

Metzneria leae Gastón & Huemer, sp. n., a new species from Spain (Lepidoptera: Gelechiidae)

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Abstract

Metzneria leae Gastón & Huemer, sp. n., a new species in the family Gelechiidae, is described from Spain and adults and genitalia of both sexes are figured. The new species differs morphologically and in the DNA barcode from all other known representatives of the genus. Until now, the new species has been confused with *Metzneria subflavella* Englert, 1974, so both species are compared in detail.

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

Metzneria leae Gastón & Huemer, sp. n., una nueva especie para España
(Lepidoptera: Gelechiidae)

Resumen

Se describe una nueva especie de la familia Gelechiidae *Metzneria leae* Gastón & Huemer, sp. n., para España y se presentan adultos y genitales de ambos sexos. La nueva especie difiere, tanto morfológicamente como en el código de barras del ADN, de todos los demás representantes conocidos del género. Hasta ahora, la nueva especie ha podido ser confundida con *Metzneria subflavella* Englert, 1974, por lo que ambas especies se comparan en detalle.

PALABRAS CLAVE: Lepidoptera, Gelechiidae, nueva especie, España.

Introduction

The European Gelechiidae have received considerable interest in recent decades, boosting the numbers of nominal species records from 617 to 865 within only 24 years (HUEMER & KARSHOLT, 2020; KARSHOLT & RAZOWSKI, 1996). Nevertheless, molecular data still indicate a considerable number of previously overlooked species, particularly in some diverse but unrevised genera, e. g. *Stomopteryx* Heinemann, 1870, *Aproaerema* Durrant, 1897, *Aristotelia* Hübner, [1825] and *Monochroa* Heinemann, 1870 (HUEMER *et al.*, 2020).

However, gaps in species lists are also evident in genera that have already been revised using standard morphological methods. *Metzneria* Zeller, 1839, is one such example. The genus was extensively revised by ENGLERT (1974), and 27 species were reviewed, including four new species. Despite a modern approach at the time with the study of type material and examination of male and female genitalia, the work has serious deficits from today's point of view. In particular, the lack of images of adults and female genitalia structures is often a considerable hurdle for correct identifications.

As a consequence, uncertainties in the correct identification of individual species of *Metzneria* are

reflected in the extremely diverse fauna of Spain. VIVES MORENO (2014) listed 24 species of *Metzneria* from Spain. Two have been synonymized, viz. *M. exposito* Vives, 2001 and *M. varennei* Nel, 1997 (HUEMER & KARSHOLT, 2020), whereas *M. neli* Huemer, 2021 was recently added to the national fauna (HUEMER, 2021). Inspired by that study JG re-examined several of his *Metzneria* and found another hitherto overlooked species, closely related to *M. subflavella* Englert, 1974, which is described below.

Material and methods

Specimen's material used for the study has been obtained by sampling at night and by day, with actinic light traps distributed in the appropriate biotopes and having the authorizations of the relevant regions. We have made our identifications based on comparative examination of the external morphological characters and, above all, on the analysis of the genital structure of the specimens. Material was traditionally set and dried or only pinned.

Genitalia preparations either followed standard techniques (ROBINSON, 1976) or slides of the male genitalia were adapted by the “unrolling technique” as described by PITKIN (1986).

Tissue samples (a single hind leg) were prepared according to prescribed standards to obtain DNA barcode sequences on a 658 base-pair long segment of the mitochondrial COI gene (cytochrome c oxidase subunit 1) 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). The obtained sequences cover the complete Spanish fauna of 24 *Metzneria* species with 159 barcodes > 500 bp and only a single short sequence of *M. subflavella* used for the Neighbour-Joining tree. The majority of sequences originate from our own samples, supplemented by public data from BOLD. Further details including complete voucher data and images can be accessed in the public dataset “DS-METZLEAE *Metzneria leae* sp. n.” dx.doi.org/10.5883/DS-METZLEAE in the Barcode of Life Data Systems (BOLD systems v. 4.0. <http://www.boldsystems.org> (RATNASINGHAM & HEBERT, 2007).

