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The African endemic species “*Nychiodes*” *tyttha* Prout, 1915 (Lepidoptera, Geometridae, Ennominae) belongs to the genus *Aphilopota* Warren, 1899

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Abstract. An extensive examination of the external and internal morphological characters of the genus *Nychiodes* shows that “*Nychiodes*” *tyttha* Prout, 1915 is incorrectly placed in this genus. The systematic position of this species was investigated by using a multigene analysis, including one mitochondrial and up to nine protein-coding nuclear gene regions, and morphological characters. These results support a re-classification of this species as *Aphilopota tyttha*, **comb. nov.** A re-description supported by illustrations of morphological characters for *A. tyttha* is provided.

Introduction

Prout (1915) described an African geometrid species *tyttha* and placed it in the genus *Nychiodes* Lederer, 1853. In his description, he mentioned the much smaller size and slight differences in venation of *N. tyttha* from other *Nychiodes* species (Prout 1915). Since size can be influenced by various parameters (e.g., the amount of available nutrition), more informative are characters such as differences in venation, widely used in Geometridae for differential diagnoses of genera (Hausmann 2001; Awmack and Leather 2002; Wanke et al. 2020).

Recently, the genus *Nychiodes* has undergone intensive integrative taxonomic revisions (Müller et al. 2019; Wanke et al. 2020). The genus contains 25 species, distributed from western Europe and North Africa to Iran, Afghanistan and Pakistan. *Nychiodes tyttha* has remained as the only species outside the mentioned distribution range, occurring in central and southern Africa (Janse 1932). The results of our investigation of morphological characters strongly support *N. tyttha* being excluded from the genus *Nychiodes* (Wanke et al. 2020). However, a suitable genus for this species could not be found until now in the absence of data allowing a molecular analysis. For this study, we aimed

to extract DNA to clarify the systematic position of *N. tyttha*. This allowed a multi-gene molecular phylogenetic analysis to be conducted together with an examination of morphological characters.

Material and methods

Specimens used in this study are deposited the following collections (acronyms after Evenhuis 2007):

| | |
|-------------------|---|
| NHMUK | Natural History Museum, London, United Kingdom; |
| HSS | Private Collection of Hermann Staude, South Africa; |
| SMNS | Staatliches Museum für Naturkunde Stuttgart, Germany; |
| ZSM (SNSB) | Zoologische Staatssammlung München (Staatliche Naturwissenschaftliche Sammlungen Bayerns), Germany. |

Morphological examination

For the documentation of external characters, a Visionary Digital photography system (LK Imaging System, Dun. Inc., equipped with a Canon EOS 5DSR camera), an Olympus E3 digital camera, as well as a Leica digital microscope (Z16 APO) were used. Standard techniques were followed for the preparation of genitalia (e.g. Robinson 1976) and eversion of the vesica took place following the method described by Sihvonen (2001). Finally, genitalia were embedded in Euparal as permanent slides and photographed with a Keyence VHX-5000.

Molecular data generation

Extraction of DNA and amplification of the “DNA barcode” fragment (658 base-pairs of the 5’ terminus) of the mitochondrial Cytochrome-C Oxidase I of the holotype of *Nychiodes tyttha*, was carried out at the Canadian Centre for DNA barcoding (CCDB, Guelph), in the framework of the Lepidoptera Campaign of the international Barcode of Life program (iBOL; www.lepbarcoding.org), using a protocol for old museum specimens based on Next-Generation-Sequencing (Hausmann *et al.* 2016; Prosser *et al.* 2016). Extraction and amplification of non-type specimens were also carried out at the Canadian Centre for DNA barcoding (CCDB, Guelph) using standard protocols (e.g., Ivanova *et al.* 2006). *Nychiodes tyttha* specimens used for analysis of the “barcode” fragment and metadata are available on BOLD. Sample ID numbers are: BC ZSM Lep 106645 (holotype); BC ZSM Lep 13914; BC ZSM Lep 98802. As the holotype’s DNA was extracted in Canada no extract was left after DNA barcoding for genomic DNA analysis. Sample BC ZSM Lep 98802 was repatriated from Guelph and amplification of further genes was done at the molecular laboratory in Finnish Museum of Natural History “Luomus”, (Helsinki) using the DNeasy Blood and Tissue kit (Qiagen), following the manufacturer’s protocol. DNA amplification and sequencing were carried out following protocols proposed by (Wahlberg and Wheat 2008; Wahlberg *et al.* 2016). One mitochondrial (cytochrome oxidase subunit I, COI) and up to ten protein-coding nuclear gene regions, Arginine Kinase (ArgK), carbamolphosphate synthetase (CAD), sarco/endoplasmic reticulum calcium ATPase (Ca-ATPase), Elongation factor 1 alpha (EF-1 α), glyceraldehydes-3-phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (IDH), cytosolic malate dehydrogenase (MDH), sorting nexin-9-like (Nex9), ribosomal Protein (RpS5), and wingless (*wgl*), were sequenced for phylogenetic analyses.

Multiple sequences were aligned using Muscle algorithms as implemented in MEGA11 (Tamura *et al.* 2021) for each gene including other sequences of Boarmiini (see Appendix 1, Fig. A1) retrieved from the local VoSeq database (Peña and Malm 2012). For the phylogenetic hypothesis of Boarmiini,

a total of 300 taxa, from Murillo-Ramos et al. (2019) were incorporated into our dataset, of which two geometroid species (Sematuridae: *Mania lunus* (Linnaeus, 1758) and Uraniidae: *Urania leilus* (Linnaeus, 1758)) served as outgroups. The newly produced DNA sequences through this study were managed with the VoSeq database. The final dataset included a total length of 7662 bp including gaps, and missing data made up 34% of the final data matrix. The sequences described here are accessible via GenBank with the following accession numbers: ON980557–ON980558; ON982490–ON982496. All GenBank accession numbers of the 300 taxa are provided in the Suppl. material 1.

DNA Barcoding analyses

Three different analyses were performed. First, COI fragments of “*Nychiodes*” *tyttha* (sequences of holotype and two non-type specimens) were compared to available sequences in the Barcode of Life Datasystems (BOLD) identification engine to search for the genetically nearest neighbor. Second, a neighbor-joining tree (K2P on BOLD) was constructed with the sequence of the holotype of “*Nychiodes*” *tyttha* and 99 samples suggested by BOLD as related taxa to find the genetically nearest neighbor. Finally, the minimum p-distance of “*Nychiodes*” *tyttha* from *Nychiodes dalmatina* was calculated, to calculate the distance to the genus *Nychiodes*.

Phylogenetic analysis

The molecular data set partitioned by gene and codon position was analysed using maximum likelihood as implemented in IQ-TREE 2.1.3 (Minh et al. 2020). Best-fitting substitution models were selected by ModelFinder (Kalyaanamoorthy et al. 2017) with “-m MFP+MERGE” option. The best-fit models were chosen as follows: GTR+F+I+G4 for ArgK, COI, Nex9, and wingless; TIMe+I+G4 for Ca-ATPase; TIM2+F+I+G4 for CAD and IDH; SYM+I+G4 for EF-1 α , GAPDH, MDH, and RpS5. The phylogenetic analysis was carried out with “-spp” option (edge proportional) that allows each partition to have its own evolutionary rate. We evaluated the node supports with ultrafast bootstrap approximations (UFBoot2) and the SH-like approximate likelihood ratio test (Guindon et al. 2010; Hoang et al. 2018) using the “-B 1000 -alrt 1000” option. To reduce the risk of overestimating branch supports in ultrafast bootstrap approximation analysis, we used the “-bnni” option, which optimizes each bootstrap tree using a hill-climbing nearest-neighbour-interchange (NNI) search. The resulting tree was rooted and visualized in FigTree v1.4.2 (Rambaut 2015).

Results

The comparison of the COI fragments only of “*Nychiodes*” *tyttha* (holotype and two non-type specimens) with data from the BOLD database, suggested that the genetically nearest neighbors are in the genera *Jankowskia* Oberthür, 1884, *Tephronia* Hübner, 1825 and *Peribatodes* Wehrli, 1943 (genetic distances of 6.4–7.9%). When a neighbor-joining tree (K2P on BOLD) was constructed using the holotype DNA barcode sequence with the 99 nearest samples provided by BOLD, an Australian species, *Aeolochroma* sp. ANIC1 (BOLD:AAV4042), which is 8.33% divergent by p-distance, separated “*N.*” *tyttha* from the above and other genera. Sequences from the other two specimens of “*N.*” *tyttha* (BC ZSM Lep 106645, BC ZSM Lep 98802) were 1.23–1.39% divergent. By contrast, the minimum p-distance (COI, K2P, BOLD gap analysis) from *Nychiodes dalmatina* is 10.4%.

Additionally, five out of the eleven target genes of a single non-type specimen of “*Nychiodes*” *tyttha* were successfully amplified and sequenced (COI-1, COI-2, wgl, Ca-ATPase, Nex9). In the multi-gene phylogenetic analysis “*Nychiodes*” *tyttha* clustered as sister to other species of *Aphilopota*

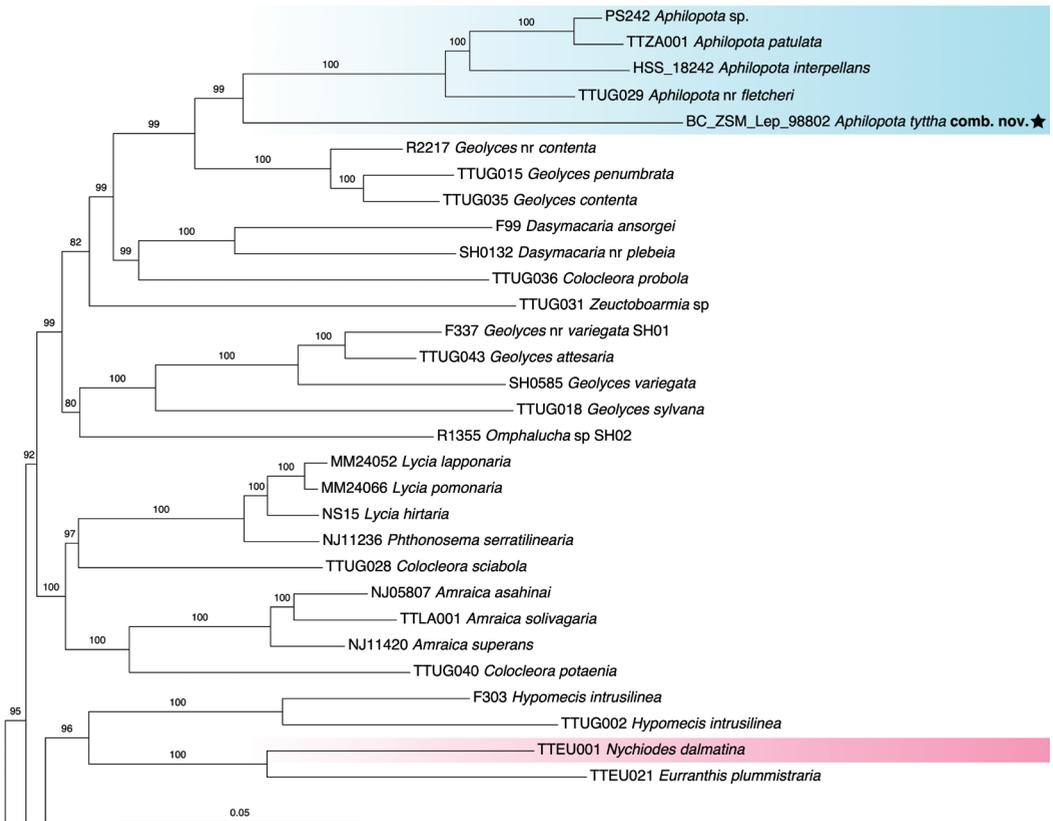


Figure 1. Phylogenetic position of *Aphilopota tyttha*, comb. nov. (marked with a star) within the tribe Boarmiini, supporting the tentative combination in genus *Aphilopota*. The numbers above the branches are the bootstrap values of the maximum likelihood IQ-TREE analysis. The complete tree is shown in Appendix 1, Fig. A1.

Warren, 1899 (Fig. 1, Appendix 1, Fig. A1). Moreover, the results of our morphological examination served as an additional line of evidence and revealed that “*Nychiodes*” *tyttha* has the diagnostic generic characters of *Aphilopota*, supporting its affiliation to this genus (for detailed comparison see the taxonomy part). The species is re-described in the taxonomic part of the discussion.

Discussion

Systematics

The results of our multi-gene molecular phylogenetic analysis show that “*Nychiodes*” *tyttha* groups as sister to *Aphilopota* (UFB = 97%). The phylogenetic analysis would allow us either to classify “*N.*” *tyttha* in a monotypic genus as sister to *Aphilopota*, or to combine it with other *Aphilopota*. The classification as sister to *Aphilopota* may not hold when more species of this genus are added to the dataset. Currently the genus *Aphilopota* consists of 44 species, distributed exclusively in Africa and Madagascar (Scoble 1999; Scoble and Hausmann 2007), but the genus urgently needs taxonomic revision. The detailed morphological investigation of the taxon *tyttha* in

the framework of the present study supports its combination with *Aphilopota*. Consequently, we transfer “*N.*” *tyttha* to the genus *Aphilopota*.

In the following diagnosis (see taxonomy part), the morphological characters of *Aphilopota tyttha* comb. nov. are compared to the type species of the genera *Aphilopota* (*A. interpellans* (Butler, 1875)) and *Nychiodes* (*N. obscuraria* (Villers, 1789)), which support the new combination.

Taxonomy

Aphilopota tyttha (Prout, 1915), comb. nov.

Figs 2–11, 17, 18, 21

Nychiodes tyttha Prout, 1915. Novitates zoologicae: a journal of zoology in connection with the Tring Museum, 22, 363. Holotype ♂ (Eritrea, Caraii). Deposited in NHMUK (examined).

Type material examined. Holotype, ♂, ERITREA, Caraii, 21.xi.1905, N. Beccari, Geometridae genitalia slide No. 4976, Rothschild Bequest B.M. 1939-1, NHMUK010920109, DNA barcode sample ID BC ZSM Lep 106645, DNA barcode process ID GWOTZ396-19, BIN BOLD:AAW8833 [579 bp]; in NHMUK

Additional material examined. 1 ♀, ERITREA, Caraii, 21.xi.1905, N. Beccari, Geometridae genitalia slide No. 4977, NHMUK014173598; 1 ♂, KENYA, Kitale, 14.9.[19]25, leg. G.W. Jeffery, NHMUK010920119; 1 ♂, [Namibia], Sissekab, N.W. of Otavi, 1300 m, leg. K. Jordan, 11.xi.1933, NHMUK010920120; all in NHMUK. 1 ♂, SOUTH AFRICA, Mkuze Chaos, 27°39.490'S, 032°00'E, 28.xii.1990, 220 m, leg. H.S. Staude; in HSS. 1 ♂, ETHIOPIA, Bahar Dar, iii.1969, Lichtfang, leg. Schäubfele, g.prep. 0732/2020 D. Wanke; in SMNS. 1 ♂, Äthiopien [Ethiopia], Awassa, Awassa Lake, Reg. Bale, 07°02.886'N, 038°27.491'E, 23.–24.v.1999, leg. R. Beck & M. Hiermeier; 1 ♂, S. ETHIOPIA – SN, Arba Minch, Nechisar NP, 2.75 km SW headquarter, 1170 m (lux), 06°00'13.6"N, 37°33'23,4"E, 22.ii.2012, leg. Hacker & Schreier; all in ZSM.

Remark. The genus *Aphilopota* needs taxonomic revision, based on a broad integrative taxonomic approach. Therefore, a comparison with other species of this genus, except of the type species *A. interpellans*, is not possible and also not necessary here.

Diagnosis. In *A. tyttha* labial palpi thin, about two thirds of the diameter of the eye (labial palpi thick, about one diameter of the eye in *A. interpellans* and *N. obscuraria*) (Fig. 2). Proboscis reduced (similar in *A. interpellans* and *N. obscuraria*) (Fig. 2). In the forewing venation of *A. tyttha* R1 arising from the cell, not reaching costa, R2 fused with R1 (similar in *A. interpellans*; arising from the cell, R1 and R2 share a common stalk in *N. obscuraria*) (Fig. 4). In the male genitalia (Figs 17–20) of *A. tyttha* valva thin, without any ampulla or harpe (similar in *A. interpellans*; valva equipped with the two main processes ampulla superior and ampulla inferior in *N. obscuraria*). Juxta of *A. tyttha* forked, large and straight, reaching up to the gnathos (juxta forked, large and tip thick, reaching far beyond the gnathos, apex bent in *A. interpellans*; juxta anchor-shaped in *N. obscuraria*). Aedeagus in *A. tyttha* thin tapered, with one long and sclerotized cornutus (aedeagus funnel-shaped, without strong cornutus in *A. interpellans*; thickness of aedeagus variable with one sclerotized cornutus variable in length in *N. obscuraria*). In female genitalia (Figs 21–24) of *A. tyttha* ovipositor telescopic and strongly elongated (similar in *A. interpellans*; not elongated in *N. obscuraria*). Corpus bursae of *A. tyttha* arched, tube-like elongated (arched, long in *A. interpellans*; round membranous in *N. obscuraria*). Signum absent in *A. tyttha* (similar in *A. interpellans*; signum stellate in *N. obscuraria*).

Tribal assignment. According to the molecular phylogenetic analysis (Fig. 1, Appendix 1, Fig. A1) and morphology (Figs 2–24), *Aphilopota tyttha* is classified in Boarmiini.



Figure 2. Head structures of male specimens of **A, B.** *Aphilopota tyttha* (Prout), comb. nov., **C, D.** *Aphilopota interpellans* (Butler), and **E, F.** *Nychiodes obscuraria* (Villers). **A, C, E.** Head in ventral view; **B, D, F.** Lateral view.

Re-description. Wingspan ♂ 21–25 mm, ♀ 28 mm, average length of forewing 11.2 mm ($n = 7$). Antennae bipectinate in both sexes. Frons weakly convex, just reaching over the eyes, densely scaled. Labial palpi thin, about two third of the diameter of the eye. Proboscis reduced, represented by barely visible rudimentary slats (Fig. 2A). Chaetosemata as two small patches, each located between the eye margin and the antennal base (Fig. 2B). Foreleg epiphysis approximately as long as tibia. Base of the epiphysis starting after one fourth of tibia. Mesotibia with one pair of spurs, hindtibia with two pairs of spurs (Fig. 3). Head, thorax and abdomen concolorous with wings.

Ground colour of wings beige brown, transverse lines present in dark brown to black. Terminal line continuous, concolorous with transverse lines. In forewing antemedial line curved towards termen. Postmedial line curved between R5/M1 and M2. Medial area with more darker scales

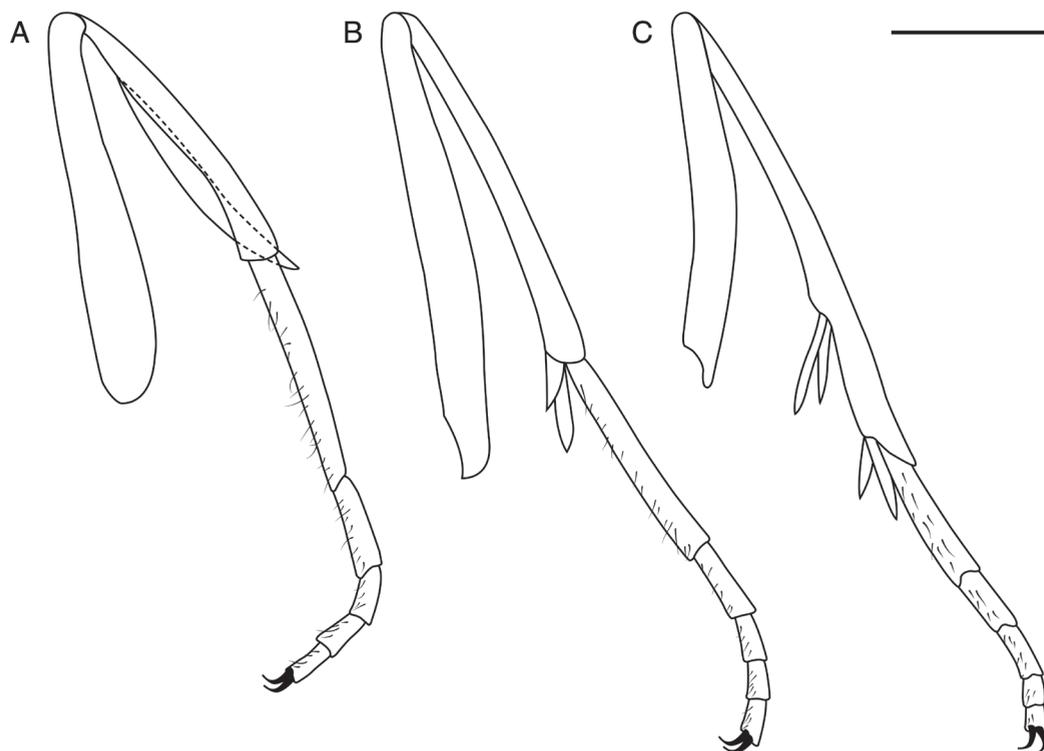


Figure 3. Drawings of the legs of *Aphilopota tyttha* (Prout) comb. nov. **A.** Foreleg; **B.** Midleg; **C.** Hindleg.

intermixed. In hindwing antemedial line curved towards termen on M1. Discal spots only present on underside (Figs 5–11).

In forewing, vein R1 arising from the cell, not reaching costa, R2 merged with R1, R3–5 with a common stalk arising from the cell. In hindwing Sc+R1 strongly curved in basal area, approximating to the cell, M2 absent, A3 and A1+2 originating separately (Fig. 4).

In male genitalia uncus strongly sclerotized, short, basally broad and triangular, apically pointed. Gnathos well developed and strongly sclerotized, triangular. Costa of valva sclerotized, valva thin, without any ampulla or harpe. Juxta forked and big, reaching up to gnathos. Saccus tapering. Aedeagus thin tapered, carrying one long and sclerotized cornutus. Cornutus almost same length as aedeagus (Figs 17–18).

Female genitalia thin and long, with strongly elongated ovipositor. Apophyses posteriores very long, apophyses anteriores 1/3 length of apophyses posteriors. Antrum sclerotized. Ductus bursae short, bend. Corpus bursae tube-like, elongated. Signum absent (Fig. 21).

Phenology. Adults observed from November to May.

Biology. Unknown.

Habitat. Investigated specimens collected at elevations from 220 to 1300 m in dry savanna ecoregions.

Distribution. In East Africa (Eritrea, Ethiopia, Kenya), south-western Africa (Namibia) and South Africa (Janse 1932).

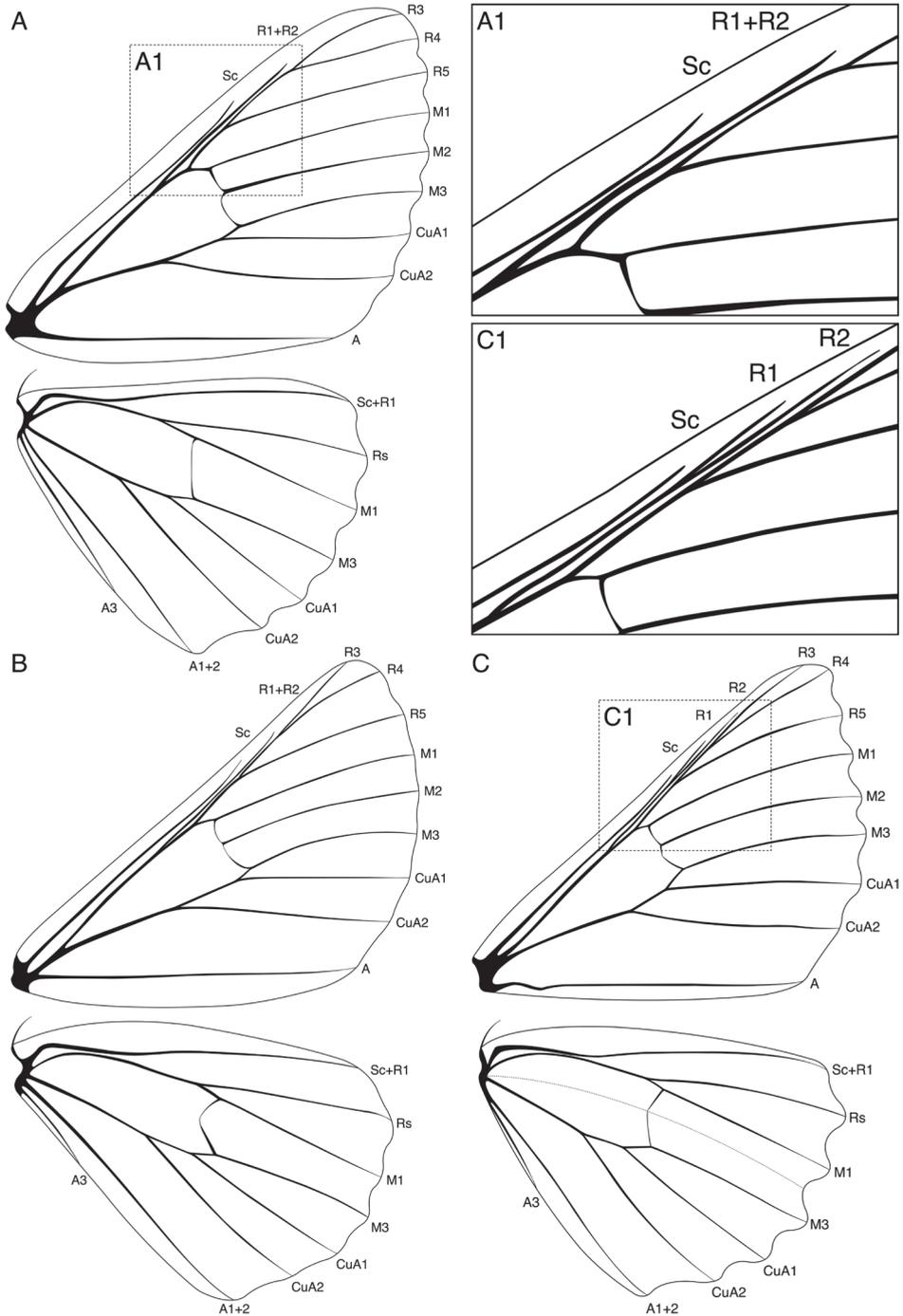
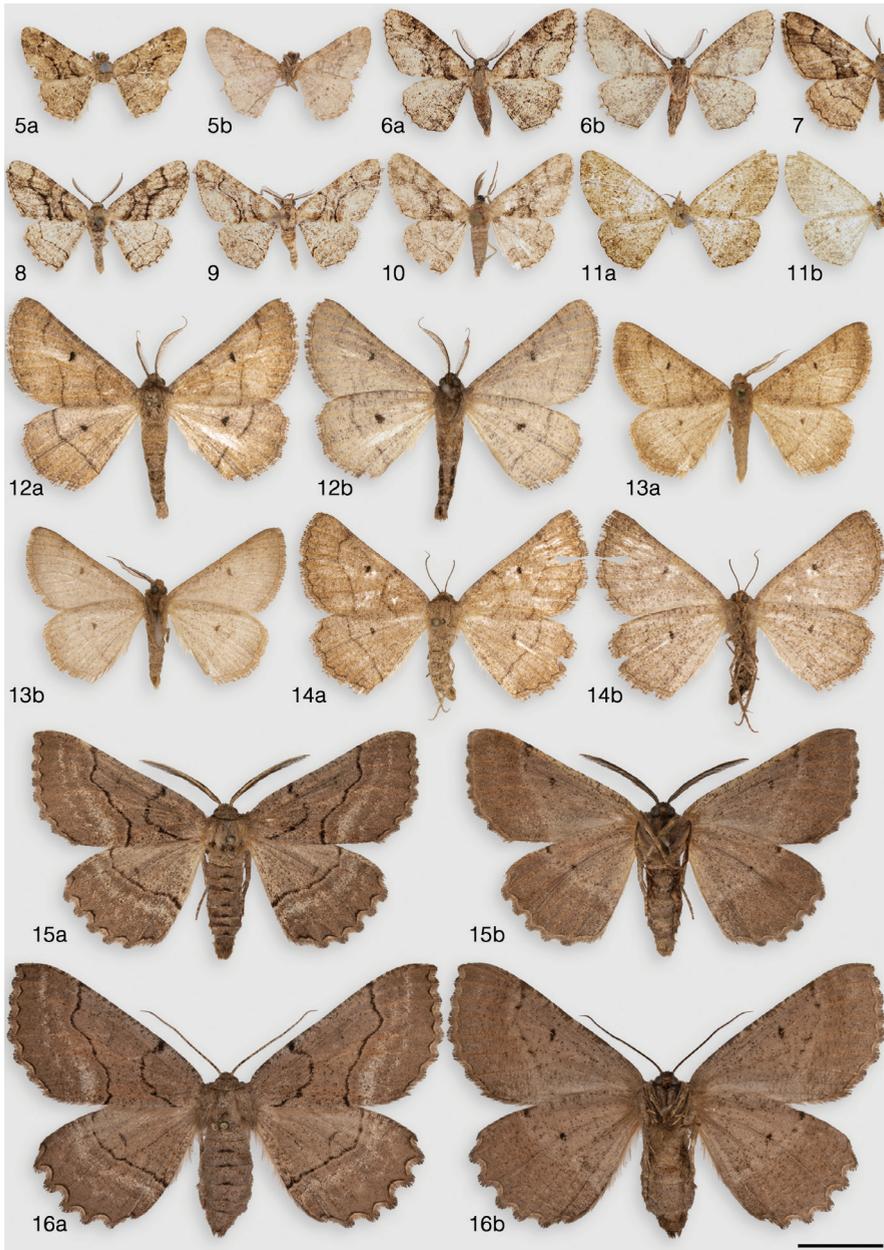
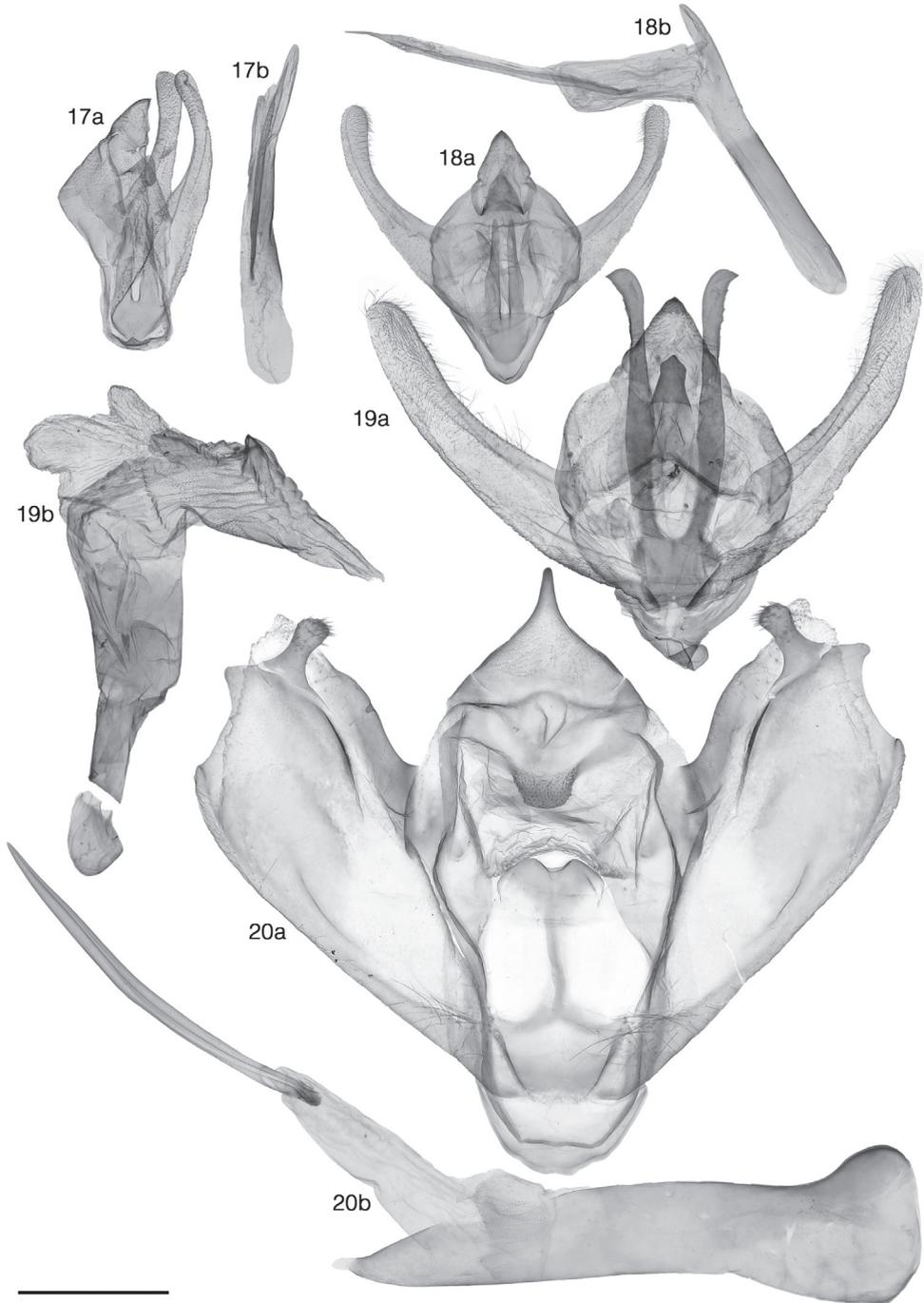


Figure 4. Wing venation drawings of male specimens of **A.** *Aphilopota tyttha* (Prout) comb. nov.; **B.** *Aphilopota interpellans* (Butler), and **C.** *Nychiodes obscuraria* (Villers). In the forewing of *A. tyttha* and *A. interpellans* vein R2 is fused with R1 (rectangle A1), vein R1 and R2 share a common stalk in *N. obscuraria* (rectangle C1). Remark: as in the genus *Nychiodes*, the veins R1 and R2 are on a common stalk. This suggests that the veins R1 + R2 are fused to one vein in the genus *Aphilopota*; therefore, here we name this vein R1+R2.



Figures 5–16. Wing pattern of *Aphilopota tyttha* (Prout), comb. nov., *Aphilopota interpellans* (Butler) and *Nychiodes obscuraria* (Villers). **5–11.** *A. tyttha* (**5.** Holotype, Eritrea, Carai, g.prep.4976, NHMUK010920109; **6.** Ethiopia, Awassa, Awassa Lake; **7.** Kenya, Kitale, NHMUK010920119; **8.** [Namibia], Sissekab, N.W. of Otavi, NHMUK010920120; **9.** Ethiopia, Bahar Dar, g.prep. 0732/2020 D. Wanke; **10.** South Africa, Mkuze Chaos; **11.** Eritrea, Carai, NHMUK014173598); **12–14.** *A. interpellans* (**12.** South Africa, KwaZuluNatal, Vryheid, g.prep. 1314/2022 D. Wanke; **13.** [South Africa], Transvaal, Bords du Limpopo, g.prep. 1316/2022 D. Wanke; **14.** South Africa, KwaZuluNatal, Vryheid, g.prep. 1315/2022 D. Wanke); **15–16.** *N. obscuraria* (**15.** Spain, Albarracin, g.prep. 2096/2017 H. Rajaei; **16.** Spain, Albarracin, g.prep. 2097/2017 H. Rajaei); a = upperside; b = underside. Scale bar: 1 cm.



Figures 17–20. Male genitalia of *Aphilopota tyttha* (Prout), comb. nov., *Aphilopota interpellans* (Butler) and *Nychiodes obscuraria* (Villers). **17–18.** *A. tyttha* (**17.** Holotype, Eritrea, Carai, g.prep. 4976, NHMUK010920109; **18.** Ethiopia, Bahar Dar, g.prep. 0732/2020 D. Wanke); **19.** *A. interpellans* (South Africa, KwaZuluNatal, Vryheid, g.prep. 1314/2022 D. Wanke); **20.** *N. obscuraria* (Spain, Albarracin, g.prep. 2096/2017 H. Rajaei). a = genitalia capsule; b = aedeagus. Scale bar: 1 mm.



Figures 21–24. Female genitalia of *Aphilopota tyttha* (Prout), comb. nov., *Aphilopota interpellans* (Butler) and *Nychiodes obscuraria* (Villers). **21.** *A. tyttha* (Eritrea, Carai, g.prep. 4977, NHMUK014173598); **22–23.** *A. interpellans* (South Africa, KwaZuluNatal, Vryheid, g.prep. 1315/2022 D. Wanke; **22.** Ventral view; **23.** Lateral view); **24.** *N. obscuraria* (Spain, Albarracin, g.prep. 2097/2017 H. Rajaei). Scale bar: 1 mm.

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Appendix 1

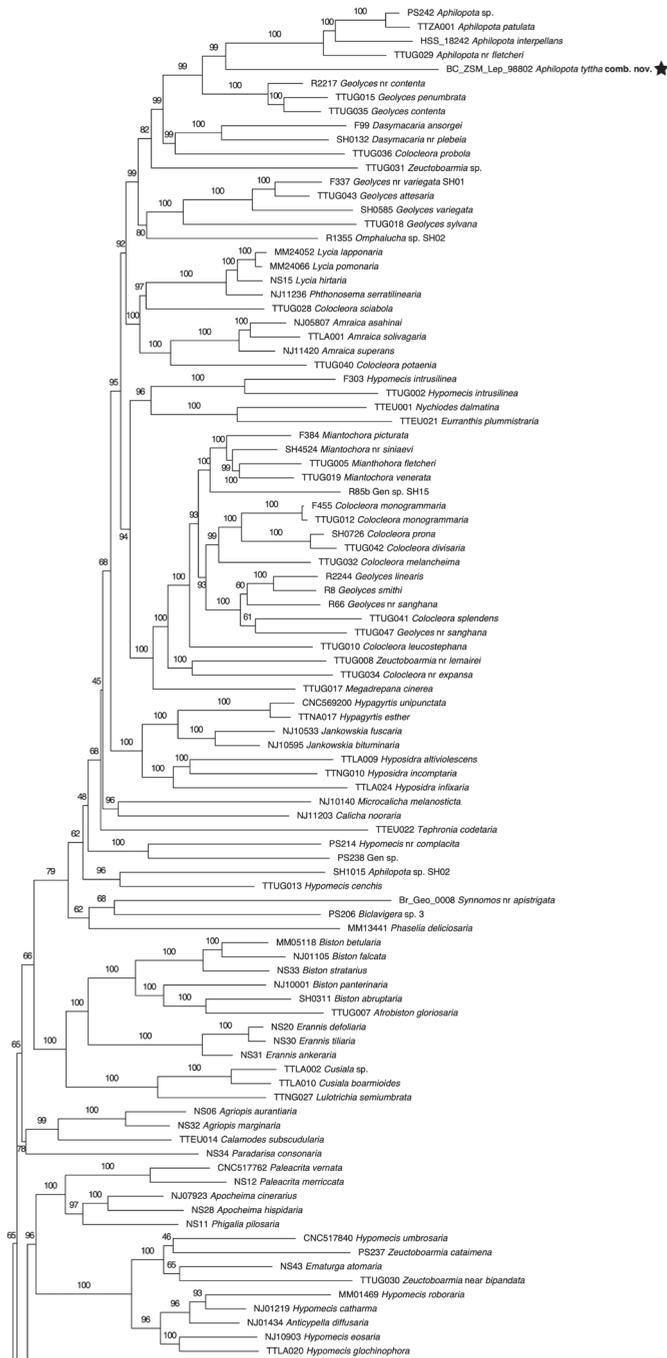


Figure A1. Complete phylogenetic analysis from IQ-TREE, showing the phylogenetic position of *Aphilopota tyttha* comb. nov. (marked with a star) within the tribe Boarmiini. Support values are indicated above the branch. Node confidence values were estimated based on 1000 ultrafast bootstrap replicates.

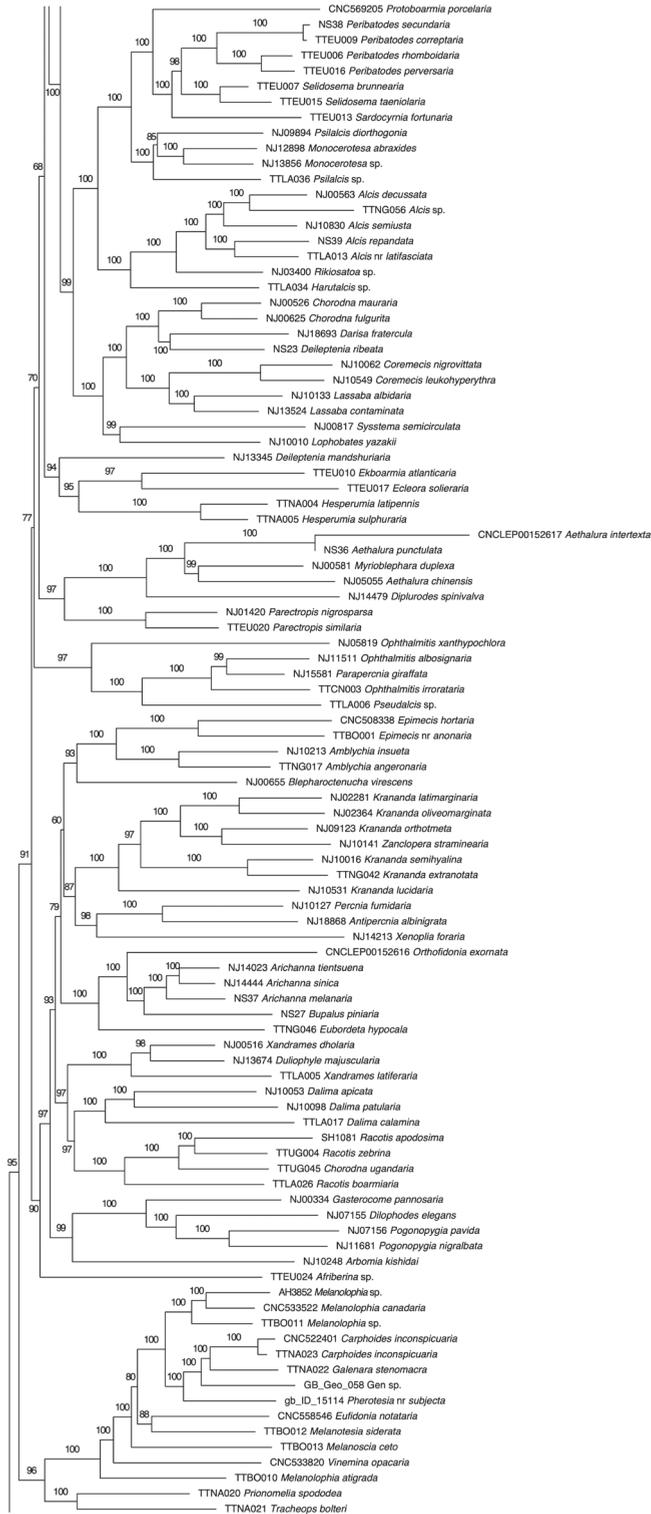


Figure A1. Continued.

