (IBER); 1 ♂, 1 ♀ Crete, Petres, 1.1km S, 35°20'56''E, 24°21'20''E, 35 m, leg. P. Huemer; 1 ex., Crete, Chania, Langos, Fassatal, 400 m,13.v.2003, leg. W. Ruckdeschel; 3 ex. Crete, Chania, Xekollimenos, Patellari, 20 m, 17.v.2003, leg. W. Ruckdeschel, DNA Barcode TLMF Lep 16959); 2 ex., ditto, but 15.v.2003; 1 ex., Crete, Chania, Xekollimenos, Kirtomados, 50–70 m, 9.v.2000, leg. W. Ruckdeschel; 8 ex., ditto, but 8.v.2000; 3 ex., ditto, but 20–30 m, 9.v.2000, DNA Barcode TLMF Lep 16942; 2 ex., ditto, but 8.v.2000; 1 ex., Crete, Rethymon, Kissou Kampos, 460 m, 20.iv.2008, leg. Ruckdeschel, DNA Barcode TLMF Lep 16931 (TLMF). Tunisia: 1 ♂ Tunis, Gammarth, Gammarth forest, 50 m, 10.iv2016, leg. G. Leraut, DNA Barcode TLMF Lep 32756 (Research Collection of G. Leraut, Paris).

Diagnosis. The species resembles *E. cana* but externally differs by the forewing ground colour being reddish brown; in *E. cana* it is variable (from pale ochre to brown). The ratio length/width of sternite 7 of the female is 0.72 in *E. subvittana* and 0.60 in *E. cana*; lateral incisions of the sterigma are more expressed in *E. cana* (Fig. 4). The members of the *Eucosma hohenwartiana* species group are with more or less similar forewing pattern but can be distinguished by lack of dark lines on the veins.

Redescription. Adult (Fig. 1A–H). Sexual dimorphism not detected. Male. Head: frons grey; vertex reddish brown; antenna filiform with scape and pedicellus reddish brown; labial palpus grey. Thorax: patagium reddish brown; mesonotum pale reddish brown with darker median and lateral longitudinal lines; tegula pale reddish brown; metanotum grey; thorax ventrally pale grey; legs dark grey; forewing length 7.4–8.7 mm (mean 8.0, n=7), costal fold 0.4 length of forewing, with two types of scales within: long and small elliptical; upperside ground colour reddish brown, paler in the distal area, with darker longitudinal lines on veins and sometimes between them, basal elements of wing pattern ill-defined, sub-basal fascia occurs only on dorsal margin as a darker spot, sub-basal interfascia merely a paler dorsal interfascial spot ("dorsal patch" sensu Razowski 2003), median fascia ill-defined, costal pairs of strigulae 5–9 whitish, connected with confluent whitish lines reaching ocellus, ocellus bordered by lead-coloured lines and with black dashes on veins, terminal line grey, cilia creamy with black admixture in apical area; underside uniformly dark grey with costal strigulae repeating those of upperside. Variation: more or less contrasting pattern elements; sometimes pairs of strigulae 5–6 suffused, not distinguishable. Hindwing upperside grey with dark grey terminal line and pale grey cilia; underside concolour with forewing underside or paler. Abdomen. Grey. Male genitalia (Fig. 2A–G). Uncus small, rounded. Socii drooping, subconical. Sacculus slightly convex, with more or less rounded angle. Neck of valva relatively long, with parallel dorsal and ventral margins. Cucullus small, with rounded dorsal angle and distinct ventral lobe. Phallus short, with endophallite and bundle of c. 30 long, sinuate, deciduous cornuti. Variation: neck of valva sometimes narrower; cucullus with broader or narrower dorsal angle and sometimes with narrower ventral lobe. Female. Colouration and wing pattern as in male. Forewing length 8.2–8.6 mm (mean 8.4, n=3). Female genitalia (Fig. 3A–C). Papillae anales narrow, elongate. Apophyses anteriores and apophyses posteriores of equal length. Sterigma inclined to left anteriorly, with deeper round incision on left side and slight round incision on right side. Sternite 7 hexagonal, ratio length/width 0.67–0.75 (mean 0.72, n=3). Ductus bursae with relatively long cingulum at middle. Two signa of almost equal size.