Degrees of intra- and interspecific variation of DNA barcode fragments were calculated using the Kimura 2 parameter model on BOLD systems v. 4.0. (<http://www.boldsystems.org>). A COI Neighbour-Joining tree was constructed using the Kimura 2 parameter model in MEGA6 (TAMURA *et al.*, 2013). A three-letter code (ISO 3166-1 alpha-3, https://en.wikipedia.org/wiki/ISO_3166-1_alpha-3) was used to abbreviate country names in this tree. DNA Barcode Identification Numbers (BINs) are automatically calculated for records in Bold Data Systems that are compliant with the DNA Barcode standard (RATNASINGHAM & HEBERT, 2013).

Photographic documentation: For the photographic documentation of the genitalia, NIKON Eclipse E400 microscopes and NIKON D3100 digital cameras have been used. For photographic documentation of the adults, SONY α 100 DSLR-A100K digital camera have been used with an AF MACRO, 100 mm 1:2.8 (32) lens. The photographic retouches have been made in the Adobe Photoshop © program.

Abbreviations:

RCFG	Research collection of Friedmar Graf, Bautzen, Germany
RCJG	Research collection of Javier Gastón, Getxo, Spain
RCJJ	Research collection of Jari Junnilainen, Vantaa, Finland
RCTM	Research collection of Toni Mayr, Feldkirch, Austria
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
JG	Javier Gastón
PH	Peter Huemer
gen. prep.	genitalia preparation
sp. n.	new species

Results

MOLECULAR ANALYSIS

DNA sequencing analysis is based on barcode sequences > 600 bp for 157 specimens, two sequences > 500 bp and one with only 261 bp, covering all 24 species known from Spain. Sequences of the majority of species reveal moderate intraspecific mean distances of 0.97% but significantly higher interspecific distances with mean distances of 6.37% to the nearest neighbours (Table 1). Exceptionally large intraspecific distances beyond 3% are observed in *M. aprilella* (Herrich-Schäffer, 1854), *M. artificella* (Herrich-Schäffer, 1861), *M. diffusella* Englert, 1974, *M. metzneriella* (Stainton, 1851) and *M. neuropterella* (Zeller, 1839) and all these taxa should be re-assessed for potential cryptic diversity (HUEMER *et al.*, 2020). All species cluster in one or several unique BINs and molecular analysis also clearly supports the specific status of *M. leae* and its nearest neighbour *M. subflavella* (Fig. 1).

Table 1.– Intraspecific mean K2P (Kimura 2 Parameter) divergences, maximum pairwise distances, nearest species, nearest neighbour and distance to nearest neighbour (in %) of *Metzneria* species recorded from Spain.

Species	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Distance to NN
<i>Metzneria aestivella</i>	0.77	1.98	<i>Metzneria castiliella</i>	7.86
<i>Metzneria agraphella</i>	N/A	0	<i>Metzneria lappella</i>	9.64
<i>Metzneria aprilella</i>	2.55	4.61	<i>Metzneria ehikeella</i>	5.72
<i>Metzneria artificella</i>	2.56	4.62	<i>Metzneria diffusella</i>	6.78
<i>Metzneria campicolella</i>	1.36	1.36	<i>Metzneria castiliella</i>	8.23
<i>Metzneria castiliella</i>	0.8	1.43	<i>Metzneria aprilella</i>	6.08
<i>Metzneria diffusella</i>	2.16	3.57	<i>Metzneria neuropterella</i>	5.46
<i>Metzneria ehikeella</i>	1.04	2.03	<i>Metzneria staehelinella</i>	4.59
<i>Metzneria hilarella</i>	0.71	1.87	<i>Metzneria staehelinella</i>	5.91
<i>Metzneria intestinella</i>	0.93	0.93	<i>Metzneria tristella</i>	6.18
<i>Metzneria lappella</i>	0.18	0.77	<i>Metzneria leae</i>	6.4
<i>Metzneria liitorella</i>	0.41	0.62	<i>Metzneria subflavella</i>	7.91
<i>Metzneria metzneriella</i>	2.5	4.47	<i>Metzneria ehikeella</i>	4.76
<i>Metzneria neli</i>	2.19	2.19	<i>Metzneria metzneriella</i>	5.21
<i>Metzneria neuropterella</i>	1.63	4.12	<i>Metzneria diffusella</i>	5.46
<i>Metzneria paucipunctella</i>	0.6	1.88	<i>Metzneria ehikeella</i>	5.75
<i>Metzneria riadella</i>	0.31	0.31	<i>Metzneria liitorella</i>	8.09
<i>Metzneria santolinella</i>	1.29	2.03	<i>Metzneria ehikeella</i>	5.07
<i>Metzneria leae</i>	0.62	0.93	<i>Metzneria subflavella</i>	5.58
<i>Metzneria staehelinella</i>	N/A	0	<i>Metzneria ehikeella</i>	4.59
<i>Metzneria subflavella</i>	N/A	0	<i>Metzneria leae</i>	5.58
<i>Metzneria tenuiella</i>	0	0	<i>Metzneria ehikeella</i>	8.58
<i>Metzneria torosulella</i>	0.74	0.74	<i>Metzneria neuropterella</i>	7.56
<i>Metzneria tristella</i>	N/A	0	<i>Metzneria ehikeella</i>	5.83