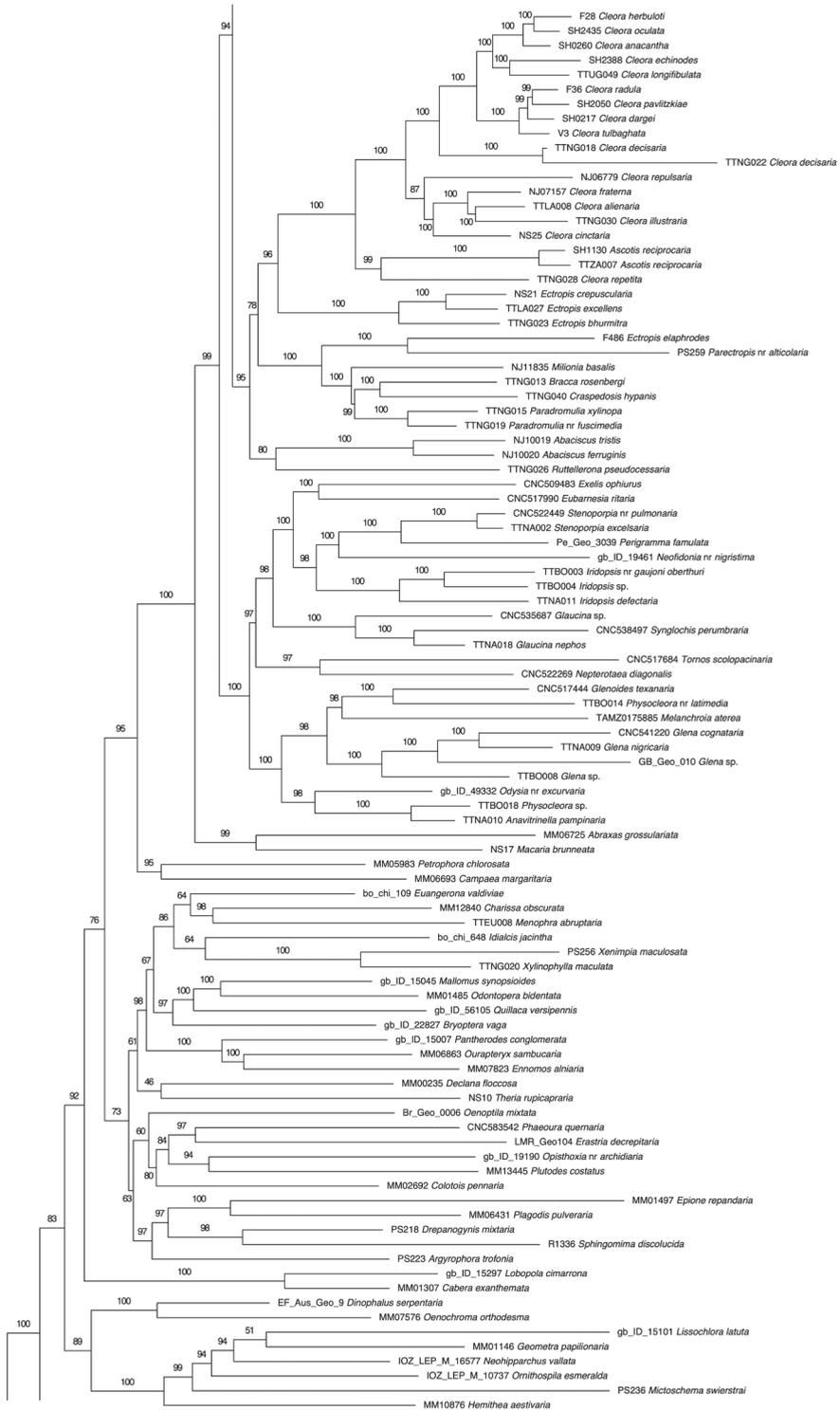


Figure A1. Continued.

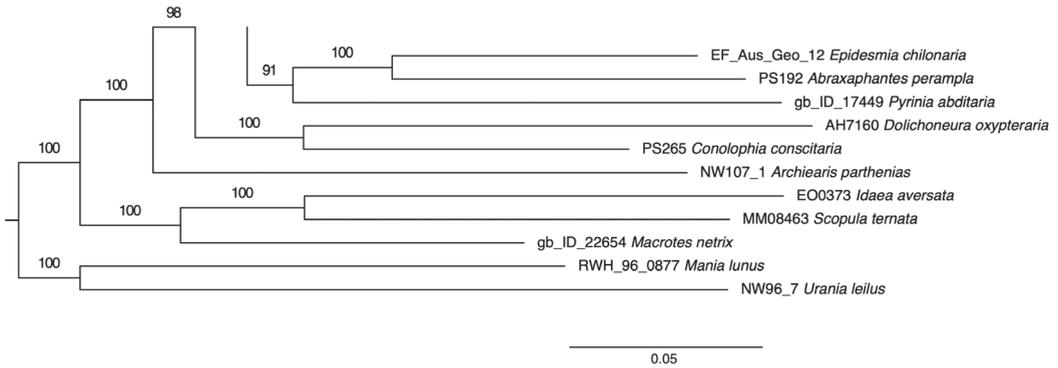


Figure A1. Continued.

Supplementary material 1

Taxa used in this study

Authors: Dominic Wanke, Axel Hausmann, David C. Lees, Kyung Min Lee, Geoff Martin, Pasi Sihvonen, Hermann Staude, Hossein Rajaei

Data type: table (excel file).

Explanation note: Taxa used in this study, with identification, process code, and GenBank accession numbers for each gene. Data from Murillo-Ramos et al. 2019 & Wanke et al. (current paper).

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Link: <https://doi.org/10.3897/nl.46.94940.suppl1>

Commatarcha galicicae Tokár & Srnka, sp. nov., and a genus new for Europe (Lepidoptera, Carposinidae)

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<https://zoobank.org/B10754BB-C496-479C-BE05-C945FC127160>

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Abstract. *Commatarcha galicicae* Tokár & Srnka, sp. nov. is described from specimens taken in two south-eastern European localities, the Galičica Mountain in North Macedonia, and Dubova in Banat, Romania. The new species belongs to the genus *Commatarcha* Meyrick, 1935, which is new for the European Lepidoptera fauna. It resembles some Asian species from this genus and also the American species *Bondia comonana* (Kearfott, 1907) in external characters. In the genitalia of both sexes, it is similar to *C. oresbia* Diakonoff, 1989 but differs markedly in the colour and pattern of the forewing. The immature stages of the new species are unknown. Colour photographs of the adults and of the habitat are included with illustrations of the female and male genitalia.

Introduction

The family Carposinidae is widespread on all continents, with approximately 290 described species in 32 genera, rich in the Oriental and Australian regions, and with remarkable diversity on some island groups (e.g. Hawaii, Macaronesia) (Diakonoff 1989; Ponomarenko 1999). In Europe, only the one genus *Carposina* Herrich-Schäffer, [1854] with seven species has been known hitherto (Karsholt and Nieuwerkerken 2017). Five of these are endemic, described from Madeira and the Canary Islands. Only two species are known from continental Europe: *C. berberidella* Herrich-Schäffer, [1854], and *C. scirrhosella* Herrich-Schäffer, [1854]. Species from the family occurring in the Palaearctic region were revised by Diakonoff (1989). The North American species were reviewed by Davis (1969).

In late June 2016, while surveying the nocturnal Lepidoptera fauna of the Galičica Mountain in North Macedonia, the second author found two females of an unknown species at elevations between 1500–1600 m. A few years later, in July 2021, he was successful in collecting additional specimens of the species, two males and two females, at the same locality. A detailed study of the specimens by the first author showed that they represent a hitherto undescribed species in the genus *Commatarcha* Meyrick, 1935. After the first revision of our article, we received information from Peter Buchner that the brothers Zoltán and Sándor Kovács had found two specimens of a species

unknown to them near the village of Dubova in south-western Romania also in 2021. After studying the adults and the female genitalia slide, we concluded that they belong to our new species.

The genus *Commatarcha*, with *C. palaeosema* Meyrick, 1935 as the type species, consists of 15 currently known species that are distributed in the Eastern Palearctic, most of them in China (Li 2004; Qian and Li 2018). The closest relative to our new species in male and female genitalia is *C. oresbia* Diakonoff, 1989, a species found in the mountains of north-west Pakistan. This species is also geographically closest to our new species. In external appearance, the new species resembles several Chinese *Commatarcha* species, e.g. *C. acidodes* Diakonoff, 1989, *C. convoluta* Li, 2018, *C. fanjingshana* Li, 2004, and the American species of the genus *Bondia* Newman, 1856, *B. comonana* (Kearfott, 1907). American *Bondia* species are morphologically very close to *Commatarcha* species, indeed closer than to the Australian *Bondia nigella* Newman, 1856, the type species of its genus.

Materials and methods

All of the examined specimens were taken as adults, having been attracted to light. The genitalia were dissected following the usual procedure for small Lepidoptera (Robinson 1976) except that three preparations of the paratypes were temporarily stored in glycerol in small plastic vials. The drawings of the genitalia were made with Indian ink and water-soluble paint on transparent sheets of drawing paper. Photographs of the adult and the type locality were taken using digital cameras Canon PowerShot G11 and Panasonic Lumix DMC-LS85. A Nikon D 3100 camera, a Nectaris stereomicroscope with a microshift and a 4× planachromatic lens were used to photograph the heads.

Molecular analysis

We sent a leg of one North Macedonian specimen of the new species together with legs of two specimens of *Carposina* species (*C. berberidella*, *C. scirrhosella*) for DNA barcoding to Shandong Normal University (China) and COI barcodes were obtained from the tissue of these legs. Protocols for total DNA extraction and mitochondrial COI gene amplification followed Liu and Wang (2017). We supplemented the sampling with public and private records of *Bondia* spp., *Commatarcha* spp. and some other related species available in BOLD database (see Acknowledgements). The DNA barcodes of the new species along with the two *Carposina* (*C. scirrhosella*, BOLD:ADJ0833, *C. berberidella*, BOLD:ACG2435) have been deposited in a dataset DS-CARPBOND (dx.doi.org/10.5883/DS-CARPBOND) in the Barcode of Life Database (BOLD) (Ratnasingham and Hebert 2007). The results of DNA analysis of two Romanian specimens, sequenced in the Canadian Centre for DNA Barcoding (CCDB; University of Guelph) using standard protocols, were also stored in this database. We compared the sequence initially with all records accessible to us and then selected a representative of each species and BINs (Barcode Index Numbers) of *Bondia* and *Commatarcha* for further analyses, particularly tree reconstructions. The genetic distance estimations were done using BOLD analytics and the Neighbor Joining and the Maximum likelihood trees were made in MEGA 10.0.5 (Kumar et al. 2016) under the Kimura 2-Parameter model for Neighbor Joining and GTR (General Time Reversible) model for Maximum likelihood. Node supports were estimated based on 500 bootstrap replicates. The Neighbor Joining tree was rooted to *Meridarchis excisa* (Walsingham, 1900) (Carposinidae).

Taxonomy

Commatarcha galicicae Tokár & Srnka, sp. nov.

<https://zoobank.org/2F6F62E4-31B6-41F6-AE84-7554B1BA44DD>

Material. Holotype: NORTH MACEDONIA: ♀, pinned, genitalia in a separate slide. Original labels: “Macedonia, Galičica Planina, 1570 m, 27–29.vi.2016, lgt. L. Srnka”, “Gp. Z. Tokár ♀ 14081”, “DNA sample SDNU.ZT_21 BUCCN” (blue label), “HOLOTYPE *Commatarcha galicicae* Tokár & Srnka” (red label), coll. L. Srnka (to be deposited in the National Slovak Museum in Bratislava). Coordinates of the type locality: 40°58'N, 20°51'E.

Paratypes: NORTH MACEDONIA: 1 ♀, same locality and data as holotype, Gp. Z. Tokár ♀ 13777, coll. Z. Tokár; 2 ♂, 2 ♀, same locality as holotype, 14.vii.2021, L. Srnka leg., Gp. Z. Tokár ♂ 14170, 14171, ♀ 14169, coll. L. Srnka & Z. Tokár; Romania: 2 ♀, Banat, Dubova, 44°37'N, 22°15'E, 200–300 m, 17.vi.2021, TLMF Lep 32643, TLMF Lep 32675, leg. & coll. S. & Z. Kovács, Gp. Z. Tokár ♀ 14390. All paratypes with red labels “PARATYPE *Commatarcha galicicae* Tokár & Srnka”.

Description. Adult, female (Figs 1, 4). Forewing wingspan 14.5–19 mm, length 6.5–9 mm. Head: frons golden yellow, vertex with golden yellow to light brown scale tufts. Proboscis well-developed. Maxillary palpi not visible. Labial palpi straight, second segment $1\frac{1}{4}$ times as long as the eye diameter, outer side dark brown, most of the inner surfaces light golden yellow, apical segment short, dark brown, golden yellow at apex. Antennal flagellomeres each ringed alternately dark brown and light golden yellow. Ventral surface of flagellomeres covered with fine sensilla, length approximately half the segment diameter. Thorax tegulae dark brown to black, abdomen the same colour tinged with yellow on some segments. Forewing oblong, gradually widening. Ground colour light yellow to golden yellow. Markings dark brown to black: transverse band at base, extending towards costa, narrow strip along costal margin, irregular band approximately from $\frac{1}{2}$ of costa to $\frac{2}{3}$ of dorsum with extended part at discal cell pointed towards termen and distinct triangular tornal spot, broad arched band covering outer third of forewing, and small scattered dark spots or scales throughout. Dark markings brightened or interrupted by light spots or groups of scales. Fringe dark brown, yellow basally. Hindwing dark grey, fringe same colour, yellow basally.

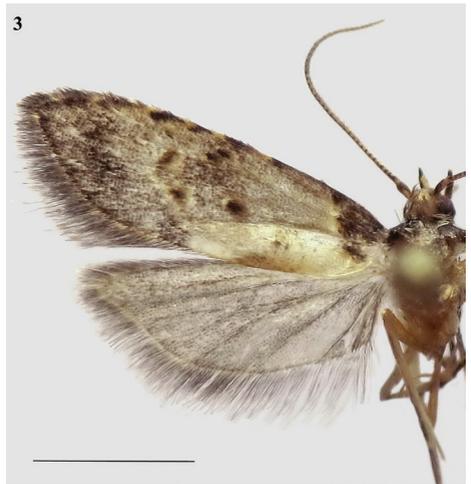
Male (Figs 2, 3, 5). Forewing wingspan 14.5–16.5 mm, length 6.5–7.8 mm. Labial palpi and antennae sexually dimorphic. Second segment of labial palpi $1\frac{1}{4}$ times longer than the eye diameter. Fine, elongate sensilla, covering ventral surface of antennal flagellomeres, length approximately 1.5 times the segment diameter. Forewing markings as female but less pronounced.

Female genitalia (Fig. 6). Ovipositor moderately elongate, lobes soft; apophyses posteriores elongate, approximately 1.6 times length of apophyses anteriores. Abdominal segment VIII moderately sclerotised, covered with long bristles and spinulose; ostium bursae margins curved inwards; antrum cup-shaped, distally covered with small thorns. Sterigma laterally dilated. Sclerotised part of ductus bursae (colliculum) funnel-shaped, slightly concave, widening into a membranous, twisted swelling posterior to a slender, finely papillate area of ductus bursae. Ductus bursae gradually widening to form suboval corpus bursae; signa absent.

Male genitalia (Figs 7, 8). Uncus indistinct, fused to tegumen. Gnathos absent. Valvae broad at base with protuberant lobe, outer half deeply divided into two curved digitate processes of unequal size and width. Medial process slightly shorter but twice as wide and more sclerotised than the lateral process. Ectophallus well-developed, stout and arched. Juxta ellipsoid. Vinculum and saccus well-developed, V-shaped. Phallus slender, vesica with a pair of rows of stout cornuti of various sizes.



Figure 1. *Commatarcha galicicae* Tokár & Srnka, sp. nov., female, holotype, Galičica Planina, 27–29. vi.2016. Scale bar: 3.0 mm.



Figures 2, 3. *Commatarcha galicicae* Tokár & Srnka, sp. nov., males, paratypes, Galičica Planina, 14.vii.2021. Scale bar: 3.0 mm.

Diagnosis. The male and female genitalia of *Commatarcha galicicae* Tokár & Srnka, sp. nov. closely resemble those of *C. oresbia*. The male genitalia of the new species differ from *C. oresbia* mainly in having an indistinct uncus, and the vinculum and saccus wide and V-shaped, whilst in the latter the uncus is a small, sclerotised triangle, and the vinculum and saccus is long and rather slender. In the female genitalia, the new species can be distinguished from *C. oresbia* by the different characters of the ostium and ductus bursae; posterior margin of the ostium bursae and the margins of the colliculum being concave, whilst in *C. oresbia* they are convex.



Figures 4, 5. *Commatarcha galicicae* Tokár & Srnka, sp. nov., heads **4.** Female, holotype, Galičica Planina, 27–29.vi.2016; **5.** Male, paratype, Galičica Planina, 14.vii.2021.

In addition, both species differ significantly from each other in external appearance. Externally the new species is somewhat similar to several Chinese *Commatarcha* species (*C. acidodes*, *C. convoluta*, *C. fanjingshana*), but perhaps the most similar looking species is the American species *Bondia comonana*. However, all these species differ considerably in the structure of the genitalia of both sexes.

Distribution. So far only known from the two localities, the Galičica Mountain, North Macedonia, and Dubova, Banat region, Mehedinți County, Romania.

Biology. Adults of the new species were on the wing in the second half of June and in July. The habitat of the locality in the Galičica Mountain has a forest-steppe character. We observed the following tree/shrub species there: *Fagus sylvatica* L., *Corylus* sp., *Prunus* sp., *Rubus* sp., *Juniperus* sp., *Acer* sp., *Abies* sp., *Quercus* sp., *Amelanchier* sp., *Aria* sp., and others. This biotope is shown in Fig. 9. It is worth noting that specimens of another carposinid species *Carposina scirrhosella* were found in the same biotope and at the same time as our new species.

The Romanian specimens were collected in a limestone area near Dubova covered with grassy vegetation and plenty of *Syringa* L., and *Cotinus coggygria* Scop. bushes, and the presence of a mixed forest dominated by *Carpinus betulus* L., *Quercus* sp., and also with *Acer campestre* L. (Zoltán and Sándor Kovács pers. comm.).

The biology of *Commatarcha* species is very little known. The life history of *Commatarcha palaeosema* Meyrick, 1935 was described by Yano (1959) from Japan. According to the author the larva of the species feeds under the bark of the trunks and branches of *Castanopsis cuspidata* (Thunb.) Schottky, *Quercus glauca* Thunb., and *Q. serrata* Murray, and considerably injures them producing a remarkable protuberance or gall-like swelling. He observed that usually a number of larvae bore into the same swellings and eject small reddish pellets of frass and woody fragments. Of the closely related American species included in the genus *Bondia*, the best known is *Bondia comonana*, which can form stem galls on *Prunus* or *Quercus* trees (Powell and Opler 2009; Robinson et al. 2010). We can only surmise that the larva of our new species also develops in a similar way in the galls of some tree or shrub species occurring in the type localities.

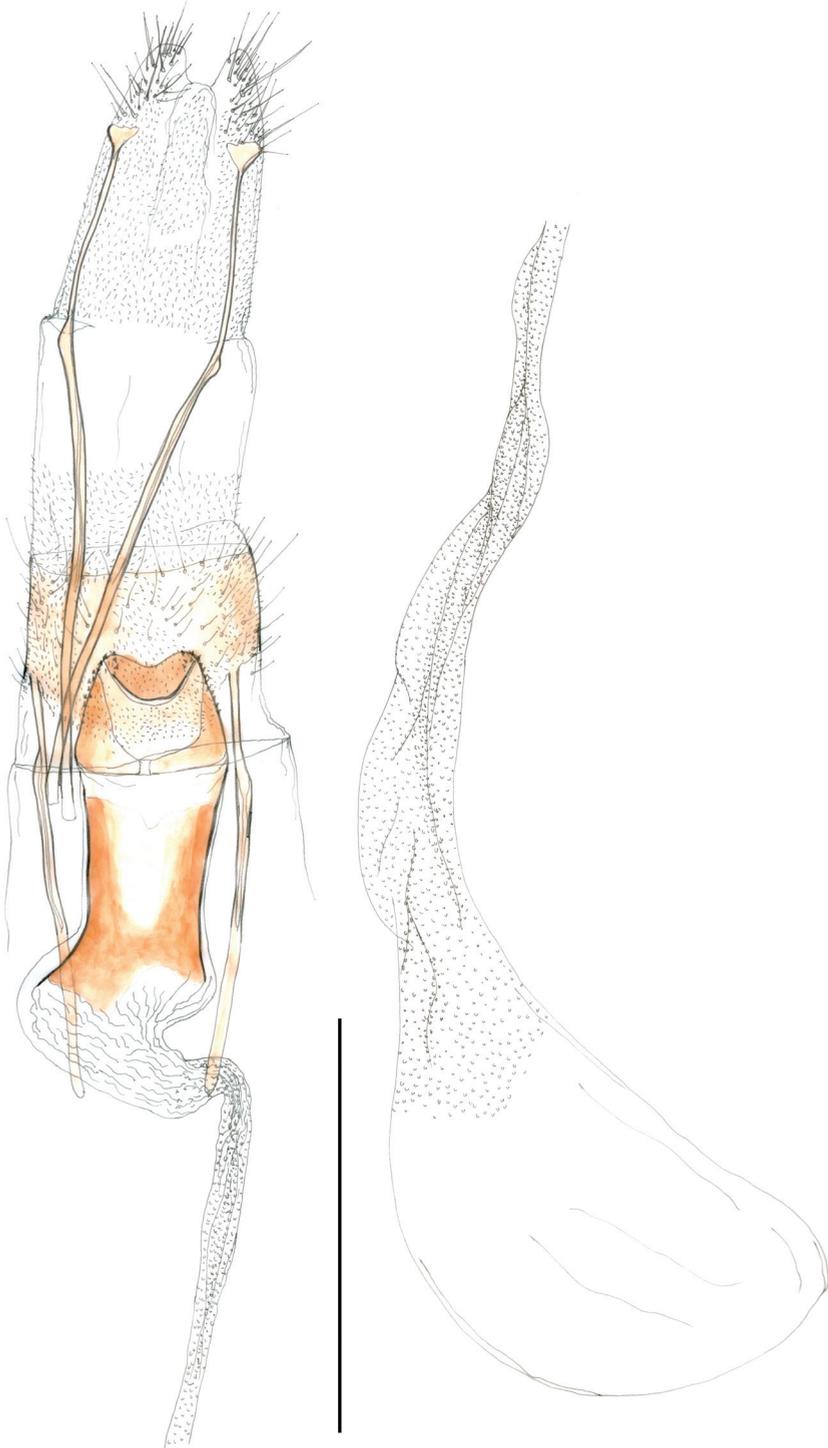
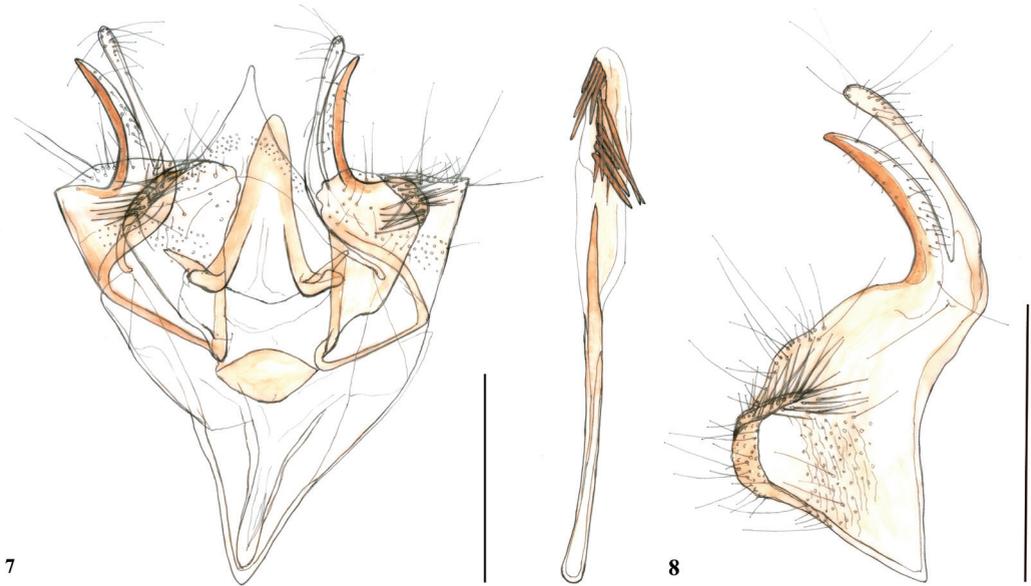


Figure 6. Female genitalia of *Commatarcha galicicae* Tokár & Srnka, sp. nov., paratype, Gp. Z. Tokár ♀ 13777, dorsal view. Scale bar: 1.0 mm.



Figures 7, 8. Male genitalia of *Commatarcha galicicae* Tokár & Srnka, sp. nov., paratypes **7.** Gp. Z. Tokár ♂ 14170, dorsal view. Scale bar: 0.5 mm; **8.** Gp. Z. Tokár ♂ 14171, valva. Scale bar 0.5 mm.



Figure 9. Type-locality of *Commatarcha galicicae* Tokár & Srnka, sp. nov. in the Galičica Mountain.

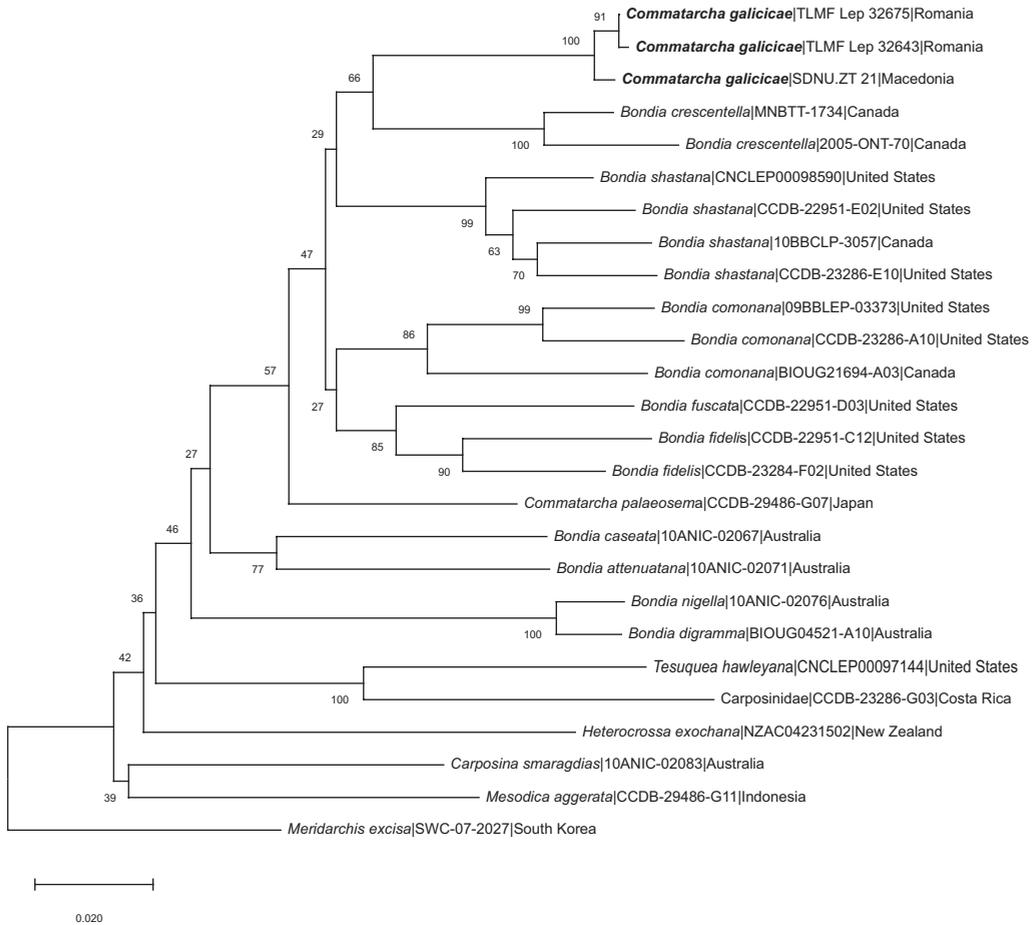


Figure 10. A Neighbor Joining tree of *Commatarcha galicicae* Tokár & Srnka, sp. nov. (shown in bold face) and selected closely related species based on COI sequences. Numbers are bootstrap percentages. Scale bar represents 2% K2P genetic divergence between sequences.

Etymology. The specific name *galicicae*, a noun in the genitive case, is derived from the Galičica Mountain, where the first specimens of the new species were discovered.

Molecular data (Figs 10, 11). The DNA barcoded specimens form a unique BIN: BOLD:AEH8633. Sequences in this BIN are highly divergent to other BINs with a minimum p-distance of 8.05% to the closest BIN (*Bondia crescentella* (Walsingham, 1882)). The Macedonian specimen shows 4–5 nucleotide substitutions compared to the two Romanian specimens, which differ from each other by one substitution.

Discussion. As mentioned in the Introduction, the Palearctic *Commatarcha* species are very close to American *Bondia* species morphologically. According to the molecular data, these two genera are closely related but not reciprocally monophyletic in the Maximum likelihood tree, although this finding is based on a single mitochondrial marker only. None of the analyses based on COI-5P show threshold support (e.g. >70% bootstrap) for major geographic clusters. However,

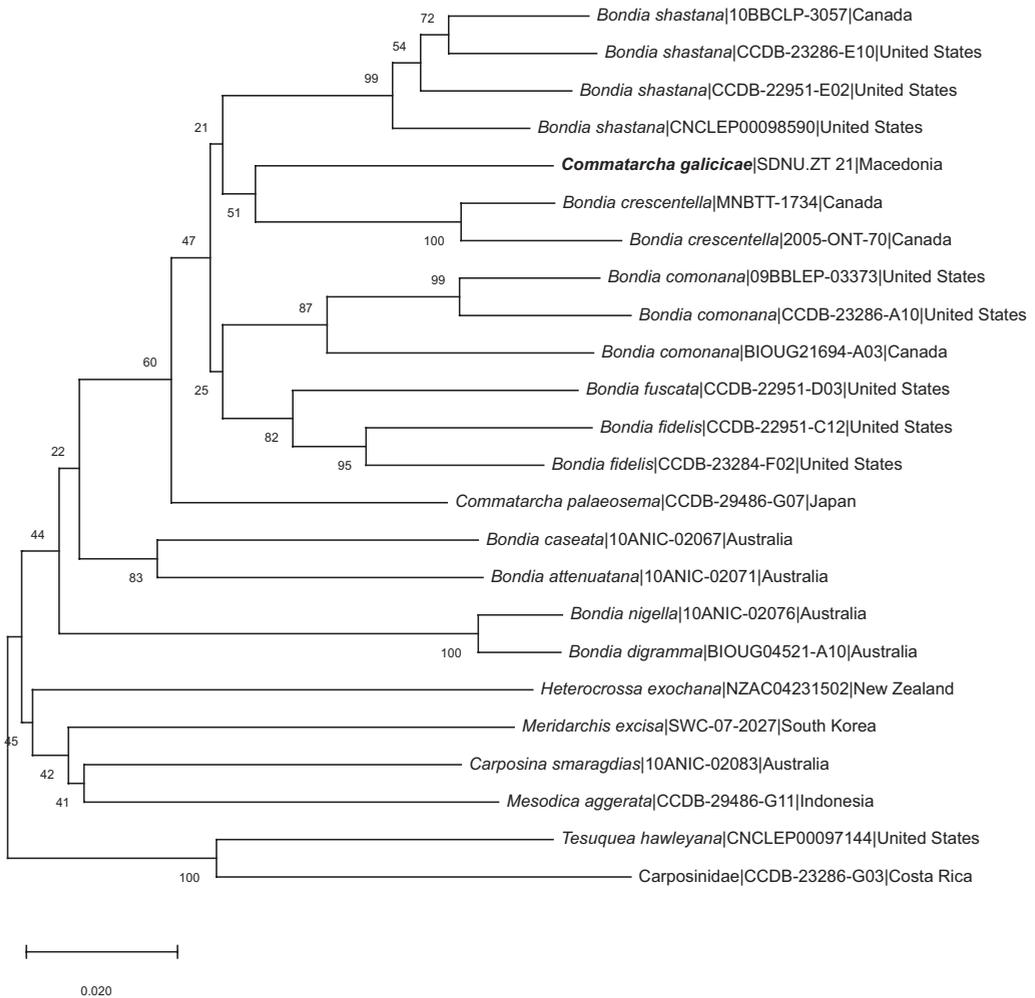


Figure 11. A Maximum Likelihood tree of *Commatarcha galicicae* Tokár & Srnka, sp. nov. (shown in bold face) and selected closely related species based on COI sequences. Numbers are bootstrap percentages. Scale bar represents 2% difference (GTR model) between sequences.

for species of *Commatarcha* very little genetic data are available, for which reason confirmation of this observation should be verified by broader genetic and taxonomic sampling. It was the incomplete sampling of *Commatarcha* and genetic similarity of the American species of *Bondia* that led us in the wrong direction when we initially placed our new species in the genus *Bondia*. Only a more thorough comparison of male and female genitalia of *C. galicicae* Tokár & Srnka, sp. nov. showed that it is actually morphologically closer to some Asiatic species of *Commatarcha*. Generally, species of Palearctic *Commatarcha* and American *Bondia* have many morphological features in common, including sexually dimorphic antennae, the uncus of the male genitalia with a small conical lobe, the absence of a gnathos, the valva with processes, a well developed and

usually arched ectophallus and the female genitalia with a heavily sclerotized colliculum and signa absent. By contrast, the Australian *B. nigella*, the type species of the genus, has the valva without processes in the male genitalia and the corpus bursae with signa in the female genitalia, representing significant differences from the above mentioned groups. Moreover, Australian *Bondia* species form a separate cluster in the DNA barcoding trees (Figs 10, 11). Davis (1969) considered that it may be decided after an adequate study of all the species has been completed that the Australian species and North American species of *Bondia* require separate generic or subgeneric placements but he did not have sufficient data to change their generic position at that time. Diakonoff (1989) showed on morphological grounds that Asiatic species, previously placed in the genus *Bondia*, are not congeneric with the Australian type species, and moved them all to *Commatarcha*. He also supported Davis' doubts about the similarities of *Bondia* from the American and Australian continents but this was not followed up with taxonomic actions. According to current knowledge we are convinced that the Palaearctic *Commatarcha* and American *Bondia* groups are congeneric and therefore a comprehensive revision of Australian and American *Bondia* species would be required.

The discovery of *Commatarcha galicicae* Tokár & Srnka, sp. nov. shows that the fauna of the Galičica Mountain is still insufficiently studied and can provide discoveries of hitherto unknown species. For example, the new species *Platyptilia galicicaensis* has recently been described from there (Junnilainen and Kaitila 2017). Galičica is characterized by a high incidence of endemic plants (Matevski et al. 2011), which also indicates the presence of endemic insect species there. After the first discovery, we assumed that our new species might be endemic to this area. However, the subsequent findings in south-western Romania point to a wider distribution of the species. The question arises as to whether *C. galicicae* represents another newly introduced or recently spread species from the regions of Western or Central Asia, or if it is an "old" European species that had remained undiscovered until now. The two nearly simultaneous discoveries from two different areas in the same year suggest that the species is a recent addition to the fauna of this region.

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Detection of the Large White butterfly *Pieris brassicae* (Linnaeus, 1758) (Lepidoptera, Pieridae) in Rapa Nui (Easter Island)

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Abstract. The Large White butterfly (*Pieris brassicae* (Linnaeus, 1758)) originally from the Palearctic and previously introduced to South Africa and Chile is detected on Rapa Nui (Easter Island). The records correspond to all stages of the life cycle: eggs, caterpillars, pupa and adults. Caterpillars were found feeding on *Tropaeolum majus*. The introduction is likely to have occurred from mainland Chile, as the species has not been reported in French Polynesia. A dark green coloration present on the underside of the wings is congruent with the one reported from the Chilean population. The origin of colonisation still remains to be tested using molecular tools.

Introduction

The Large White butterfly (*Pieris brassicae* (Linnaeus, 1758)) is a Palearctic species also introduced to South Africa, Chile and with scattered records elsewhere (<https://www.gbif.org/species/1920506>). As its name suggests, this butterfly feeds on plants from the family Brassicaceae (Cruciferae nom. cons.). Among those is *Brassica oleracea* L. from which different vegetables are derived (cabbage, cauliflower, broccoli, Brussels sprouts, etc.) (CABI 2022). The newly hatched caterpillars can completely defoliate a cabbage leaf leaving the veins intact. In its native range, the population is controlled by the parasitic Hymenoptera *Cotesia glomerata* (Linnaeus, 1758) (Bracoonidae) and *Pteromalus puparum* (Linnaeus, 1758) (Pteromalidae) (Herreros 1971).

This species was first recorded in Chile in the city of Viña del Mar in November, 1970; a few months after that record, other occurrences were found near Valparaíso (Herreros 1971). In 1972, the butterfly was detected in Santiago, which is about 100 kilometers inland from the original record (Gardiner 1974). Chilean entomologist Luis Peña suggested that the species came from Eastern Europe (Gardiner 1974). Since that initial introduction the species has expanded to the north into the Antofagasta region and to the south into the Los Lagos region (Benyamini et al. 2014; Zúñiga-Reinoso and Mardones 2014; Contreras 2020). The species has not been previously recorded on Rapa Nui (Holloway 1990; Tennent 2006).

Several natural enemies have been reported in Chile (Neira et al. 1989; Prado 1991). The caterpillar is parasitized by *C. glomerata* and *Apanteles* sp. (Braconidae) (Neira et al. 1989; Araya et al. 2005) and by *Incamyia chilensis* Aldrich, 1928 and *Incamyia spinicosta* Aldrich, 1928 (Tachinidae) (Prado 1991). The pupal stage is subject to various degrees of parasitism including from *P. puparum* (Pteromalidae), *Coccygomimus fuscipes* (Brullé, 1846) (Ichneumonidae) and infection by the fungus *Beauveria brongniartii* (Sacc.) Petch, 1926 (Cordycipitaceae) (Neira et al. 1989). Overall, in Chile it is considered only as a secondary pest (Araya et al. 2005).

Methods

Specimens were found and photographed in the field. Two additional records were retrieved from the platform iNaturalist. Taxonomic identification followed Gardiner (1974) and Zúñiga-Reinoso and Mardones (2014).

Results

We report the first detections of the species on Rapa Nui (Isla de Pascua/Easter Island; 27°07'S, 109°22'W). The records correspond to all stages of the life cycle: eggs (on cabbage (Brassicaceae) on 19.viii.2021. Fig. 1a), caterpillars (on *Tropaeolum majus* L. (Brassicales: Tropaeolaceae) on 15.vii.2021. Fig. 1b), a pupa (20.vii. 2021. Fig. 1c) and adults (on cabbage on 19.viii.2021. Fig. 1d; nectaring on *Lantana camara* L. on 15.vii.2021. Fig. 1e; 10.viii.2021). In addition, at the platform iNaturalist, there are two independent records of adults of the species present on the island on 21.v.2021 (Ramírez 2021) and on 01.x.2022 (Barnard 2022).

Due to the extent of dark pigmentation on the forewings, and the very different morphology and gregarious behavior of the caterpillars it can be distinguished from *Pieris rapae* (Linnaeus, 1758). *P. rapae* is originally from Europe and has been introduced to every continent except for South America and Antarctica (Ryan et al. 2019).

Discussion

The introduction of *P. brassicae* to Rapa Nui (butterflies are locally referred to as “Pepeka”) is likely to have occurred from mainland Chile due to the constant movement of goods and produce via air and sea transportation. The frequency of the flights to the island vary depending on the season. Normally, it is one or two per day, but it can go up to three during exceptionally high travel season. Between 2020 and 2021, the commercial flights almost completely stopped due to the COVID pandemic. However, some chartered flights with goods from the continent continued. The island is also supplied by ships, with three regular arrivals every month and a half, and a fourth one sporadically. These two routes represent the most likely entry mechanism for the species, but it is difficult to ascertain which one. Tahiti in the Society Islands is the other regular air connection to the island, but the species has not been reported in French Polynesia (Tennent 2006; GBIF 2022). A similar introduction mechanism could explain the record of the species in a patch of native forest on Robinson Crusoe island (specimen 571_UCCC_MZUC_LEP_2015; Museo de Zoología de la Universidad de Concepción) on 20.i.1996. This is another volcanic island belonging to the Juan Fernández archipelago off the coast of central Chile.

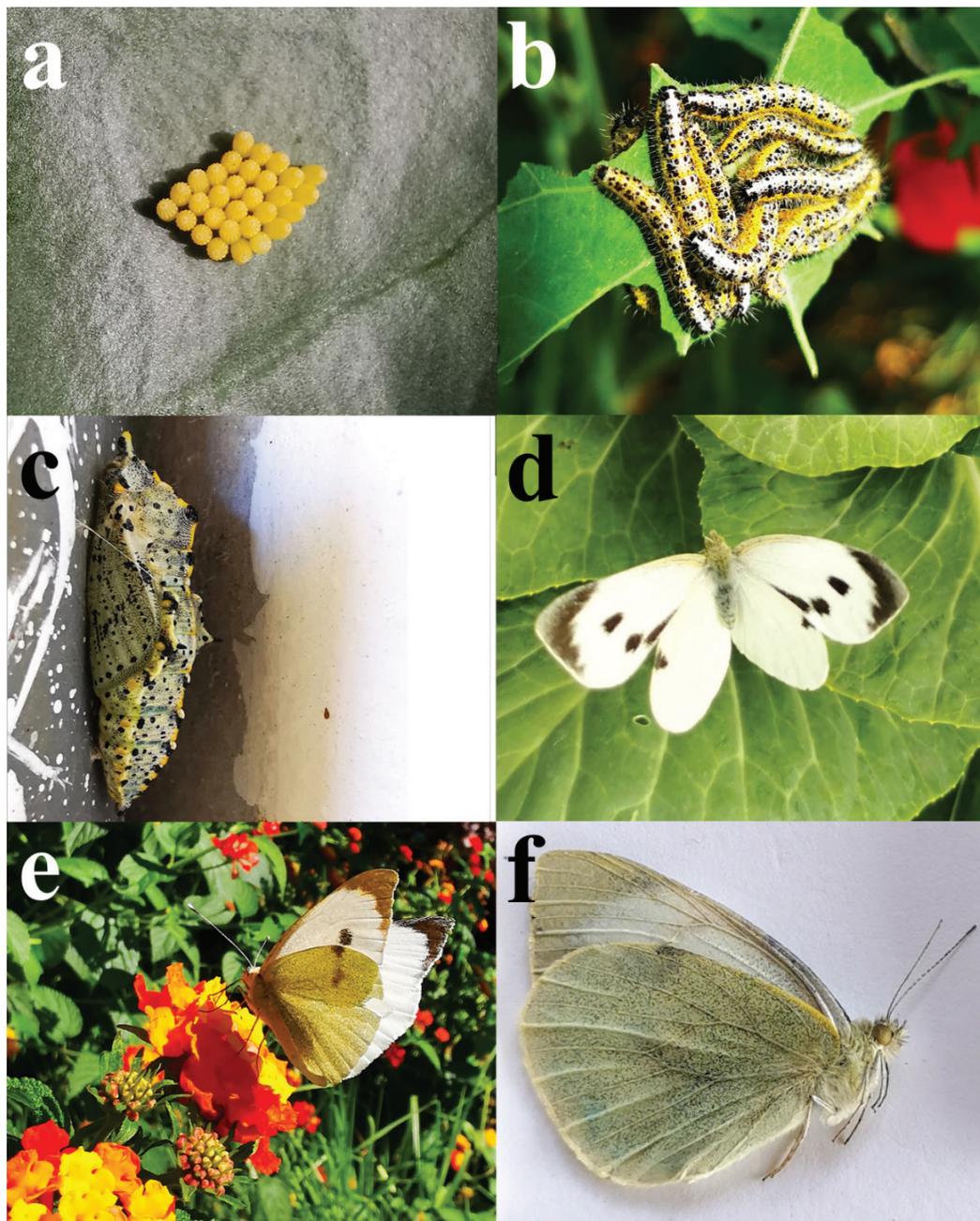


Figure 1. Different life stages of *P. brassicae* documented in Rapa Nui. **a.** Eggs; **b.** Aggregation of caterpillars; **c.** Pupa; **d.** An adult female on a cabbage plant; **e.** An adult male on a *L. camara* flower and **f.** The underside of the wings of an adult showing dark greenish coloration.

