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# A revision of the *Phyllonorycter ulicicolella* species group with description of a new species (Lepidoptera: Gracillariidae)

Z. Laštůvka, A. Laštůvka & C. Lopez-Vaamonde

## Abstract

Diagnostic morphological and biological characteristics of all known species of the *Phyllonorycter ulicicolella* species group are presented. Results of DNA barcoding analysis are shown. One new species of this group, *Phyllonorycter erinaceae* Laštůvka & Laštůvka, sp. n. living on *Erinacea anthyllis* is described from Spain. *Phyllonorycter scorpius* Laštůvka & Laštůvka, 2006 is synonymized with *Ph. echinosparti* Laštůvka & Laštůvka, 2006, and *Ph. lobeliella* Nel, 2009 with *Ph. baetica* Laštůvka & Laštůvka, 2006. New faunistic and biological observations (host plant use) are added. Questionable cases are considered. Important diagnostic morphological characters (forewings, right valvae and female genitalia) are illustrated.

KEY WORDS: Lepidoptera, Gracillariidae, *Phyllonorycter*, COI, DNA Barcoding, new species.

## Una revisión de las especies del grupo *Phyllonorycter ulicicolella* con la descripción de una nueva especie (Lepidoptera: Gracillariidae)

## Resumen

Se definen las características morfológicas y biológicas de todas las especies conocidas del grupo *Phyllonorycter ulicicolella*. Se muestran los resultados del análisis del código de barras de ADN. De este grupo, se describe una nueva especie de España: *Phyllonorycter erinaceae* Laštůvka & Laštůvka, sp. n., viviendo en *Erinacea anthyllis*. *Phyllonorycter scorpius* Laštůvka & Laštůvka, 2006 se sinonimiza con *Ph. echinosparti* Laštůvka & Laštůvka, 2006, y *Ph. lobeliella* Nel, 2009 con *Ph. baetica* Laštůvka & Laštůvka, 2006. Se presentan nuevas observaciones faunísticas y biológicas. Se comentan los casos dudosos. Se ilustran los caracteres morfológicos importantes en el diagnóstico (ala anterior, valva derecha y la genitalia de la hembra).

PALABRAS CLAVE: Lepidoptera, Gracillariidae, *Phyllonorycter*, COI, ADN codigos de barras, nueva especie.

## Introduction

With over 400 species described worldwide, the leaf-mining moth genus *Phyllonorycter* Hübner, [1822] (Gracillariidae) is one of the most hyperdiverse genera of the order Lepidoptera (DE PRINS & DE PRINS, 2005, 2011). Despite being fairly well known taxonomically new species are being discovered regularly even in well-studied Europe (LAŠTŮVKA & LAŠTŮVKA, 2006; TRIBERTI, 2007, etc.).

The *Phyllonorycter* species living on Fabaceae constitute a large and relatively homogeneous unit, but they appear to have colonized this plant family several times independently through evolutionary time (LOPEZ-VAAMONDE *et al.*, 2003, 2006). European species of Fabaceae-feeding *Phyllonorycter* have been provisionally divided into three groups, *Phyllonorycter haasi*, *Ph. fraxinella* and *Ph. ulicicolella* species groups, based mainly on the male genitalia morphology (LAŠTŮVKA &

LAŠTŮVKA, 2006). However, some species, such as *Phyllonorycter telinella* Laštůvka & Laštůvka, 2006, *Ph. phyllocytisi* M. Hering, 1936, *Ph. eugregori* Laštůvka & Laštůvka, 2006 and *Ph. nevadensis* (Walsingham, 1908) occupy a relatively isolated position or they do not comfortably fit into one of these groups. In this study we focus on the *ulicicolella*-group, which is probably the most homogeneous and taxonomically difficult of them. This group currently contains 12 described species, but the validity of some of them requires confirmation and new species are likely to be discovered. The last three of the species mentioned above (*Ph. phyllocytisi*, *Ph. eugregori* and *Ph. nevadensis*) were preliminarily included in the *ulicicolella* species group (LAŠTŮVKA & LAŠTŮVKA, 2006), but further morphological and molecular evidence (unpublished) suggests they do not belong to this group, hence their exclusion from the present study.

The aims of this contribution are: 1) to state diagnostic characters in the male genitalia; 2) to combine present knowledge based on morphology with DNA barcodes (HEBERT *et al.*, 2003) and test the efficiency of this genetic marker to discriminate and identify taxa belonging to this species group; 3) to assess host plant specificity using rearing data; 4) to describe a new species whose larvae have been found mining thorns of *Erinacea anthyllis*.

Detailed descriptions of known species and biological data are given by LAŠTŮVKA & LAŠTŮVKA (2006, 2009a,b, 2011) and supplemented with description of the new species *Ph. lobeliella* by NEL (2009). Therefore only important and new data are given here (see also DE PRINS & DE PRINS, 2005, 2011, PÉREZ, 2012, or the original descriptions of the individual species mentioned below).

## Material and methods

The material was mainly collected by night at light (8 to 25 W fluorescent tubes, UV lamp 125 W) or during the day in bushes of the host plant; mines with larvae were also collected and reared. All the studied material is listed here for clarity, including those samples from previous publications (cf. LAŠTŮVKA & LAŠTŮVKA, 2006). Genitalia of both sexes were prepared in the usual way (e.g. ROBINSON, 1976). The male genitalia of all species have already been illustrated by LAŠTŮVKA & LAŠTŮVKA (2006). The diagnostic characters are especially on the right valva, therefore only these valvae have been figured here. The right valva was separated during dissection of genitalia and illustrated in non-deformed state. The genitalia are later preserved in glycerine tubes. The length of papillae anales has been measured together with apophyses, i.e. from the top of papillae to the end of apophyses posteriores.

DNA was extracted and barcodes were sequenced at the Canadian Centre for DNA Barcoding (CCDB, Guelph) using standard high-throughput protocols described in IVANOVA *et al.* (2006). A total of 45 specimens were sequenced (Table 1) producing full-length DNA barcodes (658 bp) for 38 specimens, and shorter sequences for 7 specimens. Barcode data were analysed using the analytical tools of the Barcode of Life Data Systems (BOLD; [www.boldsystems.org](http://www.boldsystems.org); RATNASINGHAM & HEBERT, 2007) to compute mean and maximum intraspecific variation as well as mean and minimum distances between species. Besides the distance analyses computed on BOLD, we also analyzed the COI sequences using neighbor-joining and maximum-parsimony approaches in PAUP \* 4.0b8a (SWOFFORD, 2002). All genetic distances were calculated using Kimura's 2 parameter model (K2P), unless stated otherwise. The level of support for individual nodes was evaluated by nonparametric bootstrapping with 1000 replicates MP analyses (Fig. 36).

Records for all 45 specimens barcoded are gathered within the project 'Gracillariidae – PUBLIC records' (code GRPUB) in the Published Projects section of BOLD. Information on specimen vouchers (field data and GPS coordinates) and sequences (nucleotide composition, trace files) can be found in this project. The *ulicicolella* dataset is registered under DOI number [dx.doi.org/10.5883/DS-ULICI](https://doi.org/10.5883/DS-ULICI). Sequences are also available on GenBank with accession numbers KC567625 to KC567660 (Table 1).

The nomenclature of host plants is based on the world database ILDIS (2005).