TAXONOMY

Metzneria leae Gastón & Huemer, sp. n.

Material examined: Holotype, 1 ♂, SPAIN, CASTELLÓN, Coves de Vinroma, Mas de la Bassa, 22-V-2004, J. Domínguez leg., ADN genetic sequence number TLMF Lep 06958, gen. prep. 9050JG, deposited in Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria (TLMF).

Paratypes 3 ♂♂, 3 ♀♀: SPAIN, TERUEL, Albarracín, 1100 m, 2 ♂♂, 30-V-1997, J. Gastón leg. and

coll., gen. prep. 4641JG and 8971JG; idem. 2 ♀♀, gen. prep. 7655JG and 8972JG; idem, 1 ♀, 24-VI-2019, F. Graf leg. and coll., ADN genetic sequence number TLMF Lep 26266; idem, Sierra de Cucalón, 1275 m, 1 ♂, 21-VI-2017, J. Junnilainen leg. and coll., ADN genetic sequence number MM26896.

Description of adult (Figs 2-3): Wingspan 17 mm (n=5). Head with compact pale ochreous scales on frons and vertex becoming black along margin of eye. Labial palpus well developed, long second segment directed forwards and slightly curved upwards, densely covered with reddish ochreous scales, distally light cream, short third segment strongly curved upwards and covered with very short reddish ochreous scales, pale cream scales at apex; antennae filiform, dark brown and ringed paler. Thorax and tegulae pale ochreous; abdomen slightly paler ochreous; femur and tibia of all legs with reddish ochreous hairs mixed with pale cream hairs. Forewing with acute apex, characteristic of the genus; pale yellowish ochre, reddish yellow in fresh specimens and especially females, broad, very dark ochreous, costal streak from base to apex, on the longitudinal veins bordering cell and in the subterminal area; discal spot dark and diffuse, sometimes obscure; hindwing narrow, grey, cilia grey, basally pale ochreous.

Male genitalia (Fig. 8): Uncus wide, short, distally with two triangular projections and broadly V-shaped median emargination; tegumen much broader than long, with rounded posterior margin; small pedunculi; valva short narrow base, strongly dilated, digitiform distal part, a pronounced and pointed tooth at apex, dorsal margin convex and nearly straight ventral margin; sacculus triangular, broad basally, abruptly narrowing to pointed apex; saccus sub-triangular, short; phallus wide, bulbous and short, apex with short finger-like subtriangular projection; subapical area of inverted vesica membranous covered with minute spinules, mixed with somewhat larger and very slightly sclerotised spines, vesica with three to four medium-long and strongly sclerotised cornuti in middle.

Female genitalia (Fig. 9): Papilla analis sub-oval, weakly setose; apophysis posterioris about two and a half times length of papilla analis; segment VIII slightly sclerotised, short with a row of setae on posterior margin; apophysis anterioris of the same length as apophysis posterioris; antrum membranous, funnel-shaped; entrance of membranous ductus bursae with a short, strongly sclerotised colliculum, anteriorly with an inconspicuous tongue-shaped projection; ductus bursae membranous, narrow and long; corpus bursae membranous, ovoid, small, without signum.

Diagnosis: *Metzneria leae* sp. n. is very similar to *M. subflavella* in the male and female genitalia. It differs from that species in several characters in the adult, especially in the forewing pattern in having mostly dark ochreous scales on the costal margin, and a poorly defined dark spot at the distal end of the cell, which is absent in *M. subflavella* (Figs 4-6). In the male genitalia of *M. subflavella* (Fig. 10) the valva is less dilate distally and the sacculus has a broader base with the dorsal margin curved upwards, whilst in *M. leae* sp. n., the base is narrower and both margins are straight. In the vesica of the phallus of *M. subflavella* a group of 3 or 4 medium-long and strongly sclerotised cornuti is present with several small and weakly sclerotised cornuti, which are absent in *M. leae* sp. n. The female genitalia of *M. subflavella* (Fig. 11) are very similar to *M. leae* sp. n. but with a significantly shorter ductus bursae.

