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# Three new species of Fancy Case caterpillars from threatened forests of Hawaii (Lepidoptera, Cosmopterigidae, *Hyposmocoma*)

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## Abstract

The endemic Hawaiian moth genus *Hyposmocoma* includes 348 described species and perhaps twice as many that remain undescribed. The genus is unusual within Lepidoptera in that its larvae create distinctive silk cases in which they perambulate while protected and camouflaged. An extraordinary diversity of case types exists, and to date more than ten different types have been identified, each corresponding roughly to a separate evolutionary lineage. In this study, we describe three new species of *Hyposmocoma*: *H. ipohapuu* **sp. n.** from Big Island*, H. makawao* **sp. n.** from Makawao Forest Reserve in Maui and *H. tantala* **sp. n.** from Mt. Tantalus, Oahu*,* all of which produce tubular purse cases during their larval stage. We also describe the female of *H. inversella* Walsingham, which was previously undescribed, and re-describe two closely related species*, H. auropurpurea* Walsingham and *H. nebulifera* Walsingham, neither which have been formally described in recent years. We present for the first time, primer sequences for a 705 bp fragment of CAD, designed for *Hyposmocoma* and relatives. The molecular phylogeny based on mitochondrial and nuclear loci demonstrates that all are distinct species. The discovery of a new, endemic species from Mt. Tantalus, an area with many invasive species, suggests that even relatively degraded areas in Hawaii would be worthy of active conservation efforts.

## Keywords

Case-bearing, endemism, Hawaii, conservation, Microlepidoptera, moth, new species

## Introduction

*Hyposmocoma* Butler, 1881 includes 348 described species endemic to the Hawaiian Islands. The archipelago is one of the most threatened species "diversity hotspots" in the world, and large endemic radiations, including *Hyposmocoma*, are thought to have already lost many of their species (e.g. Carson et al. 1990; Freed et al. 1987; Jordan et al. 2003; Zimmerman 1978). The total number of *Hyposmocoma* species may be greater than the radiation of *Drosophila* (Kaneshiro 1997), as many species in this moth genus appear to be endemic to a single valley or volcano within an island (Rubinoff and Schmitz 2010). It is therefore imperative that taxonomic and biodiversity studies describe and catalog the remaining *Hyposmocoma*, as they likely provide a unique and important perspective on diversification on isolated island systems.

Unlike most cosmopterigids which are internal feeders of leaves, seeds and stems (Hodges 1998), *Hyposmocoma* feed externally. Species in the genus have extraordinary, in some cases globally unique, life-histories and behavior. Some caterpillars are carnivorous, feeding on tree snails (Rubinoff and Haines 2005) and others are amphibious living above and under water along streams (Rubinoff and Schmitz 2010; Schmitz and Rubinoff 2011b). Larvae in the genus create silk "cases" in which they perambulate while protected and camouflaged. An extraordinary diversity of larval case types exists, and to date, more than ten different types have been identified (e.g., Rubinoff and Schmitz 2010; Schmitz and Rubinoff 2008; 2009; 2011a; Zimmerman 1978). In this paper, we focus on the "tubular-purse" case type, a monophyletic group basal to most other lineages, such as the burrito and cone cases (Rubinoff and Schmitz 2010).

The total diversity of tubular-purse case *Hyposmocoma* remains largely unknown because historic sampling has focused primarily on the adult and there are very few records of larvae. From our examination of specimens in the Bishop Museum, Honolulu (BPBM), University of Hawaii Insect Museum (UHIM) and Smithsonian National Museum, Washington D.C. (USNM), there are seven species of tubular purse case *Hyposmocoma* that have been described prior to this study. These include, but may not be limited to the following species on the following islands: *H. auropurpurea* Walsingham, 1907 (Oahu), *H. ekemamao* Schmitz & Rubinoff, 2009 (Laysan), *H. fuscopurpurea* Walsingham, 1907 (Maui), *H. inversella* Walsingham, 1907 (Oahu), *H. mokumana* Schmitz & Rubinoff, 2009 (Necker), *H. nebulifera* Walsingham, 1907 (Oahu), and *H. rubescens* Walsingham, 1907 (Kauai).

Here we describe three new species of tubular purse case *Hyposmocoma*, *H. ipohapuu* Kawahara & Rubinoff, sp. n.*, H. makawao* Kawahara & Rubinoff, sp. n. and *H. tantala* Kawahara & Rubinoff, sp. n. from the islands of Oahu, Maui and Hawai'i. While it is best to incorporate new species descriptions as part of a thorough revision, we have chosen to describe new species separately because of the sheer diversity of *Hyposmocoma* and the urgent conservation need of the genus. We present the first molecular phylogeny of purse-cased *Hyposmocoma* to shed light on the relationships between purse-cased species and assess its phylogenetic placement. We chose an integrative approach of combining traditional morphological data with molecular characters

(Dayrat 2005; Rubinoff 2006; Will et al. 2005). This is part of an ongoing project to document the extraordinary diversity and ecology of *Hyposmocoma* across the Hawaiian Islands (e.g. Rubinoff 2008; Rubinoff and Haines 2005; Rubinoff and Schmitz 2010; Schmitz and Rubinoff 2008; 2009).

## Methods

### Specimen preparation, dissection, and description

Genitalia preparation techniques and morphological terminology follow previous work on *Hyposmocoma* (e.g. Rubinoff and Haines 2006; Schmitz and Rubinoff 2008; 2009). The abdomen of the dried moth was placed in a 20% KOH solution at room temperature overnight. When fats were not fully dissolved, the KOH solution and abdomen was heated on a hot plate. Genitalia were dissected and transferred to a solution of lactic acid with Orange G to stain hard cuticle. Membranes of the female genitalia were subsequently stained with Chlorazol Black, fixed in a sequence of 30%, 70%, and 95% ethyl alcohol, and mounted on slides with Euparal. Genitalia of two specimens, a female of *H. ipohapuu* and a male of *H. makawao* were prepared at the beginning of this study and were stained only with Orange G. Forewing and case lengths were measured with a scale in the ocular of a Leica MZ16 stereomicroscope. Adult moths were photographed with a Canon D50 digital camera mounted to the microscope; genitalia were photographed with an Olympus QColor3 Camera mounted on an Olympus SZX10 stereomicroscope. Types of previously described *Hyposmocoma* were examined from the collections of the Bernice P. Bishop Museum (BPBM) and the University of Hawaii Insect Museum (UHIM) on the University of Hawaii Manoa campus. Non-type specimens were examined from these museums and the US National Museum of Natural History (USNM). Types of the new species described here are deposited in the UHIM. All specimens in this study were reared from larvae, which were fed carrots and fish flakes and stored in Petri dishes until emergence.

### Molecular phylogenetic analysis

We included sequences of eight species of tubular purse-cased *Hyposmocoma*: *H. auropurpurea*; *H. ekemamao*; *H. inversella*; *H. mokumana*; *H. nebulifera*; and three new species: *H. ipohapuu*, *H. makawao* and *H. tantala*. We also included sequences for six outgroups: *H. alliterata* Walsingham, 1907; *H. carnivora* Schmitz & Rubinoff, 2011a; *H. eepawai* Schmitz & Rubinoff, 2011b; *H. laysanensis* Schmitz & Rubinoff, 2009; *H. nihoa* Schmitz & Rubinoff, 2009; and *H. nohomaalewa* Schmitz & Rubinoff, 2011a; all known to be distantly related to purse-cased *Hyposmocoma* (Rubinoff and Schmitz 2010). Taxa were sequenced for three genes, the mitochondrial *Cytochrome c Oxidase I* (COI; 762 bp), and two nuclear genes: the *Carbomoylphos-*

*phate Synthase* domain of CAD (705 bp), and *Elongation factor 1-alpha* (EF-1α; 771 bp), a concatenated sequence totaling 2,238 bp. We included data from both nuclear and mitochondrial genomes as they have different processes of selection, recombination and inheritance (Rubinoff 2006; Rubinoff and Holland 2005). All sequences included in this study are listed along with Genbank accession numbers in Table 1.

Genomic DNA was extracted from all specimens using the DNeasy™ animal blood and tissue extraction kit following recommended protocols (Qiagen, Inc., Valencia, CA). The tissue was digested at 56° C for 24 hours, 200 ml of EB buffer was used to elute the

**Table 1.** Taxa sequenced for the present study along with UH log, extract, and GenBank accession numbers. An asterisk indicates a species that constructs a tubular case.

| Species | UH Log No. | Extract No. | CAD | EF-1α | COI |
|---|---|---|---|---|---|
| *H. alliterata* Walsingham, 1907 | DR08J7H | DN112 | GU560396 | GU560714 | GU560555 |
| *H. auropurpurea* Walsingham, 1907* | DR09A3I | DN1315 | JQ182760 | - | JQ231052 |
| | DR09J1A | DN1219 | JQ182756 | JQ231030 | JQ231047 |
| | DR09J1A | DN1329 | JQ182763 | JQ231038 | JQ231058 |
| | DR09J1A | DN1331 | - | JQ231040 | JQ231060 |
| | DR09K1A | DN1202 | JQ182754 | JQ231028 | JQ231045 |
| | DR09K1A | DN1330 | - | JQ231039 | JQ231059 |
| | DR10C17B | DN1262 | JQ182758 | JQ231032 | JQ231049 |
| | DR10C17B | DN1328 | JQ182762 | JQ231037 | JQ231057 |
| *H. carnivora* Schmitz & Rubinoff, 2011a | DR08F5A | DN50 | GU560342 | GU560660 | GU560501 |
| *H. eepawai* Schmitz & Rubinoff, 2011b | DR09B7B | DN156 | JQ182764 | JQ231041 | JQ231061 |
| *H. ekemamao* Schmitz & Rubinoff, 2009* | DR07I2F | PS09 | GU560311 | GU560631 | GU560472 |
| | DR09H6A | DN1193 | JQ182753 | JQ231027 | JQ231044 |
| *H. inversella* Walsingham, 1907* | DR09A3I | DN1314A | JQ182759 | - | JQ231050 |
| | DR09A3I | DN1314B | - | - | JQ231051 |
| *H. ipohapuu* Kawahara & Rubinoff, sp. n.* | DR08K8C | DN121 | GU560405 | GU560723 | GU560564 |
| | DR08K8C | DN1321 | - | JQ231036 | JQ231056 |
| | DR08K8C | KY2 | - | - | JQ231063 |
| *H. laysanensis* Schmitz & Rubinoff, 2009 | DR07I2D | DN31 | GU560320 | GU560640 | GU560481 |
| *H. makawao* Kawahara & Rubinoff, sp. n.* | DR08K12A | DN114 | JQ182752 | JQ231026 | JQ231043 |
| | DR08K12A | DN1320 | - | JQ231035 | JQ231055 |
| *H. mokumana* Schmitz & Rubinoff, 2009* | DR04I1 | CP96 | GU560267 | GU560601 | GU560442 |
| | DR04I1 | CP97 | GU560268 | GU560602 | GU560443 |
| | DR04I1 | CP98 | GU560269 | GU560603 | GU560444 |
| *H. nebulifera* Walsingham, 1907* | DR09J2B | DN1220 | JQ182757 | JQ231031 | JQ231048 |
| | DR09A3K | DN118 | GU560402 | GU560720 | GU560561 |
| | DR09A3K | DN161 | JQ182765 | JQ231042 | JQ231062 |
| *H. nihoa* Schmitz & Rubinoff, 2009 | DR07G10 | TT05 | GU560312 | GU560632 | GU560473 |
| *H. nohomaalewa* Schmitz & Rubinoff, 2011a | DR09B12D | DN1215 | JQ182755 | JQ231029 | JQ231046 |
| *H. tantala* Kawahara & Rubinoff, sp. n.* | DR10H2I | DN1317 | JQ182761 | JQ231033 | JQ231053 |
| | DR10H2I | DN1319 | - | JQ231034 | JQ231054 |

DNA and extracts were stored at -20° C. COI was amplified in a single fragment: the forward primer Jerry (CAA CAT TTA TTT TGA TTT TTT GG) and reverse primer Pat-2 (TCC AAT GCA CTA ATC TGC CAT ATT A; Simon et al. 1994), and for EF-1α, a single fragment with the forward primer Coma (GGC CCA GGA AAT GGG CAA AGG) and reverse primer Toes (GGA GTC WCC AGC KAC GTA ACC; Rubinoff 2008), and for CAD the primers Cue (GGG ATT TAG CAA AAT TCA AAC GC) and Eh (CCT ATC GCT CAT ATC ATA ATC GG). The primers Cue and Eh were not published previously and thus described here. Amplifications were performed in a total volume of 25 μl. Thermal profiles for COI and CAD (94°C for 1 min; 51°C for 1 min; 72°C for 2 min), differed from EF-1α (94°C for 1 min; 53°C for 1 min; 72°C for 2 min). Each reaction started with 2 min of denaturation at 94°C, for 34 cycles, and followed by a final elongation for 12 min at 72°C. All PCR-products were visualized on 1% agarose gel and purified using QIAquick® spin columns (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. Sequencing was conducted with an Applied Biosystems 3130xl Genetic Analyzer at the Greenwood Molecular Biology Facility, Pacific Biosciences Research Center, the University of Hawaii at Manoa. For each sample, both sense and anti-sense strands of PCR products were sequenced. Multiple sequence alignments were conducted using MAFFT ver. 6.843 (Katoh 2010) and assembled in Geneious ver. 5.1 (Drummond et al. 2010). Genbank accession numbers are listed in Table 1.

Phylogenetic analyses were conducted with maximum likelihood (ML) as implemented in GARLI 1.0 (Zwickl 2006), and in a Bayesian framework with the software MrBayes ver. 3.1.2 (Ronquist and Huelsenbeck 2003). We used jModelTest (Posada 2008) to determine the best substitution model which was always the General-Time-Reversible (GTR) model (Lanave et al. 1984; Tavaré 1986), incorporating among-site rate heterogeneity modeled according to a gamma (G) distribution (Yang 1994) while allowing for a proportion of invariable sites (I) (Gu et al. 1995). One thousand ML and bootstrap tree searches were conducted as implemented in GARLI. To expedite tree searches, we used Grid computing (Cummings and Huskamp 2005) through The Lattice Project (Bazinet and Cummings 2009). GARLI analyses were conducted using the Lattice Grid portal (Bazinet and Cummings 2011). Bayesian analyses were conducted for $5 \times 10^7$ generations with default settings and $3 \times 10^7$ generations were treated as burn-in and removed. Chain convergence was assessed with MrBayes and AWTY Online (Wilgenbusch et al. 2004).

## Species descriptions

### *Hyposmocoma auropurpurea* Walsingham, 1907
http://species-id.net/wiki/Hyposmocoma_auropurpurea
Figs 1, 11, 18

**Diagnosis.** This species is unique among species of *Hyposmocoma* because it has metallic purple wings with a narrow, diagonal orange band near the wing apex.

**Re-description.** Male. (n = 2; Fig 1). Forewing length 4.8–5.0 mm. Head with a mixture of copper and metallic purple colored scales arranged radially from compound eye. Haustellum with light brown scales. Maxillary palpus reduced. Labial palpus curved with copper and metallic purple colored scales, scale color similar on all surfaces of palpus. Antenna brown with a mosaic of metallic purple scales. Thorax mostly copper; dark brown/purple scales present along anterior margin. Foreleg coxa with brown and metallic purple scales; femur, tibia, and tarsomeres mostly dark brown. Midleg as foreleg, but spurs covered in a mixture of dark and light-brown scales. Hindleg as midleg, but longer with long hairs on dorsal margin of tibia. Forewing metallic with a narrow diagonal orange band near wing apex. Diffuse orange patch in proximal region of FW along anal margin. Fringe orange and brown/metallic purple, longer scales tending to be brown/metallic purple, shorter ones orange. Hindwing brown with brown fringe. Abdomen dorsally dark brown; ventrally metallic brown/purple, with tuft of long dark brown scales covering lateral surface of genitalia.

**Male genitalia** (Fig. 11). Right brachium of uncus sickle shaped and curved at 1/2, tapering gradually, heavily sclerotized. Left brachium small, not sclerotized. Tegumen wide and sclerotized. Valvae roughly symmetrical, two long thin setae arising from approximately 2/3 length along dorsal margin of both valvae. Dense row of fine, hair-like setae disposed comblike along inner ventral margin of valva. Phallus stout, heavily sclerotized, open ventrally, tapered, and bent ventrad at about 1/2 of length; vesica without spines or cornuti. Anellus with two symmetrical rounded lobes with short setae.

**Female.** (n = 4). Externally as male, forewing length 5.2–5.5 mm.

**Female genitalia.** (Fig 18). Papillae anales very short and setose. Anterior and posterior apophyses slender, long, posterior apophyses about same length as anterior apophyses. Ostium bursae small, heavily sclerotized, externally protruding, roughly triangular, not snail-shaped. Corpus bursae oval and elongate, with very light scobination; signum absent. Ductus bursae long and narrow, slightly twisted, about equal in length of corpus bursae. Apical margin of tergum VIII with median emargination.

**Larval case** (n = 7). Dark brown, smooth, 7–9 mm in length and 1.7–2 mm wide.

**Material examined.** (2♂, 4♀). 1♂, 1♀: HI: Oahu, Waianae Range, Palikea trail, "purse" case 15-I-09, em. 23-III-2009, #DR09A3I, coll. P. Schmitz, D. Rubinoff, W. Haines, J. Eiben. 1♂, 1♀: HI: Oahu, Waianae Mountains, Palikea area, ~ elev. 850 on *Pittosporum* sp. leaves, 23-X-2009, em. 22-I-2010, #DR09J1A, coll. P. Krushelnycky. 1♀: HI: Oahu, Waianae Mountains, Palikea area, ~elev. 850 m, 5-XI-2009, em. 28-I-2010, #DR09K1A, coll. P. Krushelnycky. 1♀: HI: Oahu, South Waianae Mountains, Palikea area, 800–925 m, 30-III to 8-IV-2010, em 15-V-2010, #DR10C7B, coll. P. Krushelnychy and OANRP staff. All specimens from the UHIM.

**Specimens sequenced.** Eight specimens from Oahu, Hawaii, USA (UH log numbers DR09A3I (1), DR09J1A (3), DR09K1A (2), DR10C17B (2)), extraction codes DN1202, DN1219, DN1262, DN1315, DN1328, DN1329, DN1330, and DN1331.

**Biology.** Case-making larvae were collected from October to April. Larvae were typically found in leaf litter, but in some cases were found on leaves such as *Pittosporum* sp. (Pittosporaceae).

**Distribution.** Known only from the Waianae Mountain Range, Oahu.
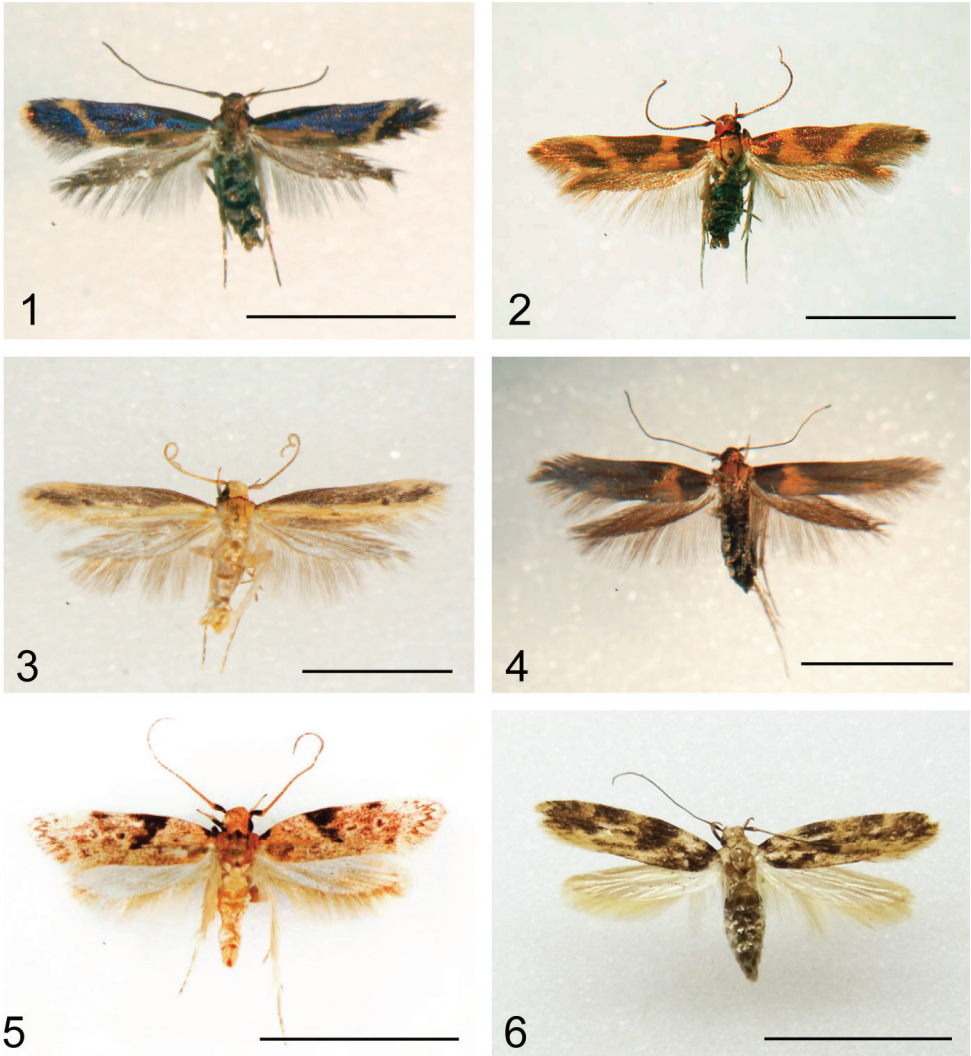
**Figures 1–6.** Purse-cased *Hyposmocoma*. **1** *Hyposmocoma auropurpurea*, male **2** *Hyposmocoma inversella*, male. **3** *Hyposmocoma ipohapuu* sp. n. holotype male **4** *Hyposmocoma makawao* sp. n. holotype male **5** *Hyposmocoma nebulifera*, male **6** *Hyposmocoma tantala* sp. n. holotype female. In order to examine genitalia, the abdomen of each was removed after photographs were taken. Scale bar = 5 mm.

### *Hyposmocoma inversella* Walsingham, 1907
http://species-id.net/wiki/Hyposmocoma_inversella
Figs 2, 17

**Diagnosis.** This species has a large, orange, "V"-shaped marking on the forewing found in no other described species of *Hyposmocoma*.

**Re-description.** Male. (n = 1; Fig 2). Forewing length 4.9 mm. Head with copper-colored scales arranged radially from compound eye. Haustellum with light brown

scales. Maxillary palpus reduced. Labial palpus curved with copper colored scales, scale color similar on all surfaces of palpus. Antenna brown with a mosaic of copper scales. Thorax mostly copper; dark brown scales present along anterior margin. Foreleg coxa with brown and copper scales; femur, tibia, and tarsomeres mostly dark brown with light brown ring at distal margin of femur, tibia, and tarsomeres I-V. Midleg as foreleg, but spurs covered in a mixture of dark and light-brown scales. Hindleg as midleg, but longer with long hairs on dorsal margin of tibia. Forewing brown, two transverse orange fascia form a "V". Proximal orange band widens along anal margin, distal band uniform in width. Fringe orange and brown, longer scales tending to be brown, shorter ones orange. Hindwing brown with brown fringe. Abdomen dorsally dark brown; ventrally copper, with tuft of long dark brown scales covering lateral surface of genitalia.

**Male genitalia.** Right brachium of uncus sickle shaped, slender, heavily sclerotized, widening at 1/2 length, slightly twisted to left. Left brachium small, not sclerotized. Tegumen wide and sclerotized. Four long thin setae along dorsal margin of left valva, three shorter setae at same location of right valva. Left valva slightly wider than right. Dense row of fine, hair-like setae disposed along inner ventral margin of valva. Phallus stout, blunt tipped, heavily sclerotized, open ventrally, and bent ventrad at about 1/2 of length; vesica without spines or cornuti. Anellus with two symmetrical rounded lobes with short setae.

**Female.** (n = 1). Externally as male, forewing length 4.7 mm.

**Female genitalia.** (Fig 17). Papillae anales short and setose. Anterior and posterior apophyses slender, long, anterior apophyses slightly longer than posterior apophyses. Ostium bursae small, heavily sclerotized, externally protruding, roughly triangular, not snail-shaped. Corpus bursae oval and elongate, with very light scobination; signum absent. Ductus bursae long and narrow, slightly twisted, about equal in length of corpus bursae. Apical margin of tergum VIII with median emargination.

**Larval case.** (n = 2). Dark brown, smooth, 7.8–8.5 mm in length and 1.8–2 mm wide. The larval case is very similar to that of *H. auropurpurea*. Though both species are found in the Waianae Mountains, adult morphology and DNA sequence confirms these are distinct species.

**Material examined.** (1♂, 2♀). 1♂: HI: Oahu, Waianae Range, Palikea Trail, "purse" case, 15-I-2009, emergence. 16-III-2009, #DR09A3I, coll. P. Schmitz, D. Rubinoff, W. Haines, J. Eiben. Specimen in perfect condition. Male genitalia slide #AK100. 1♀: same data as male. Female genitalia slide #AK101. 1♀: HI: Oahu, Pahole NAR. Northern Waianae Mts. Elev. 500 m, on *Pipturus albidus* (Urticaceae). 14-XII-2010, #DR10L1C, em. 24-III-2011, coll. P. Krushelnycky. Additional voucher collection #AR0803, spec/lot #PKSP11751. Molecular ID: AK-259-11. All specimens from the UHIM.

**Specimens sequenced.** Two specimens from Oahu, Hawaii, USA (UH log number DR09A3I), extraction codes DN1314A, DN1314B.

**Biology.** Case-making larvae were collected in the Waianae Mountains of Oahu on the Palikea Trail during December and January. Larvae were found in leaf litter and on rotting logs. Adult emergence occurred between March and April. Because we have

collected fairly extensively in the Waianae Mountains, we predict that this species univoltine, with larvae active during the winter months and adults emerging in the spring.

**Distribution.** Known only from the Waianae Mountain Range, Oahu.

### *Hyposmocoma ipohapuu* Kawahara & Rubinoff, sp. n.

urn:lsid:zoobank.org:act:DBFC2894-9314-41EE-81BB-F19198CA3F6F
http://species-id.net/wiki/Hyposmocoma_ipohapuu
Figs 3, 7, 14, 19

**Diagnosis.** *Hyposmocoma ipohapuu* has a forewing pattern that differs from any other described species in the genus. A wide pale yellow band is present along the anal margin of the forewing and curves proximally at the wing margin towards the costa. A small, round, dark brown mark is present approximately two thirds of the way from the wing base to the apex.

**Description. Male** (n = 2; Fig. 3). Forewing length 5.0 mm. Head with pale brown scales radiating from compound eye. Haustellum with a mixture of light and dark brown scales. Maxillary palpus reduced. Labial palpus recurved and covered in pale brown scales, which are dark laterally. Antennal flagellum pale brown, with a ring of dark brown scales extending from the proximal margin of each flagellomere. Thorax mainly pale brown, dark brown at cervical margin and near posterior margin of notum. Foreleg dark brown with pale brown ring at distal margin of femur. Midleg as foreleg, but with pale brown ring at distal margin of tibia and tarsomeres I-V, spurs pale brown. Hindleg as midleg but longer with long pale brown hairs. Forewing pale brown, with dark brown band extending from costal margin of wing base to apex, mark forming a convex arch near apex. A separate brown band extends along anal margin of forewing. Fringe pale brown. Hindwing and hindwing fringe grayish brown. Abdomen dorsally dark brown; ventrally pale brown with a tuft of long pale brown scales on either side of genitalia.

**Male genitalia.** (Fig. 14). Right brachium of uncus sickle shaped, rather thick, heavily sclerotized, slender at apical 1/3, slightly twisted to left. Left brachium small, not sclerotized. Tegumen wide and sclerotized. Valvae asymmetrical, left valva slightly wider at middle than right, with three long sclerotized club-shaped setae along dorsal margin curving posteriorly. Dorsal margin of right valva smooth, lacking setal sockets. Phallus stout, blunt tipped, heavily sclerotized, and bent ventrad at about 1/2 of length; vesica without spines or cornuti. Anellus with two symmetrical rounded lobes, thin until apex, both adorned with few small setae.

**Female.** (n = 1). Externally as male, but with longer forewing length (5.8 mm) and small dark brown mark medially below dark band on forewing.