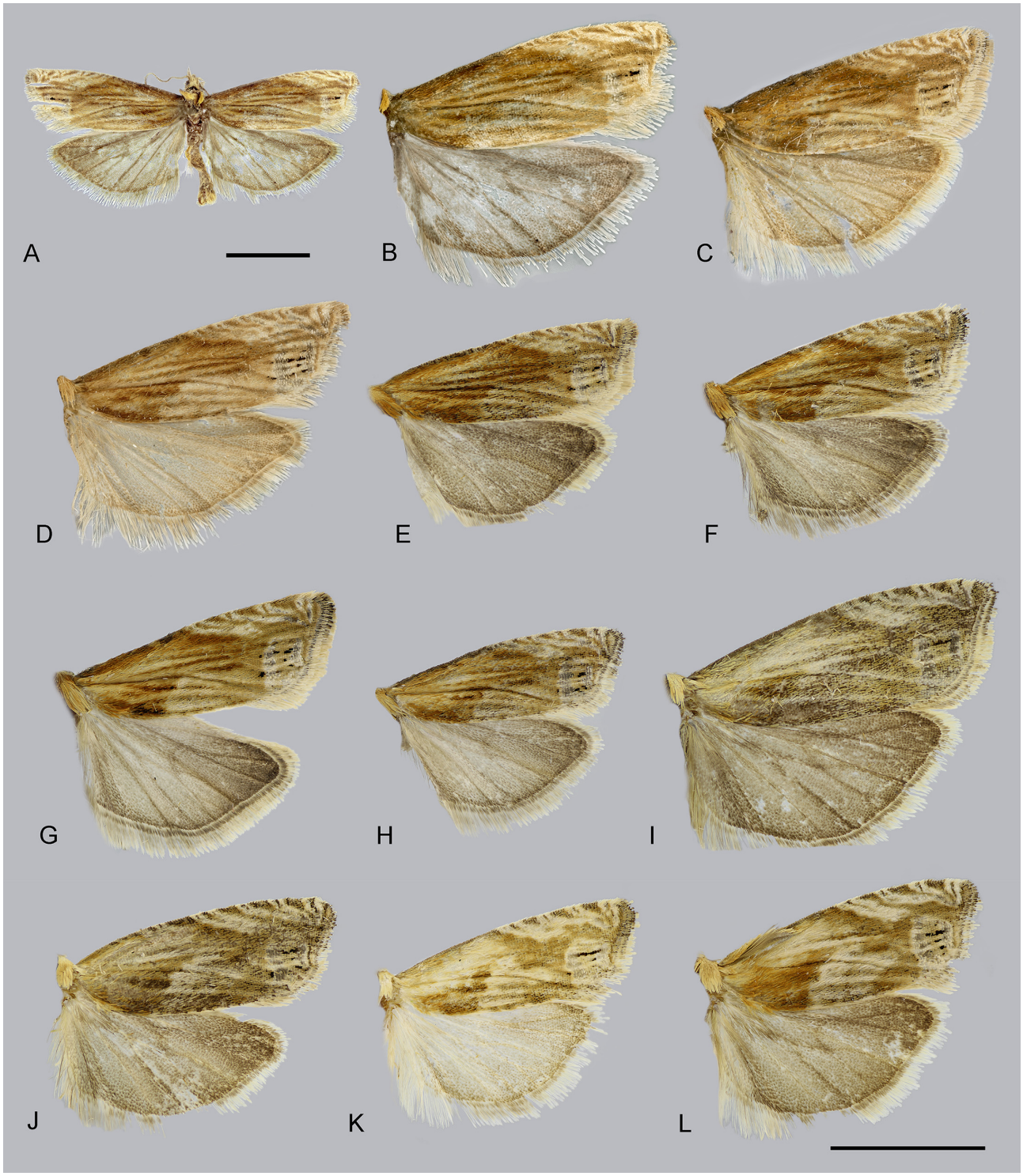

FIGURE 1. *Eucosma subvittana* and *E. cana*. A. Lectotype ♂of *E. subvittana*, Tunisia. B–I Wing pattern. B. *E. subvittana* lectotype. C. *E. subvittana* paralectotype ♂, Spain, Barcelona (left wings mirrored digitally). D. *E. subvittana* paralectotype ♀, Italy, Sardinia. E. *E. subvittana* ♀, Crete, Agia Galini, 25.iv.2022. F. *E. subvittana* ♀, ibid. G. *E. subvittana* ♂, ibid. H. *E. subvittana* ♂, Crete, Georgioupoli, 4.v.2022. I. *E. cana* ♂, Bulgaria, Pirin Mts, 1730 m, 17.vii.2010. J. *E. cana* ♀, Bulgaria, Sredna Gora Mts, 720 m, 28.vii.2012. K. *E. cana* ♂, Bulgaria, Black Sea coast, 6.vi.2013. L. *E. cana* ♂, Bulgaria, Yambol reg., 29.vi.2012. Scale bars 5 mm, B–L to scale; A–D (c) Museum für Naturkunde Berlin, with permission.

Molecular data. BIN: BOLD:ACT0042. The intra-BIN average distance of the barcode region is 0.53%, the maximum distance is 1.44% (p-dist) (n=14). The minimum distance to the Nearest Neighbor *E. cana* (BIN: BOLD: AAB4296) (n=86) is 2.09% (Fig. 5).

