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Two European *Cornus* L. feeding leafmining moths, *Antispila petryi* Martini, 1899, sp. rev. and *A. treitschkiella* (Fischer von Röslerstamm, 1843) (Lepidoptera, Heliozelidae): an unjustified synonymy and overlooked range expansion

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Abstract. *Antispila treitschkiella* (Fischer von Röslerstamm, 1843) and *A. petryi* Martini, 1899, **sp. rev.** were regarded as synonymous since 1978, but are shown to be two clearly separated species with different host-plants, life histories, DNA barcodes and morphology. *Antispila treitschkiella* feeds on *Cornus mas* L., is bivoltine, and has, by following its ornamentally planted host, greatly expanded its range in north-western Europe. In contrast *A. petryi* feeds on the widespread native *C. sanguinea* L., is univoltine, and is one of only two *Antispila* species previously resident in the British Isles, the Netherlands and northern Europe. Consequently, the increase in abundance of *A. treitschkiella* in the Netherlands since the early 1990s and in Great Britain in recent years must be regarded as part of a recent expansion into north-western Europe, whereas the native *A. petryi* is hardly expanding and less abundant. In Britain, detailed surveys of parks and living collections confirmed the monophagy of these two species. A search of British herbarium samples provided no evidence for an earlier date of establishment. Information on recognition of all stages, including DNA barcodes, and distribution is provided, and these two species are compared with the third European *Cornus* L. leafminer, *A. metallella* (Denis & Schiffermüller, 1775).

Introduction

The leafmining family Heliozelidae has about 125 named species and is especially diverse in Australia and the Americas (van Nieukerken et al. 2012b; van Nieukerken and Geertsema 2015; Milla et al. 2017). In a preliminary phylogeny, the earlier suggestion that *Antispila* is an artificial, polyphyletic entity was confirmed: species of *Antispila* s.l. occur in three clades interspersed with

morphologically rather disparate groups (van Nieukerken et al. 2012b; Milla et al. 2017). In the monophyletic genus *Antispila* s. str. (sensu van Nieukerken et al. 2012b), we recognise 24 named and a few unnamed species from North America, Europe, South Africa and Asia. Most species feed either on Cornaceae or Vitaceae, with the current global total of *Cornus*-feeding species being ten, including three species in North America (Lafontaine 1973), three in Japan (Kuroko 1961) and one in China (Liu and Wang 2017).

In Europe, Heliozelidae are poorly represented, with only ten species recorded, including two recent invasives from North America: *Antispila oinophylla* van Nieukerken & Wagner, 2012 and *Coptodisca lucifluella* (Clemens, 1860) (van Nieukerken 2012; van Nieukerken et al. 2012b; Bernardo et al. 2015). Amongst the eight indigenous heliozelids, two *Antispila* species feeding on *Cornus* L. (Cornaceae) are commonly recognised: the larger *A. metallella* (Denis & Schiffermüller, 1775) and the smaller *A. treitschkiella* (Fischer von Röslerstamm, 1843). However, during most of the 20th century, three *Antispila* species feeding on *Cornus* were known. In addition to *A. treitschkiella*, feeding on *Cornus mas* L., *A. petryi* Martini, 1899 had been recognised as a second, slightly smaller species feeding on *C. sanguinea* L. Martini (1899) described morphological differences between *A. treitschkiella* and *A. petryi* in the larva, forewing colour pattern and wing venation. In his description, Martini was advised by the foremost German Microlepidoptera morphologist at that time, O. Hofmann (of Regensburg), who examined wing venation and the male genitalia, and considered slight differences observed in the latter noteworthy ("beachtenswerth"). Hofmann retained original Martini specimens (syntypes) in his collection, which is now in NHMUK, London. Martini's findings were later partly confirmed and extended by Dziurzyński (1952), who also noted intraspecific variation in some of these characters, although he downplayed the diagnostic importance of wing venation. Rather surprisingly, because Fischer von Röslerstamm had already described *A. treitschkiella* from what he called "*Cornus mascula*", the European Cornel, the same author described yet another species, *A. stachjanella* Dziurzyński, 1948 from *C. mas* (Dziurzyński 1948). In Hering's standard keys for the European leafmines (Hering 1957), all four species were recognised. He found the characters to separate *A. petryi* from the other species reliable, but had doubts regarding the separation of *A. stachjanella* from *treitschkiella*. In the UK, the influential lepidopterist Emmet (1976), probably following Hering, correctly identified the smaller dogwood miner as *A. petryi*, and not *A. treitschkiella*. However, these three species (*A. treitschkiella, A. stachjanella* and *A. petryi*) were subsequently regarded as one variable taxon on the basis of unpublished research by Janus Wojtusiak (cited in Razowski 1978). This view was not challenged since and has been followed in almost all major European faunas and checklists (Karsholt and Razowski 1996; Leraut 1997; Gaedike and Heinicke 1999; Bengtsson et al. 2008; Swisslepteam 2010; Karsholt and van Nieukerken 2012; Agassiz et al. 2013; Huemer 2013; Aarvik et al. 2017; Gaedike et al. 2017; Kimber 2017; Lepiforum E.V. 2017). The recent use of the name *A. petryi* for Portuguese specimens (Corley 2015) was based on information from EvN to Martin Corley. Only Emmet (1988) had maintained the use of *A. petryi*, and openly disagreed with the synonymy (Emmet 1981), but more recent British lists nonetheless followed the synonymy. In a local checklist of Lepidoptera, Laštůvka et al. (1993) still used the name "*petryi*", but referred to Razowski's synonymy in a note, and dismissed the name in later checklists (Laštůvka and Liška 2011).

Antispila treitschkiella was reported to expand suddenly in distribution and abundance on planted *C. mas* in parks in the Netherlands (Kuchlein and Frankenhuyzen 1999; van As and Ellis 2004; Huisman et al. 2005). One of the remarkable observations was that the park populations on *C. mas*

were invariably bivoltine, whereas the rare populations on the native *C. sanguinea* had previously been reported as univoltine, like populations in Poland and Great Britain (Dziurzyński 1948, 1952; Emmet 1976; Huisman et al. 2005). A satisfactory explanation for the biological differences within *A. treitschkiella* could not be given prior to our studies. Incidentally, when checking DNA barcodes of a number of Heliozelidae, we noticed that barcodes from European *Antispila* larvae that had been collected on *Cornus* fell into three groups rather than two. After sequencing an adult *A. metallella* from the Netherlands, it was also clear that the sequences published as *A. metallella* from Italian larvae on *C. sanguinea* (used as such in van Nieukerken et al. 2012b), had been misidentified.

While a predecessor of the present manuscript was in preparation, a slightly worn small female moth with a six mm wingspan and a golden median fascia with two other golden forewing spots was caught, on 23.viii.2016, in an MV Robinson moth trap in the Wildlife Garden of the Natural History Museum in South Kensington (UK, London). DCL identified this moth provisionally as an *Antispila* species, a genus not previously reported from the South Kensington area. This determination was confirmed by EvN from a photograph, a finding that struck DCL as peculiar since the *Antispila* species known in Britain generally fly in May–June (*A. metallella*) or June–July (*A. treitschkiella* sensu auctorum). The specimen was too small to correspond to *A. metallella*, nor had it the typical size and markings, thus ruling out this species. Surprisingly, no mines could be found on *C. sanguinea* in the NHM Wildlife Garden, nor on adjacent beds containing *C. alba* L. A few vacated mines were subsequently found on old plants of *C. sanguinea* in nearby Kensington Gardens. This find led later to the discovery of many *C. mas* feeding *Antispila* in London.

Here we analyse material from throughout Europe and show that *A. treitschkiella* as currently used is a composite species: *A. petryi* should be re-instated as a valid, well separated species. In order to recognise the three European *Cornus* feeding species, they are briefly redescribed and diagnostic differences are presented.

Material and methods

Material. Much of the material for this paper was collected or observed by the authors; additional specimens from the following collections are considered.

Abbreviations for depositories

NHMUK	Natural History Museum, London, UK (previously known as BMNH).
RMNH	Naturalis Biodiversity Center, former Leiden Zoology collections, Leiden, Netherlands.
UPI	University of Padova, Dept. of Environmental Agronomy and Crop Science, Italy
ZMA	Naturalis Biodiversity Center, former Zoological Museum of Amsterdam collections, Leiden, Netherlands.
ZMUO	Zoological Museum University of Oulu, Finland.

Details of specimens examined are provided in the Suppl. material 1, in the text we merely list countries and numbers of examined specimens.

Rearing. Collected leaves were kept in polystyrene jars or bags, with some moss and/or paper tissue added, until the larvae had prepared their leaf-epidermis-shield cases in the fourth instar. It was often necessary to remove the cut-out cases manually from the leaves, after which the leaves were taken from the rearing jars and dried as vouchers.

Table 1. Material of Heliozelidae used for DNA barcodes, all in dataset DS-ANTITR, doi: 10.5883/DS-ANTITR.

Identification	BIN	Sample ID	Process ID	Hostplant	GenBank	Institution
Antispila metallella	ACG8679	RMNH.INS.18463	HELA015–13	*Cornus sericea*	MG191951	RMNH
Antispila metallella	ACG8679	RMNH.INS.24371	HELA016–13	*Cornus sericea*	KP697814	RMNH
Antispila oinophylla	AAI4367	BVS03	ANTVI029–11	*Vitis vinifera*	JQ412531	UPI
Antispila oinophylla	AAI4367	RMNH.INS.24038	ANTVI009–11	*Vitis vinifera*	JQ412537	RMNH
Antispila petryi	AAV5055	RMNH.INS.18803	HELA023–13	*Cornus sanguinea*	MG191953	RMNH
Antispila petryi	AAV5055	RMNH.INS.24372	HELA024–13	*Cornus sanguinea*	MG191950	RMNH
Antispila petryi	AAV5055	TER1CS	ANTVI035–11	*Cornus sanguinea*	JQ412517	UPI
Antispila petryi	AAV5055	TER2CS	ANTVI036–11	*Cornus sanguinea*	JQ412518	UPI
Antispila petryi	AAV5055	RMNH.INS.29304	HELA025–13	*Cornus sanguinea*	MG191937	RMNH
Antispila petryi	AAV5055	RMNH.INS.30614	HELA142–16	*Cornus sanguinea*	MG191936	RMNH
Antispila petryi	AAV5055	RMNH.INS.30615	HELA143–16	*Cornus sanguinea*	MG191947	RMNH
Antispila petryi	AAV5055	DL-AP1	HELA150–17	*Cornus sanguinea*	KY613518	NHMUK
Antispila treitschkiella	AAU1917	CLV2597	GRPAL724–11	*Cornus mas*	MG521913	INRA
Antispila treitschkiella	AAU1917	TER3CM	ANTVI037–11	*Cornus mas*	JQ412553	UPI
Antispila treitschkiella	AAU1917	RMNH.INS.11818	ANTVI040–11	*Cornus mas*	JQ412554	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.11856	ANTVI038–11	*Cornus mas*	JQ412555	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.11857	ANTVI039–11	*Cornus mas*	JQ412551	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.11858	ANTVI019–11	*Cornus mas*	JQ412552	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.29300	HELA029–13	*Cornus mas*	MG191955	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.544573.1	LEAFN053–13	*Cornus mas*	MG191949	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.544573.2	LEAFN054–13	*Cornus mas*	MG191954	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.544573.3	LEAFN055–13	*Cornus mas*	MG191952	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.544585	LEAFN079–13	*Cornus mas*	MG191941	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.552214.1	LEAFN389–13	*Cornus mas*	MG191945	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.552214.2	LEAFN390–13	*Cornus mas*	MG191939	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.552214.3	LEAFN391–13	*Cornus mas*	MG191944	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.30611	HELA144–16	*Cornus mas*	MG191942	RMNH
Antispila treitschkiella	AAU1917	RMNH.INS.30613	HELA145–16	*Cornus mas*	MG191948	RMNH
Antispila treitschkiella	AAU1917	DL-AT1	HELA148–17	*Cornus mas*	KY613515	NHMUK
Antispila treitschkiella	AAU1917	DL-AT2	HELA149–17	*Cornus mas*	KY613516	NHMUK
Antispila treitschkiella	AAU1917	DL-AT3	HELA147–17	*Cornus mas*	KY613517	NHMUK
Antispilina ludwigi	AAW5935	RMNH.INS.11852	HELA034–13	*Bistorta officinalis*	MG191940	RMNH
Antispilina ludwigi	AAW5935	RMNH.INS.11855	HELA033–13	*Bistorta officinalis*	MG191943	RMNH
Coptodisca lucifluella	AAV9339	RMNH.INS.24368	HELA047–13	*Juglans regia*	KJ427013	RMNH
Coptodisca lucifluella	AAV9339	RMNH.INS.24462	COPIN003–14	*Juglans regia*	KJ427007	RMNH
Heliozela hammoniella	AAK4551	MM15506	LEFIG642–10		JQ412566	ZMUO
Heliozela hammoniella	AAK4551	MM15507	LEFIG643–10		HM876306	ZMUO
Heliozela resplendella	AAI6721	MM06188	LEFID308–10		HM873105	ZMUO
Heliozela resplendella	AAI6721	RMNH.INS.24359	HELA058–13		MG191938	RMNH
Heliozela sericiella	AAK4533	MM05954	LEFIA1161–10		JQ412568	ZMUO
Heliozela sericiella	AAK4533	MM05955	LEFID174–10		HM872981	ZMUO
Heliozela sp. Lesbos	ACO3181	RMNH.INS.24606	HELA102–14		MG191946	RMNH
Holocacista rivillei	AAU2507	AVS09	ANTVI027–11	*Vitis vinifera*	JQ412569	UPI
Holocacista rivillei	AAU2507	RMNH.INS.24626	HELA107–14	*Vitis vinifera cv Pinot Grigio*	KP697786	RMNH

Morphology. Methods for preparation of the genitalia follow van Nieukerken et al. (2012b). We usually embedded the total genitalia in dorso-ventral position, or removed the phallus. In some cases we unrolled the genitalia, but refrained from spreading the valvae, as this distorts the genitalia too much. For staining male genitalia, we used phenosafranin. Wings were cleaned and descaled in ethanol 70%, stained with phenosafranin and mounted in euparal. Larval pelts remaining after DNA extraction were also stained with phenosafranin and embedded in euparal. Photographs of moths, leafmines, genitalia slides and larval slides in Leiden were taken with a Zeiss AxioCam digital camera attached, respectively, to a Zeiss Stemi SV11 stereo-microscope, a motorized Zeiss SteREO Discovery.V12 or a Zeiss Axioskop H, using Carl Zeiss AxioVision software. Photos by RB were taken with a Canon EOS 5D, Mark II with a Canon MP-E 65mm lens. Photos of complete leaves and leafmines were taken with a Canon EOS 60D on a stand with back lighting, using a light box. Drawings of the genitalia were prepared by JCK with a compound microscope using the camera lucida method (Koster and van Nieukerken 2017). Morphological terminology for adults, including interpretation of wing veins, follows recent Heliozelidae treatments (van Nieukerken and Geertsema 2015).

Measurements of genitalia and forewing details (Table 2) were taken from photographs, using the measurement tools in AxioVision ® software. Measurements of wingspan and forewing length in descriptions were taken with a Zeiss Stemi SV11 stereo microscope, using an eyepiece graticule scale. Measurements in descriptions are given as range with mean ± standard deviation in brackets, followed by sample size (e.g.: 2.7 ± 0.2, 12) when the sample size is 5 or larger.

DNA extraction and sequencing. DNA was extracted using the Qiagen Blood and Tissue kit, either destructively from larvae or adult specimens preserved in 96% or 100% ethanol or non-destructively from the abdomen of voucher specimens, which were then used to prepare genitalic dissections (partly according to protocol in Knölke et al. 2005), or non-destructively from larvae. A 665 bp or a 658 bp fragment of the mitochondrial COI gene was amplified using the Lep primers (Hebert et al. 2004) or the Folmer primers (Folmer et al. 1994), or a combination thereof and sequenced bidirectionally (van Nieukerken et al. 2012a; Doorenweerd et al. 2016).

Data selection and phylogenetic analysis. Since many molecular data on Heliozelidae have recently been published or will soon be published (van Nieukerken et al. 2012b; van Nieukerken and Geertsema 2015; Milla et al. 2017), we restrict molecular data here to European species. Neighbor Joining (NJ) trees based on DNA barcode sequences of all available specimens were prepared with tools provided by the Barcode of Life Data Systems (Ratnasingham and Hebert 2007). Genetic distance calculations were performed both using the Kimura two-parameter (K2P) model and uncorrected P distance, the former being more commonly used but the latter being more biologically appropriate (Srivathsan and Meier 2012). Maximum Likelihood analyses of the aligned COI barcode regions were performed using RAxML v8 (Stamatakis 2014), searching for the best scoring tree and a multiparametric bootstrap analysis with automated halting following the extended majority rule criterion. The bootstrap support values were subsequently plotted onto the best scoring tree.

The sequence data generated and used in this study have been deposited in the public BOLD dataset ("*Antispila treitschkiella and petryi in Europe* " [DS-ANTITR], doi: 10.5883/DS-ANTITR and GenBank (Table 1).

Hostplant nomenclature and recognition. For the nomenclature we follow in principle Govaerts (2017), which also is the source for The Plant List (2013) and the Catalogue of Life (Roskov et al. 2017).

Table 2. Comparison of measurements of forewing and costal spot in *Antispila petryi* and *A. treitschkiella* males and females. Measured on photos, specimens from RMNH collection. Forewing length here does not include fringe, and thus differs from measurements in descriptions. The ratio length–width of the costal spot differs significantly between the species (marked in bold), but still shows an overlap.