**Female genitalia.** (Fig 19). Papillae anales long and setose. Anterior and posterior apophyses thin and straight, slightly enlarged at posterior end, posterior apophyses about 2x length as anterior apophyses. Ostium bursae small, heavily sclerotized, externally protruding into a triangle, not snail-shaped. Corpus bursae oval with light
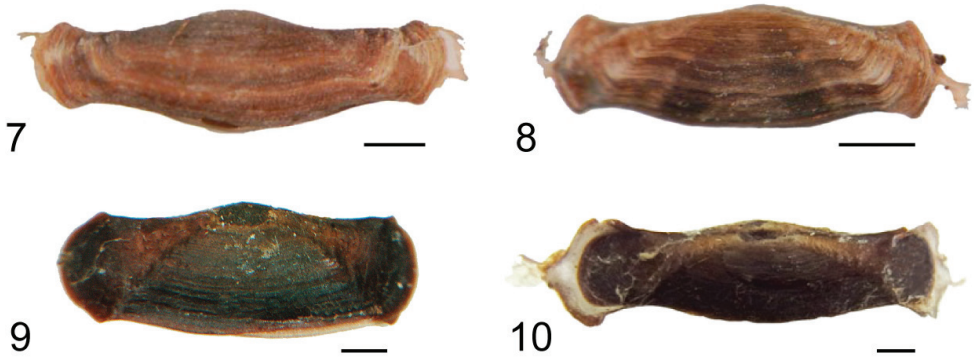
**Figures 7–10.** Larval cases of some purse-cased *Hyposmocoma*. **7** *H. ipohapuu* sp. n. **8** *H. makawao* sp. n. **9** *H. nebulifera* **10** *H. tantala* sp. n. Scale bar = 1 mm.

scobination; signum absent. Ductus bursae thick, approximately 1/2 length of corpus bursae. Apical margin of tergum VIII with median emargination.

**Larval case.** (n = 5; Fig 7). The mature case is 5.9–6.6 mm in length and 1.2–1.5 mm wide, smooth with banding that follows the length of the case. The case widens slightly at both ends.

**Material examined. Holotype:** ♂, [1] Printed white label: 'H[AWAI]I: Hawaii, Volcano village | Pearl ave[nue], on tree fern | "purse" case, X-30-08 | em[ergence]. III-13-[20]08, #DR08K8C | coll[ector]. J[esse]. Eiben, M[elissa]. Dean'; [2] Printed red label: 'HOLOTYPE | *Hyposmocoma ipohapuu* Kawahara & Rubinoff'. Male genitalia slide #AK110. Specimen in perfect condition. **Paratypes:** (1♂, 1♀). 1♂: same data as holotype but emergence 2-March-2009. 1♀: Data same as male paratype, but emergence 13-March-2009. Female genitalia slide #AK114. Cases (5): same data as holotype. All pinned specimens and slides deposited in the UHIM.

**Specimens sequenced.** Three specimens from Big Island, Hawaii, USA (UH log number DR08K8C), extraction codes DN121, DN1321 and KY2. This species was tentatively called "Hsp69" before given this formal name.

**Etymology.** This species is named "ipohapuu", which means "tree-fern lover" in Hawaiian.

**Biology.** Case-making larvae were collected on the abaxial surface of old fronds of a Hawaiian tree fern (Hāpuʻu, *Cibotium glaucum* (Sm.) Hook. & Arn., Cibotiaceae) in the rain forest at Volcano Village near Hawaii Volcano National Park, Hawaii (Big) Island. The habit of residing in old tree fern fronds, still attached to the stump, is typical of several purse case species including *H. filicivora* Meyrick, 1935.

**Distribution.** Known only from Volcano Village, Hawaii Island. Probably restricted to the rainforest areas around Hawaii Volcanoes National Park, where it appears to be uncommon. The elevation for the type locality is approximately 1200 m.

## *Hyposmocoma makawao* Kawahara & Rubinoff sp. n.

urn:lsid:zoobank.org:act:98EE02FA-5A90-4675-A4E2-A2236F8787FC
http://species-id.net/wiki/Hyposmocoma_makawao
Figs 4, 8, 15

**Diagnosis.** *Hyposmocoma makawao* differs from any other species in the genus. No other species has a single, thick, transverse orange band near the base of the forewing.

**Description. Male.** (n = 1; Fig 4). Forewing length 4.8 mm. Head brown with iron-red scales near outer margin of eye; scales large near vertex. Haustellum pale brown. Maxillary palpus reduced. Labial palpus curved with pale brown scales, scales dark brown along lateral margin of labial palpus. Antennal flagellum dark brown along dorsal surface, lighter brown ventrally. Thorax reddish brown, laterally brown with patches of lighter brown scales below forewing. Foreleg and midleg with brown scales, scales dark brown laterally. Hindleg same as midleg, but with long scales along dorsal margin. Spines on legs light brown. Forewing dark brown with a single, wide fiery red-brown transverse fascia one fourth from the base of the wing to apex and narrowing towards costal margin. Abdomen covered in dark brown scales dorsally, light brown scales covering ventral surface.

**Male genitalia.** (Fig 15). Right brachium of uncus sickle shaped, slender, heavily sclerotized, widening slightly at 1/2 length, slightly twisted to left. Left brachium small, not sclerotized. Tegumen wide and sclerotized. Valvae asymmetrical, left valva slightly wider than right, valvae without large sclerotized setae or sockets along dorsal margin. Dense row of fine, hair-like setae along inner ventral margin of both valvae. Phallus stout, blunt tipped, heavily sclerotized, open ventrally, and bent ventrad at about 1/2 of length; vesica without spines or cornuti. Anellus with two symmetrical rounded lobes with fine setae.

**Female.** (n = 1). Same as male, but with forewing length 4.9 mm.

**Larval case.** (n = 7; Fig 8). The case is 4.1–5.0 mm in length and 1.2–1.6 mm wide, smooth with banding that follows the length of the case. Two wide, dark bands form a "V" that crosses over the central region of the case.

**Material examined. Holotype:** ♂, [1] Printed white label: 'H[AWAI]I: Maui, Makawao Forest Reserve | elev[ation]: 3500 ft, on Koa branches | "purse" case, I-30-[20]09, #DR08K12A | coll[ector]. W[illiam]. Haines'; [2] Printed red label: 'HOLO-TYPE | *Hyposmocoma makawao* Kawahara & Rubinoff'. Male genitalia slide AYK103. Specimen in perfect condition. **Paratype:** 1♀: same data as holotype, but emergence date 13-Feb-2009, abdomen missing. Cases (7): same data as holotype. All specimens stored in the UHIM.

**Specimens sequenced.** Two specimens from Maui with UH log number DR-08K12A, extraction codes DN114, DN1320. The specimen from which extract DN114 was taken was tentatively called "Hsp67" before being given this formal name.

**Etymology.** This species is named "*makawao*" after its type locality, Makawao Forest Reserve, Maui.

**Biology.** Case-making larvae were collected at Makawao Forest Reserve (MFR), Maui. Samples were collected using a beat-sheet, placed under branches of the koa tree (*Acacia koa* Gray).

**Distribution.** Known only from the MFR, which harbors an extraordinary diverse natural fauna and flora, and is the only known locality for several species of *Hyposmocoma*, including *H. domicolens* (Butler, 1881), *H. molluscivora* Rubinoff & Haines, 2005, *H. opuulaau* Schmitz & Rubinoff, 2011, *H. pukoa* Schmitz & Rubinoff, 2011, and *H. pupumoehewa* Schmitz & Rubinoff, 2011. Unfortunately, the native habitat has been in sharp decline, even during the short course of this project, due to ongoing damage from invasive ungulates. If conservation action is not soon taken, it is likely that many of the rare plants and animals that remain in this mixed mesic forest will disappear.

### *Hyposmocoma nebulifera* Walsingham, 1907
http://species-id.net/wiki/Hyposmocoma_nebulifera
Figs 5, 9, 12

**Diagnosis.** *Hyposmocoma nebulifera* is similar to *H. rubescens* from Kauai, but differs in having a larger dark brown C-shaped forewing mark, and much smaller spurlike specialized setae on the valva. It is also similar to *H. ekemamao* but is larger, and has two central round spots on the forewing, while *H. ekemamao* only has one.

**Re-description. Male.** (n = 3; Fig 5). Forewing length 5.8 – 6.0 mm. Head light brown with scales near outer margin of eye, scales large near vertex. Haustellum pale brown. Maxillary palpus reduced. Labial palpus curved with pale brown scales, scales dark brown at terminus. Antennal flagellum light with dark brown bands. Thorax light brown, with lighter brown scales on tegula. Foreleg and midleg with brown scales and bands of light brown. Hindleg same as midleg, but with long scales along dorsal margin formed into a brush-like patch. Spines on legs light brown. Forewing light brown with a C-shaped dark brown mark 1/3 of distance to forewing apex, dark brown spot at base of forewing. One dark brown mark with light border at center of wing, another mark about 1/3 distance between first mark and apex. Abdomen covered in brown scales.

**Male genitalia.** (n = 1; Fig. 12). Right brachium of uncus sickle shaped, thin, long, and slightly twisted to left. Tegumen wide and sclerotized. Valvae asymmetrical, left valva medially slightly wider than right. Valvae without large sclerotized setae, but adorned with fine hair-like setae disposed comblike on inner surface of ventral margin. Phallus stout, blunt tipped, heavily sclerotized, and gradually curved ventrad at approximately 2/3 of length; vesica without spines or cornuti. Anellus with two symmetrical rounded lobes, thin until apex, both adorned with few small setae.

**Female.** (n = 2). Externally as male, but with larger wing span (6.5–7.2 mm).

**Female genitalia.** Papillae anales short and setose. Anterior and posterior apophyses thin, slightly curved, posterior apophyses slightly longer than anterior apophyses. Ostium bursae heavily sclerotized, externally protruding, C-shaped curled left, not triangular. Corpus bursae oval with light scobination; signum absent. Ductus bursae
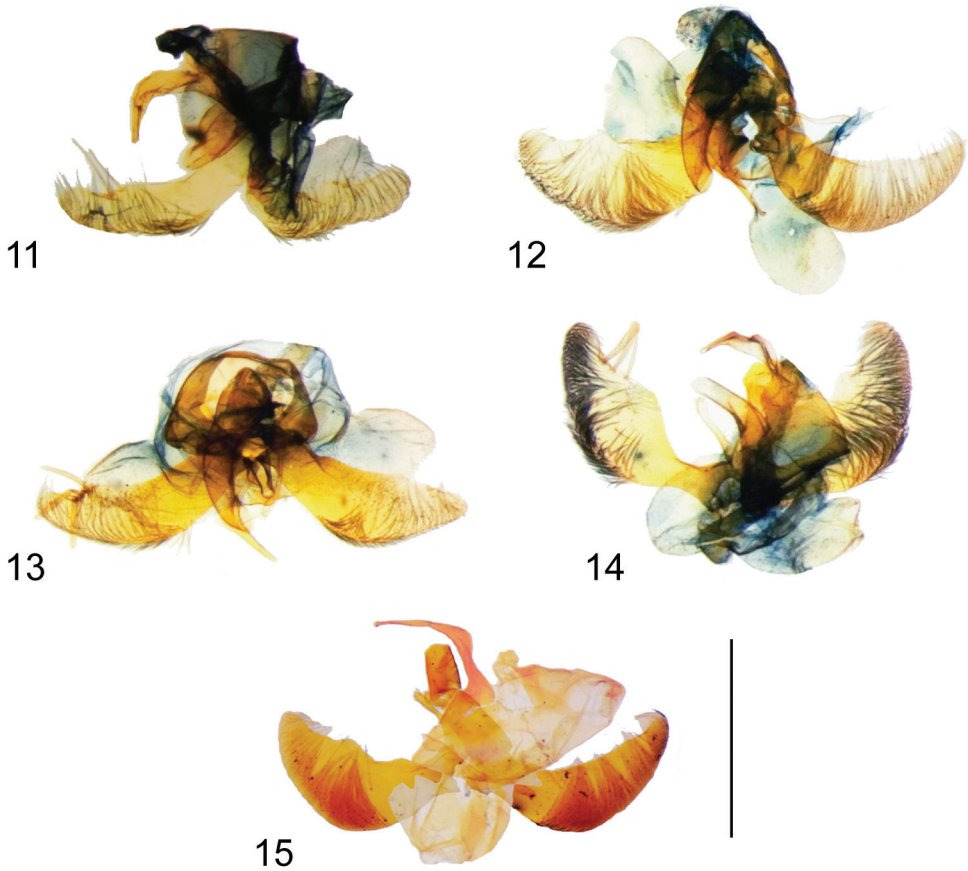
**Figures 11–15.** Male genitalia. **11** *Hyposmocoma auropurpurea* **12** *H. nebulifera* **13** *H. tantala* sp. n. **14** *H. ipohapuu* sp. n. **15** *H. makawao* sp. n. Scale bar = 1 mm. Figs **11–14** stained with Chlorazol Black, Fig. **15** stained with Orange G.

long, narrow, and approximately 2/3 length of corpus bursae. Apical margin of tergum VIII with median emargination.

**Larval case.** (n = 1; Fig. 9). Dark brown, smooth, 8.1 mm in length and 2.5 mm wide.

**Material examined. Paratype:** ♀, [1] Printed white labels: '[HAWAII, Oahu,] Waianae Mts. | 3000 ft. OAHU | Hawaiian Is. | IV. 1892. | Perkins. 225133'; [2] 'Fauna Hawaiiensis | Collection'; [3] Hand written and printed white label: 'Hyposmocoma | nebulifera | Wlsm. | PARA-TYPE 1/3'; [4] BPBM Paratype pink label: 'No 32412 | Hawaiian Coll. | BISHOP Museum' (BPBM). **Non-type material:** 1♂, HI: Oahu, Waianae Mountains, Palikea trail, 15 January 2009, emergence 13 February 2009, #DR09A3K, coll. P. Schmitz, D. Rubinoff, W. Haines, J. Eiben, male genitalia slide #AK113 (UHIM). 1♂, 1♀: HI: Oahu, Waianae Mountains, Palikea area, elev: 850 m, in leaf litter, extracted in Berlese funnel, "purse case", emergence 29 October 2009, #DR09J2B, coll. P. Krushelnycky, female genitalia slide #AK109 (UHIM).

**Specimens sequenced.** Two specimens from Palikea, Oahu, UH log number DR09A3K, extraction codes DN118, DN161. One specimen from Oahu, UH log number DR09J2B, extraction code DN1220. The specimen from which extract DN118 was obtained was tentatively called "Hsp68".

**Distribution.** Known only from the Waianae Mountain Range, Oahu. With nearly thirty described species of *Hyposmocoma* (Zimmerman 1978), the Palikea preserve in the Waianae mountains has a very high diversity of *Hyposmocoma*, and the area is also one of the best-preserved on Oahu. The rich species diversity of *Hyposmocoma* may reflect the diversity throughout the island before human colonization.

### *Hyposmocoma tantala* Kawahara & Rubinoff, sp. n.
urn:lsid:zoobank.org:act:5B0155F3-0795-4706-895B-B728E4C575FC
http://species-id.net/wiki/Hyposmocoma_tantala
Figs 6, 10, 13, 16

**Diagnosis.** *Hyposmocoma tantala* is similar to *H. nebulifera*, but differs in having a dark forewing background color and much thicker dark forewing markings. The male genitalia has large sclerotized spines on the left valva that are absent in *H. nebulifera.*

**Description. Male.** (n = 1). Forewing length 5.5 mm. Head light brown with light brown scales near outer margin of eye; scales large near vertex. Haustellum pale brown. Maxillary palpus reduced. Labial palpus curved with pale brown scales, scales dark brown at terminus. Antennal flagellum light with dark brown bands. Thorax light brown, with lighter brown scales on tegula. Foreleg and midleg with brown scales and bands of light brown. Hindleg same as midleg, but with long scales along dorsal margin formed into a brush-like patch. Spines on legs light brown. Forewing light brown with a large dark brown mark extending from costal margin 1/3 of distance to forewing apex and a smaller dark brown mark at 2/3 of distance to apex. Abdomen covered in brown scales.

**Male genitalia** (Fig. 13). Right brachium of uncus thick and curved ventrad, smooth, gradually tapering, slightly twisted to left. Left brachium small, not sclerotized. Tegumen wide and sclerotized. Shape of valva largely symmetrical, but left valva with three long tapered narrow spurlike setae along dorsal margin near apex; right valva without large sclerotized setae. Dense row of fine, hair-like setae disposed along inner ventral margin of both valvae. Phallus stout, blunt tipped, heavily sclerotized, open ventrally, and bent ventrad at about 1/2 of length; vesica without spines or cornuti. Anellus with two symmetrical rounded lobes with fine setae.

**Female.** (n = 2; Fig 6). Externally as male, but with larger wing span (6.2 mm).

**Female genitalia.** (Fig 16). Papillae anales short and setose. Anterior and posterior apophyses thin and straight, posterior apophyses slightly longer than anterior apophyses. Ostium bursae heavily sclerotized, externally protruding, and C-shaped curled left. Ductus bursae long and of small girth. Corpus bursae roughly kidney-shaped, with light scobination; signum absent. Apical margin of tergum VIII with median emargination.
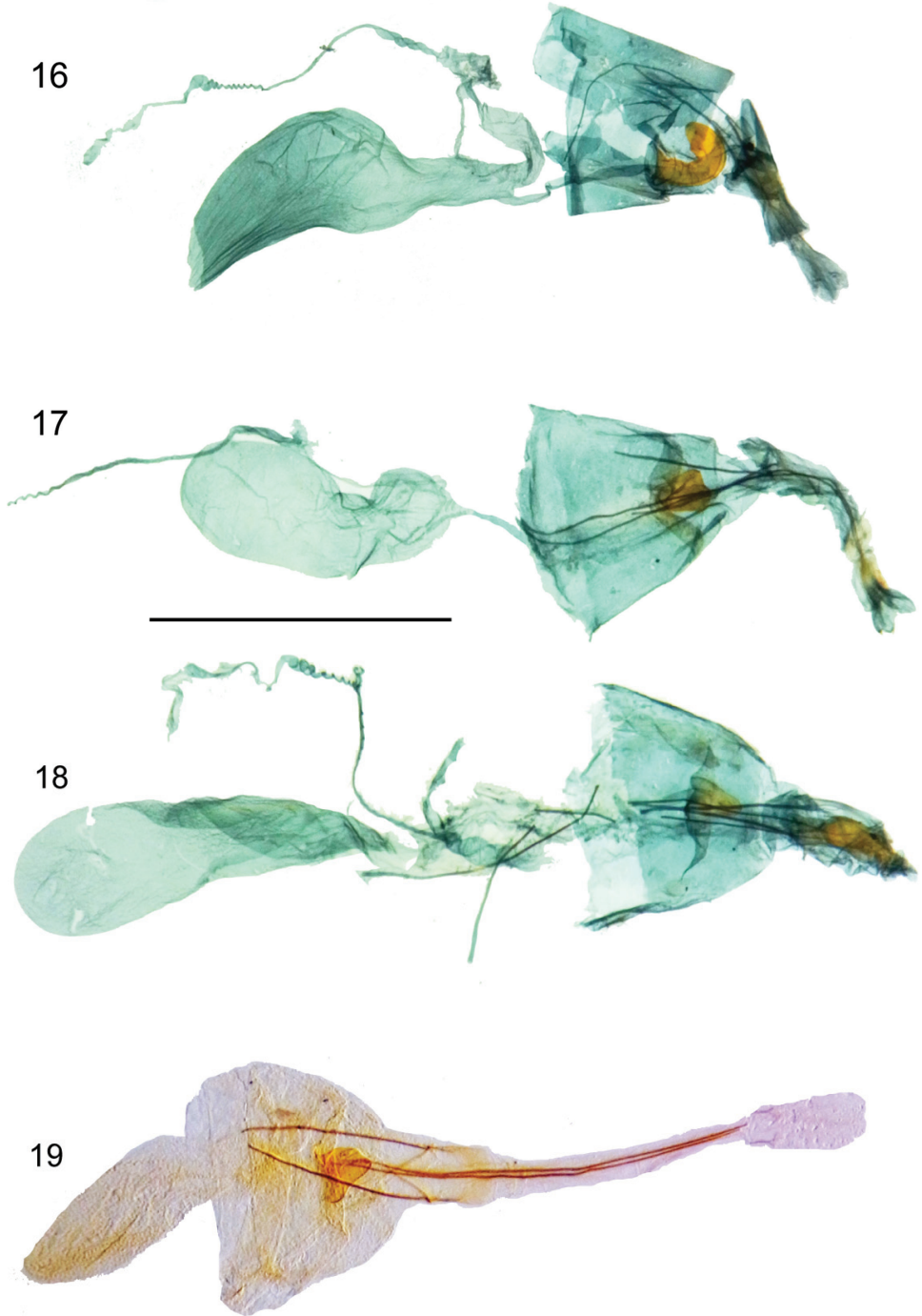
**Figures 16–19.** Female genitalia. **16** *Hyposmocoma tantala* sp. n., lateral view **17** *H. inversella*, ventral view **18** *Hyposmocoma auropurpurea*, ventral view **19** *Hyposmocoma ipohapuu* sp. n., ventral view. Scale bar = 1 mm. Figs **16–18** stained with Chlorazol Black, Fig. **19** stained with Orange G.
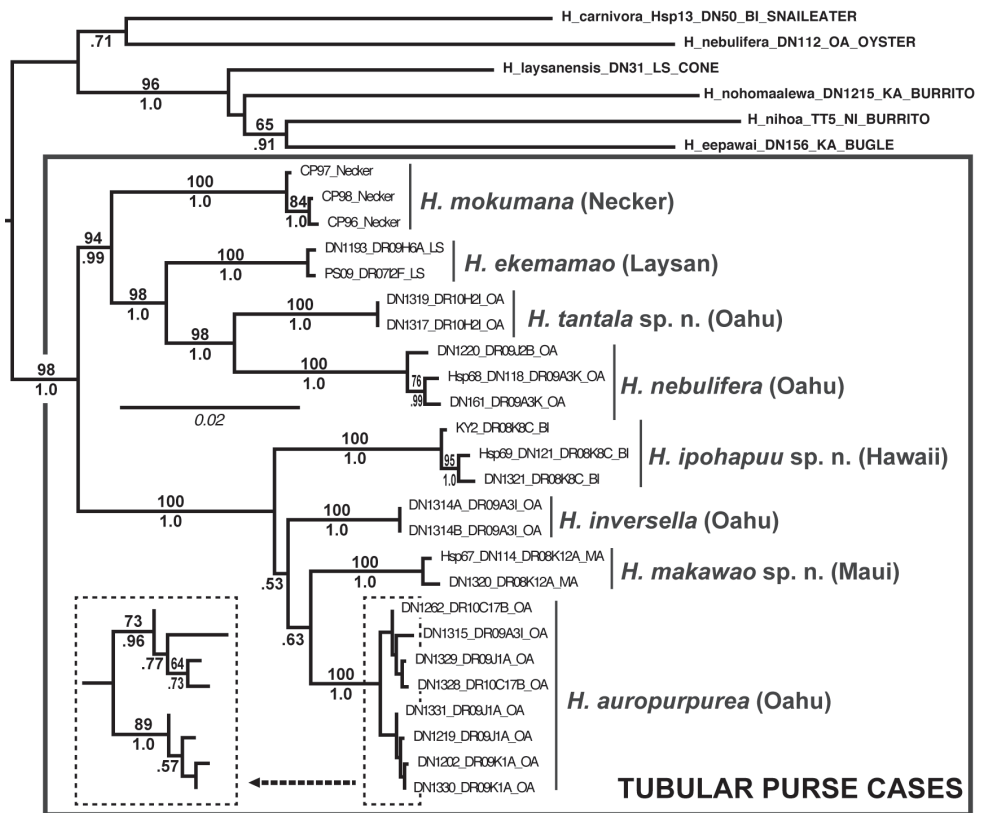
**Figure 20.** ML phylogeny of tubular purse-cased *Hyposmocoma* and relatives. Numbers above branches are ML bootstrap values, numbers below are Bayesian posterior probabilities. Scale bar = 0.02 substitutions/site.

**Larval case.** (n = 1; Fig 10). Dark brown, smooth, 9 mm in length and 2 mm wide.

**Material examined. Holotype:** ♀, [1] Printed white label: 'H[AWAI]I: Oahu, Tantalus, Manoa | Cliffs Trail, n[ea]r. Round Top Dr[ive]. | Purse Case DR10H2I [in bold type] | 22-Aug-2010, em[ergence]. 25-Oct[ober]-2010 | A. Y. Kawahara, W. Haines, | C. Yee, C. Atta collectors; [2] Printed red label: 'HOLOTYPE | *Hyposmocoma tantala* Kawahara & Rubinoff'. Specimen in perfect condition. Female genitalia slide # slide AK108 (UHIM); **Non-type material:** 1♂, HI: Oahu: Mt. Tantalus, 4 km N of Manoa, 600 m., 8-VI-1991, coll. W. E. Steiner et al. (USNM).

**Specimens sequenced.** Two specimens from Oahu (UH log number DR10H2I), extraction codes DN1317, DN1319.

**Etymology.** This species is named "*tantala*" after Mt. Tantalus, from where the type specimen was collected. While Mt. Tantalus has experienced a tremendous amount of destruction from invasive species in the past century, it has historically been a locality with very high endemism, and the type locality of several other endemic insects, including flies (Hardy 1960; 1964), beetles (e.g. Liebherr and Zimmerman 2000), and at least two other species of *Hyposmocoma* (Zimmerman 1978). We wish to recognize this

historically noteworthy site and draw attention to the fact that some endemic species, worthy of conservation, still persist even after long periods of disturbance.

**Biology.** Adults were reared from case-making larvae collected on bark of a damp dead tree covered partially with lichen.

**Distribution.** Known only from Mt. Tantalus, Oahu.

## Conclusion

Our morphological investigation coupled with molecular sequence data supports the separation of all three species described as new in this study. Interspecific genetic divergence in COI for sister-species for other Lepidoptera range from slightly less than 1% to nearly 4% (e.g. Landry et al. 1999; Rubinoff and Powell 2004; Sperling and Hickey 1994, but see also Mitter et al. 2011), suggesting that species of *Hyposmocoma* studied here exhibit relatively high levels of interspecific divergence. This may be due to lineage-specific rates of evolution, and/or differential rates of colonization and extinction in the Hawaiian islands. Future research will focus on the evolutionary processes behind the distribution and diversity of purse-cased *Hyposmocoma* and their relatives.

While we have sampled *Hyposmocoma* broadly on all Hawaiian islands, all species in this study and those previous appear to be largely restricted to one volcano on one island. Unfortunately, restricted ranges pose challenges for conservation since many species are vulnerable to extinction because of widespread habitat destruction. Mount Tantalus on Oahu, the only known locality for *H. tantala*, is the type locality for endemic flies, beetles and at least two other species of *Hyposmocoma*. The area is heavily infested with invasive species, but does retain some endemism, though the native habitat is still declining. The discovery of a new, endemic species from Mt. Tantalus suggests that even relatively degraded areas would be worthy of active conservation efforts to control invasive species. The same is true for the Makawao Forest Reserve on Maui that straddles an area between rainforest and dry forest, and thus supports a particularly diverse, highly unique endemic flora and fauna. This forest reserve is also the type locality for six species of *Hyposmocoma*. As such, native species in these refugia continue to decline, and this must include endemic species of *Hyposmocoma*, all of which are found nowhere else. We hope this information will encourage more active conservation, not only to protect these moths, but also the immense diversity of geographically restricted species displaying amazingly specialized life histories and the remnant native habitats that sustain them.

## Acknowledgements

## References

Bazinet AL, Cummings MP (2009) The Lattice Project: a Grid research and production environment combining multiple Grid computing models. In: Weber WHW (Ed) Distributed & Grid Computing - Science Made Transparent for Everyone Principles, Applications and Supporting Communities Tectum. Marburg, 2–13.

Bazinet AL, Cummings MP (2011) Computing the Tree of Life - Leveraging the power of desktop and service grids. Fifth workshop on desktop grids and volunteer computing systems, PCGrid.

Carson HL, Lockwood JP, Craddock EM (1990) Extinction and recolonization of local populations on a growing shield volcano. Proceedings of the National Academy of Sciences USA 87: 7055–7057.

Cummings MP, Huskamp JC (2005) Grid computing. EDUCAUSE Review 40: 116–117.

Dayrat B (2005) Towards integrative taxonomy. Biological Journal of the Linnean Society 85: 407–415.

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A (2010) Geneious v. 5.1. http://www.geneious.com/

Freed LA, Conant S, Fleischer RC (1987) Evolutionary ecology and radiation of Hawaiian passerine birds. Trends in Ecology and Evolution 2: 196–203.

Gu X, Fu Y-X, Li W-H (1995) Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Molecular Biology and Evolution 12: 546–557.

Hardy DE (1960) Insects of Hawaii. Volume 10, Diptera: Nematocera-Brachycera (except Dolichopodidae). University of Hawaii Pres, Honolulu, 368 pp.

Hardy DE (1964) Insects of Hawaii. Volume 11, Diptera: Brachycera, Family Dolichopodidae, Cyclorrhapha, series Aschiza, Families Lonchopteridae, Phoridae, Pipunculidae, and Syrphidae. University of Hawaii Press, Honolulu, 458 pp.

Hodges RW (1998) The Gelechioidea. In: Kristensen NP (Ed) Lepidoptera, Moths and Butterflies 1 Hanbuch der Zoologie/Handbook of Zoology IV/35. Walter de Gruyter, Berlin and New York.

Jordan S, Simon C, Polhemus D (2003) Molecular systematics and adaptive radiation of Hawaii's endemic Damselfly genus *Megalagrion* (Odonata: Coenagrionidae). Systematic Biology 52: 89–109.

Kaneshiro KY (1997) Perkins' legacy to evolutionary research on Hawaiian Drosophilidae (Diptera). Pacific Science 51: 450–461.

Katoh K (2010) MAFFT ver. 6.843. http://align.bmr.kyushu-u.ac.jp/mafft/software/

Lanave C, Preparata G, Saccone C, Serio G (1984) A new method for calculating evolutionary substitution rates. Journal of Molecular Evolution 20: 86–93.

