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Taxonomic history and invasion biology of two *Phyllonorycter* leaf miners (Lepidoptera: Gracillariidae) with links to taxonomic and molecular datasets

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Abstract

This paper deals with two European species, *Phyllonorycter mespilella* (Hübner, 1805) and *P. trifasciella* (Haworth, 1828), that have colonized the subtropical Canary Islands. The Rosaceae leaf miner, *P. mespilella*, is recorded for the first time from Lanzarote and La Palma, while the Caprifoliaceae leaf miner, *P. trifasciella*, is recorded from Tenerife. We present the diagnoses of these species based on morphology, a preliminary DNA barcode (COI) library of congeneric and confamilial species, and discuss the taxonomic position of the colonizers within the *blancardella* and *trifasciella* species groups. The recent intensification of anthropogenic disturbance likely accounts for their range expansion, an event that may impact the relict flora present on the Canary Islands.

Key words: Canary Islands, leaf-miner, *Lonicera*, invasive species, *Malus*, new distribution records, *Phyllonorycter,* pest species

Introduction

In the last decade there has been a massive increase in research on invasive insects (Tsutsui *et al*. 2000; Mooney & Cleland 2001; Lockwood *et al*. 2007; Davis 2009; Roques *et al*. 2010; Blackburn *et al*. 2011). The dramatic growth of data and its importance to conservation issues has led the entomological community to question what is a native species (Pyšek *et al*. 2004; Roques *et al*. 2010), and what to call populations established in an area where the species was formerly unknown (Lockwood *et al*. 2007; Richardson *et al*. 2011). Most prior studies on alien species within Europe have investigated taxa from another continent or of unknown origin (Blackburn 2011; van Nieukerken *et al*. 2012a, b). However, few investigators have examined colonization events from continental Europe to subtropical areas.

Species of the gracillariid subfamily Lithocolletinae provide some of the best examples of how alien species can rapidly spread across vast areas (Gilbert & Grégoire 2003; Gilbert *et al*. 2004, 2005; Šefrová 1999, 2001, 2002a, b; Šefrová & Laštůvka 2001; Valade *et al*. 2009; Lopez-Vaamonde *et al*. 2010; Péré *et al*. 2010a; Davis & De Prins 2011), even colonizing new host plants (Gregor *et al*. 1998; Péré *et al*. 2010b). *Phyllonorycter* leaf-miners, due to their small size and life history (Vári 1961; Emmet *et al*. 1985; Davis & Deschka 2001; Bengtsson & Johansson 2011; De Prins & Kawahara 2012), disperse readily with their host plants. When conditions are favourable, these moths are multi-voltine, sometimes creating outbreaks on native plants (Carter 1984; Sarto i Monteys & Vives de Quadras 1991; Blommers & Vaal 1996; Kuznetzov 1999; Kuznetzov & Baryshnikova 2004). This paper reports the establishment of two species of European leaf miners in the Canary Islands—*Phyllonorycter mespilella* (Hübner, 1805) and *Phyllonorycter trifasciella* (Haworth, 1828). The Rosaceae feeding species, *P. mespilella,* shows wide ecological plasticity and now has a range that spans Europe, North Africa (Morocco,

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Tunisia), Middle Asia (Turkey), and North America (Canada, the USA). The Caprifoliaceae feeding *P. trifasciella* is widely distributed in Europe and the Middle East (Turkey), but has not been recorded outside these areas.

This paper reports the colonization of the subtropical Canary Islands by these two *Phyllonorycter* species. The taxa not only have the potential to become pests, but may invade sub-Saharan Africa, triggering novel interactions that might disrupt local floras. This paper discusses the taxonomic position of these two species, presents diagnoses for their identification based on morphological and molecular characters, and provides references to supplementary taxonomic information.

Material and methods

Collecting and rearing. An adult of *P. mespilella* was observed on *Malus domestica* Borkh. [Rosaceae] in La Palma on 1st November 2006 in the evergreen "laurisilva" forest in the northwestern region of this island. A sampling effort in October 2007 (25–27.x.2007) revealed additional mines of *Phyllonorycter* on *M. domestica* at a site 2 km S Barlovento, 28°49'N 17°46'W, at an altitude of 270 m. Leaves with these mines were kept outdoors in polystyrene jars with moistened tissue until adults (5 males, 6 females) emerged a few days later.

While selecting specimens for DNA barcoding, three males of *P. trifasciella*, reared from *Lonicera periclymenum* L. [Caprifoliaceae], were rediscovered in the collection of Willy and Jurate De Prins. These specimens derived from mines collected by the second author and Francis Coenen in Tenerife, Los Cristianos, at 10 m altitude, from 3–6.i.1982. These specimens indicate that the Canary Islands have been invaded by a second European species. Several additional mines of this species were observed in gardens within La Laguna (Tenerife) in 1993 where *L. periclymenum* L. is planted as a decorative plant (European Information Platform for Plants http://www.plantaeuropa.org). Because the *Phyllonorycter* fauna of the Canary Islands was extensively studied by outstanding microlepidopterists like Rebel (1896, 1939), Walsingham (1908), Hering (1927), Deschka (1968, 1970), Klimesch (1979), it is unlikely that they overlooked this easily recognizable European species if it occurred in the Canary Islands at that time.

Morphological dissections and imaging. Adults were examined using a Leica MZ12.5 stereomicroscope with a digital micrometer to make measurements. Images were taken with a Canon EOS 550D camera and its image grabbing system EOS Utility. A composite image was obtained using CombineZ5 software to assemble several shots taken in different focal planes. Genitalia were prepared following Robinson (1976) with some modifications. Each abdomen was macerated in 10% KOH for 18 hours, and then cleaned in deionized water before male genitalia were stained with Eosin B, while female genitalia were stained with a 1% chlorazol black E solution and embedded in Euparal on slides. Genital morphology was examined at 75× and 100× using a Leica DMLB microscope before slides were photographed with a Q imaging Micropublisher 5.0 RTV camera and Auto-Montage Syncroscopy to produce a composite image. Genital terminology follows Vári (1961), Klots (1970), Kumata (1963, 1993, 1995), Kristensen (2003), and De Prins & Kawahara (2012). Measurements are in millimeters for adults and in micrometers for genitalia and pupae.

Scanning electron microscopy (SEM) was used to examine some pupal exuviae and male genitalia of *P. mespilella* (Hübner, 1805). These structures were dried in HMDS, gold coated using a Jeol JFC-1300 Auto fine Coater and examined with a Jeol 6480 LV electron scanning microscope. Pupal terminology follows Wilson (1985), Davis (1987, 1994), Kawahara *et al.* (2009), and Davis & De Prins (2011).

Under the taxonomic section in this publication, we designate a neotype and a lectotype and indicate different labels by numbers in square brackets (e.g. [1], [2]). A forward slash "/" indicates the end of a line; the depositories for the neotype and lectotype are indicated by the fixation of the name-bearing type. Specimens from the Canary Islands and genitalia preparations are deposited in the collection of Willy & Jurate De Prins (WJDP).

Abbreviations for collections:

BMNH	Natural History Museum, London, UK;
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria;
WJDP	Collection of Willy and Jurate De Prins, Leefdaal, Belgium;
YMUK	Yorkshire Museum and Gardens, York, UK;
ZMHB	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany.

Molecular protocols. A preliminary DNA barcode library was constructed using adult specimens from the WJDP collection. DNA was extracted from dry abdomens including one *P. mespilella* collected in La Palma, the Canary Islands (Voucher DP09053). Standard analytical protocols at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) were used to obtain DNA barcodes, a 658 bp fragment near 5' terminus of the COI gene (deWaard *et al.* 2008; www.dnabarcoding.ca/pa/ge/research/protocols). The 'DNA barcode' region of COI was amplified with the primer set LepF1/LepR1 (Hebert *et al*. 2004). All PCR amplifications were performed according to the standard PCR reaction protocol used in CCDB (Hajibabaei *et al.* 2005). PCR products were checked on a 2% E-gel® 96 Agarose (Invitrogen). All full-length amplicons were bidirectionally sequenced, while shorter fragments obtained using the internal primers MLepR1 and MLepF1 were unidirectionally sequenced. The sequencing reactions followed CCDB protocols (Hajibabaei *et al.* 2005), with products subsequently purified using Agencourt® CleanSEQ protocol (Agencourt, Beverly, MA, USA). The sequences were managed in SeqScape version 2.1.1 (Applied Biosystems, Foster City, CA, USA) and Sequencher 4.5 (Gene Code Corporation, Ann Arbor, MI, USA) and aligned using MEGA4 (Tamura 2007).

Records for the specimens are available in the Dataset 'Gracillariidae Molecular Dataset' (code DS-GRAC3) in the Public Data Portal of the Barcode of Life Data systems (BOLD; www.barcodinglife.org) (Ratnasingham & Hebert 2007) accessible via the DOI dx.doi.org/10.5883/DS-GRAC3.