	Antispila petryi			***Antispila treitschkiella***		
	Range	**Mean ± sd**	**#**	**Range**	**Mean ± sd**	**#**
Males						
Forewing length	2.19–2.70 mm	2.52 ± 0.19	7	2.54–3.11 mm	2.78 ± 0.19	11
Costal spot width	0.23–0.39 mm	0.33 ± 0.07	7	0.35–0.44 mm	0.37 ± 0.03	11
Costal spot length	0.34–0.56 mm	0.44 ± 0.07	7	0.36–0.45 mm	0.41 ± 0.03	11
Ratio spot l/w	**1.179–1.625**	**1.373 ± 0.154**	7	**0.923–1.222**	**1.092 ± 0.096**	11
Ratio spot w/fw l	0.098–0.148	0.129 ± 0.019	7	0.116–0.157	0.135 ± 0.014	11
Females						
Forewing length	2.16–2.91 mm	2.61 ± 0.23	7	2.52–2.82 mm	2.65 ± 0.10	10
Costal spot width	0.25–0.44 mm	0.35 ± 0.06	7	0.35–0.44 mm	0.40 ± 0.03	10
Costal spot length	0.35–0.46 mm	0.42 ± 0.04	7	0.32–0.43 mm	0.39 ± 0.03	10
Ratio spot l/w	**1.045–1.517**	**1.245 ± 0.165**	7	**0.780–1.171**	**0.974 ± 0.127**	10
Ratio spot w/fw l	0.110–0.167	0.132 ± 0.019	7	0.124–0.169	0.151 ± 0.013	10

In Europe, there are three widespread native species of *Cornus*, two shrubs *C. mas* and *C. sanguinea*, and a herb, *C. suecica* L. In addition *C. alba* L. occurs natively in Russia, North and East of Moscow, through Siberia to Korea and NE China (Sokolov et al. 1986), but is also planted elsewhere and locally naturalised. The North American *C. sericea* L. (some authors use the name *C. stolonifera* Mitch.) is widely planted and becoming an invasive shrub locally in Europe (Bačič et al. 2015; van Valkenburg et al. 2017). Most European floras (e.g. Ball 1968; Jonsell 2010) treat these four species, but some do not separate the closely related *C. sericea* and *C. alba*, which apparently also form hybrids and are sometimes considered subspecies, resulting in the name *C. alba* subsp. *stolonifera* (Michx.) Wang for the North American subspecies (Schulz 2012). However, recent North American treatments do not follow this (Murrell and Poindexter 2016).

For *C. sanguinea* in Europe there usually are two subspecies recognised: *C. sanguinea* subsp. *australis* (C.A.Mey.) Jáv. from SE Europe (but often planted elsewhere!), and the nominal subspecies *sanguinea*. They can be separated by the type of hairs on leaf-underside: normal hairs in *sanguinea*, so-called compass hairs or medifixed hairs (also the common type of hairs in other *Cornus* spp.) in subsp. *australis* (see Suppl. material 2: Fig. S1). Some authors also recognise a *C. sanguinea* subsp. *hungarica* (Karpati) Soo, but according to Verloove (2017) that is a hybrid of the other two subspecies.

In our experience, many records of hostplants of *Antispila* are either incomplete by just mentioning the genus *Cornus*, and quite a few are incorrect or questionable. We encourage collectors and observers to try to identify the host accurately to species level, in order to get a better impression of the host distribution of the *Antispila* species. Identification is usually not difficult when fruits are present, which may be found on the ground later in the season. Identification of single plucked leaves may be difficult, but often not impossible, and it is best to make notes and photographs and take a voucher (herbarium sample) of the plant where possible for future reference. As a host identification aid, we list some of the vegetative characters of the four species, and add the Japanese Cornelian Cherry, *C. officinalis* Torr. ex Dur., since *A. treitschkiella* mines can be found on botanic living collections in Europe seemingly misidentified as that species, or of hybrid origin:

Cornus mas. Stems green, rarely reddish. Leaves 4–10 cm long, greatest width below middle. Veins 3–5 pairs, often with tufts of white hairs in vein axils, most hairs medifixed, straight (Suppl. material 2: Fig. S1c).

Cornus officinalis. Stems green or red. Leaves ovate to elliptic, 4–12 cm long. Veins 4–7 pairs, with dense tufts of rusty red to brown (not white) hairs in vein axils. Otherwise closely resembles *C. mas*. (Suppl. material 2: Fig. S1e)

Cornus sanguinea subsp. *sanguinea*. Stems at least partly red. Leaves 3–10 cm long, largest width in middle. Veins 3–4 pairs, no tufts of hairs, but hairs spread on green underside (becoming red in autumn), only few medifixed hairs (Suppl. material 2: Fig. S1a).

Cornus sanguinea subsp. *australis*. As nominal subspecies, but most hairs are medifixed (Suppl. material 2: Fig. S1b).

Cornus sericea. Stems red at sun side, forming roots when touching ground (stoloniferous). Leaves 6–13 cm long. Veins 5–7 pairs, underside often hairy, pale coloured (glaucous), both sides with medifixed hairs (Suppl. material 2: Fig. S1d).

Cornus alba. Stems always red to purple, rather straight, not forming roots when touching ground. Leaves 4–10 cm. Veins 5–7 pairs, underside often hairy, pale coloured (glaucous), both sides with medifixed hairs.

Living collections study. A few parks and living botanic collections in the UK were studied in detail in 2016–2017 in order to address the reliability of findings regarding monophagy of *A. treitschkiella* and *A. petryi* in particular. Because *A. treitschkiella* was found to be commonly established in the London area as well as in Cambridge, reaching outbreak levels on some trees of *C. mas*, and *Cornus* species were often planted in clusters, this seems a reasonable assay of hostplant repertoire at this time. This survey was conducted in the UK in October and November 2016 within the distribution range so far known for *A. treitschkiella*, guided mostly by garden maps printed by botanical garden staff. The survey was mainly conducted at Royal Botanic Gardens, Kew on 28.ix.2016 and at Royal Horticultural Society, Wisley on 5.x, with a visit to Cambridge Botanical Gardens on 17.ix. Depending on different plantings at these gardens, examination was conducted of *C. mas* and *C. sanguinea* (including several cultivars of both species), and in addition for specimens of *C. sericea*, *C. rugosa* Lam., *C. alba*, *C. amomum* Mill., *C. brachypoda* C.A. May, *C. schindleri* Wangerin, *C. drummondi* C.A. Mey, *C. darvasica* (Pojark.) Pilip., *C. kousa* F. Buerger ex Hance (incl. "Eddie's White Wonder", *C. walteri* Wangerin (=*C. coreana* Wangerin), *C. controversa* Hemsl., *C. bretschneideri* L. Henry, *C. capitata* Wall., *C. x arnoldiana* Rehder (= *C. racemosa* Lam. x *C. obliqua* Raf.), *C. quinquinervis* Franch. (= *C. paucinervis* Hance) and *C. officinalis*. A single specimen of *C. mas* was also examined at Chelsea Physic Garden in x.2016 and Oxford University Parks on 30.vii.2017, as well as a single specimen in Oxford Botanical Garden (*C. mas* var. *variegata*). Additional negative records on *C. mas* from other observers elsewhere in the UK are reported under results.

Herbarium study. In the UK in 2016, herbaria in Kew and Wisley and at NHMUK (the AMC herbarium of native and naturalised species) were examined and *Cornus* was surveyed, including all available *C. mas*, with the hope of determining origins of invasion of *A. treitschkiella* in the UK and within Europe. It was hoped that this approach would prove informative as it was for *Cameraria ohridella* Deschka & Dimič (Lees et al. 2011). Wisley was potentially useful because of its focus on cultivated specimens. Specimens of *C. mas* in flower but not in leaf were ignored.

Parasitoids. We have tried to trace all literature records of parasitoids of these species. As basis we used the databases for Chalcidoidea (Noyes 2003) and Ichneumonoidea (Yu et al. 2011b; Yu

2012) and subsequently traced original references to check for the identity of the host species. We assumed that hosts identified as "*Antispila treitschkiella*" were *A. petryi* when reared from *C. sanguinea*, and *A. treitschkiella* when reared from *C. mas*. Hosts identified as *Antispila* sp. could only be identified to species when the host was given as *C. mas,* because *Antispila* on *C. sanguinea* can refer to both *A. petryi* and *A. metallella*. In addition a few records of specimens reared by DCL are added (Table 3), which were identified by N. Dale-Skey, J. Noyes and C. Hansson.

Results

Recognition of the species

Adult morphology. Martini (1899) wrote that the species have only small external differences ("*Diese neue der* Treitschkiella *äußerst ähnliche Art zeigt in Färbung und Zeichnung nur geringe Unterschiede*"), but he found differences in the distal costal spot: longer than wide and almost triangular in *A. petryi* and almost square and shorter in *A. treitschkiella*. Dziurzyński (1952) confirmed this character, but by his many measurements he also showed that the character is not fully reliable. We agree that in many cases this character works as a first indication, particularly in females, as the ratio length/width is significantly different in our measurements (see Table 2), but there is some overlap.

Dziurzyński (1952) also mentioned that the fascia is straighter in *A. treitschkiella*, never interrupted, while it is often interrupted in *A. petryi* and more curved, with the concave margin at the base. Checking the photographs of many specimens we cannot support this character, both species seem to be variable in this respect.

Venation. Martini (1899) was convinced about the difference in the venation, particularly the position of the M stem in the discoidal cell. However, Dziurzyński (1952) showed the variability of this character. We only prepared one pair of wings of each species (Figs 9–10) and these wings indeed support Martini´s difference, but we cannot attest to its reliability. The usefulness of venational characters at species level has been challenged in other moths such as Elachistidae (Albrecht and Kaila 1997), whereas at generic level of Heliozelidae, the larger differences in venation appear to be phylogenetically informative (van Nieukerken et al. 2012b; van Nieukerken and Geertsema 2015).

Genitalia. Martini (1899) mentioned that genitalia were studied, in a time when almost nobody did so with microlepidoptera, but he did not provide details or differences. In two weighty papers, Dziurzyński (1948, 1952) described the genitalia of the species in great detail, with many illustrations. These provided sufficient characters for separation of the species, including the characteristic "horseshoe-shaped" anellus in *A. petryi* (Fig. 14) that is not developed in *A. treitschkiella* (Fig. 19). Other differences are found in the uncus, the shape and number of spines on the phallotheca and the shape and length of the sublateral process of the transtilla. These differences were considered to fall within the intraspecific variation as considered by Wojtusiak (In: Razowski 1978), but we refute that here: the characters are constant and identify at least the males easily.

In the female genitalia, Dziurzyński (1952) could not find differences, but we see a small difference in the length of the apophyses, it is possible that the tip of the oviscapt shows a tiny difference, being more indented in *A. treitschkiella* (Figs 22, 25, 31, 32), and it seems that the tip of segment 8 is more bilobed in *A. petryi* (Figs 23, 26), but the shown differences may be partly due to the preparation process. We only examined very few slides of female genitalia.

Larval characters. In the original description of *A. petryi*, Martini (1899) suggested that it can be separated from *A. treitschkiella* by the number of black (melanised) spots on the dorsal side of thorax

Table 3. Parasitoids recorded from *Cornus* feeding *Antispila* species. Where possible, primary sources were checked, indicated by the catalogue databases (Noyes 2003; Yu et al. 2011a; Yu 2012).

Taxa	Locality	Host-plant	Original identification	Reference
Antispila petryi				
Eulophidae				
Pnigalio pectinicornis (Linnaeus, 1758)	UK	*C. sanguinea*	*A. treitschkiella*	Askew and Shaw 1974
Pnigalio cf. *soemius* (Walker, 1839)	UK, Surrey, Priest Hill,	*C. sanguinea*	*A. petryi*	DCL reared
Antispila treitschkiella				
Braconidae				
Colastes flavitarsis (Thomson, 1892)	HU: Pécs, 17.xi.1971, 27.x.1974	*C. mas*	*A. treitschkiella*	Papp 1975; Papp 2008
Eulophidae				
Chrysocharis budensis (Erdős, 1954)	HU: Budapest, Budakeszi, leg. Szőcs	[interpreted as *C. mas*, since Szőcs recognised both *Antispila* spp.]	*A. treitschkeella*	Erdős 1954; 1956; Szőcs 1959
Chrysocharis budensis (Erdős, 1954)	PL: Woj. Poznan	Not given	*A. stachjanella*	Hansson 1985
Cirrospilus diallus Walker, 1838	CZ: Koda near Beroun, 3.1954	*C. mas*	*A.* sp	Bouček 1959
Cirrospilus viticola (Rondani, 1877) [as *subviolaceus* Thomson]	CZ: Koda near Beroun, 3.1954	*C. mas*	"a leafminer" [interpreted as *treitschkiella*]	Bouček 1959
Cirrospilus vittatus Walker, 1838	CZ	*C. mas*	*A.* sp	Bouček 1959
Derostenus gemmeus (Westwood, 1833)	HU: Normafa, 14.vii.1954	[interpreted as *C mas*, since Szőcs recognised both *Antispila* spp.]	*A. treitschkeella*	Erdős 1956
Minotetrastichus frontalis (Nees, 1834)	UK, SE London, Kelsey Park, x.2016	*C. mas*	*A. treitschkiella*	DCL reared
Minotetrastichus frontalis (Nees, 1834)	UK: London, Victoria Embankment, 30.vi.2017	*C. mas*	*A. treitschkiella*	DCL reared
Antispila metallella				
Eulophidae				
Chrysocharis budensis (Erdős, 1954)	NL: Simpelveld [as Simpelweld]	Not given	*A. pfeifferella*	Hansson 1985
Chrysocharis budensis (Erdős, 1954)	PL: Torún	*C. sanguinea*	*A. pfeifferella*	Vidal and Buszko 1990
Host unidentifiable *Antispila*				
Colastes braconius Haliday, 1833	UK	*Swida sanguinea*	*A.* sp.	Shaw 1983
Colastes braconius Haliday, 1833		Not given	*A. treitschkiella*	Hedwig 1955; Belokobylskij and Tobias 1986
Colastes flavitarsis (Thomson, 1892)	HU: Pécs, Üszög,1.x.1973	*C. mas*	*A. pfeifferella* [unlikely]	Papp 2008 1
Chrysocharis budensis (Erdős, 1954)	DE: München	Not given	*A. treitschkiella*	Hansson 1985
Pnigalio cristatus Ratzeburg, 1848	[primary source not seen]	Not given		Noyes 2003
Zagrammosoma variegatum Masi, 1907	[primary source not seen]	Not given		Noyes 2003

and abdomen: nine in *A. petryi* and eight in *A. treitschkiella*, missing the spot on the mesothorax. However, Dziurzyński (1952) refuted this character as the number of these dots and their presence on the mesothorax are variable. We found this differential character to be correct; it can be used in many cases (Figs 39, 41). However, the amount of melanisation indeed varies, leading to a lower number of observed dots in some cases even though they are present. In microscopic preparations these differences are always apparent and these spots stain when phenosafranin is used (Figs 33–37).

Dziurzyński (1952) described another larval character on the dorsal side of the 8th segment: a belt of dark "warts", termed by him the "cingulum macropapillare". The difference between the species is that *A. petryi* has 4–10 warts in a single row or two rows, whereas *A. treitschkiella* (as *stachjanella*) has many more small wartlets in (at least) three rows. We here confirm the usefulness of this character (Figs 35, 38, 40, 42). This structure has been described in more detail from the related North American *A. nysaefoliella* Clemens, 1860 as "bumps", that were shown to generate essentially substrate-born vibrations that can even be heard by the human ear (Low 2008, 2012). This alleged stridulatory behaviour whose function was hypothesised by Low to deter parasitoids has not been studied in European species, but seems very likely also to occur here.

Mines. The shape of the mines is variable, and both earlier authors were unable to point out clear differences, other than the hostplant species. The size of the cut-out is on average slightly smaller in *A. petryi* than *treitschkiella*, but without diagnostic value (Figs 43–48, 55, 56).