Gardiner (1974) described a dark green coloration on the underside of the wings of the individuals from the Large White population present in continental Chile. That trait was also found in the butterflies recorded from Rapa Nui (Fig. 1f). The colouration of the eggs, caterpillar and pupa, as

well as the gregarious behaviour of the caterpillar (Fig. 1b) are also congruent with what has been previously reported from mainland Chile (Gardiner 1974). However, a hypothesis of an origin from continental Chile remains to be tested using molecular tools.

A summer diapause was described in populations of the southwest distribution of the native range of *P. brassicae* in the Iberian Peninsula (Spieth et al. 2011) and for the introduced population in Chile (Benyamini 1995). In Chile, this local adaptation has been associated with a physiological strategy to avoid the dry summers of the Mediterranean climate where there is a lack of food sources (Benyamini 1995). This summer diapause is followed by an increase in abundance of the adults which peaks in May before the winter time. Indeed, in the first record of the species in Chile there is a reference to an increase in population density towards May and an abrupt collapse in June as the southern winter starts (Herreros 1971). In Rapa Nui, by contrast, *P. brassicae* appears to have retained the summer diapause, but has a continuous activity throughout the winter months (Yancovic Pakarati pers. obs.).

Regarding the previously reported enemies of the species, in Rapa Nui a potential braconid parasitoid from the genus *Apanteles* has been recorded (Rojas 1981; Ripa et al. 1995). Therefore, its populations might be controlled by a parasitoid species already present on the island. However, there is no evidence of the existence of that interaction.

Conclusion

This record is relevant as a baseline for monitoring the species, assessing its impact on the local biodiversity of Rapa Nui and evaluating its management strategy. *P. brassicae* was successfully eradicated from New Zealand 6.5 years after its original detection and following four years of an active integrated pest eradication strategy (Phillips et al. 2020). An early detection of introduced arthropod species into oceanic islands is key (Cotoras et al. 2017; González et al. 2020; Pérez-González et al. 2020) to preventing larger ecological and economic damage, as well as in minimizing management costs.

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Stigmella naturnella (Klimesch, 1936) (Lepidoptera, Nepticulidae) a fast-spreading European leafminer of *Betula*, with a revised key to linear leafmines on *Betula*

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Abstract. *Stigmella naturnella* (Klimesch, 1936), a leafminer of *Betula*, is here recorded as new for France, Croatia, Ukraine, Belgium, and the Netherlands. Since 2018, it has expanded its range into the last two countries, partly based on numerous online observations. Its distribution history is reviewed, the species is diagnosed and its life history is described. A lectotype is designated for *Nepticula naturnella* Klimesch, 1936. The species is widespread in the Palearctic, from Japan to the North Sea, with a maximum of 1.63% variation in its DNA barcode. Legacy leafmine records for Germany are reviewed, resulting in the confirmation of its occurrence in Baden-Württemberg already in 1935, but other old records are rejected. It is one of the few Nepticulidae species that hibernate as adult, a possible contributing factor to its expansion. As the leafmines may be confused with other *Betula* mining species, a revised key to the leafmines of European *Stigmella* species on *Betula* is provided. *Stigmella glutinosae* (Stainton, 1858) and *S. alnetella* (Stainton, 1856), usually feeding in *Alnus*, are both recorded to occur occasionally on *Betula* and are included in the key.

Introduction

As for many other organisms, spread and invasions have been recorded recently for several leafmining moth species. A number of species have invaded Europe from other continents, aided by human activity, and have often spread quickly over the continent, hostplant availability permitting. Examples are *Macrosaccus robiniella* (Clemens, 1859) and *Parectopa robiniella* Clemens, 1863 that invaded Europe probably with the aid of airplanes in the 1970s or 1980s of the last century, and which have since spread over a large part of Europe, finding their hostplant *Robinia pseudoacacia* L. widely distributed (Mally et al. 2021). Two North American leafminers of walnuts and related Juglandaceae were recently noted for the first time respectively in Italy (*Coptodisca lucifluella* (Clemens, 1860)) (Bernardo et al. 2011, 2015) and Hungary (*C. juglandiella* (Chambers, 1874)) (Takács et al. 2020), and both have since also been found in neighbouring countries, suggesting spread through dispersal (Takács et al. 2020; Tomov 2020; Haslberger and Segerer 2021; Huemer 2021; Laštůvka et al. 2021).

Further examples in leafmining Lepidoptera are seen in European species that have expanded their range due to the widespread planting of their hosts in parks and gardens beyond their native habitat: for example *Antispila treitschkiella* (Fischer von Röslerstamm, 1843) on *Cornus mas* L.

(van Nieukerken et al. 2018), *Phyllonorycter leucographella* (Zeller, 1850) on *Pyracantha* species (Šefrová 1999; Walczak et al. 2010); and several *Acer* feeding species (van Nieukerken et al. 2006b; Corver et al. 2011; Huisman and Muus 2020).

Whereas in these cases climate change may have been a major factor in the spread, other causes are more complex to explain. Spread of leafmining species on hostplants that have always been native in the region are probably better indicators of climate change, such as *Stigmella nivenburgensis* (Preissecker, 1942) on *Salix* species and several *Phyllocnistis* species feeding on *Salix* and *Populus* that have expanded recently in NW Europe (van Nieukerken et al. 2017; Prick et al. 2018; van Nieukerken and Wullaert 2018a, b; Schulz and Fähnrich 2019; Wullaert 2019). Also the oak leafmining nepticulid *Ectoedemia quinquella* (Bedell, 1848) is shifting its distribution northwards in the Netherlands (Alders and Donner 1992; van As and Scheffers 2013).

On *Betula* until now, we did not see any clear northward shifts of leafmining species, which is to be expected, as *Betula* itself is a more northerly genus (San-Miguel-Ayanz et al. 2015) with few southern herbivore specialists (Ellis 2022). An exception may be *Stigmella sakhalinella* Puplesis, 1984, of which there are indications of a northward shift as seen from relatively recent first records in northern Europe, Norway in 1988 (Aarvik et al. 1997), Sweden 1990 (Svensson 1992), Lithuania 2004 (Anisimov and Stonis 2008), Denmark 2009 (Buhl et al. 2011), Estonia 2009 (Jürivete 2011; Jürivete and Õunap 2020), Ireland 2015 (Langmaid and Young 2016) and Finland in 2016 (Aarvik et al. 2021) (details unpublished, mines collected by Mikhail Kozlov, identified by the author, see Fig. 63 and BOLD record RMNH.INS.31157).

Stigmella naturnella (Klimesch, 1936) is the only *Betula* feeding species of Nepticulidae in Europe with a more southerly distribution. It was described from the southern Alps in Italy, South Tyrol from the warm valleys of the Vintschgau (Venosta) (Klimesch 1936). For many years very little information was added about this species, apart from a treatment by Klimesch (1948), who described the male genitalia, and a few odd records of leafmines (Skala 1937, 1939; Buhr 1940a, b) that were subsequently mostly neglected or believed to be incorrect. The species was rarely recorded from Central Europe and considered to be a rare and thermophilous species (Szöcs 1973; Laštůvka and Laštůvka 1991, 1997). The fact that the leafmines of *S. naturnella* were considered to be almost impossible to distinguish from similar ones such as *S. confusella* (Wood & Walsingham, 1894) added to the poor state of knowledge of the species.

Meanwhile *S. naturnella* was described again as *Astigmella dissona* Puplesis, 1984 from the Far East of Russia (Puplesis 1984a, b), a synonymy that was later recognised when the species appeared to be common in the Volga area in European Russia (van Nieukerken et al. 2004). Only recently a few odd records were published from Europe north of the Alps: one record in Poland and three in Germany (Baran 2013; Guggemoos et al. 2018; Sobczyk et al. 2018; Segerer et al. 2019). After learning how to recognise mines of this species from my work on the Russian leafmines, I found *S. naturnella* mines in South Tyrol, and to my surprise in 2017 in western France, far from the Alps. An even bigger surprise was the discovery of photographs of leafmines resembling this species on the Netherlands' observation platform waarneming.nl. Although I was sceptical at first that they could be attributed to *S. naturnella*, I set out in the autumn of 2020 to search for such *Betula* mines in the Netherlands. This led to the discovery that *S. naturnella* had entered the Netherlands unobserved over the last few years and had already established populations throughout its southern half, often having become one of the commonest leafminers on birch. The Belgian observation platform waarneming.be also contained several misidentified mines that



Figure 1. *Stigmella naturnella*, live female, The Netherlands, Rijssen, emerged 14.x.2022 (photo Gerwin van de Maat).

could be attributed to this species and a few photos of live adults were identified as *S. naturnella*. These observations were announced on two online platforms (van Nieukerken 2020a, b). In this paper all records are reviewed, together with a history of the species spread, and a diagnosis of the species is provided. As the leafmines can easily be confused with other species, special attention is paid to the diagnostics of *Stigmella* mines on *Betula*, aided by the provision of a key.

Material and methods

Collecting

Material collected by me before 2020 was found during general leafmine searching, not focussed particularly on *Betula*. In autumn 2020 (September-October) collecting in the Netherlands was devoted to *Betula* miners specifically to study the distribution of *Stigmella naturnella*. Six full days of field work were carried out in the provinces of Utrecht, Gelderland, Noord-Brabant and Zuid-Holland; in addition some observations were made during other activities in Limburg; in 2021 and 2022 a few further records were obtained. Mature and juvenile trees and seedlings were searched for leafmines, and samples of all species of Nepticulidae were collected, whether vacated or with larvae. Leafminers of other groups than Nepticulidae were noted and only occasionally collected. In most cases photographs were taken of the various species in the field. Leaves with mines were gathered in plastic bags or small containers. Some larvae, especially those that had died or looked in poor condition, were dissected from their mines and preserved in 96% ethanol for molecular and morphological studies. Samples of leaves with leafmines were dried using a plant press and subsequently stored in glassine envelopes.

Rearing

Collected leaves with occupied leafmines were kept in small jars or polystyrene bags, with some moss, earth and/or paper tissue added, until the larvae had left their mines. After that the leaves were taken from the rearing jars and pressed and dried as vouchers. Adults were reared

from the cocoons in the same containers, occasionally adding some moisture. *Stigmella naturnella* adults emerged within a few weeks, no hibernation occurred, and rearing jars were kept indoors until emergence. Rearing jars with cocoons of other species were kept in a refrigerator at ca. 4 °C between November and early March and then brought indoors until emergence. Emerged adults were mounted freshly after allowing them to harden for at least a day, or overnight.

Materials and observations

In Leiden all larval specimens, samples of leafmines and the majority of adults received a registry number in the form RMNH.INS.#####. Most field collections and observations were also registered on the observation platform waarneming.nl and received a field number (EventId) in the form EvN no 2020#### (year and serial number between 001 and 999) for each combination of locality and hostplant species (or as short form EvN2020####). For multiple species on a single host, an extra number follows a dash, letters indicate destination of each sample (K=breeding, M=molecular tissue sample, H=herbarium, pressed leaves with vacated or unfinished mines). Examples are EvN no 2020062-1K, EvN no 2021152-3H.

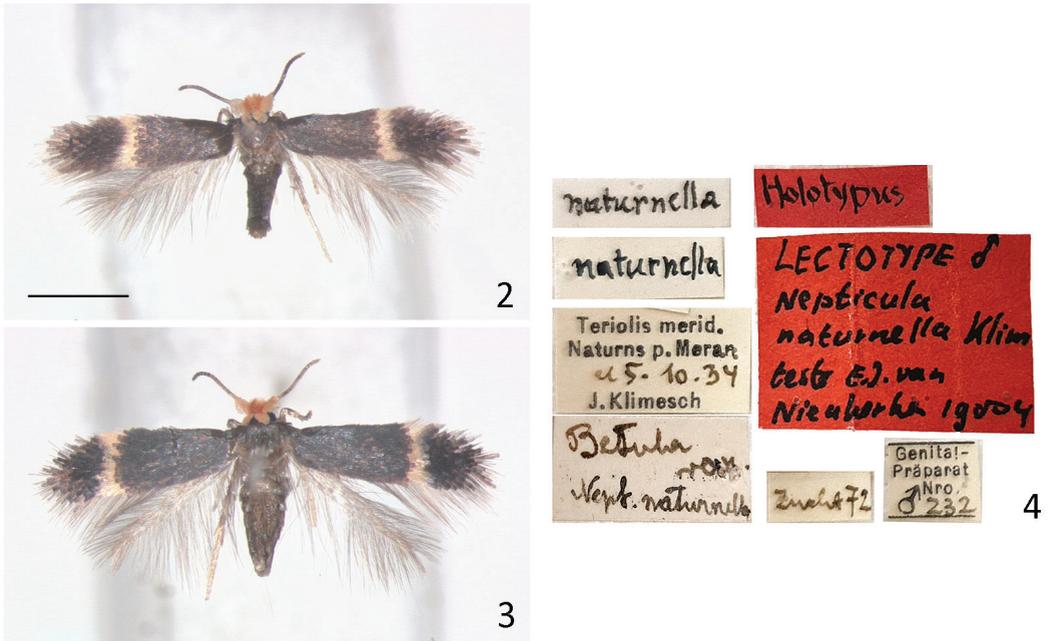
Some material, both adults and leafmines, was borrowed from other institutes or received from various colleagues in identification loans over the course of many years. Some data were obtained from databases: viz. the Netherlands Lepidoptera database “Noctua” (via Dutch Butterfly Conservation) and the database of the Tiroler Landesmuseen Ferdinandeum (via Peter Huemer). Many records were obtained from the Dutch and Belgian online observation platforms (respectively <https://waarneming.nl/>, Observation International and local partners 2022a and <https://waarnemingen.be/>, Observation International and local partners 2022b), often originally registered under different identifications. Some records were also found on the international platform <https://www.inaturalist.org/> (iNaturalist community 2022), but none on <https://observation.org/>. Many of these records could be re-identified or confirmed if suitable photographs were uploaded, and I was able to validate or correct records from <https://waarneming.nl/> and <https://www.inaturalist.org/>, Carina van Steenwinkel validated records on <https://waarnemingen.be/>. Records were used only when validated, or verified by me. Obvious duplicate records were omitted. Data were included up to November 2022.

Records of DNA barcoded specimens were downloaded from BOLDSYSTEMS (Ratnasingham and Hebert 2007) and are brought together in a publicly available dataset (<https://doi.org/10.5883/DS-STIGNATU>) and in Suppl. material 1.

A detailed dataset of material, observations and literature records was uploaded via NLBIF to GBIF (<https://doi.org/10.15468/9u5f59>). The material listed below includes only that which has been examined by me. This material is organised by country, and for those countries with much information also by province. Data of material for other species, referred to in the text and figures is listed in the appendix, and also in the GBIF dataset.

Molecular methods

DNA barcoding followed the procedures at our laboratory as described by van Nieuwerkerken et al. (2012) and Doorenweerd et al. (2015). Genbank accession codes were added to the datasets cited above and in Suppl. material 1. A few DNA barcode sequences originated from other sources, viz. projects by Peter Huemer (TLMF), Christian Wieser (Landesmuseum Kärnten, Austria), Andreas Segerer (ZSM) and from the Centre for Biodiversity Genomics, Guelph. Specimens from the last source were collected with Malaise traps, for a description of the protocol see deWaard et al. (2019)



Figures 2–4. *Stigmella naturnella* 2. male, The Netherlands, Wolfheze, RMNH.INS.172151; 3. female, Goirle, The Netherlands, RMNH.INS.17214; 4. Lectotype labels (photo Andreas Seegerer). Scale bar: 1 mm (2, 3).

The Neighbor Joining tree was prepared with tools provided by BOLDSYSTEMS (Ratnasingham and Hebert 2007) and edited with Adobe Illustrator CS5.

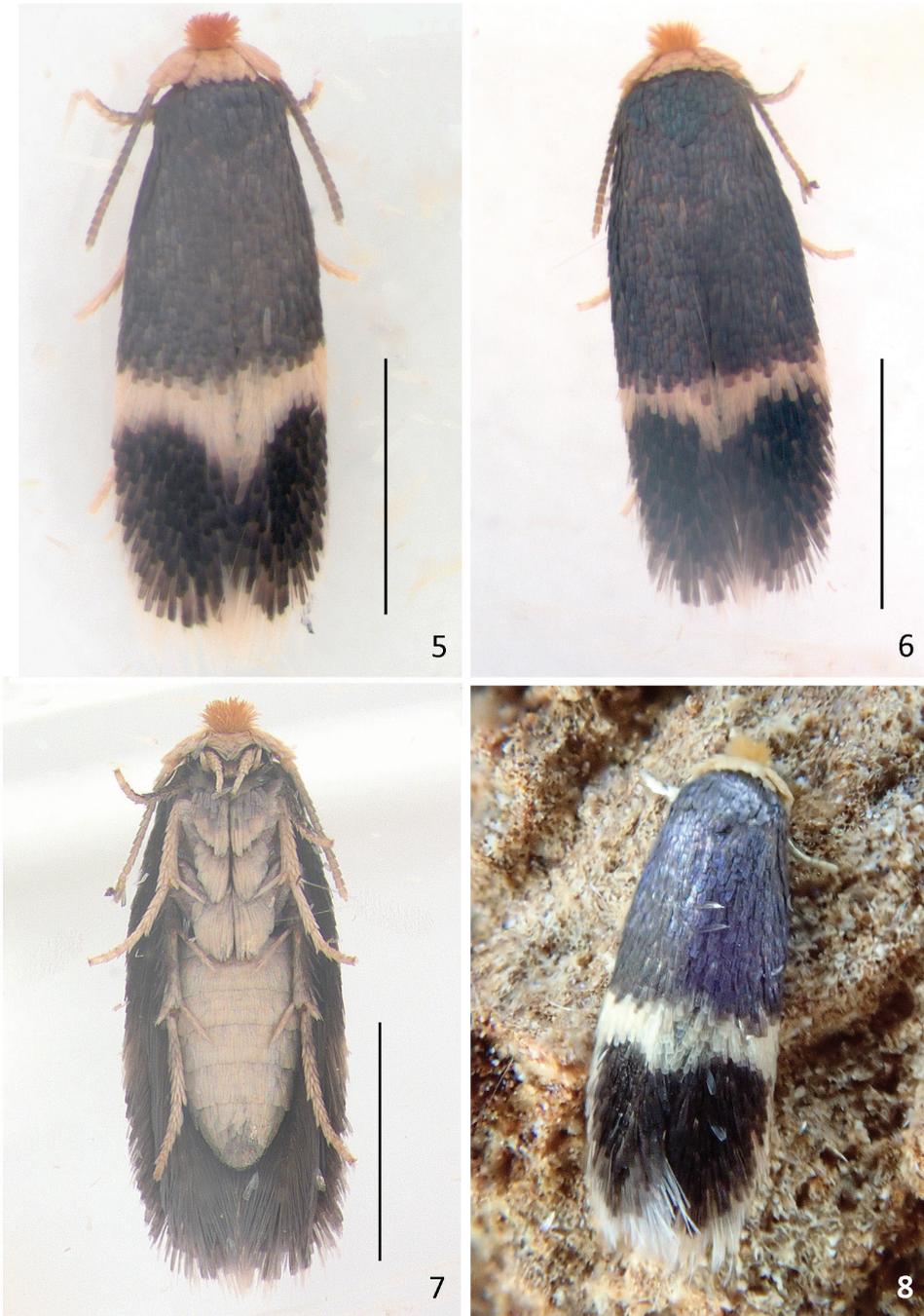
Morphology

Genitalia were prepared according to our standard procedures, usually including DNA extraction, as described earlier in detail (van Nieukerken 1985; van Nieukerken et al. 2010).

Measurements of moths, genitalia and leafmines were taken with measuring tools in Carl Zeiss AxioVision software on photographs, see below. For a sample size of 4 and higher, mean, standard deviation and sample size are provided between brackets. Details of measurements are given in Suppl. material 2.

Illustrations

Unless otherwise mentioned, all photographs were taken by the author. Photographs of moths, leafmines, genitalia slides and larval slides were taken with a Zeiss AxioCam digital camera attached, respectively, to a Zeiss Stemi SV11 stereo-microscope, a motorized Zeiss SteREO Discovery V12 or a Zeiss Axioskop H, using Carl Zeiss AxioVision software version 4.8 or 4.9. Field photographs were taken with a Canon EOS 600D prior to 2020, in 2020 with a Nikon D70 or a Mobile phone and in 2021 with a Canon EOS 850D. For photographing leafmines with the Zeiss set up, dark field illumination was used. Images were edited with Photoshop CS5, mainly to obtain a more even background, better lighting and some sharpening was added; some illustrated photographs were composed from a number of photographs by Photoshop photomerge. Plates were composed with Photoshop CS5. The distribution maps were prepared with QGIS 3.10.



Figures 5–8. *Stigmella naturnella*, live adults **5.** Male, 4.x.2020, Netherlands, Wekerom, RMNH.INS.25268; **6, 7.** Female, 1.x.2020, Netherlands, Soest RMNH.INS.17204; **8.** Hibernating adult under bark of *Platanus*, Belgium, Antwerp, 6.ii.2020 (photo Stijn Baeten), <https://waarnemingen.be/observation/185190974/>. Scale bars: 1 mm.

Abbreviations

| | |
|------|--|
| EvN | Erik J. van Nieukerken; |
| HNHM | Hungarian Natural History Museum (Termesztudományi Múzeum), Budapest, Hungary; |
| JE | Herbarium Haussknecht, Friedrich Schiller University Jena, Germany; |
| NHMK | Natural History Museum London, UK; |
| RMNH | Naturalis Biodiversity Center, Leiden, Netherlands; |
| SMNS | Staatliches Museum für Naturkunde, Stuttgart, Germany; |
| TLMF | Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria; |
| ZIN | Zoological Institute, St Petersburg, Russia; |
| ZMUC | Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark; |
| ZSM | Zoologische Staatssammlung München, Germany. |

Results

Stigmella naturnella Klimesch

Local names. zuidelijke berkenmineermot (Dutch), südliche Birkenminiermotte (German), szőrösnyír-törpemoly (Hungarian).

Nepticula naturnella Klimesch, 1936: 205. Lectotype ♂ (here designated), Italy: “Teriolis merid., Naturns p. Meran, el 5.10.34, J. Klimesch / Betula verr., Nept. naturnella / Zucht 72/ Genital-Präparat Nro ♂ 232/ Holotypus”, [larvae collected September 1934] (labels Fig. 4) (ZSM) [examined].

Astigmella dissona Puplesis, 1984: 112. Holotype ♂, Russia, Primorskiy Krai, 20 km E Ussuriysk, Gornotayezhnoe, 28.v.1983, leg. R. Puplesis, genitalia slide no. AG 403 (ZIN) (synonymised by van Nieukerken et al. 2004a: 133).

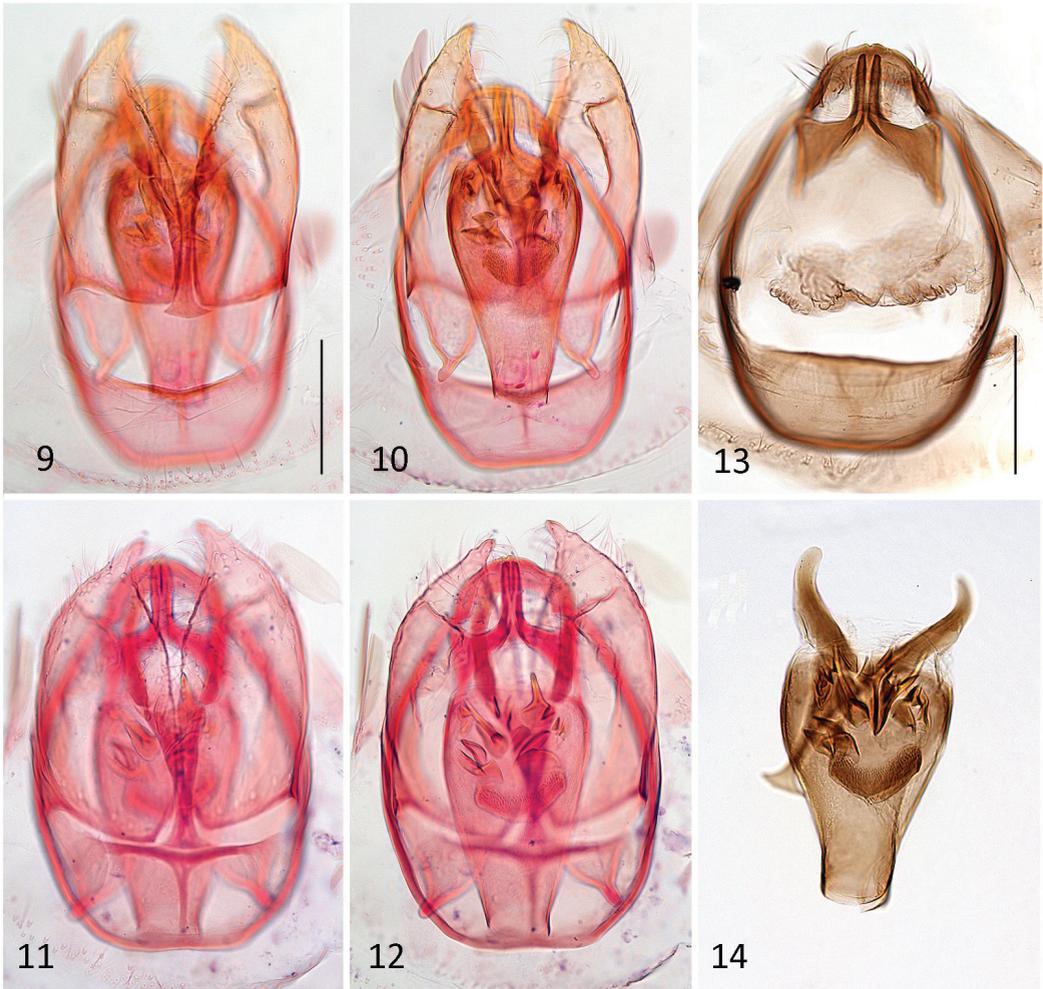
“*Nepticula argentifasciella* Klimesch”: Skala 1936: 11. Unavailable name and Junior Homonym of *Nepticula argentifasciella* Braun, 1912.

Stigmella naturnella Klimesch 1948: 65 [recombination, male genitalia], van Nieukerken et al. 2004 [Synonymy, European Russia]; Stonis and Rocienė 2013: 2, 3 [photographs male genitalia holotype *A. dissona*].

Stigmella dissona Puplesis 1994: 58 [recombination, redescription].

Diagnosis. *Stigmella naturnella* adults resemble most other *Stigmella* species with a fascia, pale head and white collar superficially, including other *Betula* miners in the *S. betulicola* group. Characteristic is the combination of a distinct fringe line, white fringe, rather shining fascia, and a relatively short antenna in both sexes, reaching only halfway to the fascia. The basal part of the forewing may vary from grey to black with a blue iridescence. Those with grey can be confused with *S. tityrella* or *S. carpinella*, but these have usually the last part before the fascia darker and the antennae distinctly longer, reaching the fascia. Species in the *Stigmella betulicola* group do not have a fringe line and the males have longer antennae. Male genitalia are very characteristic by shallow uncus, connected gnathos arms and shape and number of cornuti, female genitalia much less so, but the very long posterior apophyses are notable. For mines and larvae see below.

Description. Male (Figs 2, 5). Forewing length 1.7–2.2 mm (2.0 ± 0.2 , n=6), wingspan ca. 3.9–4.8 mm. Head. Frontal tuft pale orange, collar conspicuous, cream white. Scape large, cream white. Antenna short, reaching slightly more than halfway between wing base and fascia; with 18–21 articles (19.0 ± 1.2 , n=5). Thorax and forewings basal to fascia shining dark grey to almost black; a narrow silvery white fascia slightly beyond middle, usually constricted in middle; apical area contrasting darker black compared to wing base; a fringe line of black scales separates the silvery white terminal fringe. Hindwing grey. Abdomen black, no visible anal tufts.

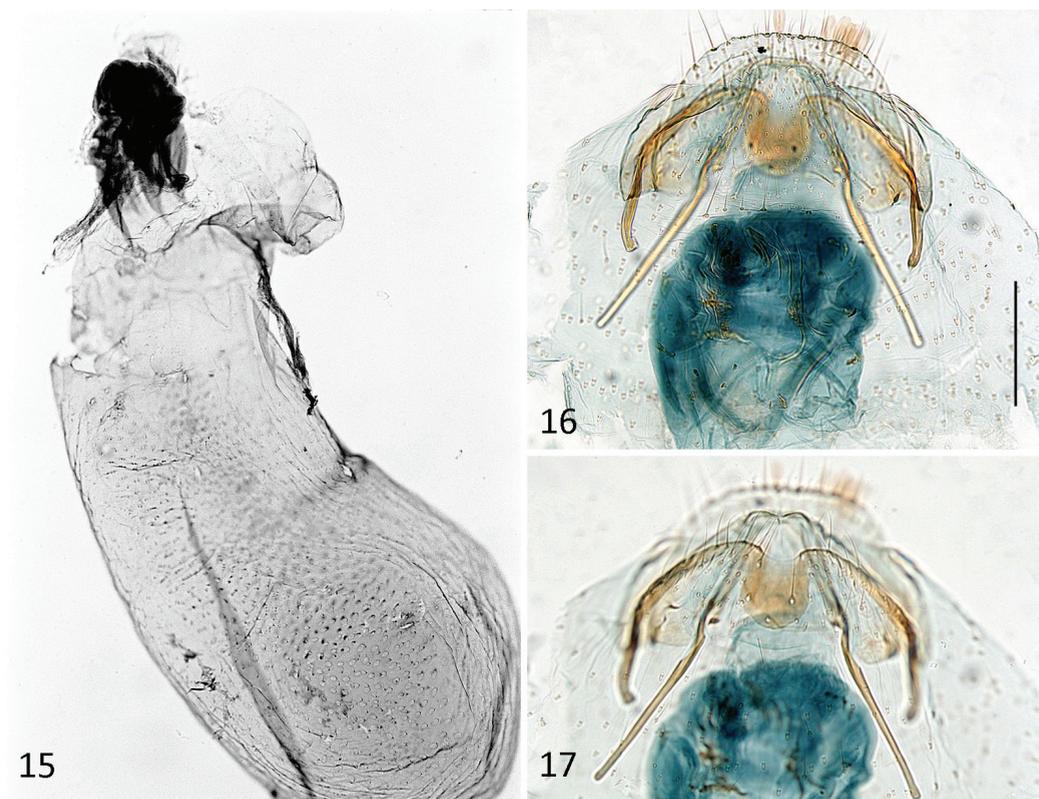


Figures 9–14. *Stigmella naturnella*, male genitalia, ventral aspect. **9, 10.** Slide EvN5268; **11, 12.** Slide EvN5341; **13.** Genitalia without valvae and phallus, slide EvN3302; **14.** Phallus, slide EvN3303. Scale bars: 100 μm (9–12 top left, 13, 14 top right).

Female (Figs 1, 3, 6, 7). Forewing length 2.1–2.3 mm (2.2 ± 0.1 , $n=6$), wingspan ca. 4.4–5.0 mm. Antenna very short, reaching less than halfway between wing base and fascia; with 17–18 articles (17.2 ± 0.4 , $n=6$). Abdomen slightly tapering.

Male genitalia (Figs 9–14). Vinculum with narrow ventral plate; tegumen band-shaped; uncus slightly indented; gnathos with distal arms very close, appearing almost as single structure. Valvae broad, slightly acuminate. Juxta anteriorly arrow shaped, posteriorly ending in two arms. Phallus distally widened, with ca. 8–10 strong cornuti.

Female genitalia (Figs 15–17). Ovipositor blunt. No visible anal papillae. Tergum 8 with indented posterior margin, with central sclerotised plate, few setae along margin. Narrow posterior

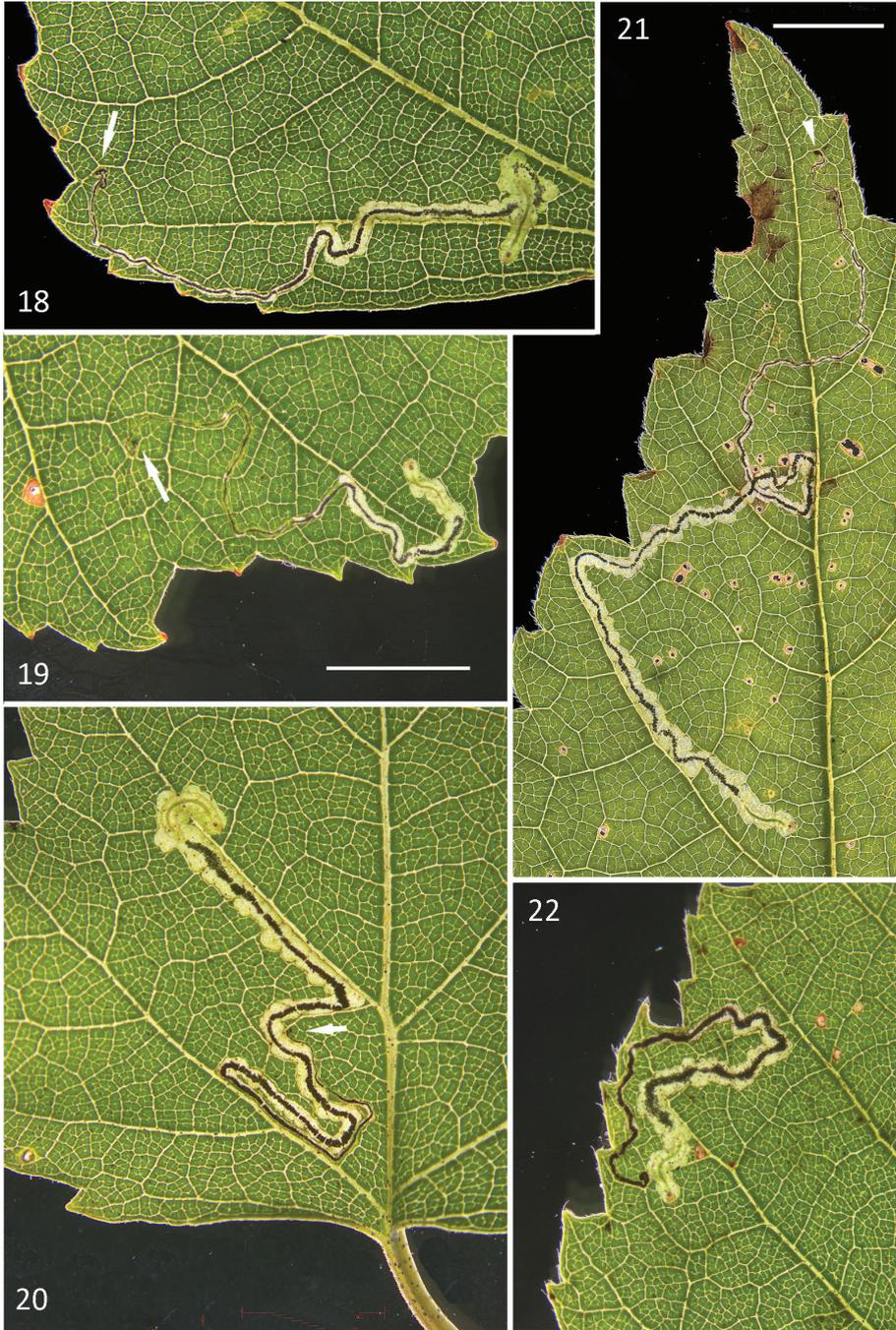


Figures 15–17. *Stigmella naturnella*, female genitalia, ventral aspect **15**. Bursa copulatrix, slide VU1874; **16**, **17**. Abdominal tip, respectively more ventrally and more dorsally in focus, slide EvN5342. Scale bar: 200 μ m (**16**, **17**).

apophyses reaching distinctly beyond anterior apophyses in abdomen. Vestibulum folded, staining strongly with chlorazol. Ductus spermathecae not coiled. Bursa copulatrix rather long, covered with small spines and pectinations.

Biology. Host plants. In Europe *Betula pendula* subsp. *pendula* Roth and *B. pubescens* Ehrh. and their hybrids, from East Asia reported from *B. pendula* subsp. *mandshurica* (Regel) Ashburner & McAll. (*B. platyphylla* Sukaczew) and *B. dahurica* Pall. (adults found on trunks, Puplesis 1984a, b). In the botanical garden of Linz, leafmines were also observed on the eastern Palearctic taxa *B. pendula* subsp. *mandshurica* and *B. utilis* D. Don subsp. *jacquemontii* (Spach) Ashburner & McAll. (see Klimesch (1990) and material examined). Much more frequently observed on *B. pendula* than on *B. pubescens*, also most literature references cite *B. pendula* (often under the old name *B. verrucosa*), but although Baran (2013) suggested that his record from *B. pubescens* was a new host record, it had been recorded from *B. pubescens* before (Skala 1939; Wörz 1958), records that are here partly confirmed.

Egg deposited on leaf upper- or underside, 67% were found on the underside (n=315), but the percentages differ per population, although almost always both positions occur when ten or more



Figures 18–22. *Stigmella naturnella*, occupied leafmines, seen from above, with dark field illumination; all from The Netherlands **18, 19, 22.** Wekerom, De Valouwe, 16 Sep. 2020, *Betula pendula*, EvN no 2020062-1; **20.** Goirle, Gorp en Roover, 30 Sep. 2020, *B. pendula*, EvN no 2020083-1; **21.** Soest, Korte Duinen, 23 Aug. 2021, *B. pubescens*, EvN no 2021121. Scale bars: 5 mm (all but 21 on same scale), arrows point at egg position (upper- or underside of leaf).

mines per population are examined. The egg may be deposited at any place of the leaf, but most frequently away from the midrib or large veins; the egg capsule is conspicuous and dark brown after the larva has hatched (Figs 25, 26).

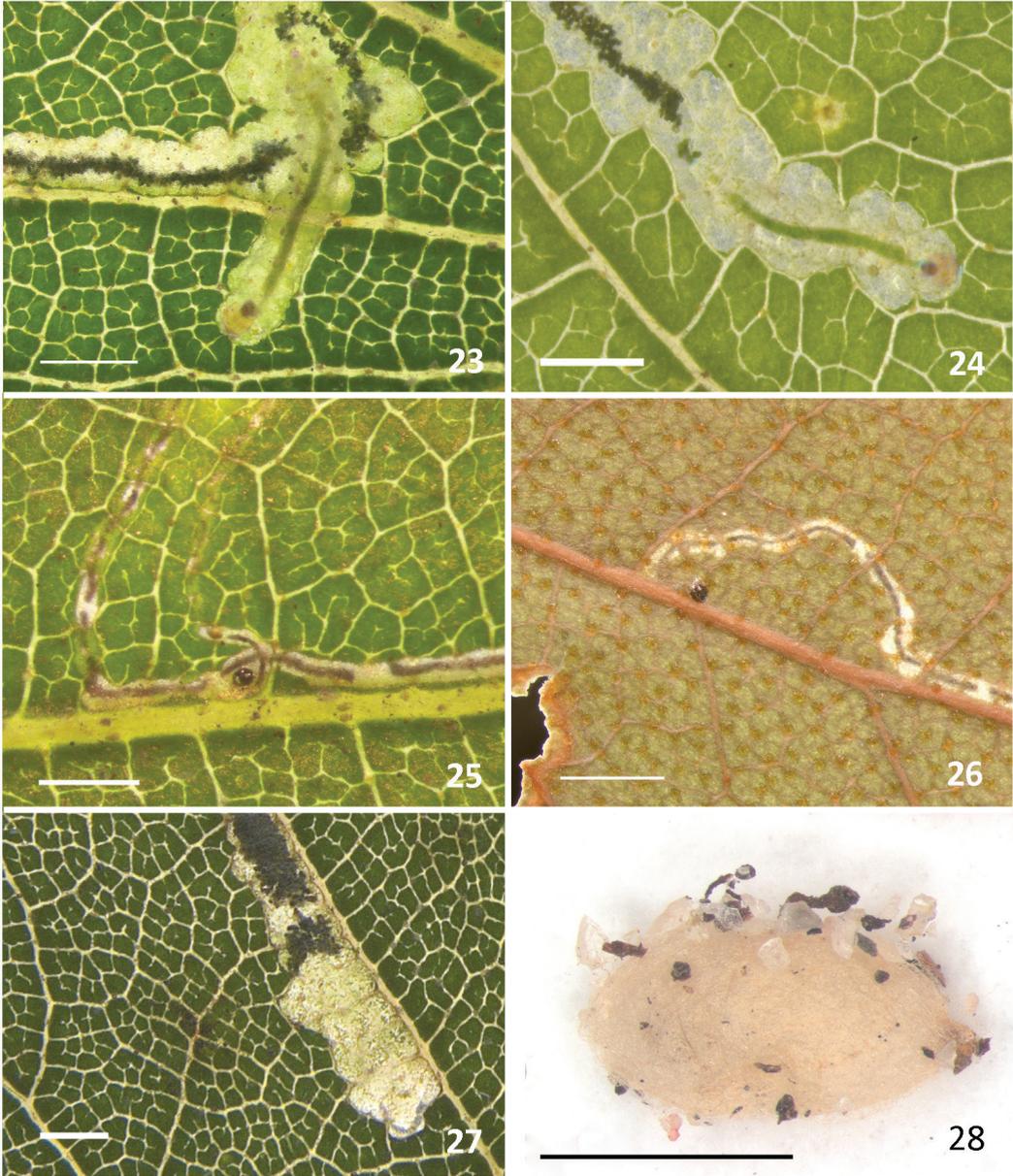
Leafmine (Figs 18–27, 29–34). A linear or gallery mine with variable length and frass disposition. From the egg the mine often runs straight away, or makes a single loose bend around the egg. The mine often does not follow parts of veins or the leaf margin, but some mines do follow veins for a shorter or longer stretch. The early mine starts in the spongy parenchyma layer, often resulting in the initial part of the mine appearing green from above, outside the frass line. This arrangement is particularly frequent on *Betula pubescens*, but many mines do not exhibit the green appearance. Frass varying from a narrow central line to a wider band of dispersed frass; in the early part of the mine it occasionally fills its entire width. The mine may be rather contorted, only occasionally crossing itself, and rarely crossing the midrib. The exit slit is invariably on the upper side of the leaf in nature. The very few mines (2–3) where an underside slit was observed, where mines that were completed by the larva in captivity after collecting; in these cases the leaf probably did not stay in its natural position, light conditions were poor, which may explain why the larva left the mine at the leaf underside. The final larval chamber often is buckled. Mines with an upperside egg and a green part at the start are the easiest to determine as *S. naturnella*, see below. Total length of mine 22.0–52.9 mm (34.0 ± 7.8 , $n=27$), width of final larval chamber 0.9–1.4 mm (1.0 ± 0.1 , $n=27$).

Larva (Figs 23, 24). The larva feeds venter upwards. Colour white to faintly pale yellow, head capsule brown; ventral nerve chord and ganglia invisible, apart from the conspicuous brown circular suboesophageal ganglion, which is a decisive diagnostic character; in actively feeding larvae the green intestine is also conspicuous.