Process ID	Sample ID	Species	Country, province	Accession number (NCBI GENBANK)
GRACI412-09	D08andal	<i>Ph. andalusicus</i>	ES, Granada	KC567653
GRACI411-09	D07andal	<i>Ph. andalusicus</i>	ES, Granada	KC567625
GRACI424-09	E08baet	<i>Ph. baetica</i>	ES, Cuenca	KC567649
GRACI423-09	E07baet	<i>Ph. baetica</i>	ES, Cuenca	KC567654
GRACI271-08	E12pumil	<i>Ph. baetica</i>	ES, Cuenca	KC567634
GRACI425-09	E09vuel	<i>Ph. baetica</i>	ES, Avila	KC567648
GRACI255-08	E10baeti	<i>Ph. baetica</i>	ES, Almería	KC567656
GRACI545-09	E02nbaet	<i>Ph. baetica</i>	ES, Ávila	HM392510
GRACI413-09	D09vuel	<i>Ph. baetica</i>	ES, Ávila	KC567638
GRACI247-08	E09baeti	<i>Ph. baetica</i>	ES, Almería	KC567639
GRACI426-09	E10vuel	<i>Ph. baetica</i>	ES, Ávila	KC567636
PHLAD392-11	TLMFLep03567	<i>Ph. lobeliella</i>	FR, Var	KC567640
PHLAD391-11	TLMFLep03566	<i>Ph. lobeliella</i>	FR, Var	KC567657
PHLAD390-11	TLMFLep03565	<i>Ph. lobeliella</i>	FR, Var	KC567633
GRACI223-08	E06vuel	<i>Ph. echinosparti</i>	ES, Ávila	KC567642
GRACI546-09	E03echin	<i>Ph. echinosparti</i>	PT, Beira Baixa	HM392511
GRACI416-09	D12scorp	<i>Ph. scorpius</i>	ES, Zaragoza	KC567659
GRACI420-09	E04polya	<i>Ph. echinosparti</i>	PT, Algarve	KC567645
GRACI532-09	D01phsta	<i>Ph. echinosparti</i>	PT, Baixo Alentejo	HM392503
GRACI531-09	C12phsta	<i>Ph. echinosparti</i>	PT, Baixo Alentejo	HM392502
GRACI239-08	E08echin	<i>Ph. echinosparti</i>	PT, Beira Alta	KC567631
GRPAL852-12	LF5spAra	<i>Ph. scorpius</i>	ES, Teruel	KC567641
GRACI215-08	E05vuel	<i>Ph. echinosparti</i>	ES, Ávila	KC567650
GRACI231-08	E07echin	<i>Ph. echinosparti</i>	PT, Beira Alta	KC567651
GRACI536-09	D05pheri	<i>Ph. erinaceae</i>	ES, Almería	HM392506
GRACI535-09	D04pheri	<i>Ph. erinaceae</i>	ES, Almería	HM392505
LNOUD2116-12	CLV5212	<i>Ph. etnensis</i>	IT, Sicily	KC567626
GRPAL834-12	LD11etne	<i>Ph. etnensis</i>	IT, Sicily	KC567629
GRACI216-08	D05hespe	<i>Ph. hesperiella</i>	ES, Cádiz	KC567630
GRACI208-08	D04hespe	<i>Ph. hesperiella</i>	ES, Cádiz	KC567652
GRACI462-09	H10spn	<i>Ph. lapadiella</i>	CR, Dalmatia	KC567644
GRACI258-08	B10lapad	<i>Ph. lapadiella</i>	CR, Istria	KC567627
GRACI266-08	B11lapad	<i>Ph. lapadiella</i>	CR, Istria	KC567628
GRACI530-09	C11phsp	<i>Ph. lapadiella</i>	CR, Dalmatia	HQ956763
GRACI417-09	E01pumil	<i>Ph. pumila</i>	ES, Cuenca	KC567658
GRACI263-08	E11pumil	<i>Ph. pumila</i>	ES, Cuenca	KC567647
GRPAL221-11	CLV2379	<i>Ph. ulicicolella</i>	FR, Indre et Loire	KC567635
GRPAL220-11	CLV2378	<i>Ph. ulicicolella</i>	FR, Indre et Loire	KC567632
GRACI415-09	D11ulic	<i>Ph. ulicicolella</i>	ES, Granada	KC567646
GRPAL219-11	CLV2377	<i>Ph. ulicicolella</i>	FR	JN280225
GRPAL1070-12	CLV5812	<i>Ph. ulicicolella</i>	FR	KC567637
GRACI414-09	D10ulic	<i>Ph. ulicicolella</i>	ES, Granada	KC567643
LNOUC1148-11	ALvuel1	<i>Ph. vueltas</i>	ES, Ávila	KC567655
LNOUC1149-11	Alvuel2	<i>Ph. vueltas</i>	ES, Ávila	KC567660
GRACI544-09	E01vuel	<i>Ph. vueltas</i>	ES, Ávila	HM392509

**Table 1.**– Specimens used for the DNA barcoding analysis. Both the Process ID and sample ID codes are unique identifiers linking the record in the BOLD database and the voucher specimen from which the sequence is derived. Additional collecting and specimen data are accessible in BOLD’s public project GRPUB, as well as all sequence data.

***Phyllonorycter ulicicolella*-group**

The *Phyllonorycter ulicicolella* species group was defined by LAŠTŮVKA & LAŠTŮVKA (2006) on the basis of the following diagnostic characters. All known species are characterized by the general (underived) “*Phyllonorycter*” forewing pattern. They are very difficult to distinguish externally from each other and often also in their genital morphology. The right valva is of equal breadth along its whole length or broadest in the middle, usually without a hair pencil in its basal part and with a distinct, but relatively short stout seta before its end; vinculum is broad, with an inner band and saccus very short, or nearly absent. Their larvae develop in stem or thorn mines on shrubby Fabaceae.

*Phyllonorycter etnensis* Laštůvka & Laštůvka, 2006 (Figs. 1, 15, 25)

*Acta Univ. Agric. Silvic. Mendel. Brun.*, **54**(5): 72; type locality: Italy, Sicily, Etna, Monte Arso

Material: Italy, Sicily, Etna, Monte Arso, 9 ♂♂, 1 ♀, 21-VI-2000 (holotype and paratypes), 4 ♂♂, 1 ♀, 17-VI-1995, all leg. A. & Z. Laštůvka, coll. A. Laštůvka.

Diagnostic characters: Externally similar to other species of this group, especially to *Ph. echinosparti*, *Ph. ulicicolella* and *Ph. andalusicus*. It resembles several species living on *Salix*, e.g. *Ph. dubitella* (Herrich-Schäffer, 1855) and *Ph. salicicolella* (Zeller, 1846), by the rigid seta in the basal part of the right valva. The right valva 0.86-0.90 mm, of uniform breadth, slightly narrowing to the end, with the distinct rigid seta (thorn) in its basal part. The left valva 0.88-0.90 mm, 2.4-2.72 times longer than broad. Papillae anales 0.43 mm, ostium bursae evenly concave caudally, with long lateral edges, similar to that of *Ph. retamella*.

Biology: Larva in stem mine on *Genista aetnensis* (Biv.) DC., probably IX-V (not found), adult VI; voltinism unknown.

Distribution: Sicily.