Updated checklist of European Heliozelidae

Heliozela Herrich-Schäffer, 1853
1. *hammoniella* Sorhagen, 1885
2. *resplendella* (Stainton, 1851)
3. *lithargyrellum* (Zeller, 1850)
4. *sericiella* (Haworth, 1828) [type species]

Antispila Hübner, 1825
5. *metallella* (Denis & Schiffermüller, 1775) [type species]
6. *treitschkiella* (Fischer von Röslerstamm, 1843)
7. *petryi* Martini, 1899

Provisionally in *Antispila*, but not belonging there
8. *oinophylla* van Nieukerken & Wagner, 2012*
   ampelopsifoliella auctt. [misidentification]

Antispilina Hering, 1941
9. *ludwigi* Hering, 1941 [type species]

Holocacista Walsingham & Durrant, 1909
10. *rivillei* (Stainton, 1855) [type species]

Coptodisca Walsingham, 1895
11. *lucifluella* (Clemens, 1860)*
* species introduced from North America

Key to adults of European Heliozelidae

1 Forewing shiny grey to brown, with only one or two spots along dorsum..... .....***Heliozela*** [not further treated here]

– Forewing with pale spots at both costal and dorsal margin, often a fascia present as well..... **2**

2 Forewing basally silvery white, distally dorsal area black and costal area yellow, with two conspicuous triangular silvery spots. Very small, wingspan 3.5–4.7 mm, introduced on Juglandaceae: walnut species (*Juglans* L.) and pecan (*Carya illinoinensis* (Wangenh.) K.Koch) in Italy (Bernardo et al. 2015).....***Coptodisca lucifluella***

– Forewing more or less uniform brown or fuscous, with a pale fascia, sometimes broken, at 1/3 and two opposite spots at 2/3 .....**3**

3 Forewing with an additional white silvery spot in apex, not touching the wing margins. Head silvery white. Wingspan 5.5–6.2 mm. Introduced on Vitaceae: grapevine (*Vitis vinifera* L.) and Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch.) in Italy (van Nieukerken et al. 2012b).....***Antispila oinophylla***

– Forewing without an additional apical spot, head not white, various tinges of grey, brown or leaden. Native species.....**4**

4 Small species, wingspan 4.0–4.7 mm. Venation reduced, no cell in forewing. Antenna with 15–20 segments. Leafminers on *Vitis* or *Bistorta* Scop. ***5***

– Larger species, wingspan at least 5.0 mm, usually more. Venation complete with discoidal cell (Figs 9, 10). Antenna with 19 segments or more. Leafminers on *Cornus* **6**

5 Forewing fascia usually divided into two spots, spots relatively large, antenna with 15 segments. Male foretibia with small epiphysis. Mediterranean species, mines on *Vitis* (van Nieukerken and Geertsema 2015)..... ***Holocacista rivillei***

– Forewing fascia not divided in two spots, spots small, antenna with 20 segments. Male foretibia without epiphysis. Rare Central European species, mines on *Bistorta officinalis* Delarbre (Polygonaceae) (Hering 1941).....***Antispilina ludwigi***

6 Smaller species, wingspan 4.8–7.0 mm; forewing and thorax dark fuscous to black, head greyish; male forewing underside with bunch of yellow androconial scales (Figs 7, 8). Antenna with ca 19–22 segments **7**

– Larger species, wingspan 6.8–8.5 mm, usually larger than 7.0 mm; wings and thorax coppery, head golden; male forewing underside without androconial scales. Antenna with ca 25 segments..... ***Antispila metallella***

7 Forewing costal spot usually triangular, ratio length/width: 1.18–1.63 in males, 1.05–1.52 in females; slightly smaller, wingspan 4.8–6.1 mm. Best distinguished by male genitalia, larva and hostplant: *C. sanguinea*.....***A. petryi***

– Forewing costal spot usually trapezoid to almost square, ratio length/width: 0.93–1.22 in males, 0.78–1.17 in females; slightly larger, wingspan 5.7–7.0 mm. Best distinguished by male genitalia, larva and hostplant: *C. mas* .....***A. treitschkiella***

Key to male genitalia of European *Antispila* feeding on *Cornus*

1 Uncus medially blunt, central lobe reduced or absent. Valva at inner margin with spinelike process (Figs 57, 59).....***A. metallella***

– Uncus with prominent central lobe. Valva without spinelike process **2**

2 Horseshoe-shaped anellus present between the valvae (Fig. 11). Uncus laterally of medial lobe hardly indented (Figs 14, 27). Lateral process of transtilla distally clearly widened (Fig. 14).....***A. petryi***

– No anellus between valvae visible (Fig. 16). Uncus laterally of medial lobe distinctly indented (Figs 19, 28). Lateral process of transtilla distally not or hardly widened (Fig. 16).....***A. treitschkiella***

Key to the leafmines and larvae of European *Antispila*

1 Near start of mine 2–7 small brown scars made by ovipositing female ("test punctures") (Figs 62, 63, 65). Larva without dark sclerotised plates (Fig. 64). Vacated mine with large cut-out of ca. 5.5–7 mm length. Mines on *C. sanguinea, C. alba* or *C. sericea*.....***A. metallella***

– No scars near start of mine. Larva with row of dorsal black sclerotised plates (note: larvae usually feed with ventral side upwards) (Figs 39, 41). Vacated mine with small cut-out of ca. 4–5 mm length. Mines on *C. sanguinea* or *C. mas* **2**

2 Mine on *C. mas*. Full grown active larva whitish, with maximum of 8 black dorsal plates (Fig. 41), often less, no plate on mesothorax. Abdominal segment 8 dorsally at anal end with 2–3 rows of more than 20 black warts of different sizes (Fig. 42) ...***A. treitschkiella***

– Mine on *C. sanguinea*. Full grown active larva greyish, with maximum of 9 black dorsal plates, often less visible (plates not always melanised), plate on mesothorax present (Fig. 39). Abdominal segment 8 dorsally at anal end with one row of ca 5 large black warts (Fig. 40) .....***A. petryi***

Taxonomic descriptions

Antispila petryi Martini sp. rev.

Figs 1, 2, 7, 10–15, 21–23, 27, 29, 31, 33–35, 39, 40, 43–45, 49–52, 55

Antispila petryi Martini, 1899: 398. Syntypes: **Germany**: 12–14 adults, Thüringen, Sachsenburg, caterpillars 18.viii.–8.ix.1895, *Cornus sanguinea*, emerged 6–24.vi.1896, 1898, Martini; ditto, caterpillars mid ix.–3.x.1897, no emergence date given (at least seven syntypes in NHMUK via Hofmann collection, examined by DCL).

Antispila petryi; Spuler and Meess 1910: 471; Martini 1917: 158 (records Thüringen); Hering 1932: 18 (key); Toll 1947: 31 (Poland); Dziurzyński 1952: 1 (monograph , Poland); Bentinck 1951: 331 (corrected id Dutch record); Hering 1957: 325 (leafmine); Gozmány 1965: 47 (footnote, key, Hungary); Hering 1968: 120 (letter to Klimesch of 1952); Szőcs 1973: 452 (Hungary); Maček 1974a: 56 (Slovenia); Maček 1974b: 94 (Slovenia); Emmet 1976: 306 (description, England); Lempke 1976: 14 (Netherlands, checklist); Szőcs 1977: 121 (leafmine key); Kuznetsov 1978: 73 (keys); Szőcs 1979: 46 (Hungary: Börzsöny); Szőcs 1981: 210 (Hungary: Budapest); Szabóky 1982: 8 (Hungary: Bakony); Emmet 1988: 38 (biology); Laštůvka et al. 1993: 36 (Czech Republic: Moravia); Corley et al. 2015: 60 (Portugal, checklist); Corley et al. 2016: 619 (Portugal).

Elachista treitschkeella [Misidentification, Unjustified emendation]; Stainton 1851: 9 (England).

Elachista treitschkiella [Misidentification]; Stainton 1854: 250 (England); Fologne 1860: 109 (Belgium, probably *petryi*).

Antispila treitschkiella [Misidentification]; Healy 1864: 126 (life history); Stainton et al. 1870: 318 (England); Wocke 1874: 88 (Schlesien, now Poland); Heinemann and Wocke [1876]: 515 (description, Germany); Glitz 1877: 40 (Germany: Hannover); Sand 1879: 192 (France); Frey 1880: 405 (Switzerland); Rössler 1881:323 (Germany: Hessen); Meyrick 1895: 684 (England); Buhr 1935: 158 (Germany: Mecklenburg); Doets 1949: 416 (the Netherlands); Lhomme 1963: 1157 (France, partim); Wojtusiak 1976: 12 (partim, key); Razowski 1978: 96 (Poland, partim, *petryi* synonymised); Steuer 1984: 102 (Germany: Thüringen); Klimesch 1990: 77 (partim, Austria); Svensson 2007: 44 (Sweden); Bengtsson et al. 2008: 288 (key, description, Sweden); Jürivete 2012: 2 (Estonia).

Material examined. Total 23♂, 27♀: France (leafmines), Germany (2♂, 1♀, leafmines), Greece (1♂, larvae, leafmines), The Netherlands (14♂, 12♀, larvae, leafmines), Switzerland (5♂, 14♀, larvae, leafmines), United Kingdom (1♂, larvae). Details in Suppl. material 1.

Differential diagnosis. *Antispila petryi* and *A. treitschkiella* differ from *A. metallella* by their smaller size (wingspan 4.8–7.0 mm against 6.8–8.5 mm) and in male by presence of a tuft of yellow androconial scales on forewing underside. *A. petryi* differs from *A. treitschkiella* by the usually smaller and more triangular costal spot at 2/3, and *A. petryi* is on average smaller than *A. treitschkiella*, but there is some overlap (wingspan 4.8–6.1 against 5.7–7.0 mm). In the male genitalia, the indentations in the uncus are shallower in *A. petryi* than in *A. treitschkiella*, the lateral process of the transtilla is straight and widened and the shorter phallus bears two types of spines externally. The horseshoe-shaped sclerotized anellus is characteristic for *A. petryi*, an anellus is undeveloped in *A. treitschkiella*. Separation by female genitalia not reliable.

Larva easily separated from *A. metallella* by presence of a row of dorsal black dots, in contrast to *A. treitschkiella*, a total of nine dots, including the mesothorax, but some of these may be poorly melanised, making this character not always useable; abdominal segment 8 with a single row of five black warts. Leafmines in principle not separable without larva or when hostplant species is not known.

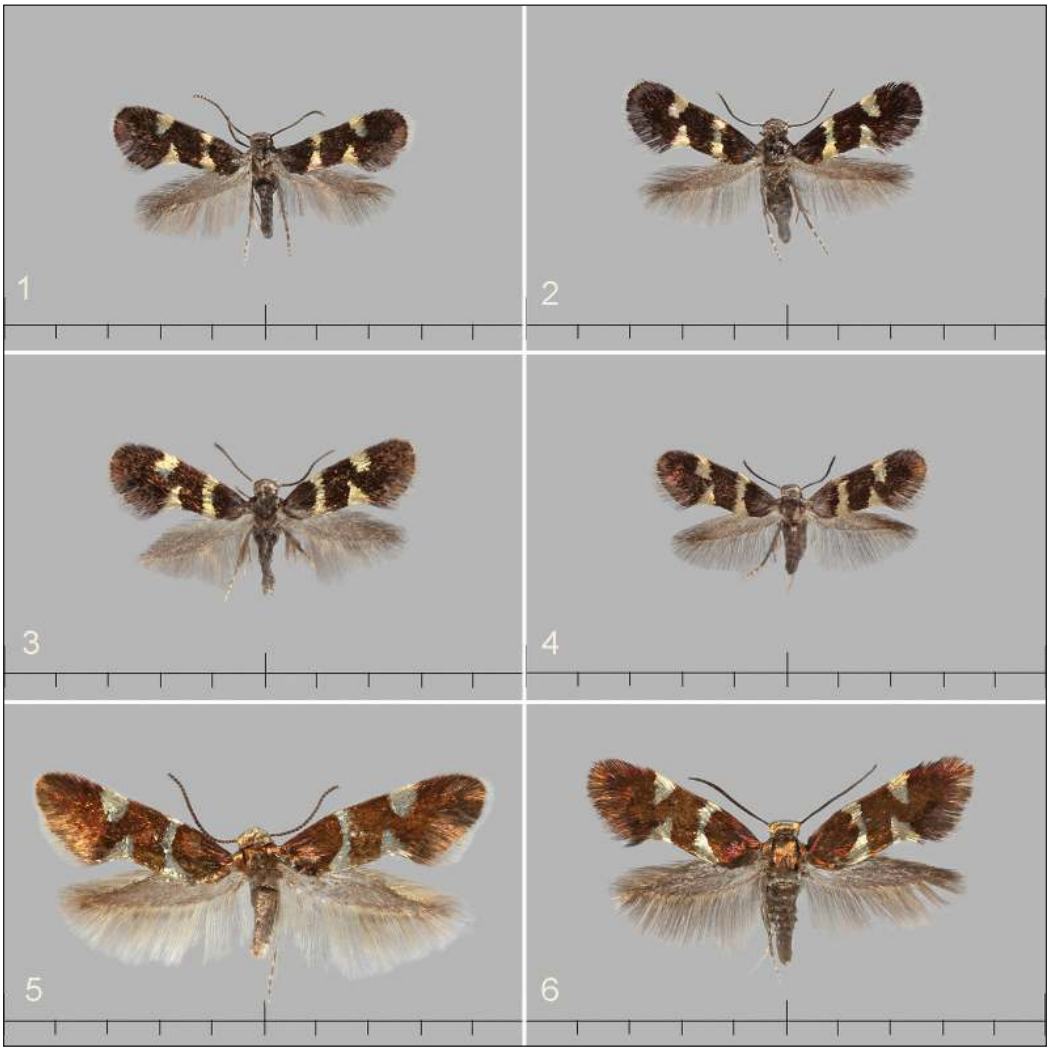
Description. Male (Figs 1, 49, 50). Head, face and vertex covered with appressed lead-grey scales. Antenna fuscous, clearly ringed, particularly near tip. Thorax dark fuscous, concolorous with forewings. Legs grey, tarsi ringed white at tip, spurs and undersides paler. Forewing dark fuscous to almost black with silver-golden patterning; an outwardly oblique fascia at ca 1/3, narrowing in middle, sometimes broken, dorsal edge slightly wider than costal; dorsal spot slightly beyond middle of posterior margin, triangular, reaching hardly to middle of wing, a similar triangular costal spot at 2/3, slightly longer than wide; fringe line distinct. Terminal fringe paler. Hindwing rather dark grey. Underside of wings fuscous, close to base a yellow to orange tuft of androconial scales. Abdomen lead-coloured, including vestiture on external genitalia.

Female (Fig. 2). Similar to male, androconial scales absent. Abdomen with slightly protruding ovipositor.

Measurements, male: forewing length 2.3–2.9 mm (2.7 ± 0.2, 12), wingspan 4.8–6.1 mm, 19–20 antennal segments (n=8); female: forewing length 2.3–3.0 mm (2.7 ± 0.2, 7), wingspan 4.8–6.1 mm, 19–20 antennal segments (n=3). For costal spot see Table 2.

Male genitalia (Figs 11–15, 27, 29). Uncus with two shallow setose lateral lobes and a more prominent central lobe, however, not reaching beyond a line between the lateral lobes; shallowly indented between lobes. Vinculum 335–350 µm long, anteriorly almost truncate. Valva length 230–255 µm, basally broad, more or less triangular, narrowing towards digitate tip; pecten on pedicel, with 15–16 comb teeth (Fig. 15); anellus a strongly sclerotised horseshoe-shaped band between valvae (this structure was termed juxta by Kuroko 1961 in *Cornus* feeding species); transtilla plate-like, deeply indented anteriorly, sublateral processes distinct and widened at tips. Juxta anteriorly spade-shaped, about half as long as phallus. Phallus 375–385 µm long, phallotheca with groups of many scaly spines an less larger pointed spines; clearly two types of spines.

Female genitalia (Figs 21–23, 31). Anterior apophyses 950–985 µm, posterior apophyses 1025–1085 µm (n=4). Oviscapt with two large lateral cusps and two smaller ones more distally, tip shallowly indented. Sternum 8 indented in middle. Internal genitalia not examined in detail, no sclerotisations visible.



Figures 1–6. *Antispila*, adult habitus, all from Switzerland**. 1** *A. petryi*, male, Mont Vully, Sugiez, e.l. 08.v.2016 **2** *A. petryi*, female, Mont Vully, Sugiez, e.l. 30.iv.2016 **3** *A. treitschkiella*, male, Gampelen, e.l. 17.iv.2016 **4** *A. treitschkiella*, female, Le Landeron, e.l. 17.iv.2016 **5** *A. metallella*, male, Muntelier, 30.iv.1990 **6** *A. metallella*, female, Cudrefin, e.l. 18.iii.2015. All to same scale, scale line 10 mm. Photos: Rudolf Bryner.

Larva (33–35, 39, 40). Pale grey translucent, head capsule brown, prothorax with large black tergum and sternum. In instar IV, the final feeding instar, mesothorax, metathorax and abdominal segments 1 to 7 dorsally each with a central black spot, with fuzzy outline, more or less rhomboid, spots becoming smaller from segment 5 to 7; ventrally up to 5 spots on metathorax and segments 1 to 4. Abdominal segment 8 dorsally with a swollen hump, at the anal end lined with a single row of 5 black warts. Anal segment black. More details on earlier instars are given by Dziurzyński (1952). The fifth instar is a non-feeding prepupal instar that is reached after one moult inside the case.

Figures 7–8. *Antispila*, male forewing underside with tuft of yellow androconial scales, Switzerland. **7** *A. petryi*, Mont Vully, Sugiez, e.l. 14.v.2016 **8** *A. treitschkiella*, Le Landeron, e.l. 22.iii.2007. Photos Rudolf Bryner.

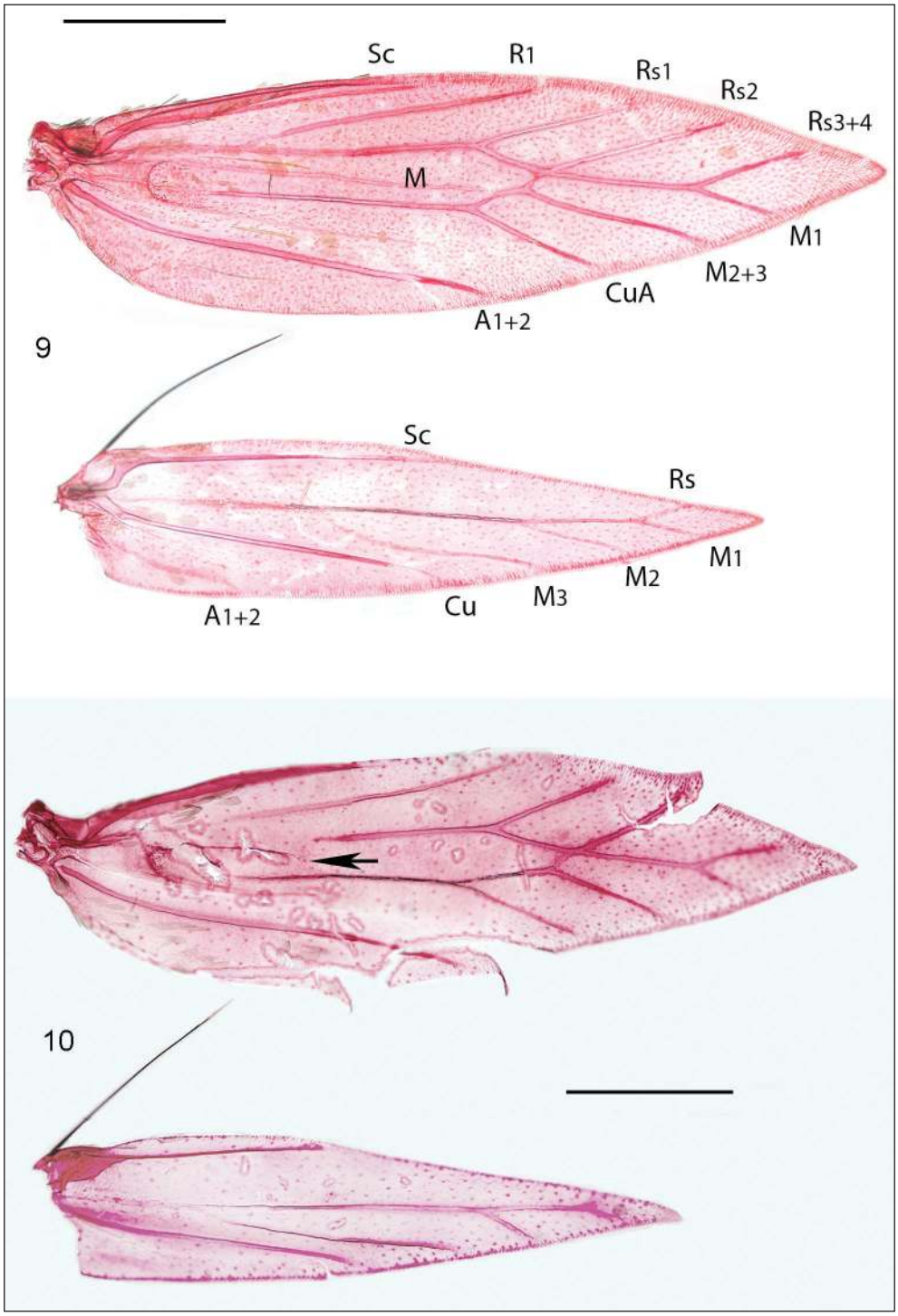
Biology. ***Hostplants.*** *Cornus sanguinea*, both subsp. *australis* and *sanguinea*, and incidentally on cultivated *C. alba* (Wocke cited in Martini 1899 and see under Living collections). Martini (1899) reported also *C. mas* as a rare host, but did that on venational characters of moths alone, which are unreliable; we thus consider these records for now as unlikely to be correct.