Cocoon (Fig. 28). White and rather flimsy.

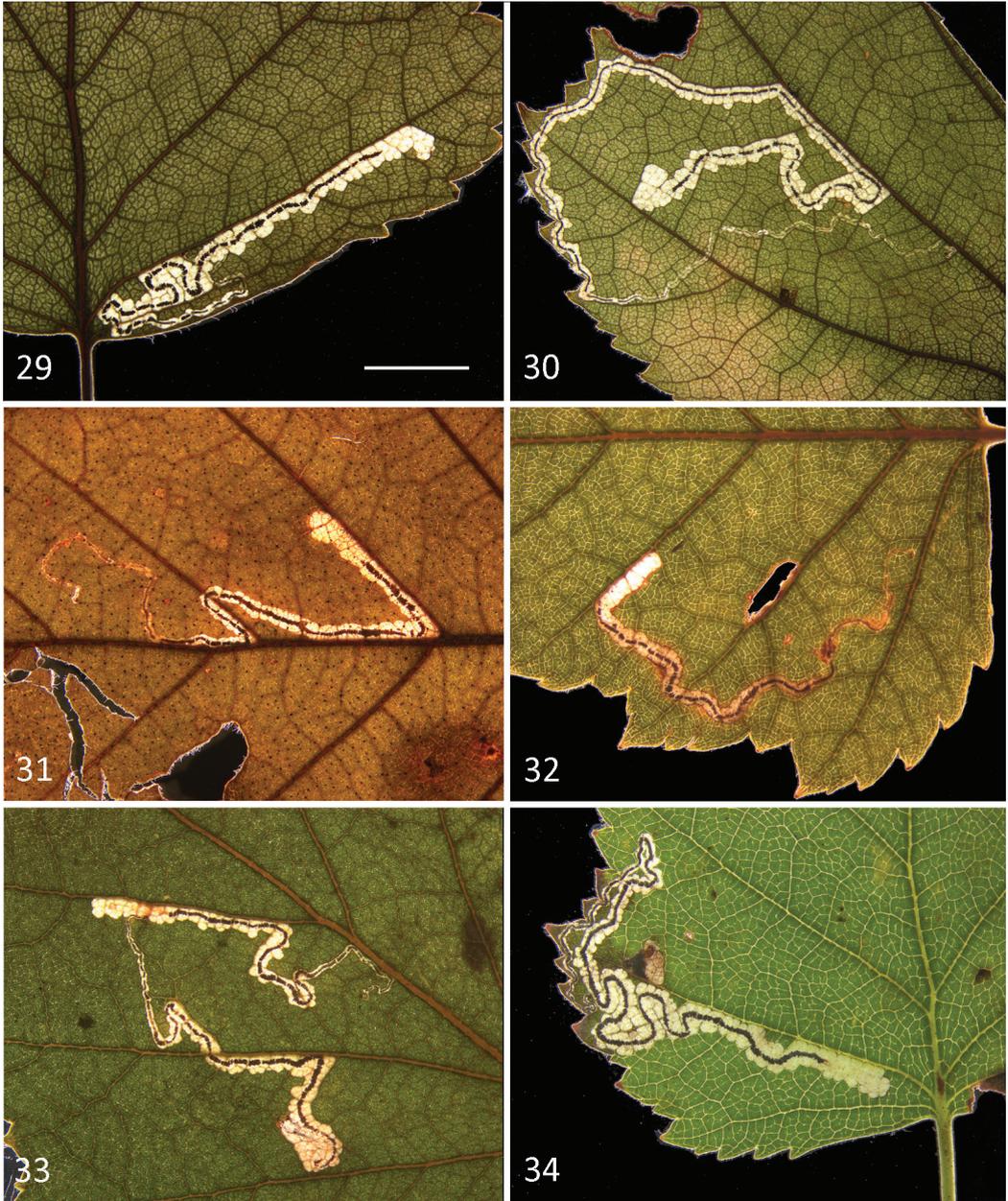
Life history. Larvae have been recorded from late-May to mid-July, and again from mid-August until October, with a single record from November. It is one of the earliest Nepticulidae larvae occurring on *Betula*, only *S. lapponica* can also be found in May and early June. Larvae seem to be most abundant in August, in the autumn only single larvae are found amongst large numbers of vacated mines. Adults have been collected or observed, after hibernation, from early April to early May, and again from 25 June almost continuously to 10 October, with a single record on 3 November. Hibernating adults have been found under the bark of trees, often *Platanus* (Fig. 8), but also on oaks, in November, January, February and March in Belgium and the Netherlands (Table 1) and in Russia (Ul'yanovsk) under bark of *Betula* on 19 April (van Nieukerken et al. 2004). Adults reared from larvae usually emerge within 2–3 weeks after collecting; in our material from the Netherlands from 17–23 days after collecting the larva (19.09 ± 2.07 , $n=11$). Klimesch (1936, 1948) reported a pupal stage between 10–12 days, Laštůvka and Laštůvka (1991) reported 13 days between collecting and emergence; but only Sobczyk et al. (2018) reported a longer period of 32 days. Note that many of these data are from indoor rearing in the autumn, when temperatures outside, especially at night were gradually becoming lower than those inside. From these data it appears that *S. naturnella* has at least two generations, maybe more in some cases, but from a large part of the distribution area hardly any data are available.

Habitat and ecology (Figs 35, 36). Most localities visited by me were on sandy soil, relatively dry forests or forest margins, either dry forest with *Pinus sylvestris*, or other planted *Pinus* species, or with *Quercus robur* and *Betula* often in the undergrowth. Mines were found sometimes in *Calluna* heathland that was becoming overgrown with juvenile *Betula* trees. *Betula pendula* was usually the most



Figures 23–28. *Stigmella naturnella*, biological details, all on *Betula pendula* **23.** Larva, detail of Fig. 18; **24.** Larva, detail of Fig. 21; **25.** Early part of leafmine, with upperside egg, Wekerom, data as Fig. 18; **26.** Early part of leafmine from underside, with underside egg; Italy, Naturno, 1935, coll. Wörz; **27.** Final part of mine with exit slit on leaf upperside; Soest, Korte Duinen, 23 Aug. 2021, EvN no 2021120; **28.** Cocoon, Wekerom, data as Fig. 18. Scale bars: 1 mm.

abundant birch, but *B. pubescens* was also present. In the Netherlands I often heard the cricket *Nemobius sylvestris* (Bosc, 1792) singing in these localities; this species has a comparable habitat preference. Table 2 shows the accompanying leafminer species in 36 localities with sufficient data, most



Figures 29–34. *Stigmella naturnella*, vacated leafmines, dried samples **29**. Italy, Naturno, Jun. 1935, *Betula pendula*, coll. Wörz; **30**. Italy, Naturno, Sep. 1934, *B. pendula*, coll. Wörz; **31**. Germany, Stuttgart, Willd-park, Sep. 1935, *B. pubescens*, coll. Wörz; **32**. Germany, Badenweiler, Sep. 2001, *B. pendula*, ZMA.INS. MIG.07497; **33**. Russia, Voronezh, Aug. 2017, unfinished mines, one larva barcoded, *B. pubescens*, RMNH. INS.46142; **34**. Netherlands, Wekerom, De Valouwe, Sep. 2020, *Betula pendula*, EvN no 2020062-1. Scale bar: 5 mm (all on same scale).



Figures 35, 36. Habitat of *Stigmella naturnella* **35 (top)**. The Netherlands, Goirle, Gorp en Rovert – Noord, 30 Sep. 2020, EventId: EvN no 2020083, many mines of *S. naturnella* on *Betula pendula* shrub; **36 (bottom)**. Germany (NRW), Hövelhof, NSG Moosheide, 19 Aug. 2021, in 2021 only one mine on *B. pendula* (photo Dieter Robrecht).

common were *Stigmella sakhalinella*, *S. continuella* and *S. betulicola*. In more southern localities in France, Italy and Russia, *S. naturnella* was the dominant species with no accompanying nepticulids, or just one (*S. sakhalinella* or *S. glutinosae*). Species such as *Lyonetia clerkella* (Linnaeus, 1758) or

Table 1. Observations of hibernating adults of *Stigmella naturnella* in the period 2017–2022 in Belgium and the Netherlands.

| Date | Locality | # | site | observer | Link |
|--------|--------------------|----|----------------------|-----------------------------------|---|
| 02-Jan | BE: Antwerpen | 1 | <i>Platanus</i> bark | G. Logghe | https://waarnemingen.be/observation/231441880/ |
| 12-Jan | BE: Antwerpen | 1♂ | <i>Platanus</i> bark | R. Hendrickx | https://waarnemingen.be/observation/232262916/ |
| 23-Jan | BE: Brugge | 1♂ | <i>Platanus</i> bark | S. Stevens | https://waarnemingen.be/observation/232781643/ |
| 05-Feb | BE: Antwerpen | 6 | <i>Platanus</i> bark | S. Baeten | https://waarnemingen.be/observation/185190974/ |
| 12-Feb | NL: Bergen op Zoom | 1 | <i>Platanus</i> bark | V. Vandenbulcke & G. Groeneweg | https://waarneming.nl/observation/167485152/ |
| 09-Mar | NL: Putte | 2 | <i>Quercus</i> trunk | G. Dekkers | https://waarneming.nl/observation/135289746/ |
| 29-Nov | BE: Booischoot | 1 | <i>Platanus</i> bark | W. Veraghtert | https://waarnemingen.be/observation/260148887/ |

Table 2. Species of Nepticulidae and Bucculatricidae found together with *Stigmella naturnella*, in 36 samples of *Betula* in the Netherlands (25), France (4), Italy (2) and Russia (5). The numbers are the samples where the species was present. For details see Suppl. material 3.

| Species | # samples |
|---|-----------|
| <i>Stigmella sakhalinella</i> Puplesis, 1984 | 27 |
| <i>Stigmella continuella</i> (Stainton, 1856) | 18 |
| <i>Stigmella betulicola</i> (Stainton, 1856) | 13 |
| <i>Stigmella luteella</i> (Stainton, 1857) | 10 |
| <i>Bucculatrix demaryella</i> (Duponchel, 1840) | 8 |
| <i>Stigmella glutinosae</i> (Stainton, 1858) | 3 |
| <i>Ectoedemia occultella</i> (Linnaeus, 1767) | 3 |
| <i>Stigmella lapponica</i> (Wocke, 1862) | 2 |
| <i>Stigmella confusella</i> (Wood & Walsingham, 1894) | 1 |
| <i>Ectoedemia minimella</i> (Zetterstedt, 1839) | 1 |

Agromyza alnibetulae Hendel, 1931 and some sawflies were often seen, but not consistently noted by me. Nepticulidae that prefer moister habitats and have a more northern distribution, e.g. *Ectoedemia minimella*, *S. lapponica* or *S. confusella* were rarely found together with *S. naturnella*, except on 10.x.1983 when J.J. Boomsma found *S. naturnella* in the Naturno area (Italy, Bolzano) together with an outbreak of hundreds of vacated mines of *S. lapponica*.

Distribution (Figs 37, 38). Central and West Europe: Austria (throughout), Belgium (new record: north-western part), Croatia (new record: Brodsko-Posavska), Czechia (Bohemia and Moravia), France (new record: Sarthe, Savoie), Germany (Baden-Württemberg, Bayern, Brandenburg, Nordrhein-Westfalen [new record], Saarland [new record], Sachsen), Hungary, Italy (Bolzano, Torino [new record], Trento), the Netherlands (new record: southern two thirds), Poland, Slovakia, Switzerland (Graubunden, Valais), Ukraine (new record: Chernikhiv Oblast), Russia, from European part to Primorye (van Nieuwerkerken and Sinev 2019, 2022) and Japan, Honshu (Hirano 2013). For further details, references and history see below.

DNA barcodes (Fig. 39). DNA barcode data are available for a total of 35 specimens across the distribution area between the Netherlands and Japan. All fall within Barcode Identification Number BOLD:AAV8372, with an average distance of 0.72% and a maximum distance of 1.63%. The nearest neighbour, with 5.9% distance, is the North American *Stigmella nigriverticella* (Chambers, 1875), in the *Stigmella saginella* group. However, barcodes of the closely related



Figure 37. Distribution records of *Stigmella naturnella* in Europe and western Siberia.

Eastern Palearctic species *S. mirabella* Puplesis, 1984 are still unknown. There is some geographical pattern visible in the NJ tree (Fig. 39, see also the haplotype network given on the BIN page, http://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAV8372). All Dutch, French and western German DNA barcodes belong to the same cluster, together with some Austrian records, whereas the remaining Austrian records and one from Germany, Sachsen, group with an Italian and several Russian records. DNA barcodes, especially those from easternmost Russia, Primorsky Krai, show most variation, but it should be noted that these DNA barcodes were on average a bit shorter than the others (total length 510–618 base pairs). However for the region covered by the missing base pairs, the other sequences show only variability in three sites.

Nomenclature and lectotype designation. Klimesch originally intended to name the species “*Nepticula argentifasciella*”, as can be seen on some of his original mines in the collection Würz, examined by me. However, he had crossed out that name on the herbarium sheets and replaced it by “*naturcella*”, presumably having realised that *N. argentifasciella* was a homonym of the North American *Nepticula argentifasciella* Braun, 1912 (now *Stigmella argentifasciella*). The name, however, entered the literature as a *nomen nudum*, as it was cited by Skala (1937).

Nepticula naturnella was described from an unspecified number of specimens, without selecting a holotype. I designate as lectotype the male that bears Klimesch’s dissection number 232 (labels see Fig. 4).

Remarks. *Stigmella naturnella* was placed in the *Stigmella lapponica* group by van Nieukerken (1986a) on the basis of the gnathos shape, which was later shown to be a homoplasious character (Doorenweerd et al. 2016). Puplesis (1984a, b) based his genus *Astigmella* Puplesis, 1984 on

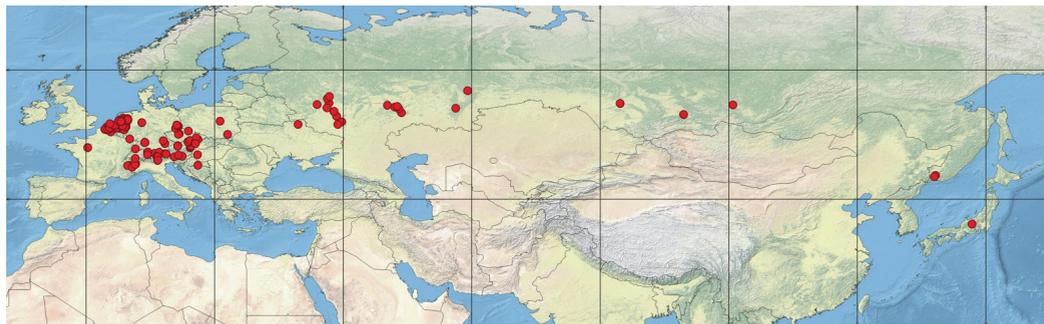


Figure 38. Global distribution records of *Stigmella naturnella*, from France to Japan (Honshu). Grid interval 20 degrees.

the synonym *A. dissona*. He separated that genus from *Stigmella* by the shorter Cu vein in the forewing and the characteristic genitalia. *Astigmella* was later synonymised with *Stigmella* (van Nieuwerkerken 1986a). Currently *S. naturnella* is considered to form a separate species group with the East Palearctic *S. mirabella* (Puplesis, 1984), falling within the large “Non-Core” *Stigmella* clade, partly on the basis of unpublished molecular data. The *naturnella* group is close to some Asiatic *Ficus* mining species, and relatively close to the *S. ulmivora* and *S. saginella* groups.

Material examined. 23 ♂ 11 ♀ 1 sex unknown, cocoons. *B.* = *Betula*. All in RMNH, unless otherwise mentioned.

AUSTRIA • 1 ♂; Nordtirol, Fliess; 25 Jun. 2008; 47.117°N, 10.632°E; alt. 1000 m; P. Skou & D. Nilsson leg.; Genitalia slide: JCK8488; ZMUC.

GERMANY • 1 ♂; Saarland, Fraulautern, TrÜbpl.; 49.325°N, 6.7854°E; 04 Sep. 2020; A. Werno leg.; Genitalia slide: EvN5341; RMNH.INS.25341; Werno, A., personal collection. • 1 ♀; Sachsen, Königswartha, Deichgebiet; 51.3193°N, 14.3527°E; 01 Jul. 2020; A. Werno leg.; Genitalia slide: EvN5342; RMNH.INS.25342; Werno, A., personal collection.

HUNGARY • 1 ♀; Veszprem, Uzsa, Nyires; 46.897°N, 17.333°E; 27 Aug. 1968; J. Szöcs leg.; *B. pubescens*; emerged 13 Sep. 1968; Genitalia slide: VU1874; HHNM.

JAPAN • 1 ♀; Honshu, Nagano ken, Yamagata-mura, Karasawa; 36.158°N, 137.846°E; 13 Jun. 2009; Nagao Hirano leg.; *B. pendula* subsp. *mandshurica*; emerged 25 Jun. 2009; EventId: Host-2733; Genitalia slide: EvN4747; RMNH.INS.24747.

NETHERLANDS – **Gelderland** • 1 ♂ 2 ♀, 2 cocoons (plus exuviae); Wekerom, De Valouwe, Immenkampweg; 52.08977°N, 5.71459°E; 16 Sep. 2020; EvN leg.; *B. pendula*; emerged 04 Oct. 2020; EventId: EvN no 2020062-1K; Genitalia slide: EvN5268; RMNH.INS.25268, RMNH.INS.17206–17208. • 2 ♂ 1 ♀, 4 cocoons (plus exuviae); Wekerom, Wekeromse Zand, near Hoge Valksedijk; 52.09188°N, 5.67616°E; 16 Sep. 2020; EvN leg.; *B. pendula*; emerged 03–04 Oct. 2020; EventId: EvN no 2020063-1K; RMNH.INS.17209–17212. • 1 ♂; Wolfheze, Wolfhezerbos, Oude Kloosterweg; 51.997°N, 5.79882°E; 07 Oct. 2020; EvN leg.; *B. pendula*; emerged 25 Oct. 2020; EventId: EvN no 2020100-1K; RMNH.INS.17215. – **Limburg** • 1 ♀; Leudal, Sint Elisabeth, Roggelse Beek valley; 51.25462°N, 5.93057°E; 21 Sep. 2020; EvN leg.; *B. pendula*; emerged 08 Oct. 2020; EventId: EvN no 2020071-1K; RMNH.INS.17213. – **Noord-Braabant** • 1 ♀; Goirle, Gorp en Rovert - Noord; 51.50795°N, 5.07473°E; 30 Sep. 2020; EvN leg.; *B. pendula*; emerged 20 Oct. 2020; EventId: EvN no 2020082-1K; RMNH.INS.17214. – **Utrecht** • 1 ♀; Soest, Hees, Wiexloterweg, Heitje; 52.15885°N, 5.27867°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; emerged 13 Sep. 2021; EventId: EvN no 2021123-K; RMNH.INS.17451. • 1 ♀, 2 cocoons (plus exuviae); Soest, Korte Duinen S. edge; 52.15107°N, 5.32399°E; 10 Sep. 2020; EvN leg.; *B. pendula*; emerged 01 Oct. 2020; EventId: EvN no 2020056-1K; RMNH.INS.17204–17205. • 1 ♀;

Soest, Korte Duinen S. edge; 52.15112°N, 5.32402°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; emerged 11 Sep. 2021; EventId: EvN no 2021121-1K; RMNH.INS.17448. • 1 ♀; same locality data; *B. pendula*; emerged 11 Sep. 2021; EventId: EvN no 2021122-1K; RMNH.INS.17449.

RUSSIA – **Primorsky Krai** • 1 ♂; 20 km E Ussurijsk, GTS [Gornotayezhnoye, Mountain taiga station]; 43.692°N, 132.164°E; 02 Aug. 1982; R. Puplesis leg.; Genitalia slide: JCK8123. – **Samara Oblast** • 1 ♂; Zhiguli, Bakhilova Polyana, S.; 53.4°N, 49.07°E; 04 May. 1992; S.A. Sachkov leg.; Genitalia slide: EvN3303; Zolotuhin, V., personal collection. – **Ulyanovsk Oblast** • 6 ♂; Ul'yanovsk N., Pobeda forest Park; 54.37°N, 48.42°E; 19 Apr. 1995; V. Isajevy leg.; under trunks of *B. pendula*; Genitalia slide: EvN3302; RMNH.INS.23302. • 7 ♂; same data; Zolotuhin, V., personal collection.

SWITZERLAND • 1 ♂; Graubunden, GR, Trimmis, Hag; 46.9124°N, 9.560464°E; alt. 560 m; 13 Jul. 2005; A. Kopp leg.; Genitalia slide: AK5.095; Kopp, A., personal collection.

UKRAINE • 1 ♂; Chernihiv Oblast, Korop; 51.58°N, 32.98°E; 24–31 Jul. 2009; K.E. Lundsten & Bo Wikström leg.; Genitalia slide: EvN5196; RMNH.INS.25196.

Larvae and leafmines (in collection). *B.* = *Betula*. All in RMNH, unless otherwise mentioned.

AUSTRIA • 3 mines; Oberösterreich, Linz-Bauernberg, Botanischer Garten; 48.297°N, 14.277°E; 13.vi.1976; J. Klimesch leg.; *Betula jacquemontii* [= *B. utilis* subsp. *jacquemontii*]; RMNH.INS.47930.

CROATIA • 10 mines; Brodsko-Posavska, Nova Gradiška, in town; 45.254°N, 17.387°E; 17 Oct. 1983; van Nieukerken & Boomsma leg.; *B. pendula*; EventId: VU no 83505-H; ZMA.INS.MIG.11575.

CZECHIA • 2 mines; Bohemia centr., Prague; 50.12208°N, 14.49573°E; 16 Sep. 2010; V. Lanta leg.; *B. pendula*; RMNH.INS.45394. • 3 mines (photo examined); Moravia, Kotojedy, Obora; 49.264°N, 17.402°E; 30 Aug. 1961; H. Zavřel leg.; *B. pendula*; BMNH(E)425547; NHMUK.

FRANCE – **Sarthe** • 1 larva (slide, DNA barcoded), 7 mines; Le Mans, Arche de la Nature, Bois de Changé; 47.9885°N, 0.2604°E; alt. 85 m; 07 Oct. 2017; EvN & S. Richter leg.; *B. pendula*; EventId: EvN no 2017146-2M/H; RMNH.INS.31042(P), RMNH.INS.44072, RMNH.INS.44073. • 1 larva (ethanol, tissue collection), 5 mines; same locality data; *B. pubescens*; EventId: EvN no 2017147-1H/M; RMNH.INS.31044, RMNH.INS.44075, RMNH.INS.44077. • 11 mines; Le Mans, Arche de la Nature, Bois de Changé; 47.9888°N, 0.25835°E; alt. 83 m; 07 Oct. 2017; EvN & S. Richter leg.; *B. pendula*; EventId: EvN no 2017150-3H; RMNH.INS.44085. – **Savoie** • 4 mines; Avrieux, along D215; 45.22262°N, 6.72683°E; alt. 1375 m; 24 Sep. 2018; EvN leg.; *B. pendula*; EventId: EvN no 2018158-4H; RMNH.INS.46341. • 11 mines; Saint-Martin-d'Arc, Les Fontaines; 45.20722°N, 6.46972°E; alt. 900 m; 21–24 Aug. 2017; M. Kozlov & V. Zverev leg.; *B. pendula*; ecological sample 50; RMNH.INS.46127.

GERMANY – **Baden-Württemberg** • 4 mines; Badenweiler, Sophienruhe; 47.7977°N, 7.6749°E; 28 Sep. 2001; AC & WN Ellis leg.; *B. pendula*; ZMA.INS.MIG.07497. • 4 mines; Stuttgart, Willdpark; 48.77°N, 9.1°E; 01 Sep. 1935; Wörsz leg.; *B. pubescens*; SMNS. • 2 mines; Zuffenhausen, Lemberg; 48.82°N, 9.14°E; [no date]; Wörsz leg.; *B. pendula*; SMNS. – **Nordrhein-Westfalen** • 4 mines; Wegberg, Forst Meinweg; 51.15875°N, 6.19451°E; 23 Sep. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021152-3H; RMNH.INS.48753. – **Sachsen** • 1 mine; Pirna, Copitz, Camping; 50.98168°N, 13.92177°E; alt. 120 m; 28 Jul. 2014; EvN leg.; *B. pendula*; EventId: EvN no 2014067-3H; RMNH.INS.47934.

ITALY – **Bolzano** • 4 mines (on 2 sheets); Südtirol, Naturno bei Meran, 46.656°N, 11.00200°E; "Ende 09.34, imagines e.l. A.10.34, Ende 06.35, imagines e.l. A.7.34 [recte 35]"; J. Klimesch leg.; *B. pendula* [*Betula verrucosa*]; SMNS (coll. Wörsz). • 22 mines; Naturno, 3 km SE, N. slope; 46.62964°N, 11.02486°E; alt. 1000 m; 10 Oct. 1983; J.J. Boomsma leg.; *B. pendula*; EventId: VU no. 83437; RMNH.INS.46938. • 2 mines; Naturno, N. slope; 46.62987°N, 11.01181°E; alt. 800 m; 10 Oct. 1983; J.J. Boomsma leg.; *B. pendula*; EventId: VU no. 83433; RMNH.INS.46937. • 3 larvae, mines; Naturno, near Canal; 46.63°N, 11.025°E; 24 Jun. 1985; J.J. Boomsma leg.; *B.*; RMNH.INS.12248. • 10 mines; Völlan, 2 km S Lana; 46.59373°N, 11.15197°E; alt. 670 m; 10 Jul. 2005; EvN leg.; *B. pendula*; EventId: EvN no 2005064-H; RMNH.INS.41069. – **Torino** • 4 larvae (ethanol, tissue collection, DNA barcoded), 55 mines; Issiglio, along SP61; 45.44865°N, 7.73024°E; alt. 750 m; 02 Oct. 2018; EvN leg.; *B. pendula*; EventId: EvN no 2018242-1M/H; slide: RMNH.INS.31263–31266, RMNH.



Figure 39. Neighbor Joining tree of partial COI sequences (DNA barcodes) of *Stigmella naturnella*, under the KP2 model. The labels provide data on Sample Id, Host (if known), Collection date, Country, Province.

INS.46501–46502. • 10 mines; Valle del Chisone, Perosa Argentina, rivervalley N of city; 44.96208°N, 7.18461°E; alt. 627 m; 30 Sep. 2018; EvN leg.; *B. pendula*; EventId: EvN no 2018230-H; RMNH.INS.46487. • 11 mines; Valperga, Sacro Monte di Belmonte; 45.36688°N, 7.63067°E; alt. 685 m; 01 Oct. 2018; EvN leg.; *B. pendula*; EventId: EvN no 2018238-1H; RMNH.INS.46497.

NETHERLANDS – **Gelderland** • 1 mine; Ede, Edese Heide, Koeweg; 52.05867°N, 5.69665°E; 16 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020064-2H; RMNH.INS.48336. • 6 mines; Ede, Planken Wambuis, Mosselse Pad; 52.07214°N, 5.7576°E; 16 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020058-2H; RMNH.INS.48308. • 3 mines; Ugchelen, Leesterheide, t Leesten; 52.16887°N, 5.90794°E; 07 Oct. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020097-1H; RMNH.INS.48432. • 1 mine; same locality data; *B. pubescens*; EventId: EvN no 2020098-1H; RMNH.INS.48437. • 3 larvae (ethanol, tissue collection, DNA barcoded, slide), 22 mines; Wekerom, De Valouwe, Immenkampweg; 52.08977°N, 5.71459°E; 16 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020062-1H/K/M; RMNH.INS.31449–31450, RMNH.INS. 48317–48319. • 10 mines (larvae reared); Wekerom, Wekeromse Zand, near Hoge Valksedijk; 52.09188°N, 5.67616°E; 16 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020063-1H/K; RMNH.INS. 48327–48328. • 4 mines (larva reared); Wolfheze, Wolfhezerbos, Oude Kloosterweg; 51.997°N, 5.79882°E; 07 Oct. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020100-1H/K; RMNH.INS.48445–48446. – **Limburg** • 1 mine; Epen, Onderste Bos; 50.7657°N, 5.89338°E; 20 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020067-2H; RMNH.INS.48342. • 1 mine; Epen, Geuldal, Cottessen; 50.76232°N, 5.93053°E; 20 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020068-2H; RMNH.INS.48346. • 1 larva (DNA barcoded, slide), 6 mines; Leudal, Nunhem - Sint Ursula, Zelsterbeek valley; 51.25424°N, 5.9512°E; 21 Sep. 2020; EvN leg.; *B. pubescens*; EventId: EvN no 2020073-1H/M; RMNH.INS.31460, RMNH.INS.48359–48360. • 5 mines; same locality data; *B. pendula*; EventId: EvN no 2020072-1H; RMNH.INS.48357. • 1 larva, 10 mines; Leudal, Sint Elisabeth, 2 km E Heythuysen; 51.24649°N, 5.92678°E; 21 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020070-1H/M; RMNH.INS.31458, RMNH.INS. 48350–48351. • 4 mines (larva reared); Leudal, Sint Elisabeth, Roggelse Beek valley; 51.25462°N, 5.93057°E; 21 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020071-1H/K; RMNH.INS. 48354–48355. – **Noord-Brabant** • 14 mines (2 larvae reared); Goirle, Gorp en Rovert - Noord; 51.50795°N, 5.07473°E; 30 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020082-1H/K; RMNH.INS. 48381–48382. • 2 larvae (ethanol, tissue collection, DNA barcoded, slide), 22 mines (3 larvae reared); Goirle, Gorp en Rovert - Noord; 51.50512°N, 5.08499°E; 30 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020083-1H/K/M; RMNH.INS.31465–31466, RMNH.INS. 48389–48391. • 22 mines; Goirle, Regte Heide; 51.52008°N, 5.03386°E; 30 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020081-1H; RMNH.INS.48377. • 9 mines; Leende, Leenderbos, Parking Strijperpad; 51.35075°N, 5.51628°E; 19 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020065-1H; RMNH.INS.48337. • 10 mines; Tilburg, Kaaistoep Oost; 51.54092°N, 5.02876°E; 30 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020079-1H; RMNH.INS.48370. • 4 mines; Tilburg, Wilhelminakanaal, East banks; 51.52175°N, 5.14482°E; 30 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020084-1H; RMNH.INS.48398. • 2 mines; same locality data; *B. pubescens*; EventId: EvN no 2020085-1H; RMNH.INS.48402. • 2 mines; Tilburg, De Sijsten, Heidebaan; 51.54111°N, 5.00389°E; 30 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020080-1H; RMNH.INS.48375. – **Overijssel** • 4 mines; Lemele, Lemelerberg; 52.46178°N, 6.39946°E; 3 Aug. 2022; EvN leg.; *B. pendula*; EventId: EvN no 2022005-1H; RMNH.INS.48842. – **Utrecht** • 13 mines (rearing failed); Leersum, Dartheide; 52.02°N, 5.4083°E; 25 Sep. 2020; Ben van As leg.; *B. pendula*; EventId: EvN no 2020087-K/H; RMNH.INS.48407. • 1 mine; Leusden, Den Treek, Hazenwater; 52.12511°N, 5.37929°E; 10 Sep. 2020; EvN leg.; *B. pubescens*; EventId: EvN no 2020052-5H; RMNH.INS.48278. • 5 mines; Leusden, Den Treek-Henschoten, t Waswater; 52.11789°N, 5.37407°E; 10 Sep. 2020; EvN leg.; *B. pubescens*; EventId: EvN no 2020054-1H; RMNH.INS.48281. • 19 mines; Soest, Korte Duinen S.; 52.15137°N, 5.3261°E; 04 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020046-1H; RMNH.INS.48250. • 2 mines; Soest, Korte Duinen S.; 52.15131°N, 5.32578°E; 04 Sep. 2020; EvN leg.; *B. pubescens*; EventId: EvN no 2020047-4H; RMNH.INS.48259. • 1 larva (ethanol, tissue collection), 49 mines; Soest, Korte Duinen S.; 52.15122°N, 5.32729°E; 10 Sep.

2020; EvN leg.; *B. pendula*; EventId: EvN no 2020055-1H/M; RMNH.INS.31444, RMNH.INS.48288, 48290. • 10 mines; Soest, Korte Duinen S.; 52.15131°N, 5.32675°E; 23 Aug. 2021; EvN leg.; *B. pendula*; EventId: EvN no 2021120-1H/K; RMNH.INS.48692–48693. • 3 larvae (ethanol, tissue collection, DNA barcoded, slide), 25 mines (larvae reared); Soest, Korte Duinen S. edge; 52.15107°N, 5.32399°E; 10 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020056-1H/K/M; RMNH.INS.31441–31443, RMNH.INS.48297–48299. • 13 mines; Soest, Korte Duinen S. edge; 52.15112°N, 5.32402°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021121-1H/K; RMNH.INS.48696–RMNH.INS.48697. • 9 mines; Soest, Korte Duinen S. edge; 52.15112°N, 5.32402°E; 23 Aug. 2021; EvN leg.; *B. pendula*; EventId: EvN no 2021122-1H/K; RMNH.INS.48699–48700. • 1 larva (DNA barcoded, slide), 8 mines; Soest, Lange Duinen E.; 52.15116°N, 5.30074°E; 04 Sep. 2020; EvN leg.; *B. pendula*; EventId: EvN no 2020049-1H/M; RMNH.INS.31436, RMNH.INS.48264–48265. • 1 larva, 1 mine; Soest, Lange Duinen S.; 52.14687°N, 5.28791°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021130-M/H; RMNH.INS.31659, RMNH.INS.48713. • 1 mine; Soest, Op Hees, W border; 52.15582°N, 5.25182°E; 23 Aug. 2021; EvN leg.; *B. pendula*; EventId: EvN no 2021125-H; RMNH.INS.48705. • 3 mines; Soest, Op Hees, W border; 52.15582°N, 5.25182°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021126-1H; RMNH.INS.48706. • 6 mines; Soest, Op Hees/ Willem Arntzbos; 52.15152°N, 5.25401°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021127-H/K; RMNH.INS.48708–RMNH.INS.48709. • 2 mines; Soest, Hees, Wieksloterweg, Heitje; 52.15885°N, 5.27867°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021123-K; RMNH.INS.48704. • 5 mines; Soest, Soesterberg, nr Wildwissel, along railway; 52.14528°N, 5.26222°E; 23 Aug. 2021; EvN leg.; *B. pubescens*; EventId: EvN no 2021128-H/K; RMNH.INS.48710–48711.

POLAND • 5 mines; Mazowieckie, Walendów; 52.08816°N, 20.8445°E; 16 Sep. 2010; V. Lanta leg.; *B. pendula*; RMNH.INS.45444.

RUSSIA– **Irkutsk Oblast** • 1 larva (DNA barcoded, slide), 2 mines; Tulun; 54.60111°N, 100.64°E; alt. 510 m; 22 Aug. 2009; V. Chepinoga leg.; *B. pendula subsp. mandshurica*; EventId: Kozlov-3-leaf1; RMNH.INS.29880, RMNH.INS.46693. – **Kaluga Oblast** • 1 mine; Kondrovo Distr., Gorbienki; 54.6589°N, 35.9385°E; 13 Aug. 2013; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45634. – **Krasnoyarsk Krai** • 1 larva (DNA barcoded, slide); village Tanzybei, foothill of Sayan Mts; 53.1199°N, 92.9672°E; 17 Jun. 2017; N. Kirichenko leg.; *B. pendula*; EventId: NK-13-17; RMNH.INS.31140. – **Lipetsk Oblast** • 1 mine; Krasnoe Distr., Jablonovo; 52.8318°N, 38.9817°E; 11 Aug. 2014; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.47960. • 2 mines; Krasnoe Distr., Leski; 52.8729°N, 38.97°E; 15 Jul. 2013; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45783. • 1 larva (DNA barcoded, slide), 61 mines; Usman Distr., Usman; 51.983°N, 39.783°E; alt. 165 m; 28 Jul. 2017; V. Zverev leg.; *B. pendula*; RMNH.INS.31160, RMNH.INS.44331, RMNH.INS.44341, RMNH.INS.45010. • 46 mines; same locality data; *B. pubescens*; RMNH.INS.44337–44338. – **Moscow Oblast** • 1 mine; Serpukhov, Svinenki; 54.9°N, 37.8°E; alt. 130 m; 16 Sep. 2009; M. Brynskikh leg.; *B. pendula*; RMNH.INS.46670. – **Novosibirsk Oblast** • 1 larva (DNA barcoded, slide); Novosibirsk, Central Siberian botanical garden; 54.82°N, 83.10389°E; alt. 155 m; 10 Jul. 2012; N. Kirichenko leg.; *B. pendula*; EventId: CD13121; RMNH.INS.30247. • 7 mines; same locality data; 14 Sep. 2013; N. Kirichenko leg.; *B. pendula*; EventId: NK# 68_12; RMNH.INS.40809, 40810. – **Sverdlovsk Oblast** • 1 mine; Revda; 56.8075°N, 59.3625°E; alt. 375 m; 15 Aug. 2009; E. Belskaya leg.; *B. pubescens*; RMNH.INS.46719. – **Tula Oblast** • 2 mines; Kurkino Distr., Danilovka; 53.5926°N, 38.5499°E; 26 Jul. 2006; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45931. • 1 mine; Kurkino Distr., Vodyanoe Pole; 53.6176°N, 38.5766°E; 17 Jul. 2009; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45973. • 2 mines; Leninski Distr., Inshinsky, 10 km W. Tula; 54.1436°N, 37.4738°E; 26 Sep. 2009; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45976. • 1 mine; Shchyokino Distr., Yasnaya Polyana, 14 km S Tula; 54.0893°N, 57.5101°E; 01 Aug. 2009; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.46010. – **Ulyanovsk Oblast** • 6 mines; Surskoe Distr., 10 km WNW vill. Lava; 54.55°N, 46.883°E; 07 Jul. 2019; V. Zolotuhin leg.; *B. pubescens*; EventId: VZ19_13-Betula pubescens; RMNH.INS.46845. • 1 mine; Ulyanovsk city; 54.3°N, 48.38°E; 09 Sep. 2002; students Uljanovsk State Pedagog. Univ. leg.; *B. pendula*; RMNH.INS.27861. • 1 larva (DNA barcoded, slide), 82 mines; Ulyanovsk city S., Vinnovka

forest-park; 54.27°N, 48.03°E; Jul.– Aug. 2002; A. Mistchenko leg.; *B. pendula*; slide: EvN3566; RMNH.INS.23566, RMNH.INS.27857–27860. • 4 mines; Ulyanovsk Oblast, Ulyanovsk city S., Vinnovka forest-park; 54.27°N, 48.03°E; 21 Sep. 2002; A. Mistchenko leg.; *B. pendula*; RMNH.INS.27862, RMNH.INS.27866 [the latter was misidentified as *S. betulicola* by van Nieukerken et al. 2004]. • 1 mine; Ulyanovsk city S., Vinnovka forest-park; 54.27°N, 48.03°E; 30 Sep. 2002; A. Mistchenko leg.; *B. pendula*; RMNH.INS.27863. – **Voronezh Oblast** • 1 larva (DNA barcoded, slide), 3 mines; Voronezh; 51.583°N, 39.167°E; alt. 150 m; 28 Aug. 2017; V. Zverev leg.; *B. pubescens*; RMNH.INS.31325, RMNH.INS.46142.

SLOVAKIA • 2 mines; Západoslovenský Kraj, Sekule, 6 km SW Kúty; 48.614°N, 17.009°E; 04 Oct. 1992; EvN leg.; *B. pendula*; EventId: EvN no 92075; RMNH.INS.48289.

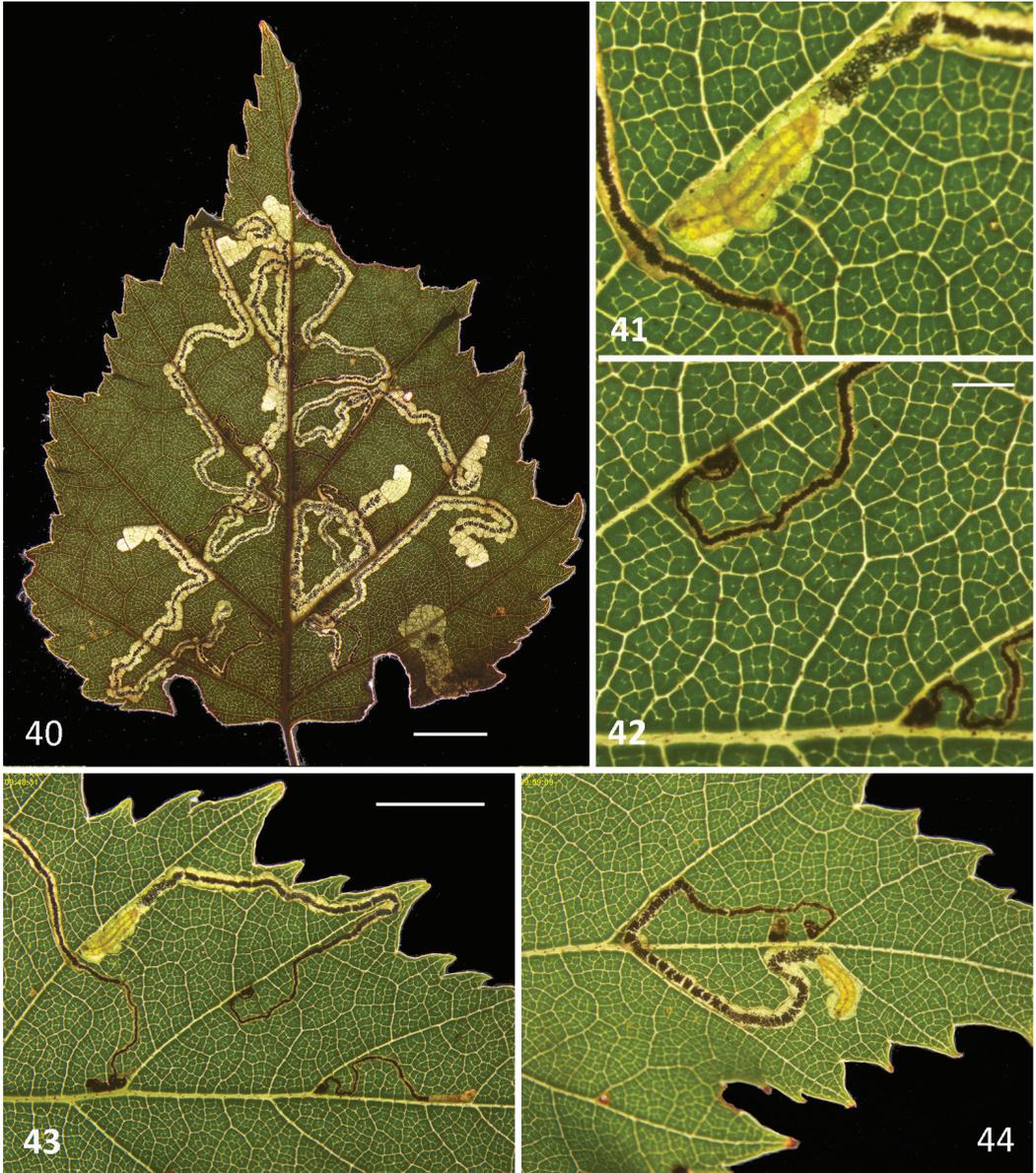
Leafmine diagnostics

Although most leafmines occurring on *Betula* in Europe can be identified from the leafmine pattern and larval characters with several sources (Hering 1957; Pitkin et al. 2019; Edmunds 2022; Ellis 2022), identification of linear or corridor mines is still a challenge, especially now, since *S. naturnella* appears to have become widespread, while the two *Stigmella* species normally found on *Alnus* have been recorded on *Betula* several times in more southern parts of Europe. To assist identification, diagnostic notes are provided for all *Stigmella* species occurring on *Betula* in Europe followed by an identification key.