*Phyllonorycter baetica* Laštůvka & Laštůvka, 2006 (Figs. 2, 16, 26)

*Acta Univ. Agric. Silvic. Mendel. Brun.*, **54**(5): 70; type locality: Spain, Almería, Sierra de Baza, Padilla

*Phyllonorycter lobeliella* Nel, 2009; *Rev. Assoc. Roussillon. d'Ent.*, **18**(1): 42; type locality: France, Var, Plan d'Aups, Hôtellerie; **syn. n.**

Material: Spain, Almería, Sierra de Baza, Padilla, 2000 m, 1 ♂, 9 ♀♀ (holotype and paratypes), 25-VI-2006, Las Piletas, 3 ♂♂, 11-VI-2008; Ávila, Las Vueltas, mines on *Genista cinerascens*, 25-VI-2007, 2 ♂♂ ex p., VI-2007, 1 ♂, 25-VI-2007, 1 ♂, 22-VI-2008, Navacepedilla de Corneja, mines on *Genista cinerascens*, 9-VI-2010, 4 ♂♂, 3 ♀♀ ex l., VI-2010; Cuenca, La Toba, 1 ♂, 26-VI-2007, 2 ♂♂, 23-VI-2008, both in the bushes of *Genista versicolor* subsp. *pumila*; Granada, Sierra de Baza, thousands of specimens in the growth of *Genista versicolor*; 16-VI-2007, 25-VI-2008, all A. & Z. Laštůvka leg., coll. A. Laštůvka.

Diagnostic characters: Less distinct forewing strigulae, head with black hairs, the shape of the right valva with a comb of setae in the distal third are characteristic. The right valva 0.76-0.78 mm, one or two separate spines in the middle and a continuous row in the last third which could be slightly raised. The left valva 0.71-0.74 mm, 2.29-2.38 times longer than broad, with the short row of spines in its last third. Papillae anales 0.4 mm, with short setae at the apex. The caudal margin of the VIIth segment only slightly convex. Ostium bursae evenly concave caudally.

Biology: Larva in thorn or stem mine on *Genista cinerascens* Lange, *G. versicolor* Boiss., *G. versicolor* subsp. *pumila* (Hervier) Fern. Casas, *Genista lobelii* DC., up to VI, adult VI-VII.

Distribution: Spain (new province record for Cuenca), southern France.

Remarks: We found no morphological differences between *Ph. baetica* and *Ph. lobeliella*, a species which cannot be distinguished either by its DNA barcode from specimens of *Ph. baetica* from

the type locality in Andalusia (P. Huemer, unpubl.). Therefore *Ph. lobeliella* is synonymized here with *Ph. baetica*. However, other specimens of *Ph. baetica* from various parts of the Iberian Peninsula and from different host plants show relatively large genetic heterogeneity (up to 1.61 or 2.89 % respectively in COI). It is difficult to decide without further study of more specimens and genes, whether it is a genetically heterogeneous species or a group of cryptic species, trophically or geographically limited.

***Phyllonorycter erinaceae* Z. Laštůvka & A. Laštůvka, sp. n.** (Figs. 13, 14, 17, 27)

Material: Holotype ♂, Spain, Almería, Sierra de los Filabres, Calar Alto, 2100 m, thorn mine on *Erinacea anthyllis*, 20-VI-2009, ex p. VI-2009, A. & Z. Laštůvka leg., coll. A. Laštůvka; Paratypes 29 ♂♂, 20 ♀♀, Spain, Albacete, Riopar, Las Espineras, 2 ♂♂, 25-VI-2011; Almería, Sierra de los Filabres, Calar Alto, 2100 m, mines on *Erinacea anthyllis*, 20-VI-2009, 2 ♂♂, 2 ♀♀ ex p., VI-2009, all A. & Z. Laštůvka leg., coll. A. Laštůvka, Sierra de los Filabres, Alto del Calar del Gallinero, 1900-2020 m, 2 ♂♂, 17-18-VI-2007, J. Šumpich leg. et coll., Sierra de los Filabres, 1850 m, 1 ♀, 8-VII-2010, Z. Tokár leg. et coll.; Granada, El Molinillo, 1 ♀, 27-VI-2004, 1 ♂, 1 ♀, 18-VI-2007, 1 ♂, 1 ♀, 23-VI-2011, Puebla de Don Fadrique, Nablanca, 1 ♂, 22-VI-2012, Sierra de Baza, Padilla, 2050 m, mines on *Erinacea anthyllis*, 17-VI-2010, 5 ♂♂, 2 ♀♀ ex p., VI-2010, Sierra de Guillimona, Puerto del Pinar, 1650 m, mines on *Erinacea anthyllis*, 21-VI-2010, 4 ♂♂, 6 ♀♀ ex p., VII-2010, mines on *Erinacea anthyllis*, 25-VI-2011, 10 ♂♂, 7 ♀♀ ex p., VII-2011; Teruel, Alcalá de la Selva, Masia del Altico, 1 ♂, 26-VI-2012, all A. & Z. Laštůvka leg., coll. A. Laštůvka, 2 ♂♂, 2 ♀♀ coll. A. Vives / Museo Nacional de Ciencias Naturales, Madrid.

Diagnosis: Externally similar to *Phyllonorycter* species with the general wing pattern, as e.g. *Ph. cerasicolella* (Herrich-Schäffer, 1855), which all distinctly differ in their genitalia. The similarly large *Ph. andalusicus* has narrow basal streak, less distinct strigulae and long right valva. It differs from the variable *Ph. echinosparti* by narrower left valva and by slightly enlarged right valva in the middle.

Description: Wingspan 9.2-10.0 mm; head orange-brown, with white frons and white hairs on vertex; antenna whitish grey, darker distally, slightly ringed; thorax ochreous orange, with a distinct white central line; forewing ochreous orange, with golden sheen; strigulae distinct, white; the basal streak long, almost straight, slightly enlarged in its distal half; four costal strigulae bordered with black scales on their inner margins; two dorsal strigulae, the first one long, connected with the white wing margin basally, bordered with only a few black scales distally; the second dorsal strigula triangular, distinctly bordered with black scales on its inner margin; the black scales occasionally absent in the first costal and dorsal strigulae; an elongate group of black scales in apex; cilia line distinct; hindwing greyish brown, more whitish in female, cilia ochreous; legs whitish with dark scales in distal parts of tarsal segments, and with dark spines; abdomen dark brown in male and brownish ochreous in female, the last segment whitish ochreous.

Male genitalia: The right valva short, 0.71-0.78 mm, slightly enlarged in the middle dorsally, the row of spines begins distinctly before its middle; the left valva 0.74-0.78 mm, narrower, 3.21-3.3 times longer than broad, the row of spines begins in its first third.

Female genitalia: Papillae anales 0.54 mm, with dense long setae nearly on its whole surface; ostium bursae broadly concave, with slightly elongate lateral edges.

Biology: Larva in thorn mine on *Erinacea anthyllis* Link., up to VI, adult VI-VII; probably univoltine.

Distribution: Spain: Andalusia, Castilla la Mancha, Aragón.

Etymology: Named after its host plant (a noun in genitive in apposition).