Taxonomic notes on *Phyllonorycter mespilella* (Hübner, 1805)

Although Rosaceae-feeding *Phyllonorycter* species have been studied since the 18th century (e.g. Fabricius 1781; Hübner 1796), taxonomic divisions of this economically important group of leaf-miners are recent. Basing his conclusions on adult characters, Triberti (2007a) divided the Palaearctic taxa feeding on Rosaceae into nine species groups: 1) *blancardella,* 2) *corylifoliella,* 3) *japonica,* 4) *kumatai,* 5) *leucographella*, 6) *ringoniella,* 7) *salicifoliella,* 8) *spinicolella*, 9) *trifasciella,* and *nomina dubia*. Three of these groups (*blancardella*, *corylifoliella* and *spinicolella*) share similar genital morphology—the presence of a basal process of the valva, and were formerly placed in the same group. This group also included the *Alnus*-feeding *Phyllonorycter rajella* (Linnaeus, 1758), the type species of the genus. However, Triberti (2007a) discovered additional diagnostic characters within the *rajella* group which allowed him to erect three additional groups: *blancardella*, *corylifoliella,* and *spinicolella* (Table 1). He placed *P. mespilella* in the *blancardella* group which now includes 15 Palaearctic species: *P. aino* (Kumata, 1963), *P. anceps* Triberti 2007, *P. blancardella* (Fabricius, 1781), *P. cerisolella* (de Peyerimhoff, 1871), *P. cydoniella* ([Denis & Schiffermüller], 1775), *P. deschkai* Triberti, 2007, *P. gerasimowi* (Hering M., 1930), *P. hostis* Triberti, 2007, *P. malella* (Gerasimov, 1931), *P. malicola* (Kuznetzov, 1979), *P. mespilella* (Hübner, 1805), *P. oxyacanthae* (Frey, 1855), *P. pyrifoliella* (Gerasimov, 1933), *P. sorbi* (Frey, 1855), and *P. sorbicola* (Kumata, 1963) and two Nearctic species: *P. crataegella* (Clemens, 1859) and *P. elmaella* Doğanlar & Mutuura, 1980. Kuznetzov & Baryshnikova (2006) subsequently placed *P. gerasimowi* into the *ulmifoliella*-group of the subgenus *Phyllonorycter* Hübner, 1822.

The allied apple-feeding species of *Phyllonorycter* were reviewed by Landry & Wagner (1995), and Olivella (1997a, b) while the *blancardella* species group was diagnosed by Triberti (2007a: 160) (for species identification keys refer to Landry & Wagner 1995: 609–610; Triberti 2007: 160–161). Molecular data (28S rDNA) are available for seven of the 15 Palaearctic species in the *blancardella* group and for both Nearctic species (Lopez-Vaamonde *et al*. 2003, 2006). These nine species all belong to the *crataegella*-*pyrifoliella* clade with *P. junoniella* as the sister taxon (Lopez-Vaamonde *et al*. 2006). Molecular vouchers for the taxon *P. pomonella* (sensu Lopez-Vaamonde *et al*. 2003, 2006) need re-investigation and re-identification since *Lithocolletis pomonella* Zeller 1846 is a senior subjective synonym of *Lithocolletis oxyacanthae* Frey, 1855 (see Opinion 2205 of the ICZN (ICZN 2008: 227–228), see also Baldizzone 2007: 126; De Prins & De Prins 2007: 126–127; Landry 2007: 127; Triberti 2007b: 96–99). *P. oxyacanthae* and *P. pomonella* are placed by Lopez-Vaamonde *et al*. (2003, 2006) in two separate subclades as two different taxa.

TABLE 1. Diagnostic characters of the *blancardella, corylifoliella* and *spinicolella* groups according to Triberti (2007a) with minor additions.

Species-group	Shape of valva	Symmetry of basal processes	Place of origin of basal processes	Length and shape of apical spine
blancardella	long, narrow, more or less curved, apically rounded*	asymmetrical**	transtilla/sacculus***	short, stiff
corylifoliella	wide, almost rectangular, slightly curved	symmetrical	sacculus	short, stiff
spinicolella	straight, long, thin, apically pointed, with one or two small crests on apical area	symmetrical	sacculus	long, as long as valva or longer, very flexible

* except *P. oxyacanthae* (Frey, 1855). The valva in *P. oxyacanthae* is straight, slightly narrowed medially, pointed apically (see Triberti 2007a: 197, fig. 41).

** *P. malicola* (Kuznetzov, 1979) lacks the left basal process.

*** *P. gerasimowi* (Hering, 1930), *P. pomiella* (Gerasimov, 1933) and *P. pyrifoliella* (Gerasimov, 1933) lack basal processes of valva but according to the habitus of adult and the structure of male and female genitalia these three species are placed by Triberti (2007a) into the *blancardella* group.

Phyllonorycter mespilella **(Hübner, 1805)**

(Figs 1–33, 38, 40, 41)

Tinea mespilella—Hübner J. 1796–1838: pl. 39, fig. 272 (for a list of citations see Triberti (2007a: 176). Type locality: [Germany]. Type specimens of this species are missing. For full synonymy, see Landry & Wagner (1995); De Prins & De Prins (2005, 2013) and Triberti (2007a).

Designation of the neotype. Horn & Kahle (1935: 119) provided the last information on the location for the type specimens in the Hübner collection as: "Hübner, Jacob (1761–1826), Lepidopt.-Typen via V. A. v. Mazzola, an Naturhist. Mus., Wien." However, this type material was apparently destroyed during the Second World War.

The following issues were considered in the designation of the neotype:

i) since no syntype survives from the Jacob Hübner collection and no name-bearing type specimen of *Tinea mespilella* Hübner, 1805 is believed to be extant, we assign as neotype (ICZN, Recommendation 75A) a male topotypic specimen, in good condition, studied, illustrated and identified as *Phyllonorycter mespilella* (Hübner 1805) by Triberti (2007a);
ii) the revisionary study on the Palaearctic Rosaceae-feeding *Phyllonorycter* conducted by Triberti (2007a) revealed a complex of 17 very similar species belonging to the *blancardella* group showing considerable intraspecific variability. As a result, the designation of a neotype is critical to objectively define the nominal taxon *Phyllonorycter mespilella* (Hübner, 1805);
iii) the neotype of *Tinea mespilella* Hübner, 1805 is designated with the express purpose of clarifying the status of this taxon, an important matter because this species is a potential pest in subtropical and tropical orchards;
iv) following the recommendation of the ICZN 76A, the collection locality for the neotype is in southern Germany in close proximity to the location where the original type series was collected. The specimen chosen for the neotype was reared from *Cydonia vulgaris* Pers. which is a synonym of *Cydonia oblonga* Mill. (Rosaceae), the main host plant for *P. mespilella* (De Prins & De Prins 2005, 2013; see also ICZN 75.3.6);
v) the specimen selected as a neotype was studied, identified, and illustrated as *Phyllonorycter mespilella* (Hübner, 1805) in a major revision (Triberti 2007a: 178*)*;
vi). the designated neotype is morphologically consistent with what is known of *Phyllonorycter mespilella* (Hübner, 1805) from the original illustration (Fig. 38) and subsequent sources.

Neotype ♂ (Figs 31–33), designated here, [1] (printed) 'Württemberg / Marbach-N. / L. Süssner / (handwritten in black Indian ink) e.p. iv. [19]69 / *Cydonia* / *vulg*.'; [2] (printed) '*Phyllonorycter* / *mespilella* Hb. / det. P. Triberti

/ slide 3175♂'. The neotype of *Phyllonorycter mespilella* is in the collection of the Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria (TMLF). This collection has extensive holdings of Alpine Lepidoptera and proper facilities for preserving name-bearing types which are accessible for study (ICZN, Art. 75.3.7). Before proposing designation of this neotype, we consulted Peter Huemer, the curator of the Microlepidoptera collection at the TMLF, and received his support for this nomenclatural act (ICZN, Recommendation 75B).

Identification. *Adult* (Figs 01, 02, 31). Very similar to the other 16 species in the *blancardella* group. Because intraspecific variability is extensive (Emmet 1985; Landry & Wallace 1995), identification based on external characters is impossible.

FIGURES 01–02. *Phyllonorycter mespilella*, adult, Canary Islands, La Palma, 31.x.2007. 01, male. 02, female. Scale bar 1 mm.

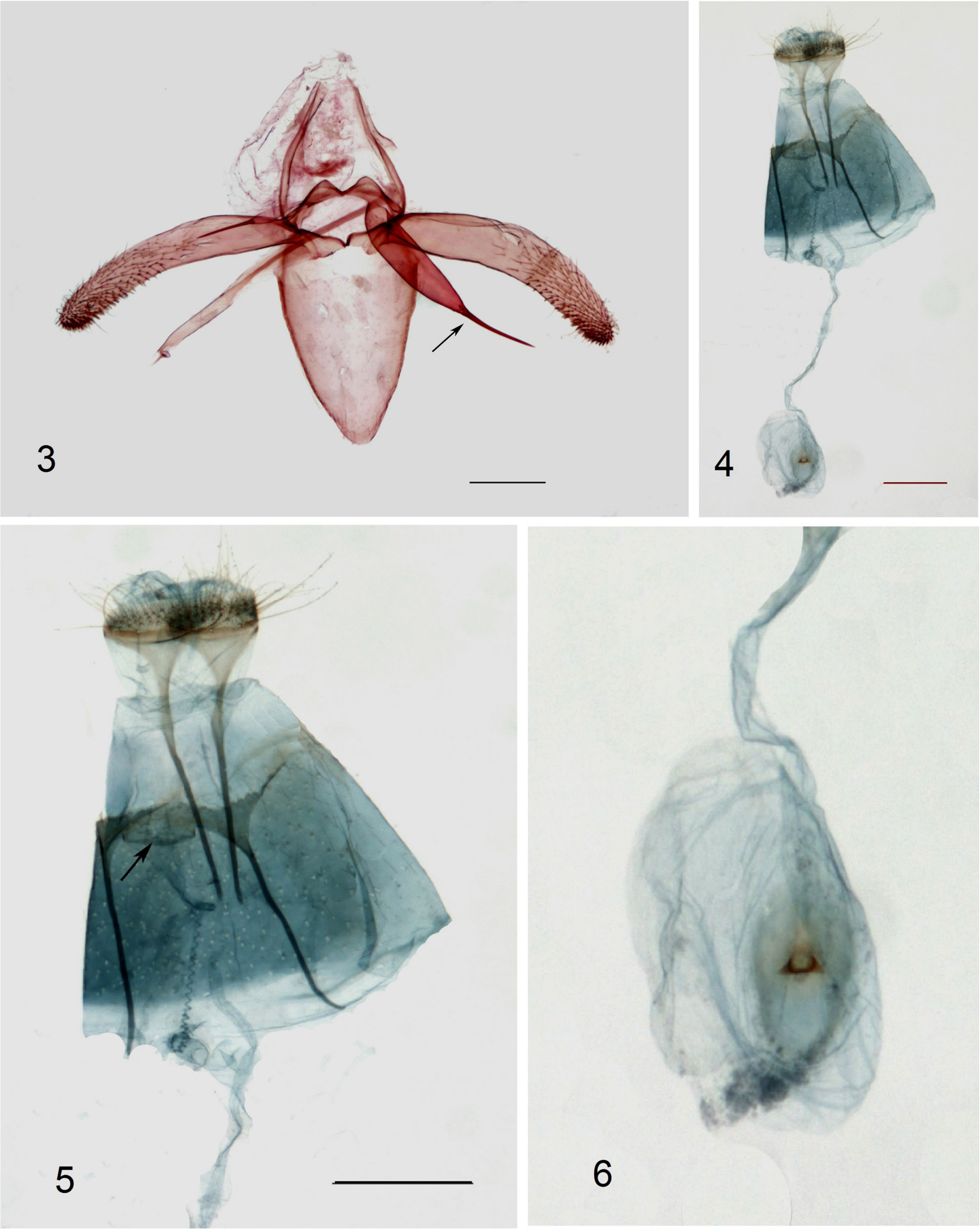
Male genitalia (Figs 03, 07, 08, 33). Similar to *P. malella* and *P. malicola* for the broad right valval process. However they can be differentiated because the first species has a very curved spine at the end of this process and the second is lacking of the left basal process. This broad process differentiates *P. mespilella* from the other species of *blancardella* group (Triberti 2007a).