Landry B, Powell JA, Sperling FAH (1999) Systematics of the *Argyrotaenia franciscana* (Lepidoptera: Tortricidae) species group: evidence from mitochondrial DNA. Annals of the Entomological Society of America 92: 40–46.

Liebherr JK, Zimmerman EC (2000) Insects of Hawaii. Volume 16. Hawaiian Carabidae (Coleoptera), Part 1. Introduction and Tribe Platynini. University of Hawaii Press, Honolulu, 504 pp.

Mitter K, Larsen TB, DePrins J, DePrins W, Collins S, vande Weghe G, Sáfián S, Zakharov EV, Hawthorne DJ, Kawahara AY, Regier JC (2011) The butterfly subfamily Pseudopontiinae is not monobasic: marked genetic diversity and morphology reveal three new species of *Pseudopontia* (Lepidoptera: Pieridae). Systematic Entomology 36: 139–163.

Posada D (2008) jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25: 1253–1256.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Rubinoff D (2006) DNA barcoding evolves into the familiar. Conservation Biology 20: 1548–1549.

Rubinoff D (2008) Phylogeography and ecology of an endemic radiation of Hawaiian aquatic case-bearing moths (*Hyposmocoma*: Cosmopterigidae). Philosophical Transactions of the Royal Society of London, B Biological Sciences 363: 3459–3465.

Rubinoff D, Haines WP (2005) Web-spinning caterpillar stalks snails. Science 309: 575.

Rubinoff D, Haines WP (2006) *Hyposmocoma molluscivora* description. Science 311: 1377.

Rubinoff D, Holland BS (2005) Between two extremes: mitochondrial DNA is neither the panacea nor the nemesis of phylogenetic and taxonomic inference. Systematic Biology 54: 952–961.

Rubinoff D, Powell J (2004) Conservation of fragmented small populations: endemic species persistence on California's smallest channel island. Biodiversity and Conservation 13: 2537–2550.

Rubinoff D, Schmitz P (2010) Multiple aquatic invasions by an endemic, terrestrial Hawaiian moth radiation. Proceedings of the National Academy of Sciences 107: 5903–5906.

Schmitz P, Rubinoff D (2008) Three new species of *Hyposmocoma* (Lepidoptera: Cosmopterigidae) from the Hawaiian Islands, based on morphological and molecular evidence. Zootaxa 1821: 49–58.

Schmitz P, Rubinoff D (2009) New species of *Hyposmocoma* (Lepidoptera, Cosmopterigidae) from the remote Northwestern Hawaiian Islands of Laysan, Necker, and Nihoa. Zootaxa 2272: 37–53.

Schmitz P, Rubinoff D (2011a) Ecologically and morphologically remarkable new Cosmet Moth species of the genus *Hyposmocoma* (Lepidoptera: Cosmopterigidae) endemic to the Hawaiian Islands, with reference to the spectacular diversity of larval cases. Annals of the Entomological Society of America 103: 1–15.

Schmitz P, Rubinoff D (2011b) The Hawaiian amphibious caterpillar guild: new species of *Hyposmocoma* (Lepidoptera: Cosmopterigidae) confirm distinct aquatic invasions and complex speciation patterns. Zoological Journal of the Linnean Society.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651–701.

Sperling FA, Hickey DA (1994) Mitochondrial DNA sequence variation in the spruce budworm species complex (Choristoneura: Lepidoptera). Molecular Biology and Evolution 11: 656–665.

Tavaré S (1986) Some probablistic and statistical problems on the analysis of DNA sequences. Lectures on Mathematics in the Life Sciences 17: 57–86.

Wilgenbusch JC, Warren DL, Swofford DL (2004) AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. http://ceb.csit.fsu.edu/awty.

Will K, Mischler BD, Wheeler QD (2005) The perils of DNA barcoding and the need for integrative taxonomy. Systematic Biology 54: 844–851.

Yang Z (1994) Maximum-likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. Journal of Molecular Evolution 39: 306–314.

Zimmerman EC (1978) Insects of Hawaii. Volume 9. Microlepidoptera, Part 2. Gelechioidea. University of Hawaii Press, Honolulu.

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. The University of Texas at Austin, Ph.D. dissertation.

RESEARCH ARTICLE

# A new species of genus *Syntactus* Förster (Hymenoptera, Ichneumonidae, Ctenopelmatinae) with a key to Oriental and Eastern Palearctic species

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## Abstract

A new species, *Syntactus jiulianicus* Sun & Sheng, **sp. n.** belonging to the tribe Pionini of the subfamily Ctenopelmatinae (Hymenoptera, Ichneumonidae), collected from Jiangxi Province, China, is reported. Illustrations of the new species and *S. delusor* (Linnaeus, 1758), *S. minor* (Holmgren, 1857) and *S. varius* (Holmgren, 1858) are provided. A key to the species of *Syntactus* known from the Oriental and Eastern Palaearctic Regions is given.

## Keywords

Pionini, *Syntactus*, new species, Key, taxonomy, Oriental Region, Palaearctic Region, China

## Introduction

*Syntactus* Förster, 1869, belonging to the tribe Pionini of the subfamily Ctenopelmatinae (Hymenoptera, Ichneumonidae), comprises six described species (Horstmann 2007, Kasparyan and Khalaim 2007, Yu et al. 2005), all of them distributed in the Palaearctic Region, three of which are from the Eastern Palearctic.

The European species of *Syntactus* Förster were catalogued by Aubert (2000). Kasparyan and Khalaim (2007) provided a key to the species of the Russian Far East. Two species in Yu et al. (2005), *S. aigneri* (Kiss, 1926) and *S. croaticus* (Kiss, 1926), were subsequently synonymized with, respectively, *Phobetes leptocerus* (Gravenhorst, 1820) and *Phobetes atomator* (Muller, 1776) by Horstmann (2007). One species, *Syntactus delusor* (Linnaeus, 1758), was recorded from Shanxi, China (Chao 1976, Uchida 1952). So far, no species of the genus have been described from the Oriental Region.

In this article a new species belonging to *Syntactus*, collected in Jiangxi Province, situated at the northern border of the Oriental part of China, are reported.

## Materials and methods

Specimens were collected with entomological nets in the forests of Jiulianshan Natural Reserve, Longnan County, Jiangxi Province (CHINA). The forest composed of mixed deciduous angiosperms and evergreen conifers, mainly including *Castanea* spp., *Castanopsis fabri* Hance, *Cinnamomum* spp., *Dalbergia hupeana* Hance, *Dendropanax dentiger* (Harms) Merr., *Machilus* spp., *Quercus* spp., *Tsoongiodendron odorum* Chun, *Pinus massoniana* (Lamb.).

Images of whole bodies were taken using a CANON Power Shot A650 IS. Other images were taken using a Cool SNAP 3CCD attached to a Zeiss Discovery V8 Stereomicroscope and captured with QCapture Pro version 5.1. Specimens of *Syntactus delusor* (Linnaeus, 1758), *S. minor* (Holmgren, 1857), *S. varius* (Holmgren, 1858) and *S. fusiformis* (Thomson, 1894), preserved in Zoologische Staatssammlung München, Germany, (ZSM), were checked.

The morphological terminology is mostly that of Gauld (1991). Wing vein nomenclature is based on Ross (1936) and the terminology on Mason (1986, 1990).

Type specimens are deposited in the Insect Museum, General Station of Forest Pest Management, State Forestry Administration, People's Republic of China.

## Taxonomy

### *Syntactus* Förster, 1869
http://species-id.net/wiki/Syntactus

*Syntactus* Förster, 1869. Verhandlungen des Naturhistorischen Vereins der Preussischen Rheinlande und Westfalens, 25(1868):210. Type-species: *Ichneumon delusor* Linnaeus; designated by Perkins 1962.

*Tromopoea* Förster, 1869:210. Type-species: *Catoglyptus minor* Holmgren; designated by Perkins 1962.

*Brischkea* Kriechbaumer, 1897:167. Type-species: (*Brischkea parvula* Kriechbaumer) = *delusor* Linnaeus; monobasic.

**Diagnosis.** *Syntactus* can be distinguished from all other genera of Pionini by the combination of the following characters: Apical margin of clypeus blunt. Clypeal foveae open, extraordinarily impressed. Subbasal portion of lower margin of mandible sharp, its outer face without a basal impression. Upper end of epicnemial carina reaching front margin of mesopleuron. Areolet absent. Nervellus subvertical. Glymma absent. Ovipositor thin, needle-like, straight or slightly upcurved.

### Key to species of *Syntactus* Förster known in Oriental and Eastern Palaearctic Regions

1        Mesopleuron without wrinkles, with indistinct or distinct punctures (Figure 3) ...................................................................................**2**
–        Mesopleuron with distinct, dense and oblique wrinkles ...........................**4**
2        Face with dense and distinct punctures. Upper tooth of mandible slightly longer than lower tooth ..............................................***S. varius* (Holmgren)**
–        Face almost smooth and without puncture, or with weak punctures. Upper tooth of mandible evidently shorter than lower tooth (Figure 2a) .............. **3**
3        Area superomedia longer than wide (Figure 4), costula connecting in front of its middle. Mesopleuron and mesosternum yellow. Metapleuron reddish brown (female) or yellow (male). Hind legs reddish brown ..........................
        ..........................................................***S. jiulianicus* Sun & Sheng, sp.n.**
–        Area superomedia approximately as long as wide, costula connecting at its middle. Mesopleuron, mesosternum, metapleuron and hind legs black........
        .................................................................................. ***S. leleji* Kasparyan**
4        Face almost smooth, punctures indistinct. Hind femora reddish brown. (Figure 7)..................................................................***S. delusor* (Linnaeus)**
–        Face with dense punctures. Hind femora black or brownish black. (Figure 8)...............................................................***S. minor* (Holmgren)**

### *Syntactus jiulianicus* Sun & Sheng, sp.n.
urn:lsid:zoobank.org:act:B09BEBC3-C706-48F6-B7B6-2EAF91361025
http://species-id.net/wiki/Syntactus_jiulianicus
Figures 1–6

**Etymology.** The name of the new species is based on the type locality.
        **Types.** *Holotype*, Female, CHINA: Jiulianshan Natural Reserve, Longnan County, 629 m, Jiangxi Province, 27 April 2011, leg. Mao-Ling Sheng. Paratype: 1 male, same data as holotype.
        **Diagnosis.** *Syntactus jiulianicus* can be distinguished from all other species of *Syntactus* by the combination of the face and clypeus smooth and shining, sparsely and finely punctuate; gena and frons impunctate; face, mesopleuron and mesosternum yellow; first to third terga of female reddish brown, male yellow.
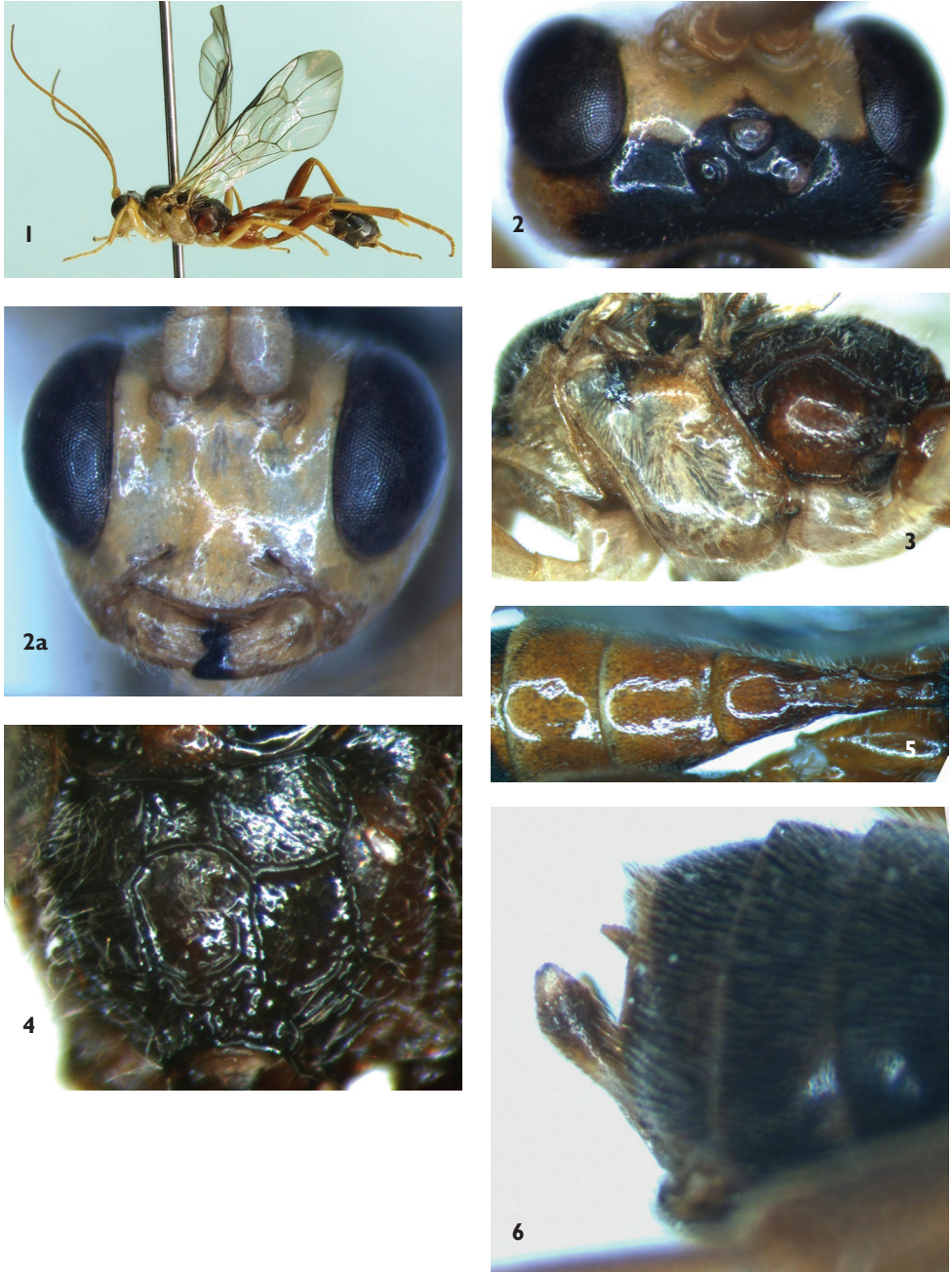
**Figures 1–6.** *Syntactus jiulianicus* Sun & Sheng, sp.n. Holotype. Female **1** Body, lateral view **2** Head, dorsal view 2a Head, anterior view **3** Mesosoma, lateral view **4** Propodeum **5** Terga 1 to 3, dorsal view **6** Apical portion of metasoma, lateral view.

**Description.** Female. Body length approximately 8.5 mm. Fore wing length approximately 7.0 mm. Antenna length approximately 7.5 mm.

**Head.** Face and clypeus almost smooth (Figure 2a). Face approximately 1.9 times as wide as long, with indistinct, uneven and fine punctures; median portion weakly and longitudinally convex. Clypeus gradually raised towards apical margin, with very sparse, fine and distinct punctures. Median portion of mandible with longitudinal wrinkles; upper tooth of mandible evidently shorter than lower tooth. Cheek nearly smooth, without punctures. Malar space approximately 0.6 times as long as basal width of mandible. Gena and vertex (Figure 2) and frons smooth and shining. Gena impunctate, hind portion slightly convergent posteriorly, in lateral view approximately 0.9 times as long as width of eye. Posterior-lateral portion of vertex with indistinct fine punctures, hind-median portion slightly concave. Interocellar area slightly convex. Postocellar line about 0.7 times as long as ocular-ocellar line. Frons impunctate; upper-median portion weakly convex; lower portion slightly concave. Antenna with 37 flagellomeres. Ratio of length from first to fifth flagellomeres: 2.0:1.8:1.4:1.3:1.3. Occipital carina complete, dorsomedian portion concave, lower end reaching base of mandible.

**Mesosoma.** Anterior portion of pronotum smooth, lateral concavity with dense oblique fine wrinkles, dorsal-porsterior portion with fine punctures. Epomia present, weak. Mesoscutum with dense and fine punctures. Notauli present, anterior 0.3 sharp. Scutoscutellar groove with weak longitudinal wrinkles. Scutellum convex, with fine punctures, basal 0.3 with lateral carina. Postscutellum weakly convex, approximately quadrate. Subalar prominence strongly convex. Mesopleuron (Figure 3) and metapleuron smooth and shining. Lower portion of mesopleuron with sparse and indistinct fine punctures. Epicnemial carina strong, upper end reaching subalar prominence. Posterior portion of metapleuron with fine oblique wrinkles. Submetapleural carina strongly, anterior portion evidently convex. Wings slightly brownish, hyaline, apical portions smoky-gray. Fore wing with vein 1cu-a distal of 1/M by 0.2 times as long as 1cu-a. Vein 2-Cu approximately 1.8 times as long as 2cu-a. Hind wing vein 1-cu about 1.5 times as long as cu-a. Apical edge of fore tibia with a small tooth at outer side. Hind coxa smooth, with sparse and fine punctures. Ratio of length of hind tarsomeres 1:2:3:4:5 is 5.3:2.7:1.9:1.1:1.4. Propodeum (Figure 4) evenly convex. Area basalis slightly longer than wide, smooth, convergent anteriorly. Area superomedia longer than wide, costula connecting in front of its middle, combined with area petiolaris, combined area smooth, nearby apical margin with distinct transverse fine wrinkles, from costula to apex evidently convergent posteriorly. Area externa with fine punctures and gray hairs. Area lateralis with dense oblique wrinkles. Pleural carina distinctly curved at the level of propodeal spiracle, with carina between pleural carina and propodeal spiracle. Propodeal spiracle approximately 2.6 times as long as wide.

**Metasoma.** Terga almost smooth. First tergum approximately 2.3 times as long as apical width, strongly convergent toward base, median dorsal carinae indistinct.

**Figure 7.** *Syntactus delusor* (Linnaeus, 1758). Female. Body, lateral view.



**Figure 8.** *Syntactus minor* (Holmgren, 1857). Female **8** Body, lateral view.

Dorsolateral carinae weak, subbasal portion near spiracle indistinct. Ventrolateral carinae complete. Spiracle convex, located slightly before middle of first tergum. Second tergum trapeziform, approximately 0.7 times as long as apical width. Third tergum approximately 0.5 times as long as apical width. Ovipositor sheath approximately 0.7 times as long as apical depth of metasoma, subapical portion distinctly wider than basal portion (Figure 6). Ovipositor very thin.

**Figure 9.** *Syntactus varius* (Holmgren, 1858). Female. Body, lateral view.

**Color.** (Figure 1). Main body and legs yellow, except the following. Flagellum reddish brown. Apical teeth of mandibles, vertex, collar, mesoscutum, lateral portions of scutellum and postscutellum, axillary troughs of mesonotum and metanotum, a small spot beneath subalar prominence, fourth to seventh terga except narrow hind margins black. Median portions of scutellum and postscutellum red. Propodeum darkish brown, lateral portion fuscous. Terga 1 to 3 reddish brown. Posterior-lateral portions of third and fourth terga with longitudinal brownish black spots. Metapleuron and hind legs reddish brown. Stigma blackish brown. Veins dust-colored.

**Male.** Body length approximately 7.0 mm. Fore wing length approximately 5.6 mm. Antenna length approximately 7.0 mm. Antenna with 35 flagellomeres. Ventral profiles of hind coxae, metapleuron, terga 1 to 3 and hind portion of tergum 4 yellow.

## References

Aubert JF (2000) Les ichneumonides oeust-palearctiques et leurs hotes. 3. Scolobatinae (=Ctenopelmatinae) et suppl. aux volumes precedents. Litterae Zoologicae 5: 1–310.

Bauer R (1958) Ichneumoniden aus Franken (Hymenoptera, Ichneumonidae). Beiträge zur Entomologie 8: 438–477.

Chao HF (1976) An outline of the classification of the Ichneumon-flies of China (Hymenoptera: Ichneumonidae). Scientific Publisher, Beijing, China, 413pp.

Gauld ID (1991) The Ichneumonidae of Costa Rica, 1. Introduction, keys to subfamilies, and keys to the species of the lower Pimpliform subfamilies Rhyssinae, Poemeniinae, Acaenitinae and Cylloceriinae. Memoirs of the American Entomological Institute 47: 1–589.

Hedwig K (1944) Verzeichnis der bisher in Schlesien aufgefundenen Hymenopteren. V. Ichneumonidae. Zeitschrift für Entomologie. Breslau 19(3): 1–5.

Horstmann K (2007) Typenrevisionen der von Kiss beschriebenen Taxa der Ctenopelmatinae (Hymenoptera, Ichneumonidae). Linzer Biologische Beiträge 39: 313–322.

Kasparyan DR, Khalaim AI (2007) Ctenopelmatinae. In: Lelej AS (Ed) 'Key to the insects of Russia Far East. Vol.IV. Neuropteroidea, Mecoptera, Hymenoptera. 5. Vladivostok: Dalnauka 1052, 474–559.

Kiss ZA (1926) Ichneumoniden aus der Sammlung des Ungarischen National-Museums. Annales Musei Nationalis Hungarici 24: 237–286.

Mason WRM (1986) Standard drawing conventions and definitions for venational and other features of wings of Hymenoptera. Proceedings of the Entomological Society of Washington 88: 1–7.

Mason WRM (1990) Cubitus posterior in Hymenoptera. Proceedings of the Entomological Society of Washington 92: 93–97.

Meyer NF (1936) Parasitic Hymenoptera in the family Ichneumonidae of the USSR and adjacent countries. Part 5. Tryphoninae. Opredeliteli Faune SSSR 21(5): 1–340.

Ross HH (1936) The ancestry and wing venation of the Hymenoptera. Annals of the Entomological Society of America 29: 99–111.

Townes HK (1970) The genera of Ichneumonidae, Part 3. Memoirs of the American Entomological Institute 13(1969): 1–307.

Uchida T (1952) Ichneumonologische Ergebnisse der japanischen wissenschaftlichen Shansi-Provinz, China-Expedition im Jahre 1952. Mushi 24: 39–58.

Yu DS, Horstmann K (1997) A catalogue of world Ichneumonidae (Hymenoptera). Memoirs of the American Entomological Institute 58:1–1558.

Yu DS, van Achterberg K, Horstmann K (2005) World Ichneumonoidea 2004. Taxonomy, Biology, Morphology and Distribution. Taxapad, CD/DVD, Vancouver, Canada. http://www.taxapad.com

RESEARCH ARTICLE

# *Antispila oinophylla* new species (Lepidoptera, Heliozelidae), a new North American grapevine leafminer invading Italian vineyards: taxonomy, DNA barcodes and life cycle

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## Abstract

A grapevine leafminer *Antispila oinophylla* van Nieukerken & Wagner, **sp. n.**, is described both from eastern North America (type locality: Georgia) and as a new important invader in North Italian vineyards (Trentino and Veneto Region) since 2006. The species is closely related to, and previously confused with *A. ampelopsifoliella* Chambers, 1874, a species feeding on Virginia creeper *Parthenocissus quinquefolia* (L.) Planchon., and both are placed in an informal *A. ampelopsifoliella* group. Wing pattern, genitalia, and DNA barcode data all confirm the conspecificity of native North American popula-

tions and Italian populations. COI barcodes differ by only 0–1.23%, indicating that the Italian populations are recently established from eastern North America. The new species feeds on various wild *Vitis* species in North America, on cultivated *Vitis vinifera* L. in Italy, and also on *Parthenocissus quinquefolia* in Italy. North American *Antispila* feeding on *Parthenocissus* include at least two other species, one of which is *A. ampelopsifoliella*. Morphology and biology of the new species are contrasted with those of North American *Antispila* Hübner, 1825 species and European *Holocacista rivillei* (Stainton, 1855). The source population of the introduction is unknown, but cases with larvae or pupae, attached to imported plants, are a likely possibility. DNA barcodes of the three European grapevine leafminers and those of all examined Heliozelidae are highly diagnostic. North American Vitaceae-feeding *Antispila* form two species complexes and include several as yet unnamed taxa. The identity of three out of the four previously described North American Vitaceae-feeding species cannot be unequivocally determined without further revision, but these are held to be different from *A. oinophylla*. In Italy the biology of *A. oinophylla* was studied in a vineyard in the Trento Province (Trentino-Alto Adige Region) in 2008 and 2009. Mature larvae overwinter inside their cases, fixed to vine trunks or training stakes. The first generation flies in June. An additional generation occurs from mid-August onwards. The impact of the pest in this vineyard was significant with more than 90% of leaves infested in mid-summer. Since the initial discovery in 2006, the pest spread to several additional Italian provinces, in 2010 the incidence of infestation was locally high in commercial vineyards. Preliminary phylogenetic analyses suggest that *Antispila* is paraphyletic, and that the *Antispila ampelopsifoliella* group is related to *Coptodisca* Walsingham, 1895*, Holocacista* Walsingham & Durrant, 1909 and *Antispilina* Hering, 1941, all of which possess reduced wing venation. Vitaceae may be the ancestral hostplant family for modern Heliozelidae.

## Introduction

There are several cases known of leafmining Lepidoptera developing into important agricultural pests, such as *Phyllocnistis citrella* Stainton, 1856 (Gracillariidae) on citrus, now a worldwide problem (Heppner and Dixon 1995) and *Leucoptera coffeella* Guérin-Méneville, 1842, *L. meyricki* Ghesquière, 1940 and related species (Lyonetiidae) on coffee, that are amongst the more important coffee pests (Le Pelley 1973). Leafmining moths apparently often disperse easily, possibly due to their small size, and some have shown rapid invasions over large areas, e.g.: *Cameraria ohridella* Deschka & Dimić, 1986, *Phyllonorycter leucographella* (Zeller, 1850)*, P. issikii* (Kumata, 1963) and *Macrosaccus robiniella* (Clemens, 1859) (Šefrová 1999; Hellrigl 2001; Šefrová 2002a, b; Šefrová and Laštůvka 2002; Davis and De Prins 2011).

Lepidopteran leafminers of grapevine (*Vitis vinifera* L.) have not yet developed into serious pests in Europe, although one North American species did recently invade European vineyards; *Phyllocnistis vitegenella* Clemens, 1859 (Lepidoptera: Gracillariidae) became established in Italy and elsewhere in Europe around 1995 (Posenato et

al. 1997). The only native European leafminer of grape is *Holocacista rivillei* (Stainton, 1855) (Lepidoptera: Heliozelidae) (Hering 1957), a minor pest in vineyards in southern Europe and western Asia (see references below). *Holocacista rivillei* was described from Malta and later reported from Italy. It develops two to three generations annually (Mariani 1942; Marchi 1956; Camporese and Marchesini 1991; Dal Rì and Delaiti 1992; De Tomaso et al. 2008; Baldessari et al. 2009). Infestations leading to damage are infrequent, probably because pest populations are controlled by a complex of eulophid parasitoids (Hymenoptera) (Camporese and Marchesini 1991; Alma 1995). European populations of *Phyllocnistis vitegenella* occur in northern Italy (Marchesini et al. 2000; Villani 2002; Reggiani and Boselli 2005; Duso et al. 2011), Slovenia (Seljak 2005) and Switzerland (Cara and Jermini 2011). It can produce up to four generations annually and has given rise to local outbreaks in northeastern Italy (Posenato et al. 1997; Marchesini et al. 2000). The larvae of both moths produce characteristic mines in grapevine leaves; in *H. rivillei* a narrow initial gallery leads subsequently to an oval full-depth blotch, from which the larva cuts out an oval pupal case or shield, in which it pupates, leaving an oval hole in the leaf. *P. vitegenella* makes a long tortuous gallery mine in the upper epidermis, with a distinct dark central frass line that ends in a pupal chamber. Both species can easily be detected in a vineyard based on the presence of their diagnostic leafmines.

In the summer of 2007, leafmines similar to those caused by *H. rivillei* were observed in a vineyard in northeastern Italy (Borgo Valsugana, Trento province). However, the initial gallery mine was immediately enlarged into a larger blotch, indicating a different species. Adults reared from these mines differed from *H. rivillei* in size and wing pattern. On their external characters they were identified as belonging to the genus *Antispila* Hübner, [1825], but not to one of the two species currently known in Europe, i.e., *Antispila treitschkiella* (Fischer von Röslerstamm, 1843) and *A. metallella* ([Denis & Schiffermüller], 1775) (Karsholt et al. 1995; Ellis 2010; van Nieukerken 2011) which both feed on dogwood (*Cornus* spp.). Thus, we determined that we had either an undescribed species or an alien species introduced from another continent. This pest has been reported previously as *Antispila* sp. (Baldessari et al. 2009; Duso et al. in press). Because taxonomic knowledge of the family Heliozelidae is poor, very few species being described up to modern standards, and because many of the known *Antispila* species are associated with *Vitis* species or related Vitaceae, it took some time to establish that this species was an undescribed, but common, North American species, hitherto confused with the North American *Antispila ampelopsifoliella* Chambers, 1874, described from Virginia creeper *Parthenocissus quinquefolia*. Unfortunately this confusion has already led to the incorrect introduction of the name *A. ampelopsifoliella* into European literature (Laštůvka 2009; van Nieukerken 2011; van Nieukerken et al. 2011a). In this paper we describe the species as *Antispila oinophylla* van Nieukerken & Wagner, sp. n., provide a diagnosis for its identification, and characterize its geographic distribution and life cycle. We also sequenced a part of the cytochrome C oxidase subunit I (COI) gene (DNA barcode) (Hebert et al. 2003b; Hebert et al. 2003a)

as well as those of a selection of other Heliozelidae and the other two European grape leaf-mining micro-moths (*H. rivillei* and *P. vitegenella*). DNA barcode data played important roles in revealing the original source of the infestation and unravelling the taxonomy of the new grape pest.