*Phyllonorycter echinosparti* Laštůvka & Laštůvka, 2006 (Figs. 4, 5, 6, 20, 28)

*Acta Univ. Agric. Silv. Mendel. Brun.*, **54**(5): 70; type locality: Portugal, Guarda, Pinzio

*Phyllonorycter scorpius* Laštůvka & Laštůvka, 2006; *Acta Univ. Agric. Silv. Mendel. Brun.*,

**54**(5): 71; type locality: Spain, Teruel, Albarracín; **syn. n.**

Material: Spain, Ávila, Las Vueltas, mines on *Genista cinerascens*, 25-VI-2007, 2 ♂♂ ex p., VI-2007, Ávila, Navacedilla de Corneja, mines on *Genista cinerascens*, 9-VI-2010, 1 ♂, 3 ♀♀ ex l., VI-2010; León, Penalba de Cilleros, pupae in *Genista ? obtusiramea*, 14-VI-2012, 2 ♂♂ ex p., VI-2012, Pinilla de la Valdería, 2 ♂♂ and pupae in *Genista hystrix*, 16-VI-2012, 3 ♂♂ ex p., VI-2012; Teruel, Albarracín, Valdevecar, 1 ♂, 25-VI-2012; Zaragoza, Aguarón, pupae in *Genista scorpius*, 10-VI-2012, 1 ♂ ex p., VI-2012; Portugal, Algarve, Odelouca, 1 ♂, 3 ♀♀, in the growth of *Genista polyanthos*, 14-VI-2010; Baixo Alentejo, Setúbal, Ameiras de Baixo, 1 ♂, 2 ♀♀, in the growth of *Stauracanthus genistoides*, 15-VI-2009; Beira Alta, Pinzio, mines with pupae on *Echinospartum lusitanicum* 17-VI-2006, 2 ♂♂, 11 ♀♀, ex p. VI-2006 (holotype and paratypes), the same locality, 2 ♂♂, 1 ♀, 17-VI-2006; Beira Baixa, Serra da Estrela, Penhas da Saúde, larvae on *Genista florida*, 11-VI-2010, 2 ♂♂, 2 ♀♀ ex l., VI-2010; Trás-os-Montes, Morais, 2 ♂♂, 1 ♀, 19-VI-2012, all leg. A. & Z. Laštůvka, coll. A. Laštůvka; specimens previously published as *Ph. scorpius*: Spain, Guadalajara, Casería El Monte, 1 ♂, 13-VI-2006 (paratype); Teruel, Albarracín, 4 ♂♂, 26-VI-2001 (holotype and paratypes), Ejulve, 3 ♂♂, 29-VI-2005, Montalbán, 1 ♂, 15-VI-2003, Royuela, 1 ♂, 16-VI-2003, Vivel del Río, 4 ♂♂, 3 ♀♀, 5-VII-1993, 1 ♂ 21-VI-1994, all leg. A. & Z. Laštůvka, coll. A. Laštůvka.

Diagnostic characters: Very variable in forewing ground coloration and in number of black scales; the basal streak occasionally connected with the first costal or dorsal strigula. Apical strigulae often without white colour, only indicated by black scales. The right valva long, 0.78-0.86 mm, of more or less uniform breadth, its last third slightly enlarged, the row of spines in more than the last third. The left valva 0.78-0.82 mm, narrower, 3.04-3.07 times longer than broad, the row of spines in more than the last third. Papillae anales 0.45 mm, with long setae also in their apex. Ostium bursae narrow, distinctly concave caudally. *Phyllonorycter andalusicus* with similar right valva, has broader left valva and narrower basal streak.

Biology: Larva in stem or thorn mine on *Echinospartum lusitanicum* (L.) Rothm., *Genista cinerascens* Lange, *G. florida* L., *G. hystrix* Lange, *G. polyanthos* Willk., *G. scorpius* (L.) DC. and *Stauracanthus genistoides* (Brot.) Samp., IX-V, adult V-VII.

Distribution: Portugal, W and C Spain (new province records for León and Zaragoza in Spain and for Trás-os-Montes in Portugal).

Remarks: *Phyllonorycter scorpius* is synonymized with *Ph. echinosparti* here because no clear morphological differences were found in extensive material studied, and because DNA barcoding data available for specimens considered to represent the typical *Ph. echinosparti* (E07echin & E08echin) and the typical *Ph. scorpius* (D12scorp & LF5spAra) do not reveal any genetic isolation between these. *Ph. echinosparti* is oligophagous and some external differences were observed between specimens obtained from different host plants (see also Discussion).

*Phyllonorycter pumila* Laštůvka & Laštůvka, 2006 (Figs. 3, 18, 29)

*Acta Univ. Agric. Silv. Mendel. Brun.*, **54**(5): 71; type locality: Spain, Cuenca, La Toba

Material: Spain, Cuenca, La Toba, 2 ♂♂ (holotype and paratype), 14-VI-2006, the same locality, 5 ♂♂ (paratypes), 27-VI-2006, the same locality, 3 ♂♂, 1 ♀ and mines with pupae, 24-VI-2012, 1 ♂, 2 ♀♀ ex p., VI-2012, all leg. A. & Z. Laštůvka, coll. A. Laštůvka; Zaragoza, Coscojar, mines in thorns of *Genista versicolor* subsp. *pumila*, 13-VI-2006, leg. A. & Z. Laštůvka.

Diagnostic characters: Externally very similar to *Ph. baetica* and *Ph. ulicicolella*, but the forewing ground colour is darker and strigulae more distinct and broader. The right valva short, 0.68-0.72 mm, of uniform breadth, the row of spines begins in the middle. The left valva 2.39-2.46 times longer than broad, similar to that in *Ph. ulicicolella*, but without concave ventral edge. The row of spines less distinct, approximately from the middle. Papillae anales 0.4 mm, with long hairs also at the apex. Ostium bursae similar to that in *Ph. baetica*.

Biology: Larva in thorn mine on *Genista versicolor* subsp. *pumila* (Hervier) Fern. Casas, VIII-VI, adult VI, phenologically later than *Ph. baetica*; mines more inflated, whitish and therefore more visible than those in *Ph. baetica*.

Distribution: C Spain.

*Phyllonorycter ulicicolella* (Stainton, 1851) (Figs. 7, 19, 30)

*Suppl. Cat. Brit. Tin. & Pter.*: 12 (*Lithocolletis*); type locality: England, Bristol, Durdham Downs.

Material: France, Loire, La Baule, mines on *Ulex europaeus*, 1 ♀ ex l., 10-V-1943, 1 ♂ ex l., 18-V-1943, leg. H. Buhr, coll. ZMHB; Spain, Granada, El Molinillo, 1 ♀, 27-VI-2004, 4 ♂♂, 3 ♀♀, 18-VI-2007, 1 ♂, 3 ♀♀, 13-VI-2008, pupae on *Ulex parvijlorus*, 13-VI-2008, 4 ♂♂, 5 ♀♀ ex p., 15-VI-2008, 10 km N Otívar, 3 ♂♂, 5 ♀♀, 14-VI-2008; Málaga, Jubrique, 1 ♀, 16-VI-2008, all A. & Z. Laštůvka leg., coll. A. Laštůvka.

Diagnostic characters: The right valva short, 0.66-0.67 mm, nearly straight, of uniform breadth, the row of spines begins in its middle. The left valva 0.64-0.68 mm, 2.28-2.61 times longer than broad, with row of spines from the middle. Papillae anales 0.40 mm, without very long setae. Ostium bursae broad, similar to that in *Ph. erinaceae*.

Biology: Larva in thorn mine on *Ulex* spp., up to VI, adult VI-VII.

Distribution. England, W France, Spain, Gibraltar.