Identification is easiest for either completed, vacated mines, in fresh condition (or dried when fresh), or mines with active larvae in their final instar. Old and withered mines should preferably be left on the tree, only with experience can they sometimes be distinguished. Moreover, mines with dead or parasitised larvae may be more difficult or even impossible to identify, as are mines containing young larvae. It is important to check whether the position of the egg is on the leaf upper- or underside as it is for the position of the exit slit where the larva has left the mine. These characters can only be seen effectively with magnification, at least a loupe in the field is necessary or a stereo microscope in the laboratory. For photographic recording detailed images are needed, and especially photos with back lighting, which shows frass and larva better.

Larvae of Nepticulidae usually are situated in their mines with the ventral side at the leaf upper-side, but all species belonging to “Core *Stigmella*” (Doorenweerd et al. 2016: 279) have the dorsum upwards. This character, often overlooked, is helpful in separating the species belonging to core *Stigmella* (here *S. continuella*, *S. lapponica*, *S. confusella* and *S. tristis*) from the rest. The larval head capsule, especially in the final instar, is longer at the dorsal side than at the ventral side (high magnification needed; see e.g. Gustafsson and van Nieukerken 1990), dorsally the paired brains may be visible (e.g. Fig. 69), no other ganglia, whereas in species with venter upwards often the ventral nerve chord is visible (e.g. Fig. 41), but this may be obsolete, and in *S. naturnella* only the suboesophageal ganglion is visible (Figs 23, 24).

Stigmella naturnella (Klimesch, 1936) (Figs 18–27, 29–34). Egg: on leaf under- or upperside, usually away from major veins, rarely against a vein, more often in the area near the margin. Exit: leaf upperside. Larva: venter upwards, pale whitish, with distinct brown circular suboesophageal ganglion, but no other ventral ganglia visible. Early mine: starts directly away from egg, sometimes with single bend around egg; early mine often appearing green as larva eats only sponge parenchyma. Later mine: rather variable, rarely very straight, but sometimes with straight parts; frass often rather narrow, but can be much wider and forming clumps.



Figures 40–44. *Stigmella betulicola*, leafmines **40**. 7 vacated mines, France, Le Mans, 7 Oct. 2017, *Betula pubescens*, 7 adults emerged Mar. 2018, EvN no. 2017150-K; **41–44**. Occupied mines, The Netherlands, Goirle, 30 Sep. 2020, *B. pendula*, EvN no. 2020083-2; **41**. Detail of larva; **42**. Details of coiled mine start. Scale bars: 1 mm (to **41** and **42**); 5 mm (**43** and **44** on same scale).

Occurrence: usually on mature trees, occasionally on juvenile trees, rarely more than one or two mines per leaf. Prefers dryer habitats in open forests and *Betula pendula*, but can be found in many other habitats as well. Larvae of second generation occur from August, often in low numbers.

Note: mines with upperside egg and/or green early part are easy to recognise, vacated mines without these characters may be difficult to separate especially from those of *S. confusella*, but mine of *S. naturnella* is usually narrower (final larval chamber 0.9–1.4 mm wide) and shorter (length 22–53 mm, small overlap with *S. confusella*) and frass appears more broken. Some isolated mines may be unidentifiable, so studying a series of mines is best for a certain identification.

Stigmella betulicola (Stainton, 1856) (Figs 40–44). Egg: on leaf underside, against a major vein. Exit: leaf underside. Larva: venter upwards, deeply yellow, with chain of ventral ganglia clearly visible. Early mine: starts contorted with close coils in a very confined area (Fig. 42), never green. Later mine: rather variable, frass width variable, between 1/3 and 3/4 mine width, sometimes filling early mine completely, mine rather long or shorter in thicker leaves.

Occurrence: most frequent on low growth, seedlings, juvenile trees, often gregarious with many mines on one leaf.

Note: mines of *S. luteella* are often mistaken for *S. betulicola* when the green part of the mine is absent. The larger contorted part and small differences in frass deposition may help if no larva is present, but some mines remain unidentifiable. Mines of *S. glutinosae* may be very similar to *S. betulicola*, but do not have coils at the start of the mine.

Stigmella luteella (Stainton, 1857) (Figs 45–52). Egg: on leaf underside (rarely on upperside), against a major vein. Exit: leaf underside. Larva: venter upwards, pale yellow, ventral ganglia hardly or not visible. Early mine: starts with a distinctly contorted part (Fig. 52), area larger than in *betulicola*; frequently the early part of the mine is in the spongy parenchyma and therefore appearing green from above. Later mine: rather variable, frass either in a very thin line or the line is broader (up to 3/4 the width of the mine) and the frass line is broken, mine rather long, shorter in thicker leaves; sides of mine often scalloped.

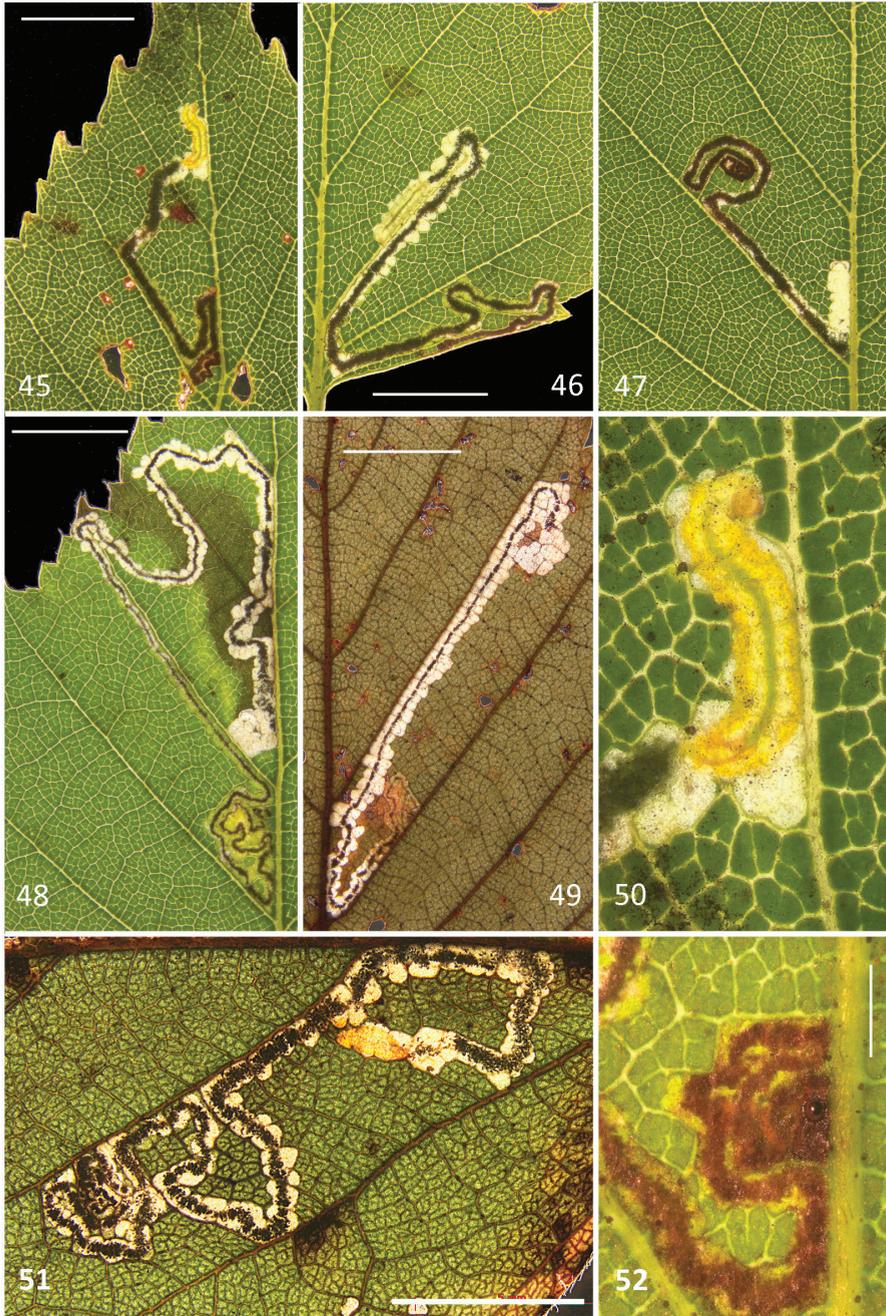
Occurrence: usually on mature trees, occurring later in the season than most *Stigmella* species, frequently found still feeding in green islands in fallen leaves in October–November. Rarely gregarious.

Note: mines of *S. luteella* are often mistaken for *S. betulicola* when the green part of the mine is absent, especially in northern Europe. The larger contorted part and small differences in frass deposition may help identification if no larva is present, but some mines will remain unidentifiable.

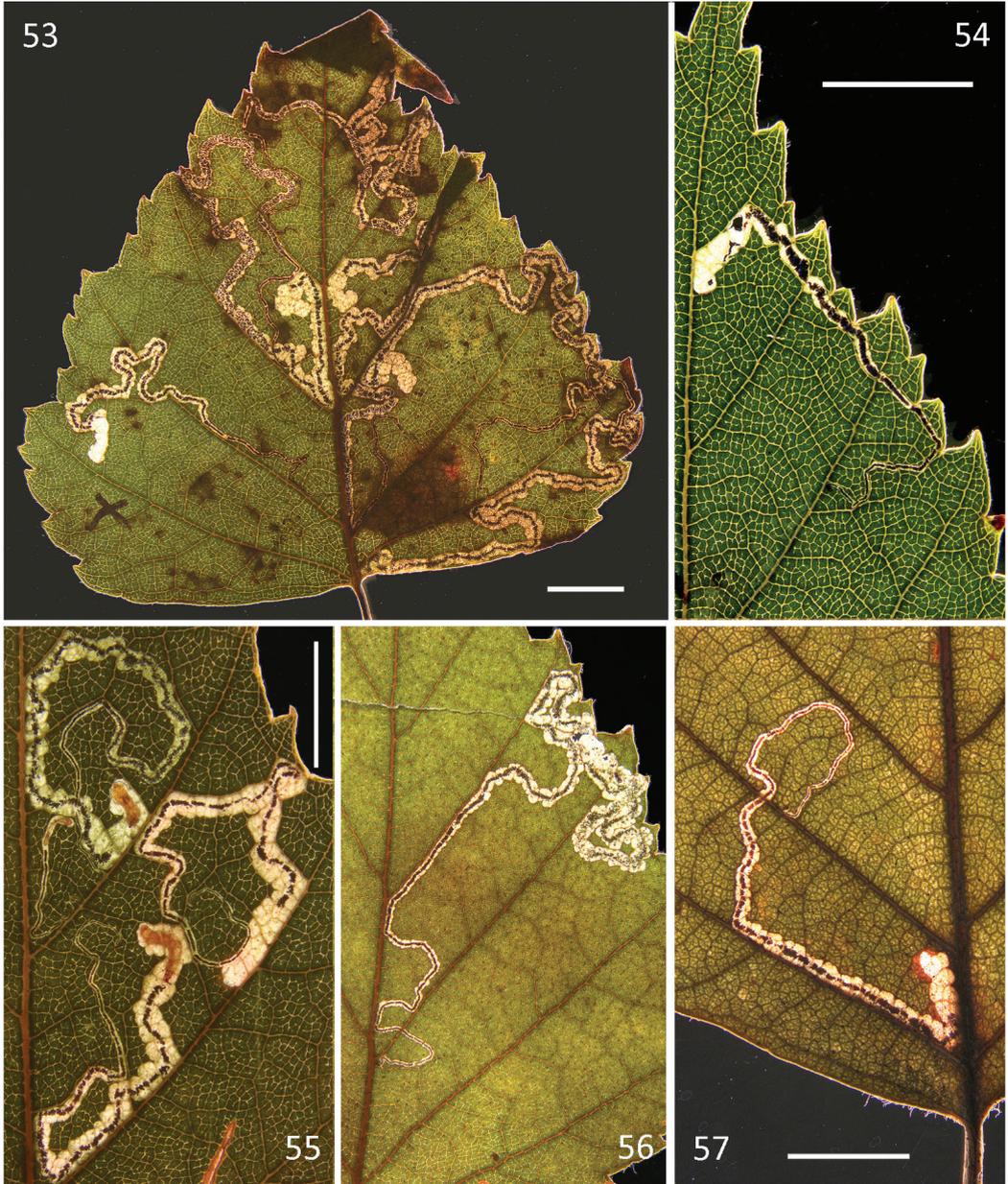
Stigmella glutinosae (Stainton, 1858) (Figs 53–57). Egg: usually on leaf underside, against a major vein, in some cases on upperside (about 20% of 51 mines examined, but all upperside eggs were in one sample). Exit: usually leaf underside, in some cases on upperside. Larva: venter upwards, pale yellow, ventral ganglia hardly or not visible. Early mine: runs straight away from egg. Later mine: rather variable, frass either in a very thin line, or a broader line with frass dispersed, and width up to 2/3 of the mine, mine rather short, total length 23.2–41.4 mm (34.5 ± 6.4 , 6), final larval chamber 1.0–1.4 mm wide.

Occurrence: when on *Betula* often on seedlings and juvenile trees, but also on mature trees. Occasionally gregarious (Fig. 53). More frequent on *Alnus*, but in southern parts of Russia and in France several times recorded on *Betula*, proven by rearing and DNA barcodes. Some leafmines from Germany (Fig. 57) and the Netherlands probably also belong to *glutinosae*, but independent confirmation is lacking.

Note: mines of *S. glutinosae* may resemble those of *naturnella*, but the egg position on a vein and larval exit on underside usually separate the two, as does the green early mine in many *naturnella*. Some mines remain unidentifiable. Some of the mines from Ulyanovsk figured as *S. naturnella* by van Nieukerken et al. (2004: fig. 10) fit *S. glutinosae* better, suggesting that the mine sample was a



Figures 45–52. *Stigmella luteella*, leafmines **45–47**. 2 Occupied mines and one vacated mine, The Netherlands, Ede, 16 Sep. 2020, *Betula pubescens*, EvN no 2020058-1K; **48**. Vacated mine, The Netherlands, Wekerom, 16 Sep. 2020, *B. pendula*, EvN no 2020062-2; **49**. Vacated mine, Germany, Hamburg, 28 Sep. 1932, *B. utilis*, published as *Nepticula naturnella* by Buhr (1940b: 229); **50**. detail of larva in Fig. 45; **51**. mine with dead larva, barcoded, Russia, Karelia Rep., Nadvoitsy, 26 Aug. 2012, *B. pubescens*, RMNH.INS.29973; **52**. data as Fig. 48, early part of mine and egg, leaf underside. Scale bars: 5 mm (**45–49**, **51**); 1 mm (**52**); no scale for **50**.



Figures 53–57. *Stigmella glutinosae*, leafmines on *Betula*, confirmed by DNA barcoding, except 57. **53.** France, Le Mans, 7 Oct. 2017, *B. pubescens*, 6 mines, 4♂ 1♀ emerged, left mine, near cross, incomplete, larva taken for barcoding, EvN no 2017150-3K/M; **54.** France, Villeneuve-sur-Cher, 30 Jul. 2009, larva taken from mine, *B. pendula*, EvN no 2009039-M; **55.** Russia, Voronezh, 28.viii.2017, *B. pendula*, dried mines with larvae, larva taken from right mine, RMNH.INS.31158; **56.** Russia, Lipetsk Obl., Osman, 28 Aug. 2017, *B. pendula*, RMNH.INS.31159; **57.** Vacated mine, Germany, Berlin, Botanischer Garten, 17 Aug. 1936, *B. papyrifera* (as *B. excelsa*), published as *Nepticula naturnella* by Buhr (1940b: 229). Scale bars: 5 mm (55–57 on same scale).

mixture of the two. It is highly likely that the leafmine reported as *S. confusella* from Ukraine, the Crimea by Navickaitė et al. (2014) also belongs to *S. glutinosae*.

Stigmella alnetella (Stainton, 1858) (Figs 58, 59). Only two mines on *Betula* are available that are proven by DNA barcodes, to be from *S. alnetella*. Egg: on leaf underside, against a major vein. Exit: leaf underside. Larva: venter upwards, pale yellow, ventral ganglia hardly or not visible. Early mine: slightly coiled. Later mine: with frass coiled, in a rather broad line in both the mines examined. Probably more variable, as in the leafmines on *Alnus*.

Occurrence: both mines on *Betula* were on mature trees. Common on *Alnus*, the two larvae bar-coded from *Betula* were from Italy; reared once in Sweden (Johansson and Nielsen 1990).

Note: Considering the difficulty of separating mines of *S. alnetella* from those of *glutinosae* on *Alnus*, we expect to experience the same difficulty on *Betula*. The two mines examined resemble somewhat those of *S. sakhalinella*, but distinguishing features are: egg on vein, early mine with narrow linear frass and exit on leaf underside. These two Italian mines were found amongst a large number of vacated mines resembling those of *S. glutinosae*, that could belong to either species, but are tentatively identified as *S. glutinosae*.

Stigmella sakhalinella Puplesis, 1984 (in older European literature under the name *S. distinguenda* auct.) (Figs 60–63). Egg: usually on leaf underside, but in several cases on leaf upperside, most frequently near leaf margin, but other positions over the whole leaf have been observed. Exit: leaf upperside. Larva: venter upwards, dark yellow, ventral ganglia clearly visible. Early mine: starts rather contorted, filled with dark frass. Later mine: usually somewhat contorted, or following leaf margin, frass black, coiled, usually almost filling width of mine, leaving narrow borders; occasionally the frass line is narrower, but still coiled (Fig. 61).

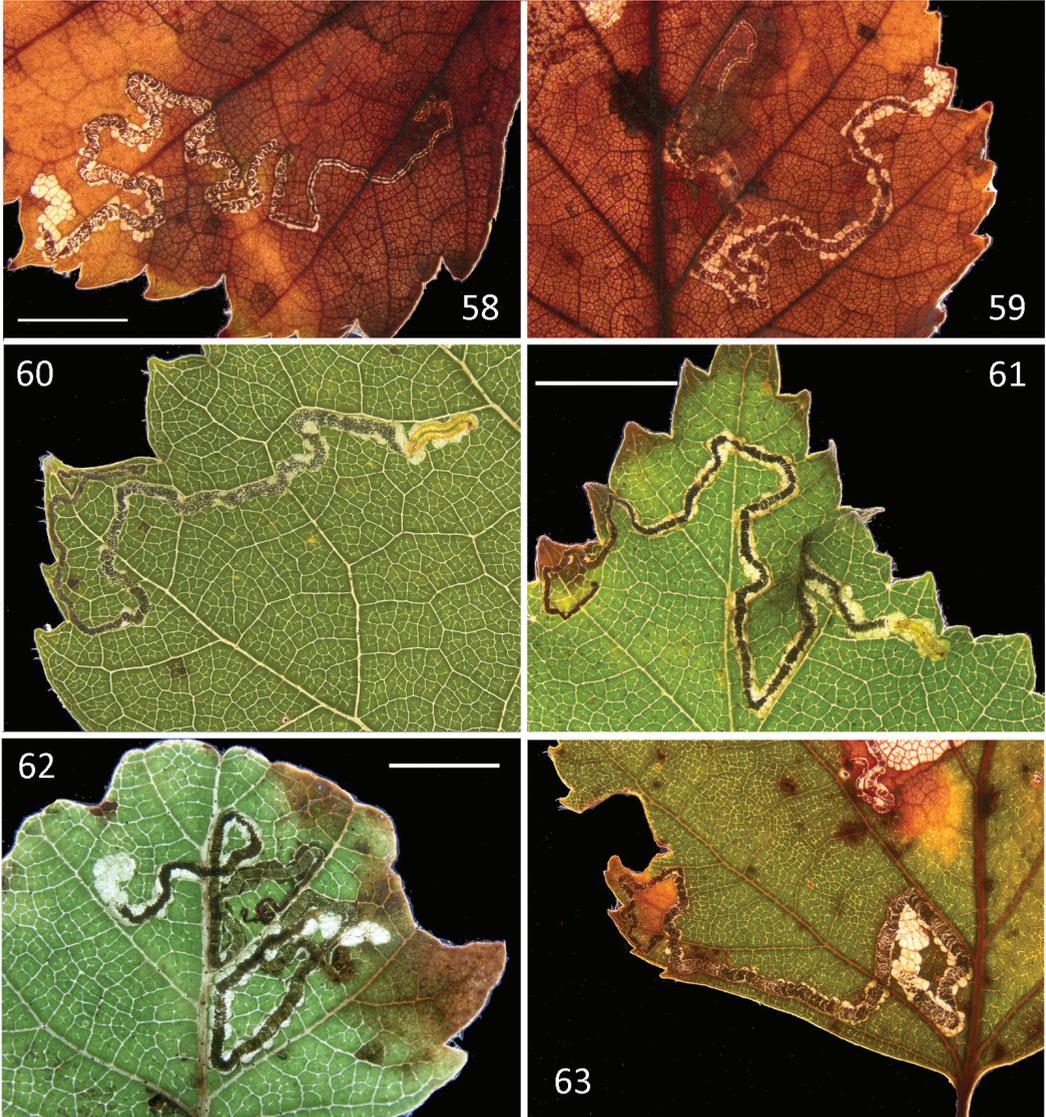
Occurrence: usually on mature trees, but also on juvenile trees, occasionally gregarious.

Note: mine unmistakable, although *S. alnetella* when rarely on *Betula* has some resemblance in the second part of the mine. Young mines sometimes confused with *S. betulicola*.

Stigmella confusella (Wood & Walsingham, 1894) (Figs 64–69). Egg: on leaf underside, usually close to a vein. Exit: leaf upperside. Larva: dorsum upwards, greenish whitish, the bilobed brain clearly visible from upper side, differing from the (ventral) circular suboesophageal ganglion in *S. naturnella*, no other ganglia visible from above. Mine: often with long straight stretches, following veins partly, occasionally partly more contorted (as in Figs 64, 65), especially at start; overall the mine has an angular appearance; frass throughout in a narrow central line, often continuous, only partly broken in later part of mine.

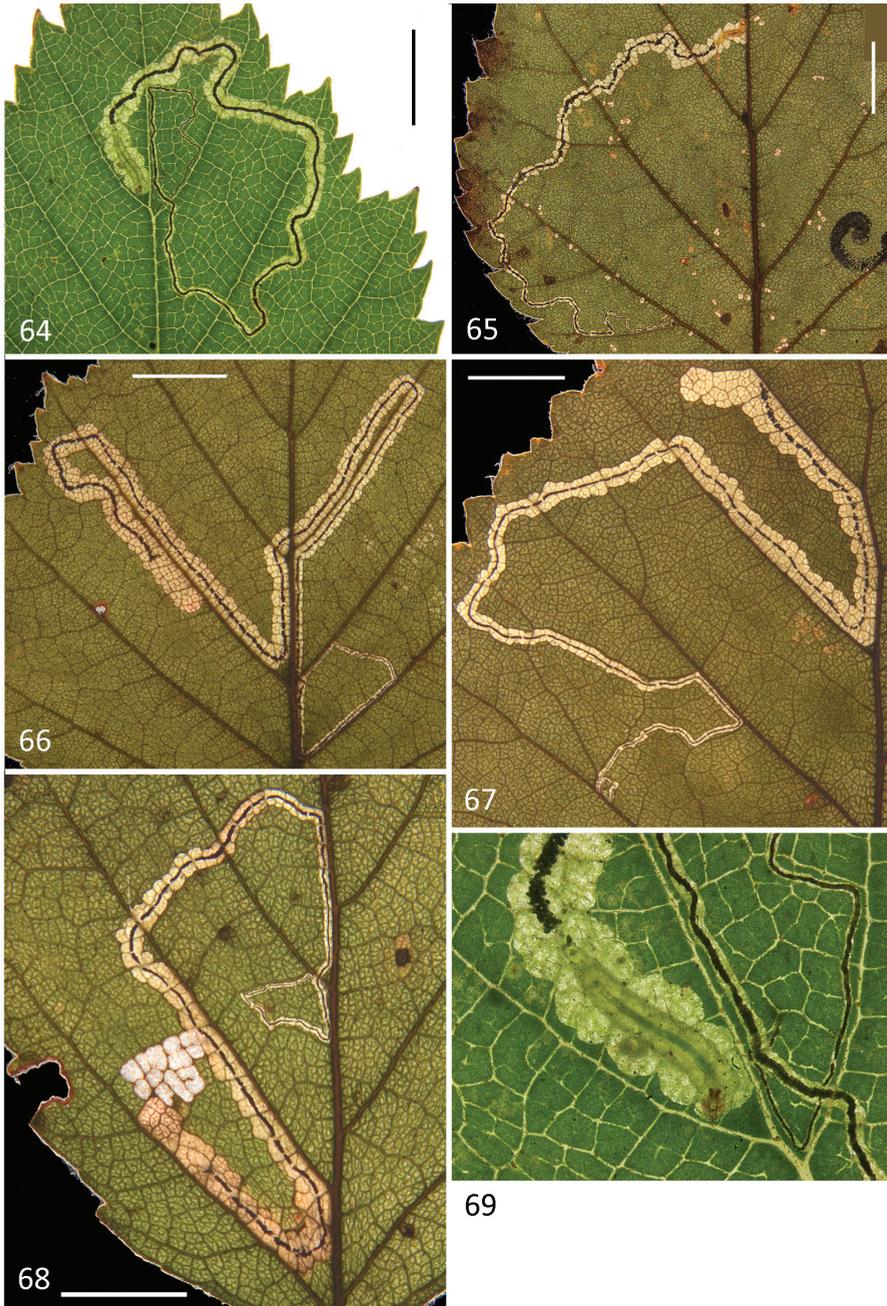
Occurrence: usually on mature trees, rarely with more than two mines per leaf. Prefers *B. pubescens* in moist habitats. Univoltine, usually occurring later than *S. lapponica*.

Note: mine most similar to *S. lapponica*, but the early frass of that species always separates the two. Less typical mines may be confused with *S. naturnella*, but the species are not often found together and *S. confusella* has a considerably longer mine: 41–91 mm ($68.9 \text{ mm} \pm 15.6$, $n=12$), with only a small overlap with *S. naturnella*. Also, the final larval chamber is wider: 1.3–2.0 mm (1.6 ± 0.2 , $n=12$), and usually straight, whereas that of *S. naturnella* is often buckled. However, some unfinished mines (without distinct larval remains) in areas where both species occur may be inseparable. Vacated mines of *Lyonetia clerkella* are sometimes mistaken for *S. confusella*, especially where photographs are used for determination, but the extreme length of the mine, its sinuous condition and the absence of a visible egg are diagnostic.



Figures 58–63. **58, 59.** *Stigmella alnetella*, vacated leafmines on *Betula*, confirmed by DNA barcoding, Italy, Roma, Manziano, 17 Sep. 2005, *B. pendula*, EvN no 2005115-3M **60–63.** *Stigmella sakhalinella*, leafmines. **60.** Occupied mine, Netherlands, Soest, 23 Aug. 2021, *B. pendula*, EvN no 2021122-2K; **61.** Occupied mine, Netherlands, Wassenaar, 10 Oct. 2018, *B. pubescens*, EvN no 2018263-2; **62.** Vacated mine, Netherlands, Wassenaar, Meijndel, 11 Oct. 2007, *B. pubescens*, EvN no 2007122-1M; **63.** Vacated mine, Finland, Helsinki, Ojakkala, 1 Sep. 2017, *B. pendula*, RMNH.INS.44352. Scale bars: 5 mm (**58 & 59**, **60 & 61** and **62 & 63** each on same scale).

Stigmella lapponica (Wocke, 1862) (Figs 70–72). Egg: on leaf underside, usually close to a vein. Exit: leaf upperside. Larva: dorsum upwards, greenish whitish, the bilobed brain clearly visible from upper side, differing from the circular suboesophageal ganglion in *S. naturnella*, no other ganglia visible from above. Early mine (made by first 3 instars) filled entirely with green or brown



Figures 64–69. *Stigmella confusella*, leafmines **64.** Occupied mine, Netherlands, Leidschendam, 11 Aug. 2013, *Betula pubescens*, EvN no 2013098-3; **65.** Occupied mine, dried with larva (barcoded), Russia, St. Petersburg, 1 Jul. 2016, *Betula* sp., RMNH.INS.31461; **66.** Vacated mine, Netherlands, Goirle, 12 Sep. 1982, *B. pubescens*, VU no 82417; **67.** Vacated mine, Norway, Stryn, Kjenndalen, 28 Jul. 2000, *Betula* sp., EvN no 2000115-1H; **68.** Vacated mine, Finland, Harjavalta, 14 Sep. 2009, *B. pendula* **69.** Detail larva of Fig. 64. Scale bars: 5 mm.

frass, in last instar frass arranged in a narrow central line, often continuous, only partly broken in later part of mine. Mine: often with long straight stretches, partly following veins, occasionally rather more contorted, the mine has an angular appearance.

Occurrence: usually on mature trees, rarely with more than two mines per leaf. Prefers *B. pubescens* in moist habitats, occurs earlier than *S. confusella*, univoltine.

Note: unmistakable by the aberrant early frass. In Nordic mines (northern Fennoscandia, Russia) the frass in the early part differs still from the second part, but often less conspicuously than in more southern populations. Otherwise the same diagnostic characters as in *S. confusella* apply.

Stigmella continuella (Stainton, 1856) (Figs 73, 74). Egg: on leaf underside, almost always on midrib. Exit: leaf upperside. Larva: dorsum upwards, yellow, but in mine often appearing green, no ganglia visible from upper side. Early mine a narrow gallery, much contorted in a zigzag fashion, with windings usually lying against each other, in some cases the windings more separate; earliest part sometimes with narrow linear frass for about 1 mm, later filled with brown frass, early mine forming a distinct brown spot on the leaf, and the leaf tissue between the windings turning brown; later mine a long and rather broad gallery, may follow veins and sometimes angular, filled with green frass pellets, often coiled, arranged in zigzags, frass green when fresh and then almost inseparable from green tissue of leaf, later turning brown.

Occurrence: regularly on seedlings and juvenile trees, but also on mature trees, sometimes with more than two mines per leaf. No host preference, in various habitats, but common in heathland.

Note: unmistakable from the brown blot at the start and the green frass, which completely fills the mine.

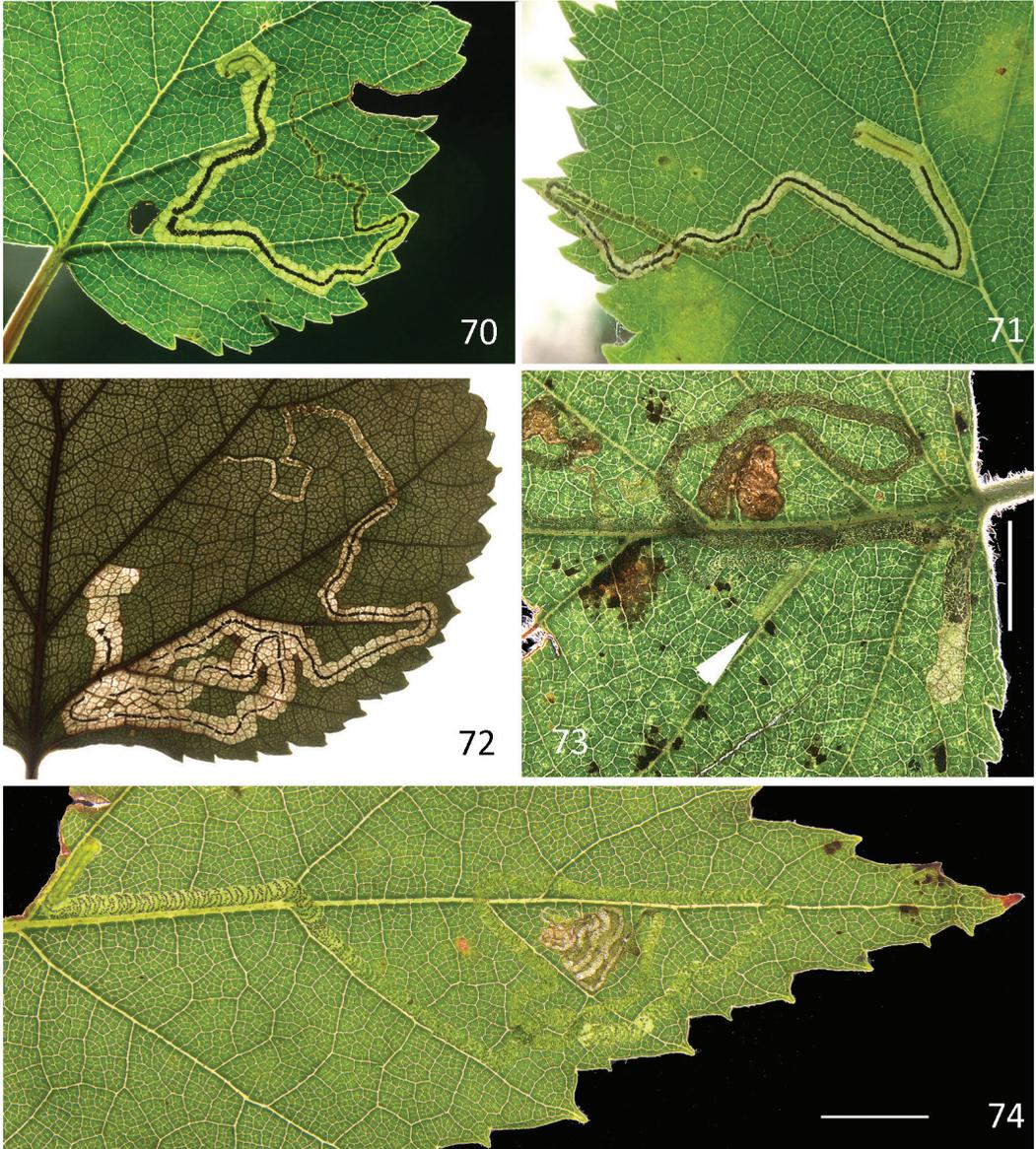
Other linear miners on *Betula*

Stigmella tristis (Wocke, 1862) is an arctic species, confined to *Betula nana* and unlikely to occur sympatrically with *S. naturnella*. It is the only other *Betula* mining *Stigmella* species with the egg on leaf upperside (Johansson and Nielsen 1990).

Mines of *Lyonetia clerkella* are often confused with *Stigmella* mines. The extremely long and narrow mines run through the leaves independent of the veins or leaf margins; frass deposited as a broken, central line of variable width. There is no eggshell on the leaf, but an oviposition scar, the larva is very long, with conspicuously constricted segments and distinct legs, visible from the leaf upperside as six black dots. Vacated mines have a very long final chamber without frass. Often on young leaves, seedlings, juvenile trees. Apart from *Betula* also common on Rosaceae trees.

Bucculatrix demaryella (Duponchel, 1840). Regularly confused with young mines of *Stigmella* species, usually *S. luteella*. Egg on leaf underside. Mine is usually short (up to 1 cm), but may be considerably longer in thinner leaves, always starting on the midrib or a larger vein, in a vein angle; usually with blackish frass in a broad line or filling the mine. Larva leaves the mine early, later feeding externally, causing windows on leaf underside. Larval chamber relatively long, three times as long as wide, often bent. The presence of small silken moulting cocoons of young larvae on the leaf are a sign of *Bucculatrix* mines.

Other linear mines are rather different and easier to identify with online keys (Ellis 2022), e.g. the dipteran *Agromyza alnibetulae*, the weevil *Anoplus plantaris* (Næzén, 1794). The early linear



Figures 70–74. *Stigmella lapponica*, leafmines **70**. Occupied mine, England, Copley, North Dean wood, 7 Jun. 2018, *B. pubescens*, photo Charlie Streets; **71**. Occupied mine, Netherlands, Noordhollands Duinreservaat, 15 Jun. 2018, *Betula* spec., photo Wouter Bol; **72**. Vacated mine, Portugal, NP Gerez, 7 Jul. 1980, *B. pubescens*. **73, 74.** *Stigmella continuella* **73**. Vacated and occupied mine (larva arrowed), Netherlands, Afferden, 24 Sep. 2008, *B. pubescens*, EvN no 2008107-1K; **74**. Occupied mine, Netherlands, Wekerom, 16 Sep. 2020, *B. pendula*, EvN no 2020062-4K. Scale bars: 5 mm; no scales available for Figs 70–72.

mines of *Eriocrania sparrmannella* (Bosc, 1791), *E. salopiella* (Stainton, 1854) and *Phylloporia bistrigella* (Haworth, 1828) are sometimes confused with *Stigmella* mines before the blotch part is formed.

Key to mines of *Stigmella* on *Betula* in Europe

- 1 Mine almost completely filled with dispersed frass in distinct coils (“zigzag”), sometimes leaving narrow white margins (Figs 58–63, 73, 74).....**2**
- Mine with frass in a central line of variable thickness, at least in second half of mine, may be broken, and filling up to two thirds of mine width, but never in coils**4**
- 2 Frass in fresh mines green, filling the width of the mine, rendering it almost invisible, later frass turning brown. Early mine narrow brown and much contorted, forming a brown blot (Figs 73, 74). Egg always underside, usually on or near midrib. Larva with dorsum upwards, no ganglia visible.....***S. continuella***
- Frass in fresh mines brown to black, usually leaving narrow white margins. Early mine usually blackish, with few or no coils. Egg position variable, usually underside, sometimes upperside. Larva with venter upwards.....**3**
- 3 Egg away from midrib, anywhere in leaf, often near margin. Exit slit on leaf upperside. Larva deep yellow with conspicuous chain of ganglia visible (Figs 60–63) ***S. sakhalinella***
- Egg against midrib or another major vein. Exit slit usually on leaf underside. Larva pale yellow, ganglia not or hardly visible. Rare on *Betula* (Figs 58, 59)..... ***S. alnetella***
- 4 Early mine completely filled with green or sometimes brown frass, later mine angular with narrow central line of frass, the change in frass deposition is abrupt, complete mine long (Figs 70–72). Exit slit on leaf upperside, egg on underside. Larva with dorsum upwards***S. lapponica***
- Early mine may be filled with frass, but without abrupt change between early and later parts of mine, or with narrow frass line. Mine length, exit slit and egg position variable.....**5**
- 5 Egg on leaf upperside**6**
- Egg on leaf underside**8**
- 6 Leafmine on *Betula nana* in northern Europe, mine rather short..... ***S. tristis***
- Leafmine on other species of *Betula*, mine rather long.....**7**
- 7 Egg anywhere on leaf, but usually away from major veins. Early mine often on underside, appearing green. Exit slit on leaf upperside. Larva pale whitish, with distinct circular suboesophageal ganglion, but no other ventral ganglia visible ***S. naturnella***
- Egg against midrib or another major vein. Early mine never green. Exit slit usually on leaf underside. Larva pale yellow, ganglia not or hardly visible, but prothorax with square brown plate. Occasionally on *Betula*, common in some places (Figs 53–57)..... ***S. glutinosae***
- 8 Early mine contorted in a small area. Egg against midrib or another major vein. Exit slit on leaf underside. Larva with venter upwards.....**9**
- Early mine running away from egg, not contorted, sometimes with a bend around it. Egg position variable. Exit slit variable. Larva with venter or dorsum upwards**10**
- 9 Early mine usually conspicuously contorted, and often appearing green. Sides of mine often scalloped, scallops usually free of frass. Larva pale yellow, without visible ganglia. Usually on trees, not on seedlings (Figs 45–52)..... ***S. luteella***
- Early mine briefly tightly contorted, never green. Sides of mine rather straight. Larva deep yellow with conspicuous chain of ganglia. Mines often gregarious on seedlings or saplings, rarely on trees (Figs 40–44)..... ***S. betulicola***
- 10 Egg usually away from major veins. Early part of mine may be green or not. Mine not very long (length 22–53 mm), not particularly angular, frass central, of variable thickness; width of final larval chamber 0.9–1.4 mm. Exit slit on upperside. Larva with venter up-

- wards, pale whitish, with distinct circular suboesophageal ganglion, but no other ventral ganglia visible.....*S. naturnella*
- Egg against midrib or another major vein. Early mine never green. Mine rather short (23–41 mm), somewhat angular; width of final larval chamber 1.0–1.4 mm. Exit slit on leaf underside, exceptionally on upperside. Larva with venter upwards, pale yellow, ganglia not or hardly visible, prothorax with square brown plate. Occasionally on *Betula*, common in some places (Figs 53–57).....*S. glutinosae*
 - Egg usually close to a vein. Early mine never green. Mine very long (41–91 mm), usually distinctly angular, with long parts following veins; width of final larval chamber 1.3–2.0 mm. Exit slit on leaf upperside. Larva with dorsum upwards, greenish whitish, the bilobed brain clearly visible from upper side (Figs 64–69).....*S. confusella*

History of distribution and spread of *Stigmella naturnella*

The original specimens were collected in 1934 and 1935 around the villages of Naturno and Stava in South Tyrol (Italy, prov. Bolzano), where the mines were reported as common, but with few larvae (Klimesch 1936). A few years later Joseph Klimesch collected the species in the Trento region (Klimesch 1948, 1951). Here we report that the species was still common in the Naturno area in 1983 and 1985 (collected by J.J. Boomsma), and in Bolzano province in 2005. Hugo Skala, who was in contact with Klimesch, soon reported more records of this species from Austria, Germany and Czechia (Skala 1937, 1939), but all were based on leafmines alone, without confirmation from reared specimens, which made Klimesch (1948) reluctant to accept these records. The oldest of these records were leafmines collected by Albert Wörz from the Stuttgart area, Württemberg, Germany, in 1935. Later Wörz (1958) repeated these records in detail. Wörz cited Klimesch’s opinion on these mines as “höchstwahrscheinlich” (“most likely”). Buhr (1940a, b) reported leafmines of this species, all identified by Skala, from various places in Germany, especially Berlin (botanical garden) and Mecklenburg. These old records have never been re-evaluated after the original publications. Hering (1957) did not cite them, but the Stuttgart records were cited in the German checklists, albeit with doubts in the second edition (Gaedike and Heinicke 1999; Gaedike et al. 2017). I have been able to study most of these leafmines; the majority of Buhr’s leafmines from Berlin are still available in his leafmine collection (Herbarium Hausknecht, Jena) and Wörz’s leafmines are available in his collection in Stuttgart. Buhr’s mines were re-identified by me as belonging to respectively *Stigmella* cf. *glutinosae* and *S. luteella*, but Wörz’s leafmines (six in total) from the Willdпарк in Stuttgart and Lemberg were indeed correctly identified as *S. naturnella*.

The old record from Czechia in 1937, from Mladé Buky (Jungbuch) (Skala 1939; Haase 1942), also identified by Skala, was given a question mark by Haase and the remark hier muß das Zuchtergebnis erst volle Gewißheit bringen [here must the rearing results provide certainty]. The identity of this record therefore remains uncertain, but is not impossible that it is of *S. naturnella*.

The only other old record that has been confirmed was from Austria, Linz, Bauernberg (Botanical Garden), from mines collected by Skala in 1936 (Skala 1937). Two of his leafmines are housed in the collection of Rebel in the Vienna Museum, of which detailed published photographs show enough detail to consider them as correctly identified (Lödl and Gaal-Haszler 2010) (although it is unfortunate that no photos with back lighting were made); Klimesch (1990) found the species here again in the 1970s, and we have some duplicate mines in RMNH. Karl Burmann also found the species in Innsbruck in 1940 (Hartig 1964), and again in 1950 and 1960. For these we have no confirmations, but they are probably correct.