## Family Heliozelidae

The family Heliozelidae (superfamily Adeloidea) comprises 123 described species in 12 genera (van Nieukerken et al. 2011b), with the greatest diversity in North America and Australia. Larvae of the Heliozelidae produce leafmines (rarely galls) in various trees and vines, rarely herbs, and typically cut-out an oval case or shield from the leafmine, in which they moult once into a non-feeding final instar or prepupa, and finally pupate in the leaf litter or on plant parts. All are thought to overwinter in temperate regions as prepupae. Eight species of Heliozelidae occur in Europe (van Nieukerken 2011), belonging to four genera: *Antispila*, *Antispilina* Hering, 1941, *Heliozela* Herrich-Schäffer, 1853 and *Holocacista* Walsingham & Durrant, 1909. Four species of Heliozelidae were previously known from Italy: *Heliozela lithargyrellum* (Zeller, 1850), *H. sericiella* (Haworth, 1828), *Holocacista rivillei* and *Antispila treitschkiella* (Fischer von Röslerstamm, 1843) (Karsholt et al. 1995), but it is likely that the fauna is incompletely sampled, and that most European species occur in Italy as well. In addition to *A. oinophylla*, we record here *A. metallella* ([Denis & Schiffermüller], 1775) as new from Italy (see Appendix B).

The grapevine family Vitaceae comprises an important group of hosts for the genus *Antispila* worldwide; out of 32 *Antispila* species for which host plants are known, 13 feed on Vitaceae, of which at least ten are associated with the genus *Vitis* (Table 1). Several more unnamed species are also associated with Vitaceae. In North America, *Heliozela aesella* Chambers, 1877 makes galls in leaves and shoots on *Vitis* (McGiffen and Neunzig 1985). There are only a few previous records of Heliozelidae as minor pests on grape: in addition to *H. rivillei*, as mentioned above, *Antispila uenoi* has been recorded as a pest in Japan (Kuroko 1987; Ueno et al. 1987). *Antispila viticordifoliella* Clemens, 1860 is listed by McGiffen and Neunzig (1985) as occurring on bunch grape leaves, but not as a pest.

## Material and methods

### Material

*Antispila oinophylla* adults were collected from Borgo Valsugana for sequencing and larvae were collected and reared for morphological studies. *Holocacista rivillei* and *Phyllocnistis vitegenella* adults were also collected from northeastern Italy (Appendix B). To obtain material of the new species and related species from North America for com-

**Table 1.** Heliozelidae species associated with Vitaceae, type country and hostplant species. For the American species where the identity is not fully established we added [cf] between genus and species name.

| Species | Type country | Hostplants | source |
|---|---|---|---|
| *Antispila oinophylla* | USA | *Vitis aestivalis, V. labrusca, V. riparia, V. vinifera, V. vulpina,* [*Parthenocissus*] | this paper |
| *Antispila ampelopsifoliella* Chambers, 1874 | USA | *Parthenocissus quinquefolia* | Chambers 1874a, this paper |
| *Antispila* sp. "vitis1" | (USA) | *Vitis aestivalis* | this paper |
| *Antispila* [cf] *isabella* Clemens, 1860 | USA | *Vitis aestivalis, V. labrusca, V. riparia* | Clemens 1860, this paper |
| *Antispila* sp. "vitis2" | (USA) | *Vitis aestivalis, Vitis riparia* | this paper |
| *Antispila viticordifoliella* Clemens, 1860 | USA | *Vitis vulpina* | Clemens 1860, this paper |
| *Antispila* cf *viticordifoliella* Clemens, 1860 | (USA) | *Parthenocissus quinquefolia* | this paper |
| *Antispila voraginella* Braun, 1927 | USA | *Vitis arizonica* | Braun 1927, DLW |
| *Antispila ampelopsia* Kuroko, 1961 | Japan | *Ampelopsis brevipedunculata, Vitis flexuosa* | Kuroko 1961 |
| *Antispila inouei* Kuroko, 1987 | Japan | *Vitis coignetiae, V. labruscana* | Kuroko 1987 |
| *Antispila iviella* Kuroko, 1961 | Japan | *Parthenocissus tricuspidata* | Kuroko 1961 |
| *Antispila orbiculella* Kuroko, 1961 | Japan | *Ampelopsis brevipedunculata* | Kuroko 1961 |
| *Antispila tateshinensis* Kuroko, 1987 | Japan | *Vitis coignetiae* | Kuroko 1987 |
| *Antispila uenoi* Kuroko, 1987 | Japan | *Vitis coignetiae, V. labruscana* | Kuroko 1987 |
| *Antispila argostoma* Meyrick, 1916 | India | *Cayratia trifolia* | Meyrick 1916, Fletcher 1920 |
| *Antispila aristarcha* Meyrick, 1916 | India | *Vitis* sp. | Meyrick 1916, Fletcher 1920, Fletcher 1933 |
| *Antispila isorrhythma* Meyrick, 1926 | India | *Vitis* sp. | Meyrick 1926 |
| *Antispila* species | Indonesia, Borneo | *Leea indica* | EJvN |
| *Antispila* species | Australia | *Cissus antarctica* | Common 1990 |
| *Holocacista rivillei* Stainton, 1855 | Malta | *Vitis vinifera* | see text |
| *Heliozela aesella* Chambers, 1877 | USA | *Parthenocissus quinquefolia, Vitis vulpina, Vitis* sp. | McGiffen and Neunzig 1985 |

parison, various *Antispila* mines and larvae were collected by EJvN and CDo during a field trip September-October 2010 in the states of Georgia and Tennessee and by EJvN in September 2011 (partly with DLW) in Connecticut, Massachusetts, Vermont and New York state. Other material included in the taxonomic and DNA analyses was collected by DLW, who has been collecting and rearing *Antispila* and other leafmin-

ers from across North America for three decades (Appendix B). Further material was studied or borrowed from the following collections.

Abbreviations for depositories:

**ANSP**     Academy of Natural Sciences in Philadelphia, Pennsylvania, USA
**CNC**      Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada
**DLW**      Research collection of David L. Wagner, Storrs, Connecticut, USA
**MCZ**      Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA
**RMNH**     Netherlands Centre for Biodiversity Naturalis, former Leiden Zoology collections, Leiden, Netherlands
**UMDC**     University of Maryland, College park, USA
**UPI**      University of Padova, Department of Environmental Agronomy and Crop Science, Italy
**ZMUO**     Zoological Museum University of Oulu, Finland

### Rearing

Collected leaves were kept in polystyrene jars or bags, with some moss and or tissue added, until the larvae had prepared the shields. It was often necessary to remove the cut/out shields from the leaves, which were then removed from the breeding jars and dried as vouchers. Breeding jars were kept during winter in an outbuilding, and brought indoors in March, where they were kept until emergence of adults. Specimens collected during fall 2011 were still in hibernation diapause when this manuscript was accepted.

### Morphology

Methods for preparation of the genitalia follow Nielsen (1980a) and van Nieukerken (1985), with some minor changes. Nielsen's unrolling technique does not work well for Heliozelidae, so we usually embedded the total genitalia in dorso-ventral position. For staining male genitalia we used (Mayers) haemaluin or phenosafranin. Wings were stained with phenosafranin and mounted in euparal. Photographs of moths, leafmines, genitalia slides and wing slides were taken with a Zeiss AxioCam digital camera attached, respectively, to a Zeiss Stemi SV11 stereo-microscope, a motorized Zeiss Ste-REO Discovery.V12 (only Figs 1, 40, 41) or a Zeiss Axioskop H, using Carl Zeiss AxioVision software.

    The Distribution Map for North America was prepared with DMap 7.0 (Morton 2000).

## Molecular analysis

DNA was extracted destructively from larvae or adult specimens preserved in 96% or 100% ethanol or extracted in a non-destructive fashion from the abdomen of voucher specimens, which were then used to prepare genitalic dissections (protocol in Knölke et al. 2005). From some larvae used for DNA extractions, the cuticle was also cleared and saved. In Padova, total DNA was extracted applying a salting-out protocol (Patwary et al. 1994). In Leiden extractions were carried out with the Qiagen DNeasy Blood and Tissue kit (QIAGEN), using the protocol "purification of total DNA from animal tissues (spin-column protocol)."

A 665 bp or a 658 bp fragment of the mitochondrial COI gene was amplified using the following primers: in Padua LCO1490 and HCO2198 (Folmer et al. 1994), in Leiden the Lep primers (Hebert et al. 2004), often tailed with T7 promotor and T3 tails in the shorter (amplifying 665 bp) and longer versions (amplifying 658 bp): T-LepF1-short and T-LepR1-short or T-LepF1 and T-LepR1, or when not tailed LepF1-short and LepR1-short. For some older museum specimens, the DNA was too degraded for amplifying sections over 400 bp long. For these we used internal primers (Hajibabaei et al. 2006). For details of primers see the BOLD site (http://www.barcodinglife.com/).

In Padova, amplification was carried out in 20ml volumes containing 2ml from the nucleic acid extract, 200mM dNTPs, 0.5mM of each primer, 4mM 10x PCR buffer, 2.5 mM $MgCl_2$ and one unit of Taq polymerase (Promega). The reaction was performed in an INC PTC-100 thermal controller (MJ Research Inc.). Amplification conditions were as follows: the first period of denaturation was 94°C for 5 min, followed by 38 cycles of denaturation at 94°C for 1 min, annealing at 48°C for 1 min, and extension at 72°C for 1 min; the final extension cycle had a step at 72°C for 5 min. A negative control with no template was included for each series of amplifications, to detect instances of contamination. The amplified products were separated on a 1% agarose gel and visualized under UV following staining with Sybr Safe (Invitrogen). PCR products were purified with the ExoSAP-IT kit (Amersham Biosciences).

In Leiden, amplification was performed in volumes of 25 μl. The PCR cycle consisted of 3 min initial denaturation at 94°C, 15 sec cycle denaturation at 94°C, 30 sec cycle at 50°C, 40 sec cycle extension at 72°C for 40 cycles. After all cycles had finished, a final extension was performed at 72°C for 5 min. The amplified products were separated on a 1% agarose gel and visualized under UV following staining with ethidium bromide.

The sequencing at Padova was performed at the BMR Genomics Service (Padova, Italy) in an ABI PRISM automatic DNA sequencer (Applied Biosystems), in both forward and reverse direction, but for some samples only in forward direction. In Leiden PCR clean-up and sequencing was outsourced to MACROGEN on an ABI 3730XL, all samples were sequenced in both forward and reverse direction. The chromatograms were checked with Sequencher (Gene Codes Corporation) and the resulting sequences were aligned by eye in BIOEDIT 7.0.9.0 (Hall 2004).

### Tree analysis

Neighbor-joining (NJ) trees based on DNA barcode sequences of all available specimens were reconstructed with Paup* 4.0b10 (Swofford 2003). Genetic distance calculations were performed both using the Kimura two-parameter (K2P) model and uncorrected P distance (Srivathsan and Meier 2011). After initial analyses with barcodes of Italian *Phyllocnistis vitegenella*, we excluded this gracillariid from subsequent analyses (because it was so divergent from focal Heliozelidae: minimum K2P distance being greater than 18%). A Genbank sequence of *Incurvaria masculella* (Denis & Schiffermüller, 1775) (Incurvariidae), another member of the superfamily Adeloidea, was used as the outgroup. Bootstrap values were calculated with 10,000 replicates.

Phylogenetic trees based on maximum parsimony were generated with PAUP using a heuristic search, 1,000 replicates, with tree-bisection-reconnection (TBR) as the branch-swapping algorithm. A bootstrap analysis was run with TNT (Goloboff et al. 2008), a program made available with sponsorship of the Willi Hennig Society, for 10,000 replicates. From the dataset we selected one sequence for all barcode clusters with less than 2% intraspecific distance, but we included four specimens of our target species *A. oinophylla*, two from Italy and two from the USA.

A Bayesian Analysis was carried out with the same dataset. Model selection was performed using jModeltest 0.1.1 (Posada 2008). The best-fit model was chosen based on AIC value (Posada and Buckley 2004). Bayesian analyses were run in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Each analysis was run twice, starting from random starting trees, for 20 million generations and sampling every 1000 generations. Two partitioning schemes were explored: first, each codon position was given a separate partition and rate multipliers, while the second scheme combined first and second codon positions into a single partition with respect the third codon positions (Shapiro et al. 2006). Convergence of the Markov Monte Carlo chains was assessed by plotting the likelihood scores in Tracer v1.5 (Rambaut and Drummond 2007). A conservative burn-in of 5 million generations was chosen.

The sequence data generated and used in this study have been deposited in the public BOLD database (project "*Antispila* Vine introduction" [ANTVI] and GenBank (Appendix B).

### Field observations

Surveys were carried out from 2007 to 2011 to investigate the *A. oinophylla* distribution in northeastern Italy. We sampled commercial vineyards but also isolated vine rows and plants of Virginia creeper, *Parthenocissus quinquefolia*.

Observations on *A. oinophylla* phenology and behaviour were carried out in 2008 and 2009 in Borgo Valsugana (Trentino Regione). The vineyard was planted with a Chardonnay cultivar and was trained with the local "pergola" system. The vineyard

received a number of fungicide treatments but insecticides were not applied. In 2008, a total of 180 leaves (30 plants, six leaves per vine) were sampled six times during the season, from May to September. In 2009, a total of 100 leaves (five replicates of ten plants, two leaves per plant) taken from the mid part of the shoots were sampled across ten dates, from May to September. In both years the number of mines produced by *A. oinophylla* larvae was assessed on each leaf. In 2009, active mines containing living larvae were distinguished from those vacated by the larvae (mines with larval cut-outs).

## Results

### Identification

To identify the new Italian *Antispila*, we checked all descriptions of the Vitaceae miners, as well as all other known *Antispila* species. Unfortunately, outside Europe, genitalia have been illustrated and described only for Japanese species of *Antispila*, including all five Vitaceae miners (Kuroko 1961; Kuroko 1987). For the North American fauna, only a revision for three Cornaceae-feeding *Antispila* (with genitalia illustrations), has been published (Lafontaine 1973). The genitalia of the Italian populations (Fig. 9) did not match any published illustrations. An important external character of the moths is the silver apical spot on the forewing (Figs 1–2), a feature found in just a few members of the genus, whereas the other pattern elements that we examined are more general across the genus. Similarly-sized subapical spots were only noted in descriptions of some *Antispila* from the New World, although larger subapical patches occur in Japanese species, such as *A. orbiculella* Kuroko, 1961. After excluding a poorly known species from Brazil as a less likely candidate, two North American Vitaceae miners with this spot were studied in more detail: *A. voraginella* Braun, 1927, occurring in Arizona and southern reaches of the Rocky Mountain area, and *A. ampelopsifoliella*, which occurs widely across eastern North America. The genitalia of the male holotype of *A. voraginella* did not match, but several specimens identified as *A. ampelopsifoliella* and reared from *Vitis*, had almost identical genitalia as the Italian populations. However, all specimens of *A. ampelopsifoliella* reared from *Parthenocissus*, were consistently different (*A. ampelopsifoliella* was described by Chambers from leafmines that he collected on *Parthenocissus* in Kentucky). Leafmines that we collected in 2011 in northeastern United States on *Parthenocissus* further showed that at least two species with different mines occur on that host. DNA barcoding results discussed below demonstrated that the Italian and North American examples from *Vitis* belong to the same species, and that American *Parthenocissus* feeders belong to two different barcode clusters, supporting our morphological and biological findings that two *Antispila* species, co-occurred on *Parthenocissus* in eastern North America. Material from the Chambers collection (see below) was insufficient to confirm the identity

of *A. ampelopsifoliella*. Here we restrict the name *A. ampelopsifoliella* to one of the two species feeding on *Parthenocissus*. The *Vitis* miner from North America, previously misidentified in collections as being *A. ampelopsifoliella*, is unnamed, morphologically identical to the Italian population, and described below.

## Taxonomy

### *Antispila* Hübner

*Antispila* Hübner, [1825]: 419. Type species *Antispila stadtmuellerella* Hübner, [1825]: 419 (a junior synonym of *A. metalella* ([Denis & Schiffermüller], 1775), subsequent designation by ICZN (1988).

### *Antispila oinophylla* Van Nieukerken & Wagner, sp. n.
urn:lsid:zoobank.org:act:F58A029E-A856-4414-B4EA-D7CAA6151948
http://species-id.net/wiki/Antispila_oinophylla
Figs 1–6, 9–29, 62, 63

*Antispila* sp.; Baldessari et al. 2009: 68 [first record for Italy]; Duso et al. in press [pest status].
[*Antispila ampelopsifoliella*; Needham et al. 1928: 289 [partim]; Davis 1983: 4 [partim]; van Nieukerken 2011: Fauna Europaea database; Laštůvka 2009: S57; van Nieukerken et al. 2011a: 51. Misidentifications].
[*Antispila ampelopsiella*; Dyar et al. 1903: 539 [partim]; Barnes and McDunnough 1917: 181 [partim]; Forbes 1923: 226 [partim]; McDunnough 1939: 91 [partim]; Brower 1984: 29 [partim]. Misidentifications].

**Type material. Holotype** ♂, **USA:** Georgia, Murray Co., Chattahoochee Nat. Forest, E of Chatsworth, GA rd 52, 523 m, 34.74066N, 84.71852W, hardwood forest along highway, leafmines on *Vitis aestivalis* var. *aestivalis*, 14.x.2010, EvN2010266, emerged 14.iv–4.v.2011, E.J. van Nieukerken & C. Doorenweerd, Genitalia slide EJvN 4204, RMNH.INS.24204 (RMNH).

    **Paratypes.** 32♂, 31♀. **Italy:** 1♂, 3♀ (all dissected), Trento, Borgo Valsusana, leafmines 2007, on *Vitis vinifera*, emerged 1.iii–26.iv.2008, M. Baldessari; 3♀, same locality, 13.viii.2008; 10♂, 1♀ (1♂ RMNH.INS.23920 dissected & DNA barcode), same locality, 18.viii.2008; 17♂, 18♀ (1♂ RMNH.INS.24038, 1♀ RMNH. INS.24039 dissected & DNA barcode), same locality, 29.vi.2009, leafmines on *Vitis vinifera*, EvN no 2009903, emerged in Leiden, 14.vii–6.viii.2009, M. Baldessari (all RMNH). **Canada:** 1♂, **Ontario**, Ottawa, mines on *Vitis,* rearing 57–112, emerged 31.iii.1958, Freeman & Lewis (CNC); 1♀, **Quebec**, Hull, mines on *Vitis,* rearing 55–228, emerged 26.vi.1956, T.N. Freeman (CNC). **USA**: 1♂, **Con-**
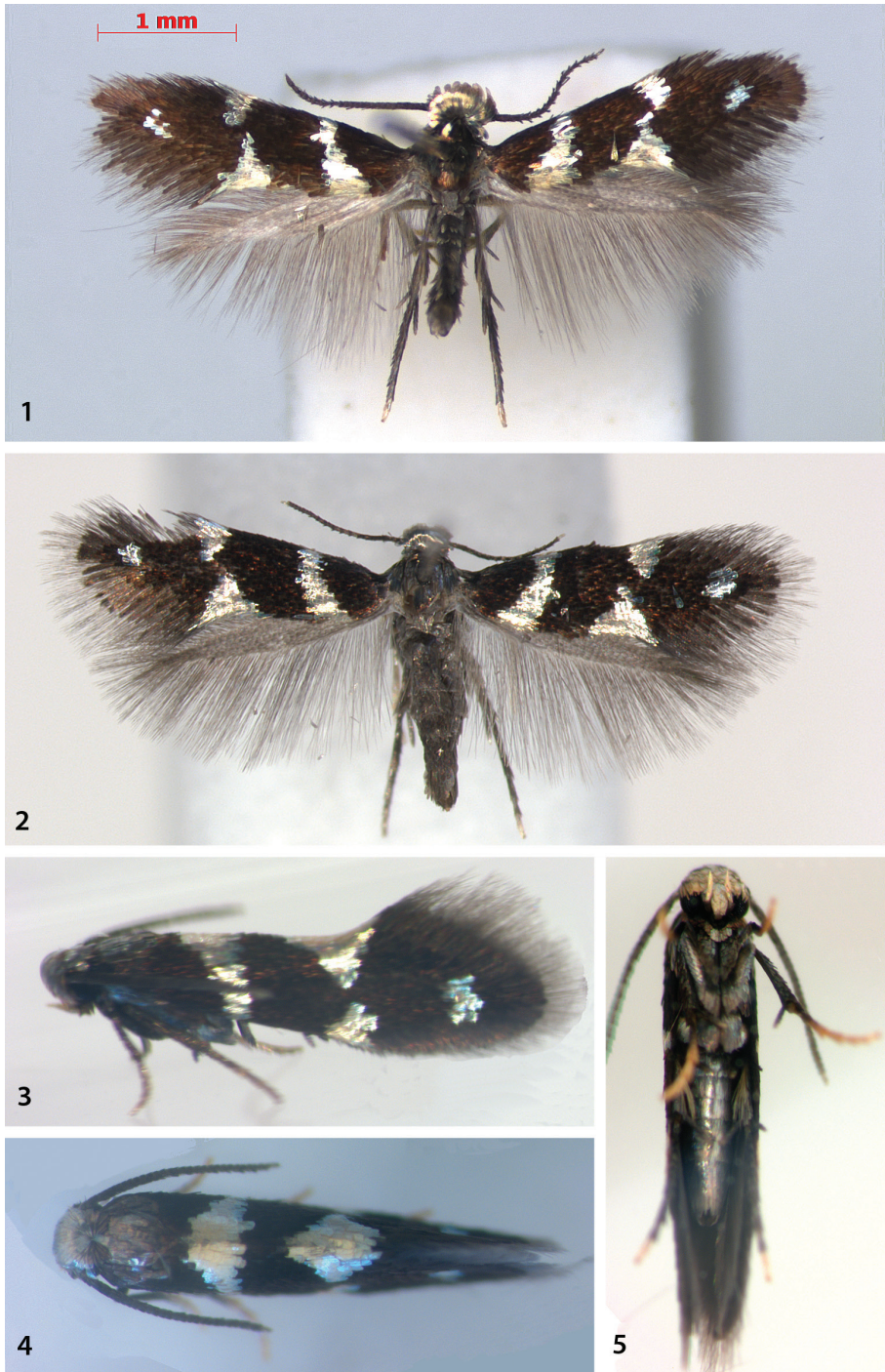
**Figures 1–5.** *Antispila oinophylla*, adult habitus. **1** Male holotype, RMNH.INS.24204 **2** Female paratype, RMNH.INS.24039, Italy, Borgo Valsusana. **3–5** Alive male, Georgia, paratype, emerged 29.iv.2011.

necticut, Tolland Co., Mansfield, 22.viii.1989, leafmines on Vitis, DLW89H37 breeding, emerged 4.v.1990, D.L. Wagner (DLW); 1♀ (dissected), **Connecticut**, Windham Co., Hampton, 916 Pudding Hill Rd., leafmines on *Vitis* 1–5.ix.1988, DLW 88J7, emerged 20.vii.1989, D.L. Wagner (DLW); 1♂ 1 ♀, **Georgia**, same data as holotype; 1♀, **Georgia**, Murray Co., Chattahoochee Nat. Forest, Cohutta Overlook, 730 m, 34.785356N, 84.627323W, shrub in forest clearing, leafmines on *Vitis aestivalis* var. *bicolor*, 14.x.2010, EvN2010270, emerged 19.iv.2011, E.J. van Nieukerken & C. Doorenweerd (RMNH); 1♀ (dissected, EvN 4211), **Kentucky**, [Covington], bred, [19th century], Chambers, "pseudotype," MCZ Type 1367 (MCZ); 1♂, 1♀ (♂ dissected), **Vermont**, Chittenden Co., South Burlington, leafmines on *Vitis* 11.viii.1988, DLW 88H23, emerged 30.iii–15.v.1989, D.L. Wagner (DLW).

**Non-type material** (all in RMNH). **Italy:** leafmines & larvae, Borgo Valsusana, 29.vi.2009, on *Vitis vinifera*, EvN no 2009903, M. Baldessari. **USA:** 1 larva, **Connecticut**, Tolland Co., Storrs campus, on *Vitis labrusca*, 185 m, 8.ix.2011, EvN2011168, B. Gagliardi; leafmines and larvae (being reared), **Connecticut**, New London Co., Connecticut College Arboretum, 34 m, 41.37929N, 72.11121W, on *Vitis labrusca*, 10.ix.2011, EvN2011193, E.J. van Nieukerken; leafmines and larvae (being reared), **Connecticut**, New Haven Co., West Rock Ridge SP, 125 m, 41.33353N, 72.96423W, on *Vitis aestivalis* var *aestivalis*, 10.ix.2011, EvN2011198, E.J. van Nieukerken; leafmines & larvae (DNA barcode RMNH.INS.18394), **Georgia,** same data as holotype; leafmines & larvae (DNA barcode RMNH.INS.18392), **Georgia**, Murray Co., Chattahoochee Nat. Forest, Cohutta Overlook, 730 m, 34.78535N, 84.62732W, shrub in forest clearing, leafmines on *Vitis aestivalis* var. *bicolor*, 14.x.2010, EvN2010270, E.J. van Nieukerken & C. Doorenweerd (RMNH); leafmines & 2 larvae (DNA barcode RMNH.INS.18533), **Massachusetts**, Berkshire Co., Beartown State forest, SW margin, 480 m, 42.19814N, 73.28928W, on *Vitis riparia*, 12.ix.2011, EvN2011208, E.J. van Nieukerken; leafmines & larvae (DNA barcode RMNH.INS.18558), **New York**, Essex Co., Hwy 9N, 3.5 km WSW Keeseville, 142 m, 44.49233N, 73.52042W, on *Vitis riparia*, 14.ix.2011, EvN2011237, E.J. van Nieukerken; leafmines & larvae (DNA barcode RMNH. INS.18555), **New York**, Essex Co., Wilsboro, Noblewood Park, 62 m, 44.35216N, 73.36435W, on *Vitis riparia*, 14.ix.2011, EvN2011244, E.J. van Nieukerken; leafmines & larvae (DNA barcodes RMNH.INS.18298, 18300), **Tennessee**, Blount Co., NP Great Smoky Mts, Rich Mountain Gap, 619 m, 35.64557N, 83.80537W, rich forest on limestone ridge, leafmines on *Vitis vulpina*, 2.x.2010, EvN2010119, E.J. van Nieukerken & C. Doorenweerd (RMNH); mine and larva, (DNA barcode LGSME035–06), **Tennessee**, Cocke Co., Cosby, ATBI house, 35.77771N, 83.21359W, on *Vitis* sp. 12.viii.2006, DLW 2006H55, D.L. Wagner (DLW); leafmines & larvae (being reared and DNA barcode RMNH.INS.18669), **Ver-**
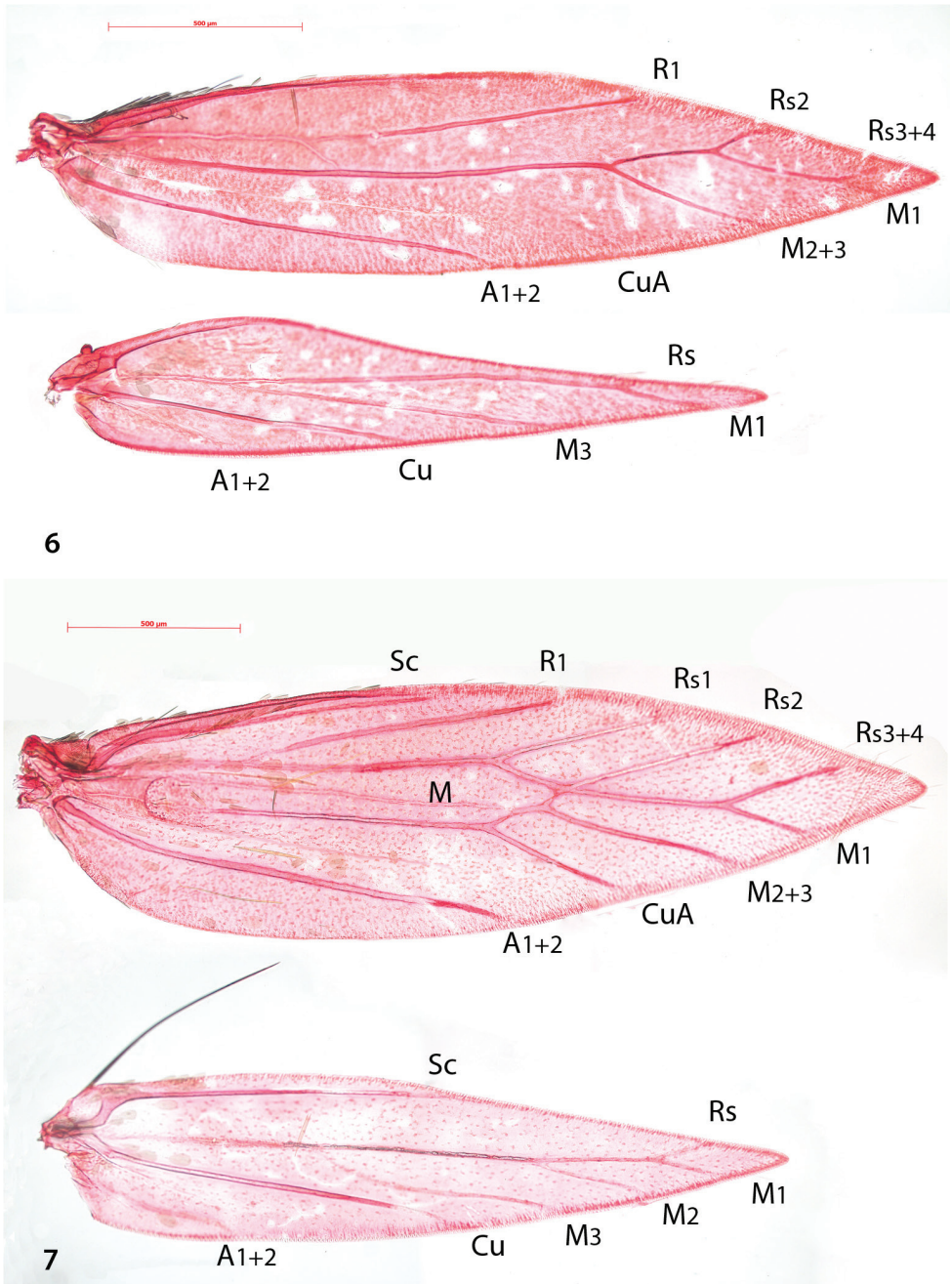
**Figures 6–7.** *Antispila*, venation. **6** *A. oinophylla*, male, Italy, RMNH.INS.24257 **7** *A. treitschkiella*, male, Netherlands, Leiden, RMNH.INS.24258.