Molecular data: BIN: BOLD:ABW1820. The intraspecific average distance of the barcode region is 0.62%, the maximum distance is 0.93% (p-dist) (n=3). The distance to the nearest neighbour in BOLD, an unidentified and unpublished gelechiid from China (BIN: BOLD:AEJ1362) which in fact belongs to the genus *Metzneria*, is 2.72%. The minimum distance to the nearest European congeneric species, *M. subflavella*, is 5.58% (BIN: BOLD:ADM8252).

Distribution (Fig. 7): *Metzneria leae* Gastón & Huemer, sp. n. is only known from a few localities in eastern Spain, in the supra-Mediterranean bioclimatic zone (RIVAS-MARTÍNEZ, 1987), but is likely to be more widely distributed on the Iberian Peninsula. The closely related *M. subflavella* is stated to occur from Western Europe to Central Asia (ELSNER *et al.*, 1999; ENGLERT, 1974; ÖZASLAN *et al.*, 2016; PASTORÁLIS *et al.*, 2018). However, several of these earlier records require verification, and uncertainties in the identification also affect previous reports from the Iberian Peninsula, i. e. Portugal (CORLEY, 2005) and Spain (DERRA & HACKER, 1982). Oleksiy Bidzilya has provided us with a photograph of a specimen captured by Ernst Arenberger on 14-VII-1967 in Beuda (Gerona,

Spain) (fig. 4) and deposited in the Staatliches Museum für Naturkunde, Karlsruhe (Germany). The habitus of this specimen corresponds to *M. subflavella*. The type series of *M. subflavella* was collected between 24-30-VI-1959 in south-east France (Durance Valley, St. Crépin) (ENGLERT, 1974).

Bionomics: Host-plants and preliminary stages are undescribed. A record of rearing *M. subflavella* from the seed-heads of *Klasea baetica* Cass., in F. Cuvier in Portugal (CORLEY, 2005) possibly refers to the new species. In Turkey *M. subflavella* has been bred from *Centaurea stapfiana* (Hand.-Mazz.) Wagenitz (ÖZASLAN *et al.*, 2016).

Remarks: A unique specimen from Iran clusters near *M. laeae* in the DNA barcode. However, morphological traits, particularly the male genitalia, do not convincingly support the conspecificity and the species requires careful re-assessment from additional samples.

Etyymology: The new species is dedicated to Lea Gastón Kitamura, first author's granddaughter.

Acknowledgements

PH is particularly grateful to Paul D. N. Hebert (Canada) and the staff at the Canadian Centre for DNA Barcoding for sequence analysis and support with BOLD. Several DNA barcodes and/or specimens were generously made available by various colleagues, particularly Kai Berggren (Norway), Jari Junnilainen (Finland), Marko Mutanen (Finland), and Friedmar Graf (Germany). Antonio Vives (Spain) for various information and editorial, to Olesksiy Bidzilya (Ukraine) for providing the photograph of the specimen of *M. subflavella* deposited in the Staatliches Museum für Naturkunde, Karlsruhe (Germany), and last but not least Robert J. Heckford (United Kingdom) for his careful language proofreading with valuable comments.

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(Recibido para publicación / *Received for publication* 16-IV-2022)

(Revisado y aceptado / *Revised and accepted* 20-V-2022)

(Publicado / *Published* 30-VI-2022)