Leafmines (Figs 43–45, 55). The egg is inserted on the leaf underside, often on leaf margin (65% of 54 mines), or less frequent away from the margin; the oviposition site is recognisable as a reddish dot (the vesicula incubatoria of Dziurzyński). The mine starts with a narrow gallery, almost straight along the leaf margin when the egg was laid there, or much contorted in other cases; it is usually filled with frass, but the width of the frass line is variable. Later mine expanding into a large full depth blotch, in some cases completely absorbing the earlier gallery; frass often in a clump near the origin of the mine and also scattered around. The larva prepares an oval cut-out of ca 4–5 mm length, usually at the other edge of the mine, lined with silk, and drops to the ground in this case. The larvae feed with ventral side up, but they start turning around in the blotch when preparing the cut-out. The gallery part of the mine is prepared during the first two instars, the blotch during instar 3 and 4.

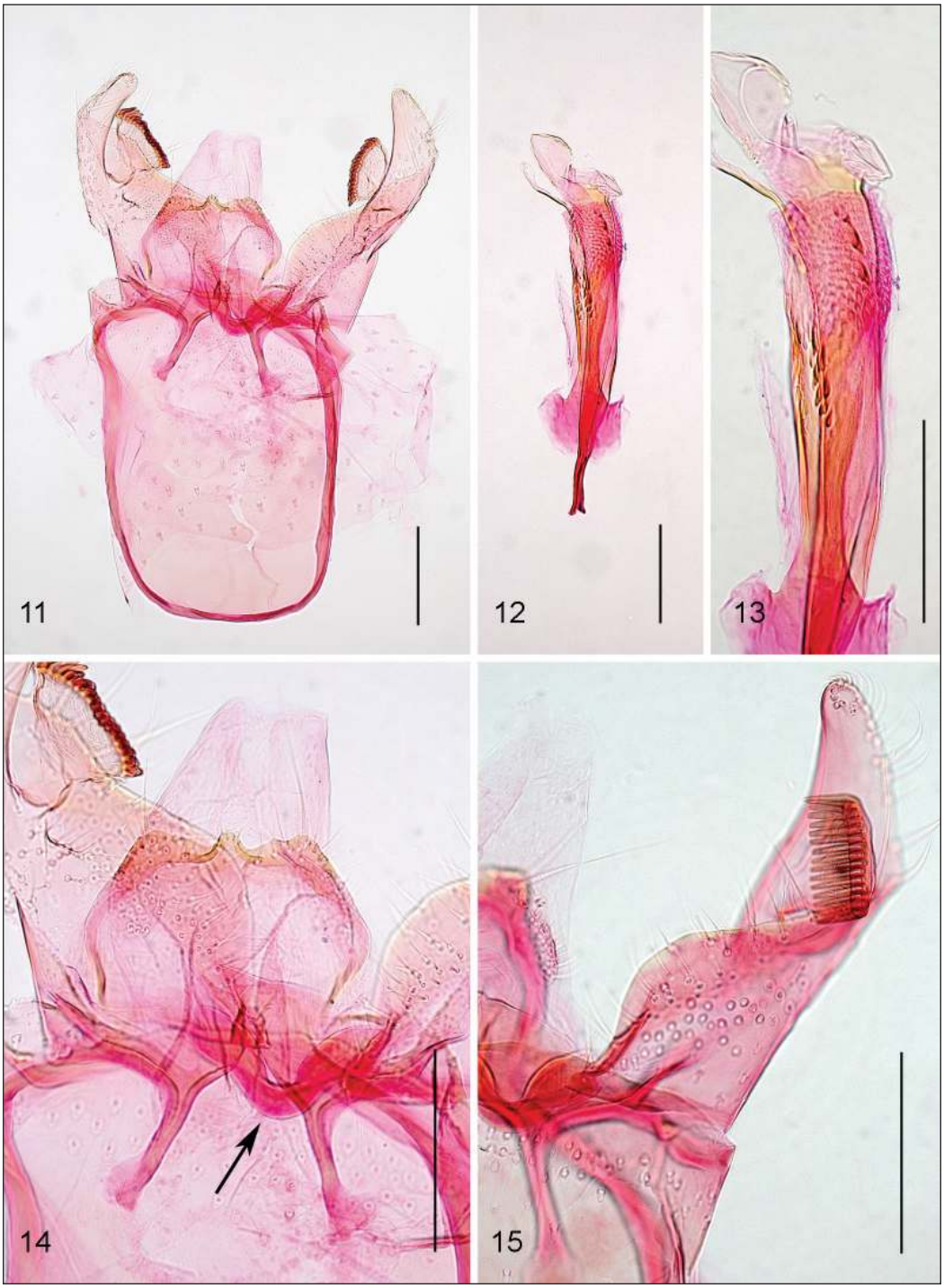
Life history. Univoltine. Larvae usually from late August until October, in Greece still active in early November, few records from early August. Adults emerge in captivity from April to June, the few specimens collected as adults being found from June to early August. We assume they are mostly active during the day, rarely collected at light, but found in malaise traps.

Distribution. Widespread in Europe, local in southern England, in the Netherlands and Belgium local in hilly limestone areas in the South and East. Throughout central and southern Europe, but not known in detail, due to confusion with *A. treitschkiella*, but correct records (on the basis of examined adults, larvae or hostplant data) exist from Germany, Poland, Hungary, Czech Republic (Z. Laštůvka, pers. comm.), Austria (as *A. treitschkiella*: Klimesch 1990), Switzerland, France, Portugal (Corley 2015), Italy, Slovenia and Greece. Recently recorded (as *A. treitschkiella*) from the island Öland in Sweden since 2006 (Svensson 2007) and the island Saaremaa in Estonia since 2010 (Jürivete 2012).

The natural distribution of *C. sanguinea* comprises most of Europe, in the north including the whole of the British Isles, southern coastal areas of Norway and Sweden (south of Stockholm), a northern limit in Estonia and in Russia below a line from the Latvian/Estonian border to Moscow,



Figures 9–10. *Antispila*, male venation. **9** *A. treitschkiella* with veins indicated, slide EvN4257. **10** *A. petryi*, slide EvN5012, the arrow indicates the curved M. Scales 0.5 mm. Photos Erik van Nieukerken.



Figures 11–15. *Antispila petryi*, male genitalia, slides JCK8056, JCK8055 (15 only). Scales 100 µm (**11–12**), 50 µm (**13–15**). Arrow indicates anellus. Photos Erik van Nieukerken.

in southern Europe including northern parts of the Iberian peninsula, all of Italy, Corsica, Sardinia, and all of the Balkans except the Greek islands; local in northern Turkey, and widespread in the Caucasus region, reaching Iran (Popescu et al. 2016).

Remarks. This species was described from an unspecified number of specimens reared by Martini from mines on *C. sanguinea*, collected as caterpillars in 1895 and 1897 in Sachsenburg (not far from Sömmerda) and with adults reared in 1896 and 1898. Martini also mentioned the species from Regensburg, Bavaria, and Breslau (now Wrocław, Poland), but he probably did not study these himself, and relied on information received from respectively Hofmann and Wocke. Issue no 10–12 (Heft IV), pages 333–429 of volume 59 of the Stettiner Entomologische Zeitschrift was published in June 1899 (see page 429: http://biodiversitylibrary.org/page/8946769). This would have allowed Martini to include the reared adults from 1898, although he did not give emergence dates for these.

Martini's study is very thorough, comparing *A. petryi* with *A. treitschkiella* and *A. metallella* ("*pfeifferella*"), including the biology and mines. Martini's collection of Palaearctic Microlepidoptera was presented to the primary school ("Volksschule") in Sömmerda, his place of residence in the German federal state of Thüringen, and is no longer traceable (Horn et al. 1990: 253). There is a series of seven potential syntypes of *A. petryi* in the Hofmann collection in London, cited in Suppl. material 1, from which a lectotype could be selected, if found necessary. For now we consider the identity of *A. petryi* sufficiently settled and refrain from lectotypification.

Wojtusiak (1976), in his keys to Polish species, lumped the *C. mas* and *C. sanguinea* feeders under *A. treitschkiella*, but he did not propose a formal synonymy, nor did he mention the names *A. petryi* or *A. stachjanella* at all. The synonymy of *A. petryi* with *A. treitschkiella* was formalized by Razowski (1978). The translation of his argumentation in Polish [the paragraph on page 97 starting with: "Uwagi. Gatunek ten ….."] for this synonymy reads roughly (edited from Google translation, kindly checked by Lukasz Przybyłowicz):

Note. This species was known under the three names listed under the synonymy [viz. *treitschkiella*, *petryi and stachjanella*]. *Dziurzyński (1948, 1952) gave some differences between* A. treitschkiella *(F. R.) and* A. petryi *Mart. and described in addition a new species. The extensive material examined by Dr. J. Wojtusiak allowed to conclude that these differences are unstable and fall within the limits of intraspecific variation.*

The male genitalia figured by Wojtusiak and reproduced by Razowski resemble more *A. petryi* than *A. treitschkiella*, but some important characters are not illustrated (transtilla, anellus). Also in the drawings in Bengtsson et al. (2008) the anellus is not figured.

Antispila treitschkiella (Fischer von Röslerstamm) Herrich-Schäffer

Figs 3, 4, 8, 9, 16–20, 24–26, 28, 30, 32, 36–38, 41, 42, 46–48, 53, 54, 56

Elachista treitschkiella Fischer von Röslerstamm, 1843 [March]: 297, pl. 100: 4. Syntypes, number and sex unspecified: **Austria**: Wien [Vienna], v–vi, leg. Mann, flying around *Cornus mascula*. One potential syntype in NHMUK without date or hostplant information [examined by DCL; not dissected].

Oecophora treitschkiella Duponchel, [1843, 4 May]: 319, pl. 77:1. Syntypes, [**Austria**: Vienna, leg. Mann], received from Pareyss (independent new description) (Paris).

Antispila stachjanella; Dziurzyński 1948: 3. Syntypes: unspecified number, both sexes: **Poland**, Kraków, 1944–1946, 1948, ex mines in *Cornus mas*; NHMUK [syntypes examined by DCL and by L. Przybyłowicz] Synonymised by Razowski 1978: 96.

Antispila treitschkiella; Herrich-Schäffer 1855: 315 (recombination, Vienna); Rebel 1911: 420 (Romania); Toll 1938: 211 (Podolia, now Ukraine); Jäckh 1942: 239 (Germany, Loreley); Hering 1957: 325 (leafmine keys); Klimesch 1961: 725 (Alps); Lhomme 1963: 1157 (France, partim); Hering 1968: 120 (letter to Klimesch of 1952); Kuznetsov 1978: 73 (keys); Razowski 1978: 96 (Poland, partim); Klimesch 1990: 77 (Austria, partim); Buszko and Beshkov 2004: 726 (Bulgaria); Patočka and Kulfan 2009: 43, Fig. 52 (Slovakia, ecology); Péré et al. 2010: 1014 (abundance, Bulgaria).

Antispila treitschkeella [unjustified emendation]; [Stainton 1851: 9 (misidentification for *petryi*)]; Spuler and Meess 1910: 471; Hering 1932: 18 (key); Wörz 1958: 271 (Germany, Württemberg); Gozmány 1965: 47 (Hungary, key); Szőcs 1973: 452 (Hungary); Szőcs 1977: 121 (leafmine key); Szőcs 1981: 210 (Hungary: Budapest); Szabó 1982: 159 (ecology); Szabóky 1982: 8 (Hungary); Tóth et al. 1992: 343 (sex attractants); De Prins 2007: 4 (Belgium).

Antispila treitschkella [misspelling]; Borkowski 2003: 114 (Polish Sudeten).

Antispila stachjanella; Hering 1957: 325 (leafmine); Gozmány 1965: 47 (key); Berestynska-Wilczek 1966a: 433 (larval habits); Berestynska-Wilczek 1966b: 455 (nervous system); Maček 1974a: 56 (Slovenia); Maček 1974b: 94 (Slovenia).

Material examined. Total 34♂, 33♀, 4 sex unknown: Albania (1 ♀), Austria (2♂, 2♀, 2 sex unknown), Bulgaria (larvae, mines), Czech Republic (6♂, 3♀, leafmines), France (leafmines), Germany (1♂, 1♀, leafmines), The Netherlands (16♂, 16♀, 2 sex unknown, larvae, leafmines), Poland (4♂, 2♀), Switzerland (5♂, 7♀, larvae, leafmines), United Kingdom (1♀, larvae, leafmines). Details in Suppl. material 1.

Differential diagnosis. See *A. petryi*. Larva see *A. petryi*, characteristic absence of black dot on mesothorax (second visible segment), making a maximum of eight dots, abdominal segment 8 with 2–3 rows comprising many black warts of different sizes.

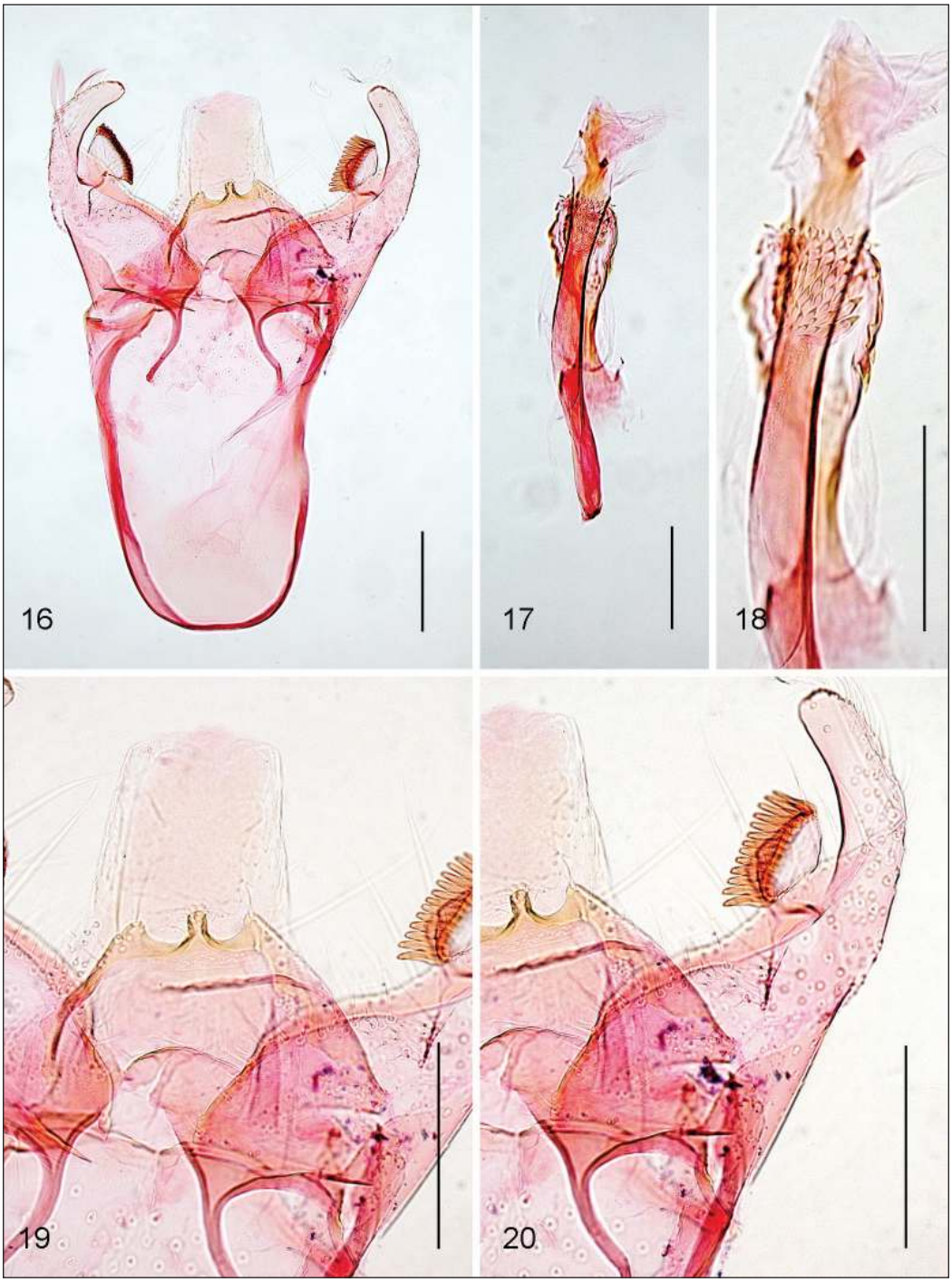
Description. Male (Fig. 3). Head face and vertex covered with appressed shiny grey scales. Antenna fuscous, ringed, particularly near tip, and better visible on underside. Thorax dark fuscous, concolorous with forewings. Legs grey, tarsi ringed white at tip, spurs and undersides paler. Forewing dark fuscous with a slight purple shine, with silver-golden patterning; an outwardly oblique fascia at ca 1/3, hardly or not narrowing in middle, dorsal edge slightly wider than costal; dorsal spot slightly beyond middle of posterior margin, triangular, reaching hardly to middle of wing, a more trapezoid or rectangular costal spot at 2/3, slightly longer than wide; fringe line distinct. Terminal fringe paler. Hindwing rather dark grey. Underside of wings fuscous, close to base a yellow to orange tuft of androconial scales. Abdomen lead-coloured, including vestiture on external genitalia.