**mont**, Addison Co., Button Bay SP, Lake Champlain borders, 44 m, 44.18154N, 73.36892W, on *Vitis riparia*, 16.ix.2011, EvN2011253, E.J. van Nieukerken.

**Differential diagnosis.** In North America, at least four other species have an apical silver spot (together forming the *ampelopsifoliella* group): *A. ampelopsifoliella* , *A. voraginella*, which has a darker head, an unnamed species from *Vitis* (here *A.* "vitis2") and *A. hydrangaeella* Chambers, 1874. The latter, which is closely similar in appearance, can be separated by the greater number of white flagellomeres at the antennal tip (six segments) and feeds on *Hydrangea arborescens* L. (Hydrangeaceae). Dissection of genitalia is needed to distinguish *Antispila oinophylla* from other members of the *ampelopsifoliella* group. Male genitalia are characterised by the long carinal spine at the phallotrema and several other details; female genitalia differ by the number of cusps on the ovipositor from at least *A. ampelopsifoliella*.

In Europe, *A. oinophylla* differs from all other Heliozelidae with a similar forewing colour pattern (species of *Antispila*, *Antispilina* and *Holocacista*) by the presence of a small silvery spot in the apical part of forewing and the distinctly white head. Some Elachistidae are superficially similar, but differ in long-pointed and upcurved palpi, longer antennae and more elongate habitus.

The leafmine of *A. oinophylla* differs from that of *H. rivillei* by its short initial gallery, which is later usually completely incorporated into the blotch, whereas the initial gallery of *H. rivillei* mines is usually as long as or longer than the blotch, and remains intact. In Eastern North America other *Vitis*-feeding *Antispila* do not show the concentric arrangement of frass that is typical for *A. oinophylla* – particularly in thinner leaves – and the mines are often larger. Mines of *A.* cf *isabella* and related species are much larger, and also have much larger cut-outs, 5 mm or longer. Since not all *Vitis* miners have been comprehensively studied, mine identification cannot yet be relied on.

**Description.** Adult (Figs 1–5). Head face and vertex covered with appressed, strongly metallic, silvery-white scales, more prominently raised in male. Palpi porrect, white; base of proboscis covered with white scales. Antenna fuscous, apical 1 or 2 flagellomeres white. Labial palp silvery white, slightly upturned. Thorax lead-coloured, shiny, contrasting with forewings. Legs grey, tarsi mostly yellowish white, especially on undersides. Forewing dark fuscous with silver-golden patterning; an outwardly oblique fascia from 1/8 of posterior margin to 1/4 of costa, narrowing towards costa; triangular (dorsal) spot at middle of posterior margin, reaching to middle of wing, smaller triangular costal spot just beyond middle, sometimes touching dorsal spot; small, silvery subapical spot in middle of wing at 3/4; fringe line distinct. Terminal fringe paler. Hindwing pale grey. Abdomen lead-coloured, including vestiture on external genitalia.

Measurements: male: forewing length 2.5–2.8 mm (2.6 ± 0.10, n=11), wingspan 5.5–6.2 mm, 25–31 antennal segments (29.1 ± 1.9, n=11); female: forewing length 2.3–2.8 mm (2.5 ± 0.16, n=10), wingspan 4.8–5.6 mm, 25–29 antennal segments (27.2 ± 1.4, n=8).

Venation (Fig. 6). Forewing with Sc barely visible. R1 a separate vein, connected by persistent trachea to Rs+M stem. Rs+M terminating in five branches, interpreted
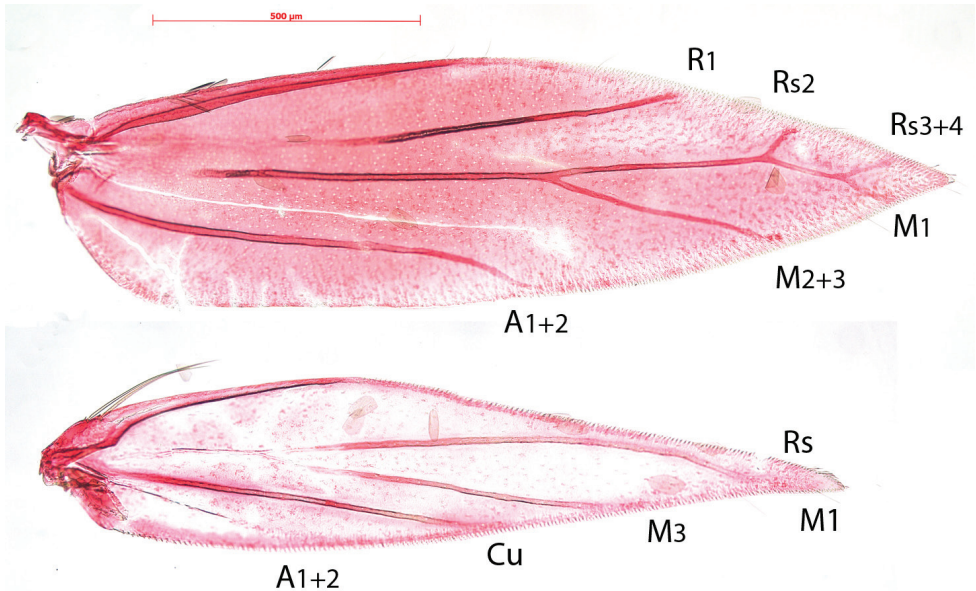
**Figure 8.** *Holocacista rivillei*, venation. Female, Italy, RMNH.INS.24259.

as Rs2 (possibly with 1) to costa, Rs3+4 to costa just before apex, M1 to dorsum just beyond apex, M2+3 to dorsum and a weakly developed CuA. A1+2 a strong separate vein. Hindwing with Sc barely or not visible, Rs+M a strong vein, bifurcate from ca. $1/4^{th}$, upper vein ending in two branches: Rs and M1, lower vein single (M3); Cu and A1+2 separate veins.

Compared to the complicate venation of many other *Antispila* species, including the type species *A. metalella*, (example in Fig. 7, *A. treitschkiella*) venation reduced with loss of forewing cell, separate M stem and connection between R1 and Rs, loss of Rs1 and in hindwing loss of M2. The venation more closely resembles that of *Holocacista rivillei* (Fig. 8), which is even more reduced and also lacks Cu in the forewing.

Male genitalia (Figs 9–16). Uncus bar-shaped, with two large setae dorsally. Vinculum very long, anteriorly rounded, posteriorly shallowly bilobed. Valva more or less triangular, pecten on pedicel, with 10–13 comb teeth (Fig. 15); inner margin of valva with setose lobe anterior to pecten pedicel; basally with a triangular protuberance, almost touching that of other valva; transtilla with trapezoid medial plate, sublateral processes relatively short. Juxta anteriorly spade-shaped, about half as long as phallus. Phallus long, anteriorly much widened, at phallotrema with a comb of about 10–12 strong teeth and at left side a very long curved process (Figs 10–12, 16).

Female genitalia (Figs 17–20). Ovipositor with 4–5 cusps at either side (Fig. 19). S8 medially indented, with many papillate setal sockets. Vestibulum with broad, indistinct sclerotization and no spines (Fig. 18).

**Biology.** Host plants. In North America reared from or found as larva on summer grape *Vitis aestivalis* Michx., both var. *aestivalis* and var. *bicolor* Deam, fox grape *V. labrusca* L., riverbank grape *V. riparia* Michx. and frost grape *V. vulpina* L. Literature
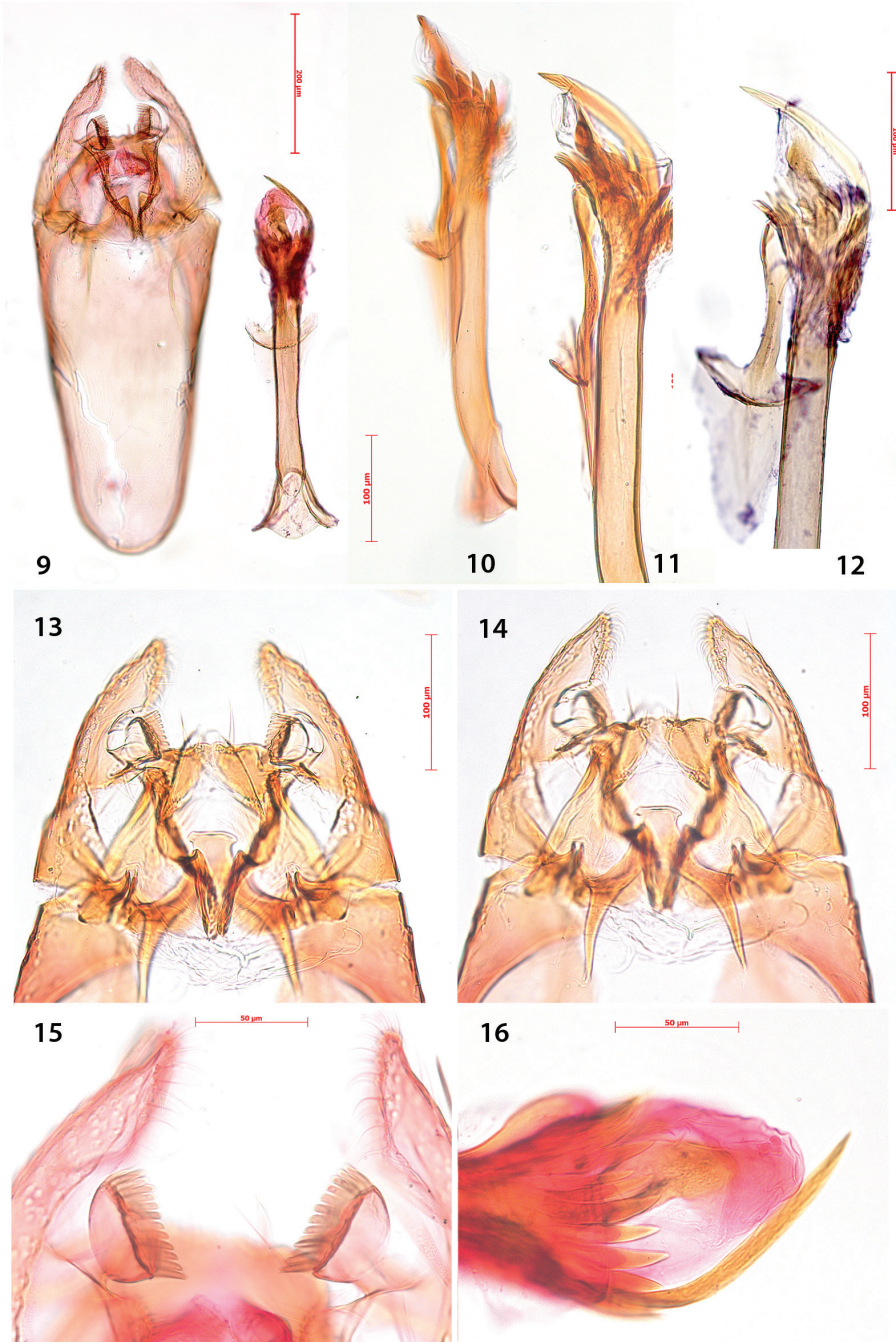
**Figures 9–16.** *Antispila oinophylla*, male genitalia. Paratype, Italy, RMNH.INS.23920 (**9, 15, 16**), Paratype, Italy, RMNH.INS.15247 (**12**), Holotype, RMNH.INS.24204 (**10, 11, 13–14**). **9** Complete genitalia with separate phallus in ventral view **10–12** Phallus and juxta in ventro-lateral view **15–16** Complex of tegumen, uncus, valvae and transtilla **15** Detail of valval tips and pectinifers **16** Detail of spines near phallotrema.
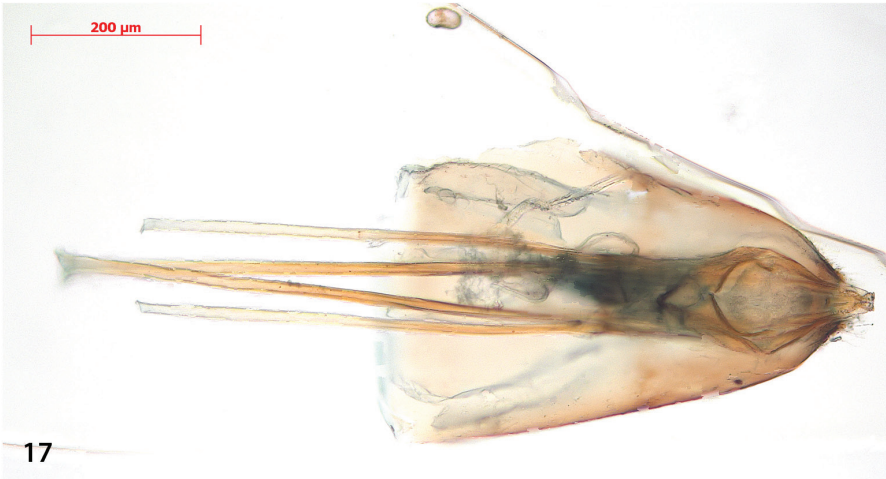
**Figures 17–20.** *Antispila oinophylla*, female genitalia. **17** Terminal segments and apophyses, ventral view, paratype, EJvN4211, USA, Kentucky (pseudotype *ampelopsifoliella*) **18** Internal genitalia, lateral view, showing sclerotisation in vestibulum, paratype, EJvN4206, USA, Connecticut **19** Ovipositor tip, dorsal view, EJvN4206 **20** Detail of S8, ventral view, paratype, Italy, RMNH.INS.15244.

records of *Antispila* "*ampelopsifoliella*" from *Vitis* or grape likely refer to this species (Chambers 1874a, b; Forbes 1923; Needham et al. 1928). We did not find any reports of this species occurring in vineyards in North America. In Italy mines produced by *A. oinophylla* were detected on various *V. vinifera* cultivars, hybrids (e.g. *Vitis riparia* x *rupestris*) and French-American grapes (e.g. Clinton). Infestation levels on the latter were comparable with those observed on commercial vineyards. A preference for some grape cultivars (e.g. Cabernet Sauvignon, Chardonnay, Muscat) is suggested from observations carried out in mixed cultivar vineyards. It is interesting that we also found active mines on Virginia creeper *Parthenocissus quinquefolia* in Italy (Levico and Caldonazzo, Trento province) (identification of larvae confirmed by DNA barcodes, no rearing attempted), whereas we have as yet no records of *A. oinophylla* from this host in North America.

Leafmines (Figs 21–28). The egg is inserted on the underside of a leaf, usually within 1–2 mm from a vein. The mine starts as a rather straight or slightly contorted gallery towards the vein, usually forms a right angle and often follows the vein for a short distance, then again turns away from the vein where it expands into a blotch. The gallery portion, of variable length, is usually later incorporated into the blotch mine. The frass is linear, usually occupies the complete mine width, but occasionally is deposited in a thin line (Fig. 27). In the blotch much of the blackish-brown frass is deposited close to the origin in semicircular concentric frass lines. This characteristic pattern is best seen in thin shade leaves (e.g., Figs 25, 26); in sun-exposed leaves the frass pattern is often obscured. The whole mine occupies as a rule an area of less than 10 × 10 mm; only in thin leaves are mines appreciably larger. The larva cuts out an elliptic case of about 3.2–4.0 mm long.

**Distribution** (Fig. 29, 62). In North America, *A. oinophylla* is known with certainty (material cited) from Canada: Ontario, Quebec; USA: Connecticut, Georgia, Kentucky, New York, Tennessee, and Vermont. Records under *A. ampelopsifoliella* from Maine, Missouri, and Ohio (Brower 1984, Forbes 1923) may partly refer to this species. In Europe introduced into northern Italy, see below. In our experience in the southern Appalachians and New England, at least in the fall, *A. oinophylla* is often the most abundant *Antispila* species occurring on *Vitis*.

**Etymology.** The epithet *oinophylla*, a noun in apposition, is from the Greek οινος (oinos = wine) and φυλλον, plural φυλλα (phyllon, phylla = leaf), "wine leaves," because the larva lives in the leaves of the grapevine from which wine is made.

**Justification for status as new species.** Four species feeding on Vitaceae have been named previously from North America. No name-bearing types are available for three species, only for *A. voraginella* is a holotype extant. The latter is clearly different from *A. oinophylla*, and restricted to western North America. For the eastern species *A. isabella, A. viticordifoliella* and *A. ampelopsifoliella*, we have only the original descriptions and subsequent interpretations to establish identities. The fact that our preliminary sampling of DNA barcodes for grape-feeding *Antispila* show great diversity, complicates matters further. Below, we will discuss these three species in the chronological order of their descriptions.
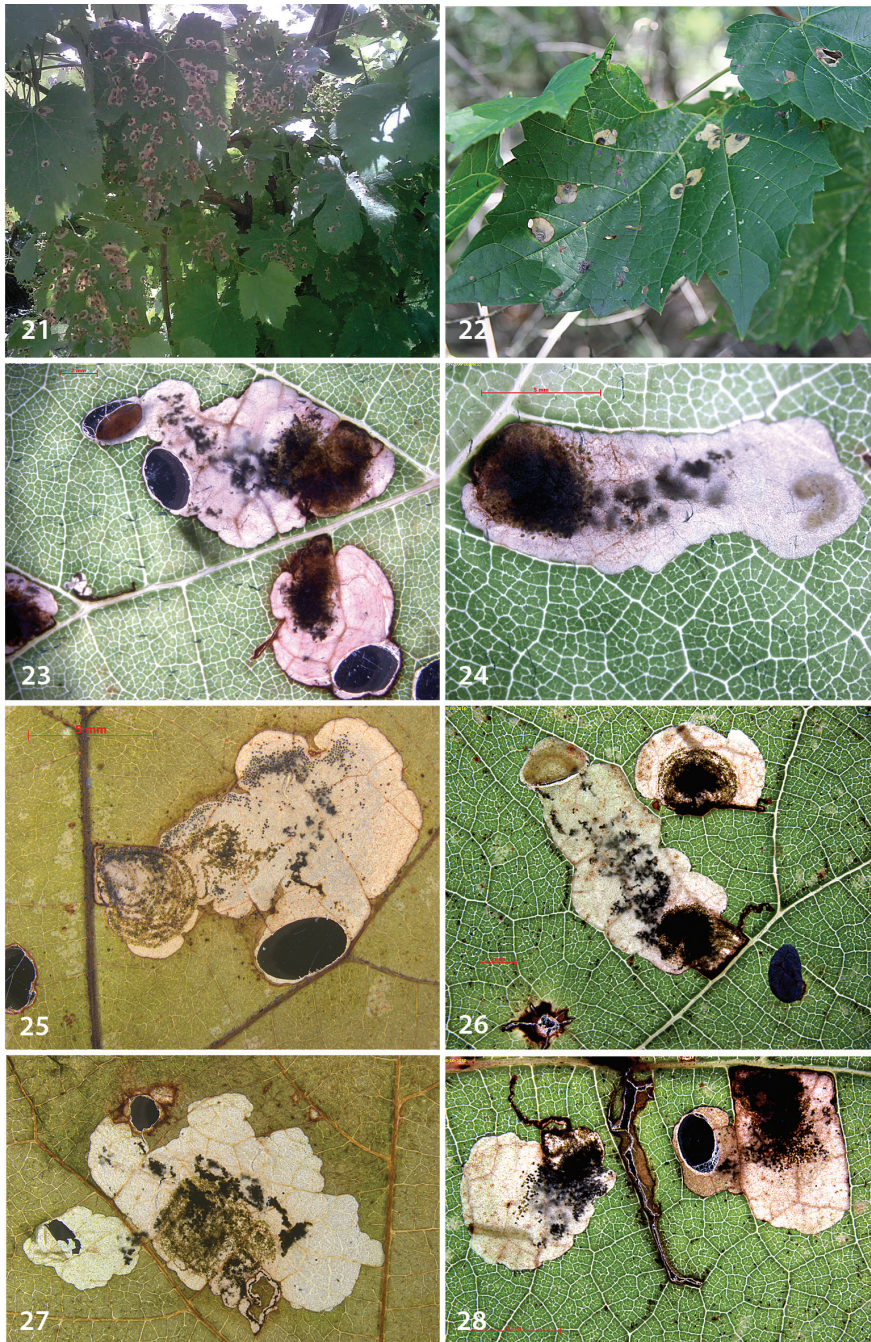
**Figures 21–28.** *Antispila oinophylla*, life history: leafmines on several species of *Vitis* and different localities. **21, 23, 24** Italy, Borgo Valsusana, *Vitis vinifera*, 25.vi.2009 **22** USA: Vermont, Button Bay SP, *Vitis riparia* 16.ix.2011 **25** USA: Tennessee, NP Great Smoky Mts, *Vitis vulpina,* 2.x.2010, mine in shade leaf **26, 28** USA: Georgia, type locality, *Vitis aestivalis* var. *aestivalis*, 14.x.2010 **27** USA: Vermont, Button Bay SP, *Vitis riparia,* 16.ix.2011, DNA barcode, RMNH.INS.18589.

*Antispila isabella* was described from mines on "Isabella grape" (a cultivar of *Vitis labrusca*) and adults (Clemens 1860). The description unequivocally describes a relatively large species without a silvery apical spot. Clemens characterizes the case (shield) as large and almost roundish – both features exclude our species. We have tentatively named one larger barcode cluster as *A.* cf. *isabella*, because mines and adults conform to this description.

*Antispila viticordifoliella* was also described by Clemens in 1860, from mines on "wild grapes" only, differing by a smaller case (shield) and a larva "without dots." Although the foodplant was not explicitly mentioned by Clemens, from the species name it is evident that the host must have been *Vitis cordifolia* Michx. (a synonym of *V. vulpina*). In fact his very brief description could fit the mines of *A. oinophylla*, but subsequently the name has always (e.g. Forbes 1923) been used in the sense of Chambers (1874a), who first described the moth (as "*viticordifoliella* N. sp.?"), without an apical spot and with several, white, distal flagellomeres. He reared that moth from the same hostplant (*Vitis cordifolia*) as Clemens did, and was not able to find the mine on any other *Vitis* (Chambers 1874a: 169). One of the species that we studied from *Parthenocissus* has similar externals, and is named here *A.* cf *viticordifoliella* (Fig. 37). Because we haven't been able to find or rear any similar adults from *Vitis* we are at the moment unable to establish if the *Parthenocissus* miner is indeed the same as *A. viticordifoliella*, but clearly it is not our species (because it lacks an apical spot). In a future revision a neotype will need to be selected to firmly anchor the identity of this species, material from the Chambers' collection (two extant "syntypes", see Miller and Hodges 1990) probably is most suitable for that goal. In collections and websites (e.g., http://moth-photographersgroup.msstate.edu/) the name *A. viticordifoliella* is often misinterpreted as the species that we call *A.* cf *isabella* or a closely related one.

*Antispila ampelopsifoliella*: Chambers (1874a: 168) only briefly described the mine and larva from "*Ampelopsis quinquefolia*" [= *Parthenocissus quinquefolia*] (and stated that he "never succeeded in breeding it."). Only a month later he described the moth under the name "*A. ampelopsisella*" [sic, considered as a subsequent incorrect spelling], writing: "Since that paper was placed in the hands of the Editor, many months ago, I have succeeded in rearing it from the mine [from *Parthenocissus*]" (Chambers 1874b). The confusion of the new species with *A. ampelopsifoliella* dates from Chambers' original description, because he also described a moth that he reared from *Vitis* and shows the external characters of both species:

"Last summer I found its leaves [referring to a *Vitis* species] mined by a larva closely resembling that of *A. ampelopsifoliella*, supra, and which I suspect to be the same. ….. From it I bred the species described below, which I do not now name, as it may prove to be identical with *A. ampelopsifoliella*." (Chambers 1874a). One month later he wrote: "but I believe it to be the same" (Chambers 1874b). Ever since these two publications, the species has been considered to feed both on *Parthenocissus* and *Vitis* (e.g., Forbes 1923; Brower 1984). However, our rearing and barcode data show that two or three species of *Antispila* are feeding on *Parthenocissus*, which show large barcode distances to *A. oinophylla* or other *Vitis* miners (Fig. 30), and thus are not identical.
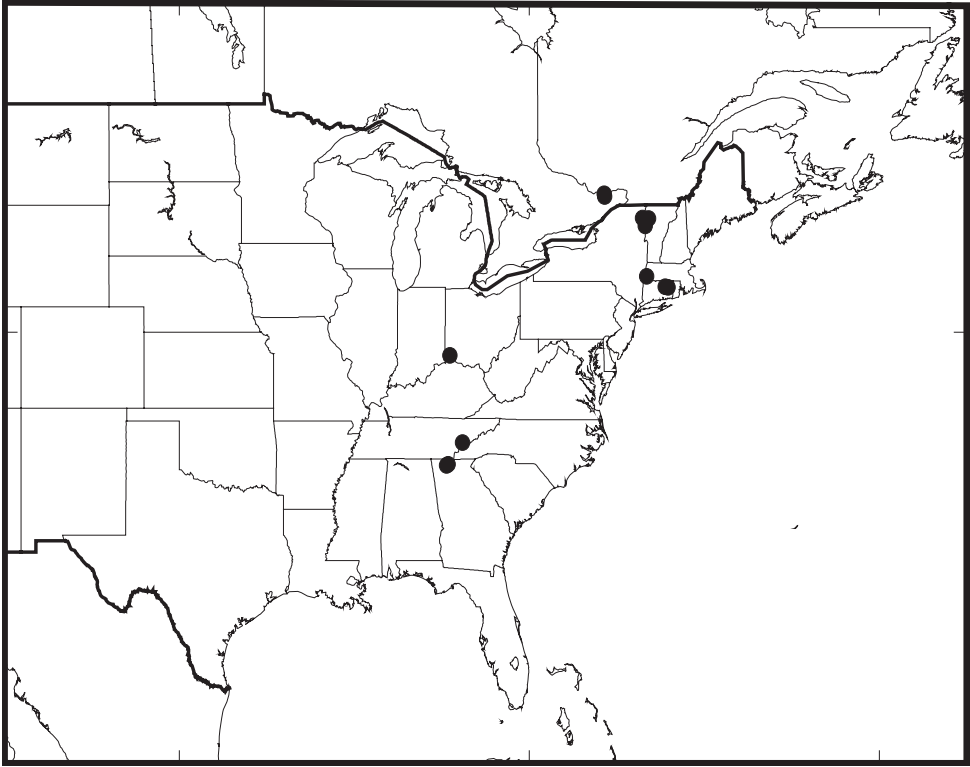
**Figure 29.** *Antispila oinophylla*, distribution in North America.

In Chambers' collection at MCZ there are three specimens under the name *A. ampelopsifoliella* that probably served as the basis for the adult description. These specimens were termed pseudotypes (Miller and Hodges 1990), since they were not available at the time of the original description, because then Chambers only had mines and larvae available. Of the three specimens, one is completely missing from the pin. The one labelled as from *Parthenocissus* unfortunately is heavily damaged, only a forewing and hindwing being present. A third specimen, a female, is complete and was dissected (Fig. 17). This specimen, however, appears to be *A. oinophylla.* This is no surprise, since Chambers (1874a, 1874b) considered the *Vitis* miner to be the same as the *Parthenocissus* miner, and thus he would have placed specimens reared from both hosts under the same name. There is no indication of the hostplant or the collecting year on this particular specimen, so it is useless for confirmation of the identity of *A. ampelopsifoliella.*

We restrict here the usage of the name *A. ampelopsifoliella* to the species feeding on *Parthenocissus*, with an apical spot (The generic name for *Parthenocissus quinquefolia* was *Ampelopsis* at the time Chambers described the species.) Although we have not obtained a DNA barcode form such an adult, the fact that an adult from the other cluster on this host (see below) does not have such a spot and is tentatively identified as *A.* cf *viticordifoliella*, we can associate *A. ampelopsifoliella* adults with one of the larval types.