*Phyllonorycter lapadiella* (Krone, 1909) (Figs. 8, 21, 31)

*Jahresber. Wien. Ent. Ver.*, **19**(1908): 130 (*Lithocolletis*); type locality: Croatia, Gravosa [Dubrovnik], Lapad Peninsula

*Lithocolletis dalmatinella* Amsel, 1951; *Redia*, **36**: 415; type locality: Montenegro, Igalo near Herceg Novi; synonymized by LAŠTŮVKA & LAŠTŮVKA (2006)

*Phyllonorycter picardi* Buvat, 1995; *Alexanor*, **19**: 15; type locality: France, Marseille, Le Roy d'Espagne; synonymized by LAŠTŮVKA & LAŠTŮVKA (2006)

Material: Spain, Gerona, La Junquera, 1 ♂, 7-VI-2009; France, Aude, Sigean, 1 ♀, 23-VI-1991; Italy, Campania, Atena Lucana, 4 ♂♂, 3 ♀♀, 25-VI-2000, Calabria, Santa Catarina delo Ionio, 2 ♂♂, 6-VI-2000; Sicily, Ficuzza, 2 ♂♂, 2 ♀♀, 16-VI-2000, Francavilla di Sicilia, 2 ♂♂, 3 ♀♀, 23-VI-2000, Mandanici, 2 ♂♂, 1 ♀ 8-VI-1995; Croatia, Istria, Pula, mines with pupae on *Spartium junceum*, 1-V-2003, 4 ♂♂ ex l., V-2003; Greece, Ioanina, Asprangeli, 1 ♂, 1 ♀, 10-VI-1997, Séres, Kalokastro, 1 ♀, 24-VI-1997, Fthiotida, Agios Haralambos, 1 ♂, 22-VI-1998, Ahaia, Kalávrita, 2 ♂♂, 1 ♀, 23-VI-1996, Messinia, Exohori, 1 ♂, 1 ♀, 10-VI-1997, Kardamili, 1 ♂, 17-VI-1998, all leg. A. & Z. Laštůvka, coll. A. Laštůvka; Montenegro, Herceg Novi, Igalo, 1 ♂, 15-IV-1938, 1 ♂, 1-V-1938, 1 ♀, 8-V-1938, paratypes of *Lithocolletis dalmatinella*, leg. H. G. Amsel, coll. A. Laštůvka (ex coll. F. Gregor); Croatia, Gravosa, Lapad, 4 ♂♂, 5-24-V, coll. Krone HNHM Budapest, lectotype and paralectotypes of *Lithocolletis lapadiella* (cf. LAŠTŮVKA & LAŠTŮVKA, 2009b).

Diagnostic characters: All strigulae distinctly bordered with black scales. The right valva 0.88-0.90 mm in the first and 0.79 mm in the second generation, slightly enlarged in its middle part, the row of spines begins in its middle; the left valva 2.6-2.7 times longer than broad. Papillae anales (0.48 mm) large, with very long setae. The caudal margin of the VIIth segment slightly convex. Ostium bursae distinctly concave, with slightly raised margins.

Biology: Larva in stem mine on *Spartium junceum* L.; IX-VIII, adult IV-IX; bivoltine or polyvoltine, the summer and autumnal generation(s) distinctly smaller.

Distribution: NE Spain, southern France, Italy, Sicily, Croatia, Montenegro, Greece.

*Phyllonorycter hesperiella* (Staudinger, 1859) (Figs. 9, 22, 32)

*Dt. ent. Ztg. Stettin*, **20**: 256 (*Lithocolletis*); type locality: Spain, Cádiz, Chiclana

Material: Spain, Cádiz, Chiclana, P.T. [type ?], 1 ♂, leg. Staudinger, coll. A. Laštůvka (ex coll. D. Povolný); Cádiz, Punta Paloma, 3 ♂♂, 5 ♀♀, 30-III-4-IV-1994, leg. H. van der Wolf, coll. A.



Laštůvka, 6 ♂♂, 5 ♀♀, 22-VI-2006, leg. A. & Z. Laštůvka, coll. A. Laštůvka; Portugal, Algarve, Cabanas, 22-VI-2006, stem mines on *Retama monosperma*, leg. A. & Z. Laštůvka; Algarve, Monte Gordo, 2 km SW Vila Real de Santo António, 10-I-1987, stem mines with very young larvae on *Retama monosperma*, leg. E. J. van Nieukerken; Algarve, Vila Real de Santo António, 14 ♂♂, 8 ♀♀, 17-VI-2009, leg. A. & Z. Laštůvka, coll. A. Laštůvka.

Diagnostic characters: The forewing coloration less distinct, brownish, elongate strigulae with brown margins. Externally similar to *Ph. retamella* whose forewings are clearer, with a large group of black scales in apex. The right valva elongate, 0.96-1.05 mm, slightly enlarged beyond half, the row of spines begins in its middle, a distinct single seta near its base. Papillae anales length 0.47 mm, ostium bursae broad, straight or slightly convex caudally (concave in *Ph. retamella*, which has longer setae on papillae anales).

Biology: Larva in stem mine on *Retama monosperma* (L.) Boiss., only empty mines observed V-VI, adult III-VI; at least bivoltine or polyvoltine.

Distribution: SW Spain (Andalusia), S Portugal; probably also in northern Africa, HERING (1957) found mines on *Retama monosperma* in Canary Islands.

*Phyllonorycter retamella* (Chrétien, 1915) (Figs. 10, 33)

*Ann. Soc. Ent. France*, **84**: 363 (*Lithocolletis*); type locality: Tunisia, Fériana

Material: Italy, Sicily, Licata, 1 ♀, 18-VI-2000, leg. A. & Z. Laštůvka, coll. A. Laštůvka.

Diagnostic characters: Externally very similar to *Ph. hesperiella*, forewings are more clear with a large group of black scales in apex. Papillae anales (0.55 mm) with long setae caudally and ostium bursae distinctly concave.

Biology: Larva in stem mine on *Retama raetam* (Forssk.) Webb; IX-VI, adults collected V-VI; probably bi- or polyvoltine.

Distribution: Tunisia, Sicily.

Remark: The species independence (or its conspecificity with *Ph. hesperiella*) is still unclear.

*Phyllonorycter andalusicus* Laštůvka & Laštůvka, 2006 (Figs. 11, 23, 34)

*Acta Univ. Agric. Silv. Mendel. Brun.*, **54**(5): 69; type locality: Spain, Granada, Diezma

Material: Spain, Almería, Los Piletas, 1 ♂, 17-VI-2010; Granada, Diezma, 1 ♂, 1 ♀, 1-VII-1992 (holotype and paratype), Granada, Caniles, 1 ♂, 2-VII-1994 (paratype), stem mines with pupae on *Genista umbellata*, 12-VI-2008, 3 ♂♂, 6 ♀♀ ex p., VI-2008; Málaga, Ronda, 2 ♀♀, 23-VI-2006, all leg. A. & Z. Laštůvka, coll. A. Laštůvka.

Diagnostic characters: The basal streak long and narrow. Similar to *Ph. echinosparti* in the male genitalia, but the right valva longer and the left one broader; the right valva long, 0.91-0.92 mm, of equal breadth, the row of spines approximately in its last third. The left valva 0.87-0.91 mm, broad, 2.35-2.84 times longer than broad, the row of spines begins in the middle. Papillae anales 0.50 mm, with very long setae, especially in the cranial part. Ostium bursae broad, slightly convex caudally, with elongate lateral margins. The caudal margin of the VIIth segment nearly straight.

Biology: Larva in stem (thorn) mine on *Genista umbellata* (L'Hér.) Poir., IX-IV, adult V-VI; probably univoltine.

Distribution: Spain, Andalusia (new province record for Málaga).