Female genitalia (Figs 04–06). Differs from all other species belonging to the *blancardella* group in that the posterior margin of segment VII in *P. mespilella* projects very slightly, and the aperture of the ostium bursae is enringed by thin sclerotization (Triberti 2007a: 177–178). This combination of characters differentiates *P. mespilella* from all other species of the *blancardella* group.

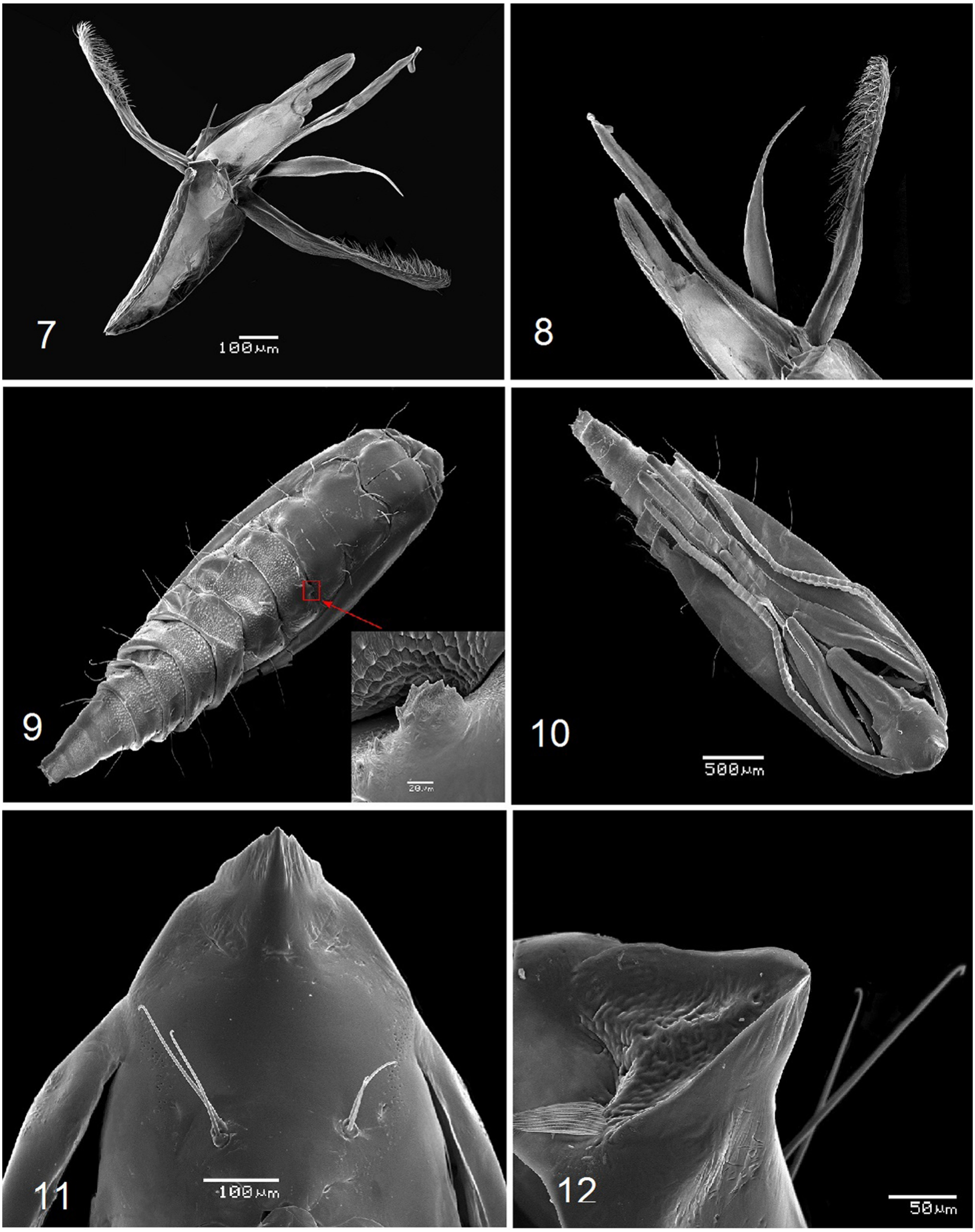
Description of pupa (Figs 09–24). Maximum length 3.9 mm; width 1.0 mm, elongate, cylindrical, narrower in the last five segments, varying in different shades of brown (Figs 09, 10, 17). Head without setae. Vertex furnished with a frontal process (cocoon cutter), which is relatively short, broadly triangular, acute, with wrinkled median surface, lateral ridges straight (Figs 11–13). Forewings long, extending to the anterior margin of abdominal segment A5, and unattached at their distal ends (Figs 10, 14, 17). The appendages of the antennae are slightly longer than forewings, and extending to anterior margin of A6, but shorter than the appendages of metathoracic future legs. The future hind legs extend to posterior margin of A6 (Figs 14, 17). The distance between the apices of the mesothoracic and metathoracic legs is approximately 1.23× the distance between the apices of the prothoracic and mesothoracic legs (Fig. 10). Abdominal segments A5–7 free in male, A5–6 in female, enabling the pupa to wriggle actively when disturbed (Figs 14, 17). Abdominal segments mostly covered dorsally and ventrally with dense minute spines (Fig. 16). A1–2 possess latero-dorsal bulbous expansions situated close to anterior margin (Fig. 09); terminal segment A10 elongate, fully covered with spinaculae, the convex area present anteriorly on dorsal surface of A8 covered with tiny erected spines, the remaining surface of segment A8 with spinaculae (Figs 14, 16). One pair of dorsal setae is present on segments TI–TII, two pairs of setae (one dorsal and one lateral) are on TIII and A1; four pairs of setae (two dorsal, one latero-dorsal and one lateral) are present on each segment of A2–6; two pairs (one dorsal and one lateral) are present on segments A7–8 (Figs 15, 17–19). Cremaster (Figs 21–24) with two pairs of hook-shaped processes; outer process ca. 2× bigger than inner process; bases of inner processes partially superimposed to outer processes (Patočka & Turčáni 2005; Triberti 2007a).

Host plant(s). Many species of Rosaceae. *Malus domestica* Borkh. in the Canary Islands (Figs 29, 30). De Prins & De Prins (2013) provide a full list of its host plants.