When adults are available for all barcode clusters, we suggest that a neotype be selected from material reared from *Parthenocissus* from the vicinity of Covington, Kentucky, to fix the identity of Chambers' name.

## DNA barcoding and species relationships

### Barcode analysis

Neighbor-joining trees of all sequenced barcodes, both based on Kimura 2P distances and uncorrected distances give highly similar results in topology and branch lengths, we illustrate here the last one (Fig. 30). All species clusters have a bootstrap value of 100, and within-species variation is usually low or absent. We caution, however, that for several species, such as *A. treitschkiella* or *H. rivillei* most sequences are from just one or two populations. Two species clusters show large intraspecific distances: the two specimens of *A. hydrangaeella* have 5.22% K2P distance and 4.99% uncorrected pairwise distance, and the species tentatively named *A.* cf *viticordifoliella* forms two clusters with around 4% distance in both methods. Although the mines of these clusters look superficially the same we have not studied the adults of one cluster, so it is possible that these clusters represent separate species.

We have 20 sequences representing *A. oinophylla*, seven of which are 100% identical, five from Italy (including one from *Parthenocissus*) and two from North America (RMNH.INS.18392 from Georgia and RMNH.INS.18558 from New York). The others are very similar, with at most five nucleotides differing from those of the core group (RMNH.INS.18394 from Georgia). The genetic distance varies from 0 to 1.23% K2P distance (1.22% uncorrected). The differences occur in 16 different positions, of which six cases are found in more than one specimen (e.g., a G instead of A in position 82 combined with a T in 316; the seven specimens forming a "clade" in Fig. 30 with RMNH.INS.18533 and BVS04; position 550: C instead of T; four specimens forming the "clade" in Fig. 30 with RMNH.INS.18533, position 634 a T instead of A in RMNH.INS.18298 and RMNH.INS.18300, both from Tennessee). Several haplotypes are found both in Italy and North America. The largest distance is between two North American specimens, one from Georgia and one from Tennessee (RMNH.INS.18394 and LGSM035–06). The genetic distance to the closest congeneric species *A. voraginella* is large: more than 10%.

### Phylogenetic analyses

The maximum parsimony analysis of the barcode sequences resulted in three shortest trees, of which the 50% majority rule tree is illustrated (Fig. 31). The semi-strict tree differs only in the position of *Heliozela aesella*, which forms a polytomy with the three main heliozelid clades in Figure 31. Of the 658 characters, 243 characters are parsimo-
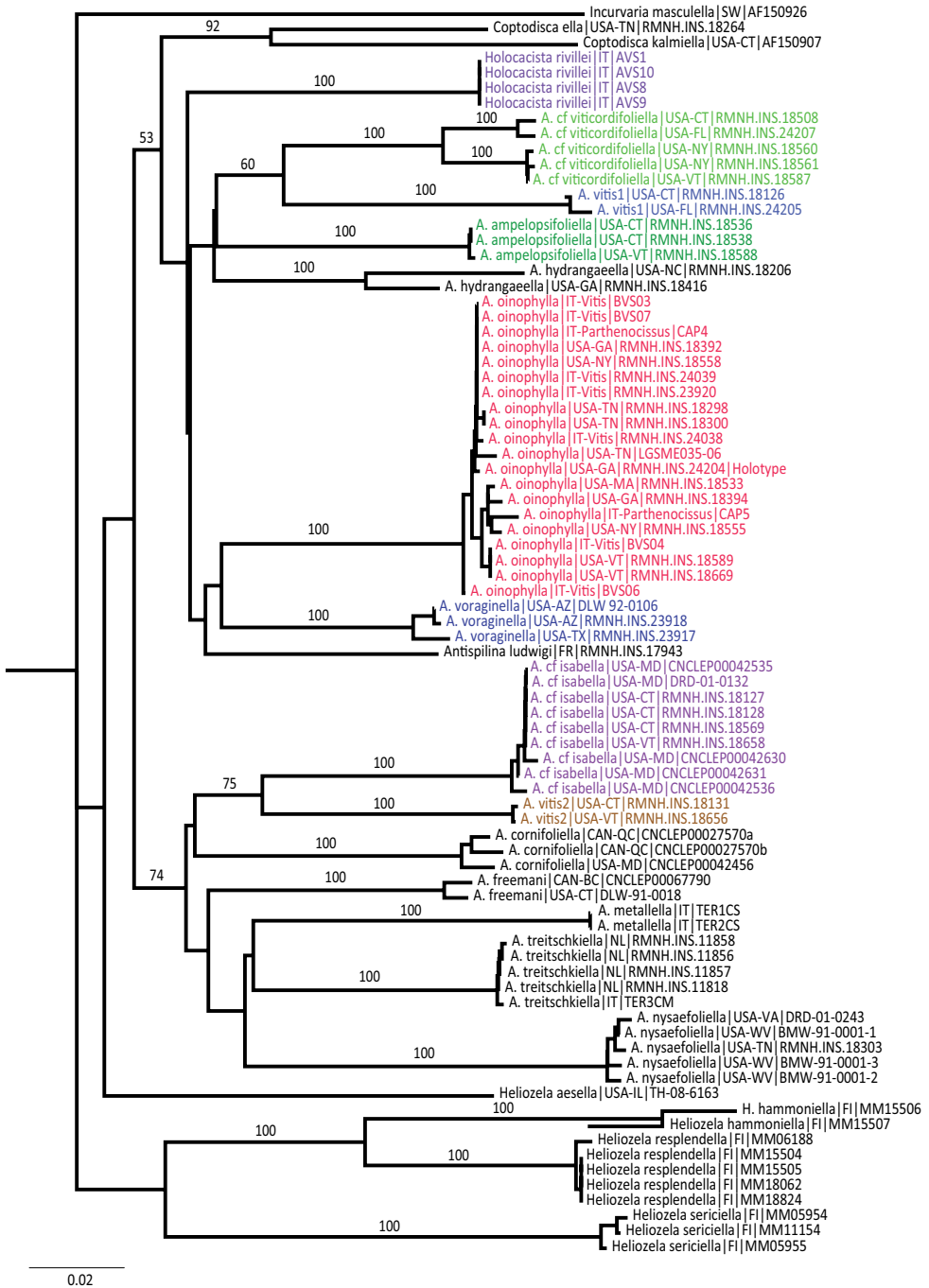
**Figure 30.** Neighbor-joining tree for heliozelid COI barcodes, based on uncorrected pairwise distances. Numbers on branches are bootstrap values, 10,000 replicates. Vitaceae-feeding clusters are coloured differently, others in black. Labels include species name or informal name, codes for country and state (in North America) and sample numbers (Genbank numbers for sequences taken from Genbank).
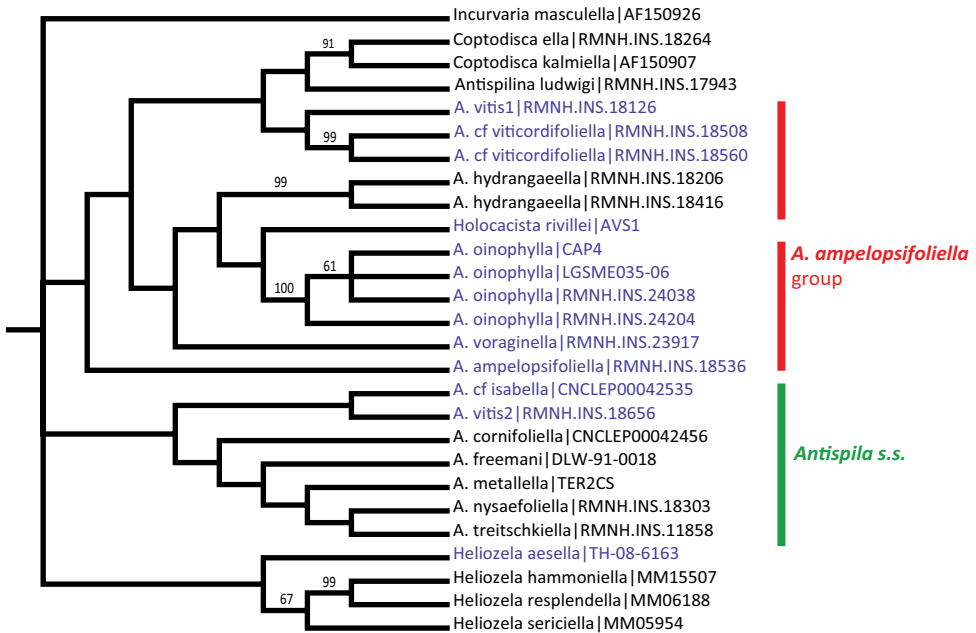
**Figure 31.** Cladogram, 50% majority rule consensus of three shortest trees from maximum parsimony analysis of COI sequences. CI = 0.361, RI = 0.456, RC = 0.168. Figures are bootstrap values from a TNT analysis (10,000 bootstrap replicates). Purple-coloured taxa are feeding on Vitaceae. The semi-strict tree differs only in the position of *Heliozela aesella* (see text).

ny informative. Bootstrap values are taken from the TNT analysis. The two Bayesian analyses of the same dataset showed few differences, we here illustrate the consensus tree based on three partitions (Fig. 32).

Both cladograms are rather similar. *Antispila oinophylla* forms a highly supported clade. Clades for *Heliozela*, a core *Antispila* grouping and a clade with several smaller genera and the *A. ampelopsifoliella* group were recovered, with strong support in the Bayesian analysis for the latter clade (0.97) and for *Heliozela* (1) and less support for core *Antispila* (0.74). Within the core *Antispila* clade, the two Vitaceae species form a clade, well supported in the Bayesian tree, nested in or sister to the Cornaceae-feeding species.

The Bayesian analysis recovered a monophyletic *A. ampelopsifoliella* group. In both analyses this group clusters with the small genera *Coptodisca, Holocacista* and *Antispilina*. These all share the reduced venation as described here for *A. oinophylla*. Relative positions of these small genera and the two clades of *Antispila* vary amongst various analyses. In the Bayesian tree there is low support for a clade of *Antispilina* and *Holocacista*. In none of the analyses was Heliozelidae recovered as a monophyletic group.
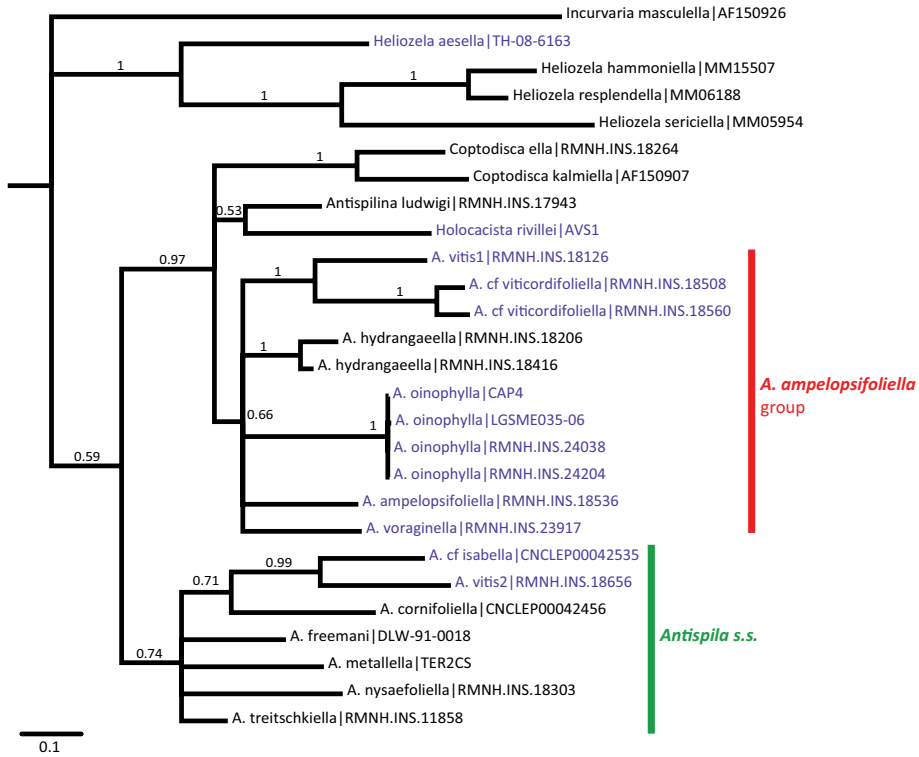
**Figure 32.** Cladogram from Bayesian analysis on three partition dataset. Figures are posterior probabilities. Purple-coloured taxa are feeding on Vitaceae.

Vitaceae-feeding taxa are indicated in the cladograms by a purple colour. If these cladograms correctly represent the phylogenetic history of the Heliozelidae, it appears that Vitaceae were the ancestral hosts for the family.

**Comparative notes to other species**

Below we will briefly treat the other Vitaceae miners amongst North American and European Heliozelidae and one other closely related species, in order to distinguish them from *A. oinophylla*. As there are several more *Antispila* species in North America than currently described, this is a preliminary treatment until a thorough revision can be completed. Because we have not yet been able to link some larval barcode clusters to their associated adults, the number of leafmine types described below is higher than the number of adult "species". Material examined for each of these "taxa" is listed in the Appendix A.

## *Antispila ampelopsifoliella* Chambers

http://species-id.net/wiki/Antispila_ampelopsifoliella
Figs 35, 42, 43, 53, 56

*Antispila ampelopsifoliella* Chambers, 1874a: 168. Syntypes: leafmines [USA: Kentucky, Covington] on *Ampelopsis quinquefolia* [= *Parthenocissus quinquefolia*], "pseudotypes", Kentucky, Covington (MCZ) [examined].
*Antispila ampelopsisella* Chambers, 1874a: 197. Subsequent incorrect spelling.
*Antispila ampelopsiella* Chambers, 1874a: 198. Subsequent incorrect spelling.
*Antispila ampelopsifoliella*; Needham et al. 1928: 289 [partim]; Davis 1983: 4 [partim].
*Antispila ampelopsiella*; Dyar et al. 1903: 539 [partim]; Barnes and McDunnough 1917: 181 [partim]; Forbes 1923: 226; McDunnough 1939 [partim]: 91; Brower 1984: 29 [partim].

**Differential diagnosis.** We cannot separate *A. ampelopsifoliella* (Fig. 35) from *A. oinophylla* based on external characters: it may average a bit smaller, but our samples are too few in number to make statistical comparisons. In the male genitalia (Figs 42–43), uncus not bilobed; valva with pecten with ca. 11–13 comb spines, base of valva with rounded lobe, not triangular; juxta rather wide, with lateral groups of spines; phallus with much shorter terminal spines and a comb of rather short triangular spines near phallotrema. Female genitalia (Fig. 53): ovipositor only with 3 cusps at either side. Vestibulum with some spines.

**Biology.** Hostplant: *Parthenocissus quinquefolia*.

**Leafmines** (Fig. 56). Egg usually inserted in leaf under- or upperside close to a vein, mine starting with a relatively long contorted gallery with thin broken frass, or when it runs along margin in a straighter course, later abruptly enlarged into elongate blotch or wide gallery; frass dispersed in middle. The early narrow gallery may be as long as the elongate blotch. The mine can be found in any part of the leaf. Larva yellowish white, black head, cut-out ca 3.5–4 mm long. The mine resembles that of *A. hydrangaeella*. It was most frequently found in the larger and thinner ground leaves of Virginia creeper.

**Distribution.** Eastern North America, confirmed from USA: Connecticut, Kentucky, New York, Vermont and Canada: Ontario.

## *Antispila voraginella* Braun

http://species-id.net/wiki/Antispila_voraginella
Figs 34, 44, 45

*Antispila voraginella* Braun, 1927: 191. Holotype male: **USA:** [Utah: Washington County] "B1206/Zion Canyon/Utah i.iv.9 [1926]- Antispila / voraginella / Type Braun.", Genitalia slide EJvN 3916 [reared from mines on *Vitis arizonica*] (ANSP) [examined].
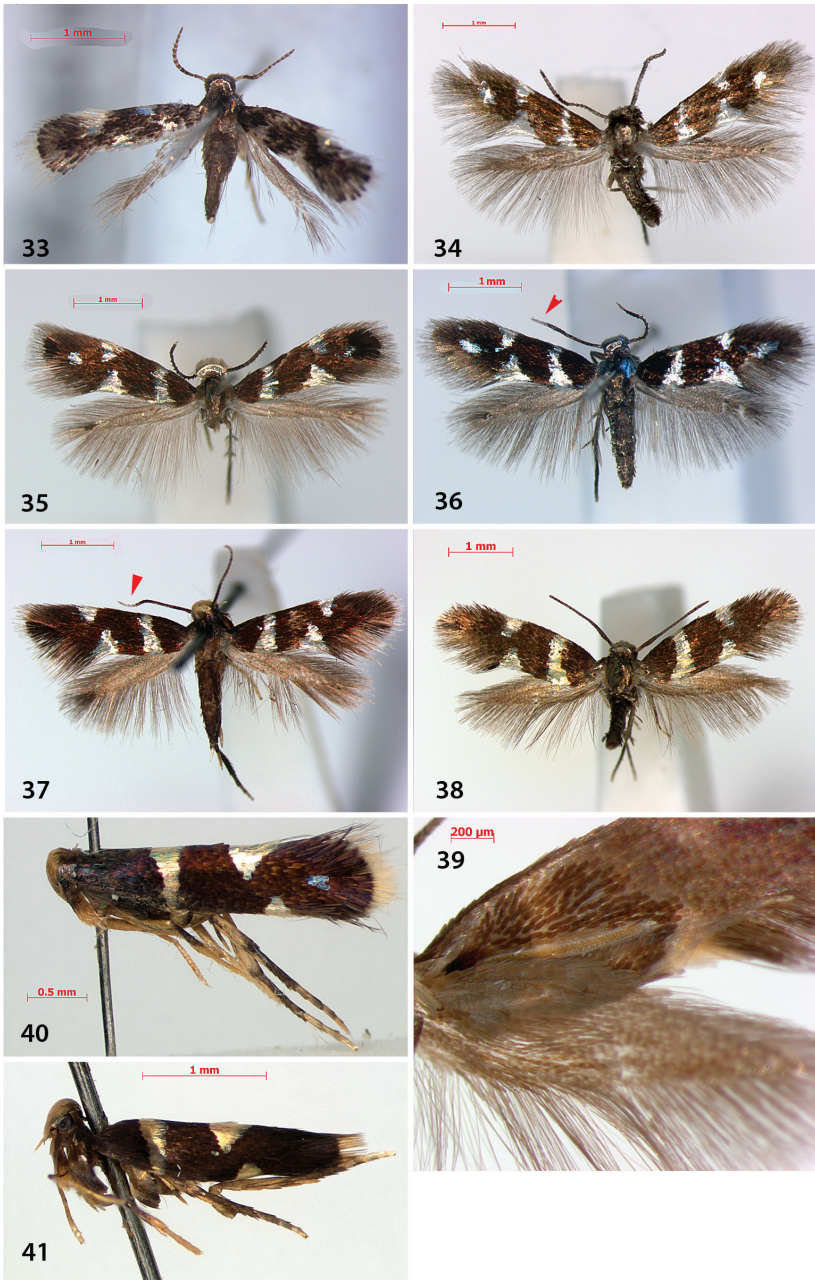
**Figures 33–41.** *Holocacista* and *Antispila* adult habitus in dorsal or lateral (**40, 41**) view. **33** *H. rivillei*, male, Italy **34** *A. voraginella*, male, USA: Arizona, genitalia slide EJvN3918 **35** *A. ampelopsifoliella*, female, USA, Vermont: Salisbury, genitalia slide JCK15220 **36** *A. hydrangaeella*, female, USA: Georgia, Chattahoochee NF **37** *A.* cf *viticordifoliella,* female, Canada: Ottawa **38, 39** *A.* cf *isabella*, male, upper and underside (39) with androconial scales, USA: Connecticut, Mansfield, DLW90J8 **40** *Antispila* "vitis1", female, USA: Florida, genitalia slide EJvN4205 **41** *A.* cf *viticordifoliella*, female, USA: Florida, genitalia slide EJvN4207. Arrows indicate white tipped antennae in *A. hydrangaeella* and cf *viticordifoliella*.

**Differential diagnosis.** Adult (Fig. 34) very similar to and about same size as *A. oinophylla*, but head and thorax covered with brassy shining scales rather than silver. In male genitalia (Figs 44–45) uncus clearly bilobed, valva with fewer pecten spines: 8–10, triangular lobe absent; transtilla with narrower central plate and phallus with rather different set of spines: the long one of *oinophylla* absent, and row along phallotrema less comb-like, whereas there is a row of many spines along both sides. Female genitalia not examined.

**Biology.** Hostplant: *Vitis arizonica*. Seems to be univoltine, larvae found in June–July northward; through September in monsoonal areas to south; adults emerging the following spring April to June.

**Leafmines.** Mine illustrated by Powell and Opler (2009: plate 59:7). Mines rather different from those of *A. oinophylla*: larvae usually gregarious with mines forming large pale blotches.

**Distribution.** Evidently allopatric to *A. oinophylla* and only recorded from the Rocky Mountains: Utah, Arizona and West Texas.

### *Antispila* "vitis1"

Fig. 40

From this barcode cluster we have just two females from Florida (Fig. 40, one barcoded) and one larva from Connecticut, of which it is unclear to what type mine it belongs. The female is indistinguishable externally from *A. oinophylla.* Almost certainly this represents another new species.

### *Antispila hydrangaeella* Chambers

http://species-id.net/wiki/Antispila_hydrangaeella
Figs 36, 47

*Antispila hydrangaeella* Chambers, 1874a: 170. Syntypes leafmines and larvae: [USA: Kentucky, Covington] on *Hydrangea arborea* [probably lost].

**Differential diagnosis.** DNA barcodes suggest that two species might be involved, and leafmines from a population in North Carolina (Smoky Mts NP) and northern Georgia do show some differences. Described adults and larvae are from the Georgia population. Externally, adult *A. hydrangaeella* (Fig. 36) is extremely similar to the other species of the *A. ampelopsifoliella* group, but it differs by the last six antennal segments being white and by genitalia and hostplant data. In male genitalia (Fig. 47) uncus only shallowly bilobed; valva with long pecten with more comb spines: *ca.* 20, triangular lobe absent, at base of valva beardlike setation; juxta rather wide, with groups of spines laterally; phallus with two very long terminal spines and many small spines near phallotrema, not forming a comb. Female genitalia not examined.
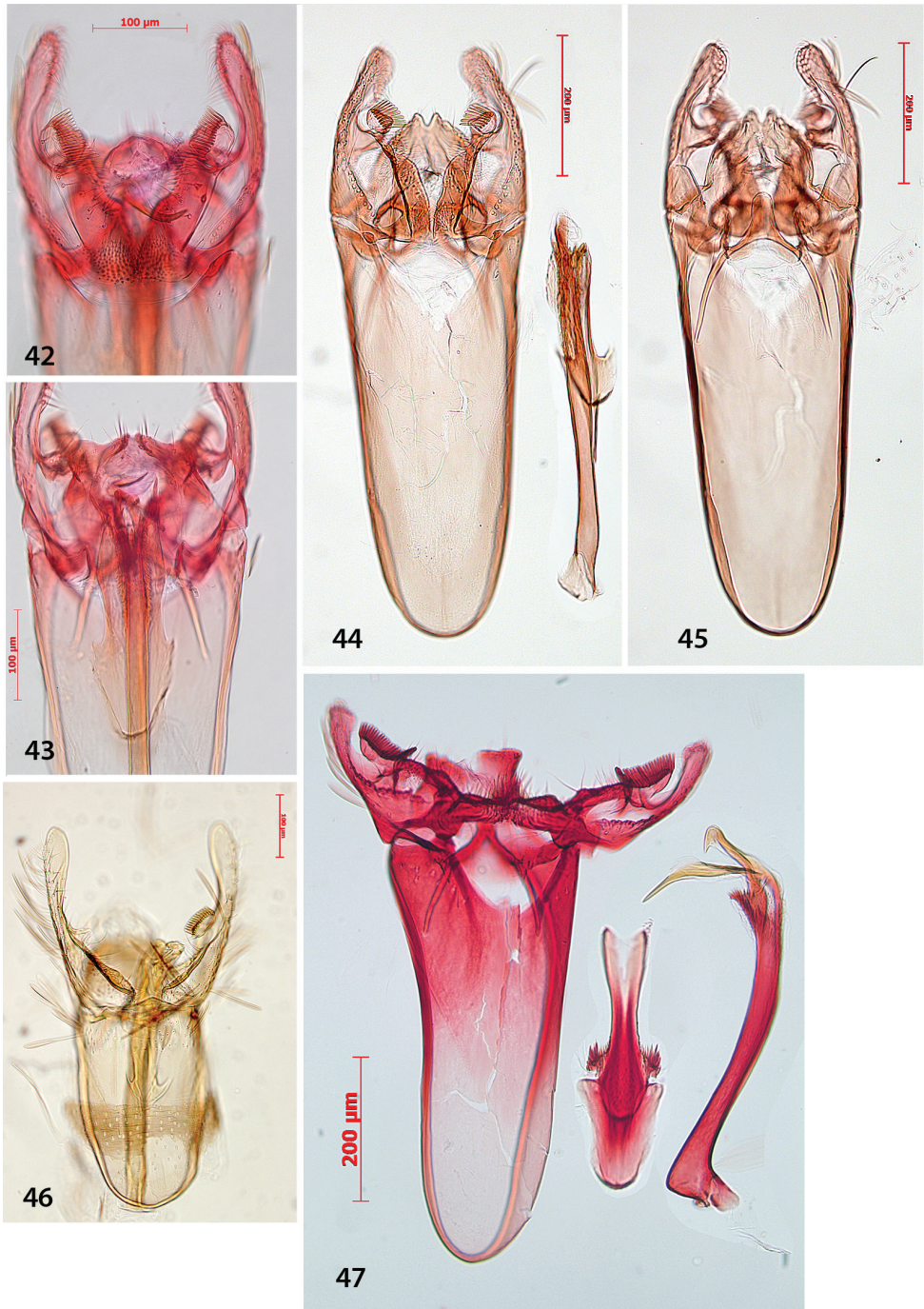
**Figures 42–47.** *Antispila* species, male genitalia. **42–43** *A. ampelopsifoliella*, USA, New York state, genitalia slide EJvN4200 **44–45** *A. voraginella*, USA, holotype, genitalia slide EJvN3916 **46** *A.* cf *isabella*, USA: Kentucky, Morehead, genitalia slide CNC MIC1859 **47** *A. hydrangaeella*, USA: North Carolina, NP Great Smoky Mts., genitalia slide EJvN4198.

**Biology.** Hostplant: *Hydrangea arborea*.

**Leafmines.** One type (North Carolina) with long gallery mines, often following a vein, ending in a blotch with greenish to brown frass. The mines from Georgia with early gallery mine much contorted in a small area, with black frass, ending in elongate mine with blackish dispersed frass.

**Distribution.** USA: Georgia, Illinois, Kentucky, North Carolina, presumably widespread in eastern United States.


### *Antispila viticordifoliella* Clemens
http://species-id.net/wiki/Antispila_viticordifoliella

*Antispila viticordifoliella* Clemens, 1860: 209. Syntype mines, larva [USA: Pennsylvania, Easton], larvae on "wild grapes" [*Vitis vulpina*], August–September, Brackenridge Clemens (ANSP if extant).
*Antispila viticordifoliella*; Chambers 1874a: 168 [first description of adult].

**Differential diagnosis.** In the interpretation of this species by Chambers (1874a), as discussed above, *A. viticordifoliella* differs from the *A. ampelopsifoliella* group in missing the apical spot on the forewing and its long white antennal tip, the latter character is shared with *A. hydrangaeella*. We have as yet not seen such specimens originating from *Vitis*.

**Biology.** Hostplant: *Vitis vulpina*. Leafmines not described in detail.

**Distribution.** USA: Kentucky, Pennsylvania. Many records are unreliable and often refer to the *isabella* complex.


### *Antispila* cf *viticordifoliella* Clemens
http://species-id.net/wiki/Antispila_cf_viticordifoliella
Figs 37, 41, 57

**Remarks.** Two females (Figs 37, 41), reared from *Parthenocissus* mines, match Chambers' (1874a) description of *A. viticordifoliella* adults. Because the possibility exists that two species with similar externals, feeding respectively on *Vitis* and *Parthenocissus*, are involved here, we cannot decide whether the *Parthenocissus* miner is conspecific with *viticordifoliella* or not, before we have studied genitalia and/or DNA barcodes from specimens originating from both hostplants (to date we have only barcodes from *Parthenocissus* miners and no males from either form). Moreover, there is a deep split in the barcodes from *Parthenocissus* miners, here tentatively identified as *A.* cf *viticordifoliella*, one cluster from New York and Vermont, the other from Connecticut and Florida. We did not see differences in mine or larva between these clusters, and thus tentatively regard them as one species.