Female (Fig. 4). Similar to male, androconial scales absent. Abdomen with slightly protruding ovipositor.

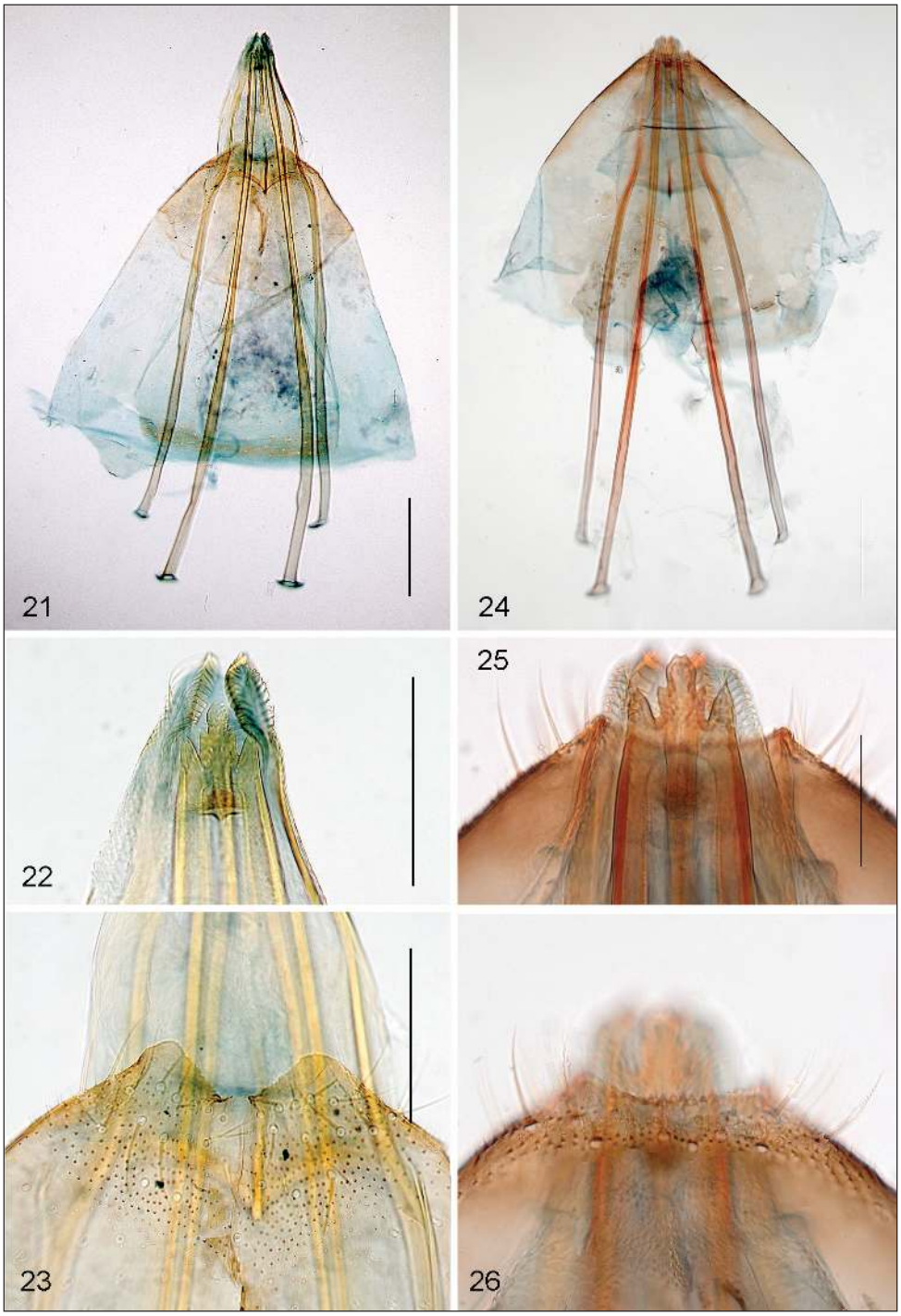
Measurements, male: forewing length 2.7–3.3 mm (2.9 ± 0.2, 13), wingspan 5.7–7.0 mm, 19–20 antennal segments (n=8); female: forewing length 2.3–3.0 mm (2.7 ± 0.2, 7), wingspan 5.7–7.0 mm, 19–20 antennal segments (n=3). For costal spot see Table 2.

Male genitalia (Figs 16–20, 28, 30). Uncus with two distinct setose lateral lobes and a more prominent central lobe that reaches beyond a line between the lateral lobes; distinctly indented between lobes. Vinculum 335–355 µm long, anteriorly almost truncate. Valva length 235–270 µm, basally broad, more or less triangular, narrowing towards digitate tip; pecten on pedicel, with 9–14 comb teeth (Fig. 20); anellus absent; transtilla platelike, in the middle emarginated anteriorly, sublateral processes long, thin and curved, hardly or not widened at tips. Juxta anteriorly spade-shaped, about half as long as phallus. Phallus 400–415 µm long, at phallotrema with group of larger pointed spines; most spines belonging to one type.

Female genitalia (Figs 24–26, 32). Anterior apophyses 1030–1080 µm, posterior apophyses 1125–1160 µm (n=3). Oviscapt with two large lateral cusps and two smaller ones more centrally, tip distinctly indented. Sternum 8 hardly indented in middle. Internal genitalia not examined in detail, no sclerotisations visible.



Figures 16–20. *Antispila treitschkiella*, male genitalia, slide JCK8059. Scales 100 µm (16–17), 50 µm (18–20). Photos Erik van Nieukerken.



Figures 21–26. *Antispila* species, female genitalia. **21–23** *A. petryi*, slide JCK8054 **24–26** *A. treitschkiella*, slide EvN5014. Scales 200 µm (21, 23), 100 µm (22–23), 50 µm (25, 26 on same scale). Photos Erik van Nieukerken.

Larva (Figs 36–38, 41, 42). Whitish translucent, head capsule brown, prothorax with large black shining tergum and sternum. In instar IV, the final feeding instar, mesothorax white, without spot; metathorax and abdominal segments 1 to 7 each with a central black spot, outline more distinct than in *A. petryi*, more or less trapezoid to almost square, spots becoming smaller from segment 5 to 7, sometimes spots lacking on segments 6 and 7 and very small on metathorax (e.g. Ellis 2017). Abdominal segment 8 dorsally with a swollen hump, at the anal end lined with 2–3 rows of more than 20 black warts of different sizes. Anal segment black. More details on earlier instars are given by Dziurzyński (1948). The fifth instar is a non-feeding prepupal instar, that is reached after one moult inside the case.

Biology. ***Hostplants.*** *Cornus mas*. Records on *C. sanguinea* probably all refer to *A. petryi*. Szabó (1982) remarked on a large population in a Hungarian oak forest:

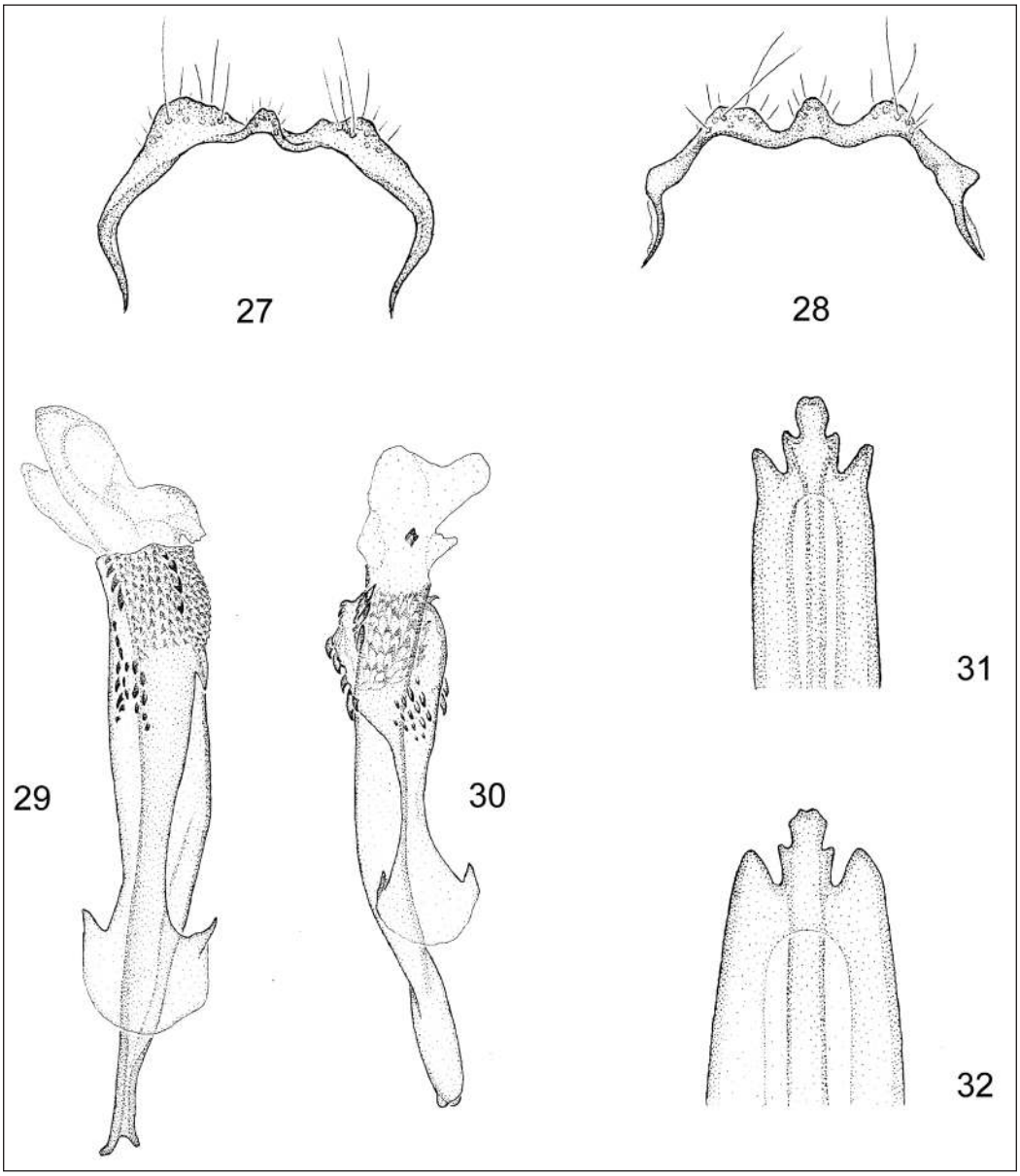
The larvae are monophagous and do not occur on the shrubs of Cornus sanguinea *L. which species is also very frequent in the forest examined.*

There are some records on the planted species *C. sericea* or *C. alba*, but the accuracy of these is questionable. May be occasionally also on *C. officinalis* (see below under Living collections).

Leafmines (Figs 46–48, 56). The egg is inserted on the leaf underside, frequently on leaf margin (82% of 135 mines), less often away from the margin; in pest densities, however, more mines appear away from the margin. The oviposition site is recognisable as a reddish dot (the "vesicula incubatoria" of Dziurzyński). The mine starts with a narrow gallery, almost straight along the leaf margin when the egg was laid there, or rather contorted in other cases; the frass line is usually rather narrow. Later mine expanding into a large full depth blotch, in many cases absorbing the earlier gallery completely (in half of the marginal mines); frass often in a clump near the origin of the mine and also scattered around. The larva prepares an oval cut-out of ca 4.5–5.5 mm length, usually at the other edge of the mine, lined with silk, and drops to the ground in this case. The larvae feed with ventral side up, but they start turning around in the blotch when preparing the cut-out. The gallery part of the mine is prepared during the first two instars, the blotch during instar 3 and 4.

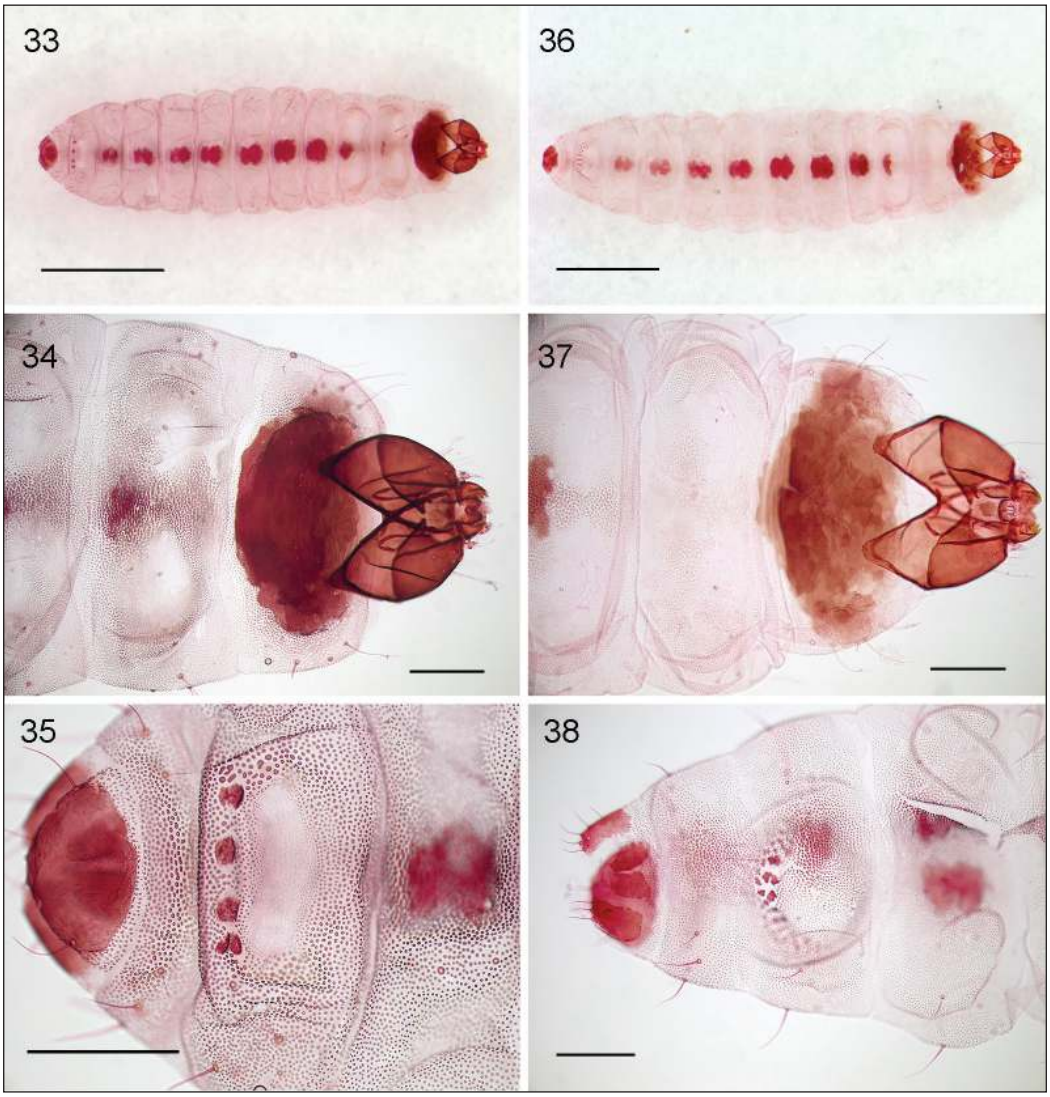
Life history. Bivoltine. Larvae of the first generation are found from June to July, a second generation from August until early November; it is possible that generations may overlap. Adults emerge in captivity from April to June (the few earlier records are almost certainly forced breedings), and again from July to late August; the few records of field caught adults agree with this pattern. The adults may swarm around the host during the day, as already was mentioned by Fischer von Röslerstamm (1843). A recent observation of ca. 100 swarming adults on *C. mas* in the Netherlands can be seen here: https://waarneming.nl/waarneming/view/139225815.

Distribution. Widespread in central and southern Europe, in the natural range of *C. mas*, and north and west of it occurring on the frequently planted trees. Positively recorded on basis of adult or larval characters, or hostplant: England, the Netherlands, Belgium, France, Germany, Poland, Czech republic, Slovakia, Hungary, Switzerland, Austria, Italy, Slovenia, Bulgaria, and Ukraine. We record it here from Albania, one specimen on BOLD, GRPAL724–11, agreeing in DNA barcode (Table 1), and from Serbia and Greece on the basis of mines in herbarium specimens (see below). For Greece, there was only a previous record of *A. treitschkiella* by Staudinger for which the identity cannot be confirmed (Gozmány 2012). *Antispila treitschkiella* has recently been expanding northwards and westwards with the widely planted trees in parks and gardens (see below). Not yet recorded from the Iberian Peninsula. Many records require verification because of confusion with *A. petryi*.