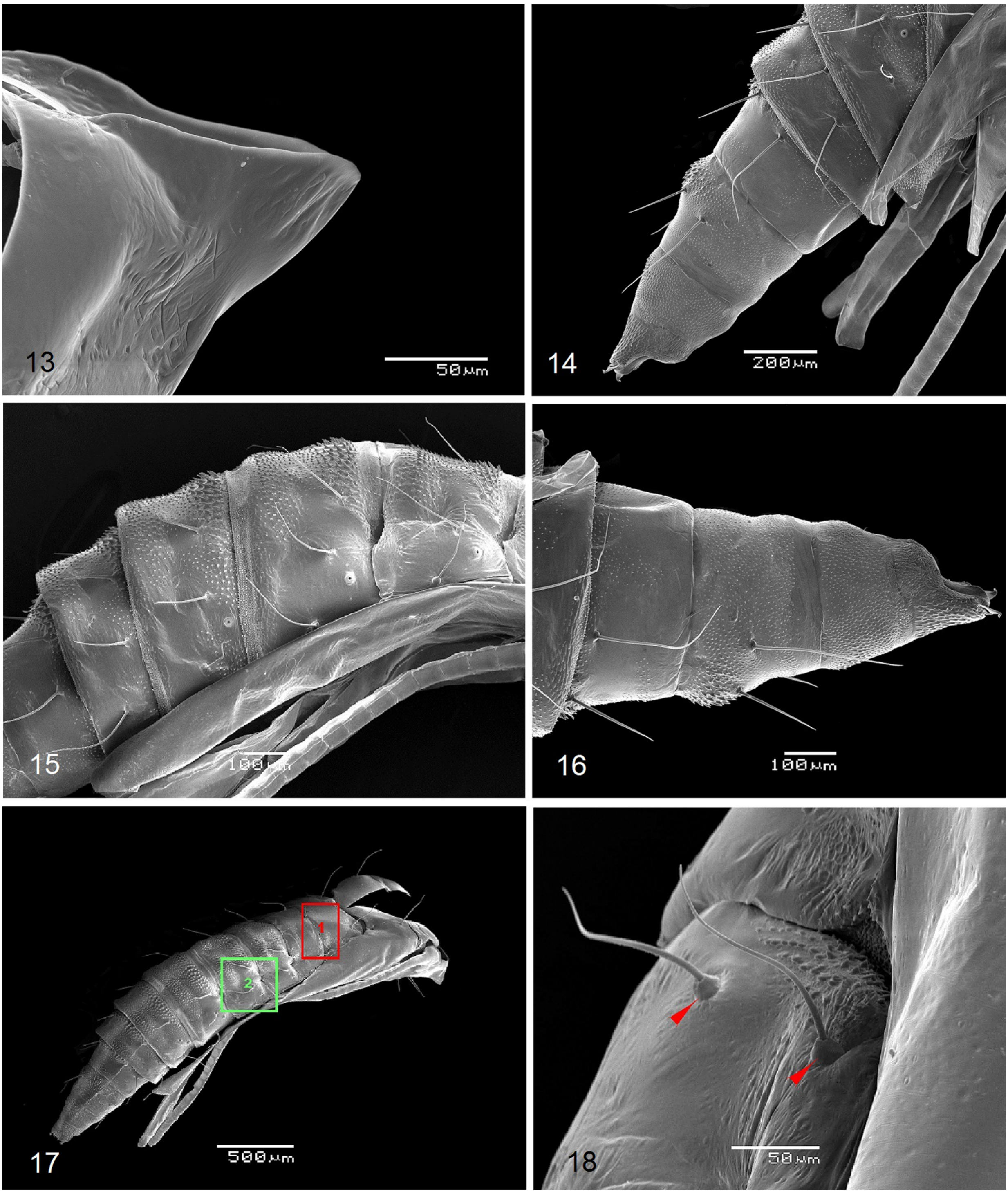
Mine. Abaxial (Figs 25–28), tentiform, parenchymal tissues are consumed in spots (Fig. 27), strongly contracted between two veins (Figs 25, 26), narrow, as long as ca. 20 mm with several longitudinal wrinkles of different length when the mine is fully developed (Fig. 26). The pupa protrudes from the mine before the emergence of the adult (Fig. 28).



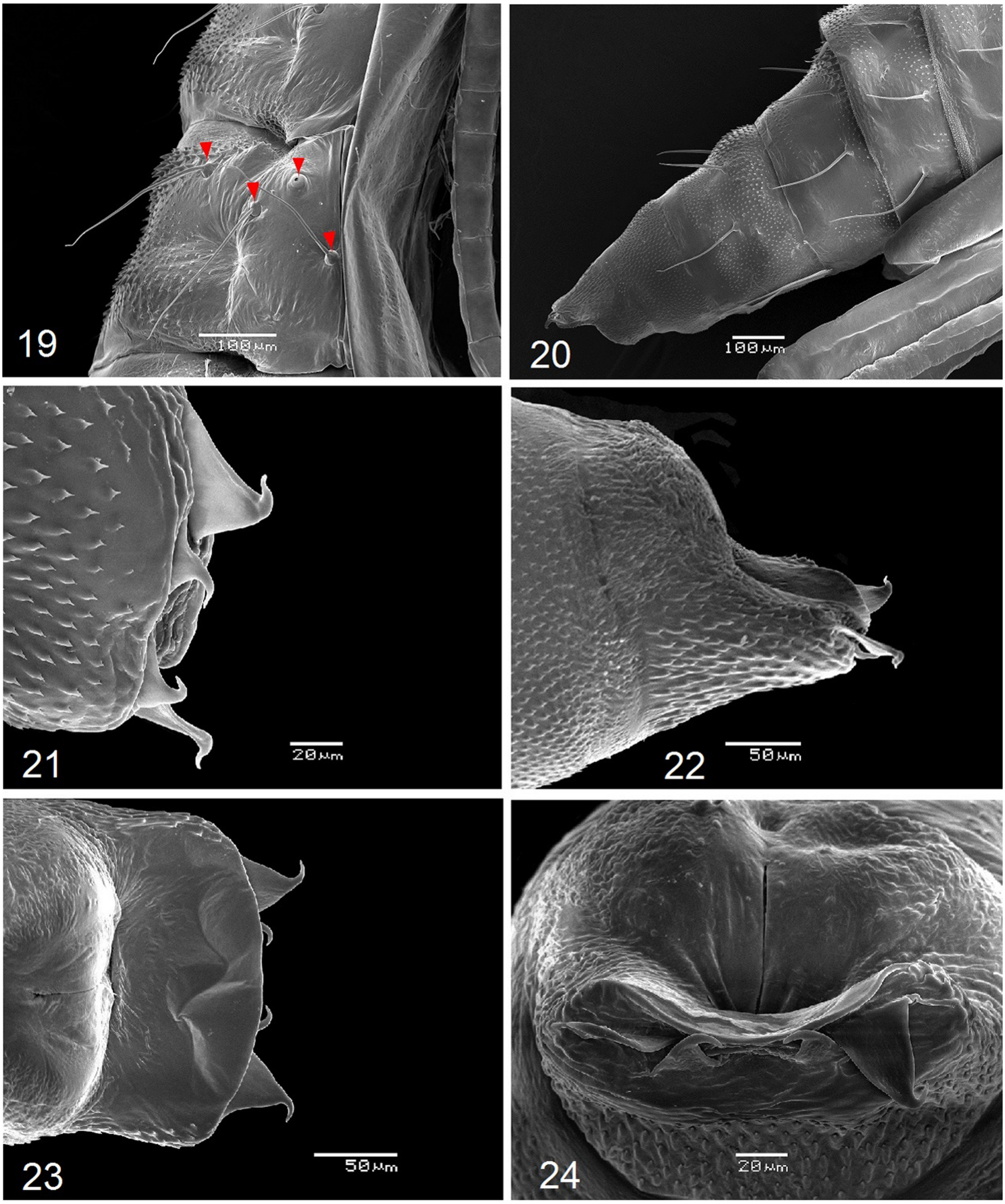
FIGURES 03–06. *Phyllonorycter mespilella,* genitalia. 03, male genitalia, gen. prep. De Prins 3804♂. An arrow indicates the asymmetrical basal process of valva with a straight spine. 04, female genitalia, gen. prep. De Prins 3805♀. 05, same preparation, segment 8, an arrow indicates ostium bursae enringed by sclerotization. 06, same preparation, corpus bursae with signum. Scale bar 200 µm.



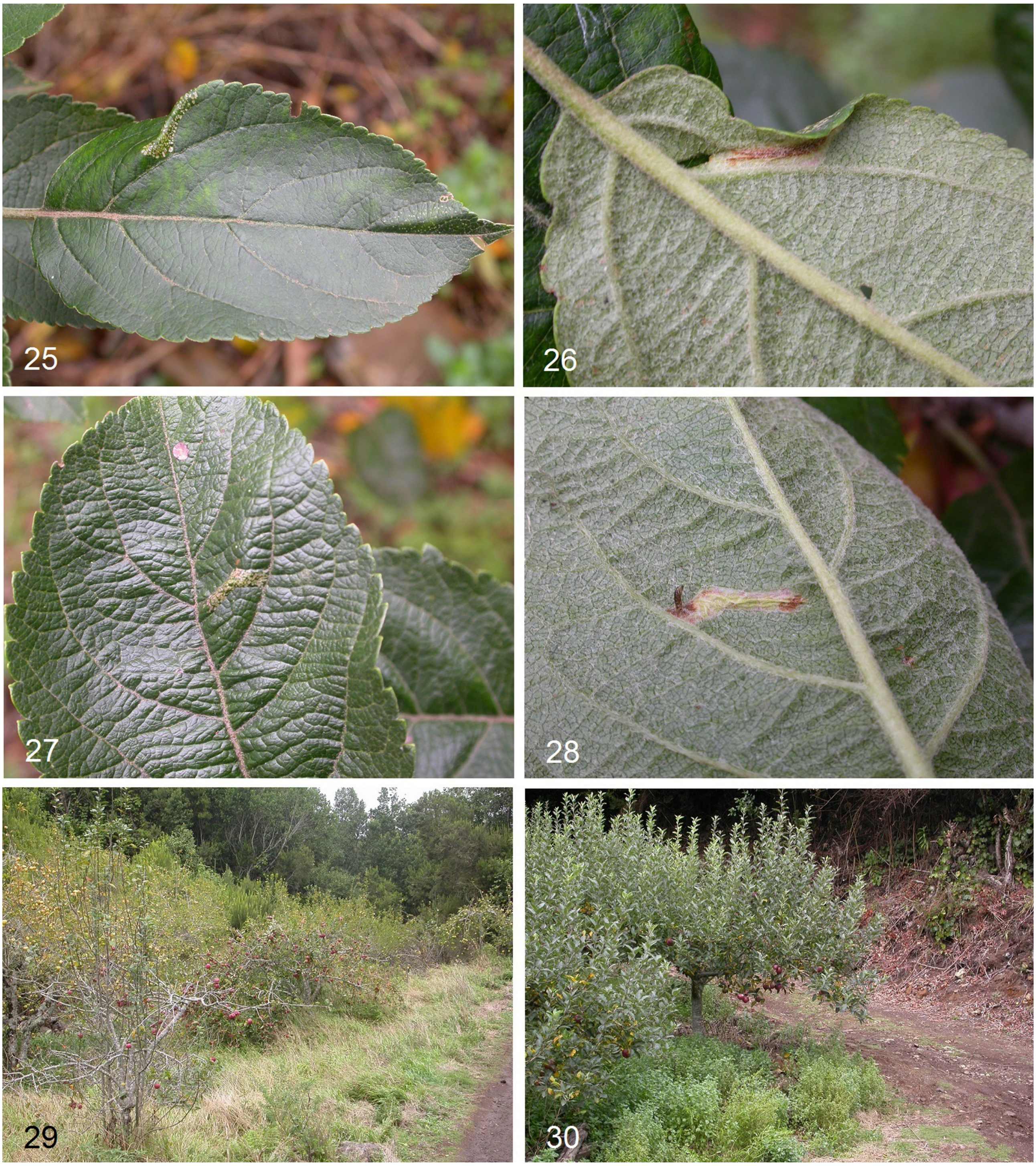
FIGURES 07–12. *Phyllonorycter mespilella*, adult male and pupa. 07, male genitalia. 08, same preparation, with enlarged basal process, valva and aedoeagus. 09, pupa, dorsal view with mapped latero-dorsal bulbous expansion on A1. 10, pupa, ventral view. 11, pupa, head, ventral view. 12, cocoon cutter, dorsal view. Scale bar as indicated.