*Phyllonorycter vueltas* Laštůvka & Laštůvka, 2006 (Figs. 12, 24, 35)

*Acta Univ. Agric. Silv. Mendel. Brun.*, **54**(5): 70; type locality: Spain, Ávila, Las Vueltas

Material: Spain, Ávila, Las Vueltas, 1 ♂, 2 ♀♀, 18-VI-2004, 6 ♂♂, 8 ♀♀, 15-VI-2005, 6 ♂♂, 17 ♀♀, 15-VI-2006 (types), 1 ♂, 22-VI-2008, mines on *Genista cinerascens*, 9-VI-2010, 1 ♂, 1 ♀ ex p., VI-2010, Ávila, Navacepedilla de Corneja, mines on *Genista cinerascens*, 9-VI-2010, 1 ♂, 1 ♀ ex p.,

VI-2010, leg. A. & Z. Laštůvka, coll. A. Laštůvka, Ávila, Sierra de Gredos, 2.5 km S Hoyos del Espino, 1 ♂♂, 4 ♀♀, 5-VIII-1986, leg. E. J. van Nieukerken, coll. RMNH, Navarredonda de Gredos, 2 ♂♂, 1 ♀♀, 4-VIII-1986, leg. E. J. van Nieukerken, coll. RMNH; Portugal, Beira Baixa, Serra da Estrela, Penhas da Saúde, mine on *Genista florida*, 22-VI-2002, 1 ♂ ex p., VII-2002; Beira Alta, Manteigas, Pousada, 3 ♂♂, 1 ♀♀, 18-VI-2006, leg. A. & Z. Laštůvka, coll. A. Laštůvka.

Diagnostic characters: Forewing with only small group of black scales in apex, less distinct strigulae, the shape of the right valva, broad ostium bursae and short setae on anal papillae are characteristic. The right valva 0.8-0.82 mm, straight, distinctly enlarged in the middle, the row of spines begins in its middle. The left valva 0.80-0.82 mm, 2.34-2.5 times longer than broad, with the row of spines from its middle. Papillae anales 0.45 mm, mostly with short setae at the apex. Ostium bursae very broad, with uneven caudal margin. The caudal margin of the VIIIth segment strongly convex.

Biology: Larva in stem mine on *Genista cinerascens* Lange (incorrectly given as *G. cinerea* by LAŠTŮVKA & LAŠTŮVKA, 2006) and *G. florida* L., up to VI, adult VI-VIII.

Distribution: Portugal, W Spain.

### Molecular data results and discussion

We obtained barcode data for 45 specimens of 10 taxa (Table 1, plus the newly synonymized *Ph. lobeliella* and *Ph. scorpius*), and representing all but one species of the *ulicicolella* group (no DNA barcode available for *Ph. retamella*). When compared with a data set of 2000 DNA barcodes of 230 European Gracillariidae species (unpublished data), all the 10 species of this group formed a monophyletic clade. The neighbor-joining (NJ) tree of the 45 barcoded specimens representing 10 taxa is shown in Fig. 36. The maximum parsimony analysis yielded 40 most parsimonious trees (L=269, CI=0.647, RI=0.894; results not shown) and the topology of the strict consensus matched that of the NJ tree.

The results of the DNA barcode analyses proved especially useful in supporting the synonymy of two species within the *ulicicolella* group. Specimens we consider as typical representatives of *Ph. lobeliella* and *Ph. scorpius* were genetically indistinguishable from *Ph. baetica* and *Ph. echinosparti* respectively. The two taxa are therefore synonymized on the basis of the absence of diagnostic genetic and morphological characters. In addition, DNA barcodes bring unequivocal support to the distinction of *Phyllonorycter erinaceae* as a new species, with as much as 5.6% p-distance between this newly described species and its nearest neighbour.

Considering this new species and the two revealed cases of synonymy, the species of the *Phyllonorycter ulicicolella* species group are all well discriminated genetically, with a minimum interspecific pairwise genetic distance of 3.1% among all species. This makes DNA barcoding an excellent tool for identifying species in this difficult group, whatever the life-stage analyzed.

The intraspecific genetic variation among specimens of the same species is generally low (mean 0.868). It is worth noting however that we found intraspecific genetic distance as high as 3.3% within *Ph. baetica*. Within this species, three specimens from La Toba, Spain found feeding on *Genista versicolor pumila* differ from the rest of *P. baetica* specimens by 2.8–3.3% and form a distinct genetic cluster (Table 1). This substantial genetic divergence, associated with the use of a different host-plant, suggests that those three specimens could represent a different species. However, morphological analysis did not reveal any significant diagnostic character so far, and further evidence is needed (e.g. nuclear genes, thorough morphological analysis of additional material) to assess if we are facing a case of cryptic species or an unusual level of intraspecific genetic variability.

Another species with relatively high intraspecific variability was *Ph. ulicicolella*, with a maximum genetic distance of 1.3% between two Spanish specimens feeding on *Ulex parviflorus* and French specimens on *Ulex minor*. Further material is needed to test whether that divergence is host related and could reflect genetically isolated lineages.

Finally, our results report a remarkable genetic homogeneity within the oligophagous species *Ph.*

*echinoparti*. This species feeds on multiple species of Genisteae (*Echinopartum*, *Genista florida*, *G. cinerascens*, *G. hystrix*, *G. polyanthos*, *G. scorpius* and *Stauracanthus*) (Table 1). Despite its oligophagy *Ph. echinoparti* shows low intraspecific genetic variability (maximum 0.61 p-distance) which does not seem to be associated to host plant use (Fig. 36). For instance, one specimen reared from *Echinopartum lusitanicum* in northern Portugal (E08echin) shows an exact barcode match to one specimen reared from *Genista polyanthos* from Algarve (E04polya) and just 0.2% from *Genista cinerascens* from the Sierra de Gredos in Spain (E06vuel). This very low genetic diversity contrasts to some slight variability in their forewing ground coloration and pattern.