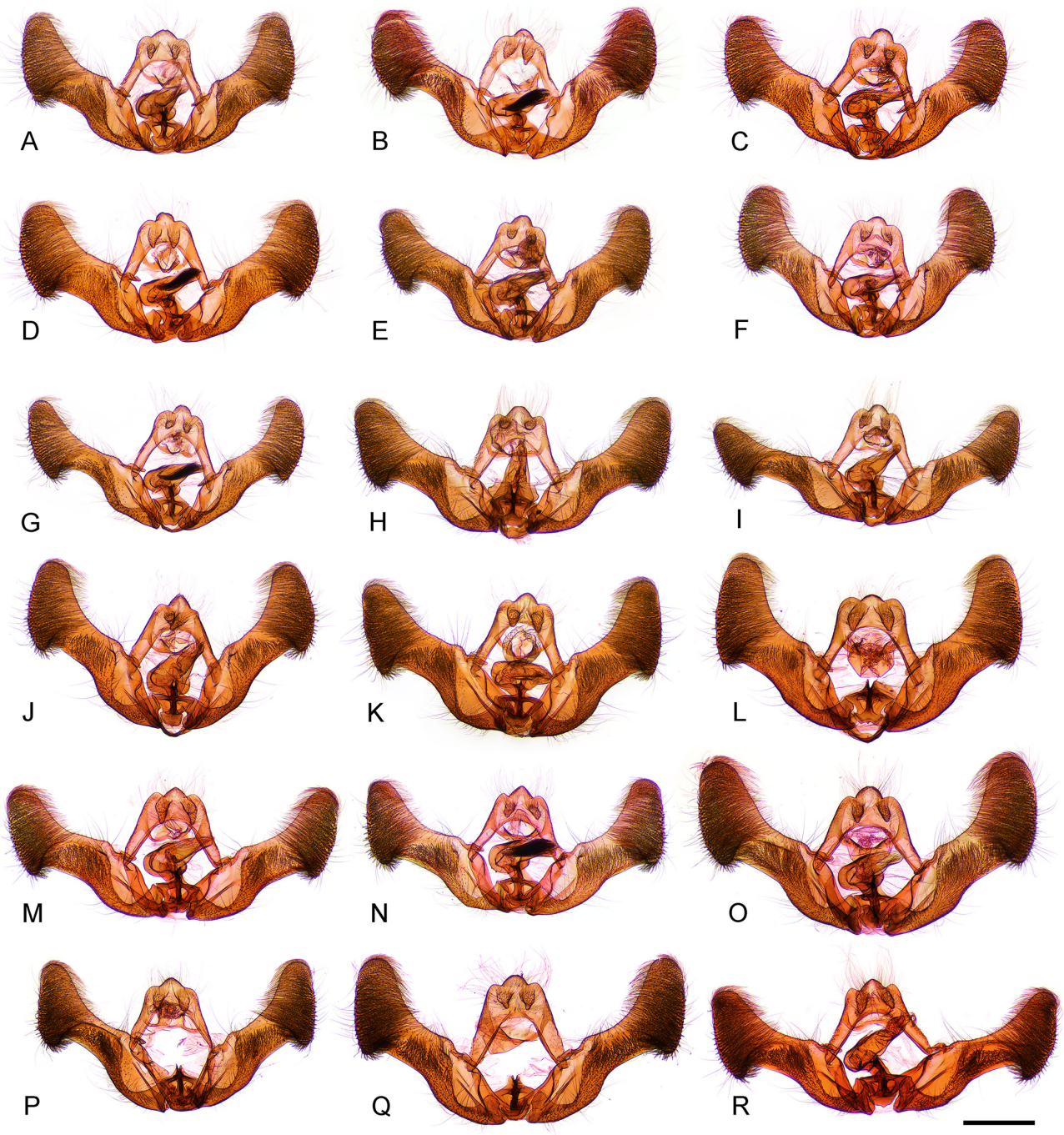

FIGURE 2. *Eucosma subvittana* (A–G) and *E. cana* (H–R), male genitalia. A. *E. subvittana*, Crete, Agia Galini, 25.iv.2022. B. *E. subvittana*, ibid. C. *subvittana*, ibid. D. *E. subvittana*, Crete, Georgioupoli, 4.v.2022. E. *E. subvittana* ibid. F. *subvittana*, ibid. G. *E. subvittana*, ibid. H. *E. cana*, Bulgaria, Balchik, 27.vi.2013. I. *E. cana*, Bulgaria, Petrich distr., 5.v.2013. J. *E. cana*, Bulgaria, Slavyanka Mts, 1550 m, 23.vii.2010. K. *E. cana*, Bulgaria, Yambol reg., 29.vi.2012. L. *E. cana*, Bulgaria, Belasitsa Mts, 1500 m, 6.vii.2010. M. *E. cana*, Turkey, Iğneada, 6.vii.2009. N. *E. cana*, Bulgaria, Black Sea coast, Karadere, 4.vii.2013. O. *E. cana*, Bulgaria, Pirin Mts, 1850 m, 8.viii.2013. P. *E. cana*, Bulgaria, Black Sea coast, Veleka, 6.vii.2013. Q. *E. cana*, Bulgaria, Pirin Mts, 1950 m, 21.vii.2010. R. *E. cana*, Bulgaria, Black Sea coast, Kranevo, 23.vii.2011. Scale bar 500 µm, all to scale.

Bionomics. Host-plants and early stages are unknown. The adults from Crete have been collected at light.

Distribution. *Eucosma subvittana* seems to be widespread in the Mediterranean. We have been able to study material from Greece (Crete), Spain (Barcelona) and Tunisia but unpublished records in BOLD indicate further records from Spain and Portugal which require verification. It remains unclear if the first record of *E. cana* from

mainland Greece (Trematerra & Colacci 2022) is a misidentification. Currently the distribution of both species seems to show an allopatric pattern with *E. subvittana* restricted to the Mediterranean.