FIGURES 13–18. *Phyllonorycter mespilella,* pupa. 13, cocoon cutter, lateral view. 14, pupa, segments A5–10, lateral view. 15, abdominal segments, lateral view. 16, minute spines on caudal segments. 17, overview with mapped details of setation. 18, same, detail 1. Arrows indicate dorsal and latero-dorsal setae on TIII. Scale bar as indicated.



FIGURES 19–24. *Phyllonorycter mespilella,* pupa. 19, same as Fig.17, detail 2. Arrows indicate dorsal, latero-dorsal and lateral setae on A2. 20, segments A7–8 with two pairs of setae, lateral view. 21, cremaster, dorsal view. 22, cremaster, lateral view. 23, cremaster, ventral view. 24, cremaster, caudal view. Scale bar as indicated.



FIGURES 25–30. The biotope and leaf mines of *Phyllonorycter mespilella* on *Malus domestica* Borkh., Canary Islands, La Palma, 31.x.2007. 25, mine, adaxial view. 26, mine abaxial view. 27, mine of the third instar larva, parenchymal tissue is consumed in spots, adaxial view. 28, pupa protrudes the mine before the emergence of the adult, abaxial view. 29, the biotope of *Phyllonorycter mespilella* in the Canary Islands, La Palma, 31.x. 2007. 30, *Malus domestica* Borkh, the host plant of *Phyllonorycter mespilella* in the Canary Islands, La Palma.

Distribution. Central southern Europe, North Africa, Canary Islands: Lanzarote (Triberti 2007: 177), La Palma (this paper) (Fig. 40), and North America. See De Prins & De Prins (2013) for a full list of the distribution records.

Remarks. Triberti (2007a: 177) provided the label data for two specimens (male and female) of *P. mespilella* collected by E. Arenberger in Lanzarote, Puerto del Carmen. The mines were collected on *Pyrus* sp. 10–15.i.1991, the adults emerged on 5.iv.1991; the depository of these specimens is ZMHB. These specimens indicate that *P. mespilella* invaded the eastern island of the Canary archipelago more than twenty years ago, although its occurrence remained unreported. Two specimens of *P. mespilella* from Lanzarote, examined by Triberti (2007a), and 11 specimens from La Palma, discussed below, are the only records of this species from the Canary Islands. Because of the frequent air transport between Europe and both the eastern (Lanzarote) and the western (La Palma) islands, *P. mespilella* may have been independently introduced to the two islands.

FIGURES 31–33. The neotype of *Phyllonorycter mespilella.* 31, male, Germany, Württemberg, iv.[19]69. Scale bar 1 mm. 32, the collecting and the male genitalia preparation labels of the neotype of *Phyllonorycter mespilella*. 33, the male genitalia of the neotype of *Phyllonorycter mespilella*, gen. prep. Triberti 3175♂. Scale bar 200 µm.

Examined specimens collected in the Canary Islands. 5♂, 6♀, Canary Islands, La Palma, 2 km S Barlovento, 270 m, 28°49'N 017°46'W, mine 31.x. 2007, leg. J. & W. De Prins; ex l. *Malus domestica* Borkh. (Rosaceae), 25–27.xi.2007. Gen. Prep. De Prins 3804♂, 3805♀, in JWDP.

In addition to genitalia examinations, a DNA barcode (GRPAL043-10) was obtained from the male specimen of *P. mespilella* collected in the Canary Islands, La Palma, 2 km S Barlovento, 270 m, 28°49'N 017°46'W, mine 31.x. 2007, leg. J. & W. De Prins; ex l. *Malus domestica* Borkh. (Rosaceae), 25–27.xi.2007 (Fig. 41). The resultant 658 bp sequence of COI was identical to four other sequences from *P. mespilella*, three collected in France (GRPAL186-11, 992-12, 229-11) and one (B01 mespi) in the Czech Republic (see BOLD; www.barcodinglife.org). This result confirms the identification of our specimen, but does not firmly establish its origin, because no sequences from Spanish populations are available.

Taxonomic notes on *Phyllonorycter trifasciella* (Haworth, 1828)

Stainton (1848) made the first attempt to group the closely related *Phyllonorycter* species. While revising the British *Argyromiges* he noted that they were divisible into several very distinct groups which the 'continental lepidopterists' (a. o. Zeller) have made into genera, one of which was *Lithocolletis.* Stainton (1848: 2079) diagnosed the genus *Lithocolletis* from two other related genera (*Opostega*, *Lyonetia*) as the genus which "has the back of the head rough, the antennae simple, with the basal joint thickened; and has an ocellated spot at the apex of the anterior wings, which are in most cases adorned with brilliant metallic colours". Stainton (1848) divided British and continental European *Argyromiges* into several sections and named the sections A, B, and C as the genus *Lithocolletis* sensu Zeller. *P. trifasciella* was placed by Stainton into section A [as no 10 in his species numeric system] together with the other 18 European *Phyllonorycter* species (Table 2). Section A was delimited by Stainton (1848) as the group of species with metallic or very glossy forewings, with paler fasciae or comma-shaped strigulae on both margins.

Pierce & Metcalfe (1935) also recognized a group of closely related species which included those currently in the *trifasciella* species group. They described the group as "group C. *Lithocolletis*, *Type trifasciella*," and diagnosed its members as having the following characteristics:"valvae symmetrical. Aedoeagus short, stumpy. The females are much alike, ovipositor a weak pad, not extensile. Signum, where it occurs, small pointed … Valva symmetrical, of various shapes. Aedoeagus short, bulbed, with expanded orifice. Saccus without tail. Ostium more pronounced than preceding group" [group B. Type *ulicicolella*] (Pierce & Metcalfe 1935: 71, 77).

Four *Phyllonorycter* species were originally placed into the *trifasciella* group: *P. scabiosella* (Douglas, 1853), *P. schreberella* (Fabricius, 1781), *P. trifasciella* (Haworth, 1828), and *P. tristrigella* (Haworth, 1828). Kumata (1963) added seven more species to this group from Japan (Table 2) and further indicated that species in the *trifasciella* group are leaf-miners of Ulmaceae with three exceptions: *P. scabiosella* mines in Dipsacaceae, while *P. lonicerae* and *P. trifasciella* mine the leaves of Caprifoliaceae. Later Kumata (1967) added *P. laciniatae* (Kumata 1967), *P. bifurcata* (Kumata, 1967), and two more Rosaceae-feeding species: *P. stephanandrae* (Kumata, 1967) from Japan and *P. rubicola* Kumata, 1973 from Nepal to this group. Kumata (1973: 5) delimitated the *trifasciella* group following Pierce & Metcalfe (1935) and added the following adult characters: "valvae usually symmetrical, rather short, without any process of costa or sacculus; aedoeagus short, stumpy, with its basal half conspicuously swollen; juxta long, heavily sclerotized".

TABLE 2. Composition of the *trifasciella*-species group in the course of the taxonomic history.

Stainton (1848)	Pierce & Metcalfe (1935)	Kumata (1963, 1967, 1973)	Kuznetzov & Baryshnikova (2006)
P. cavella (Zeller, 1846)	*P. scabiosella* (Douglas, 1853)	*P. bicinctella* (Matsumura, 1931)	*P. cephalariae* (Lhomme, 1934)
P. connexella (Zeller, 1846)	*P. schreberella* (Fabricius, 1781)	*P. bifurcata* (Kumata, 1967)	*P. loniceriphaga* Noreika, 1992
P. emberizaepenella (Bouché, 1834)	***P. trifasciella*** (Haworth, 1828)	*P. celtidis* (Kumata 1963)	*P. montanella* Bradley, 1980
P. froelichiella (Zeller, 1839)	*P. tristrigella* (Haworth, 1828)	*P. laciniatae* (Kumata 1967)	*P. raikhonae* Noreika, 1992
P. ilicifoliella (Duponchel, 1843)		*P. lonicerae* (Kumata, 1963)	*P. scabiosella* (Douglas, 1853)
P. junoniella (Zeller, 1846)		*P. pulchra* (Kumata, 1963),	***P. trifasciella*** (Haworth, 1828)
P. lantanella (Schrank, 1802)		*P. rubicola* Kumata, 1973	
P. lautella (Zeller, 1846)		*P. scabiosella* (Douglas, 1853)	
P. maestingella (Müller, 1764)		*P. schreberella* (Fabricius, 1781)	
P. mespilella (Hübner, 1805)		*P. stephanandrae* (Kumata, 1967)	
P. messaniella (Zeller, 1846)		*P. zelkovae* (Kumata, 1963)	
P. oxyacanthae (Frey, 1855)		***P. trifasciella*** (Haworth, 1828)	
P. quercifoliella (Zeller, 1839)		*P. tristrigella* (Haworth, 1828)	
P. rajella (Linnaeus, 1758)		*P. tritorrhecta* (Meyrick, 1935)	
P. schreberella (Fabricius, 1781)		*P. ulmi* (Kumata, 1963)	
P. spinicolella (Zeller, 1846)			
P. trifasciella (Haworth, 1828)			
P. tristrigella (Haworth, 1828)			
P. ulmifoliella (Hübner, 1817)			

Triberti (2007a) noted that only one feature was shared by all members of the *trifasciella* group—a short aedoeagus with a strong juxta. He further concluded that this group (sensu Kumata 1973) can be split into further groups based on valval structure. Kuznetzov & Baryshnikova (2006) placed *P. trifasciella* in its own group within the subgenus *Juxtafera* Baryshnikova, 2006 (Table 2). The group is diagnosed by five characters: i) aedoeagus short, with one pair of small flat appendices on vesica; ii) valvae rather long, narrow, slightly concave with sharp apices, directed ventrad; iii) larvae mine plants of Dipsacaceae and Caprifoliaceae; iv) mines are abaxial, tentiform; v) pupation without cocoon (Kuznetzov & Baryshnikova 2006).

