Metzneria neli Huemer, sp. n., a new species hitherto mixed with M. tristella Rebel, 1901 (Lepidoptera: Gelechiidae)

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Abstract

Metzneria neli Huemer, sp. n., a new species of the family Gelechiidae, is described from France and Spain and adults of both sexes and genitalia are figured. The new species differs both morphologically and in the DNA barcode from all other known representatives of the genus. It was formerly mixed with *Metzneria tristella* Rebel, 1901, and therefore this species is re-described here in detail.

KEY WORDS: Lepidoptera, Gelechiidae, Metzneria, new species, France, Spain.

Metzneria neli Huemer, sp. n., una nueva especie hasta ahora mezclada con M. tristella Rebel, 1901 (Lepidoptera: Gelechiidae)

Resumen

Metzneria neli Huemer, sp. n., una nueva especie de la familia Gelechiidae, se describe en Francia y España y se representan los adultos de ambos sexos y la genitalia. La nueva especie difiere tanto morfológicamente como en el código de barras del ADN de todas las demás representantes conocidas del género. Anteriormente se mezcló con *Metzneria tristella* Rebel, 1901, por lo que aquí esta especie se vuelve a describir en detalle. PALABRAS CLAVE: Lepidoptera, Gelechiidae, *Metzneria*, nueva especie, Francia, España.

Introduction

Metzneria Zeller, 1839 is a diverse genus of mainly Palearctic Gelechiidae, covering 24 described species in Europe (HUEMER & KARSHOLT, 2020). Additional taxa attached to this genus and mainly originating from Africa require revisionary work but have been questioned as congeneric (ENGLERT, 1974), whereas two species from North America (HODGES, 1983) have been introduced from Europe. *Metzneria*, contrary to several other European Gelechiidae, with the review of ENGLERT (1974), gained early attention. This paper was one of the first generic revisions of Western Palearctic Gelechiidae following a modern attempt, which, beside examination of type material, also included detailed studies of male genitalia. It is therefore not surprising that only five additional species have been described from Europe since, three of them finally turned out as synonyms of already named taxa.

Metzneria tristella Rebel 1901, originally described from Cuenca (Spain), is one of the species firstly revised by ENGLERT (1974). The seemingly unmistakable male genitalia were illustrated, but adults were not figured, and female genitalia remained unknown. These shortcomings may be responsible for the subsequent misinterpretation of the species in a later study, with description of the biology, the female sex and a new record for France (NEL, 1994). Later genetic data of alleged

M. tristella were published based on these French records (HUEMER *et al.*, 2020). However, a detailed morphological assessment of a potential new species from Spain led to the suspicion that in fact this taxon could be the true *M. tristella*. Examination of the lectotype of *M. tristella* fully supported this revised identification. Conversely, the species from France, previously identified as *M. tristella*, turned out to be undescribed. It differs from *M. tristella* and other congeners by phenotypic appearance, genitalia morphology and DNA barcodes and is here introduced as new species.

Material and methods

Specimens: Material used in this study was traditionally set and dried or, particularly more recently, pinned and subsequently spread. Earlier genitalia preparations followed standard techniques (ROBINSON, 1976) more recent slides were adapted for the male genitalia of Gelechiidae by the "unrolling technique" as described by PITKIN (1986).

DNA barcoding is based on 208 European specimens of *Metzneria* from 24 species, the majority originating from own samples, partially supplemented by public data from BOLD. DNA samples to obtain the 658 base-pair long barcode segment of the mitochondrial COI gene (cytochrome c oxidase I) (from dried legs) 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). Further details including complete voucher data and images can be accessed in the public dataset "DS-METZNERI *Metzneria* - new species" dx.doi.org/10.5883/DS-METZNERI in the Barcode of Life Data Systems (BOLD systems v. 4.0. http://www.boldsystems.org (RATNASINGHAM & HEBERT, 2007). Intra- and interspecific distances of DNA barcode fragment were calculated using analytical tools of BOLD with the Kimura 2-parameter model of nucleotide substitution. A Neighbor-joining tree of the successfully sequenced 24 species was constructed using MEGA 6 (TAMURA *et al.*, 2013) under the Kimura 2-parameter model for nucleotide substitutions.

For each species, a Barcode Identification Number (BIN) is provided, which is automatically calculated for records in Bold Data Systems that are compliant with the DNA Barcode standard (RATNASINGHAM & HEBERT, 2013).