Figures 27–32. *Antispila petryi* (27, 29, 31) and *A. treitschkiella* (28, 30, 32), comparative details of male and female genitalia. **27, 28** Uncus, slides JCK8056, JCK7888 **29, 30** Phallus, phallotheca and juxta, slides JCK8056, JCK8059 **31, 32** Tip of oviscapt, slides D1258, D1257 (D=Doets). Drawings Sjaak Koster.

The natural distribution of *C. mas* is much more restricted than that of *C. sanguinea*, its NW border being from SE Belgium to NW France, away from the coast, covering large parts of France, whereas it is scarce in isolated regions in Germany, West and South Switzerland, Austria north and east of the Alps, large parts of Italy and the whole of Southeast Europe south of southern and western Czechia, Slovakia, and southern parts of the Ukraine; also coastal areas of Turkey, Caucasus



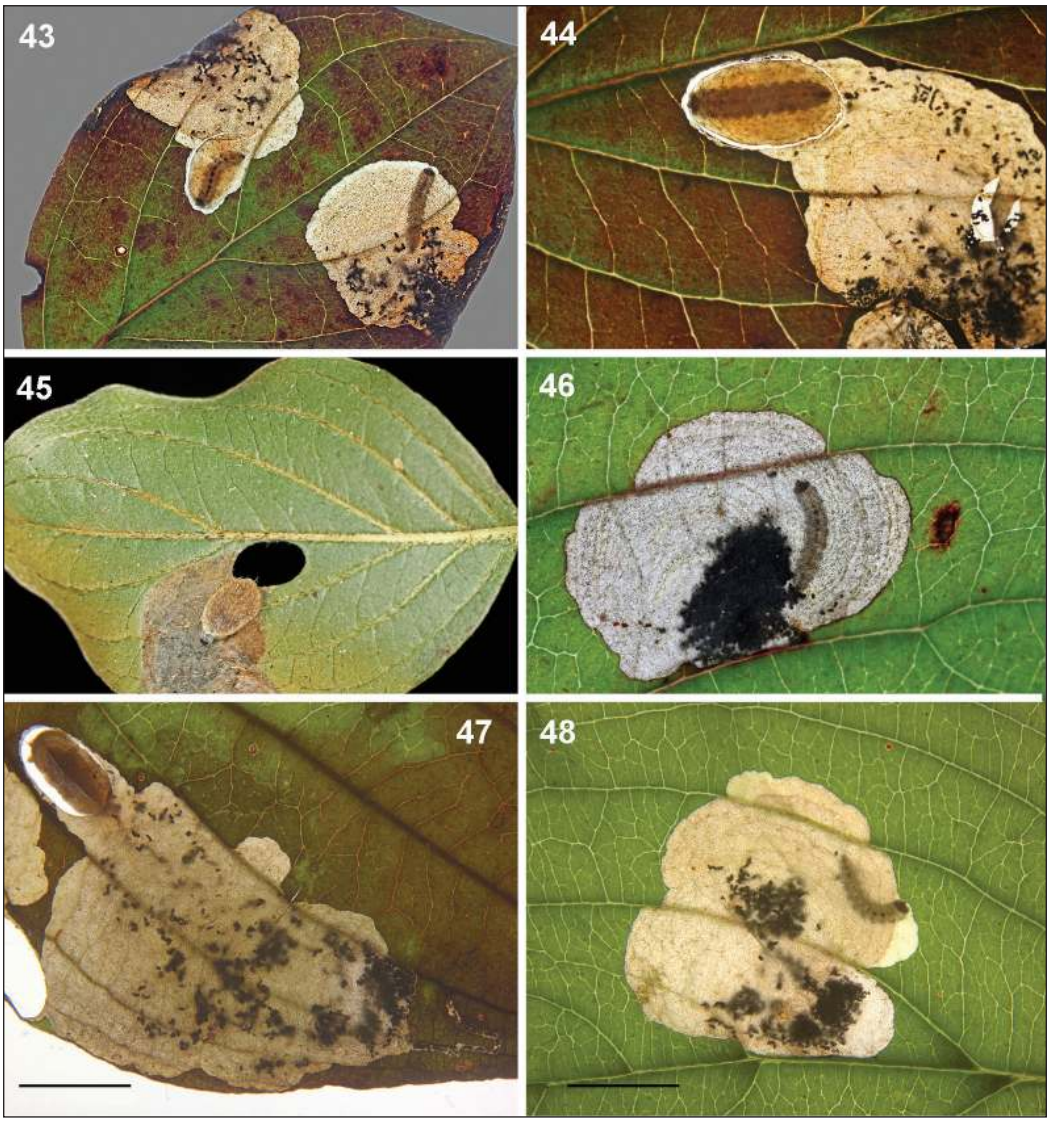
Figures 33–38. *Antispila* species, details of larvae in microscopic slides, stained with phenosafranin, Switzerland. **33–35** *A. petryi*, Le Landeron, RMNH.INS.30614 and Mont Vully, RMNH.INS.30615 (34) **36–38** *A. treitschkiella*, Le Landeron, RMNH.INS.30611 and Gampelen, RMNH.INS.30613 (38). Scales 1 mm (larvae), 200 µm (heads), 100 µm (abdominal tips). Photos Erik van Nieukerken.

region and Black Sea coast of Russia and Crimea, just reaching Azerbaijan and North Iran. *Cornus mas* is absent from the Mediterranean islands and the Iberian Peninsula, except for a very small area in the Catalonian Pyrenees (Da Ronch et al. 2016).

Remarks. The original description by Fischer von Röslerstamm (1843) of *Elachista treitschkiella* is based on an unspecified number of specimens, collected by the renowned Austrian collector J. Mann in the vicinity of Vienna, where the adults were observed flying in the afternoon and particularly swarming around sunset about bushes of "Kornelkirschensträucher (*Cornus mascula*)", i.e.

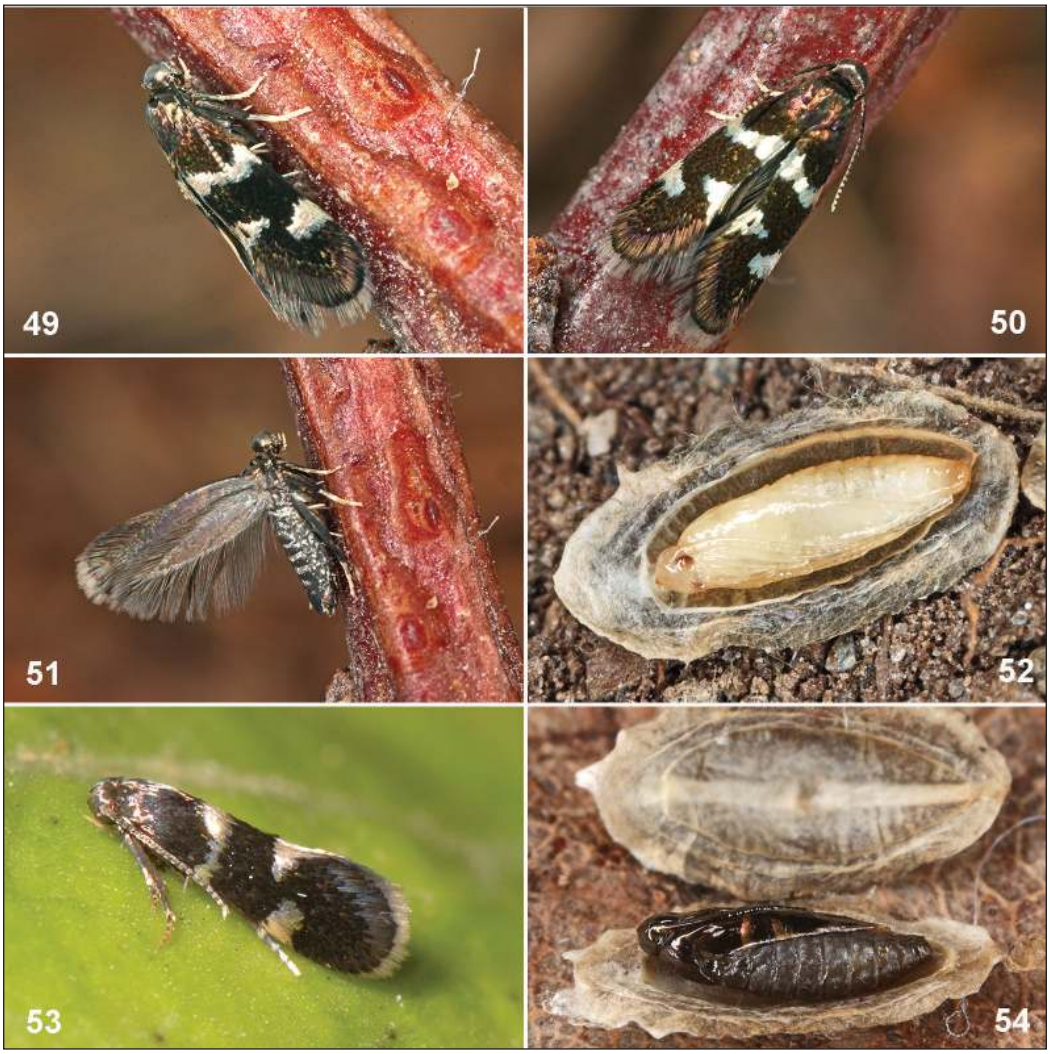
Figures 39–42. *Antispila* species, details of live larvae, Switzerland, all on 8.x.2015. **39–40** *A. petryi*, Le Landeron **41–42** *A. treitschkiella*, Le Landeron and Vully resp. Photos Rudolf Bryner.

the European cornel or *C. mas*. The hostplant and the detailed description leave little doubt as to the identity of this moth, also in the light of our DNA barcode findings. Fischer von Röslerstamm named this species after Friedrich Treitschke, who died a year earlier on 4.vi.1842, and he added a long obituary in a footnote. Duponchel [1843] described the species independently again, as *Oecophora treitschkiella*, also based on material collected by Mann, most likely in Vienna. De Joannis (1915) determined on the basis of publication dates that Fischer's name has priority (see also Fletcher and Griffin 1943). Because his name is Treitschke, Stainton (1851) emended the species name as *treitschkeella* (used as misidentification for *A. petryi*). He corrected this later (Stainton 1854), but Meess (in Spuler and Meess (1910) introduced this spelling again, which was followed by several authors, especially in Hungary, but the name is an unjustified emendation and thus not valid.



Figures 43–48. *Antispila* species, leafmines and larvae. **43–45** *A. petryi*, Switzerland, Vully, 8.x.2015 **46–48** *A. treitschkiella*, Switzerland, Gampelen, 8.x.2015 (46) and Netherlands, Leiden, 26.ix.2017. Scales 5 mm. Photos Rudolf Bryner and Erik van Nieukerken (47, 48).

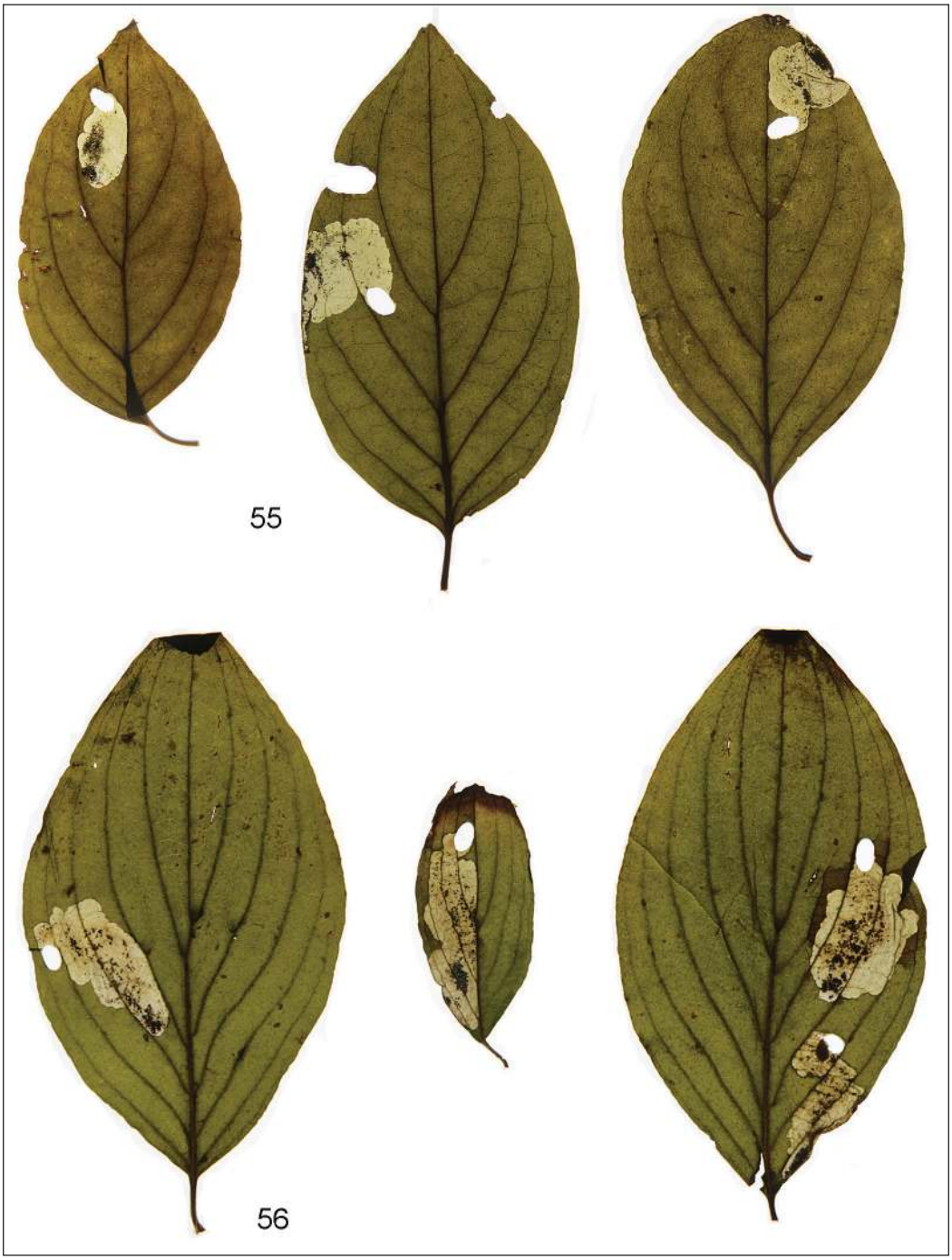
Fischer von Röslerstamm's Microlepidoptera were acquired in 1843 by Herrich-Schäffer (Horn et al. 1990: 120, 168), whose collection was split up in various ways (e.g. via Staudinger, Hofmann) so that it is now extremely difficult if not impossible to trace his original specimens. In Vienna there are no likely syntypes of *A. treitschkiella* (Sabine Gaal-Haszler, pers. comm.). There may be some FR specimens (?of *Antispila*) in the collections of contemporaries such as Zeller (now in NHMUK). A single male specimen in NHMUK (NHMUK010305384) labelled "Treitschkiella Mann. Wien" is a potential male syntype of *Elachista treitschkiella*, although unfortunately it bears



Figures 49–54. *Antispila* species, live adults and pupae, Switzerland. **49–52** *A. petryi*, Vully, male on 21.iv.2016, pupa on 21.ii.2016 **53–54** *A. treitschkiella*, Le Landeron, female, 22.iii.2007, pupa 17.iv.2016. Photos Rudolf Bryner.

no collection date. Its correspondence with the *C. mas* feeding *Antispila* needs to be established prior to potential lectotypification.

Antispila stachjanella was described from an unspecified number of specimens of both sexes, bred by Dziurzyński in 1944–1947 [1948] from larvae mining the leaves of *C. mas* in Kraków. In the Polish Academy of Sciences, Kraków are about 150 (undissected) specimens of *A. stachjanella* from Dziurzyński's rearings (1944–1948), none of which is clearly labelled as any kind of type, although at least some of them constitute a potential syntypic series (L. Przybyłowicz, pers. comm.). In NHMUK there are four specimens under *A. stachjanella*, two of which on loan, and two of which are labelled "COTYPUS", only one of which (NHMUK010305235) is apparently in the correct date range (the



Figures 55–56. *Antispila* species, leaves of *Cornus* with vacated leafmines, dry collection RMNH. **55** *C. sanguinea* with *A. petryi*, RMNH.INS.41141, Netherlands, Limburg, Geulhem, 26.viii.1949, C. Doets **56** *C. mas* with *A. treitschkiella*, RMNH.INS.40829, Netherlands, Zuid-Holland, Leiden, 17.x.2004, E.J. van Nieukerken. Photos Erik van Nieukerken.

other NHMUK010305235, is labelled 1949). The original description of *A. stachjanella* is a short diagnosis in Latin amidst a long Polish text and a rather long English summary of six pages. The Latin text does not give information on types, but there is a drawing embedded on page 5, showing a male and a wing of a female collected in Kraków Botanical Garden which emerged 26.vi.1948 ex larva (Dziurzyński 1948: 5). In Krakow there are 11 corresponding specimens which are undissected, two males and nine females (L. Przybyłowicz, pers. comm.), one of which would potentially be suitable for lectotype designation. The whereabouts of specimens giving rise to illustrations of genitalia are unknown. Both in the collections in Leiden and London there are four specimens each of *A. treitschkiella* from Kraków, collected by S. Błeszyński between 1946 and 1948. In Kraków there are no specimens currently labelled *A. petryi* and 36 specimens under *A. treitschkiella* 14 of which collected by S. Błeszyński up until 1948. Błeszyński is acknowledged by Dziurzyński (1948: 8, footnote) for his assistance. These 14 specimens could also be syntypes of *A. stachjanella*.

It is somewhat mysterious why Dziurzyński needed to introduce *A. stachjanella* in such a detailed study, while he only briefly addressed the separation from *A. treitschkiella*. A single specimen identified by Hering and collected by Toll in Podolia was his only comparative material, and he based the different identities on the fact that the antennae of his species were ringed, whereas the specimen of *A. treitschkiella* he studied did not have rings. He also cited Rebel (1891), who briefly referred to this character, but also mentioned that other authors did not cite this (Dziurzyński 1948: 6). In fact the ringing is only apparent on the upper side and may be obsolete in some specimens.