Sequence data for 28S rDNA (Lopez-Vaamonde *et al*. 2003) supports the earlier conclusion (Pierce & Metcalfe 1935) that *P. trifasciella* is the sister species to *P. scabiosella* (larvae of both species feed on Caprifoliaceae). This group [clade] included *P. tristrigella* with strong support (100%) and *P. schreberella* with weak support (57%) (Lopez-Vaamonde *et al*. 2003). Kumata's (1963) conclusion that seven Japanese species of *Ulmus* leaf-miners are phylogenetically related to the European *trifasciella* group was also partly confirmed by molecular results (Lopez-Vaamonde *et al*. 2006). *Phyllonorycter bicinctella* and *P. lonicerae* are both placed by Lopez-Vaamonde *et al*. (2006) within the *bifurcata—agilella* clade with weak support (54% and 51%). The clade also contains the European *trifasciella* group species defined by Pierce & Metcalfe (1935): *P. trifasciella*, *P. scabiosella*, *P. schreberella* and *P. tristrigella*. Past molecular analysis has only examined 28S rDNA, a gene region where indels create difficulties for alignment (Martínez-Ansemil *et al*. 2012). Trees inferred from single markers can also often reflect the evolutionary history of individual genes rather than the species (Talavera *et al*. 2012). Therefore, it is desirable to base phylogenetic conclusions on broader genetic information (Kluge 1989; Brower 1996; De Prins *et al*. 2009; Kawahara 2009; Talavera *et al*. 2012; De Prins & Kawahara 2012).

Recent attempts to split the 428 species of *Phyllonorycter* into smaller groups have largely employed ecology and morphology. For example, important revisionary taxonomic studies have delimited species groups within *Phyllonorycter* on the basis of host plant separating groups whose larvae mine on Salicaceae (Davis & Deschka 2001), Aceraceae, Ulmaceae (Kuznetzov & Baryshnikova 2004, 2006), and Rosaceae (Triberti 2007a). De Prins & Kawahara (2012) applied a different approach, delimiting species groups within a particular bio-geographical region—the Afrotropics in this case. The assumption that closely related and morphologically similar species of *Phyllonorycter* species feed on closely related plants has been confirmed in many cases (Davis & Deschka 2001; Lopez-Vaamonde *et al*. 2003, 2006; Kuznetzov & Baryshnikova 2004; Triberti 2007a; Bengtsson & Johansson 2011; De Prins & Kawahara 2012), but there are many exceptions (De Prins & Kawahara 2012). The revision, re-delimitation and the clarification of monophyly of species within the *trifasciella* species group falls beyond the scope of this paper.

Phyllonorycter trifasciella (Haworth, 1828)

(Figs 34–36, 39–41)

Tinea trifasciella—Haworth A.J. 1828: 576, nr. 56 (for list of citations see De Prins & De Prins 2013). Type locality: [United Kingdom], Coomb Wood. Type specimens: not stated in the original description (see *Designation of the lectotype* below).

Lithocolletis heydenii—Zeller P.C. 1846: 247–248, pl. 1, fig. 38. A junior subjective synonym of *Tinea trifasciella* Haworth, 1828, synonymized by Stainton (1848: 2088).

Opostega torquillaepennella Heyd. (*heydeniella*, Z.)—Bruand T. 1851: 52, nr. 1455. An unjustified emendation of *Lithocolletis heydenii* Zeller, 1846 and a junior subjective synonym of *Tinea trifasciella* Haworth, 1828, synonymized by Walker (1864: 908).

Lithocolletis heydeni Z.—Mann J. 1862: 408. An incorrect subsequent spelling.

Phyllonorycter heydenella Herrich-Schäffer 1862—Leraut P. J. A. 1997: 95. An incorrect subsequent spelling.

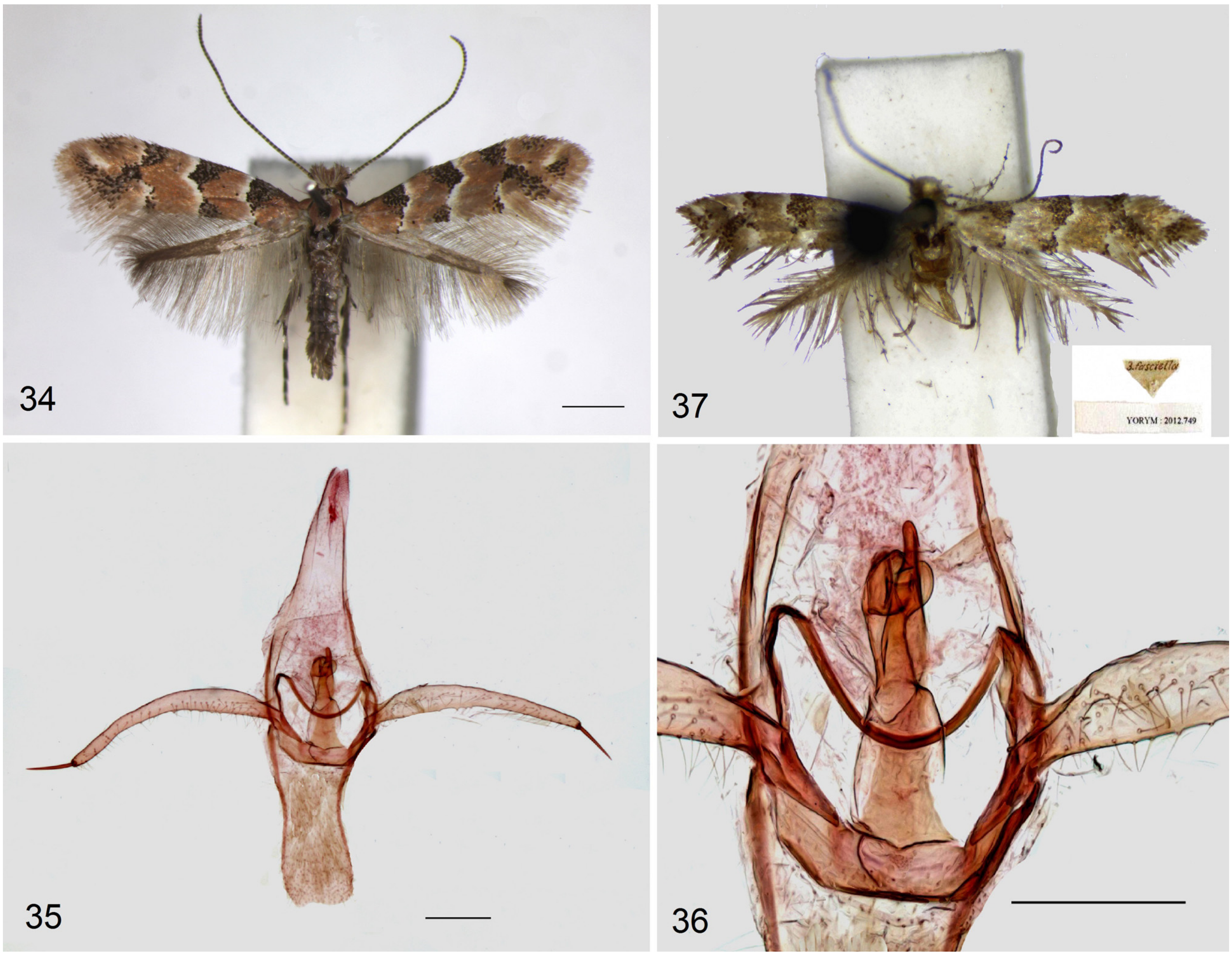
Designation of the lectotype. Haworth described species from several collections, and some specimens may subsequently have been lost (K. Tuck, pers. corr.). In 1834, a year after his death, Haworth's personal collection of British Lepidoptera was auctioned in four parts. Part 1 with some types of Microlepidoptera was obtained by the Entomological Society of London; part 2 by Lord Thomas Walsingham; part 3 by James Francis Stephens; and part 4 by Frederick William Hope (Horn & Kahle 1935). Most of these specimens are now in the Natural History Museum London, the Hope Entomological Collection of the Oxford University Museum of Natural History, and the Yorkshire Museum, York. A list compiled by staff at the BMNH in 1972 and a note of 8.x.2001 indicated that one type specimen of *Tinea trifasciella* was in the York Museum (K. Tuck, pers. corr.) (Fig. 39). Its presence was subsequently confirmed by Mr. Raymond Uffen and the first author examined and photographed this specimen.

This specimen is designated here as a lectotype, according to the ICZN (1999: Art.74.1).