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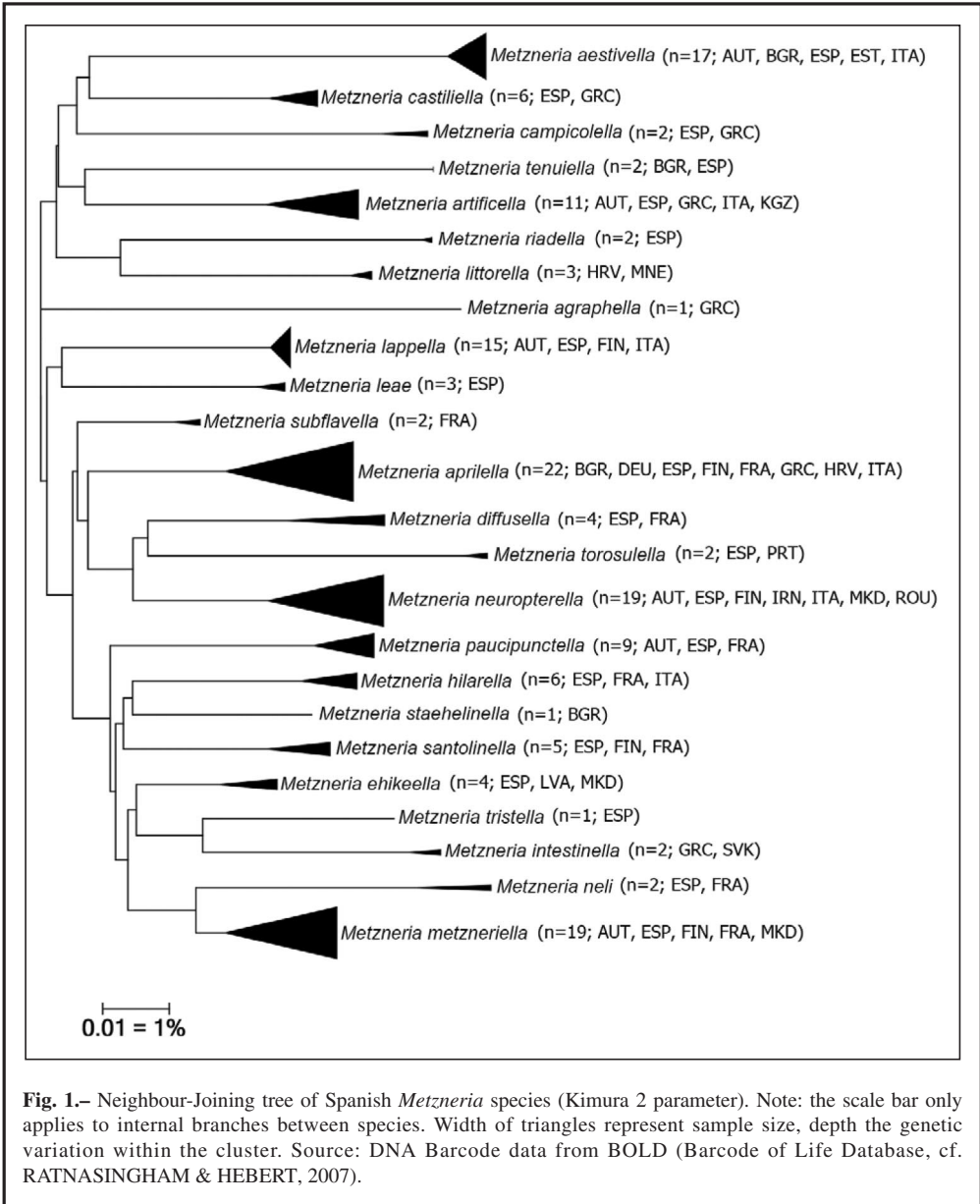