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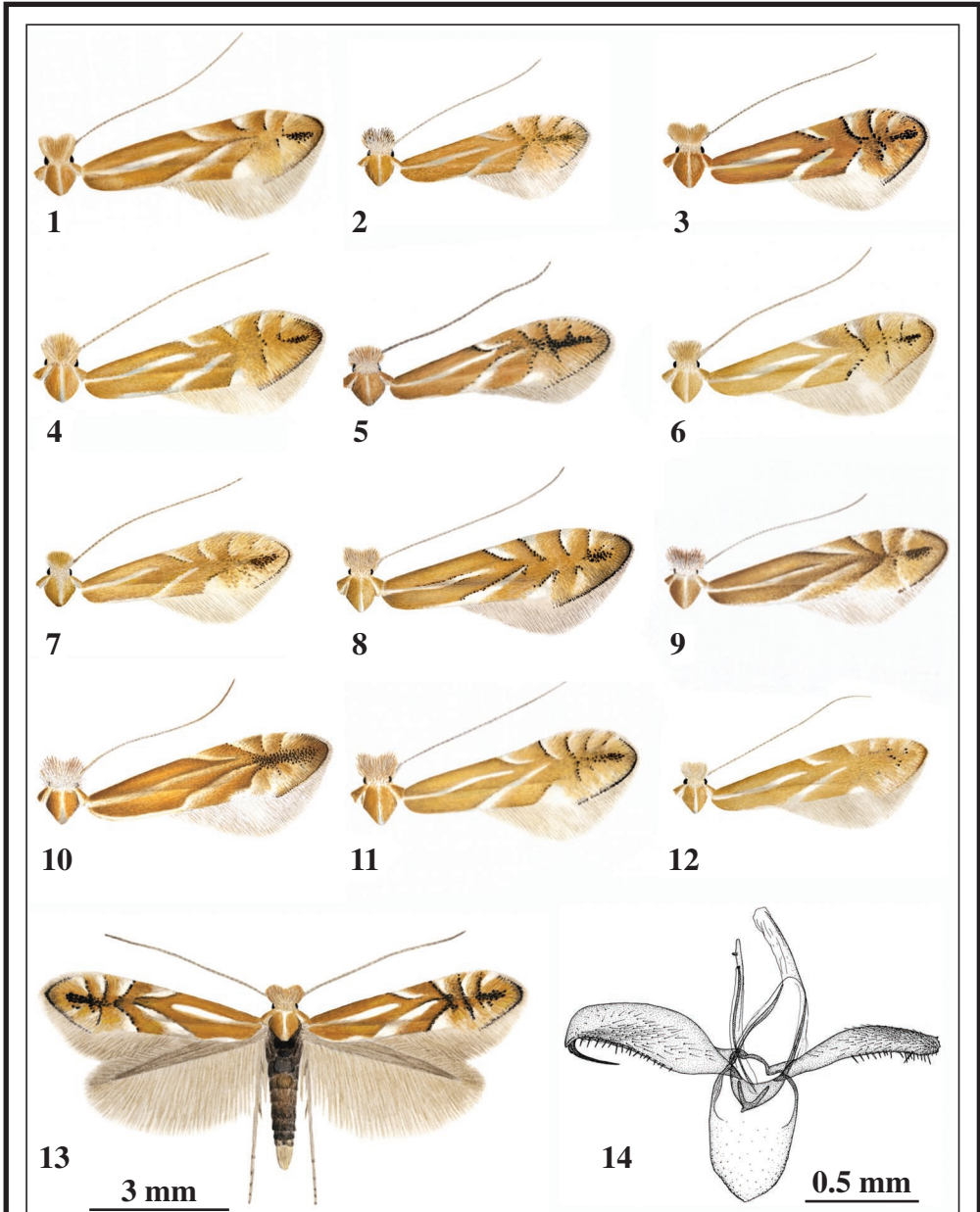
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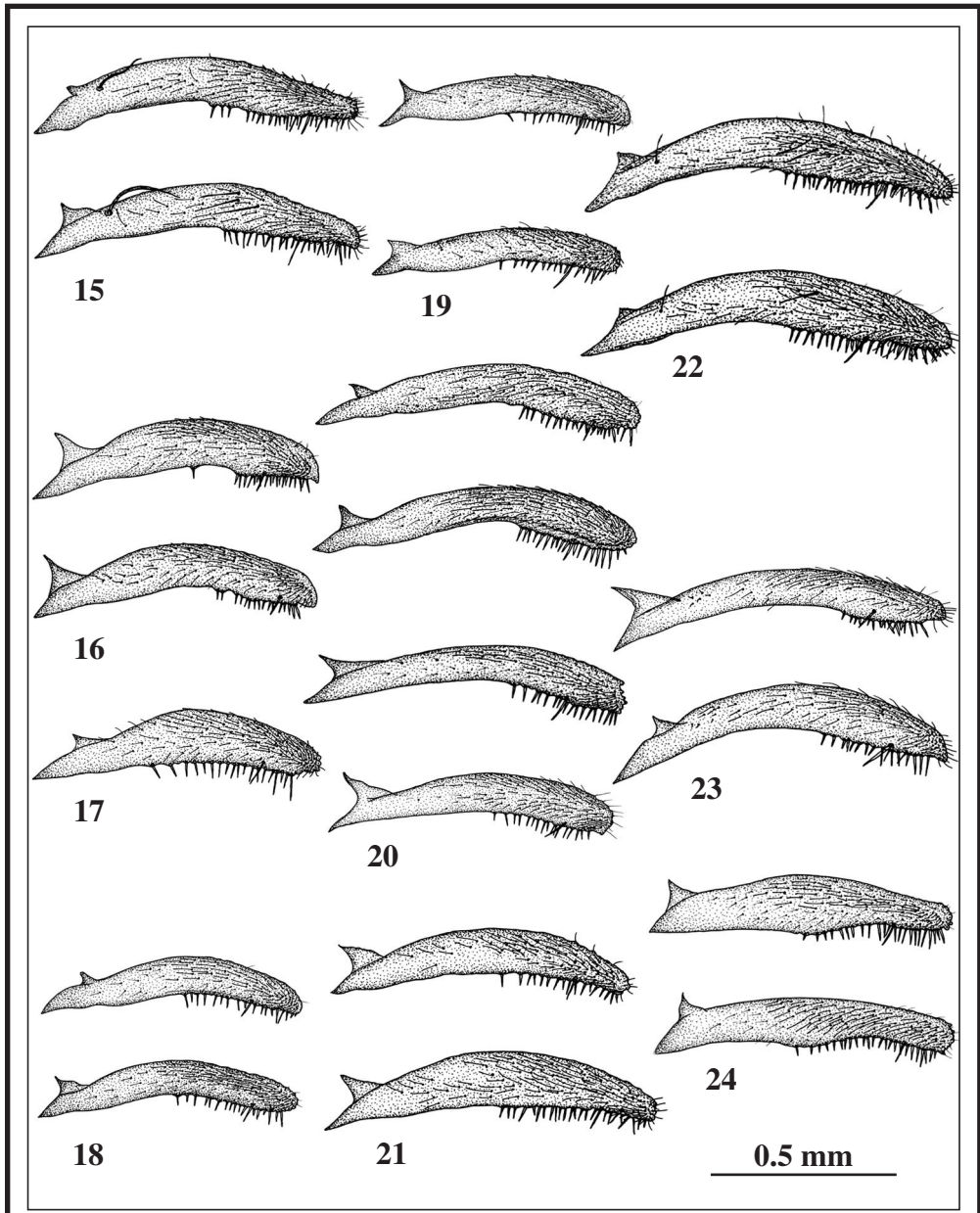
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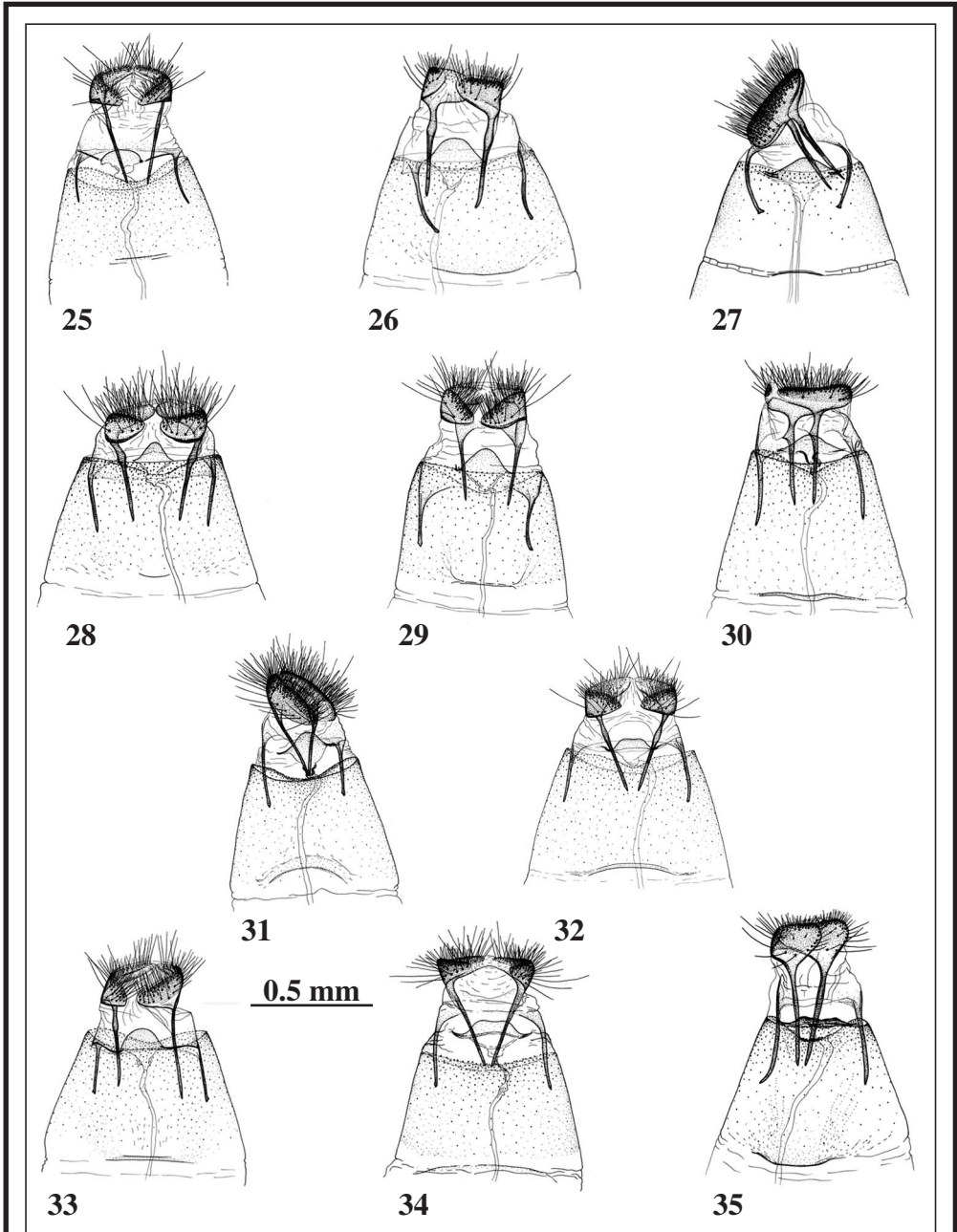
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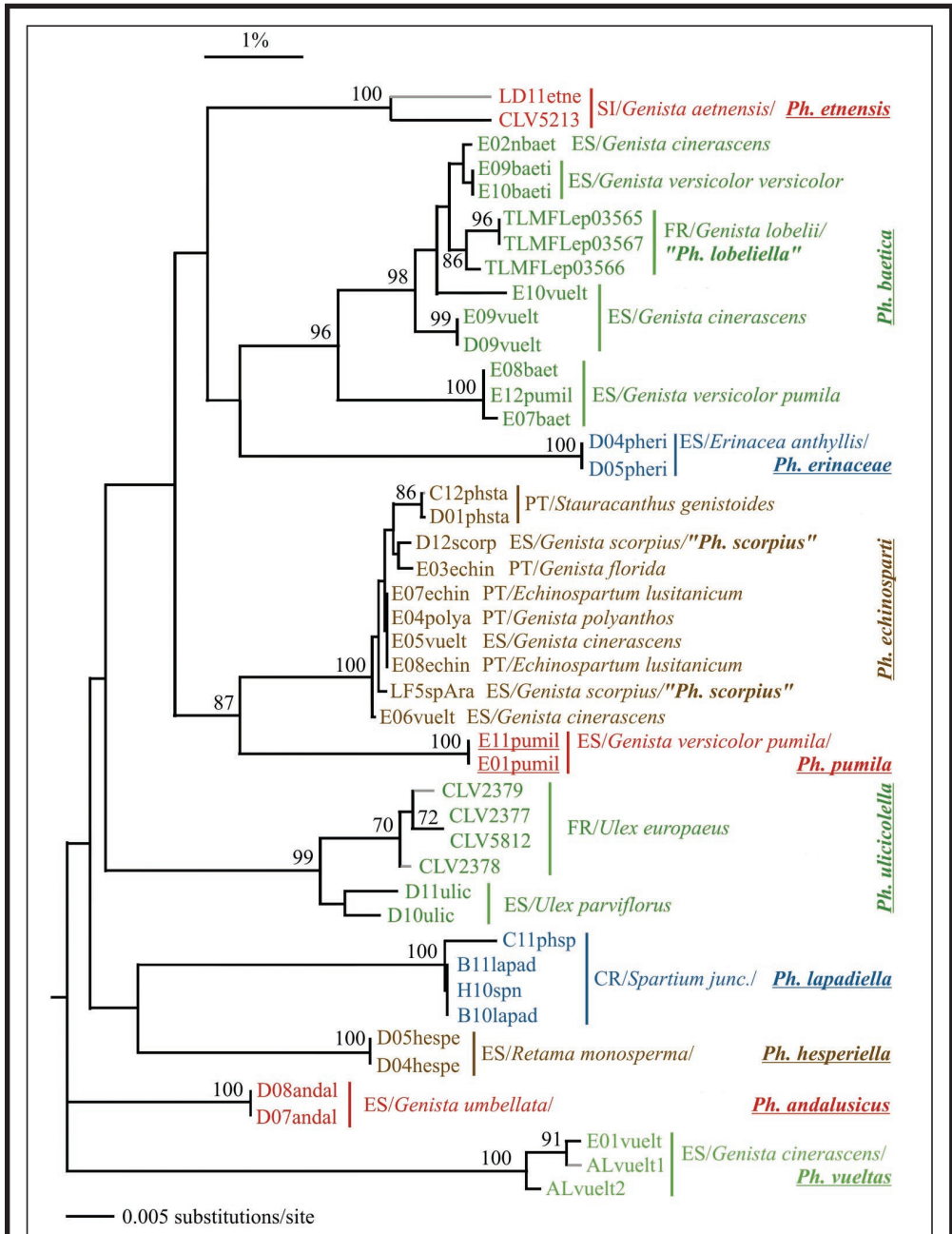
**Figs. 1-14.**— *Phyllonorycter ulicicolella*-group, thorax and forewing. 1. *Ph. etnensis*. 2. *Ph. baetica*. 3. *Ph. pumila*. 4-6. *Ph. echinospartii*, from *Echinospartum lusitanicum* (4), *Genista polyanthos* (5), *Genista scorpius* (6). 7. *Ph. ulicicolella*. 8. *Ph. lapadiella*. 9. *Ph. hesperiella*. 10. *Ph. retamella*. 11. *Ph. andalusicus*. 12. *Ph. vuellas*. 13-14. *Ph. erinaceae*. (13) Adult, male (14) Male genitalia.