After the early findings, very little information became available for *S. naturnella* for many years. An unpublished record shows that it was present in Czechia, Moravia in 1961, mines collected by H. Zavřel, present in the Hering Herbarium (NHMUK). The next published record was from Hungary in 1968 (Szöcs 1971, 1973), so far the only Hungarian record. It took 20 years for the next published records to be made from Czechia and Slovakia (Laštůvka and Laštůvka 1991; Laštůvka et al. 1992), although the species had meanwhile been found again in 1982 in Austria, in Vorarlberg (<https://www.gbif.org/occurrence/99543229>). I found leafmines of the species in 1983 in Croatia, but at that time I did not recognise them and identified these as *S. betulicola*.

Meanwhile, the species was discovered as *Astigmella dissona* in Far East Russia in the early 1980s (Puplesis 1984a, b). Only much later was it shown that *S. naturnella* is the same species, although the synonymy was already suggested by van Nieukerken (1986b). It was subsequently also found in European Russia, with the earliest record from 1992 (van Nieukerken et al. 2004, 2023 in prep).

More records were published from Austria and Czechia (Huemer 1996; Huemer and Wieser 1996; Liška et al. 2000; Laštůvka and Marek 2002; Šefrová 2005; Šumpich 2011, 2017; Wieser 2012), the first record in Switzerland was in 2005 (Kopp 2010) and we here report the first record from Ukraine in 2009.

Up to the early 2000s, most records were within a limited area, Alpine valleys and warmer areas in southern Germany, Czechia, Slovakia and Hungary, and further east throughout Russia (Dubatolov 2007; Bolshakov et al. 2008; van Nieukerken and Sinev 2019; van Nieukerken et al. 2023 in prep). My new recordings of the species in the western Alps of France and Piemonte in 2018 still largely fit this picture. Surprising new records, however, have come from much farther northwards, first from Poland in 2010 (new record of mines from Walendów) and 2011 (Baran 2013), then Germany, Sachsen in 2014 (new record of mines from Pirna), in the Oberlausitz in 2015 and 2017 and in Bavaria in 2017 and 2018 (Guggemoos et al. 2018; Segerer et al. 2019). It was earlier found again in Baden-Württemberg by Willem Ellis and mistaken for *S. luteella* as shown by leafmines in RMNH, collected 28 September 2001 (Fig. 32). In October 2017 I found many leafmines in Central France near Le Mans (Sarthe), a first record for that country; in 2018 independently Mikhail Kozlov and I collected mines in the French Alps, in Savoie.

The earliest indications for an expansion into Belgium and the Netherlands are online observations from 2017, in March and April Guus Dekker observed adults in the Netherlands, Noord-Brabant (Table 1), in August larvae and mines were observed in Belgium, Limburg, Zwarte Beek – Bakel by Carina van Steenwinkel, originally identified as *S. confusella* (<https://waarnemingen.be/observation/142508204/>, see also Ellis 2022) and in October in the Netherlands, Noord-Brabant, near Ossendrecht, originally identified as *S. luteella*. The identity of these and subsequent online records as *S. naturnella* was first recognised by me in autumn 2020. In 2018 and 2019 most records were still from Belgium, but in 2020 *S. naturnella* appeared to be common in the southern half of the Netherlands, where it was recorded in the provinces Limburg, Noord-Brabant, Utrecht, Noord-Holland (only area near Hilversum) and Gelderland, the Veluwe. In the last province I was unable to find any mines of *S. naturnella* in the Veluwe area north of highway A1 (four sites visited in 2020), whereas it was common in southern parts of the Veluwe, a sandy area of forests, heathlands and sand dunes. Yet in the area around Leiden and Wassenaar (Zuid-Holland) *S. naturnella* was still absent. The online observations fit this pattern. In 2021 the species was observed more northerly in the province of Flevoland (<https://waarneming.nl/observation/228044436/>),

confirmed again in 2022. In 2022 it was also found in the coastal dunes of Zeeland (Schouwen) and the northernmost records come from the province of Overijssel. More northern records along the coast (Noord-Holland) remain uncertain, these are vacated mines that also may belong to *S. glutinosae*. In Belgium observations originate from the following provinces: Antwerpen, Brussel, Hainaut, Limburg, Oost-Vlaanderen, Vlaams-Brabant and West-Vlaanderen.

Rechecking mines of other *Betula* feeding *Stigmella* in the rich leafmine collections of RMNH did not reveal any older misidentified mines of *S. naturnella* in the Netherlands.

In 2020 *S. naturnella* was also found in Saarland, and in 2021 and 2022 in Nordrhein-Westfalen in Germany, near Hövelhof.

Discussion

Sudden spread?

Stigmella naturnella suddenly appeared in Belgium and the Netherlands after 2017, which can be regarded as a genuine expansion. Leafminers and particularly Nepticulidae had been studied and collected intensely in the Netherlands since the late 1970s, first in our research group at the Free University, including ecological research into *Betula* miners (Boomsma et al. 1987). Later, many volunteers collected data of leafminers all over the Netherlands, especially encouraged by the appearance of the leafmine identification website developed in the early 2000s by Ellis (2022). Furthermore observation websites have led to an enormous increase of the collection/observation effort. Many of the collected leafmines of our research group between 1978 and 1985 are preserved in the RMNH collections, as well as the material collected later by Willem Ellis and collaborators from the former Zoological Museum of Amsterdam. The *Betula* mining *Stigmella* in this large collection have been re-examined by me, resulting in just a few overlooked *S. naturnella* records being cited here (from Croatia and southern Germany), but none from the Netherlands or Belgium.

The situation in other European countries is less clear: there has for instance been hardly a tradition of leafmine collecting and study in France, the few records of leafmines on *Betula* made before the faunal revision (van Nieukerken et al. 2006a) were usually identified as *S. betulicola*, most likely including misidentified leafmines of other species. No mines of *S. naturnella* were recorded from France before 2017.

It is certain that *S. naturnella* when discovered in the late 1930s, occurred in the alpine region of Italy and Austria and further north in Württemberg, an old record, often doubted, but proven to be correct here. The 1960 record in Moravia (Czechia) suggests that this record may belong to the original distribution area, which would include most of Austria and probably Hungary. In European Russia records occur since the 1990s, but before that almost nobody in Russia studied these small insects or their leafmines, so *S. naturnella* may well have been present earlier. Indeed the minimal variation in the DNA barcoding data throughout its range, with most variation being present in Russia, particularly the Far East, suggests that *S. naturnella* has spread from Siberia westwards relatively recently after the glaciations. When the current spread actually started is difficult to say, but the few records suggest that this was after 2000, from the alpine region Northwest into France and later Belgium and the Netherlands, and northward into Czechia, Poland and Germany. So far these expanding populations seem to show little or no variation in their DNA barcodes.

A fact that almost certainly contributed to overlooking the expansion of *S. naturnella* is the relatively difficult recognition of its leafmines, although Klimesch (1936, 1948) did describe them in detail, mentioning the important diagnostic features. Skala started to identify many leafmines as *S. naturnella*, but apparently made many mistakes (see above), contributing to the uncertainty of the identification of this species. The keys of Hering (1957) were of limited value, as important characters of egg position and frequent green coloration of the early mine were omitted. Another complication is the previously overlooked occurrence of *S. glutinosae* on *Betula*, a species often found together with *S. naturnella*, and with mines that are sometimes very similar. Identification of leafmines without larvae remains difficult at times, especially as it is based purely on the effect of the larva on the plant, which itself may be influenced by several external factors and the health of the larva. Caution, therefore, should be taken in using vacated leafmines for identification (especially for completely new records), i.e., without the benefits of larvae, reared adults or DNA barcodes. Plainly, studying a sample of leafmines in a population is always better than relying on a single specimen.

Fortunately, several old mine collections have been kept in good condition, notably the herbariums of Hering, Buhr and Wörz, so that it was possible to check old data and look for further records. That such collections exist is unfortunately not well known, collection managers are encouraged to register these collections online, with scans of the herbarium sheets (as e.g. done by Lödl and Gaal-Haszler (2010)). Hopefully a study of more collections like these will provide better data about the spread of *S. naturnella*.

Our current knowledge of the distribution and spread of *S. naturnella*, owes much to the many naturalists who post photographs on the observation platforms, even when the identity of the species is unknown or uncertain. Observation platforms that are open to all taxa seem to be more effective than those to a single taxon, as it invites naturalists of all persuasions to contribute records of species they may otherwise have ignored. Moreover, many naturalists have either shifted their interest, e.g. from birds to moths, or simply enlarged their scope. The presence of active groups studying leafmines in the Netherlands and Belgium probably explains the difference compared with Germany for example, where few observations of this species were posted, and most records were cited in more traditional journal articles, books or on dedicated websites.

Factors to explain the expansion

Hibernation of adult *S. naturnella* has been observed from finding moths hidden under bark in the winter months in Belgium and The Netherlands and in early spring in Russia. Another indication is the fact that adults emerge in autumn soon after larvae have been collected (also noted by Klimesch 1936). Furthermore, the flimsy nature of the cocoon seems unsuitable to protect a hibernating larva or pupa. *Stigmella naturnella* is one of the few species of Nepticulidae that hibernates as an adult, which may have been a factor explaining its northward expansion following climate change. The only other European species that hibernates as adult is *S. aceris* (Frey, 1857), which has also expanded its range considerably. In this species, however, the expansion may be affected by other factors such as the massive planting of its *Acer* hostplant (van Nieukerken et al. 2006b). Such planting is unlikely to be a cause of the spread of *S. naturnella*, as *Betula* is a common native tree, spontaneously growing from seeds in many places. It is possible that *S. naturnella* is better adapted to dry conditions, and that the longer period of its hostplant being in leaf may provide

possibilities for having several annual generations, with more offspring that can disperse when the conditions are favourable. It is interesting to observe that most hibernating adults were seen in cities on planted *Platanus* trees, although observation may partly be explained by sampling bias, as the bark of these trees, easily removed, is often studied in winter to search for hibernating insects, compared with the study of forest trees at this time of year. Nevertheless, these city locality records remain interesting, as they are often distant from *S. naturnella*'s typical habitat, and thus can only be explained by dispersal.

Effects of the expansion

The density of leafmines on *Betula* at first sight seems to be low, except in exceptional circumstances, which would suggest that interspecific competition is unlikely. Various studies in the past rejected the importance of interspecific competition between herbivorous insects, but more recently ever more studies have found that competition is an important factor influencing the performance and fitness of herbivorous insects (Kaplan and Denno 2007). In their study of the ecology of birch-feeding *Stigmella*, Boomsma et al. (1987) found an ecological segregation of the involved species, and suggested that this may be due to the limited availability of suitable leaves for oviposition and development. In this situation, the sudden appearance of a relatively abundant leafminer as *S. naturnella* might disturb the population build-up of other *Stigmella* species, or even other herbivores that use the leaves (miners, gallers, etc.). Whether this is the case can only be studied by detailed monitoring of selected populations.

Of some concern during my fieldwork in the Netherlands was that I seldomly found species with a more northerly distribution, namely *S. lapponica* and *S. confusella* which, from casual experience, seemed to be more common before 2000. Such a decline might possibly be due to the warming climate and dry spring and summer of these years. Competition seems less likely in this case, as these species are usually found more commonly in wetter places than *S. naturnella* and more often on downy birch (*B. pubescens*).

The future

Stigmella naturnella is already established in much of Northwest Europe and we may expect a further northerly spread. Even during my fieldwork between 2020 and 2022 its distribution seems to have shifted ca. 50 km northwards. Birch stands in northern parts of the Netherlands, Germany and Poland should be examined for the presence of leafmines and larvae of *S. naturnella*, and it may soon arrive in Denmark, Sweden and the Baltic States and even the UK. At the same time it is important to fill in the gaps of knowledge in other parts of its distribution area.

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Appendix 1

Material of other species examined. *B.* = *Betula*. All in RMNH, unless otherwise mentioned.

Stigmella alnetella

ITALY • 2 larvae (barcoded), 2 leafmines; Roma, Manziano, Monumente Naturale della Caldera; 42.09066°N, 12.09733°E; alt. 265 m; 17 Sep. 2005; E.J. van Nieukerken leg.; *B. pendula*; EventId: EvN no 2005115-3M; RMNH.INS.11943–11944, 41122.

Stigmella betulicola

FRANCE • 4 ♂ 3 ♀, cocoons, leafmines; Sarthe, Le Mans, Arche de la Nature, Bois de Changé; 47.9888°N, 0.25835°E; alt. 83 m; 07 Oct. 2017; E.J. van Nieukerken & S. Richter leg.; *B. pendula*; emerged 26–28 Mar. 2018; EventId: EvN no 2017150-1K; RMNH.INS.16657–16663. RMNH.INS.16931, 44082.

NETHERLANDS • 1 ♀, 1 larva, 9 leafmines; Noord-Brabant, Goirle, Gorp en Rovert - Noord; 51.50512°N, 5.08499°E; 30 Sep. 2020; E.J. van Nieukerken leg.; *B. pendula*; emerged 24 Mar. 2021; EventId: EvN no 2020083-2H/K/M; RMNH.INS.17321, 31467, 48392, 48394.

Stigmella confusella

FINLAND • 4 leafmines; Satakunta, 1 km S of Harjavalta; 61.30944°N, 22.11056°E; alt. 30 m; 14 Sep. 2009; M. Kozlov et al. leg.; *B. pendula*; EventId: HAR-1S1, 2, 4; RMNH.INS.47667.

NETHERLANDS • leafmines; Noord-Brabant, Goirle, 4 km SW, Klooster Nieuwkerk; 51.487°N, 5.0278°E; 12 Sep. 1982; E.J. van Nieukerken leg.; *B. pubescens*; EventId: VU no. 82417-H; ZMA.INS.MIG.20330. • 1 larva, leafmine; Zuid-Holland, Leidschendam, Duivenvoordse en Veenzijdse Polder, Achterbos; 52.11051°N, 4.40236°E; 11 Aug. 2013; E.J. van Nieukerken leg.; *B. pubescens*; EventId: EvN no 2013098-3M; RMNH.INS.29702.

NORWAY • leafmines; Sogn og Fjordane, Stryn, Kjenndalen, at foot of glacier; 61.74188°N, 7.03083°E; alt. 180 m; 28 Jul. 2000; E.J. van Nieukerken leg.; *Betula*; EventId: EvN no 2000115-1H/K.

RUSSIA • 1 larva (DNA barcoded), leafmines; Leningrad Oblast, St. Petersburg, Sosnovka forest; 60.02456°N, 30.34481°E; 01 Jul. 2016; M. Kozlov & V. Zverev leg.; *Betula*; EventId: U101; RMNH.INS.31461, 47988–47989.

Stigmella continuella

NETHERLANDS • 2 larvae, 2 leafmines; Gelderland, Wekerom, De Valouwe, Immenkampweg; 52.08977°N, 5.71459°E; 16 Sep. 2020; E.J. van Nieukerken leg.; *B. pendula*; EventId: EvN no 2020062-4K; RMNH.INS.48324. • 1 larva, 5 leafmines; Limburg, Afferden, Zevenboomsven; 51.64172°N, 6.03046°E; 24 Sep. 2008; E.J. van Nieukerken & C. Dooreneer leg.; *B. pubescens*; EventId: EvN no 2008107-1H/K; RMNH.INS. 12832, 42296–42298.

Stigmella glutinosae

FRANCE • 1 larva (DNA barcoded), several leafmines; Cher, Villeneuve-sur-Cher; 47.02664°N, 2.22037°E; alt. 117 m; 30 Jul. 2009; E.J. van Nieukerken leg.; *B. pendula*; EventId: EvN no 2009039-H/L/M; RMNH.INS.17941, RMNH.INS.42780–42781. • 4 ♂ 1 ♀, 1 larva (DNA barcoded), 5 leafmines; Sarthe, Le Mans, Arche de la Nature, Bois de Changé; 47.9888°N, 0.25835°E; alt. 83 m; 07 Oct. 2017; E.J. van Nieukerken & S. Richter leg.; *B. pendula*; emerged 30 Mar.– 16 Apr. 2018; EventId: EvN no 2017150-3K; RMNH.INS.16666–16669, 16749, 16933, 31076, 44086, 44196.

GERMANY • 3 leafmines; Berlin, Berlin-Dahlem, Botanischer Garten; 17 Aug. 1936; H. Buhr leg.; *B. papyrifera* [1 sample identified as synonym *B. excelsa*]; Herbarium Haussknecht. [originally as *Nepticula naturnella*, Buhr 1940: 229] [identification tentative].

HUNGARY • 9 mines; Pest, Budapest SW: Kamaraerdő; 47.43972°N, 18.98404°E; 19 Oct. 1983; van Nieuwerkerken & Boomsma leg.; *B. pendula*; EventId: VU no. 83523; ZMA.INS.MIG.11573.

ITALY • 30 leafmines; Roma, Manziano, Monumente Naturale della Caldera; 42.09066°N, 12.09733°E; alt. 265 m; 17 Sep. 2005; E.J. van Nieuwerkerken leg.; *B. pendula*; EventId: EvN no 2005115-1H; RMNH.INS.41123 [identification tentative].

NETHERLANDS • 1 leafmine; Overijssel, Weerribben, Ossenzijl, Venebosch; 52.80696°N, 5.93349°E; 27 Aug. 2011; E.J. van Nieuwerkerken leg.; *B. pubescens*; EventId: EvN no 2011117-6H; RMNH.INS.41655 [identification tentative].

RUSSIA • 2 leafmines; Lipetsk Oblast, Krasnoe Distr., Leski; 52.8729°N, 38.97°E; 11 Sep. 2014; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.47937. • 1 larva (DNA barcoded), 1 leafmine; Lipetsk Oblast, Usman Distr., Usman; 51.983°N, 39.783°E; alt. 165 m; 28 Aug. 2017; V. Zverev leg.; *B. pendula*; RMNH.INS.31159, 46143. • 2 leafmines; Lipetsk Oblast, Zadonsk Distr., Butyrki; 52.5724°N, 38.9582°E; 09 Aug. 2014; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45798. • 2 larvae (DNA barcoded); Saratov Oblast, Saratov city, botanic garden; 51.565814°N, 46.005748°E; 29 Aug. 2020; V. Anikin leg.; *B. pendula*; EventId: VVZ_230a, VVZ_230b; RMNH.INS.31642, 31643. • 1 leafmine; Tula Oblast, Belyov Distr., Staroselye; 53.965°N, 36.1505°E; 22 Jul. 2008; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45870. • 1 leafmine; Tula Oblast, Kimovsk Distr., Chebyshi; 53.7266°N, 38.6498°E; 24 Sep. 2009; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45900. • 2 larvae (DNA barcoded), 5 leafmines; Tula Oblast, Kimovsk Distr., Lupishki; 53.8966°N, 38.5335°E; 09 Aug. 2012; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.31321, 31322, 45686. • 1 leafmine; Tula Oblast, Kurkino Distr., Vodyanoe Pole; 53.6176°N, 38.5766°E; 13 Aug. 2008; L.V. Bolshakov leg.; *B. pendula*; RMNH.INS.45830. • 1 ♂ (DNA barcoded), 3 leafmines; Ulyanovsk Oblast, Ul'yanovsk: "Vinnovka" City Park; 54.27°N, 48.03°E; emerged Jul. 2006; A. Mistchenko leg.; *B. pendula*; Genitalia slide: EvN4066; RMNH.INS.24066; Zolotuhin, V., personal collection. • 3 larvae (1 DNA barcoded), 38 leafmines; Voronezh Oblast, Voronezh; 51.583°N, 39.167°E; alt. 150 m; 28 Aug. 2017; V. Zverev leg.; *B. pendula*; RMNH.INS.31158, 44342, 44344.

Stigmella lapponica

PORTUGAL • 4 leafmines; Minho, Parc Nat. de Gerez; 07 Jul. 1980; J.W. Schoorl leg.; *B. pubescens*; EventId: VU no. 80180a; ZMA.INS.MIG.11586.

Photographed leafmines (not in collection): UNITED KINGDOM • occupied mine; England, Copley, North Dean wood; 7 Jun. 2018; Charlie Streets leg.; *B. pubescens*; <http://charlielepidopteraofcalderdale.blogspot.com/2011/11/neps-1.html>. – NETHERLANDS • occupied leafmine; Noordhollands Duinreservaat, Zuiderachterveld; 52.6557°N, 4.6343°E; 15 Jun. 2018; Wouter Bol leg.; *Betula* spec.; <https://waarneming.nl/observation/158901416/>.

Stigmella luteella

GERMANY • 1 leafmine; Hamburg, Botanischer Garten Hamburg; 28 Sep. 1932; H. Buhr leg.; *Betula utilis*; Herbarium Haussknecht. [originally as *Nepticula naturnella*, Buhr 1940: 229]

NETHERLANDS • 1 ♂; 6 leafmines; Gelderland, Ede, Planken Wambuis, Mosselse Pad; 52.07214°N, 5.7576°E; 16 Sep. 2020; E.J. van Nieuwerkerken leg.; *B. pendula*; emerged 30 Mar. 2021; EventId: EvN no 2020058-1H/K; RMNH.INS.17314, 48306, 48307. • 1 larva, 4 leafmines; Gelderland, Wekerom, De Valouwe, Immenkampweg; 52.08977°N, 5.71459°E; 16 Sep. 2020; E.J. van Nieuwerkerken leg.; *B. pendula*; EventId: EvN no 2020062-2H; RMNH.INS.31451, 48320, 48321.

RUSSIA • 2 larvae (DNA barcoded), 6 leafmines; Karelia Rep., 20 km NW Nadvoitsy; 64.02893°N, 34.06971°E; 26 Aug. 2012; M. Kozlov et al. leg.; *B. pubescens*; EventId: R-64N-PUB-1; RMNH.INS.29972, 29973, 47901.

Stigmella sakhalinella

FINLAND [new record] • 1 leafmine; Helsinki, Linnanmäki park; 60.19108°N, 24.93797°E; 02 Sep. 2020; M. Kozlov & V. Zverev leg.; *Betula*; EventId: U06; RMNH.INS.47975. • 1 larva, 2 leafmines; Helsinki, Veikkola; 60.36153°N, 24.32469°E; 30 Jul. 2016; M. Kozlov & V. Zverev leg.; *Betula*; EventId: U01; RMNH.INS.47965. • 2 larvae, leafmines; Helsinki, 7 km SE Vihti, Ojakkala; 60.405°N, 24.3722°E; alt. 80 m; 01 Sep. 2017; M. Kozlov & V. Zverev leg.; *B. pendula*;

EventId: HCt; slide: 0; RMNH.INS.31157, 44352. • 8 leafmines; Salo, Hannilampi; 60.433°N, 23.433°E; alt. 94 m; 01 Sep. 2017; M. Kozlov & V. Zverev leg.; *B. pubescens*; EventId: Hki-Thu; RMNH.INS.44348.

NETHERLANDS • 12 leafmines; Utrecht, Soest, Korte Duinen S. edge; 52.15112°N, 5.32402°E; 23 Aug. 2021; E.J. van Nieukerken leg.; *B. pendula*; EventId: EvN no 2021122-2H/K; RMNH.INS.48701, 48702. • 1 larva, 4 leafmines; Zuid-Holland, Wassenaar, Hertenkamp; 52.14041°N, 4.38434°E; 10 Oct. 2018; E.J. van Nieukerken leg.; *B. pubescens*; EventId: EvN no 2018263-1H; RMNH.INS. 31282, 46553, 46554. • 2 larvae, 2 leafmines; Zuid-Holland, Wassenaar, Meijndel; 52.1374°N, 4.32566°E; 11 Oct. 2007; E.J. van Nieukerken leg.; *B. pubescens*; EventId: EvN no 2007122-1M; RMNH.INS.12647, 12648, 41480.

Supplementary material 1

DNA barcoded specimens of *Stigmella*

Authors: Erik J. van Nieukerken

Data type: table (excel file)

Explanation note: Dataset DS-STIGNATU on BOLDSYSTEMS (<https://doi.org/10.5883/DS-STIGNATU>), with details on Sample id's, Process id's, and Genbank Accession numbers of *Stigmella naturnella* and some other relevant *Stigmella* specimens, cited in this paper.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0/>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/nl.46.99360.suppl1>

Supplementary material 2

Measurements and counts *Stigmella naturnella*

Authors: Erik J. van Nieukerken

Data type: table (excel file)

Explanation note: Measurements of moths and leafmines of *Stigmella naturnella*, and relevant other species; counts of egg positions on leaves.

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Link: <https://doi.org/10.3897/nl.46.99360.suppl2>

Supplementary material 3

Synecology of *Stigmella naturnella*

Authors: Erik J. van Nieukerken

Data type: table (excel file)

Explanation note: Records of accompanying leafminer species on *Betula*, on sites where *Stigmella naturnella* was found.

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History of colonisation and updated distribution of the Monarch butterfly *Danaus plexippus* (Linnaeus, 1758) and its hostplants in mainland Portugal, Azores and Madeira

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Abstract. The first observations of the Monarch butterfly (*Danaus plexippus*) in Iberia date from 1886, although breeding records emerged almost a century later: 1960 in Spain, 1980s–1990s in Madeira and Azores, and 2003 in mainland Portugal. We reviewed the history of the colonisation of mainland and insular Portugal by the Monarch butterfly and its hostplants (*Gomphocarpus fruticosus*, *G. physocarpus* and *Asclepias curassavica*). We also compiled available historical and recent occurrence records as a basis for countrywide surveys of the butterfly and hostplants, to update their current distributions in Portugal. Locations for only a few of the older records represented newly rediscovered populations in the field, although recent occurrences were often confirmed. Hostplants were scarce and monarchs absent in northern and central mainland Portugal, but both were quite common in the southwest. In Madeira, hostplants were found in two locations, while monarchs were common and widespread. In the Azores, small hostplant patches were observed on four of seven surveyed islands, whereas monarchs were rare and restricted to two islands. Abandoned/semi-abandoned orange orchards represent the butterfly's stronghold in Portugal, with the species being increasingly scarce along rivers and road verges where hostplants are declining. Hostplant persistence is unstable, with many patches removed, while others have expanded or colonised new areas. Overall, hostplants appear to be declining, with implications for the persistence of monarch butterflies in the country.

Introduction

The Monarch butterfly and its worldwide expansion

The Monarch butterfly *Danaus plexippus* is one of the most charismatic butterfly species. This is largely because of the spectacular fall migration of its North American eastern population between southern Canada and Mexico (Urquhart and Urquhart 1978). Since the mid-19th century, the species has spread from its original North American range across the Pacific and Atlantic Oceans (Vane-Wright 1993; Zalucki and Clarke 2004), although genetics indicate a possible earlier expansion (Zhan *et al.* 2014). These authors suggested that southern USA or northern Mexico were the likely geographic origin of three independent dispersal events: (1) to Central and South America, Bermuda and Puerto Rico; (2) across the Pacific down to Australia and New Zealand; and (3) from Mesoamerica across the Atlantic to Portugal, and from there to Spain and Morocco. According to Pierce *et al.* (2014), the colonisation of the western Mediterranean must have occurred through multiple sporadic events involving few vagrant individuals.

The species became almost cosmopolitan after colonising up to 90 countries, islands or archipelagos worldwide (Fernández-Haeger *et al.* 2015; Nail *et al.* 2019), resulting in highly genetically differentiated populations globally. Throughout its new range outside of the Americas, the species became largely sedentary, though retaining some migratory behaviour in Australia (James and James 2019; Nail *et al.* 2019), and with medium-range dispersive movements out of its breeding grounds in Spain (Obregón *et al.* 2018). By becoming sedentary, monarchs lost some adaptive morphological traits of migratory populations, developing smaller forewings, lower wing loading, *i.e.*, body mass/wing area (Altizer and Davis 2010; Li *et al.* 2016) and higher flight metabolic rates (Zhan *et al.* 2014).

Monarch colonisation of the Mediterranean and Macaronesia

Monarchs have long been sighted in non-breeding areas across Europe, from Great Britain (Kirby 1896; Emmet and Heath 1990) and Denmark (Toft 1980) to northern Spain (*e.g.*, Fernández-Vidal 2002; Sabaté and Loaso 2004; Mortera and Pajuelo 2015). This has been interpreted as vagrancy from North America during particular meteorological conditions, such as strong winds across the Atlantic (Vanholder 1996; Asher *et al.* 2001). Monarchs are strong fliers known to use thermal soaring and gliding flight up to altitudes above 1200 m (Gibo 1981), so it is plausible that they travel long distances during cyclonic winds. This has also been suggested as an explanation for the colonisation of Australia from formerly well-established populations in the Pacific islands of Vanuatu and New Caledonia (Clarke and Zalucki 2004).

After the first sighting of a Monarch in Gibraltar in 1886 (Walker 1886a), the first breeding record from Iberia emerged in southern Spain in the late 1960s, after which records steadily increased in the region and the species became well established around the Strait of Gibraltar (Fernández-Haeger and Jordano Barbudo 2009). However, it may have settled in southern Spain long before being recorded by entomologists (Fernández-Haeger *et al.* 2011a). This, together with the fact that genetics revealed that monarchs established earlier on in Portugal (Zhan *et al.* 2014), does not support the speculation that their presence in Andalusia might have been the result of a deliberate introduction by British naturalists (Showler 2001). In Morocco, breeding records along the Atlantic coast and the Strait of Gibraltar increased from the end of the 20th century (TARRIER 2000; TARRIER and Delacre 2008; Fernández-Haeger *et al.* 2015). It is foreseeable that the species will expand its range along the Mediterranean coast of Spain and beyond, eventually colonising areas along the Mediterranean

coast of Europe (Sobrino et al. 2002; Zalucki et al. 2015; Obregón et al. 2018). In the archipelagos of Macaronesia, records of established populations date from much earlier on, namely from around 1880 in the Canary Islands (Wiemers 1995; Fernández-Haeger and Jordano Barbudo 2009).

Monarch hostplants in the Western Palearctic

Monarch butterfly larvae feed almost exclusively on asclepiads (Apocynaceae). Within the butterfly's original range in America, most hostplants belong to the genus *Asclepias*. Elsewhere, it relies mainly on alien Apocynaceae of the African genus *Gomphocarpus*, except for the cases of introduced *Asclepias curassavica*, which is native to Central America. The butterfly reportedly feeds mostly on *Gomphocarpus fruticosus* (e.g., Owen and Smith 1989; Wiemers 1995; Fernández-Haeger and Jordano Barbudo 2009; Nail et al. 2019) but also on *Gomphocarpus physocarpus* (Fernández-Haeger et al. 2010; James and James 2019). While the former has an extensive distribution from southern Arabia to Eastern and Southern Africa, *G. physocarpus* is mainly restricted to southern Africa (Goyder and Nicholas 2001). In Iberia and Macaronesia, Monarch larvae feed mainly on *G. fruticosus*, *G. physocarpus* and *A. curassavica* (Owen and Smith 1989; Fernández-Haeger et al. 2015), and exceptionally on the native *Cynanchum acutum* (Apocynaceae) in southern Spain (Gil-T 2006). In the Azores, larvae have also been observed feeding on *Gossypium arboreum*, Malvaceae (Neves et al. 2001).

The worldwide spread of the aforementioned hostplants was favoured by their invasiveness, likely associated with their self-compatible pollination ability (which in *G. physocarpus* seems to have been acquired during its expansion out of the native range), and with the capacity for fruiting through uniparental reproduction; invasiveness is further promoted by the observed potential for hybridisation in the case of the two *Gomphocarpus* species, through higher pollen yields and increased genetic variability (Ward et al. 2012). Moreover, the expansion and naturalisation of *G. fruticosus* in Europe and Macaronesia was probably favoured by its ancient exploitation as a textile plant (Quer 1762).

Objectives and rationale

Here, we review the history of colonisation of mainland Portugal, Azores and Madeira archipelagos, by the Monarch butterfly and its hostplants. Available historical and recent occurrence records were also compiled and used as the basis for countrywide surveys of the butterfly and hostplants, to update their distributions. Lastly, we investigated the trends in hostplant patch persistence, including the long-term persistence in old previously known locations (Palma and Bívar de Sousa 2003), and discussed their possible implications for the butterfly's populations.

Methods

Study area: – Mainland Portugal

The study comprehensively covered the localities with former records of the butterfly and its two main hostplants, *G. fruticosus* and *G. physocarpus*. This required surveying a large set of littoral and sublittoral lowland areas along the country's western and southern coasts. The study area belongs to two different Palearctic biogeographic domains (ETC 2006): the Eurosiberian, from the northern border with Spain south to the Aveiro marshlands, a temperate territory strongly influenced by the Atlantic Ocean; and the drier and warmer Mediterranean, encompassing all remaining southward coastal areas. The northern, central and Lisbon's coastal areas are heavily urbanised,

although in rural areas small property predominates. In contrast, along the southwestern coast, partially designated as a Natural Park, urban areas are smaller and scattered, and extensive farming in small to medium-sized rural property dominates, although intensive agriculture and greenhouses occupy the irrigated sectors of the coastal plateau. Along the southern coast of the Algarve, the coastline is heavily urbanised, touristic, and almost entirely bordered inland by farmland dedicated mainly to fruit production, especially oranges (LP, Pers. obs.).

Azores and Madeira

The original mesophytic vegetation of the mountainous volcanic islands of the Azores and Madeira was a laurel-type forest, with paleotropical or paleomediterranean affinities (Aguiar *et al.* 2008), denominated laurisilva. In the Azores, little remains of the original vegetation and the economy is centred on semi free-ranging dairy cattle. Madeira, on the other hand, still retains large extents of the original laurisilva, with small scale farming and villages mostly restricted to less rugged areas by the sea. As ruderal species, *Gomphocarpus* spp. occur predominantly in humanised areas such as fallow land, and in urban and suburban gardens and parks. Surveys targeted these areas, mostly in the low altitude periphery of the islands.

Compilation of historical and recent Monarch and hostplant occurrence records

We comprehensively reviewed the extant literature on monarch butterflies and their hostplants in mainland and insular Portugal, gathering reports of sightings and breeding activity for the butterfly, and introduction and naturalisation events for the hostplants.

These sources included museum herbaria, GBIF (2021) and online citizen science platforms (see below). We searched the herbarium Florae Lusitaniae of the University of Coimbra (1848–2015) and the Herbarium Lusitanicum of the University of Lisbon (1878–1886) for specimens of *G. fruticosus*. Data from Herbarium Lusitanicum were retained only if complementary to Florae Lusitaniae. Most of the older GBIF records corresponded to specimens held at Florae Lusitaniae. In the Azores and Madeira, owing to logistic and financial limitations, and to the fact that the main goal of the surveys was to collect Monarch specimens for a phylogenetic study, preliminary searches for data were more limited, and some repositories were not visited beforehand (e.g., Madeira's herbaria and museum entomological collections).

The following online citizen-science platforms were checked for recent occurrence records: (1) Biodiversity4all (<https://www.biodiversity4all.org/>); (2) Flora-on (<https://flora-on.pt/>) mostly for hostplant records (2017–2020); and (3) Observation.org (<https://observation.org/>) mostly for Monarch observations that were absent from the other platforms (2010–2017). Flora-on records proved to be largely redundant with those of Biodiversity4all. We also collected oral communications of researchers and amateur naturalists, and searched non-technical literature (e.g., Viana *et al.* 2009). For the Portuguese archipelagos, we further checked Neves *et al.* (2001) and the Azores Biodiversity Portal (<http://azoresbioportal.uac.pt/>) (Suppl. material 2).

Hostplant and Monarch field surveys: – Mainland Portugal

Surveys were conducted in 2016–18 and 2020–21. We assessed the persistence of hostplant patches in 53 (~77%) of 69 locations retrieved from the aforementioned sources, while also checking for the presence of the butterfly. Sixteen locations were not visited either because geographic references were vague or we presumed that the respective hostplant populations were very small

or had already disappeared, namely five records from the 19th century and eleven from the 20th and 21st centuries from urban areas in the centre and north of the country. Among the searched locations of older records (<1950) the plants were either absent or not found, with the exception of a 19th century record at Darque, Viana do Castelo.

Besides the previously reported locations, we systematically searched the areas where hostplant patches were more likely to be found due to their ecological requirements of water availability in summer, absence of frost and competitive vegetation induced by grazing, such as pasture fallow, under-managed or abandoned orange orchards, fences, stream banks, road verges and gardens in lowland coastal areas (Fernández-Haeger et al. 2010). Access to reported locations was made by car and on foot.

Almost half (56; ~41%) of the hostplant patches confirmed during the initial survey in 2016–18, which were mostly located within the butterfly's distribution range, were revisited in 2020–21 to verify their persistence and the butterfly's presence. Hostplant patches found only in the 2020–21 survey were visited only once (Suppl. material 2). Most of the few patches still unchecked in 2020 in the Lisbon, Setúbal and Beja districts were carefully checked in May 2021.

In order to confirm whether monarchs were present outside of the previously known range in the south of the country (Palma and Bívar de Sousa 2003), the two largest populations of *G. fruticosus* identified in central (Carnaxide, Lisbon metropolitan area, over 1000 plants) and northern Portugal (Darque, near Viana do Castelo, several hundred plants) were thoroughly examined: Carnaxide was visited four times between August and October 2018, and Darque in August 2018. Larger patches in eastern Algarve were also revisited several times to confirm the absence of the butterfly.

The areas where the butterfly's presence was previously reported (Palma and Bívar de Sousa 2003) were surveyed by car at <40 km/h, carefully searching for hostplants and/or flying monarchs along 992 km of paved and unpaved secondary roads. This was done across ~350 km² of the southwest littoral belt and ~650 km² of the western coastal Algarve, including the river valleys of the adjacent foothills. The same was carried out along ~280 km in central and eastern coastal Algarve, throughout ~350 km². This survey was carried out in September-December 2017, September-October 2018, and July-October 2020. At each patch detected, we recorded the hostplant species, geographic coordinates and overall features regarding patch geometry and density (i.e., sparse or dense, linear or compact), as well as habitat type (i.e., riverbank, pasture fallow, field fence, road verge). Additionally, we recorded the number of hostplants per patch, counting the individuals in patches with up to ~50 plants, while visually estimating the number in patches with more than 50 plants. This involved counting the plants in a small fraction of the patch, and then extrapolating to the entire patch. Apparent threats, such as mechanical clearing, pasture conversion, competition by natural vegetation, were also recorded.

In addition, patches of Lantana (*Lantana camara*), an ornamental alien plant widespread on chalet walls and in gardens, and along road verges throughout the Algarve, were systematically watched for flying monarchs while driving. Because of its high nectar yield and sugar content (Torres and Galetto 2014; Carrión-Tacuri et al. 2014) this flowering plant strongly attracts butterflies, monarchs included (Fernández-Haeger and Jordano Barbudo 2009). Indeed, wherever monarchs and lantanas were present, the butterflies were often seen feeding from the flowers (LP, Pers. obs.).

Azores and Madeira

In the Azores, the islands of Terceira, São Jorge, Pico, Faial, Flores and Corvo were surveyed once in August 2018. São Miguel Island could not be visited in August due to logistic limitations, so it was surveyed in October 2018. Field work focused on localities with previous butterfly and

hostplant occurrence data, as well as on areas with higher probability of finding both, such as public parks, private urban gardens and yards, and abandoned farms.

Madeira Island was surveyed in September 2018. The work was carried out in public parks and gardens in urban and suburban areas, where hostplants, and hence the butterflies, were more likely to be found. Localities along the coastline and some surroundings were visited, although most of the effort was spent in the capital, Funchal. Logistic constraints prevented surveys on the island of Porto Santo and on the Desertas and Selvagens Islets. Furthermore, there was no information about the presence of monarchs or hostplants on the islets, and presumably no suitable habitat.

Results

Historical background of the Monarch and hostplants in Portugal

In mainland Portugal, Cruz and Gonçalves (1973) reported monarch sightings between 1932 and 1968 in the north of the country, which they attributed to vagrancy from the Azores, Madeira or the Canary Islands. Breeding was only confirmed in 2003 and 2004 in the Algarve (Palma and Bívar de Sousa 2003; Simonson 2004), although Simonson had reported Monarch sightings in the region since 1998. It is highly likely though that the species became established in the region much earlier on, as suggested by Obregón *et al.* (2018) and inferred from genetics, which showed that monarchs settled in Portugal earlier than in Spain (Zhan *et al.* 2014), *i.e.*, before the 1960s. Curiously, its breeding was overlooked (Schmitt 2001) and attributed to immigration from North America or Macaronesia (Maravalhas 2003).

In the Azores, Godman (1870) and Walker (1886b) mentioned first sightings in 1864 on the islands of Terceira and Flores, followed by a number of irregular sightings (Neves *et al.* 2001). Cruz and Gonçalves (1973) considered the species rare but already established in the archipelago, while Neves *et al.* (2001) raised doubts about its breeding, suggesting that the records might only indicate vagrancy. The authors reported first breeding on the island of Faial in 1994, although the species was likely present there earlier, and on several other islands in 1999 and 2000 (see Suppl. material 1 for a summary of historical details).

In Madeira, monarchs appeared to be absent in Walker's time (Walker 1886b). According to Aguiar and Karsholt (2006), the Monarch was observed periodically in Madeira since 1889, but only became established in 1980. Cruz and Gonçalves (1973) considered the monarch very rare in Madeira but occasionally very common in Porto Santo. Indeed, Pereira (1989) reported large numbers of monarchs observed in Porto Santo for the first time in 1955. Swash and Askew (1982) also mentioned irregular and infrequent sightings of the Monarch in Madeira, and Jones *et al.* (1987), although mentioning the species' presence, made no reference to breeding. At the same time, however, Bívar de Sousa (1985) reported that the species was already breeding and frequently observed, and according to Meyer (1993), mostly along the island's southern shore.

Showler (2001) and Gardiner (2003) speculated about a possible introduction of monarchs and *A. curassavica* in Madeira, by a couple of British naturalists during the 1960–1970s, who had allegedly done the same in Andalusia. However, milkweeds were reportedly introduced much earlier in the island (Vieira 2002). It also seems unlikely that such a release of monarchs alone would have led to their colonisation of the archipelago, as they were already repeatedly observed in Madeira since the late 19th century and in Porto Santo since 1955 (see above). It thus seems much more likely that they became established following several natural colonisation events as defended by Pierce *et al.* (2014) concerning the Western Mediterranean.

The Central American *A. curassavica* was brought to the Iberian Peninsula in the 16th century by Spanish travellers, and *G. fruticosus* is known from coastal areas of Iberia since the 18th century, probably introduced by the Portuguese in the Azores, Madeira and mainland Portugal during the early 17th century from their former southern African colonies (Fernández-Haeger et al. 2015). In mainland Portugal, *G. fruticosus* has been recognised as naturalised since 1902 (Almeida and Freitas 2006) and is now commonly found along Mediterranean-type streams in the south of the country (Aguiar et al. 2007). *G. physocarpus* was only reported from Portugal in 2000 (Almeida and Freitas 2006) and from southern Spain (Fernández-Haeger et al. 2010; Fernández-Haeger et al. 2011a). Similarly, *A. curassavica* was quite recently (1999) reported as naturalised in mainland Portugal. The species is scarcely represented in herbaria and infrequently reported in GBIF and citizen-science platforms, reflecting its overall rareness, including within private gardens (Pers. obs.). According to Vieira (2002), *G. fruticosus* and *A. curassavica* were introduced long ago in the archipelago of Madeira for ornamental purposes. The first was introduced during the late 18th century (Menezes 1922) and the second was already present on the island in the late 19th century (Walker 1886b), both having become naturalised in uncultivated and fallow land. In Madeira, *G. physocarpus* also became naturalised in uncultivated areas and fallows, although it was only introduced in parks and gardens in the late 1970s. In the Azores, *G. fruticosus* was already reported by Godman (1870) as an occasional escape from gardens.