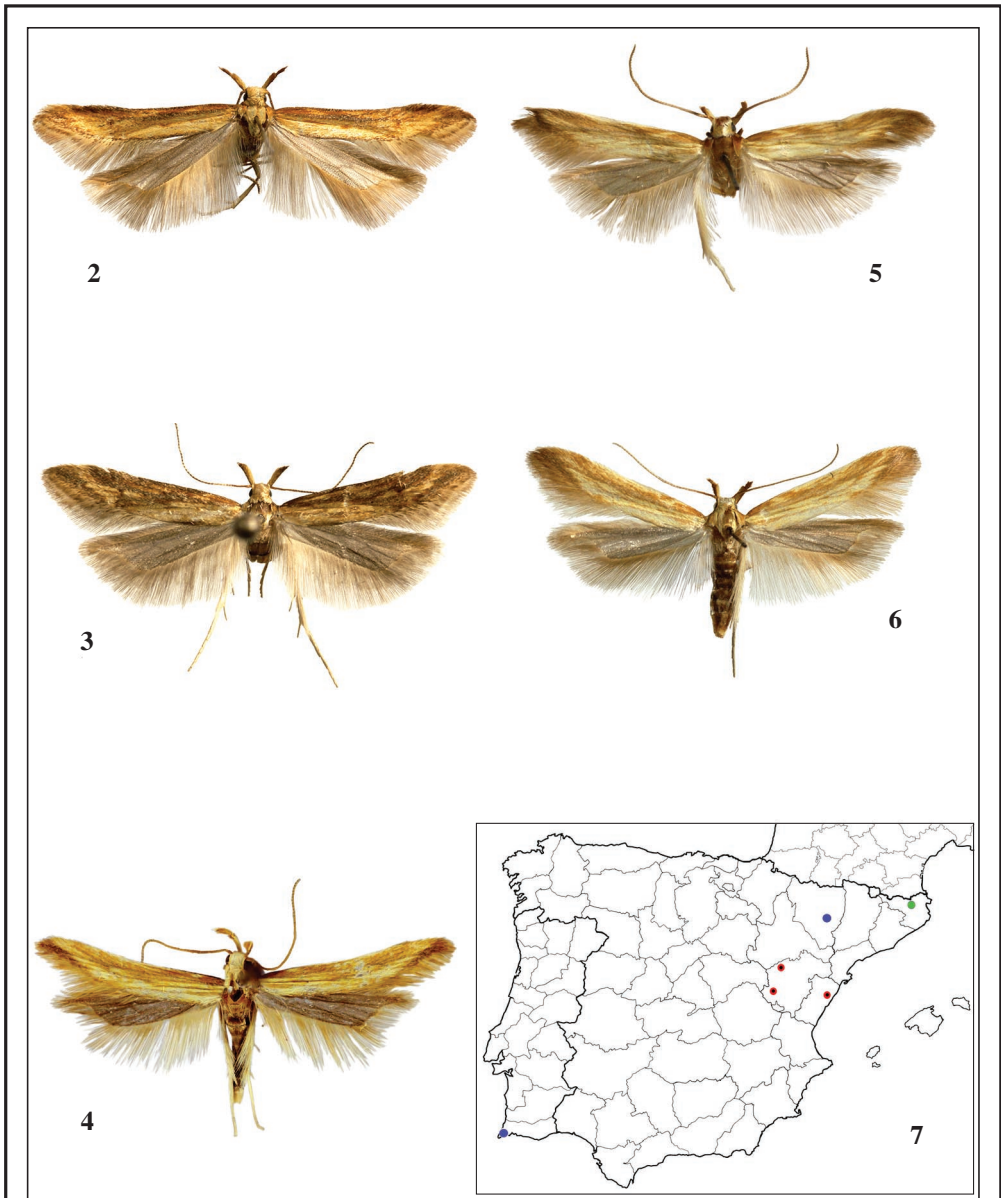
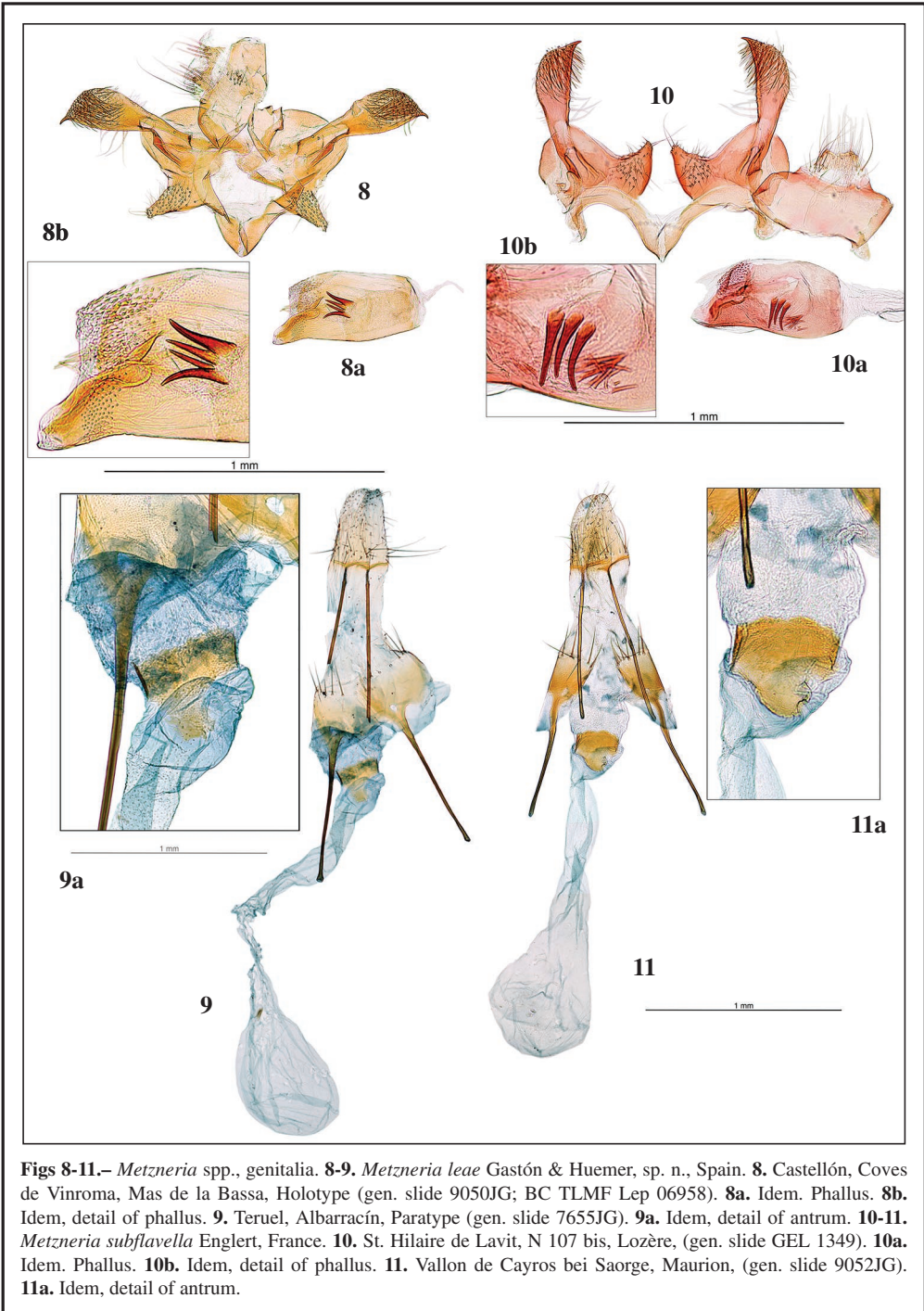