Antispila treitschkiella has been – under the name *A. stachjanella* – the subject, of two very detailed studies respectively on larval behaviour and the central nervous system (Berestynska-Wilczek 1966a, 1966b).

Antispila metallella (Denis & Schiffermüller) Koçak

Figs 5, 6, 57–65

Tinea metallella [Denis & Schiffermüller], 1775: 144 (Dunkelgoldener Schabe mit 6. Silberzeichen). Syntypes, unspecified: Austria: Wien [Vienna] [not examined, collection lost by fire]

Tinea pfeifferella Hübner, [1813]: pl. Tineae IV, Nobiles, 59: fig 398. Syntypes, unspecified, Europe [not examined]. (Lost). A primary homonym of *Tinea pfeifferella* Hübner, [1813]: pl. 63: fig. 422 (see Nielsen 1985). Synonymised by Werneburg 1864: 584.

Antispila stadtmuellerella Hübner, [1825]: 419. Syntypes, unspecified, Europe (Hübner) (Lost). [Replacement name for the pre-occupied *Tinea pfeifferella*, type species of the genus *Antispila* Hübner, 1825]

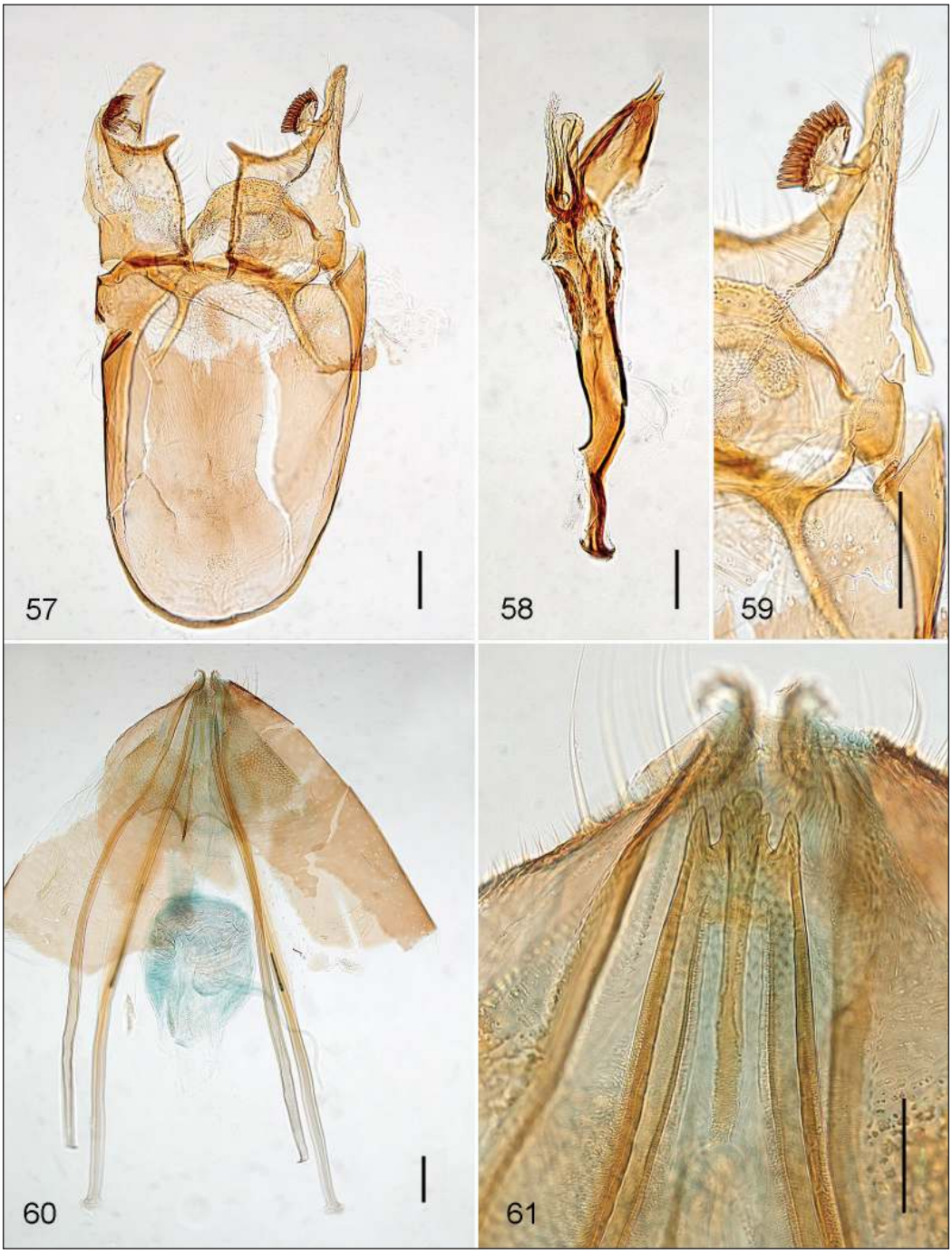
Tinea quadriguttella Haworth, 1828: 574. Synonymised by Stephens 1834: 270.

Elachista pfeifferella; Stainton 1854: 250 (England).

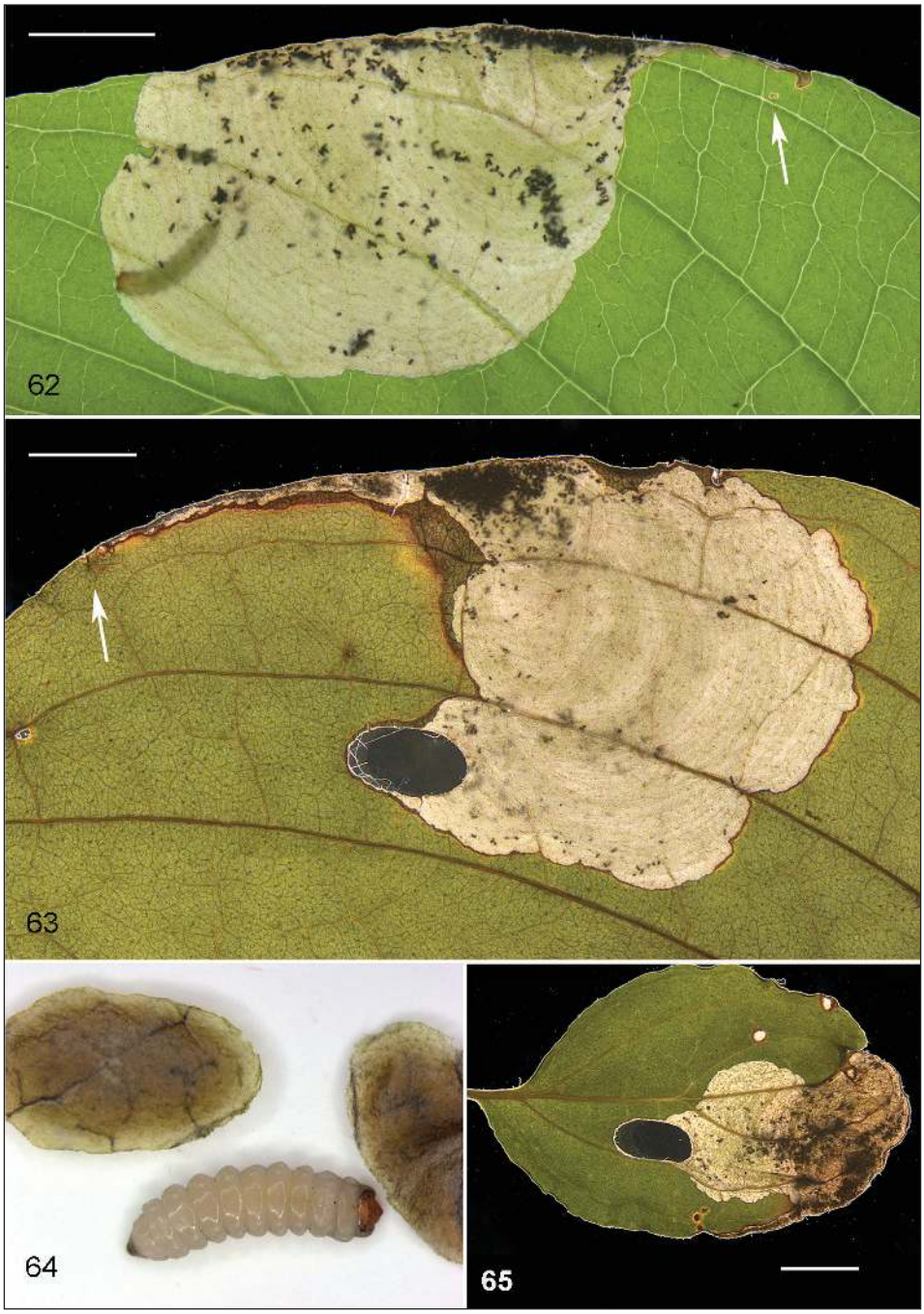
Antispila pfeifferella; Herrich-Schäffer 1855: 315 (recombination); Frey 1856: 283 (Switzerland); Stainton et al. 1870: 308; Wocke 1874: 88 (Schlesien, now Poland and Czech Republic); Heinemann and Wocke 1876: 515 (Germany); Sand 1879:192 (France); Meyrick 1895: 684 (England); Spuler and Meess 1910: 471; Grandi 1933: 178 (description larva); Toll 1938: 211 (Podolia, now Ukraine); Doets 1950: 166 (the Netherlands); Hering 1957: 325 (leafmine); Klimesch 1961: 725 (Alps); Lhomme 1963: 1157 (France); Hering 1968: 120 (letter to Klimesch of 1952); Szőcs 1977: 121 (leafmine key); Kuznetsov 1978: 73 (keys); Razowski 1978: 95 (Poland, keys).

Antispila metallella; Koçak 1984: 153 (recombination, nomenclature); Nielsen 1985: 24 (nomenclature); Emmet 1988: 38 (biology); Klimesch 1990: 77 (Austria); Bengtsson et al. 2008: 287 (keys, description, Sweden); Laštůvka and Laštůvka 2015: 635 (Spain).

Material examined. Total 8♂, 20♀: Austria (2♂, 2♀), Bulgaria (larva, leafmines), France (1♀, larva, leafmines), Germany (2♀), The Netherlands (4♂, 13♀, larvae, leafmines), Poland (1♂, 1♀), Romania (leafmines), Switzerland (1♂, 1♀). Details in Suppl. material 1.



Figures 57–61. *Antispila metallella*, male and female genitalia, slides JCK8614 (♂), JCK8615 (♀). Scales 100 µm. Photos Erik van Nieukerken.



Figures 62–65. *Antispila metallella*, leafmines and larvae. **62** Leafmine with larva, Netherlands, Oegstgeest, on *Cornus sericea*, larva RMNH.INS.18464. **63** Vacated leafmine on *C. sanguinea*, Netherlands, Valkenburg, ZMA.INS.MIG.02122. **64** Larva and halves of shield, larva RMNH.INS.18464. **65** Vacated leafmine on *C. sanguinea*, Bulgaria, Ilidentsi, RMNH.INS.42092. Scales 5 mm. Arrows indicate test punctures. Photos Erik van Nieukerken.

Figures 66–70. *Antispila treitschkiella*, invasion in the UK and herbarium specimens. **66** Leafmines of on *Cornus mas* at London, Hyde Park Corner, 22.ix.2016. **67** Leafmines on *C. mas* at London, Temple, Victoria Embankment, 28.ix.2016. **68** Herbarium specimen, RBG Kew, Verdcourt 4160, 22.viii.1964, from Mt. Pelion, Greece: *C. mas* with mines of *A. treitschkiella* **69** Detail of 68 **70** Herbarium specimen, RBG Kew, Schneider 1681, 28.viii.1907, from Rila Mt., near Samakov, Bulgaria, *C. mas* with mines of *A. treitschkiella*. Photos David Lees.

Differential diagnosis. Adults (Figs 5, 6) of *A. metallella* are easily recognised by their larger size (wingspan usually more than 7 mm, usually less in the other two species), the more coppery to bronze colour of the forewings and in the male the absence of androconial scales. Male genitalia

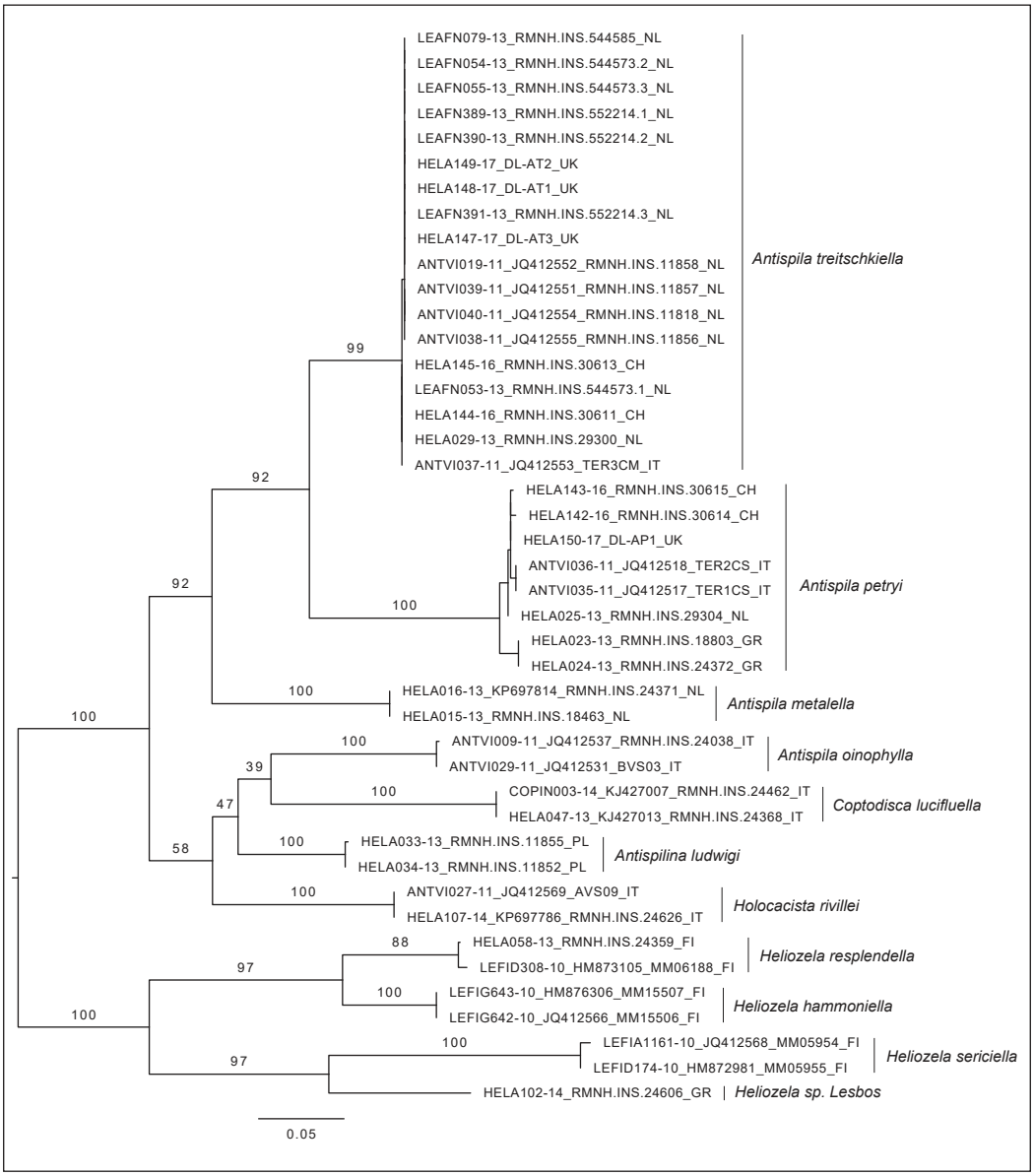


Figure 71. Maximum likelihood tree of COI barcodes of 43 European Heliozelidae belonging to 11 species. Bootstrap support values are omitted for intraspecific branching. See Table 1 and Suppl. material 1 for specimen data, Suppl. material 2: Fig. S2 provides a Neighbor Joining tree.

characterised by truncate uncus and distinct spine on inner margin of valva (Figs 57, 59). Female genitalia difficult to distinguish (Figs 60, 61).

Measurements (male). forewing length 3.7–3.9 mm (3.8 ± 0.1, 5), wingspan 7.8–8.5 mm, 24–25 antennal segments; female: forewing length 3.3–4.0 mm (3.7 ± 0.2, 11), wingspan 6.9–8.7 mm, 24–25 antennal segments.

Larva. A detailed description of a 4th instar larva was given by Grandi (1933) and (Ellis 2017). The larva differs from those of the other two species by the absence of dark plates, except on mesothorax and the last abdominal segment (Fig. 64).

Biology. ***Hostplants.*** *Cornus sanguinea*, both subsp. *australis* and *sanguinea*, rarely also recorded from planted *C. alba* and *C. sericea* (see herbarium results). In literature repeatedly recorded from *C. mas*, but in most cases without any data nor references: these records are unlikely and should be checked. Some leafmine material of *A. metallella* seen by us and labelled as from *C. mas* appeared to be misidentified, either the insect (being *A. treitschkiella*) or the plant (being *C. sanguinea*).

Leafmines. Leafmines are larger than those of the other two species, and the species can be separated by the presence of test punctures near the oviposition site (Figs 62, 63, 65), the larger cut-out in vacated mines of 5.5–7 mm) (Figs 63, 65) and the larva lacking black spots on the abdomen (Fig. 64).

Life history. Univoltine. Larvae from early June to early August, much earlier than *A. petryi* on the same hostplant. Adults fly from April to early June.