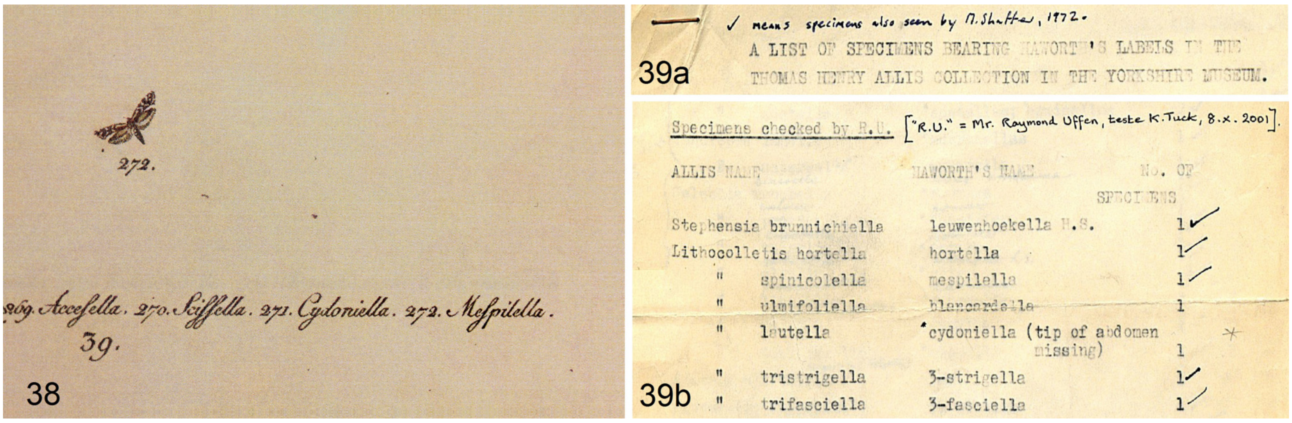
Lectotype ♀, (Fig. 37) designated here, [1] (handwritten) '3 *fasciella*'; [2] (printed) 'YORYM 2012.749'. The lectotype of *Phyllonorycter trifasciella* is the property of the insect collection of the Yorkshire Museum and Gardens (YMUK).This recognized scientific insect collection is known for preserving name-bearing types of Haworth specimens which are accessible for further study (ICZN, Art. 75.3.7). Before designating this lectotype, we consulted the curators (Kevin Tuck, Martin Honey, Stuart Ogilvy) of major natural history collections (ICZN, Recommendation 75B) and received their support for this nomenclatural act.

Identification. *Adult* (Figs 34, 37). The broad conspicuous black markings on the forewing make *P. trifasciella* easily distinguishable from all European species in the *trifasciella* group. However, it might be confused with the Nepalese *P. rubicola* (see Kumata 1973: pl. ii: fig. G). In *P. rubicola* the basal costal black patch is absent and the median white fascia is more or less straight, while in *P. trifasciella* the basal costal black patch is present and white median fascia is sharply angulated. *P. trifasciella* resembles the autumnal form of *P. tritorrhecta*, but the latter species lacks the basal costal and tornal black patches present in *P. trifasciella* (see Kumata 1963: pl. iv, fig. 54). The apical pattern of *P. trifasciella* resembles that of *P. lonicerae*, but *P. lonicerae* lacks the broad costal basal and costal median black patches that are very characteristic of *P. trifasciella* (see Kumata 1963: pl.v, fig. 59).

Male genitalia (Figs 35, 36). Slightly resembles *P. pulchra*, *P. tristrigella*, *P. tritorrhecta*, *P. ulmi,* and *P. zelkovae* because all of these species have a spine on the apical part of valva, complete transtilla, sclerotized juxta and short stumpy aedoeagus. However, the combination of the following characters: i) slightly asymmetrical, long, narrow valvae, ii) long, sharp, apical valval spine, about as long as 1/6 of valval length and iii) long, only about 1/3 shorter than tegumen, enlarged from median towards caudal part sternum VIII is unique for this species, enabling its easy identification.



FIGURES 34–37. *Phyllonorycter trifasciella*, adult. 34, male, Canary Islands, Tenerife, 30.xii.1982. Scale bar 1 mm. 35, male genitalia, gen. prep. De Prins 3806♂. 36, same preparation, aedoeagus. Scale bar 200 µm. 37, the lectotype of *Phyllonorycter trifasciella*, female, specimen No YORYM 2012.749, in the collection of the Yorkshire Museum and Gardens.



✓ means specimens also seen by M. Shaffer, 1972.

A LIST OF SPECIMENS BEARING HAWORTH'S LABELS IN THE THOMAS HENRY ALLIS COLLECTION IN THE YORKSHIRE MUSEUM.

Specimens checked by R.U. ["R.U." = Mr. Raymond Uffen, teste K. Tuck, 8.x.2001].

ALLIS NAME	HAWORTH'S NAME	NO. OF SPECIMENS
Stephensia brunnichiella	leuwenhoekella H.S.	1 ✓
Lithocolletis hortella	hortella	1 ✓
" spinicolella	mespilella	1 ✓
" ulmifoliella	~~blancardella~~	1
" lautella	*cydoniella (tip of abdomen missing)	1
" tristrigella	3-strigella	1 ✓
" trifasciella	3-fasciella	1 ✓

FIGURE 38–39. Archive references. 38, the citation and the illustration of *Tinea mespilella* Hübner, 1805 from Hübner, J. 1796–1838. *Sammlung europäischer Schmetterlinge. Achte Horde. Tineae Die Schaben; nach der Natur geordnet, beschrieben und vorgestellt*: pl. 39, fig. 272. 39a, the title page of the list of specimens bearing Haworth's labels in the Thomas Henry Allis collection. 39b, the list, indicating that the type specimen of *P. trifasciella* was examined by Mr. Raymond Uffen.

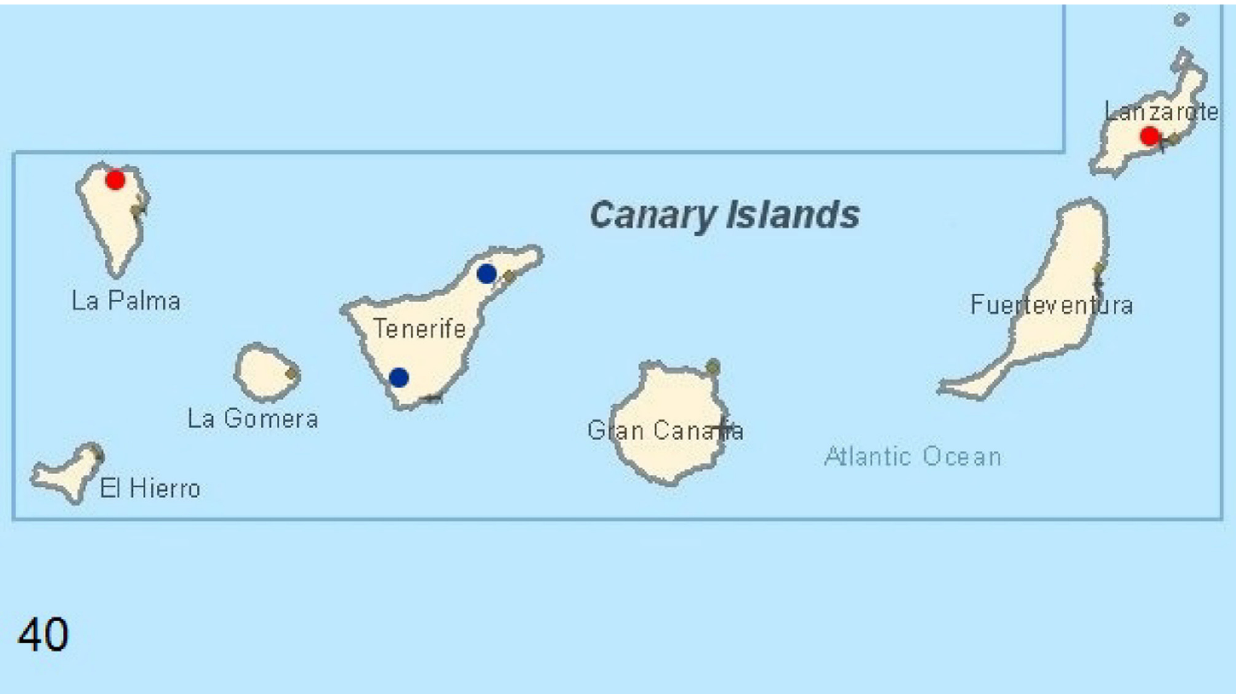


FIGURE 40. The occurrence of *Phyllonorycter mespilella* (red dots) and *P. trifasciella* (blue dots) in the Canary Islands.

Host plant(s). Many species of Caprifoliaceae. *Lonicera periclymenum* L. in the Canary Islands. For a full list of the referenced host plants refer to De Prins & De Prins (2013).

Distribution. throughout Europe. Mentioned from Turkey as *P. heydeni* (Mann 1862: 408). The Canary Islands (Tenerife) (**new record**) (Fig. 40). For a full list of the referenced localities, refer to De Prins & De Prins (2013).

Examined specimens collected in the Canary Islands. 3♂, Canary Islands, Tenerife, Los Cristianos, 10 m, mine 30.xii.1981, leg. W. De Prins; ex l. *Lonicera periclymenum* L. (Caprifoliaceae), 03–06.i.1982. Gen. prep. De Prins 3806♂, in JWDP.

The natural environment of the Canary Islands

The transformation of the natural flora of the Canary Islands has a long history, but impacts have recently intensified. The laurel forest now covers only 12% of its original extent with destruction greatest on Gran Canaria, where it now occupies just 1% of its former distribution (Del Arco-Aguilar *et al.* 2010). Consideration of the introductions of *Phyllonorycter* species from continental Europe to the subtropical Canary Islands reveals a general pattern. Firstly, these introductions have been accidental, unnoticed, and the pathways of introduction are unknown. Secondly, the introduced species became established in the Canary Islands: *Phyllonorycter platani* for 104 years, *P. trifasciella* for 32 years and *P. mespilella* for 22 years (Fig. 40). Nevertheless, the *a posteriori* studies on the European species which colonized the subtropical Atlantic Islands provides a firm background to assess which taxonomic group of *Phyllonorycter* are colonizers, which ecosystems are most at risk, and which pathways and which vectors of introduction are most important. Such information improves the assessment of invasion risk associated with particular species and ecosystems (Kenis *et al.* 2012).

TABLE 3. Species of *Phyllonorycter* known from the Canary Islands. For host plant references see De Prins & De Prins (2013).