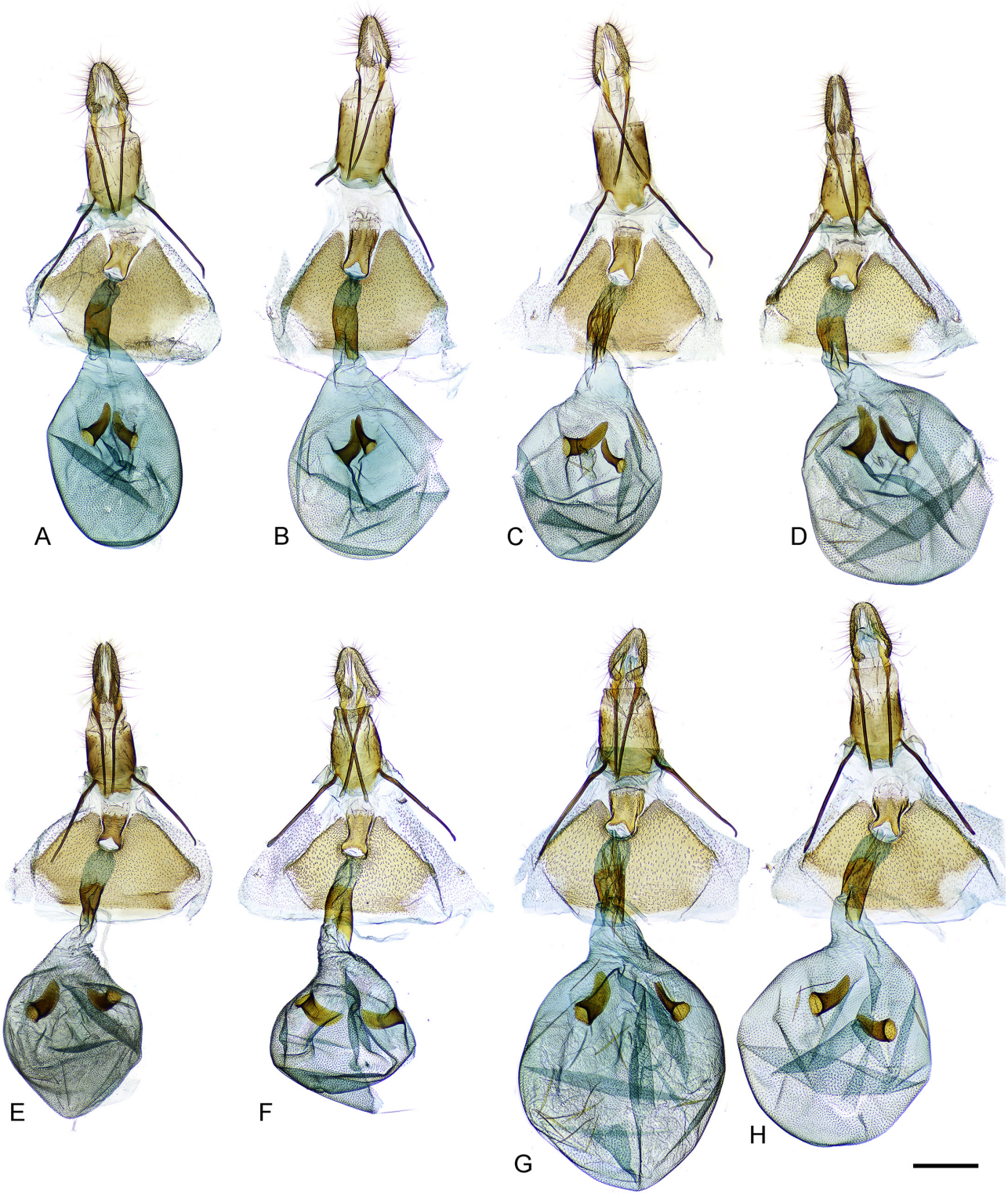

FIGURE 3. *Eucosma subvittana* (A–C) and *E. cana* (D–H), female genitalia. A. *subvittana*, Crete, Agia Galini, 25.iv.2022. B. *E. subvittana*, Crete, Georgioupoli, 4.v.2022. C. *E. subvittana*, Crete, Agia Galini, 25.iv.2022. D. *E. cana*, Bulgaria, Sredna Gora Mts, 720 m, 28.vii.2012. E. *E. cana*, Bulgaria, Shumen distr., 20.vi.2007. F. *E. cana*, Bulgaria, Petrich distr., 14.vi.2010. G. *E. cana*, Bulgaria, Emen Gorge, 16.vii.2011. H. *E. cana*, Bulgaria, Belasitsa Mts, 600 m, 28.viii.2010. Scale bar 500 µm, all to scale.