Hostplant distribution, abundance and persistence in mainland Portugal

In total, we compiled 136 locations for *Gomphocarpus* spp. across mainland Portugal (Suppl. material 2). These included the locations retrieved from the aforementioned sources as well as those newly found during fieldwork, of which 123 (89%) were visited. The great majority were located in western and central lowland Algarve (Faro district) and the sublittoral area of Odemira County (Beja district) (Fig. 1). Most of the remaining sites were distributed along the country's west coast, primarily around Lisbon and Setúbal districts (24), and the rest (11) in the coastal areas of the northern districts of Aveiro, Porto and Braga.

Gomphocarpus fruticosus was found from Minho region in the northwest to the Algarve in the south. In western Algarve (Fig. 1F), the plant was found across the sublittoral farmland and the south-flowing rivers crossing the area, in two types of habitats: 1) along river and stream banks and near road verges, from the Monchique foothills down to their lower reaches near the Atlantic; and 2) throughout the fruit growing area in the sublittoral lowlands, primarily within or at the edge of orange orchards. East of Messines, the plant is more localized, and is very rare in central-eastern Algarve. The plant was also found in a sublittoral tributary of the Mira River that flows westwards to the adjacent southwest coast.

Further north, between the Mira and Tejo Rivers, there were reports of *G. fruticosus* from six locations; three of four recent locations were visited, with the remaining two dating from the late 19th century. The species was not found in any of the three locations, suggesting that it might have disappeared from most if not the whole region. *G. fruticosus* was reported from eight locations in the Lisbon area, six of which were visited (Fig. 1E). Of these, we found it in three very small patches and two larger stands on the outskirts of Carnaxide, Oeiras County, one with ~250 plants and another with over 1000 plants. Further north, the plant was previously reported from six, mostly old, locations in the Santarém, Coimbra and Castelo Branco districts, but was absent in the three we could visit. North of the river Douro there is a large stand of hundreds of plants within

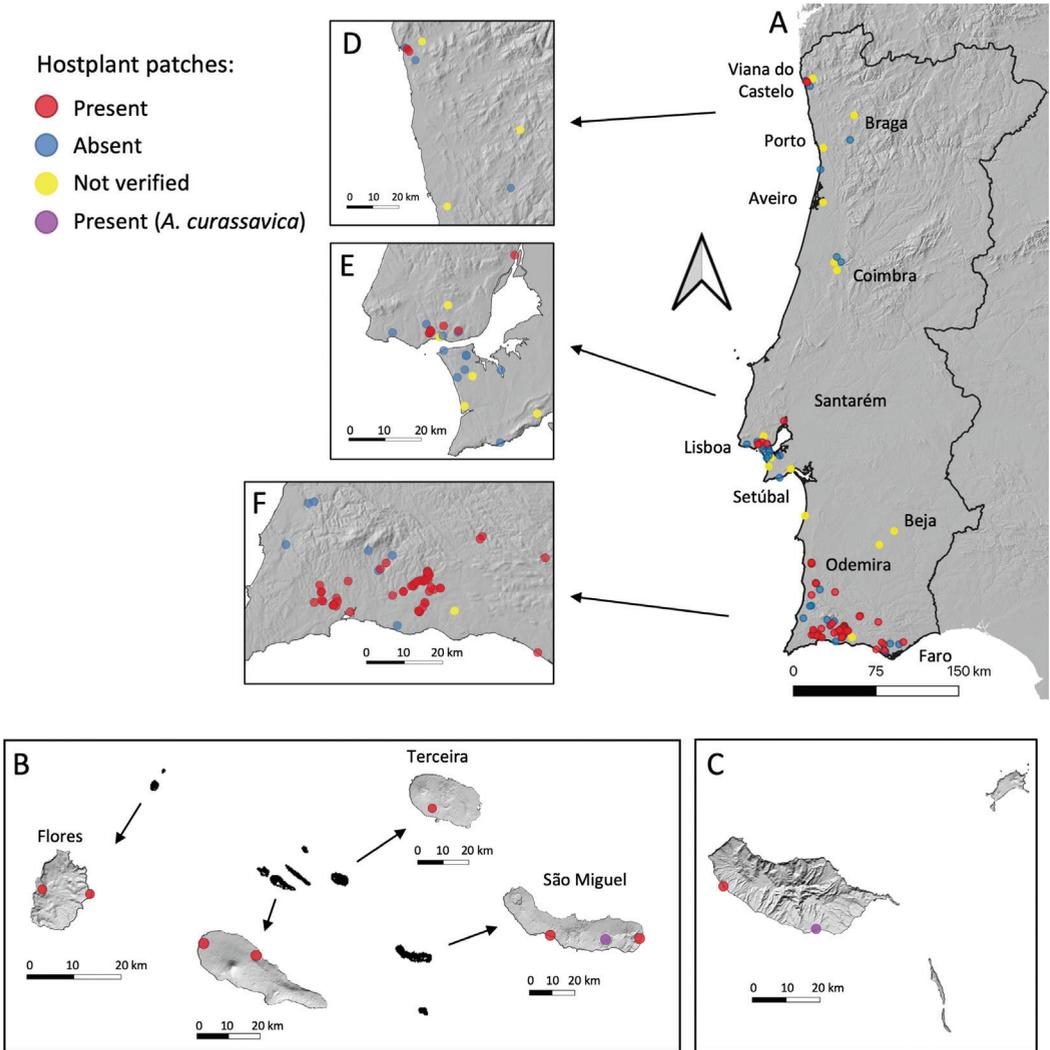


Figure 1. Distribution of the Monarch's hostplants in **A.** Mainland Portugal; **B.** Azores; and **C.** Madeira. Inserts show the detailed distribution of *Gomphocarpus* spp. **D.** In the northwest; **E.** Lisbon region and **F.** Western Algarve.

an abandoned periurban estate near Viana do Castelo (Fig. 1D). Remarkably, the first record of the species in the area dates back to 1886 (Suppl. material 1). Three of the four other locations, from the 1960s, were visited; the plant was found in only one, with 4–5 individuals remaining in a village (Fig. 1D).

Gomphocarpus physocarpus is much scarcer than *G. fruticosus* and has a dissimilar distribution. In western Algarve, the plant was found in only six stands across the orange growing area. The stands typically consisted of one to 30 and exceptionally up to 80 plants, either pure or mixed with *G. fruticosus* and morphologically apparent hybrids in variable proportions. In central-eastern Algarve, *G. physocarpus* is even rarer and occurs in very small patches, with only six former

locations known and few plants in general, mostly restricted to private gardens and backyards. A naturalised stand of ~20 plants is probably the largest one currently remaining in the region. Near the southwest coast, hundreds of individuals were known in 2017 among greenhouses and fallow ground of an intensive farm near Odemira. However, they were reduced to four plants in 2020 after land was cleared due to shifts in production.

Further north, the species is slightly more widespread though still rare (15 locations), occurring mostly in small sparsely distributed patches in urban and suburban areas of the coastal districts of Setúbal, Lisbon, Aveiro, Porto and Braga (Fig. 1D, E). Most of these records are recent (2017–2020), which is why we could only check four of them within the study timeframe. The species was not found in any. Still, in the town of Aveiro, we were able to trace back the fate of a relatively large patch of *G. physocarpus* in an abandoned yard, as photos were posted on iNaturalist in February 2016 (<https://www.inaturalist.org/photos/2996383>). Using Google Earth, we verified that a storehouse was built in its place and the plants were eradicated between 2016 and 2018. Interestingly, Monarch observations from that same neighbourhood and vicinity were repeatedly posted in Observation.org from October 2013 to January 2017. Although we thoroughly surveyed the area in July 2018, we found neither hostplant nor butterfly, which were possibly eliminated with the conversion of the yard.

Throughout the western Algarve and along the southwest coast, a large number of patches, mainly of *G. fruticosus* but also of *G. physocarpus*, disappeared or were strongly reduced between 2017 and 2020. This was mostly due to clearing for agriculture and pasture, but also due to removal of roadside vegetation and in a few cases to competition with native vegetation. This reduction was observed in 8 of 11 (~73%) of the patches revisited in the southwest coastal area and 32 of 63 (~48%) revisited in the Algarve. Conversely, a marked expansion was seen in five patches during the same period, especially in abandoned orange orchards, while resprouting was often observed in cleared stands, indicating strong metapopulation dynamics in both species.

Monarch distribution in mainland Portugal

The monarch's presence was confirmed in the majority of the patches surveyed in the western Algarve and the Mira valley (Fig. 2; Suppl. material 4). In the latter, monarchs were very abundant in 2017 when the plants were counted by the thousands, but less so during the 2020 survey after ~2/3 of the patches were almost entirely cleared for renewed cattle pasture. Still, in two remaining clusters with ~50 plants each, there were 30 and 71 individual Monarch sightings. At the time, monarchs were also present in the large patch of *G. physocarpus* further inland near Odemira, but were absent in July 2020 after the plants were cleared (see above).

Monarch populations were also formerly known between the Mira and western Algarve, especially along the Seixe valley where the species was first observed breeding in Portugal (Palma and Bivar de Sousa 2003). Several large extant patches of *G. fruticosus* along the river floodplain in 2003–2005, as well as the previously widespread and abundant monarchs, almost totally vanished due to land conversion back to cattle pasture. After the disappearance of these formerly large Monarch populations of the Seixe valley, and apparently of other small populations reported from the southwest coastal plateau (P. Canha, Pers. comm.), the Monarch population of the Mira became very isolated in relation to western Algarve. If the hostplants continue to be extensively removed there, this northernmost Monarch population may disappear.

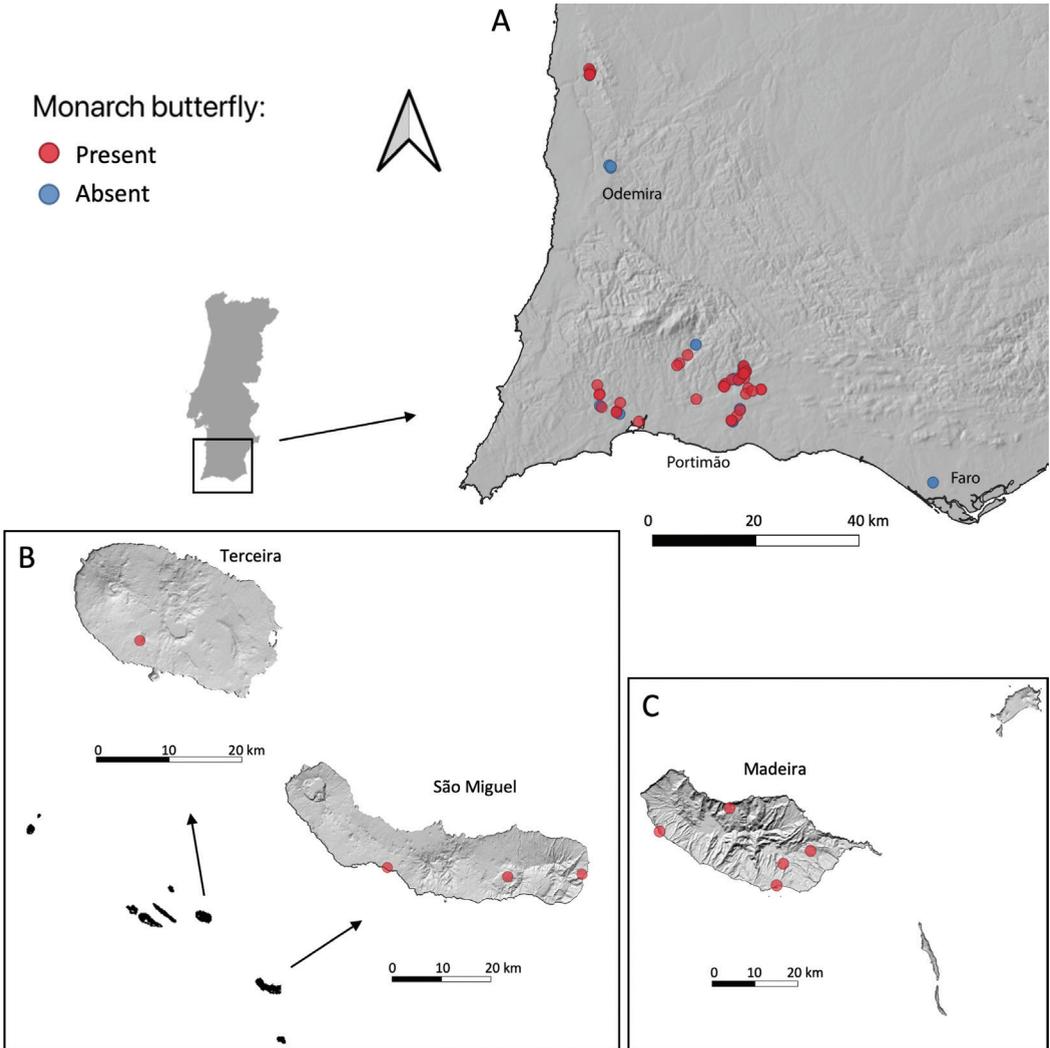


Figure 2. Distribution of Monarch butterfly in **A.** Mainland Portugal; **B.** Azores; and **C.** Madeira.

In central-eastern Algarve, we have repeatedly surveyed the largest extant patch (~20 plants) of *G. physocarpus* for the butterfly since 2018, but have not detected its presence. Furthermore, the only medium-sized patch of *G. fruticosus* (23 plants) known in the region was also lengthily inspected in mid-October 2020, with no adult butterflies or larvae observed. Until at least 2016, monarchs could be observed in small numbers in central-eastern Algarve, but became accidental at most, probably vagrants from the west. It thus appears that monarchs have not been breeding in central-eastern Algarve for some years (Fig. 2A).

Monarchs and hostplants in the Azores and Madeira

The most likely locations for hostplant and Monarch occurrence on the islands of Terceira, S. Jorge, Pico, Faial, Flores, Corvo, and S. Miguel, such as abandoned farms, public parks, private

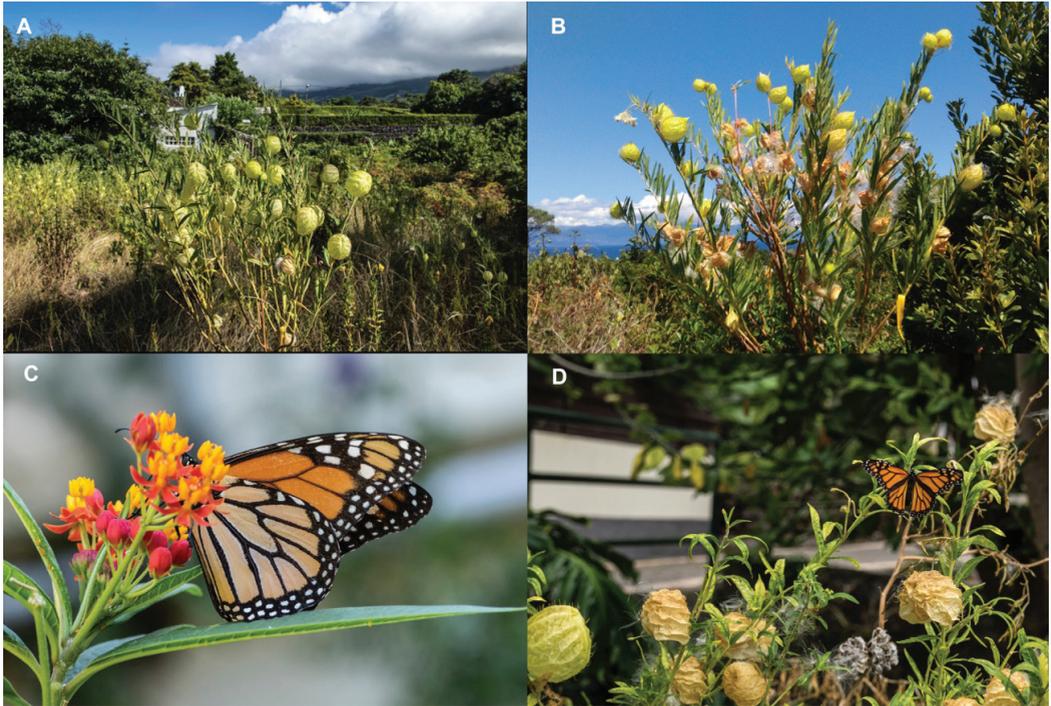


Figure 3. Photographs of the Monarch butterfly and its host plants. **A.** *G. physocarpus* on Pico island; **B.** *G. fruticosus* on Pico island; **C.** A Monarch butterfly feeding on *A. curassavica* on Madeira island; **D.** A monarch resting on *G. physocarpus* at Jardim do Mar, Madeira.

urban gardens and yards, were carefully surveyed. Hostplant species were only found on four islands, from west to east: Flores (a group of 10–20 *G. fruticosus*, and a solitary plant at a second site); Pico (abundant *G. physocarpus* and *G. fruticosus* in five closely located patches, Fig. 3A, B); Terceira (10–20 *G. physocarpus*); and S. Miguel, where patches of *G. physocarpus* and *A. curassavica* were found at three sites (Fig. 1B). Monarch butterflies were observed at three locations in S. Miguel and one location in Terceira (Fig. 2B). From the latter, additional information was provided by a local collaborator (N. Cabeceiras, Pers. comm.). The Monarch therefore seems to be relatively rare and localized in the Azores, and apparently with an irregular presence, as it appeared to be absent from the islands of Pico and Faial, where its occurrence was previously reported. Occasional oral records also pointed to yearly irregularity of Monarch occurrence. In Madeira, hostplants (Fig. 1C) were found in Funchal (*A. curassavica*, Fig. 3C) and in Jardim do Mar (*G. physocarpus*), along with butterfly imagos, larvae, pupae or eggs (Fig. 3D). Monarchs were also observed at several other locations in the Funchal area, and at six others in the SW, E and N of the island (Fig. 2C), associated with urban green areas and vegetation bordering streams, e.g. in Machico.

Discussion

We provide a review of the history of colonisation of mainland Portugal and of the Azores and Madeira archipelagos by the Monarch butterfly and its main hostplants. We have also conducted the

first countrywide surveys of both butterfly and hostplants to update their distributions in Portugal. Although the surveys in the Azores and Madeira were not systematic, they are the first extensive surveys of the Monarch and its hostplants in the archipelagos, representing a notable advance in our understanding of these species' distributions in both regions.

We found that, despite the extensive though patchy distribution of the hostplants along the coastal areas of mainland Portugal, Monarch butterflies are currently restricted to the western Algarve and more locally along the southwest coast. The Monarch's absence further north may be the result of limiting environmental factors, (e.g. climate), since potential food resources for the larvae exist, in a few cases made up of large stands of hundreds and more plants. Conversely, towards central and eastern Algarve the butterfly seems to have lost ground during the last 15 years.

Historical and current distribution of the Monarch's hostplants: – Mainland Portugal

The Monarch's hostplants are sparsely represented in museum collections, and exclusively comprise specimens of *G. fruticosus*. Those from the late 19th and early 20th centuries present in classical herbaria comprise only six locations, widely scattered from the extreme north of the country to the Lisbon area and southwestern coastal areas. This may indicate that the plant has long been widespread in the country, yet with a different distribution to the current one. However, it is not possible to judge whether it was previously common or quite localized, as past field survey conditions were incomparable to present ones. The notable exception is near Viana do Castelo, where the species was collected in 1886 (Herbarium Lusitanicum, Lisbon University) and a large population of *G. fruticosus* still exists. Only six records of this plant species from the 1900s could be found, mostly in herbaria, of which five were checked in the field. Only a small group of plants was found in one northern location.

In contrast with *G. fruticosus*, we did not find historical records of *G. physocarpus* in herbaria or elsewhere, and all records date from 2003 to 2021. This is in line with its first occurrence record in Portugal in 2000 (Almeida and Freitas 2006). Due to its scarcity in herbaria and citizen-science platforms, *A. curassavica* was intentionally not surveyed, but its rarity could be opportunistically confirmed on the ground. This rarity contrasts with the situation in Andalucía, southern Spain, where this plant is common in some areas and used as a hostplant by Monarch larvae (Fernández-Haeger and Jordano Barbudo 2009).

As expected from their ecological requirements (Fernández-Haeger *et al.* 2011a), the two *Gomphocarpus* species range along the country's littoral and sublittoral belt, mostly in lowland areas, but are patchy everywhere (Fig. 1). Although these species are present from north to south, their abundance is uneven, with both species concentrated in the western Algarve where the patches are generally small to medium-sized (Suppl. material 3), but common. Together with the Mira valley, this appears to be the area with the best ecological conditions for *G. fruticosus* in the south of the country. It is increasingly rare and localized towards the north and east of Portugal, occurring in small to very small patches (<25 plants). In central/eastern Algarve the species is largely replaced by *G. physocarpus*, which appears to better tolerate drier conditions.

The general disappearance of both *Gomphocarpus* species from most of the historical and many of the recent locations to the north of Lisbon and its environs (Fig. 1E), with remnant patches being generally very small, is probably due to the large urban expansion in these

regions. The large populations of *G. fruticosus* near Viana do Castelo and Carnaxide are the only exceptions, due to being located in an abandoned rural estate and a track or fallow rural land, respectively.

Azores and Madeira

Data on the distribution and abundance of the Monarch's hostplants in the Azores and Madeira are very scarce. Apart from the oldest references to their introduction and naturalisation (Godman 1870; Menezes 1922; Vieira 2002), there is only one specimen of *G. fruticosus* in the Coimbra herbarium, collected in Funchal in the late 19th century. More recent and detailed articles about the Monarch in the Azores and Madeira (Cruz and Gonçalves 1973; Neves et al. 2001) are also not very informative about the current distribution of its hostplants in the archipelagos. Likewise, citizen-science platforms only provide a few records of the two *Gomphocarpus* species and none of *A. curassavica*, and only the Azores Biodiversity Portal provides more detailed data on the distribution of *G. fruticosus* in these islands.

Hostplant patch persistence

The second survey (2020–2021) revealed that 48–73% of hostplant patches had disappeared since the initial survey (2016–2018). The strong extinction/recolonisation dynamics are certainly related to the ruderal nature of the plants and in the case of *G. fruticosus*, to the fact that the species is linked to the cycle of cattle grazing. Field preparation and cattle grazing contribute to the elimination of potential native competitors, such as *Rubus ulmifolius* and *Scirpus holoschoenus* that tend to replace the hostplant, but during subsequent fallow *G. fruticosus* quickly colonises the area (Fernández-Haeger et al. 2010; Fernández-Haeger et al. 2011b). Yet, when fields are converted to pasture, the plants are once more mechanically eliminated. Likewise, the periodical clearing of road edges for traffic security also contributes to their recurrent removal. On the other hand, urban expansion likely strongly affected most of the historical hostplant locations in north and central Portugal, unlike further south, where this happens much less often because the plants seldom occur within urban areas.

Geographic patterns of current Monarch occupancy in mainland Portugal

Prior to reports of Monarch breeding activity in southwest Portugal (Palma and Bívar de Sousa 2003; Simonson 2004), knowledge about the species in the country was scarce and vague. Only then were sightings of the species reported more frequently in the south of the country, in the Algarve region in particular. The large number of Monarch sightings in hostplant patches in western Algarve may be explained by the profusion of *Gomphocarpus* occurrence and the relative closeness between patches (Figs 1F, 2), especially in abandoned or semi-abandoned orange orchards and along road verges of the Silves-Messines area. In contrast, outside of the orange growing area towards the W-NW, unoccupied patches become more recurrent. This might occur because the patches located along the banks and road verges of the southerly flowing river valleys of the Monchique Mountain, are fewer and farther apart. To the east and the north of the core area of western Algarve, with the exception of the Mira valley, monarchs are absent and seemingly unable to recolonise isolated patches (Fig. 2). This contrasts with observations ~15 years ago, when despite the rareness and small size of most plant patches, monarchs were observed with relative frequency and sometimes confirmed breeding. In short, the orange growing area is currently the chief stronghold of the Monarch butterfly in Portugal.

Although the majority of the previously reported locations were searched, some hostplant patches, especially smaller ones, may have gone undetected, as some might have been in closed estates. Also, some plant patches in peripheral areas of their known ranges were found at later stages of the study and could not be visited during the Monarch survey. Nevertheless, we are confident that both cases represent a small fraction of the extant hostplant populations, and do not significantly change their overall occurrence patterns.

Future research and conservation directions

Although this study is geographically comprehensive, it provides baseline information, and much remains to be investigated about the ecology and demography of the Monarch butterfly, namely its metapopulation structure and dynamics; hostplant dispersal, extinction and recolonisation dynamics and causal factors; as well as habitat suitability modelling of both monarchs and hostplants.

Although Monarch hostplants are allochthonous and potentially invasive, their colonisation by *Danaus plexippus* in Macaronesia and the Mediterranean was a natural process. These Monarch populations are probably genetically unique (Pierce *et al.* 2014), and therefore irreplaceable, thus deserving conservation concern. However, the high removal rate of the hostplants, even if other areas are being colonised or recolonised following agricultural abandonment, is worrying because it could lead to the rarefaction or disappearance of the butterfly from a number of former locations. Although *Gomphocarpus* can be invasive, their indiscriminate control may hinder the Monarch's persistence (Fernández-Haeger *et al.* 2011b). In the case of Portugal, however, invasiveness remains at such a small scale that it should not be an impediment to the preservation of this charismatic butterfly.

Based on our study, we cannot assume that the observed clearance of hostplant patches will lead to a steady decline in hostplant availability. As in the case of the areas of the Mira and Seixe Rivers in the southwest, shifting productions or the conversion of fallows to traditional grazing, may rapidly reduce or even eliminate entire patches of *Gomphocarpus*, strongly affecting local Monarch populations. Moreover, small scale changes such as the gradual removal of plants from road verges and gardens, or their disappearance due to environmental factors (e.g. drought, competition from recovering native vegetation), could lead to the disappearance of the butterfly from large peripheral areas of its range in central-eastern Algarve, where hostplants were never abundant and have become increasingly rare. The accumulating cues for a possible and quick decline of the hostplants, and consequently of the butterfly, should thus be a matter of concern. However, given the long distance seed dispersal by wind that enables these plants to colonise remote favourable areas (Fernández-Haeger *et al.* 2010), and the strong flight ability driving the butterfly's metapopulation dynamics (Fernández-Haeger *et al.* 2011a), the observed trend should be viewed cautiously as the situation may change if conditions become more favourable.

Nevertheless, we recommend the adoption of practical solutions to avoid the potential disappearance of the Monarch butterfly due to rarefaction of its hostplants. For instance, by assessing the viability of creating a network of sizeable hostplant micro-reserves across the butterfly's core range, in partnership with collaborative landowners. Due to the butterfly's appeal, these stable patches could be promoted as touristic add-on destinies, a sort of "open-air" butterfly gardens. A similar initiative was started in Andalucía, southern Spain ("Ruta de la Mariposa Monarca de Castellar de la Frontera, Cádiz", El Giroscopio Viajero 2020). Additionally, promoting *A. curassavica* as an ornamental in suitably watered private gardens would be another means to attract the monarchs. Altogether, these actions could help elude the instability of hostplant availability and promote the persistence of *Danaus plexippus* in the country.

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Supplementary material 1

Summary of occurrence and colonisation records of the monarch butterfly and its hostplants in the Iberian Peninsula

Authors: Luís Palma, Sasha Vasconcelos, Ana Filipa Palmeirim, Juan Pablo Cancela

Data type: Occurrence records.

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Link: <https://doi.org/10.3897/nl.46.89665.suppl1>

Supplementary material 2

Georeferenced hostplant patches and patch size

Authors: Luís Palma, Sasha Vasconcelos, Ana Filipa Palmeirim, Juan Pablo Cancela

Data type: Georeferenced data.

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Link: <https://doi.org/10.3897/nl.46.89665.suppl2>

Supplementary material 3

Frequencies (%) of hostplant patch size classes arranged in geometric progression ($2\times$ ratio) (N patches = 78)

Authors: Luís Palma, Sasha Vasconcelos, Ana Filipa Palmeirim, Juan Pablo Cancela

Data type: figure.

Explanation note: This data was retrieved from Suppl. material 2.

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Link: <https://doi.org/10.3897/nl.46.89665.suppl3>

Supplementary material 4

Georeferenced butterfly relative abundance records

Authors: Luís Palma, Sasha Vasconcelos, Ana Filipa Palmeirim, Juan Pablo Cancela

Data type: Georeferenced data.

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Xenotorodor stygioxanthus gen. nov., sp. nov. (Lepidoptera, Lecithoceridae, Torodorinae), described from an established population in Spain with discussion of taxonomic placement

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<https://zoobank.org/AC23F242-7BB4-45AA-BB04-68948B04E8C3>

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Abstract. The family Lecithoceridae is not well represented in the Palaearctic region, with very few taxa in Europe. Here we describe a new genus and species of lecithocerid moth, *Xenotorodor stygioxanthus* Sterling, Lees & Grundy, **gen. nov., sp. nov.** The taxon represents a subfamily new to Europe. We consider placement of the genus within Crocanthinae or Torodorinae. We place it in Torodorinae, notwithstanding the reduced gnathos in the male genitalia. DNA barcodes suggest that the taxon belongs within a distal clade of this subfamily. They are over 9.2% pairwise divergent from any hitherto cleanly sequenced Lepidoptera taxon and over 10.1% from nearest taxonomically identified neighbours in Neighbor Joining and ML trees. Characteristics of the DNA barcode and morphology of this new taxon suggest that refinement of synapomorphies for the family and two subfamilies is needed. We have nearly 100 records for this new species since 2020, all from a small area of Southern Spain, close to the Straits of Gibraltar. The life history and early stages of the species are unknown.

Introduction

The family Lecithoceridae is a poorly known group of microlepidoptera which are found principally in the Oriental tropics, and the Australian and Afrotropical regions. Only 64 species (4.5% of the total number) are known from the Palaearctic region (Park et al. 2022) and, of these, only 10 species are reported from Europe (for these purposes the European Economic Area and other Western European countries) (<https://lepiforum.org/wiki/taxonomy/Gelechioidea/Lecithoceridae?view=0®ions=eu>). Five of these species have previously been recorded from Spain. All these European taxa belong to the Ceuthomadarinae and Lecithocerinae. Here we describe a micro moth which is genetically divergent from all hitherto DNA barcoded Lepidoptera and differs morphologically from described species of Lecithoceridae.

The taxon was discovered by Dave Grundy (DG), who found the first specimen at mercury vapour light at the research facility at the Centro Internacional de Migración de Aves (CIMA), Tarifa, Cádiz Province, Spain on 18 May 2020. Since this discovery, DG has recorded a total of 93 adult specimens from various locations within or near the research centre at CIMA and a further two adult specimens from Huerta Grande, Pelayo, Cádiz Province, also near to the coast on the

Spanish side of the Straits of Gibraltar, approximately 10 kilometres from CIMA. These have all been found at mercury vapour light or LEDs.

In seeking to identify these specimens we first considered the European taxa of Lecithoceridae (comprising two Ceuthomadarinae in the genus *Ceuthomadarus* Mann, 1864 and eight Lecithocerinae in the genera *Eurodachtha* Gozmány, 1978, *Lecithocera* Herrich-Schäffer, 1853, and *Homaloxestis* Meyrick, 1910, but here not including two Oditinae (Peleopodidae): Karsholt and Razowski 1996; Gozmány 2012; Barton 2015). However, these taxa were easily ruled out on morphological grounds. A search of images on Google revealed striking similarities in general habitus and even resting posture of the new taxon to the genera *Sisyrodonta* Meyrick, 1922 and *Protolychnis* Meyrick, 1925 especially as regards the thickened antennae and wing pattern. The arrival of Park *et al.*'s (2022) global review of Torodorinae, together with Park's previous comprehensive treatment of Crocanthinae (Park 2015), allowed us to check from a morphological perspective all possible generic affiliations for those subfamilies. To go further, we DNA barcoded three specimens and subsequently analysed all existing DNA sequences of Lecithoceridae in the public domain together with the COI data for the new taxon.

On the basis of the molecular evidence set out here and comparative morphology, using a process of elimination benefitting from the comprehensive accounts of Park (2015) and Park *et al.* (2022), as well as comprehensive searches of the Gelechioidea collection at the Natural History Museum, London (NHMUK), we conclude through careful consideration of taxonomic placement that the taxon described here represents a previously unknown European lineage in the Torodorinae and a new genus.

Materials and methods

The examined specimens for this paper were obtained live from light traps, refrigerated overnight and set on the following morning. The morphology of 16 specimens of this taxon collected by Mark Sterling (MS) and DG was examined. The illustrated material was photographed using a Canon EOS 5DSR camera and MP-E 65 mm lens equipped with a Stackshot system operated by Helicon Remote software (version 3.8.4 W); the shots were eventually stacked with Helicon Focus software (version 6.7.1), which was set up with montage controlled by Helicon using a motorised deck in about 30 to 40 steps for adults and 10 to 15 steps for genitalia and wing preparations. Genitalia dissection and mounting followed Robinson (1976). Descriptions of the genitalia follow Klots (1970) and Kristensen (2003).

The DNA from three male specimens obtained from Tarifa, Cádiz, Prov. Andalucía, Spain, NHMUK013698467–9 (details in Type Material) was extracted at NHMUK from single hindlegs, and following purification of the resulting genomic DNA, standard Sanger PCR was used to amplify COI-5P and the amplicons checked visually using a gel using the same methodology as in Sterling *et al.* (2022), see also Cuber *et al.* (2023), with a mix of the Folmer primers (HCO2198, LCO1490; Folmer *et al.* 1994) and Hebert primers (Lep-F1, Lep-R1). The following steps employed third generation sequencing technology (see Cuber *et al.* 2023 for precise procedures). As part of two 96 well plates for a range of samples, a library was prepared by ligating standard Illumina indexes (unique 20 bp tags cross-referencing sample/well to up to 658 bp COI-5P fragments) to 20 bp M13 reverse tails attaching to each DNA fragment, using the Oxford Nanopore Technologies (ONT) SQR-LSK110 ligation kit. The sample fragments were then pooled and pipetted on the loading well of a single-use 200-pore ONT Flongle flow cell (R9.4.1, FLO-FLG001) that had previously been primed by hand pipetting of the supplied buffer. The Flongle was then fitted to a GRIDION X5 benchtop sequencing machine (<https://nanoporetech.com/products/gridion>) and run for 72 hours. A single strand was read

through by the machine singly base-by-base using ion current disturbance technology. ONT barcode software (ONTbarcode v0.1.9: <https://github.com/asrivathsan/ONTbarcoder>; Srivathsan et al. 2021) was then used in the bioinformatic pipeline for retrieving the tagged DNA barcode fragments for each sample. Between 271–388 sequences were used during this demultiplexing process, to achieve a minimum coverage of N25 (25 fragments per DNA barcode at high fidelity for consensus base calls) for each 658 bp sequence. Sequences are available in the public project DS-LECITH (http://v4.boldsystems.org/index.php/MAS_Management_DataConsole?codes=DS-LECITH) with Process Ids UKMOT004-23, UKMOT005-23, UKMOT006-23 and BIN BOLD:AFA0579 on BOLD and Accession numbers OQ339151, OQ339152, OQ339153 respectively, on GenBank.

We first checked the global database of DNA barcodes on BOLD using the Identification Engine (https://v4.boldsystems.org/index.php/IDS_OpenIdEngine) and building the corresponding Neighbor Joining tree. This led to a more detailed molecular examination of the information content in DNA barcodes and tree building analyses using COI-5P.

We downloaded available Lecithoceridae from BOLD on 21/12/2022 for comparative analysis with the DNA barcode of the query taxon. We downloaded from GenBank the mixed COI and seven-gene nuclear datasets for Lecithoceridae of Kaila et al. (2011) and Wang and Li (2020) as well as three sequences for *Homaloxestis croceata* Gozmány, 1978 from the study of Regier et al. (2013) along with their DNA barcodes from BOLD. Alignment was done gene by gene using MAFFT online (Q-Ins-I option; <https://mafft.cbrc.jp/alignment/server>). We added all 17 Lecithoceridae exemplars on GenBank from these datasets (here excluding the highly divergent genus *Martyringa* Busck, 1902, as more closely related sequences, less susceptible to long branch attraction, could be used for rooting). Sequences were concatenated for the aligned genes against their sample numbers in MS Excel.

For the Lecithoceridae DNA barcodes, 435 unique BIN (Barcode Index Number) representatives that each had the longest sequence length in the corresponding ‘tsv’ file that had been downloaded from BOLD along with their GenBank accession numbers, where available, were considered and a few obvious non-members of Lecithoceridae were eliminated. We carried out a similar process for the related families Autostichidae and Xyloryctidae. Pairwise divergences were computed in this program using the Pairwise Alignment option in Bioedit 7.2.5 (Hall 1999) (‘Calculate Identity/Similarity for two sequences’) for comparable nucleotides/codons only, whereas codons were analysed using the ‘Conservation Plot’ option to a reference sequence that was edited to show the triplet ‘NNN’ for potentially informative nucleotides (reading as X for codons), and their frequencies calculated using copy/paste into a column in MS Excel. We directly examined the DNA barcodes using Bioedit alongside the DNA barcodes of the query taxon to see if there were characters linking it to particular groupings, or any character that distinguished Lecithoceridae in general. Considering these 435 BINs, six could be eliminated as obvious representatives of other families: *Rhizosthenes falciformis* Meyrick, 1935 (BOLD:AAH8698), see Wang and Li (2020: 8); the BINs BOLD:ACU2376 and BOLD:ADH8338 represent other Peleopodidae: Oditinae; BOLD:AAH3806 represents a Cosmopterigidae: Scaeosophinae; BOLD:AEA2583 represents a Cemiostomidae and BOLD:AAJ5084, NSWHP3227-19 represents a Stathmopodidae (not a *Crocantbes* Meyrick, 1886), leaving 429 BINs for further analysis. For the family level analysis we similarly considered 221 BINs in Autostichidae and 810 BINs representing Xyloryctidae.

A local BLAST was conducted in Bioedit of the (up to 658 bp) dataset constructed from these 429 sequences, to find the nearest hits, and the pairwise divergences were checked for these over comparable codons as described above.

To go further, we examined the placement of our sequences in relation to the GenBank and BOLD datasets. The idea was, when adding available DNA barcodes of Lecithoceridae, to provide a provisional scaffold for the families and subfamilies, whilst avoiding problems of paralogy by having a COI part of the dataset across all taxa, analyses that we detail here.

For the nuclear data we eliminated IDH as having only three representatives of only two subfamilies, but no Torodorinae. We analysed the resulting 5408 bp alignment (COI 1475 bp including COI-5P for 12 representatives, EF-1 α 985 bp for 15, Wingless 400 bp for 16, RpS5 600 bp for 12, CAD 850 bp for nine, MDH 407 bp for 10, and GAPDH 691 bp for six representatives). For a quick tree analysis we used Phyml 3.0 (online: <http://www.atgc-montpellier.fr/phyml>), with the GTR + G + I model selection as implemented by the Bayesian Information Criterion (BIC), and showing ABayes support.

Phyml runs were done by building datasets of publicly available DNA barcode sequences from BOLD that had been identified or were identifiable to at least generic level, alongside the 5408 bp alignment for 17 taxa, selecting where possible the longest public domain sequence within one representative of each Barcode Index Number (BIN), along with the mixed seven-gene COI and nuclear alignment. We concentrated on being as comprehensive as possible within these constraints for Torodorinae and Crocanthaenae (testing between 21 and 32 terminals), ending up with a 51-terminal dataset.

For further analyses of the 5408 bp Lecithoceridae alignment from GenBank, we used IQ-TREE (<http://www.iqtree.org>) on the 17-taxon portion of the dataset, with or without the two haplotypes of the DNA barcode of the query taxon, using a partitioning file specifying the start and ends of each of the seven genes and using automatic selection (Bayesian Information Criterion) to allocate the optimal model for each gene, displaying both ABayes and Bootstrap values (100 runs), with parameters edge-linked or edge-unlinked across the partition.

Finally, we carried out a gene-partitioned analysis of the entire dataset (51 taxa, 5408 characters) in MrBayes 3.1. This analysis implements the GTR model as in Phyml but allows rates to vary among partitions using a rate multiplier. We used the following parameter settings: *nst*=6 and *rates*=*invgamma*. In one run we specified unlinking of the parameters *statefreq*, *revmat*, *shape*, and *pinvar*, whereas in the second, they remained linked (by default). In both cases, a variable rate prior was set, the run included 10,000,000 generations under a Markov Chain Monte Carlo process, and the burnin rejected the first 25% of trees in four chains, finally verifying for convergence between two tree runs. The resulting consensus trees provide a more rigorous and direct assessment of Bayesian posterior support than is estimated using ABayes in Phyml.

Suppl. material 1 provides details of all the sequences used in Fig. 1, and where they were published for the first time, if not in this paper. The underlying alignment is also available in an online repository (<https://doi.org/10.6084/m9.figshare.22242250>).

Molecular analysis results

DNA barcode query and distances, Neighbor Joining, BLAST

Query of the DNA barcodes of the query taxon (the barcode of NHMUK013698469 is identical to that of NHMUK013698468 and differs by a C as opposed to a T in position 118 of the holotype, NHMUK013698467) on BOLD placed the taxon as more than 9.06–9.22% by p-distance from any other micromoth. However, as an exception, a single gelechiid came up as the top hit (*Ephysteris diminutella* (Zeller, 1847), Process ID LON7008-18, GenBank accession MN805721, which is

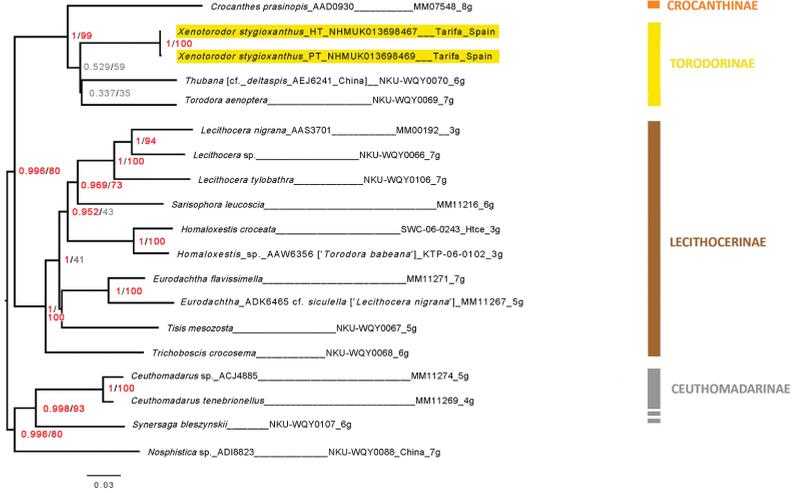
8.99% by p-distance). Of its 618 bp, 62 are ambiguously coded. When these are ignored, it is 9.0% pairwise divergent (Bioedit), whereas a clean (658 bp) sequence adjacent by its Process ID code for the same species, LON7007-18 (MN805536) is 13.07% divergent to the query taxon. This sequence should ideally be removed from consideration by BOLD. All the other ten top hits were unidentified Lepidoptera (probably lecitocerids) or identified as Lecithoceridae. The nearest identified lecitocerid species at 9.2–9.38% p-distance for two different specimens was identified as ‘*Thubana exaema*’ (i.e., *Thubana exoema* (Meyrick, 1911)) from Sri Lanka (see Discussion). The two haplotypes of the query taxon were 9.55–9.71% to the nearest two sequences identified only as a lecitocerid, and 9.68–9.82% to the nearest ones identified as a *Torodora* (BOLD:AAH3804 from Australia and BOLD:ABY1674 from Vietnam). In the corresponding NJ tree, the query taxon linked, albeit with long branches, to eight nearest terminals representing five BINs all from SE Asia, two of which were identified as Lecithoceridae and one of which as *Torodora* Meyrick, 1894, whereas it fell relatively remotely from *T. exoema* among the top 99 hits.