Photographic documentation: Photographs of the adults were taken with an Olympus SZX 10 binocular microscope and an Olympus E 3 digital camera and treated using the software Helicon Focus 4.3, Adobe Photoshop CS4, and Lightroom 2.3 softwares. Genitalia photographs were taken with an Olympus E1 Digital Camera through an Olympus BH2 microscope.

Abbreviations of collections:

NHMV = Naturhistorisches Museum, Vienna, Austria RCTM = Research collection of Toni Mayr, Feldkirch, Austria TLMF = Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria

Results

MOLECULAR ANALYSIS

DNA sequencing resulted in a full barcode fragment of 658 bp for 159 specimens and 49 sequences > 540 bp. Sequences of the COI barcode region of analysed nominal species reveal moderate intraspecific but significantly higher interspecific genetic distances. Mean distances within species are 0.90% with a maximum of 2.56%. However, species with an exceptional intraspecific divergence are to be re-assessed for potential cryptic diversity. Interspecific distances are much higher with 5.83% on average, ranging from minimum 1.12% to maximum 9.25% distance to the nearest

neighbor (Table 1). All species, except for the morphologically well separated species pair *M. fulva* Labonne, Huemer, Thibault & Nel, 2019 and *M. torosulella* (Rebel, 1893) group in one or several unique BINs thus clearly supporting the specific status of *M. tristella* and the newly described *M. neli* (fig. 1).

Table 1.- Intraspecific mean K2P (Kimura 2 Parameter) divergences, maximum pairwise distances, nearest species, nearest neighbor and distance to nearest neighbor (in %).

Species	Mean Intra-sp	Max Intra-sp	Nearest Species	Distance to NN
Metzneria aestivella	0.7	1.98	Metzneria castiliella	7.86
Metzneria agraphella	N/A	0	Metzneria lappella	9.25
Metzneria aprilella	2.13	4.62	Metzneria ehikeella	5.72
Metzneria artificella	2.56	4.62	Metzneria diffusella	6.78
Metzneria campicolella	1.75	2.18	Metzneria castiliella	8.23
Metzneria castiliella	0.73	1.43	Metzneria aprilella	6.08
Metzneria diffusella	1.73	2.83	Metzneria neuropterella	5.46
Metzneria ehikeella	1.45	3.81	Metzneria metzneriella	4.39
Metzneria fulva	0.16	0.16	Metzneria torosulella	1.12
Metzneria hilarella	0.25	0.64	Metzneria staehelinella	5.91
Metzneria intestinella	0.93	0.93	Metzneria tristella	6.18
Metzneria lappella	0.31	0.96	Metzneria ehikeella	6.55
Metzneria littorella	0.25	0.62	Metzneria riadella	7.69
Metzneria metzneriella	1.79	4.37	Metzneria ehikeella	4.39
Metzneria neli	2.19	2.19	Metzneria metzneriella	5.06
Metzneria neuropterella	1.56	4.12	Metzneria diffusella	5.46
Metzneria paucipunctella	0.6	1.88	Metzneria ehikeella	5.6
Metzneria riadella	0.31	0.31	Metzneria littorella	7.69
Metzneria santolinella	1.29	2.03	Metzneria ehikeella	4.91
Metzneria staehelinella	N/A	0	Metzneria ehikeella	4.54
Metzneria subflavella	N/A	0	Metzneria metzneriella	5.87
Metzneria tenuiella	0	0	Metzneria ehikeella	8.41
Metzneria torosulella	0.74	0.74	Metzneria fulva	1.12
Metzneria tristella	0.16	0.16	Metzneria metzneriella	5.61

Taxonomic conclusions

Metzneria neli Huemer, sp. n.

Material examined: Holotype δ , "NEL Jacques Mt-Coudon, VAR e.1. m. 15-05-1996 % *Centaurea intybaceus*" "BC TLMF Lep 06937" "P. Huemer GEL 1313 δ " (TLMF). Paratypes: FRANCE, BdR, la Ciotat, rte des Crètes, 1 δ , 15-V-1993, leg. J. Nel (gen. slide 0963 J.N.); ditto, but 1 δ , 17-V-1993 (gen. slide 0948 J.N.); ditto, but 1 \Im , 8-VI-1993 (gen. slide 0972 J.N.); ditto, but 1 δ , 1 \Im , 11-VI-1993; ditto, but 1 δ , 13-VI-1993; ditto, but 2 $\delta\delta$, 14-VI-1993; ditto, but 1 δ , 15-VI-1993; ditto, but 1 \Im , 16-VI-1993; ditto, but 1 \Im , 18-VI-1993; ditto, but 1 \Im , 19-VI-1993; ditto, but 1 \Im , 20-VI-1993 (gen. slide 01132 J.N.); ditto, but 1 \Im , 23-VI-1993; ditto, but 1 \Im , 21-VI-1995; Var, Mt-Faron, 1 δ , 17-VI-1996 e.1. (*Centaurea intybaceus*), leg. J. Nel; BdR, la Ciotat, Brusquièras de Cassis, 1 \Im , 26-IV-2003, leg. J. Nel (gen. slide GEL 1317 \Im P. Huemer) (TLMF).