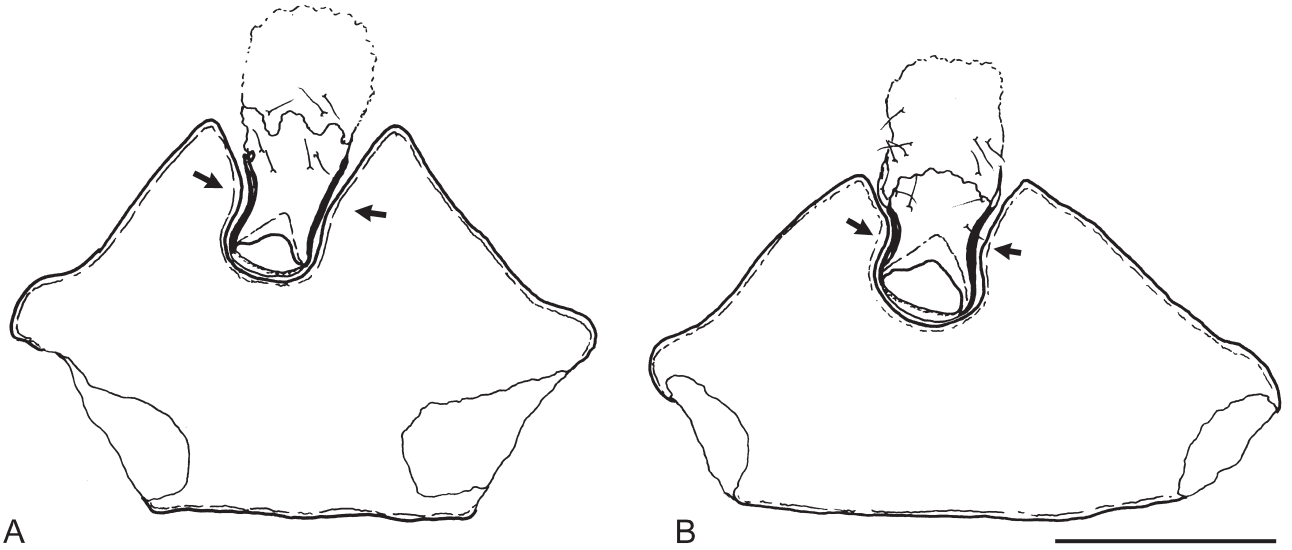

FIGURE 4. Sternite 7 and sterigma, diagnostic characters of E. *subvittana* and *E. cana*. A. *E. subvittana*, Crete, Agia Galini, 25.iv.2022. B. *E. cana*, Bulgaria, Sredna Gora Mts, 720 m, 28.vii.2012. Note the different ratio length/width of sternite 7 and lateral incisions of sterigma (arrows). Scale bar 500 µm, both to scale.

Remarks. *Grapholitha carduana* var. s*ubvittana* was described from three specimens collected in Tunisia, Italy (Sardinia) and Spain (Staudinger 1892). We have been able to examine detailed pictures of all three syntypes from MNHU which fully correspond with our extensive series from Crete. A lectotype originating from Tunisia is here designated in order to fix the identity of the species and conserve stability of nomenclature.

***Eucosma cana* (Haworth, 1811)**
(Figs 1I–L, 2H–R, 3D–H)

cana Haworth 1811 (Tortrix); Lepid. Br. (3): 456. United Kingdom: Great Britain. Syntype(s) (BMNH); unknown.
= *carduana* Guenée 1845 (*Catoptria*); Annls Soc. ent. Fr. (2) 3: 188. France. Syntype(s) (MNHN); not traced.
= *monetulana* Hübner [1814-1817] (Tortrix); Samml. Eur. Schmett. 7: pl. 41, fig. 257. Syntype(s) unknown.

Material examined.
Austria: 1 ♂ Nordtirol, Rietz NE, 47°17’48’’N, 11°02’45.7’’E, 628 m, 19.vii.2008, leg. P. Huemer (DNA barcode ID TLMF Lep 07307); 1 ♂ Nordtirol, Fließ, Gretlern, 47°06’58’’N, 10°39’48’’E, 1310-1320 m, 6.vii.2015, leg. P. Huemer (DNA barcode ID TLMF Lep 18039); 1 ♂ Nordtirol, Ellbachtal, unterer Kaiserboden, 47.539°N, 11.926°E, 780 m, 24.vii.2014, leg. P. Huemer (DNA barcode ID TLMF Lep 15030); 1 ♂ Nordtirol, Fließ, Vögele Bichl, 47°06’57’’N, 10°37’35’’E, 990 m, 6.vii.2015, leg. P. Huemer (DNA barcode ID TLMF Lep 15328); 1 ♂ Vorarlberg, Lustenau, Schweizer Ried, AZE Häusle S, 47.446°N, 9.69°E, 400 m, 25.vii.2012, leg. P. Huemer (DNA barcode ID TLMF Lep 08460); 1 ♂ Niederösterreich, Sollenau, 47.905°N, 16.266°’’E, 270 m, 6.vii.2015, leg. P. Huemer (DNA barcode ID TLMF Lep 19904) (all TLMF). Bulgaria: 1 ♂ Black Sea coast, Balchik, 43°24’16’’N, 28°12’30’’E, 60 m, 27.vi.2013, leg. B. Zlatkov & O. Sivilov; 1 ♂ Black Sea coast, Kranevo, 43°20’03’’N, 28°03’38’’E, 170 m, 23.vii.2011, leg. B. Zlatkov & O. Sivilov; 1 ♂ Black Sea coast, Chernomorets–Burgas, 42°25’48’’N, 27°32’10’’E, 10 m, 11.ix.2010, leg. B. Zlatkov & S. Beshkov; 1 ♂ Black Sea coast, Veleka river, 42°03’53’’N, 27°58’16’’E, 0 m, 6.vii.2013, leg. B. Zlatkov & O. Sivilov; 1 ♂ Black Sea coast, Karadere, 42°53’47’’N, 27°53’48’’E, 0 m, 4.vii.2013, leg. B. Zlatkov & O. Sivilov; 1 ♀ Shumen, 43°15’44’’N, 26°54’08’’, 340 m, 20.vi.2007, leg. B. Zlatkov & S. Beshkov; 1 ♀ Emen, 43°08’20’’N, 25°21’41’’E, 130 m, 16.vii.2011, leg. B. Zlatkov & O. Sivilov; 1 ♀ Stara Planina Mts, Uzana, 42°45’58’’N, 25°14’53’’E, 1270 m, 7.viii.2011, leg. B. Zlatkov & O. Sivilov; 1 ♂ Sofia, Zhiten, 42°48’06’’N, 23°15’55’’E, 520 m, 25.vii.2008, leg. B. Zlatkov; 1 ♀ Sredna Gora Mts, Golema Rakovitsa, 42°35’54’’N, 23°49’54’’E, 720 m, 28.vii.2012, leg. B. Zlatkov & O. Sivilov; 1 ♂ Osogovo Mts, Trite Buki,