**Biology.** Hostplant: *Parthenocissus quinquefolia*.
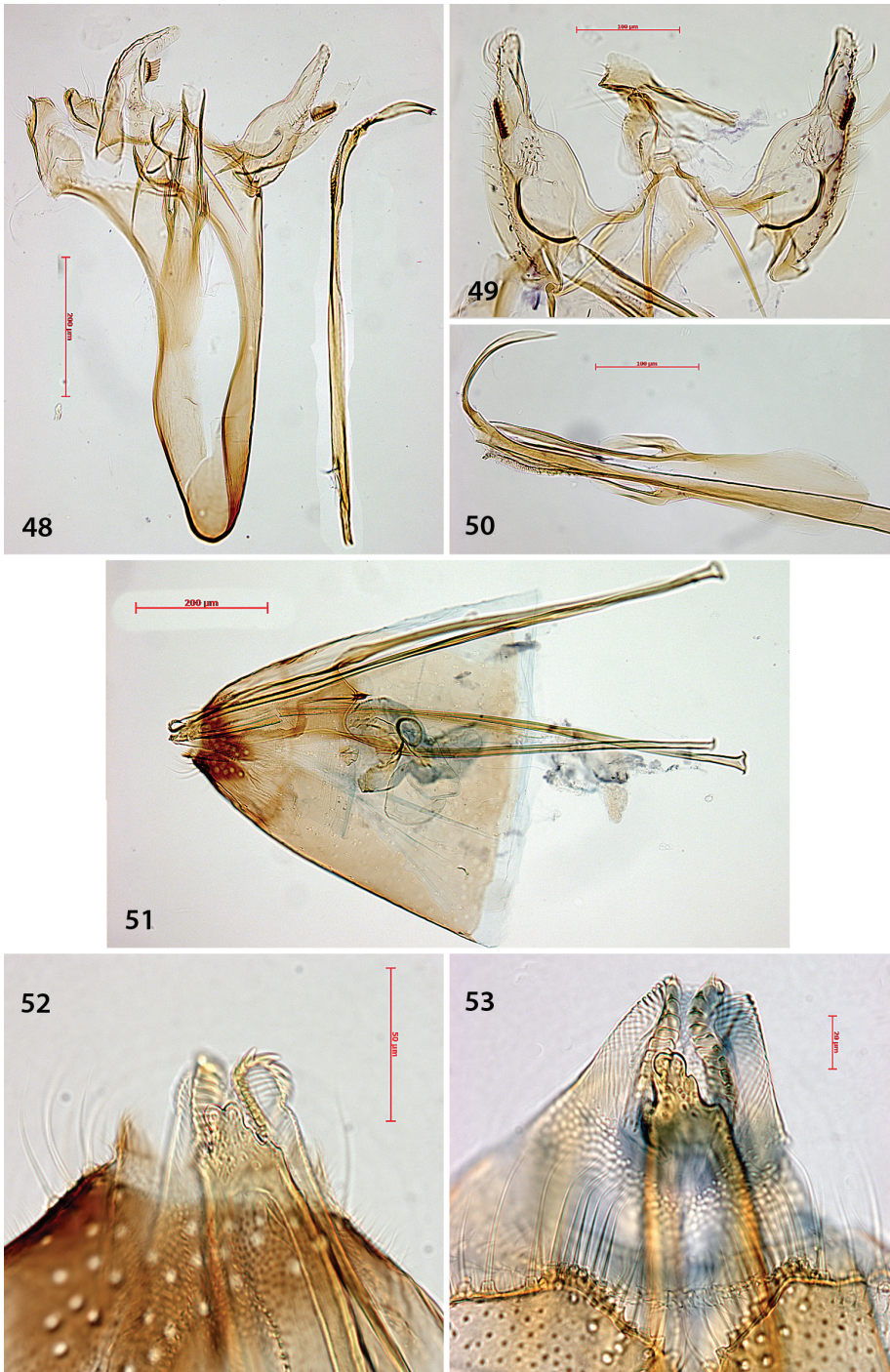
**Figures 48–53.** *Holocacista rivillei*, male and female genitalia*, A. ampelopsifoliella*, female genitalia (**53**). **48–50** Male genitalia, Italy, slides RMNH.INS.15248, 15250, 15251 **51–52** Female genitalia, slide RMNH.INS.15252 **53** Ovipositor and tergum 8, genitalia slide JCK15220.

**Leafmines** (Fig. 57). Egg often inserted on leaf margin, position often hard to find, rarely near midrib, mine without a gallery at the start, an elliptic elongate blotch mine, often running along or near leaf margin; frass sometimes grouped in a clump, more typically spread in an irregular broad line. Larva yellow with almost black head, cut-out ca 3.5–4 mm long. This mine was most frequently seen in thicker leaves borne from climbing shoots.

**Distribution.** Canada: Ontario. USA: Connecticut, Florida, New York, Vermont.

### *Antispila* **cf** *isabella* **Clemens**
http://species-id.net/wiki/Antispila_cf_isabella
Figs 38, 39, 46, 58, 60 (59, 61 *A.* "vitis2")

*Antispila isabella* Clemens, 1860: 209. Syntypes: [USA: Pennsylvania, Easton], lar-
    vae on "Isabella grape", September, adults emerged May, Brackenridge Clemens
    (ANSP if extant).
*Antispila isabella*; Chambers 1874a: 167 [redescription].

**Differential diagnosis.** Under this name there is probably a complex of species, often with conspicuous androconial scales in males. Among the barcodes we distinguish two clusters, here tentatively named as *A.* cf *isabella* and *A.* "vitis2". The adults described here do not necessarily belong to one of the described mine types.

Moths (Figs 38–39) of this species complex are easily distinguished from the *A. ampelopsifoliella* group by the missing apical spot on forewing and larger average size. Moreover males have conspicuous yellow or brown androconial scales on forewing underside (Fig. 39). The venation is also more complete (as in Fig. 7).

Male genitalia were examined of one of the species (Fig. 46) the valva is more elongate, and the pecten includes 10–13 teeth. Phallus lacks larger spines at phallotrema, but has many scale-like, small spines, and posteriorly possesses an asymmetric broad lobe; anteriorly not widened. Other individuals have not been examined; as noted above, the group is in need of revision.

**Biology.** Hostplant: *Vitis aestivalis, V. labrusca* [incl. "Isabella" grapes]*, V. riparia.*

**Leafmines.** Mines of *A.* cf *isabella* (Figs 58, 60) are relatively large mines, with the egg deposited near a vein. No gallery visible, mine a large blotch, with a roundish patch of reddish frass near beginning, probably attached to upper epidermis, and dispersed black frass throughout mine. Cut-out large, around 5 mm long.

Mines of *A.* "vitis2" (Figs 59, 61) also start on a vein, without gallery, and are relatively compact blotches, with frass concentrated in a mushroom shape or reversed triangular near beginning of mine. Cut-out large, around 4.8 mm.

**Distribution.** Canada: Ontario. USA: Connecticut, Georgia, Kentucky, New York, Pennsylvania, Vermont.

**Remarks.** Both COI sequences and external sexual secondary characters show that more species are involved. We have tentatively named the most common form as *A.* cf
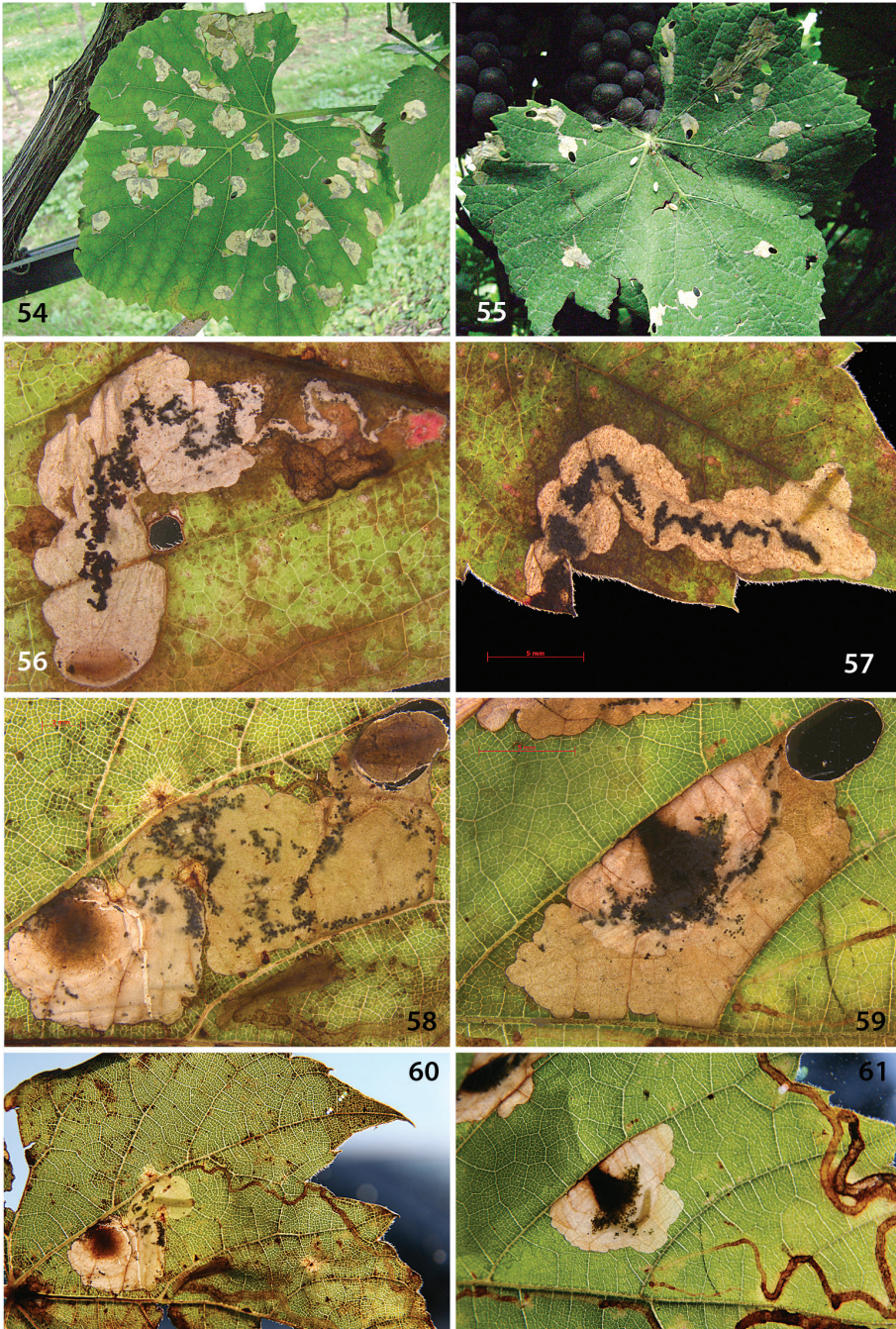
**Figures 54–61.** *Holocacista rivillei* and *Antispila* species, life history. **54–55** *H. rivillei* on *Vitis vinifera*, Italy: Rovereto **56** *A. ampelopsifoliella* on *Parthenocissus quinquefolia*, USA: Button Bay SP, 16.ix.2011 **57** *A.* cf *viticordifoliella* on *P. quinquefolia*, same locality **58, 60** *A.* cf *isabella* on *V. riparia*, USA: Button Bay SP, 16.ix.2011 **59, 61** *A.* "vitis2" on *V. riparia,* same locality. In the last four photos also parts are visible of gallery mines of *Phyllocnistis vitegenella.*

*isabella*, and research of types or material from the collections of Clemens and Chambers is needed for establishing the identities of these names.

### *Holocacista rivillei* (Stainton)

http://species-id.net/wiki/Holocacista_rivillei

Figs 8, 33, 48–52, 54, 55

[Unnamed] Godeheu de Riville 1750: 177 [extensive description from Malta].

*Alucita vitella* Vallot, 1822: 253. [Preoccupied by *Alucita vitella* Fabricius, 1775].

*Elachista rivillei* Stainton, 1855: 87. [Malta, Godeheu de Riville, 18th century, mines on *Vitis*] Renamed after Riville´s description in 1750. [types probably not existing].

*Antispila rivillei*; Stainton 1869: 310 [repetition of description by Godeheu de Riville].

*Antispila rivillella* Rondani, 1877: 288 [Redescription, parasitoids].

*Holocacista rivillei*; Walsingham and Durrant 1909: xxix [new genus, first recorded from France].

**Differential diagnosis.** Moth (Fig. 33) much smaller than *Antispila* species, with 3.5–4 mm wingspan. Forewing pattern without apical spot, costal spots further away from wingbase than dorsal spots. Male genitalia (Figs 48–50) with slightly bilobed uncus, valva more elongate, pecten with 8–10 teeth; juxta with pair of lateral teeth; phallus extremely slender and long, ending posteriorly in long curved spine and row of small spines below that. Juxta bilobed apically. Venation reduced, rather similar to that of *A. oinophylla* (see Fig. 8).

**Biology.** Hostplant: *Vitis vinifera*.

**Leafmines** (Figs. 54–55)**.** Mine beginning with relatively long, slender gallery, later a small blotch with small cut-outs. Cocoons often attached to stems or leaves.

**Distribution.** Southern Europe, western and Central Asia: Spain, France, Italy, Malta, Slovenia, Croatia, Bulgaria, Greece, Ukraine, Turkey, SE Russia, Georgia, Kazakhstan, Uzbekistan, Turkmenistan (Voigt 1931; Berro 1934; Marchi 1956; Dovnar-Zapol'skij 1969; Bournier 1977; Puplesiene 1996; Maček 1999; van Nieukerken 2011).

### Distribution of *A. oinophylla* in Italy (Fig. 62)

In Italy, *A. oinophylla* was detected for the first time in the summer of 2007 in a vineyard located in Valsugana (Borgo Valsugana, Trento province, Trentino-Alto Adige Region). Additional surveys conducted in the late summer of 2007 revealed its occurrence also in the neighbouring Vicenza and Belluno provinces (Veneto Region), particularly in neglected vineyards. In 2008, the distribution of the species did not differ greatly in the Trento province; elsewhere the insect was recorded in commercial vineyards of three provinces of the Veneto Region (Vicenza, Belluno and Treviso), sometimes at significant
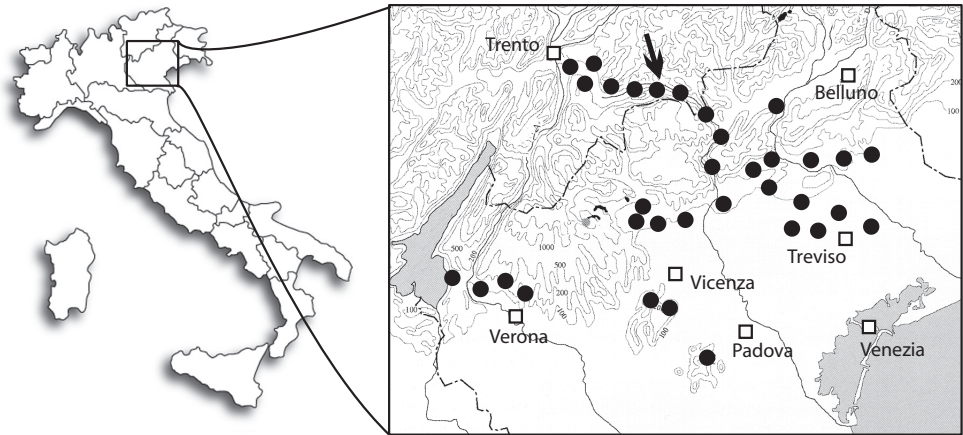
**Figure 62.** Map showing the distribution of *Antispila oinophylla* in Italy up to 2010 (filled circles = sites of occurrence; arrow = site of collection specimens for sequencing).

densities. In a number of vineyards *A. oinophylla* occurred together with *P. vitegenella*, rarely with *H. rivillei*. In 2009 and 2010, a dense infestation was detected in commercial vineyards located in the Vicenza province (Breganze), about 80 km south of Borgo Valsugana. In this area severe symptoms had been detected as early as 2006 but they were misidentified as being caused by *H. rivillei*. Since viticulture of this area is much more extensive than that around Borgo Valsugana, it is likely that *A. oinophylla* was introduced first in the Vicenza province and dispersed from there to the other areas. Also in 2010 the species was recorded in the Verona province, 90 km west of Breganze (E. Marchesini, pers. comm.). The distribution of *A. oinophylla* in Italy in 2010 is presented in Fig. 62.

**Field observations in Italy**

Observations carried out in winter 2008 showed that fully fed, final instar larvae of *A. oinophylla* overwintered inside their cases, fixed to the vine trunks or training stakes. Most larvae pupated in May and the first adults were seen in early June. Mines were detected first in the second half of June. Larvae of the penultimate instar cover the internal surface of the mine with a thin layer of silk, cut away an oval leaf section from both the upper and lower leaf surfaces, and then formed a case by joining the excised leaf sections with silk. Case-bearing larvae move slowly on the leaf surface and then descend with a silken thread until they contact a trunk, training stake, or other solid object to which they affix their case. In the experimental vineyard, the first cases were observed in the first half of July. An additional generation occurred from the second half of August onwards. In 2008, 86.9% of the leaves were infested with a density of 3.26 ± 0.25 (mean ± standard error) mines per leaf by the end of the first generation. In

late summer, by the end of the second generation, 95.6% of leaves were infested with an average of 5.44 ± 0.37 mines per leaf.

Observations carried out during 2009 in the same vineyard, confirmed the existence of two generations. Adults were detected from early June to early July. The first mines were observed in mid-June and the first cases in late June (Fig. 63). Larval densities of the first generation peaked in early July, and by late July most mines had been abandoned by the larvae. Mines of the second generation were visible beginning in the second half of August. In the first generation, 96% of leaves were infested with an average of 4.6 ± 0.53 mines per leaf. In the second generation 97% of leaves were mined with an average density of 6.67 ± 0.72 mines per leaf. Active larvae were found until mid-October.
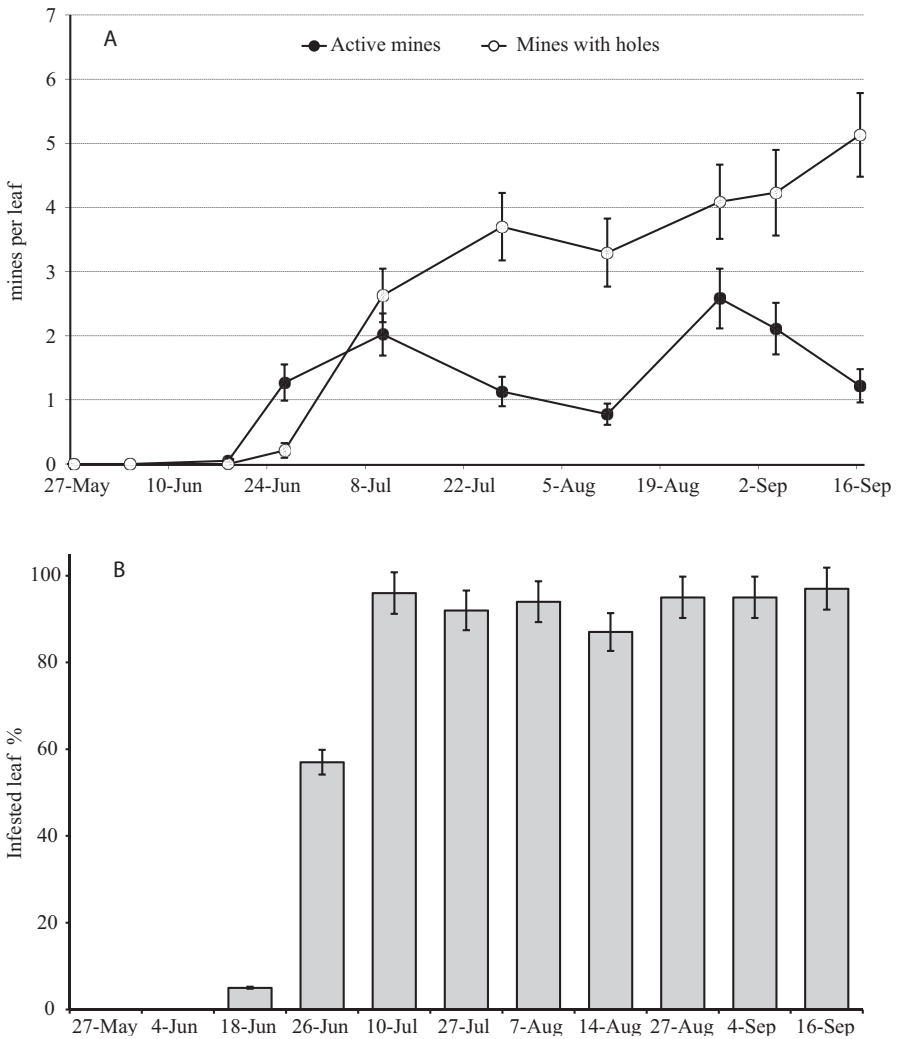


**Figure 63.** Incidence of the *Antispila oinophylla* infestation at Borgo Valsugana (Trento province, Italy) in 2009 expressed as **A** the number of mines per leaf and **B** the percentage of infested leaves (mean ± SE).

## Discussion

### Taxonomy and identification

Identification of the unknown leafminer proved to be difficult. Many groups of Microlepidoptera remain poorly studied taxonomically. Even in North America, Powell and Opler (2009) estimated that at least one third of the microlepidopteran fauna is still undescribed. There seems to be little chance of overcoming this situation, and even groups feeding on economically important plants such as *Vitis* species, remain unstudied. Although we had assembled substantial material of *Antispila*, the morphological similarity across the genus was confusing, and only after checking several genitalia slides and COI barcodes did it become clear that what was previously called "*A. ampelopsifoliella*" was composed of at least two cryptic species on different hostplants. Finding COI barcode matches, in order to rule out the possibility of a non-American sibling species, took more time, because of lack of fresh material and because the *Vitis* miners in North America are more diverse than previously thought. An initial matching of the Italian pest's barcode with that of an *Antispila* record in the BOLD identification system collected in the Great Smoky Mountains, helped focus our research efforts, and underscored the importance of a public DNA barcode reference database. In 2010 and 2011, with increased geographic and taxonomic sampling, we were able to confirm initial results and match additional sequences to those of the introduced Italian *Antispila* populations. The facts that several North American specimens show a 100% identical barcode to the majority of Italian specimens, the overall small genetic distances across all Italian specimens, and that the largest COI distance found was between two North American specimens, corroborate our position that the Italian populations represent a recent introduction from North America. All Heliozelidae species in this study differed sufficiently in their barcodes to allow reliable identification. The barcode data of North American material in addition showed us that the groups of *Vitis* and *Parthenocissus* miners are more diverse than currently recognized and that we cannot identify all taxa with certainty based strictly on morphological grounds. We also note that the North American Vitaceae-feeding *Antispila* exhibit important differences in male secondary characters and genitalia. A revision of the genus is much needed, but was not possible in the context of this study, where a name was urgently needed for a pest of grapevines. Elsewhere, for example in mainland Asia, the group of *Vitis* miners is completely unworked, and in need of taxonomic study (before new outbreaks occur).

We emphasize here the importance of combining traditional morphological descriptions with the additional dataset of DNA sequences for taxonomic groups whose identification is particularly difficult and mainly based on the description of genitalia.

An interesting observation is that we did not find any occurrence of *A. oinophylla* on *Parthenocissus* in its natural habitat in North America, although it utilizes that host in Italy. We found *A. oinophylla* mines on *Vitis* growing intertwined with *Parthenocissus* vines, that harboured two different species of *Antispila*, all occurring within a few centimetres of each other. Despite this sympatry, we did not find any indication of host shifts.

## Phylogeny

While it is generally inadvisable to rely solely on DNA barcodes for phylogenetic inferences, several recent studies suggest that some phylogenetic information could be taken from both the sequences themselves or translated amino acids (Wilson et al. 2011). Our phylogenetic results show that on the basis of the COI barcode, *Antispila* is a paraphyletic genus in relation to the genera *Holocacista, Antispilina* and *Coptodisca.* A generic revision of Heliozelidae has not been published, but the late Ebbe Nielsen made a primer to such a revision in his unpublished thesis (Nielsen 1980b) that has been examined by the senior author. Nielsen recognised three clades, one with *Heliozela* and some related genera, one with *Antispila* and *Antispilina* and a final one with *Ischnocanaba* Bradley, 1961 (from the Solomon Islands), *Holocacista*, *Coptodisca* and a new South American genus. The only difference with our findings is the position of *Antispilina.* Interestingly the clade of the *Antispila ampelopsifoliella* group with *Holocacista*, *Antispilina* and *Coptodisca*, as we find it, is characterised by the very similar reduced venation. A reduced venation has been reported before from some exotic *Antispila* (Kuroko 1961), but for instance all Japanese species seem to share the complex venation of the core *Antispila* as illustrated here (Kuroko 1961; Kuroko 1987). Another character noted by Nielsen to group *Holocacista* and *Coptodisca* is the habit of larvae to attach their cases to stems rather than the soil. This behavioural character is shared with *A. oinophylla* and other members of the *Antispila ampelopsifoliella* group. Despite the poor support for this clade on the basis of barcodes, the mitochondrial and behavioural data collectively suggest that Nielsen's groups could be good and, should such prove to be the case, the genus *Antispila* will need to be subdivided into at least two genera. Alternatively, many of the smaller genera would need to be synonymised into one large *Antispila*, or the *Antispila ampelopsifoliella* group and the smaller genera should be combined in one genus. In the latter case, the generic name would become *Coptodisca*, which is unfortunate, since the genus in its current circumscription is well recognisable both in morphology and biology. In any case, such decisions are outside the scope of the present paper and should be made after a careful phylogenetic generic revision.

Another interesting result from our provisional phylogenetic analyses is the hypothesis that Vitaceae may form the ancestral hostplants for modern Heliozelidae. For the basal genus *Plesiozela* Karsholt & Kristensen, 2003 no host plant information is available (Karsholt and Kristensen 2003). Vitaceae occupy a rather isolated position in the angiosperm phylogeny, as sister to all core rosids (Wang et al. 2009). Other Heliozelidae feed on a wide variety of angiosperm families, but most on "eudicots". *Heliozela* species feed mostly on rosids (Fagaceae, Betulaceae, Myrtaceae), *Antispila* species usually on asterids (Cornaceae, Rubiaceae), *Coptodisca* species on both rosid and asterid trees or shrubs (Davis 1998, van Nieukerken unpublished). Still, these results should be regarded as provisional hypotheses, and should be vigorously tested by analysing additional taxa and genes, as well as using morphological characters.

## Introduction in Italy

*Antispila oinophylla* is the first alien species of Heliozelidae introduced into Europe (Lopez-Vaamonde et al. 2010). Since our manuscript was finished a second species of Heliozelidae from North America was reported as introduction to Italy: a *Coptodisca* species on *Juglans* (Bernardo et al. 2011).

Factors leading to the introduction of *A. oinophylla* in Italy are unknown. *A. oinophylla* is the most recent Nearctic insect species reported to be damaging grapevines in Italy (first in Europe). Its invasion follows those of *Phyllocnistis vitegenella* (Posenato et al. 1997) and *Erasmoneura vulnerata* Fitch, 1851 (Hemiptera: Cicadellidae) (Duso et al. 2005). Trade of vines from North America to Italy is limited while that of the alternative host *Parthenocissus quinquefolia* seems to be more intense. However, the absence of records of *A. oinophylla* on *P. quinquefolia* in North America makes introduction with Virginia creeper a less likely pathway. Anyway, because the caterpillars routinely attach their cocoons to debris, stems or stakes, transport of *Antispila* cases is probably common, and thus not unlikely to have happened. With the frequency of modern air traffic even the transport of adults, and in particular gravid females is not impossible. The fact that *A. oinophylla* is an abundant and widespread species in eastern North America, together with its life history, makes such a possibility even more likely. However, it is also a warning that other species with similar life styles could be the next introduction, with an unpredictable outcome. Introduction from North America apparently occurs rather commonly; 16.5% of alien Lepidoptera species in Europe originate from North America (Lopez-Vaamonde et al. 2010).

The presence of several North American haplotypes of the DNA barcode in Italian material of *A. oinophylla* may indicate that the introduction could have involved more than a single introduction event.

## Infestation

Early observations, carried out during 2007 and 2008 in the Trento province, showed that the incidence of infestation by *A. oinophylla* was significant in vineyards not treated with insecticides. By 2009 significant infestation levels were observed in several commercial vineyards in the Trentino and Veneto Regions despite the application of insecticides. *Phyllocnistis vitegenella* is also increasingly important in commercial vineyards in northeastern Italy. Native parasitoids showed some effects in keeping *P. vitegenella* below economic thresholds (Marchesini et al. 2000). Local outbreaks could be associated with the use of broad-spectrum insecticides, probably because they knock out many egg and larval parasitoids, thereby disrupting the interactions between the pest and its natural enemies. Similar mechanisms could affect the relationships between *A. oinophylla* and its parasitoids. Knowledge of such relationships will be required to understand fully what pest status *A. oinophylla* might reach in the future. In Trento

province, presently, the role of predators and parasitoids in controlling *A. oinophylla* appears to be negligible. However, in the Veneto the situation is different, with 32 to 48% of the larvae and pupae in late summer being parasitized (C. Duso and A. Pozzebon, unpublished data). The identification of parasitoids from Italian vineyards is in progress. *Phyllocnistis vitegenella* and *H. rivillei* share a number of parasitoid species (Mariani 1942; Camporese and Marchesini 1991; Alma 1995; Marchesini et al. 2000). It is therefore likely that some of these will also be found to attack *A. oinophylla* populations.

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## References

Alma A (1995) Ricerche bio-etologiche ed epidemiologiche su *Holocacista rivillei* Stainton (Lepidoptera Heliozelidae). Redia 78: 373–378.

Baldessari M, Angeli G, Girolami V, Mazzon L, Nieukerken EJ van, Duso C (2009) *Antispila* sp. minatore fogliare segnalato in Italia su vite. L'Informatore Agrario 2009: 68–72. http://www.repository.naturalis.nl/document/133352

Barnes W, McDunnough J (1917) Check list of the Lepidoptera of boreal America. Herald Press, Decatur, Ill., viii + 392 pp.