Distribution. Widespread in central and southern Europe, distributed further north than the other species: occurs in a few localities in southern Norway and southern Sweden, the islands Gotland and Öland, the islands of Denmark, Estonia, Latvia, Lithuania, southern England to Midlands, all West and Central European Countries, just in the NE of Spain (Laštůvka and Laštůvka 2015) but has not yet been recorded from Italy (the earlier record by van Nieukerken et al. (2012b) was a misidentification of *A. petryi*), with southernmost records from Croatia, Romania, Bulgaria and more eastwards Ukraine and European Russia.

Remarks. Hübner (1813) used the name *Tinea pfeifferella* in the same work for two different species; it was his decision as first reviser to replace the name published on the earlier plate (pl. 59) rather than the one on the later (pl. 63) with "*Antispila Stadtmüllerella*" (Nielsen 1985). Although Werneburg (1864) already synonymised "*pfeifferella*" with *Tinea metallella*, this was overlooked by most authors until the 1980s (Koçak 1984; Nielsen 1985). The nomenclature of this species was extensively discussed by Nielsen (1985). Although the types of *A. metallella* and its synonyms appear lost (Horn et al. 1990: 181, 347), they are not of primary concern for this paper, since the separation of both immatures and adults of this species is quite clear. *Antispila metallella* is the type species of the genus *Antispila*, settled by the International Commission on Zoological Nomenclature (Nielsen and Nye 1986; ICZN 1988).

Further results

DNA barcoding and leafminer species relationships

We included all ten European species of Heliozelidae (39 specimens) and one unnamed candidate species in the DNA barcoding analysis. The barcodes of all group as monophyletic clusters and have much larger interspecific than intraspecific distances (Fig. 71, Suppl. material 2: Fig. S2). The closest species to *A. petryi* is *A. treitschkiella*, but in fact the closest known species by COI distance to *A. treitschkiella* is the North American *A. freemani* Lafontaine, 1973, not included in this analysis. The bootstrap support values from the maximum likelihood analysis are high for the genus *Heliozela* (100) and relatively high for *Antispila* s.str. (92), but low for the clade of the other four genera (58), that share a reduced venation (van Nieukerken et al. 2012b). Although such low support might be expected with just DNA barcode data, the grouping of these four genera is supported by morphology and has also been observed in a recent study that used four genes (Milla et al. 2017).

Identifying material based on DNA barcodes is highly reliable for this group and provides an alternative to morphology. True *A. treitschkiella* belong to the cluster with Barcode Index Number (BIN) BOLD: AAU1917 and *A. petryi* to BOLD: AAV5055, while *A. metallella* belong to BOLD: ACG8679.

Living collections

About 20 species or cultivars of *Cornus* were examined among living collections in the UK (see Methods). Every single *C. mas* tree examined by DCL had mines of *A. treitschkiella*, except for *C. mas* and *C. mas* "var. *variegata*" at Oxford Botanic Garden (where *A. treitschkiella* still seems to be absent). Perhaps surprisingly, neither *A. petryi* nor *A. metallella* were positively identified in the botanic gardens living collections survey, although it is possible that old mines on *C. sanguinea* "Annie's Winter Orange" at Wisley represented the former and on "*C. alba*" at Kew represented the latter. *A. petryi* was present on *C. sanguinea* at Priest Hill Reserve, Surrey, and along hedgerows at other countryside locations in the Cambridge area, and old mines probably of this species were present on *C. sanguinea* in Kensington Gardens, London.

At Kew and Wisley, living specimens labelled as *C. paucinervis* (a synonym of *C. quinquinervis* Franch.) with mines of *A. treitschkiella* turned out to be misidentified and were in fact referable to *C. mas* (K. McGinn, pers. comm.). The same applied to specimens labelled as *C. officinalis* (a species in the same subgenus as *C. mas*), with one exception: a tree of this species at Wisley with not a single mine of *A. treitschkiella* had the character of typical *C. officinalis*, dense rusty red hairs in the axils of the underside leaf venation. In the Wisley shop, the few living specimens for sale labelled as *C. officinalis* (without rusty hairs in the leaf axils) had one to two mines per plant; we therefore regard this record as inconclusive. There are also three specimens of *A. treitschkiella* (as *A. stachjanella*) that A. Dziurzyński reared in 1946–1949 from *C. officinalis* from the botanic garden in Kraków, in the collection of the Polish Academy of Sciences. The identity of any so-labelled plants still existing in that botanic garden would also need to be verified.

At Cambridge Botanical Garden in October 2016, all *C. mas* specimens had strong prevalence of *A. treitschkiella* mines (usually at least 1–10 per branch). In one case, an adjacent specimen of *C. amomum* subsp. *obliqua* (Raf.) J.S. Wilson (labelled as "*C. obliqua*"; number 37.0114) had a few mature mines, apparently as spillover from an adjacent *C. mas*. As these few moth individuals have not yet been DNA barcoded, nor the plant identity rechecked by botanists, we regard also this potential new hostplant record for *A. treitschkiella* so far as also inconclusive. This *Cornus* species is among the North American *Kraniopsis* Raf. (subgenus) and is either a subspecies or a good species related to *C. amomum* (Xiang et al. 2006) and, if confirmed, would be only the second hostplant record for *A. treitschkiella*.

As *C. officinalis* is the inferred sister to *C. mas* (Xiang et al. 2006), it is interesting that we could not find evidence of *A. treitschkiella* mines on any botanically verified examples of this species. Further work is needed to determine if *A. treitschkiella* always avoids *C. officinalis* or accepts plants of hybrid origin.

Herbarium study

In Wisley, the 14 specimens of *C. mas* at a suitable stage of leaf maturity collected between 1848–1997, evidenced zero mines of *A. treitschkiella*. Unfortunately, there were relatively few cultivated collections of *C. mas* preserved since the 1980s.

At NHMUK (AMC herbarium), the handful of specimens of *C. mas* of UK origin were also clear of mines. However, in this last collection, two specimens of naturalised *C. sericea* (labelled as *C. stolonifera*) from Panshanger, Hertfordshire, 1952, had clear mines of *A. metallella*, and the mature larva was extracted for verification in one case. This confirms one of the previous hostplant records for *A. metallella*; the species is, however, also reported from *C. alba* (Ellis 2017), but whether this is genuine *C. alba* or *C. sericea* is unclear, since both were treated as one species in the Flora of the Netherlands (van der Meijden 2005). A few old mines were found at RBG Kew on living collections on this last species; their identity has not yet been verified.

At Kew Gardens herbarium, only four specimens of *C. mas* were found with mines apparently of *A. treitschkiella*, all from native collections in continental Europe (Greece: Mt. Pelion; Albania: N. of Merkopele; France: Charance; Bulgaria: Rila Mts, Samakov; for details see Suppl. material 1). The identity of these mines, which do not exhibit the relatively large cut-out dimensions of *A. metallella*, has not been confirmed, but there is no reason to suspect they do not represent *A. treitschkiella*. Finally at NHMUK, two herbarium specimens of *C. mas* were found on a single sheet, the first with at least three mines and the second with one mine, presumed to be of *A. treitschkiella* (leg. C.K. Schneider, respectively from Serbia and Bulgaria).

The herbarium of the Naturalis Biodiversity Center, Leiden was briefly scanned by means of its BioPortal (http://bioportal.naturalis.nl/), but not a single sign of mines was found so far.

Discussion

Taxonomy and identification

Our results show that the *C. sanguinea* feeding *A. petryi* differs in many aspects from the *C. mas* feeding *A. treitschkiella*, and thus we conclude that the synonymy by Razowski (1978) was unwarranted. Identification is straightforward on the basis of male genitalia, larval characters, hostplant and DNA barcodes. For a separation of *A. stachjanella* from *A. treitschkiella* we, however, do not find any support, and there is thus only one taxon feeding on *C. mas*.

Literature and other records of *Antispila* "*treitschkiella*" that specifically provide the hostplant species name can be attributed to either *A. petryi* or *A. treitschkiella* s. str., but many records are inconclusive in this regard.

Hostplant relationships

All records of either species from the reciprocal principal hostplant need to be looked at with suspicion: whereas we do not a priori exclude the possibility, probably the majority are either a case of misidentified hostplant or misidentified leafminer: we did not find any convincing case of host switching between them, and we could reliably re-identify hosts of several online records. The fact that the two European *Cornus* species have a different leafminer fauna is no surprise, as the species are not closely related. *Cornus mas* belongs to the subgenus *Cornus* L. that comprises a few Asian and one European species, and is easily recognised by the cauliflory, yellow flowers appearing before the leaves, and edible large red fruits, resembling a cherry (hence the name Cornelian cherry). On the other hand, *C. sanguinea* belongs to the large subgenus *Kraniopsis* Raf., with numerous Asian and North American species: these have white flowers in umbels, appearing after the leaves, and small white or blue berries. Phylogenetically, both subgenera are clearly separate (Xiang et

al. 2006). *Cornus alba* and *C. sericea* also belong to *Kraniopsis*. Up to now, however, we have not seen evidence for *A. petryi* on these hosts and only record here *A. metallella* from *C. sericea*.

In summary, all our results support the hypothesis that the two species *A. treitschkiella* and *A. petryi* are monophagous, respectively on *C. mas* and *C. sanguinea*. In an interesting experiment, Berestynska-Wilczek (1966a) transferred larvae of *A. treitschkiella* (in her study under the name *A. stachjanella*) to empty leafmines of different insects on various other plants, including *Alnus* Mill. (Betulaceae), *Crataegus* L. (Rosaceae), but also *Cornus sanguinea*, *C. stolonifera* (= *C. sericea*) and *C. controversa*. Most larvae started feeding, but died soon thereafter, with the exception of fully developed 4th instar larvae, that appeared to be able to make a cut-out even in non-hostplants. Although her study gives no detail on the experiments with various *Cornus* species, it does lend some support to our conclusion.

Expansion

The first reports of a range expansion of *A. treitschkiella* on to planted *C. mas* was by Dziurzyński (1948), who reported it as his new species *A. stachjanella* from the Kraków region in Poland, as occurring in large numbers in parks between 1944 and 1946, with a decline in 1947 after a severe winter. The Polish records are not far north from the native distribution of *C. mas* in southern Slovakia (Da Ronch et al. 2016). For the years between 1947 and the 1990s, we have not found any literature record of expansion of this species, even though *C. mas* has been planted in many urbanised areas.

The next records are from an expansion in The Netherlands in 1996 and 1997 (Kuchlein and van Frankenhuyzen 1999), then considered to represent a sudden expansion of the native populations that live in the south of the province of Limburg on *C. sanguinea*. Large numbers of mines were found on *C. mas* in a few localities in the provinces of Gelderland and Noord-Brabant, but in many places no mines were present. It is now clear that these records represented the first Dutch records of the real *A. treitschkiella*, that since then has become numerous in many places throughout the Netherlands (van As and Ellis 2004; Huisman et al. 2005; Muus and Corver 2017), see http://www.microvlinders.nl/soorten/species.php?speciescode=60050&p=1.

In Germany the expansion was noted a bit later, and the species was recorded as new for Sachsen in Belgershain in 1999 by Stübner (Graf et al. 2001), new for Nordrhein-Westfalen in 2001 (Retzlaff 2002) and new for Niedersachsen in 2001 (ten Holt 2005). We did not find a genuine record for Hessen, because old records of *A. treitschkiella* are referable to *A. petryi* (e.g. Rössler 1881), but EvN found mines on *C. mas* in the city of Frankfurt on 20.ix.2017. Since *C. mas* is also native in some parts of Germany (Da Ronch et al. 2016), the distinction between original indigenous populations from expansions may be difficult. For instance in Thüringen *A. treitschkiella* occurs in the native range of *C. mas* and was recorded long ago by Martini (1899). Retzlaff (2002) remarked that in North Rhine-Westphalia the frequent planting of *C. mas* began in the 1960s.

In the UK, the earliest confirmed specimen still remains the adult of *A. treitschkiella* collected on 23.viii.2016 in the South Kensington area of London. Since *Antispila* are not usually recorded in light traps, it may take some effort to detect any earlier records of adults in collections. After the initial finding, we observed mines on all examined trees verifiable as *C. mas* throughout London and beyond within the presently known range of *A. treitschkiella*, in London sometimes with very heavy impact. The UK herbarium survey proved negative, based however on few recent pressings of cultivated *C. mas* (the largest such collection being 14 specimens in leaf at Wisley from

1848–1997). Absence of evidence in this case does not constitute evidence of absence, except that it was possible for botanists, at least up to about 1997, to find sections of plants fully clear of mines. Cultivated *C. mas* are not sampled every year and even European collections from its natural range are rather sparse. At Kew and NHMUK, those few preserved specimens from the wild range add, however, to distributional knowledge, assuming these mines do not represent a species other than *A. treitschkiella*. The presence of the species on even decades-old stands of *C. mas* (e.g. at Priest Hill, Surrey) in the UK, that are probably separated from other populations by many kilometres suggests either long establishment or, more likely, surprising powers of adult dispersal aided by winds or road traffic and outbreak swarmings next to major roads (the last explanation has been invoked for rapid spread of *Cameraria ohridella* Deschka & Dimić: e.g. Lees et al. (2011)). The present distribution and high local prevalence in the UK is consistent with establishment in the last one or two decades, and it seems likely that probable generalist parasitoids (notably the eulophid *Minotetrastichus frontalis*) are already recruited. The surprising overlooking of leafmines may be due to unfamiliarity with the plant species to most British lepidopterists but casual photography by passers-by or horticultural enthusiasts in London or elsewhere could provide an easy source of new records. It was hoped to detect signs of expansion in UK herbaria, but future examination of European herbaria may well turn up additional chronological information.

We have no indication of expansion of *A. petryi*, even though its hostplant is also widely planted in parks and gardens, as are the Asian and American *Cornus* relatives. However, *C. sanguinea* has a much wider natural distribution than *C. mas* (Popescu et al. 2016). An exception may be the few populations of *A. petryi* recently discovered on islands in the Baltic Sea: on Öland in Sweden since 2006 (Svensson 2007; Bengtsson et al. 2008; Bengtsson 2014), and on Saaremaa in Estonia since 2010 (Jürivete 2012), but it is of course possible that the species was overlooked earlier or that mines were confused with the more widespread *A. metallella*. The occurrence of these populations is in natural stands of the host and may represent a recent dispersal from populations in northern Poland (or Germany) across the Baltic (260 km to Öland, almost 500 km to Saaremaa), but we do not know whether *A. petryi* occurs naturally in northern Poland. The northernmost localities of *A. petryi* on the European continent that we know of are Hannover (Glitz 1877) and Neustrelitz in Mecklenburg (Buhr 1935), ca 100 km south of the coast.

In the Netherlands *A. petryi* is only known to occur in the hills of South Limburg; it is absent from *C. sanguinea* at other sites where the host is native or where it is planted in parks and gardens. In contrast, *A. metallella*, feeding on the same host, has spread all over the country since the 1980s and is now common on planted *C. sanguinea* and sometimes is found on cultivated *C. sericea* and/or *C. alba*.

While many species have been observed in recent years to spread over Europe, whether they are native in Europe or not, the expansion of *A. treitschkiella* has remained partly unnoticed, as a result of some poor taxonomical decisions. It remains a mystery as to why the very extensive and detailed descriptions by Dziurzyński (1948, 1952) have not led to a more critical evaluation of the proposed synonymy that came with limited argumentation (Razowski 1978). We think the facts that the latter paper was written in Polish, and that Dziurzyński's papers, even with extensive English summaries, are poorly known outside Poland, may have contributed to this oversight. Also the Heliozelidae, with a small European fauna, has had few taxonomic specialists (van Nieukerken et al. 2012b).

The expansion of *A. treitschkiella* to the north and west of the native range of its host on to planted trees fits the pattern observed in several other leafminers native to Europe. Especially species feeding on various *Acer* L. species (Sapindaceae) have been spreading in recent decades,

such as *Stigmella aceris* (Frey) (Nepticulidae) on *Acer campestre* L. and *A. platanoides* L. (van Nieukerken et al. 2006; Kuchlein et al. 2007) and *Caloptilia* Hübner species (Gracillariidae) on *Acer* (Corver et al. 2011). Earlier examples include *Phyllonorycter leucographella* (Zeller) on *Pyracantha* M. Roemer (Rosaceae) (Šefrová 1999) and *Ph. platani* (Staudinger) on *Platanus* L. (Platanaceae) (Šefrová 2001). The widespread planting of shrubs and trees in parks and gardens of housing development areas that proliferated after World War II has almost certainly contributed to this expansion, because it provided stepping stones in otherwise empty landscapes, and also expanded the area of the host. We have not searched for quantitative data on such horticultural expansion, but Retzlaff (2002) suggested that most such planting started in the 1960s.

It is certainly possible that climate change also has played a role in the expansion of the leafmining moths, but we have no data supporting this.

With this paper in hand it should be easy to recognise the mines of the three *Cornus* feeding species and we hope that the on-going expansion of these species will be followed by others in more detail. Posting photos of mines with accurate host data on observation sites such as https://observation.org/ is a useful way to make your records known.

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Supplementary material 1

Specimen and Locality Data *Antispila*.

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Data type: Excel file

Explanation note: Specimen data.

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Link: https://doi.org/10.3897/nl.41.22264.suppl1

Supplementary material 2

Figure S1. *Cornus* species, details of leaf underside. **a** *C. sanguinea* subsp. *sanguinea*, fresh leaf, Netherlands, Leiden, 26.ix.2017. **b** *C. sanguinea* subsp. *australis*, dried leaf, Greece, RMNH.INS.41827. **c** *C. mas,* fresh leaf, Netherlands, Leiden, 26.ix.2017. **d** *C. sericea*, dried leaf, Netherlands, RMNH.INS.41493. **e** *C. officinalis*, dried leaf, UK, Wisley 1996, Wisley herbarium.

Figure S2. NJ tree of barcodes, from BOLD.

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Data type: Adobe PDF file.

Explanation note: Tree topology for the phylogenetic hypothesis adopted, to be used as input in applications reading nexus (requires some slight previous edition).

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