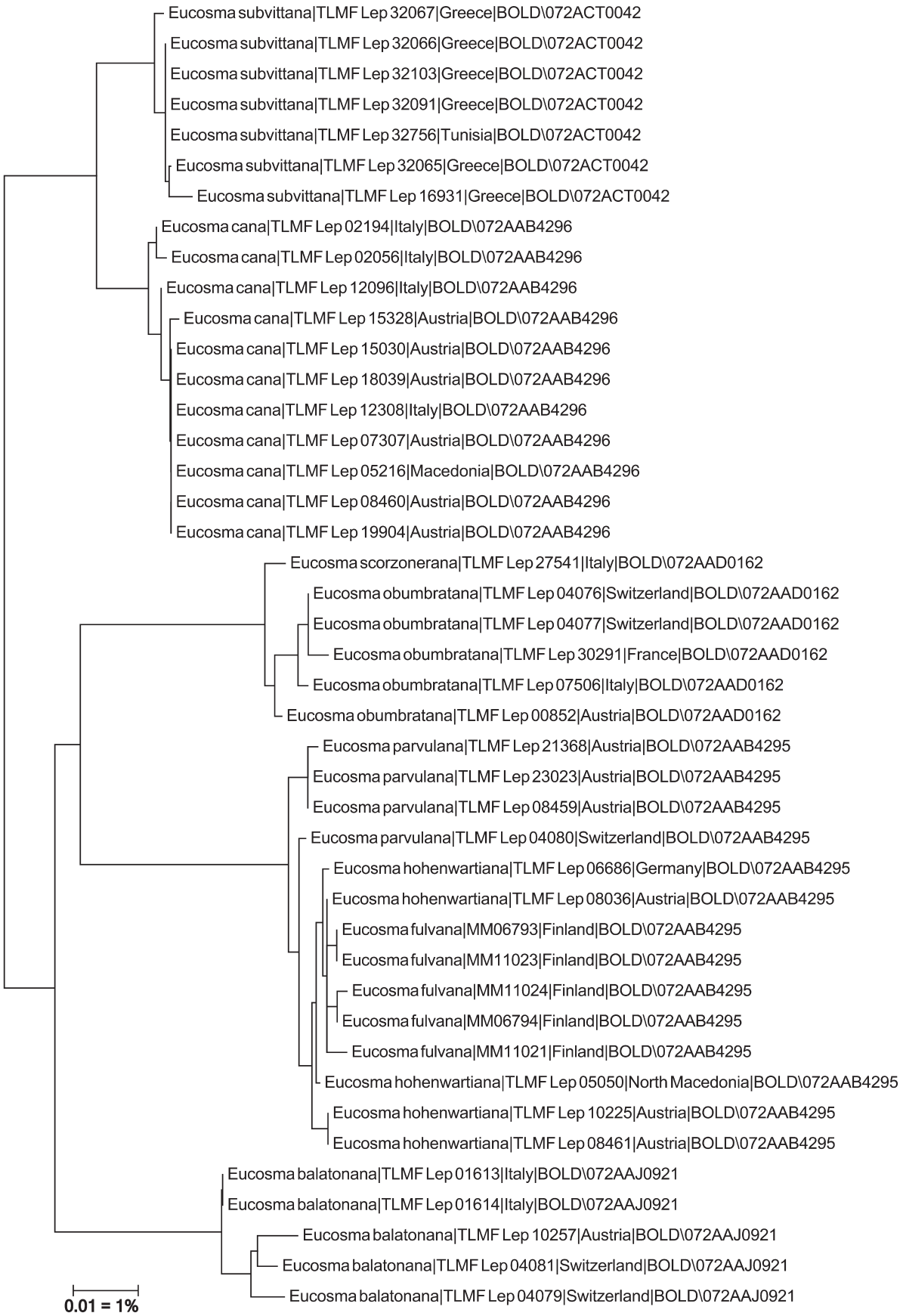

FIGURE 5. Unrooted Neighbor-Joining tree of *Eucosma* spp. (Kimura 2-parameter, built with MEGA7 (Kumar *et al*. 2016); Source: DNA Barcode data from BOLD (Barcode of Life Database; Ratnasingham 2018).