Excluded from type series: SPAIN, Alicante, Agost, 1 ^{\circ}, 21-VI-2019, leg. F. Graf, DNA Barcode ID TLMF Lep 26268 (Research collection Friedmar Graf).

Description: Adult (figs 2-3). Head pale ochreous; antenna serrate in male, filiform in female, greyish brown, weakly annulated; labial palpus dark greyish brown, with pale ochreous mottling,

particularly at upper surface of segment 2, segment 2 thickened with appressed scales, segment 3 about half length of segment 2, distinctly thickened from appressed dorsal scale brush; thorax and tegula pale ochreous with some dark mottling. Forewing length δ 6.6-7.2 mm (n=5), \Im 7.2-7.6 mm (n=5). Forewing upper side greyish brown, light orange-yellow line along fold, short orange-yellow streaks and patches along veins, particularly at discal spot and along subcostal area and wing base, oblique transverse streak at end of cell extended to costa; stigmata black, first in distal part of fold, plical and discal spots widely separated; termen weakly concave, terminal line fuscous; fringes light greyish, with distinct dark grey fringe line and dark grey apices. Hindwing narrow, grey, fringes ochreous-grey without fringe line. Underside of forewings without lighter costal spot and distinctly divided fringes.

Male genitalia (fig. 8): Uncus with rounded lateral humps; tegumen much wider than long, anterior margin weakly emarginated; pedunculi small; valva basally narrow, distal part strongly dilated to about twice width, convex dorsal and nearly straight ventral margin, with tooth-like apex pointed ventrad; sacculus evenly thorn-shaped; saccus sub-triangular, short; phallus stout, about 3 times longer than wide, subapical area with band like structure covered with spinules, apex with finger-shaped narrow projection, vesica with two moderately long and one shorter cornuti.

Female genitalia (fig. 11): Papilla analis broadly sub-oval, weakly setose; apophysis anterioris about three times length of papilla analis, rod-like, with forked posterior end; segment VIII ventromedially and dorsomedially membranous without modifications, posterior edge with long and stiff setae; entrance of membranous ductus bursae with irregular shortly funnel-shaped colliculum; corpus bursae small, membranous without any modifications.

Diagnosis: *Metzneria neli* Huemer, sp. n. from genitalia morphology is most closely related to *M. tristella*. It differs from that species by several characters of adult phenotype, i.e. the scale brush of the third segment of labial palpus, the comparatively broad and serrated antenna of the male, and the overall the lighter ground colour of the forewing with lighter and more extended orange-yellowish markings, the concave terminal excavation and the presence of a distinct fringe line. Male and female genitalia are in fact much more similar to *M. tristella* but particularly differ in the male. *Metzneria neli* Huemer, sp. n. is characterized i.e. by the comparatively longer narrow base of the valva, the evenly thorn-shaped sacculus and the phallus with long apical projection. Particularly the shape of the sacculus is unique in the genus. The much stronger and longer setae on the posterior edge of segment VIII of *M. neli* sp. n. seem to be of diagnostic value in the overall very similar female genitalia but variation is insufficiently known.

Molecular data: BIN: BOLD:ABX1726. The intraspecific average distance of the barcode region is 1.45% (p-dist) (n=2). A unique specimen from Spain corresponding with *M. neli* sp. n. from external appearance is 2.25% distant (BIN: BOLD:AEB4629) and is considered as likely conspecific. The distance to the nearest neighbor *M. metzneriella* Stainton, 1851 is 5.06%.

Distribution: Currently only known from few localities in southern France and from the above mentioned single barcoded specimen from Spain but most likely more widely distributed on the Iberian Peninsula.

Bionomics: The larva lives in the flower and sead heads of *Cheirolophus intybacea* (Lam.) Dostál where it hibernates (NEL, 1994).

Remarks: A unique specimen from Spain is considered conspecific with *M. neli* from external appearance and the similarity of the DNA barcode. However, in absence of further morphological support it is only tentatively attached to this species and not included in the type series. Following Nel (1994) the number of cornuti varies from 0 to 3.