No	Species	Host plant(s) in the Canary Islands	Host plant family	Distribution in the Canary Islands	First reference
1.	*P. bartolomella* (Deschka, 1968)	*Teline monspessulana* (L.) K. Koch	Fabaceae	**Endemic**: Gran Canaria Tenerife	 Deschka 1968: 84 Deschka 1968: 84
2.	*P. cytisella* (Rebel, 1896)	*Chamaecytisus palmensis* (H. Christ) F. A. Bitsby & K. W. Nicholls *Chamaecytisus proliferus* (L.) Link	Fabaceae	**Endemic**: Gran Canaria La Palma Tenerife	 Rebel 1939: 62 Rebel 1939: 62 Rebel 1896: 141
3.	*P. cytisifoliae* (Hering, 1927)	*Chamaecytisus palmensis* (H. Christ) F. A. Bitsby & K. W. Nicholls *Chamaecytisus proliferus* (L.) Link	Fabaceae	**Endemic**: Gran Canaria La Palma Tenerife	 Hering 1927: 422 Rebel 1939: 62 Hering 1927: 422
4.	*P. foliolosi* Walsingham, 1908	*Adenocarpus foliolosus* DC. *Adenocarpus viscosus* Webb. & Berth. *Genista canariensis* L.	Fabaceae	**Endemic**: La Palma Tenerife	 Rebel 1939: 63 Walsingham 1908: 978
5.	*P. helianthemella* (Herrich-Schäffer, 1861)	*Cistus monspeliensis* L.	Cistaceae	La Palma Tenerife	Hering 1927: 463 Walsingham 1908: 976
6.	*P. juncei* Walsingham, 1908	*Genista stenopetala* Webb & Berth. *Spartium junceum* L.	Fabaceae	Tenerife	Walsingham 1908: 978
7.	*P. klimeschiella* (Deschka, 1970)	*Arbutus canariensis* Veill.	Ericaceae	**Endemic**: Tenerife	 Deschka 1970: 30
8.	*P. mespilella* (Hübner, 1805)	*Pyrus* sp., *Malus sylvestris* Mill.	Rosaceae	Lanzarote La Palma	Triberti 2007: 177 this paper
9.	*P. messaniella* (Zeller, 1846)	*Castanea sativa* Mill., 1768 *Quercus* ilex L., *Quercus suber* L., *Quercus* sp.	Fagaceae	La Gomera La Palma Tenerife	Klimesch 1979: 161 Klimesch 1979: 161 Walsingham 1908: 976
10.	*P. platani* (Staudinger, 1870)	*Platanus orientalis* L	Platanaceae	Tenerife	Walsingham 1908: 977
11.	P. spartocytisi (Hering, 1927)	*Chamaecytisus supinus* (L.) Link. *Lygos monosperma* Boiss. var. *rhodorrhizoides* Webb & Berthel.	Fabaceae	**Endemic**: La Palma	 Hering 1927: 424
12.	*P. trifasciella* (Haworth, 1828)	*Lonicera periclymenum* L.	Caprifoliaceae	Tenerife	this paper

Phyllonorycter is known to have inhabited the Canary Islands for over a century. In fact, Rebel (1896) described the first new species from these islands; *Phyllonorycter cytisella*, indicating its host plant as *Cytisus proliferus* L. f. (Rebel 1896: 141) and its type locality as [Tenerife, Orotava]. At present 12 *Phyllonorycter* species (half endemic), are recorded from these islands (Table 3).

Linking taxonomic and molecular data

As made clear by this case study, species colonizing new areas often remain overlooked in collections for decades, but they retain value as vouchers recording these events. The linking of molecular and morphological data can greatly facilitate the detection of species invasions. Existing online taxonomic databases are excellent tools for the dissemination of information on species distributions, but they do not provide enough information to detect new invasions. The Barcode of Life Data System (http://www.boldsystems.org/), BOLD, is a public repository of specimen data, taxonomic information, and DNA barcodes. It also serves as a workbench for the collection and analysis of new barcode sequences. As the number of sequence records in BOLD grows, this platform is becoming a powerful tool for the detection of invasive species. At present, DNA barcode records are available for more than 74,000 species of Lepidoptera, nearly half of all described species in this group. Because researchers need a fast and reliable way to link global taxonomic information with DNA sequence libraries, we have provided a DNA barcode (COI) library for 124 gracillariid species (Fig. 41). These records are available in the Dataset 'Gracillariidae Molecular Dataset' accessible via the code (DS-GRAC3) in the Public Data Portal of the Barcode of Life Data systems (BOLD; www.barcodinglife.org) (Ratnasingham & Hebert 2007). It is also accessible via the following DOI, dx.doi.org/10.5883/DS-GRAC3. These molecular records are also linked with the Global Taxonomic Database for Gracillariidae (www.gracillariidae.net/).

It is important to emphasize certain points: the NJ tree (Fig. 41) should not be viewed as a reconstruction of phylogenetic relationships. The development of a strong phylogenetic hypothesis for these taxa would require sequence information from additional gene loci, including both nuclear and mitochondrial markers. The NJ tree should instead be viewed as a tool for visualizing the level of sequence divergence between species, and among specimens of a species. Examination of this tree (Fig. 41) reveals that intraspecific divergences are generally low, while divergences between species, even congeneric taxa, are considerable. For example, the 78 species of the genus *Phyllonorycter*, to which the invasive species discussed in the present paper belong, show an average divergence of 6.04%, while intraspecific divergences average just 0.94%. Similar patterns of divergence have been reported in other groups of Lepidoptera (Ratnasingham & Hebert 2007). Therefore, the DNA barcode library presented here, based on verified and validated data, is a valuable source of reference for gracillariid species recognition and correct identification. As a result, we anticipate that the incorporation of new barcode records into BOLD (www.barcodinglife.org) and linking these data with the verified, referenced and freely available online taxonomic dataset Global Gracillariidae (www.gracillariidae.net/) will accelerate the detection of invasive species, aiding the decisions required to protect vulnerable habitats.

Acknowledgements

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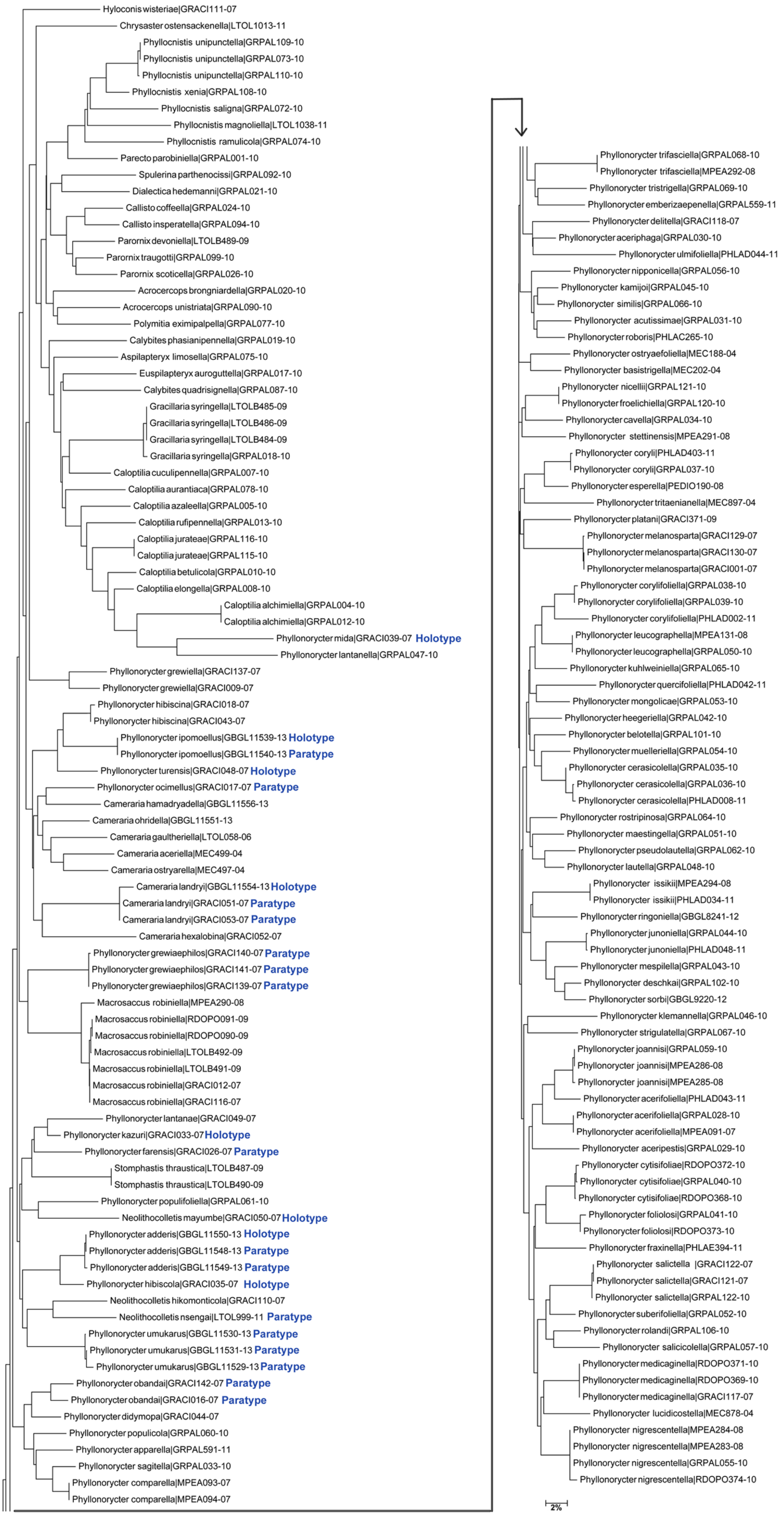

FIGURE 41. A preliminary DNA barcode (COI) library for global gracillariid species.

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