42°10’25’’N, 22°37’40’’E, 1540 m, 6.vii.2010, leg. S. Beshkov; 1 ♂ Struma valley, Kozhuh, 41°28’N, 23°15’E, 100 m, 5.vii.2006, leg. B. Zlatkov, DNA Barcode BC BZ 0279; ditto, but 8.v.2010, leg. B. Zlatkov & O. Sivilov; 1 ♂ Petrich, Novo Hodzhovo, 41°24’26’’N, 23°24’27’’E, 130 m, 5.v.2013, leg. B. Zlatkov & O. Sivilov; 1 ♀ ditto, but 14.vi.2010; 1 ♀ Belasitsa Mts, 41°22’26’’N, 23°12’01’’, 610 m, 28.viii.2010, leg. S. Beshkov; 1 ♂ Pirin Mts, Yavorov chalet, 41°49’26’’N, 23°22’47’’E, 1730 m, 17.vii.2010, leg. B. Zlatkov & O. Sivilov; 1 ♂ Pirin Mts, Orelek, 41°33’30’’N, 23°36’59’’E, 1850 m, 8.vii.2013, leg. B. Zlatkov & O. Karsholt; 1 ♂ ditto, but 41°33’46’’N, 23°37’24’’E, 1950 m, 21.vii.2010, leg. B. Zlatkov & O. Sivilov ; 1 ♂ ditto, but 41°33’13’’N, 23°36’43’’E, 1800 m, 20.vii.2012, leg. B. Zlatkov & O. Sivilov; 1 ♂ Slavyanka Mts, 41°24’58’’N, 23°36’51’’E, 1550 m, 23.vii.2010, leg. B. Zlatkov & O. Sivilov; 1 ♂ Slavyanka Mts, Livada, 41°23’23’’N, 23°36’29’’E, 1925 m, 18.vii.2012, leg. B. Zlatkov & O. Sivilov; 1 ♂ Yambol, Tarnava, 42°27’08’’N, 26°41’23’’E, 440 m, 27.vi.2012, leg. B. Zlatkov & O. Sivilov; 1 ♂ Yambol, Boyadzhik, 42°22’45’’N, 26°15’34’’E, 250 m, 29.vi.2012, leg. B. Zlatkov & O. Sivilov; Albania: 1 ♂ Tomor Mts, 40°37’11’’N, 20°11’31’’E, 1550 m, 2.vii.2015, leg. B. Zlatkov & D. Chobanov; Turkey: 1 m, Black Sea coast, Iğneada, 41°51’37’’N, 27°57’14’’E, 0 m, 6.vii.2009, leg. B. Zlatkov & R. Bekchiev (all IBER). Italy: 1 ♂ Südtirol, Ritten, Obergrünwald, 46°35’48’’N, 11°26’19’’E, 1750 m, 1.vii.2010, leg. P. Huemer (DNA barcode ID TLMF Lep 02056); 1 ♂ Südtirol, Montiggl, Kl. Priol, 46°25’37’’N, 11°17’58’’E, 600 m, 22.vii.2010, leg. P. Huemer (DNA barcode ID TLMF Lep 02194); 1 ♀ Südtirol, N Zwischenwasser/St. Lorenzen, 46.739°N, 11.873°E, 990 m, 17.vii.2013, leg. P. Huemer (DNA barcode ID TLMF Lep 12096); 1 ♂ Südtirol, Schleiser Leiten, 46.698°N, 10.517°E, 1320 m, 6.vii.2013, leg. P. Huemer (DNA barcode ID TLMF Lep 12308) (all TLMF). 1 ♂ North Macedonia, Korab, Korabsko jezero, Kobilino pole, 41.778°N, 20.582°E, 2080 m, 28.vii.2011, leg. P. Huemer & G. Tarmann (DNA barcode ID TLMF Lep 05216) (TLMF).

Remarks. The identity of *Eucosma carduana* described from France appears doubtful, and it is probably not conspecific with *Eucosma cana* and related taxa. This is indicated in particular by the reference to white forewings in the original description. Further studies, especially of potentially remaining syntype specimens, are necessary to clarify this issue.

DNA barcoding

DNA sequencing resulted in a BIN concordant DNA barcode fragment for 43 specimens from seven morphologically similar species. An extended analysis of other sequenced species in BOLD was not carried out due to multiple uncertainties in the identification of samples.

The BIN analysis shows a low mean intraspecific variation of 0.36–0.66% and a maximum intraspecific divergence of 1.44–1.88% with a high number of samples per BIN (n=14–142). In contrast, the BIN distances to the nearest neighbour are greater at 2.08–4.64% with distinct clustering (Fig. 5, Table 1). However, the examined specimens grouped in only four BINs and the morphologically very similar and partially disputed species groups *Eucosma hohenwartiana* - *E. fulvana* - *E. parvulana* and *Eucosma balatonana* - *E. scorzonerana* cannot be distinguished either by their BIN or by genetic divergence within the respective BIN. In contrast, the species pair *Eucosma cana* - *E. subvittana* is clearly differentiated genetically, reflected by a separate BIN.