Fig. 1.– Neighbour-Joining tree of Spanish *Metzneria* species (Kimura 2 parameter). Note: the scale bar only applies to internal branches between species. Width of triangles represent sample size, depth the genetic variation within the cluster. Source: DNA Barcode data from BOLD (Barcode of Life Database,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



Figs 2-7.– *Metzneria* spp., adults and maps. **2-3.** *Metzneria leae* Gastón & Huemer, sp. n., Spain. **2.** Castellón, Coves de Vinroma, Mas de la Bassa, 22-V-2004, Holotype, male (gen. slide 9050JG; BC TLMF Lep 06958). **3.** Teruel, Albarracín, 1100 m, 30-V-1997, Paratype, female (gen. slide 7655JG). **4-6.** *Metzneria subflavella* Englert. **4.** Gerona, Beuda, 14-VII-1967, male. **5.** France, St. Hilaire de Lavit, N 107 bis, Lozère, 350 m, 15-V-1993, ex larva, male (gen. slide 9051JG). **6.** France, Vallon de Cayros bei Saorge, Maurion, 670 m, 6-VII-2012, female (gen. slide 9052JG). **7.** distribution map; red circle, *Metzneria leae* sp. n.; green circle, *Metzneria subflavella*; blue circle, *Metzneria subflavella* (bibliographic citations not examined).



Figs 8-11.– *Metzneria* spp., genitalia. **8-9.** *Metzneria leae* Gastón & Huemer, sp. n., Spain. **8.** Castellón, Coves de Vinroma, Mas de la Bassa, Holotype (gen. slide 9050JG; BC TLMF Lep 06958). **8a.** Idem. Phallus. **8b.** Idem, detail of phallus. **9.** Teruel, Albarracín, Paratype (gen. slide 7655JG). **9a.** Idem, detail of antrum. **10-11.** *Metzneria subflavella* Englert, France. **10.** St. Hilaire de Lavit, N 107 bis, Lozère, (gen. slide GEL 1349). **10a.** Idem. Phallus. **10b.** Idem, detail of phallus. **11.** Vallon de Cayros bei Saorge, Maurion, (gen. slide 9052JG). **11a.** Idem, detail of antrum.