Bernardo U, Sasso R, Gebiola M, Viggiani G (2011) First record of a walnut shield bearer *Coptodisca* (Lepidoptera: Heliozelidae) in Europe. Journal of Applied Entomology: *early view*. doi: 10.1111/j.1439–0418.2011.01693.x

Berro JM (1934) La *Antispila rivillei* (Stt.) den los parrales de Almeria. Boletin de Patologia Vegetal y Entomologia Agricola 7: 60–68.

Bournier A (1977) Grape Insects. Annual Review of Entomology 22: 355–376. doi: 10.1146/ annurev.en.22.010177.002035. doi: 10.1146/annurev.en.22.010177.002035

Bradley JD (1961) Microlepidoptera from the Solomon islands: additional records and descriptions of Microlepidoptera collected in the Solomon islands by the Rennell Island Expedition 1953–1954. Bulletin of the British Museum (Natural History) Entomology 10: 111–168. http://biostor.org/reference/98466

Braun A (1927) Descriptions of new Microlepidoptera. Transactions of the American Entomological Society 53: 191–199.

Brower AE (1984) A list of the Lepidoptera of Maine, part 2. The Microlepidoptera. Section 2. Cosmopterigidae through Hepialidae. Technical Bulletin Maine Agricultural Experiment Station 114: i-x, 1–70.

Camporese P, Marchesini E (1991) Nota preliminare sulla minatrice delle foglie di vite *Holocacista rivillei* (Stainton) (Lepidoptera: Incurvariidae Heliozelinae) nel Veneto. Atti del Congresso Nazionale Italiano di Entomologia 16: 663–668.

Cara C, Jermini M (2011) La mineuse américaine *Phyllocnistis vitegenella*, un nouveau ravageur de la vigne au Tessin. Revue suisse Viticulture, Arboriculture, Horticulture 43: 224–230. http://www.revuevitiarbohorti.ch/archives_4fr.php?id_artikel=217

Chambers VT (1874a) Micro-Lepidoptera. Canadian Entomologist 6: 166–170. http://biostor.org/reference/100145; doi: 10.4039/Ent6166-9

Chambers VT (1874b) Micro-Lepidoptera. Canadian Entomologist 6: 197–198. http://biostor.org/reference/100146; doi: 10.4039/Ent6197-10

Clemens B (1860) Contributions to American Lepidopterology - No. 5. Proceedings of the Academy of Natural Sciences of Philadelphia 12: 203–221. http://biostor.org/reference/98227

Common IFB (1990) Moths of Australia. E. J. Brill, Leiden, New York etc., 535 pp.

Dal Rì M, Delaiti L (1992) Segnalata in alcuni vigneti della Bassa Vallagarina "Minatrice fogliare della vite". Terra Trentina 5: 36–39.

Davis DR (1983) Heliozelidae. In: Hodges RW, Dominick T, Davis DR, Ferguson DC, Franclemont JG, Munroe EG, Powell JA (Eds) Check list of the Lepidoptera of America north of Mexico, including Greenland. Classey, London, 4.

Davis DR (1998) The monotrysian Heteroneura. In: Kristensen NP (Ed) Lepidoptera, Moths and Butterflies, 1 Evolution, systematics and biogeography. De Gruyter, Berlin, New York, 65–90.

Davis DR, De Prins J (2011) Systematics and biology of the new genus *Macrosaccus* with descriptions of two new species (Lepidoptera, Gracillariidae). ZooKeys 98: 29–82. doi: 10.3897/zookeys.98.925

De Tomaso B, Romito A, Nicoli Aldini R, Cravedi P (2008) Minatrice fogliare della vite segnalata in Puglia. Informatore Agrario 31: 59–61.

Dovnar-Zapol'skij DP (1969) Miniruyushchie nasekomye na rasteniyakh Kirgizii i sopredel'nykh territorij. [Mining insects on plants of Kirgizia and neighbourhood territory]. Akademiya Nauk Kirgizskoj SSR, Frunze, 149 pp. [In Russian]

Duso C, Bressan A, Mazzon L, Girolami V (2005) First record of the grape leafhopper *Erythroneura vulnerata* Fitch (Hom., Cicadellidae) in Europe. Journal of Applied Entomology 129: 170–172. doi: 10.1111/j.1439-0418.2005.00944.x

Duso C, Pozzebon A, Baldessari M, Angeli G (2011) Current status of grapevine leafminers in North-eastern Italy. IOBC/wprs Bulletin 67: 203–206.

Duso C, Baldessari M, Pozzebon A, Ferrari E, Angeli G, Mazzon L, Tonina L, Nieukerken EJ van (in press) Notes on the biology and the pest status of *Antispila* sp. (Lepidoptera Heliozelidae) in North-eastern Italy IOBC/wprs Bulletin.

Dyar HG, Fernald CH, Hulst GD, Busck A (1903) A list of North American Lepidoptera and key to the literature of this order of insects. Bulletin of the United States National Museum 52: i-xix, 1–723. http://biostor.org/reference/57795

Ellis WN (2010) Bladmineerders van Europa / Leafminers of Europe. http://www.bladmineerders.nl/index.htm

Fletcher TB (1920) Life-histories of Indian insects, Microlepidoptera, 5. Heliozelidae, Heliodinidae, Glyphipterygidae, Blastobasidae and Hyponomeutidae. Memoirs of the Department of Agriculture in India, Entomological Series 6: 117–135, pls. 127–132. http://biodiversitylibrary.org/page/11511104

Fletcher TB (1933) Life-histories of Indian Microlepidoptera (second series). Cosmopterygidae to Neopseustidae. The Imperial Council of Agricultural Research, Delhi, 85 pp., 77 pls.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Forbes WTM (1923) The Lepidoptera of New York and neighboring states. Primitive forms Microlepidoptera, Pyraloids, Bombyces. Memoirs of the Cornell University Agricultural Experiment Station 68: 1–792. http://biodiversitylibrary.org/page/19819699

Godeheu de Riville (1750) Histoire d'une chenille mineuse des feuilles de vigne extraite d'une lettre écrite de Malte à M. de Reaumur. Mémoires de Mathématique et de Physique présentés a l'Académie Royale des Sciences 1: 177–189. http://biodiversitylibrary.org/page/28958314

Goloboff P, Farris J, Nixon K (2008) TNT, a free program for phylogenetic analysis. Cladistics 24:774–786. doi: 10.1111/j.1096-0031.2008.00217.x

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN (2006) A minimalist barcode can identify a specimen whose DNA is degraded. Molecular Ecology Notes 6: 959–964. doi: 10.1111/j.1471–8286.2006.01470.x

Hall TA (2004) BioEdit sequence alignment editor, 7.0.1. Isis Pharmaceuticals, http://www.mbio.ncsu.edu/bioedit/bioedit.html

Hebert PDN, Ratnasingham S, deWaard JR (2003a) Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society Biological Sciences Series B 270 (Supplement): S96-S99. doi: 10.1098/rsbl.2003.0025

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003b) Biological identifications through DNA barcodes. Proceedings of the Royal Society Biological Sciences Series B 270: 313–321. doi: 10.1098/rspb.2002.2218

Hebert PDN, Penton EH, Burns JM, Janzen DH, Hallwachs W (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America 101: 14812–14817. doi: 10.1073/pnas.0406166101

Hellrigl K (2001) Neue Erkenntnisse und Untersuchungen über die Roßkastanien-Miniermotte *Cameraria ohridella* Deschka & Dimic, 1986 (Lepidoptera, Gracillariidae). Gredleriana 1: 9–81. http://www.museonatura.it/en/publ_details_en.asp?PUBL_ID=176349

Heppner JB, Dixon WN (1995) Potential spread of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae: Phyllocnistinae) in the United States. American Entomologist 41: 110–113.

Hering M (1957) Bestimmungstabellen der Blattminnen von Europa, 3 vols. Junk, 's-Gravenhage, 1185 pp.

Hübner J [1825] (1816–1826) Verzeichniss bekannter Schmetterlinge. Augsburg, 431 + 72 pp. http://biodiversitylibrary.org/page/33045458

ICZN (1988) Opinion 1479. *Antispila* Hübner, [1825] (Insecta, Lepidoptera): *Antispila stadtmuellerella* Hübner, [1825] designated as type species. Bulletin of Zoological Nomenclature 45: 79. http://biostor.org/reference/100699

Karsholt O, Kristensen NP (2003) *Plesiozela,* gen. nov. from temperate South America: apparent sister-group of the previously known Heliozelidae (Lepidoptera: Incurvarioidea: Heliozelidae). Invertebrate Systematics 17: 39–46. doi: 10.1071/IS02047

Karsholt O, Nieukerken EJ van, Whitebread SE, Zangheri S (1995) Lepidoptera Zeugloptera, Dacnonypha, Exoporia, Monotrysia (= Micropterigoidea, Eriocranioidea, Hepialoidea, Nepticuloidea, Incurvarioidea, Tischerioidea). Checklist delle Specie della Fauna Italiana 80: 1–12. http://www.faunaitalia.it/checklist/introduction.html

Knölke S, Erlacher S, Hausmann A, Miller MA, Segerer AH (2005) A procedure for combined genitalia dissection and DNA extraction in Lepidoptera. Insect Systematics and Evolution 35: 401–409. doi: 10.1163/187631204788912463

Kuroko H (1961) The genus *Antispila* from Japan, with descriptions of seven new species (Lepidoptera, Heliozelidae). Esakia 3: 11–24, pls 14–19. http://hdl.handle.net/2324/2347

Kuroko H (1987) Three new species of the genus *Antispila* (Lepidoptera: Heliozelidae) from Japan. Tinea 12: 109–117.

Lafontaine JD (1973) Eastern North American species of *Antispila* (Lepidoptera: Heliozelidae) feeding on *Nyssa* and *Cornus*. Canadian Entomologist 105: 991–994. doi: 10.4039/Ent105991-7

Laštůvka Z (2009) Climate change and its possible influence on the occurrence and importance of insect pests. Plant Protection Science 45: S53-S62. http://www.agriculturejournals.cz/publicFiles/13969.pdf

Le Pelley RH (1973) Coffee insects. Annual Review of Entomology 18: 121–142. doi: 10.1146/annurev.en.18.010173.001005

Lopez-Vaamonde C, Agassiz D, Augustin S, De Prins J, De Prins W, Gomboc S, Ivinskis P, Karsholt O, Koutroumpas A, Koutroumpa F, Laštůvka Z, Marabuto E, Olivella E, Przyby-

lowicz L, Roques A, Ryrholm N, Sefrova H, Sima P, Sims I, Sinev S, Skulev B, Tomov R, Zilli A, Lees D (2010) Alien terrestrial arthropods of Europe, chapter 11. Lepidoptera. BioRisk 4: 603–668. doi: 10.3897/biorisk.4.50

Maček J (1999) Hiponomološka Favna Slovenije. Hyponomologische Fauna Sloweniens. Dela / Slovenska Akademija Znanosti in Umetnosti; Razred za Prirodoslovne Vede 37: 1–386.

Marchesini E, Posenato G, Sancassani GP (2000) Parassitoidi indigeni della minatrice americana della vite. L'Informatore Agrario 10: 93–96.

Marchi G (1956) Ricerche su un Lepidottero Eliozelide, l' "*Holocacista rivillei*" Staint., minatore delle foglie della Vite. Bollettino del Laboratorio di Entomologia del Reale Istituto Superiore Agrario di Bologna 22: 247–276.

Mariani M (1942) Una infestione di *Antispila rivillei* Stt. su vigneti del Palermitano. (Revisione iconografica dell' *Antispila* e descrizione di una nuova specie de *Cirrospilus* Wstw. - Hymenoptora-Chalcididae). Giornale di Scienze Naturali ed Economiche Palermo 43: 1–15, pl. 11.

McDunnough J (1939) Check list of the Lepidoptera of Canada and the United States of America, 2. Microlepidoptera. Memoirs of the Southern California Academy of Sciences 2: 1–171.

McGiffen KC, Neunzig HH (1985) A guide to the identification and biology of insects feeding on muscadine and bunch grapes in North Carolina. North Carolina Agricultural Research Service, North Carolina State University, Raleigh, North Carolina, iv, 93 pp.

Meyrick E (1916) Exotic Microlepidoptera. Exotic Microlepidoptera 2: 1–32. http://www.biodiversitylibrary.org/item/37719

Meyrick E (1926) Exotic Microlepidoptera. Exotic Microlepidoptera 3: 257–288.

Miller SE, Hodges RW (1990) Primary types of microlepidoptera in the Museum of Comparative Zoology (with a discursion[sic] on V.T. Chambers' work). Bulletin of the Museum of Comparative Zoology 152: 45–87. http://biostor.org/reference/622

Morton A (2000) DMAP for Windows, 7.0e. Alan Morton, Winkfield, Windsor, Berkshire, http://www.dmap.co.uk/welcome.htm.

Needham JG, Frost SW, Tothill BH (1928) Leaf- mining insects. Williams & Wilkins, Baltimore, viii, 351 pp. http://biodiversitylibrary.org/page/5890390

Nielsen ES (1980a) A cladistic analysis of the Holarctic genera of adelid moths (Lepidoptera: Incurvaroidea). Entomologica Scandinavica 11: 161–178. doi: 10.1163/187631280X00491

Nielsen ES (1980b) The Incurvarioid genera of the world. Unpublished thesis, Copenhagen: University of Copenhagen.

Nieukerken EJ van (1985) A taxonomic revision of the western Palaearctic species of the subgenera *Zimmermannia* Hering and *Ectoedemia* Busck s. str. (Lepidoptera, Nepticulidae), with notes on their phylogeny. Tijdschrift voor Entomologie 128: 1–164. http://biostor.org/reference/61076

Nieukerken EJ van (2011) Fauna Europaea: Heliozelidae, Nepticulidae, Opostegidae, Schreckensteiniidae, Urodidae. In: Karsholt O, Nieukerken EJ van (Eds) (2011) Lepidoptera. Fauna Europaea version 2.4. http://www.faunaeur.org/

Nieukerken EJ van, Wagner DL, Baldessari M, Angeli G, Girolami V, Mazzon L, Doorenweerd C, Duso C (2011a) The role of DNA barcoding in the identification of a wine pest:

*Antispila ampelopsifoliella.* Abstracts XVIIth European Congress of Lepidopterology: 51. http://www.soceurlep.eu/tl_files/varia/Congress2011/AbstractSEL2011Luxembourg.pdf

Nieukerken EJ van, Kaila L, Kitching IJ, Kristensen NP, Lees DC, Minet J, Mitter C, Mutanen M, Regier JC, Simonsen TJ, Wahlberg N, Yen S-H, Zahiri R, Adamski D, Baixeras J, Bartsch D, Bengtsson BÅ, Brown JW, Bucheli SR, Davis DR, De Prins J, De Prins W, Epstein ME, Gentili-Poole P, Gielis C, Hättenschwiler P, Hausmann A, Holloway JD, Kallies A, Karsholt O, Kawahara AY, Koster SJC, Kozlov M, Lafontaine JD, Lamas G, Landry J-F, Lee S, Nuss M, Park K-T, Penz C, Rota J, Schintlmeister A, Schmidt BC, Sohn J-C, Solis MA, Tarmann GM, Warren AD, Weller S, Yakovlev RV, Zolotuhin VV, Zwick A (2011b) Order Lepidoptera Linnaeus, 1758. In: Zhang Z-Q (Ed) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 212–221. http://www.mapress.com/zootaxa/2011/f/zt03148p221.pdf

Patwary MU, Kenchington EL, Bird CJ, Zouros E (1994) The use of random amplified polymorphic DNA markers in genetic studies of the sea scallop *Placopecten magellanicus* (Gmelin, 1791). Journal of Shellfish Research 13: 547–553.

Pellmyr O, Leebens Mack J (1999) Forty million years of mutualism: evidence for Eocene origin of the yucca-yucca moth association. Proceedings of the National Academy of Sciences of the United States of America 96: 9178–9183. doi: 10.1038/372257a0

Posada D (2008) jModelTest: Phylogenetic Model Averaging. Molecular Biology and Evolution 25: 1253–1256. doi: 10.1093/molbev/msn083

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53: 793–808. doi: 10.1080/10635150490522304

Posenato G, Girolami V, Zangheri S (1997) La minatrice americana, un nuovo fillominatore della vite. L'Informatore Agrario 15: 75–77.

Powell JA, Opler PA (2009) Moths of western North America. University of California Press, Berkeley, Los Angeles & London, xiii+369 pp.

Puplesiene J (1996) Chromosome numbers in four species of mining Lepidoptera (Heliozelidae, Bucculatricidae, Gracillariidae, Elachistidae). In: Jonaitis V (Ed) Lietuvos entomologu darbai (Lietuvos entomologu draugijos 30-meciui) (Research of Lithuanian Entomologist (to the thirty years of Lithuanian Entomological Society)). Lithuanian Entomological Society/Institute of Ecology, Vilnius, 90–98.

Rambaut A, Drummond AJ (2007) Tracer v1.4. http://beast.bio.ed.ac.uk/Tracer.

Reggiani A, Boselli M (2005) Espansione nel Nord Italia della minatrice americana della vite. Informatore Agrario 61: 71–72.

Rondani C (1877) *Antispila rivillella* et ejusdem parassita. Bullettino della Società Entomologica Italiana, 287–292, pl. 289 pp. http://biodiversitylibrary.org/page/33361951

Ronquist F, Huelsenbeck J (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. doi: 10.1093/bioinformatics/btg180

Šefrová H (1999) On larval morphology, biology and distribution of *Phyllonorycter leucographellus* (Zeller) (Lepidoptera, Gracillariidae). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 47: 57–63.

Šefrová H (2002a) *Phyllonorycter robiniella* (Clemens, 1859) - egg, larva, bionomics and its spread in Europe (Lepidoptera, Gracillariidae). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 50: 7–12.

Šefrová H (2002b) *Phyllonorycter issikii* (Kumata, 1963) - bionomics, ecological data and spread in Europe (Lepidoptera, Gracillariidae). Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 50: 99–104.

Šefrová H, Laštůvka Z (2002) Ausbreitung der Rosskastanien-Miniermotte *Cameraria ohridella* in Europa (Streiflichter). Gredleriana 2: 351. http://www.museonatura.it/en/publ_details_en.asp?PUBL_ID=176346

Seljak G (2005) Kačasti listni zavrtač vinske trte (*Phyllocnistis vitegenella* Clemens) že v Sloveniji [*Phyllocnistis vitegenella* Clemens occurring also in Slovenia]. SAD, Revija za Sadjarstvo, Vinogradništvo in Vinarstv 16: 13–14.

Shapiro B, Rambaut A, Drummond AJ (2006) Choosing appropriate substitution models for the phylogenetic analysis of protein-coding sequences. Molecular Biology and Evolution 23: 7–9. doi: 10.1093/molbev/msj021

Srivathsan A, Meier R (2011) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics: early view. doi: 10.1111/j.1096–0031.2011.00370.x

Stainton HT (1855) On some of the difficulties of entomological students, as exemplified by recent experience in the genus *Elachista*. Transactions of the Entomological Society of London 3: 84–89. http://biostor.org/reference/98759

Stainton HT (1869) The lost pleiad. In: Stainton HT (Ed) The Tineina of Southern Europe. John van Voorst, London, 309–319, frontispiece. http://biodiversitylibrary.org/page/21067513

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts, http://paup.csit.fsu.edu/.

Ueno W, Hayasaka T, Endo S, Shibahasi T (1987) Ecology and control of *Antispila* sp. ( Lepidoptera: Heliozelidae) infesting grape leaves. Bulletin of the Yamagata Horticultural Experimental Station 6: 1–18.

Vallot JN (1822) Des insectes qui se voient sur la vigne ou vivent à ses dépens, avec l'indication des plantes parasites de cet arbuste. Mémoires de la Société Linnéenne de Paris 1: 248–254. http://biostor.org/reference/99463

Villani A (2002) La minatrice americana della vite in Friuli Venezia Giulia. Notiziario ERSA 2002: 47–48.

Voigt G (1931) Bemerkungen über die Rebenminiermotte, *Antispila rivillei* Stt. Journal of Pest Science 8: 90–93. doi: 10.1007/BF02338870

Walsingham L, Durrant (1909) Rediscovered Micro-Lepidopteron from the south of France. Transactions of the Entomological Society of London 1909: xxix.

Wang H, Moore MJ, Soltis PS, Bell CD, Brockington SF, Alexandre R, Davis CC, Latvis M, Manchester SR, Soltis DE (2009) Rosid radiation and the rapid rise of angiosperm-dominated forests. Proceedings of the National Academy of Sciences 106: 3853–3858. doi: 10.1073/pnas.0813376106

Wilson JJ, Rougerie R, Schonfeld J, Janzen DH, Hallwachs W, Hajibabaei M, Kitching IJ, Haxaire J, Hebert PDN (2011) When species matches are unavailable are DNA barcodes correctly assigned to higher taxa? An assessment using sphingid moths. BMC ecology 11: 18. doi: 10.1186/1472-6785-11-18

## Appendix A

## Material studied for comparison with *A. oinophylla*

### *Antispila ampelopsifoliella*

**Canada**: 2♂, 2♀ (2♂, 1♀ dissected), Ontario, Normandale, mines on *Parthenocissus*, rearing 57–157, emerged 16–23.iii.1958, Freeman & Lewis (CNC). **USA:** 1♂ (dissected), New York, St. Lawrence Co., Oak Point, leafmines on *P. quinqueguttella,* 12–17.viii.1988, DLW 88N41, emerged 14.iii.1989, D.L. Wagner (DLW); 1♀, (dissected), Vermont, Salisbury, Bryant Mtn. 16 Pudding Hill Rd., leafmines on *P. quinqueguttella,* 10–11.ix.1987, DLW 87J10, emerged 29.iv.1988, D.L. Wagner (DLW).

Leafmines and larvae (barcodes RMNH.INS.18536, 38), **USA:** Connecticut, Windham Co, Windham airport, Mansfield Hollow SP, 88 m, 9.ix.2011, EvN2011178, E.J. van Nieukerken & D.L. Wagner; Leafmines and larvae: New York, Essex Co, 3 km N Keene, Lacy Rd, 250 m, 14.ix.2011, EvN 2011233, E.J. van Nieukerken; Leafmines and larvae (barcode RMNH.INS.18588), Vermont, Addison Co., Button Bay SP, Lake Champlain borders, 44 m, 16.ix.2011, EvN2011254, E.J. van Nieukerken. All on *Parthenocissus quinqueguttella* and in RMNH.

### *Antispila* "vitis1"

**USA:** Larva (barcode RMNH.INS.18126), Connecticut, Tolland Co., Mansfield, 30.viii.2009, leafmines on *Vitis*, DLW 2009H285, D.L. Wagner (DLW, RMNH); 2♀ (barcode RMNH.INS.24205), Florida, Ocala Co., Ocala, Anthony, 18.vi.2006, leafmines on *Vitis aestivalis*, DLW 2006F32vii.2006, D.L. Wagner & T. Dickel (DLW).

### *Antispila voraginella*

**USA:** 1♀, Arizona, Cochise Co., Huachuca Mts., Carr Canyon, Sierra Vista, *Vitis*, 4.vi.1987 [probably 1986], DLW 86F123, emerged 20.iv.1987, R. Wielgus (DLW); 1♂, Arizona, Cochise Co., Huachuca Mts., Miller canyon, *Vitis arizonica*, 31.vii.1986, DLW 86G15, emerged 23.v.1987, D.L. Wagner (DLW); 3♂, 3♀ (2♂ dissected and DNA barcode RMNH.INS.23918), Arizona, Santa Cruz Co., Sycamore Cany.S of Ruby, leafmines *Vitis arizonica*, 23.vii.1991, DLW 91G18, emerged 3.vi.1992, D.L. Wagner (DLW); 2♂, 1♀ (1♂ dissected and DNA barcode RMNH.INS.23917), Texas: Culberson Co., Guadalupe Mts NP, McKittrick canyon, leafmines *Vitis* 30–31. vii.1989, DLW 89G108, emerged 14–23.iv.1990, D.L. Wagner (DLW); 1♂ (holotype) 2♀ (paratypes), Utah: [Washington County], Zion Canyon, leafmines *Vitis arizonica*, 24.vii.[1925], B. 1206, emerged 12–14.iv.[1926], A.F. Braun (ANSP).

### *Antispila hydrangaeella*

**USA:** 27♂ ♀ (1♂ dissected), leafmines & larvae (DNA barcode RMNH. INS.18416), Georgia, Gilmer Co., Chattahoochee Nat. Forest, Barnes Creek Picknick Area, 760 m, hardwood forest, leafmines on *Hydrangea arborescens*, 14.x.2010, EvN 2010279, emerged 3–18.iv.2011, E.J. van Nieukerken & C. Doorenweerd (RMNH); 29♂ ♀, leafmines & larvae (DNA barcode RMNH.INS.18206), North Carolina, Haywood Co., NP Great Smoky Mts, Big Creek area, 573 m, hardwood forest along river, leafmines on *Hydrangea arborescens*, 28.ix.2010, EvN 2010073, emerged 31.iii– 9.iv.2011, E.J. van Nieukerken & C. Doorenweerd (RMNH).

### *Antispila* cf *viticordifoliella*

Canada: 1♀, Ontario, Ottawa, 14.iv.1971, "Vir. creeper" [*Parthenocissus*], 70–48, G.C. Lewis (CNC). USA: Leafmines and larvae (barcode RMNH.INS.18508), **USA:** Connecticut, Windham Co, Windham airport, Mansfield Hollow SP, 88 m, leafmines on *Parthenocissus quinqueguttella*, 9.ix.2011, EvN2011178, E.J. van Nieukerken & D.L.Wagner (RMNH); 1♀ (barcode RMNH.INS.24207), Florida, Miami-dade Co., Key Biscane, Cape Florida SP, leafmines on *Parthenocissus quinqueguttella,* 19.iv.2002, DLW 2002D6, emerged 22.v.2002, R. Wagner & D.L. Wagner (DLW); Leafmines and larvae (barcodes RMNH.INS.18560–61), **USA:** New York, Essex Co, Hwy 9N, 3.5 km WSW Keeseville,142 m, 14.ix.2011, EvN 2011238, E.J. van Nieukerken; Leafmines and larvae (barcodes RMNH.INS.18587), Vermont, Addison Co., Button Bay SP, Lake Champlain borders, 44 m, 16.ix.2011, EvN2011254, E.J. van Nieukerken. All on *Parthenocissus quinqueguttella* and in RMNH.

### *Antispila* cf *isabella*

**Canada:** 2♀ (dissected, MIC1862), Ontario, Simcoe, emerged 4.vi.1965, leafmines on *Vitis*, T.N. Freeman (CNC); 1♂, Ontario, Simcoe, emerged 10.iii.1971, leafmines on *Vitis*, T.N. Freeman (CNC); **USA:** 1♂, Connecticut, Windham Co., Hampton, 916 Pudding Hill Rd., leafmines on *Vitis*, 22.viii.1989, DLW 89H47, emerged 18–20. iii.1990, D.L. Wagner (DLW); Leafmines and larvae (barcodes RMNH.INS.18127– 28), Connecticut, Tolland Co., Mansfield, 30.viii.2009, leafmines on *Vitis*, DLW 2009H285, D.L. Wagner (DLW, RMNH); 1♂, larvae and leafmines, Georgia, Murray Co., Chattahoochee Nat. Forest, E of Chatsworth, GA rd 52, 523 m, leafmines on *Vitis aestivalis* var. *aestivalis*, 14.x.2010, EvN2010266, emerged 14.iv–4.v.2011, E.J. van Nieukerken & C. Doorenweerd (RMNH); 1♂, (dissected, MIC1859), Kentucky, Morehead, emerged 3.vi.1963, leafmines on *Vitis*, T.N. Freeman (CNC); Leafmines and larvae (barcodes)**.** Vermont, Addison Co., Button Bay SP, Lake Champlain borders, 44 m, 44.18154N, 73.36892W, on *Vitis riparia*, 16.ix.2011, EvN2011253, E.J. van Nieukerken (RMNH); 1♂, Vermont, Chittenden Co., South Burlington, 11.viii.1988, DLW 88H23, emerged 30.iii.1989, D.L. Wagner (DLW)

### *Antispila* "vitis2"

**USA:** Leafmines and larvae (barcode RMNH.INS.18131), Connecticut, Tolland Co., Mansfield, 30.viii.2009, leafmines on *Vitis*, DLW 2009H285, D.L. Wagner (DLW, RMNH); Leafmines and larvae (barcode RMNH.INS.18656), Vermont, Addison Co., Button Bay SP, Lake Champlain borders, 44 m, leafmines on *Vitis riparia*, 16.ix.2011, EvN2011253, E.J. van Nieukerken (RMNH).

### *Holocacista rivillei*

Italy: 28 adults (4♂, 1♀ dissected), Trento, Borghetto, *Vitis vinifera*, 2007, emerged i-ii.2008, M. Baldessari (RMNH).

## Appendix B

List of samples used for the DNA barcoding. (doi: 10.3897/zookeys.170.2617.app2) File format: Excel spreadsheet (xls).

**Explanation note:** List of samples used for the DNA barcoding analysis with collection site and BOLD and GenBank accession numbers. *GenBank sequences from Pellmyr and Leebens Mack (1999).