TABLE 1. BINs with intra- and interspecific mean K2P (Kimura 2 Parameter) divergences and distance to the nearest neighbour (distances in %) in selected species of *Eucosma.*

BIN	n	Species	Mean intra-sp	Max intra-sp	Dist NN	NN
BOLD:ACT0042	14	*Eucosma subvittana*	0.53	1.44	2.08	*Euosma cana*
BOLD:AAB4296	86	*Eucosma cana*	0.36	1.66	2.08	*Eucosma subvittana*
BOLD:AAB4295	142	*Eucosma hohenwartiana* *Eucosma fulvana* *Eucosma parvulana*	0.53	1.61	4.64	*Eucosma crassana*
BOLD:AAJ0921	37	*Eucosma balatonana,* *Eucosma scorzonerana*	0.66	1.88	3.66	*Eucosma alatana*

Discussion

Eucosma subvittana **stat. rev.** resembles the widely distributed Palaearctic *E. cana*. The latter is a highly variable species, not only in wing pattern but also in size and male genitalia morphology. Specimens from the Balkan Peninsula (the population with closest proximity to Crete) were used for comparison. The putatively more widespread

E. subvittana demonstrates constant wing pattern and female genitalia morphology, but the size of the moths and the male genitalia vary considerably, similarly to *E. cana*. The two species are indistinguishable in the costal fold (including the scent scales), venation and male genitalia. Nevertheless, the different ground colour, female genitalia, and considerable barcode gap from the other species of the genus support the existence of a separate species on Crete, Tunisia and other regions of the Mediterranean. Similar examples with other species of the genus *Eucosma* are known, e.g. the *E. hohenwartiana* group (Agassiz & Langmaid 2004), however, unlike *E. subvittana* - *E. cana* several of these taxa share BINs and are inseparable by DNA barcodes (Fig. 5).

The forewing pattern of the Tortricidae has caused much confusion in taxonomic descriptions and, as a consequence, numerous systems for designation of its elements can be found in the literature (e.g. Danilevskii & Kuznetsov 1968; Razowski 2003). Here we adopt the system proposed by Brown & Powell (1991) refined by Baixeras (2002) (Fig. 6). The most recognisable elements are the costal intervenular strigulae, of 9 pairs which are often fused or reduced, particularly the more basal of them. The most prominent in Eucosmini are the pairs 5–9 which can be used as landmarks for recognition of the other elements. The pairs 7, 8 and 9 are located between the ends of radial veins R_1 and R_2, R_2 and R_3, R_3 and R_4 respectively. The costal margins of the alternating fasciae and interfasciae are between each pair of strigulae and expand to the dorsal margin of the forewing. Here we designate the interfasciae in the same order as the fasciae; i.e. the most proximal fascia is between the wing base and the line determined by pair of strigulae 1, it is the basal fascia; it is followed by the basal interfascia; correspondingly the next fasciae and interfasciae are the sub-basal, median, postmedian and pre-terminal. In *E. subvittana* most of the elements are ill-defined. The most recognisable are the strigulae pairs 5–9, in some specimens also 3 and 4 are discernible, and 1 and 2 are not discernible. The white dorsal spot typical in the Eucosmini and other tribes is formed by the dorsal portion of the interfascia between the sub-basal and median fasciae (i.e. it is a part of the sub-basal interfascia, therefore designated as "interfascial spot"), and in *E. subvittana* it is only slightly paler than the surrounding area.

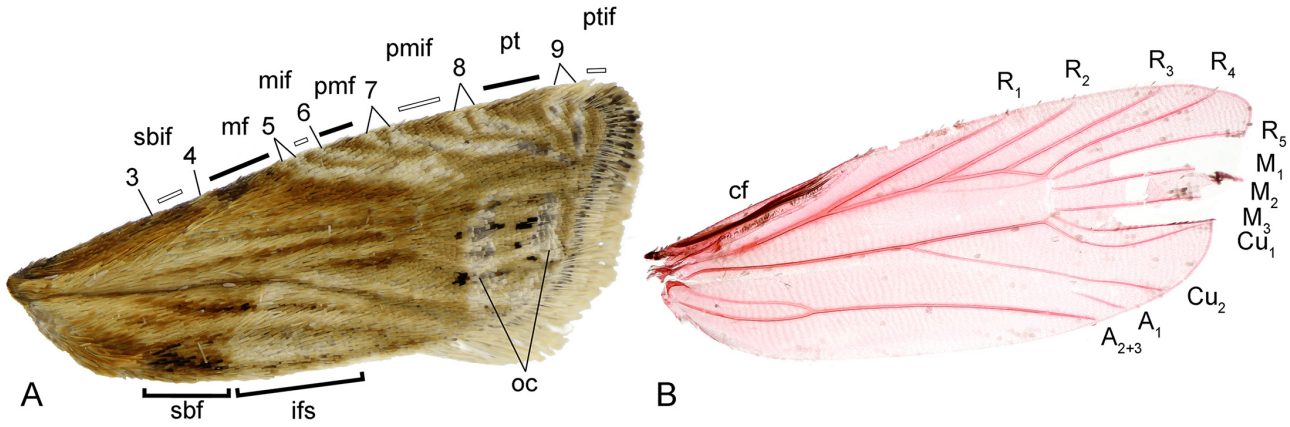

FIGURE 6. Forewing of *Eucosma subvittana* ♂. A. Elements of the wing pattern. B. Venation. The subcostal vein is located in the area of the costal fold and is not visible in the photograph. Legend: (3–9) pairs of costal strigulae; (cf) costal fold; (ifs) interfascial spot; (mf) median fascia; (mif) median interfascia; (oc) ocellus; (pmf) postmedian fascia; (pmif) postmedian interfascia; (pt) pre-terminal fascia; (ptif) pre-terminal interfascia; (sbf) sub-basal fascia; (sbif) sub-basal interfascia.

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