Derivatio nominis: The new species is dedicated to Dr. Jacques Nel (La Ciotat, France), eminent Lepidopterist and collector of the type series.

Metzneria tristella Rebel, 1901

Metzneria (Parasia) tristella Rebel, 1901. Dt. ent. Z., Iris, 13(2): 164

Material examined: Lectotype &, here designated: "Stgr. 900 Cuenca" "Metz. tristella Rbl. Type" "tristella 6212 Rbl. E. Jäckh 1971 % MUS. VIND. 723" (NHMV).

Other material: SPAIN, Aragon, Lake Riba-Roja, Mequienca, 1 & 1-2-V-2000, leg. J. Ortner (gen. slide GEL 1312 & P.H., DNA Barcode TLMF Lep 28251); Castilia - La Mancha, N Cuenca, 1065 m, 40°06.54'N, 02°10.0'W, 2 \Im , 17-V-2012, leg. T. Mayr (gen. slide GEL 1316 \Im P.H., DNA Barcode TLMF Lep 25480, DNA Barcode TLMF Lep 25481) (TLMF, RCTM).

Description Adult (figs 4-5): Head fuscous to greyish brown; antenna filiform, dark greyish brown, weakly annulated; labial palpus greyish brown, lighter at upper surface of segment 2, segment 2 thickened with appressed scales, segment 3 about half length of segment 2, weakly thickened; thorax and tegula fuscous with few lighter scales. Forewing length δ 5.8–7.0 mm (n=2), \Im 5.2–5.6 mm (n=2). Forewing upper side dull brownish grey, orange-brown line along fold, short orange-brown streaks along veins, particularly along discal spot and at subcosta; stigmata black, first in distal part of fold, plical and discal spots widely separated; terminal line fuscous; fringes fuscous, without fringe line. Hindwing narrow, grey, concolorous fringes without fringe line. Underside of wings without any markings.

Male genitalia (figs 9-10): Uncus with lateral humps; tegumen much wider than long, anterior margin weakly emarginated; pedunculi small; valva with short and narrow base, strongly dilated distal part of about twice width, convex dorsal and nearly straight ventral margin, with tooth-like apex pointed ventrad; sacculus thorn-shaped, with particularly broad base and abruptly narrowed apex; saccus sub-triangular, short; phallus stout, about 3 times longer than wide, subapical area with band like structure covered with spinules, apex with short sub-triangular projection and vesica with two moderately long cornuti.

Female genitalia (fig. 12): Papilla analis broadly sub-oval, weakly setose; apophysis anterioris about three times length of papilla analis, rod-like, with forked posterior end; segment VIII ventromedially and dorsomedially membranous without modifications, posterior edge with short setae; entrance of membranous ductus bursae with irregularly funnel-shaped colliculum; corpus bursae small, membranous without any modifications.

Diagnosis: Easily separated from other congeneric species y the dull colour of the forewing. For other diagnostic characters see above.

Molecular data: BIN: BOLD:ADM8252. The intraspecific average distance of the barcode region is 0.16% (p-dist) (n=2). The minimum distance to the nearest neighbor *M. metzneriella* is 5.81%.

Distribution: Proved specimens originate from Spain, whereas records from Portugal (CORLEY *et al.*, 2000) and Tunisia (NEL, 1994) require verification.

Bionomics: The larva has been recorded from *Cheirolophus sempervirens* Pomel (CORLEY *et al.*, 2000) but it remains uncertain if this record refers to *M. tristella* or to another related species.

Remarks: *Metzneria tristella* was described from an unspecified number of specimens collected nearby Cuenca (REBEL, 1901). The re-examined lectotype was designated by ENGLERT (1974) in order to fix the identity of the species and conserve stability of nomenclature (figs 6-7, 10).

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Fig. 1.– Neighbor-Joining tree of European *Metzneria* species (Kimura 2 parameter). Note: the scale bar only applies to internal branches between species. Width of triangles represent sample size, depth the genetic variation within the cluster. Source: DNA Barcode data from BOLD (Barcode of Life Database, cf. RATNASINGHAM & HEBERT, 2007).



Figs 2-7.– Adults: **2.** *Metzneria neli* Huemer, sp. n., *δ*, paratype. **3.** *Metzneria neli* Huemer, sp. n., *φ*, paratype. **4.** *Metzneria tristella* Rebel, 1901, *δ*. **5.** *Metzneria tristella* Rebel, 1901, *φ*. **6.** *Metzneria tristella* Rebel, 1901, *δ*, lectotype. **7.** *Metzneria tristella* Rebel, 1901, *δ*, lectotype labels.