**Figs. 15-24.**— *Phyllonorycter ulicolella*-group, right valva. **15.** *Ph. etnensis*. **16.** *Ph. baetica*, from *Genista versicolor* and *G. versicolor* subsp. *pumila*. **17.** *Ph. erinaceae*. **18.** *Ph. pumila*. **19.** *Ph. ulicolella*. **20.** *Ph. echinosparti*, from *Echinopartum lusitanicum*, *Genista polyanthos*, *Stauracanthus genistoides* and *Genista scorpius*. **21.** *Ph. lapadiella* (above valva of the 2<sup>nd</sup> generation). **22.** *Ph. hesperiella*. **23.** *Ph. andalusicus*. **24.** *Ph. vueltas*.



**Figs. 25-35.**– *Phyllonorycter ulicicolella*-group, female genitalia. 25. *Ph. etnensis*. 26. *Ph. baetica*. 27. *Ph. erinaceae*. 28. *Ph. echinosparti*. 29. *Ph. pumila*. 30. *Ph. ulicicolella*. 31. *Ph. lapadiella*. 32. *Ph. hesperiella*. 33. *Ph. retamella*. 34. *Ph. andalusicus*. 35. *Ph. vueltas*.



**Fig. 36.**– Neighbor-Joining tree (uncorrected *p*-distance) of the *ulicicolella* species group COI (DNA barcode) sequences. Each specimen is identified by its SampleID code (see Table 1). Values above branches are bootstrap supports.