As another distance approach, using a local BLAST in Bioedit of the 429 Lecithoceridae sequences representing different BINs downloaded from BOLD, the sequences of the query taxon were 9.57–9.73% pairwise divergent to a species of Lecithoceridae (BOLD:ACT7825, LNAUT3910-15, Malaysia), 9.8–9.95% to another Lecithoceridae (BOLD:ADV1376, GMPBS211-18, Pakistan), 10.18–10.33% to *Halolaguna subluxata* Gozmány, 1978 (BOLD:ABA2899, KF523781) and 10.1–10.35% to *Torodora aenoptera* Gozmány, 1978 (BOLD:AEG4946, MN852952), these representing the four top “hits” by local BLAST.

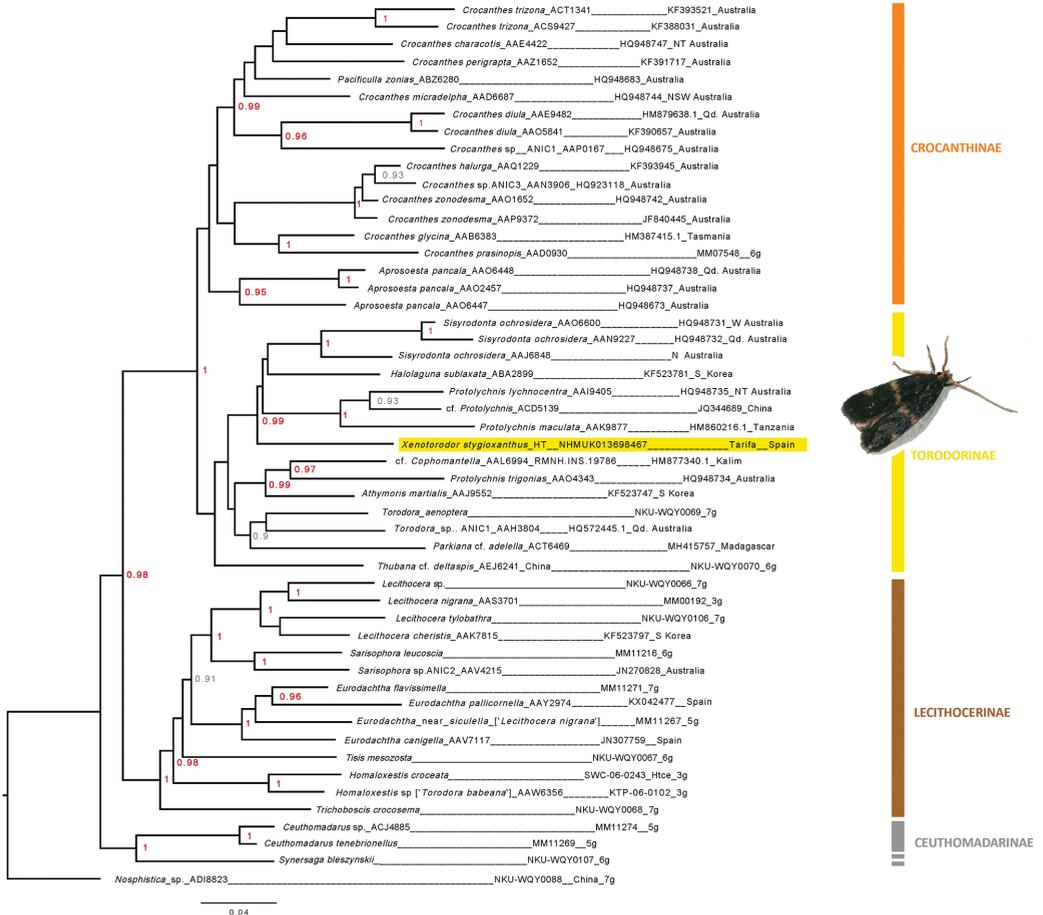
As a third approach, the top hit using nucleotide BLAST on Genbank on 29/01/2023 was *Halolaguna subluxata* (KF523779.1) at 9.86–9.71% divergence (depending on the haplotype of the query taxon). However, equally divergent was a member of Xyloryctidae, *Xylorycta cirrhophragma* Meyrick, 1921 (KF404885.1), with *H. subluxata* (KF523781.1) at 10.02%, showing weak signal to noise for this approach, which does not benefit from the DNA barcode dataset comprehensiveness of BOLD.

Inspection of sequences for synapomorphies

We then examined the amino acid translation of the DNA barcode region of the alignment, first examining the 429 BINs attributed to Lecithoceridae. The 161st complete codon has the state Asparagine (N), as opposed to Serine (S), which is more usual in Gelechioidea. This is the state in NHMUK013698467–9 and is particularly prevalent in Lecithoceridae. Among the Lecithoceridae BINs, the following states (using standard amino acid abbreviations) are represented: N = 91.8%, S = 7%, K = 0.9% and D = 0.2%. By contrast, among the 221 Autostichidae BINs the proportions of states were N = 0.9%, S = 94.6%, others = 0.45% (F, L, M, T, Y), whereas among the 810 BINs of Xyloryctidae, N = 2.5%, S = 71.7%, F = 9.5% and other states 16.3% (Y, M, A, T, Q, L, K, I). An ‘N’ (generally the triplet AAT although sometime AAC) was a feature of all the sequences identified to at least a Torodorinae genus, except that an apparent reversal (see Fig. 1b) to S (the triplet AGT) occurred locally in two of four *Sisyrodonta* BINs (BOLD:AAN9227, BOLD:AAO6600), and among those that belong to Lecithocerinae, a reversal to S was seen in the *Lecithocera nigrana* (Duponchel, 1835) complex (comprising one BIN and six closely related BINs from Greece, Madagascar, and Pakistan), and in two BINs of *Sarisophora* Meyrick, 1904 from Australia. We found no such clear synapomorphies at subfamily level for single nucleotide or codon positions, although some group-specific patterns in complete codon 94.



a



b

Figure 1. a. Phylogenetic analysis using ML as implemented in IQ-TREE (edge-unlinked), of Lecithoceridae using 17 taxa from GenBank for a matrix of 5408 characters and 3–8 nuclear genes ('3g'..'8g'), together with DNA barcodes of the holotype and a paratype of *Xenotorodor stygiioxanthus* gen. nov., sp. nov., showing a supported position in (Crocantinae + Torodorinae), and a branch with greater proximity to species of *Thubana* and *Torodora* than to the type species of *Crocantines*, *C. prasinopis*. The first support value for each node is ABayes, and the second percentage of 100 bootstraps. The tree is displayed as 'midpoint' rooted.

b. Phylogenetic analysis of Lecithoceridae in Phym 3.0 showing the placement of the holotype DNA barcode of *Xenotorodor stygiioxanthus* gen. nov., sp. nov. (an individual is shown in its 'alert' resting posture). The tree is based on a matrix of 51 taxa and 5408 characters, with all taxa represented by COI-5P. Terminal names include the last seven characters of the BOLD cluster (BIN, abbreviated from the format 'BOLD:ABC1234'), and the country of origin, where known or relevant. '3g'..'8g' specifies the number of genes used (two sections of COI and EF-1a are treated as single genes), for requisite taxa whose nuclear data is from GenBank. ABayes support values are shown to the right of nodes; values < 0.95 are not considered supported for the purposes of this analysis. The tree is rooted on *Nosphistica*, and all subfamilies are delineated, where known.

IQ-TREE analysis

Based on analysis of 17 terminals including nuclear data or 19 terminals including the two haplotypes of the query taxon, IQ-TREE automatically implemented the following partitioned model selection: COI: GTR+F+I+G4; EF-1a: Tim3e + G4; Wingless: K2P + G4; RpS5: TIM2e + G4; CAD: TIM2 + F + G4; GAPDH: TIM2u + F + I; MDH: TIM2e + G4. Although the edge-unlinked partitioned analysis in IQ-TREE provided a slightly higher log likelihood than the corresponding edge-linked analysis (-23644 to -23770; 17 terminals and -23805 +/-343 to -23939 +/- 346; 19 terminals) the standard errors overlapped and the lower Bayesian Information Criterion for the edge-linked analysis was therefore preferred (48548 vs 49261). In this analysis, when 17 taxa were run (i.e., only taxa with nuclear data), the Ceuthomadarinae node showed a value of pp = 1 /bootstrap = 100%, with the Lecithocerinae + Torodorinae + Crocantinae node with 1/64%, the Crocantinae + Torodorinae node with 1/100%, and the Torodorinae node (*Thubana* + *Torodora*) not achieving support thresholds (0.877/64%). When the two haplotypes of the query taxon were added (Fig. 1a) and 19 terminals were run, the respective nodal values became 1/100% (Ceuthomadarinae), 0.998/93% (*Ceuthomadarus* + *Synersaga*), 0.996/80% (latter including also *Nosphistica*), 1/100% (Lecithocerinae), 0.996/80% (Lecithocerinae + Crocantinae + Torodorinae), 1/99% (Crocantinae + Torodorinae), but unsupported (0.337/35%) for the existing Torodorinae, and no support either (0.529/59%) for a sister relationship between the new taxon and *Thubana* cf. *deltaspis* Meyrick, 1935 (Fig. 1a), although the branch of the DNA barcode of the query taxon fell closest to the torodorine rather than crocantine branch.

Phym analysis of full dataset

We attempted various phylogenetic analyses of available sequences using Phym 3.0, an instance of which is shown (Fig. 1b). We based the last analysis on a matrix of 51 taxa and the 5408 nucleotide positions, including the 17 terminals from GenBank. This dataset is contiguous for all taxa across 483 positions of COI-5P (with all but 10 exemplars contiguous over 658 bp), using the GTR + G + I model that was selected by BIC. For these 17 taxa, dispersed across the subfamilies, COI-3P and 3–7 nuclear genes were added from the GenBank dataset referred to in Materials and Methods in order to provide a general framework for rooting and for the subfamilies (number of genes shown in Fig. 1b, otherwise just COI-5P).

The Phym1 analyses never placed the new taxon within the Ceuthomadarinae nor the Lecithocerinae. These groups each appear to be monophyletic according to Wang and Li 2020 and our analysis (see Park *et al.* 2022 regarding the relationship of “*Torodora babeana*” to *Homaloxestis* Meyrick, 1910). Rather, they consistently placed the DNA barcode of the new taxon within a distal group of Lecithoceridae, *i.e.*, the subfamilies Torodorinae + Crocanthinae (in the last case comprising representatives of the genera *Crocantnes*, *Aprosoesta* Turner, 1919 and *Pacificulla* Park, 2013). Consistently the DNA barcode of the query taxon fell in a group or clade (pp = 0.99 in Fig. 1b with *Sisyrodonta* ‘*ochrosidera*’—a complex of BINs, three BINs within *Protolychnis*, and *Halolaguna subluxata*. BOLD places *Sisyrodonta* in Lecithocerinae, while Common 1990 has it in the Lecithoceridae, but our analyses always placed it in this grouping of Torodorinae. However, our analyses always failed to provide a significant support level for a sister genus or the sister group of the branch of the query taxon.

The Phym1 3.0 analysis only showed separation of Crocanthinae and Torodorinae in cases when taxon/BIN sampling was relatively dense (instance shown in Fig. 1b). More often with fewer terminals (especially among *Crocantnes*, which include several multi-BIN species on BOLD), resulting trees had Crocanthinae and Torodorinae partially intermingled, occasionally with the query taxon grouping with *C. diula* and *C. prasinopis*. When the sampling of identified taxa from BOLD among Torodorinae plus Crocanthinae was improved, Torodorinae formed a separate grouping as did Crocanthinae, albeit without support. The topology of Lecithocerinae was similar to that shown by Wang and Li (2020: 5, S2, S3) except for the position of *Homaloxestis*. The query taxon fell in a supported grouping (p = 0.99) with the three *Protolychnis*, three *Sisyrodonta* and *Halolaguna subluxata*.

MrBayes analysis of full dataset

The MrBayes analyses on the 51-terminal 5408 bp dataset using a paratype sequence of the query taxon (NHMUK013698469, OQ339153) provided a 50 percent majority rule consensus tree (shown in Suppl. material 2, unlinked and Suppl. material 3, linked). These analyses show support for Ceuthomadarinae (pp = 1), but for *Ceuthomadarus* + *Synersaga* only in S3 (pp = 0.96). Also, they show support for Lecithocerinae (pp = 0.99 and 1 respectively), but a lack of resolution for Torodorinae + Crocanthinae. Within this “bush”, there were a number of unsupported groupings that include the query taxon (+ *Halolaguna* + three each of *Sisyrodonta* and *Protolychnis* with pp = 0.97, Suppl. material 3 or without support, Suppl. material 2), (*Athymoris* Meyrick, 1935 + *Cophomantella* Fletcher, 1940 + *Protolychnis trigonias*, pp = 0.95, Suppl. material 3 or without support, Suppl. material 2), in each case with unsupported groupings of *Crocantnes* and *Aprosoesta* ‘*pancala*’).

Morphological systematics

Xenotorodor Sterling, Lees & Grundy, gen. nov.

<https://zoobank.org/75DA3064-908C-4764-B2EC-1E4AD58C887C>

Type species. *Xenotorodor stygioxanthus* Sterling, Lees & Grundy, sp. nov.

***Xenotorodor stygioxanthus* Sterling, Lees & Grundy, sp. nov.**

<https://zoobank.org/02DC9393-4978-4D54-B25C-29DFDE08B3E5>

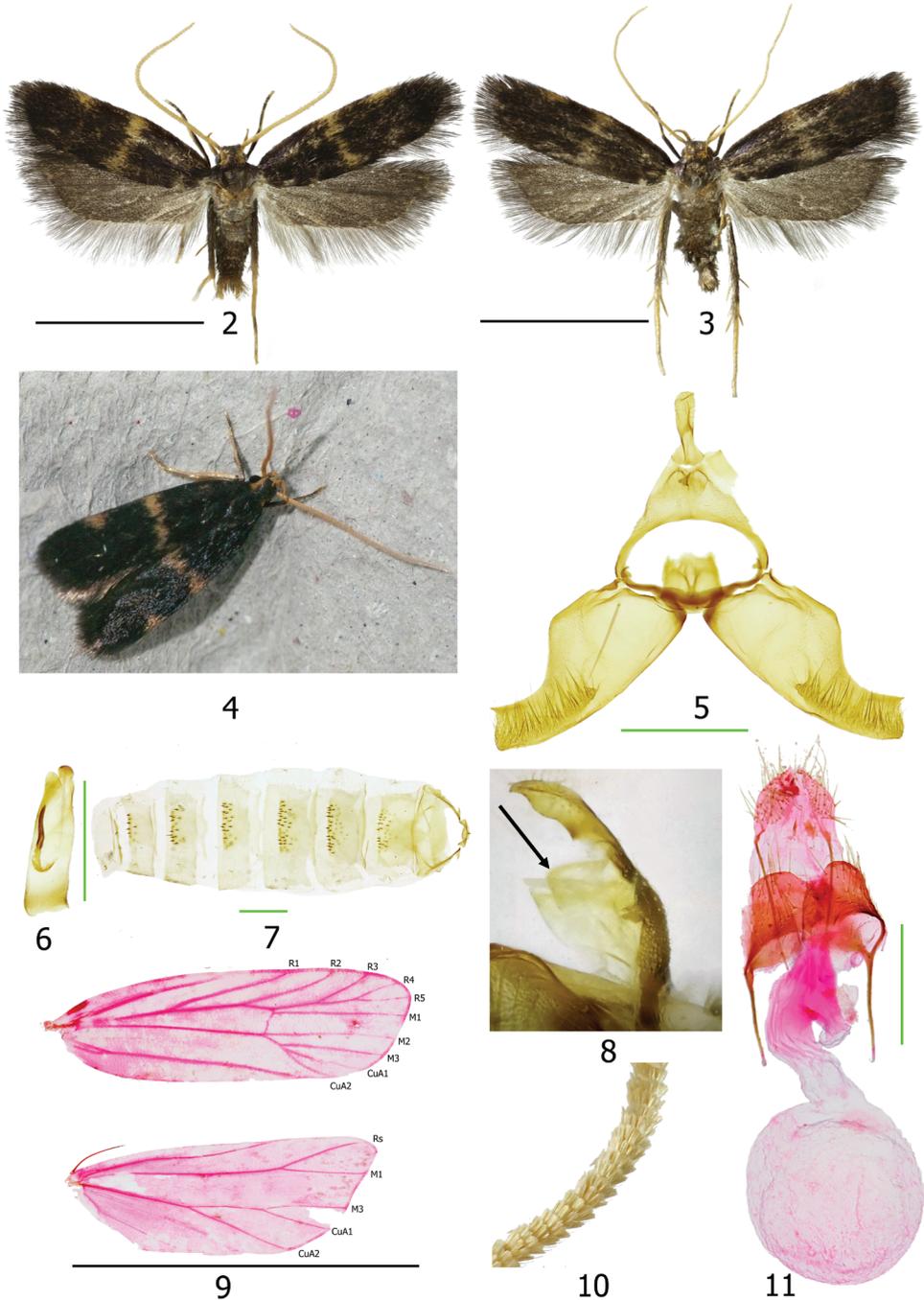
Type material. *Holotype* ♂ Spain, Cádiz, Tarifa, CIMA, Site 1, MV light, 24.v.2022, 36.0167, -5.5864, 60 m, Leg. M. Sterling, D. Grundy, specimen no. NHMUK013698467, slide no. NHMUK014331156, wingspan 14 mm, fwl 6.25 mm.

Paratypes (12♂, 3♀) 2♂, collection data as holotype, specimen number NHMUK013698468, specimen number NHMUK013698469; 1♀, 27.05.2022, otherwise same collection data for Site 1, specimen no. NHMUK013699868, slide no. NHMUK014331160. 3♂ Spain, Cádiz, Tarifa, CIMA, Site 2, MV light 25.v.2022, 36.0166, -5.5858, 75 m, specimen no. NHMUK013699866, slide no. NHMUK014331157, specimen no. NHMUK013699867, slide no. NHMUK014331158, specimen no. NHMUK013698979, slide no. NHMUK014331159; 1♀ 27.v.2022, otherwise same collection data for Site 2, specimen no. NHMUK013698523, slide no. NHMUK014331162. 3♂, Spain, Cádiz, Tarifa, CIMA, Site 3, MV light 27.05.2022 36.0148, -5.5871, 85 m, specimen no. NHMUK013699869, specimen no. NHMUK013699871, specimen no. NHMUK013698522; 1♀, same collection data as site 3, specimen no. NHMUK013699870. 3♂ Spain, Cádiz, Tarifa, CIMA, Site 4, MV light, 29.v.2022, specimen no. NHMUK013698524, specimen no. NHMUK013698525, specimen no. NHMUK013698526, 1♂ Site 4, 30.v.2022, specimen number NHMUK013699872. The holotype and 12 paratypes have been deposited at the NHMUK. In accordance with the terms of the collecting permit, the three last mentioned paratypes have been deposited with the Museo Nacional de Ciencias Naturales, Madrid.

Diagnosis. Forewings black with a purplish sheen, a narrow yellow bar from costa to dorsum before $\frac{1}{2}$ and a yellow costal spot at $\frac{3}{4}$ (Figs 2–4), antenna and labial palps yellow, antenna of male thickened with rings of large yellow scales projecting outwards from base of each flagellomere (Fig. 10). R3, R4 and R5 of forewing with a common stalk. M2 present in forewing but absent in hindwing. M3, CuA1 and CuA2 of forewing with a common stalk, CuA1 and CuA2 stalked in forewing (Fig. 9). Male genitalia with small membranous gnathos and very small sclerotised mesial process (Figs 5, 8), vinculum semicircular, sclerotised and melanised (Fig. 5). Female genitalia with appendix bursae, corpus bursae small and rounded without signum (Fig. 11).

Description. Male. Wingspan 13–15 mm. **Head.** Ocellus absent, frons dark grey, vertex dark grey with thick lateral tufts of dark grey and dark yellow scales, yellow scaling continuing above the eye; maxillary palps very small, grey, pilifers with thick brushes of short yellowish bristles. Labial palps long, thin, strongly recurved, approximately 3× diameter of eye, dark yellow, basal segment paler than other segments, second and third segment same length. Antenna same length as forewing, yellow, long scape with appressed scales, rings of thick yellow scales pointing outwards from base of each flagellomere give antenna a thickened appearance (see Fig. 10). Haustellum with basal half scaled whitish. **Thorax.** Thorax and tegulae black. Foreleg dorsally dark grey, ventrally yellow, small tibial epiphysis, middle leg yellow with fine dark grey scales, tarsus of hind leg with fine dark grey scales, tibia and femur yellow with some darker scaling. **Wings.** Venation: Forewing with R3 stalked with stalk of R4 and R5, R4 and R5 stalked, M1 present, M2 and M3 almost parallel, M3 stalked with stalk of CuA1 and CuA2, CuA1 and CuA2 stalked. Hindwing with M2 absent (Fig. 9). Forewing costa slightly arched, apex rounded, termen angled inwards, tornus obtusely angled; ground colour black with a purplish sheen, thin yellow bar before $\frac{1}{2}$ from costa to dorsum, yellow patch on costa at $\frac{3}{4}$, cilia long, black. Hindwing with apex slightly projecting, dark grey, unmarked, cilia long, concolorous.

Female. Similar (including labial palps). Rings of antennal scales shorter and colour of antenna paler than in male.



Figures 2–11. *Xenotorodor stygioxanthus* gen. nov., sp. nov. **2.** ♂ Holotype. **3.** ♀ Paratype. **4.** ♂ Habitus. **5.** Male genitalia. **6.** Aedeagus. **7.** Pre-genital abdomen (**5–7.** ♂ holotype slide no. NHMUK014331156). **8.** ♂ Holotype lateral image prior to mounting, gnathos indicated. **9.** Wing venation slide no. NHMUK014331163. **10.** ♂ Holotype, highly magnified section of antenna. **11.** ♀ Paratype, female genitalia, slide no. NHMUK014331160. Scale bars: 5 mm (black); 0.5 mm (green). Figs **4**, **8** and **10** not to scale.

Male genitalia. Uncus directed caudally, narrow, elongate, slightly spatulate posteriorly, with narrow elongate lateral flanges. Gnathos fused to tegumen, small, box like, membranous, a very small sclerotised projection from tegumen medially. Tegumen broad anteriorly, tapering and indented posteriorly, weakly sclerotised, rough textured; pedunculi short, slightly curved. Vinculum semicircular, thin, strongly sclerotised, melanised. Saccus short and very broad. Juxta short and broad, almost square in shape, without caudal projections. Valva weakly sclerotised, costal margin bulging medially, sharply converging towards ventral margin postmedially, cucullus rectangular, small pointed projection at apex, cucullar region with dense broad setae, small digitate process near inner margin of setae. Aedeagus short, straight, weakly sclerotised, with a small rounded projection posteriorly, and a single broad arrow headed cornutus medially, strongly sclerotised posteriorly, hooked anteriorly.

Female genitalia. Papillae anales short and broad. Apophyses posteriores over 1.5× length of apophyses anteriores. Eighth tergite rectangular. Eighth sternite indented posteriorly. Ostium circular. Antrum short, broad, membranous. Ductus bursae short, broad and membranous, appendix bursae present. Corpus bursae rounded, without signum.

Biology, behaviour and early stages. Collection of samples of leaf litter for early stages in May 2022 proved unsuccessful and the early stages are unknown. Since its discovery in 2020, the adult has been recorded in May, June, August, September and November. The principal emergences are in late May/June and August with 20 records between 18 May and 21 June 2020, 48 records between 24 May and 11 June 2022 and 22 records between 17 and 22 August 2021. Recording in May/June 2021 was not possible due to COVID restrictions. Both sexes are attracted to mercury vapour light and have been found flying around MV lights in the two hours after darkness. When the adult is resting the antennae are projected upwards and sideways (see Fig. 4) in an alert posture and tucked under the costa of the forewing when fully resting. The wings are always posteriorly flat to the resting surface (Fig. 4).

Distribution. The taxon is known principally from various localities in an area consisting of a patchwork of Mediterranean scrub, dry cattle grazed pasture, wild olive groves and some *Eucalyptus* sp. plantations, approximately 0.5 kilometres from the coast around the research centre at CIMA near the town of Tarifa in Southern Spain. Two specimens (not retained) were found in June 2022 at Huerta Grande, Pelayo (36.081, -5.503, 250 m) approximately 10 kilometres from CIMA and slightly further inland. Huerta Grande has been well recorded by DG in the flight period of this species for the last 10 years and the moth has never been recorded there before, so this is believed to be a new arrival. The climate in the presently known range is wet and warm in winter (temperatures not usually below freezing) and almost completely dry in summer.

Etymology. *Xenotorodor* from *xenos*, gr., meaning, among other things, stranger or outsider. This is a reference to the unusual combination of morphological features for a species of *Torodorinae* in the new taxon, and the substantial geographical extension of the range of the subfamily. The gender of the genus name is male. The specific name *stygioxanthus* is from *stygios*, gr., meaning among other things extremely dark; and *xanthos*, gr., meaning yellow, a reference to the blackish forewings marked with yellow.

Material examined. Type material and six unset and unpinned specimens in tubes with the following data, which are excluded from the type material: 4♂ Spain, Cádiz, Tarifa, CIMA, MV light, 17–21.viii.2021, leg. D. Grundy; 2♂ Spain, Cádiz, Tarifa, CIMA, MV light, 05–13.xi.2021, leg. D. Grundy. The remainder of the specimens recorded were not retained although photographs of 20 of these specimens, taken by DG, were examined.

Discussion

Family placement

Park *et al.* (2022: 12, 14) note the following [syn-]apomorphies for Lecithoceridae: gnathos fused to tegumen, antennae usually longer than forewing and mesial process of gnathos of the male genitalia always downturned and laterally compressed. In the new taxon the gnathos is fused to the tegumen, the antennae are the same length as the forewing (Park *et al.* (2022: 15) note that a recent study of the Afrotropical fauna has confirmed that the antenna is not always longer than the forewing in several genera, including *Protolychnis*) but the gnathos of the new taxon is much reduced (Figs 5, 8). However, the subfamily Crocanthinae is defined as having the autapomorphic character with the gnathos always absent or reduced in the male genitalia (Park 2015: 252; Park *et al.* 2022: 16). At a family level, the reduced gnathos in the new taxon is therefore not inconsistent with a diagnosis of Lecithoceridae. The wing venation, including the stalking of R3, R4 and R5 and the stalking of CuA1 and CuA2 in the forewing and the absence of M2 in the hindwing is typical of a number of lecithocerid genera, as is the presence of tergal spines in the pre-genital abdomen. In the male genitalia, the narrow thorn like uncus, thin strongly sclerotised vinculum and the vestigial saccus are also often found in Lecithoceridae. The rings of outwardly pointing yellow scales arising from each flagellomere on the male antenna are also potentially synapomorphic with *Protolychnis* and *Sisyrodonta*, if those two taxa are found to belong to a clade not including *Halolaguna* (but see Fig. 1b).

The results from the DNA barcode query and the analyses considering distance and Neighbor Joining support a diagnosis of Lecithoceridae for the new taxon. Also, from our search of the sequences for synapomorphies, we consider the state Asparagine (N) in the 161st complete codon of the DNA barcode to represent a ground plan synapomorphy of Lecithoceridae, which is reversed in a few genera and species (for example distally in the genus *Sisyrodonta* and in *Lecithocera nigrana* and its widely dispersed species complex). The new taxon exhibits this synapomorphy. This is a groundplan feature of all lineages of Lecithoceridae *sensu* Wang and Li (2020), apart from their outgroup, *Martyringa* (USA-SE Asia), which exhibits either a Phenylalanine (F) or Serine (S) for this position, whereas *Ceuthomadarus*, *Synersaga* and *Nosphistica* all show an Asparagine in the homologous position.

Subfamily placement

According to Park (2022) the subfamily Ceuthomadarinae can be distinguished from the other subfamilies based on the absence of a proboscis and the subfamily Lecithocerinae can be defined and distinguished from Torodorinae by the presence of a bridge-like structure connecting the tegumen and the valval costa of the male genitalia. The new taxon has a well developed proboscis and does not have the bridge-like structure in the male genitalia used to distinguish Lecithocerinae from Torodorinae. The new taxon is therefore the first record of a new subfamily of Lecithoceridae for Europe. However, placement, on morphology, between the current concepts of Crocanthinae and Torodorinae is not as straightforward. On the basis of Park's definition of Crocanthinae, it would appear that the new taxon should be placed in Crocanthinae because it has a reduced gnathos. However, apart from the reduced gnathos, there is little in the adult morphology to connect the new taxon to Crocanthinae.

The suggested synapomorphies of Crocanthinae, apart from the state of the gnathos, include relatively bright coloured wings and hindwing often with similar markings like those of the forewing

(Park et al. 2022: 17). The new taxon has neither wing synapomorphy. Park (2015) redefines *Crocantthes* (the type genus of Crocanthinae) with the following morphological characters: labial palpus with dimorphism, male with second segment long, thickened with rough scales, and third segment absent, aborted or shortened, but female with normal slender third segment. Forewing normally elongate, with usually well-developed postmedian fascia; costa gently curved beyond 2/3; apex acute or normally produced; termen usually concave medially or slightly convex; venation with R2 usually free or sometimes short-stalked with R3 and R4; R5 absent; M2 absent; CuA1 and CuA2 short-stalked; cell opened. Hindwing usually unicolorous, as wide as forewing, apex acute; termen slightly concave or strongly oblique; venation with M2 absent; M3 and CuA1 stalked basally; CuA2 arising from the ½ length of the wing. Apart from the configuration of the hindwing venation, the new taxon displays few of these characters. The labial palps are not dimorphic, in the male the second segment is not thickened with rough scales and the third segment is not absent, aborted or shortened, the forewings are not elongate and do not have a postmedian fascia and the apex is rounded. In the forewing venation, R3 is on a common stalk with R4 and R5, R5 is present and stalked with R4. M2 is present, the stalk of CuA1 and CuA2 is substantial and the cell is closed. In addition, the antennae in *Crocantthes* are not (with the exception of *C. diula*) thickened with outwardly projecting rings of scales. In the male genitalia of *Crocantthes*, the vinculum is U shaped, the juxta usually has caudal projections, the sacculus is developed and the cornuti in the aedeagus are complex.

In addition, the general appearance (externally and in the male and female genitalia) of the new taxon is substantially different from any species currently described within Crocanthinae, which are almost exclusively only known from the island of New Guinea and Australia, although a few genera reach other parts of Indonesia and the Philippines.

Only three existing genera of Crocanthinae are represented here (Fig. 1b) of which *Aprosoesta* tended to be the earliest diverging, and *Crocantthes* is also represented by its type species. The new taxon fell away from the sampled members of Crocanthinae when taxon sampling of identified terminals from BOLD was enhanced (as in Fig. 1b).

The subfamily Torodorinae is defined (Park et al. 2022) by the absence of a bridge-like structure connecting the tegumen and the valva and by the uncus usually thorn-like, directed caudally in the male genitalia. The present taxon has both these synapomorphies (although these are also present in most Crocanthinae). A comparison of the features of the new taxon with Park et al. (2022) shows that, although it has an unusual combination of features, it is the case that taxa with similarity to one or more characters among palps, antennae, wingshape, wing pattern, forewing venation and male genitalia, can be found within Torodorinae. In our view it is not inconsistent for a lecitocerid moth with a reduced gnathos to fall within Torodorinae. We also note that Yu et al. (2022) have recently noted 12 species of *Torodora* (the type genus of the subfamily) in which the gnathos is without a mesial process.

Moving to the molecular data, in interpreting the IQ-TREE analysis on the 19-terminal dataset (Fig. 1a) and based on the Phyml (Fig. 1b) and MrBayes analyses (Suppl. materials 2, 3) of the full datasets, parts of the trees with good coverage of nuclear data (Lecithocerinae in particular with 10 taxa) show relatively good resolution, whereas other parts predominated by COI-5P (in which only *Thubana* cf. *deltaspis*, *Torodora aenoptera* and *Crocantthes prasinopsis* included nuclear data) show relatively weak phylogenetic signal.

The IQ-TREE analysis (Fig. 1a) provides support for placement of the new taxon within a combined clade of Torodorinae + Crocanthinae (1/99), and no support for its placement in Lecithocerinae

or Ceuthomadarinae nor for a clade of *Xenotorodor* plus Crocanthinae (the last grouping occurred in the unlinked analysis, but without support). The analyses of the full dataset (Fig. 1b, Suppl. materials 2, 3) similarly provide no support for placement of the new taxon in Lecithocerinae or Ceuthomadarinae and, although the supporting data is almost all COI-5P, provide posterior support for the placement of the new taxon in a subclade of Torodorinae rather than Crocanthinae.

However, as noted in Park (2022), the subfamilial relationship of Lecithoceridae still reveals some problems. We confirm monophyly of Ceuthomadarinae (although inclusion of *Synersaga* or even *Nosphistica* is equivocal) and Lecithocerinae, although the relationship of these subfamilies varied among analyses and were sensitive to parameterisation, but linked analyses produced relationships consistent with that shown by Wang and Li (2020), with Lecithocerinae sister to Torodorinae + Crocanthinae. However, further work needs to be done to demonstrate that Torodorinae really constitutes a monophylum without the inclusion of all or part of Crocanthinae (i.e., whether proximal, distal, or sister to Crocanthinae in trees). Both the 17- and 19-terminal edge-unlinked IQ-TREE analysis showed support for a Crocanthinae + Torodorinae clade but the Torodorinae-only clade was unsupported by bootstrap in either analysis (or in Fig. 1b).

Generic placement

The morphological differences between *Xenotorodor* and *Crocantthes* are dealt with above. Also, the new taxon is morphologically divergent from all existing genera within Torodorinae and is genetically divergent from those eight traditional torodorine genera (plus *Sisyrodonta*) whose DNA barcode sequences were available for analysis. It displays some distinctive characters shared with some genera (including *Sisyrodonta* and *Cophomantella* which are not currently placed in either Torodorinae or Crocanthinae), but to place it in an existing genus would involve polyphyletic expansion of the concept of that genus. The genera with which the present taxon displays some potential synapomorphies (and the reason for rejecting placement in those genera) are:

1. *Protolychnis* Meyrick, 1925 (type species *Lecithocera maculata* Walsingham, 1881, from South Africa). The antenna of the type species was described by Lord Walsingham (Walsingham 1881) as thick yellowish ochreous. This character is present in most if not all species within the genus. The palps of the members of this genus are yellow and the antenna shorter than the forewing (Park *et al.* 2022: 189). The forewing pattern of *P. trigonias* (Meyrick, 1904) and *P. chlorotoma* (Meyrick, 1914) have some similarities to the present taxon (although the hindwings are pale in *P. trigonias*) and the forewing and hindwing venation are similar. However, in the male genitalia, the gnathos is developed with a large mesial process, and in the type species and most other species the saccus is developed, the juxta has caudal projections, the aedeagus is slender, usually longer than the valva and the female genitalia has a horseshoe shaped signum (Park *et al.* 2022: 190). The female genitalia also lack an appendix bursae. Further, although the venation is similar, M2 and M3 are approximated in the forewing in *Protolychnis* whereas in the present taxa they are almost parallel (see Park and De Prins (2019), Park and Koo (2020, 2022) and Park *et al.* (2022) for further information). DNA barcodes of *Protolychnis* show a phylogenetically close, but not sister relationship with the new taxon (Fig. 1b). A DNA barcode has been identified as the type species (*P. maculata*; BOLD:AAK9877; HM860216.1), and there are at least two closely related taxa widespread in Africa on BOLD (BOLD:ADT8222, not shown, is 4.1% pairwise divergent from the *P. maculata* sequence). Nevertheless, the sequence of the new taxon OQ339151 (NHMUK013698467) exhibits a 11.4% pairwise divergence to HM860216.1. This seems a consid-

erable distance for a potential congeneric. It is greater than for the highly allopatric *P. lychnocentra* from Australia (which is clearly a *Protolychnis* according to Park et al. 2022) and which differs from *P. maculata* by 10.3% and *X. stygioxanthus* by 11.52%, and a likely member of the genus from China (BOLD:ACD5139; Fig. 1b) which differs from *P. lychnocentra* by 6.5% and *P. maculata* by 9.6% (Fig. 1b). *P. trigonias* (Meyrick, 1904) (BOLD:AAO4343; also Australia) did not group with the two other identified *Protolychnis* in trees; this taxon is 12.64% pairwise divergent from *X. stygioxanthus*; (610 bp) and 13.1% pairwise divergent from *P. maculata*; rather, it groups with *Athymoris martialis* Meyrick, 1935 and a species of “*Cophomantella*” (BOLD:AAL6994) (Fig. 1b, Suppl. material 2). It therefore seems doubtful that *P. trigonias* represents a true *Protolychnis*, although the missing abdomen of the type (*Styloceros trigonias* Meyrick, 1904) has made morphological assessment difficult (Park et al. 2022: 196).

2. *Sisyrodonta* Meyrick, 1922 (type species *Sisyrodonta ochrosidera* Meyrick, 1922, from Australia). This is currently a monotypic genus known from Western Australia, although DNA barcodes indicate (e.g. Fig. 1b) that there is a species complex widely spread through Australia (BOLD:AAJ6848, BOLD:ACK2022; identified as a *Crocantbes*, BOLD:AAN9227, BOLD:AAO6600). The forewing pattern of *S. ochrosidera* is black (with a purplish sheen) and yellow, the antenna and labial palps are yellow and each flagellomere of the male antenna is ringed with large yellow scales. The antenna is more lamellate than in the present taxon and Meyrick considered it to be unique (Meyrick 1922) but in our view the antennae are similar to those of the new taxon. However, the hindwings are pale in both the type species and undescribed members of the complex. More importantly, CuA1 is absent in the forewing in the genus and CuA2 and M3 are stalked in the type species. In the male genitalia, the uncus of *S. ochrosidera* is broad at base and strongly sclerotised throughout, the gnathos has a large, strongly sclerotised medial section which is strongly beaked and strongly projected posteriorly and in the specimen of *Sisyrodonta* sp. from Western Australia (NHMUK013698527; Slide no. NHMUK014331161) which we have examined, the aedeagus is thin and elongate and without a cornutus. *Sisyrodonta* was included in the ‘Gelechiidae’ by Fletcher (1929: 204). It was transferred to the Lecithoceridae by Sattler (1973: 250). The genus was transferred to the Xyloryctidae from the Lecithoceridae by Gozmány (1978: 263). However, Common (1990: 264) considered that the wing venation, the presence of the supplementary wing-coupling setae, and the behaviour of the adults leaves little doubt that it belongs in the Lecithoceridae. *Sisyrodonta* was also treated in the Lecithoceridae by Nye and Fletcher (1991: 277). We consider that *Sisyrodonta* is correctly placed in the Lecithoceridae although the strongly sclerotised medial section of the gnathos is unusual for the family. We place the genus, which in the full dataset analyses (see Fig. 1b, Suppl. material 3) fell in a small grouping that included *Protolychnis* and *Halolaguna*, as well as the new taxon, in the Torodorinae.

3. *Cophomantella* Fletcher, 1940 (type species *Onebala elaphopis* Meyrick, 1910, from India). *Cophomantella* was established as an objective replacement name for *Cophomantis*, Meyrick, 1925, a junior homonym of a frog, *Cophomantis* Peters, 1870 (Nye and Fletcher 1991). As a result of various combinations, “*Cophomantella*” applies to a few remaining species and Park et al. (2022: 24) recommend that the type species should be transferred to a genus in Gelechiidae. We have examined the type of *C. elaphopis*, including the wing preparation and male genitalia contained on the type slide (JFGC 8911), and apart from the reduced gnathos and somewhat reduced venation, it exhibits many of the typical features of a torodorine including the tergal spines on the abdomen, the thorn-like uncus directed caudally, the shape and orientation of the valva and the large, complex cornuti in the aedeagus. We therefore consider *Cophomantella* to be a further

example of a genus within Torodorinae with a reduced gnathos, and we transfer it back to Lecithoceridae. The new taxon is not, however, within *Cophomantella*. The palps of *C. elaphopis* are dark, the scaling on the antennae of the male is not projected outwards, the forewing markings are different and in the forewing venation, R3 and R4 are stalked, R5 is absent and M3 is absent. Also, in the male of *C. elaphopis* there is an expansible pencil of hairs from the base on the forewing ventral surface along the costa (Meyrick 1910). This is not present in the new taxon. In the male genitalia of *C. elaphopis*, the vinculum is U shaped and the tegumen and valvae are also a different shape from those of the new taxon. The one South East Asian BIN on BOLD attributed to the genus (BOLD:AAL6994) that we analysed fell with posterior support together with *Protolychnis trigonias* from Australia (Fig. 1b; but see also Suppl. material 3).

4. *Torodora* Meyrick, 1894 (type species *Torodora characteris* Meyrick, 1894, from Myanmar). This is a genus which comprises more than 200 described species (Park *et al.* 2022: 271) and seems very likely to be polyphyletic. It is considered here on the basis of the resemblance of several features in the male genitalia, including the shape of the uncus, juxta, vinculum, saccus and valva and the rough texture of the tegumen in *T. meifengensis* Park, 2015; *T. octavana* (Meyrick, 1911); and *T. umbriella* Park & Heppner, 2022 (Fig. 5, Park *et al.* 2022 plates 113C, 118E, 132A). The genus is generally defined by the wing venation as follows: Forewing with R3, R4 and R5 usually on a common stalk, CuA1 and CuA2 stalked, and M2 present in both wings (Park *et al.* 2022, 271). The present taxon satisfies these criteria except that M2 is absent in the hindwing, although Park *et al.* (2022: 272) notes that venation is variable and that generic assignment should be based on a combination of characters. According to Park, the male genitalia of *Torodora* are characterised by the hooked gnathos, the foot shaped or variously elongated valva and the strong spinous zones on the tergites of the abdomen (Park *et al.* 2022: 272), although Yu *et al.* (2022) give mesial process of the gnathos as present or absent as a generic character and identify 12 species of *Torodora* in which it is absent. The latter two characters are present in the new taxon (the second occurs in the ground plan of all Lecithoceridae, Autostichidae and Xyloryctidae) but the new taxon lacks the hooked mesial process of the gnathos (which is present in the three species of *Torodora* referred to above). In addition, the wing pattern and thickened antenna in the male would be unusual for *Torodora*. The one identified species we analysed, *T. aenoptera*, fell in an analysis (Fig. 1b) within Torodorinae, next to a species from Australia (BOLD:AAH3804), and a species of *Parkiana* from Madagascar (see Park *et al.* 2020), however, with no support for its placement.

5. *Thubana* (Type species *Thubana bisignatella* Walker, 1864, from Borneo). Although it is 9.2–9.38% pairwise divergent, *T. exoema* (Meyrick, 1911) is the nearest identified sequenced species of Lecithoceridae to the present taxon in the NJ tree resulting from the BOLD search, while the one *Thubana* species analysed (Figs 1a, 1b) also shows no sister relationship to it. According to Park *et al.* (2022: 212), the genus is characterised by the following synapomorphic characters: the forewing commonly having a large creamy-white or light-orange triangular costal patch, with a few exceptions, and the venation with M3, CuA1, and CuA2 on a common stalk; R3 stalked with R4 and R5; R5 absent or often present; the hindwing with M2 present and closely approximated to the stalk of M3 and CuA1; the abdomen with dense spinous zones on tergites. The present taxon does not have the triangular costal patch, R5 is present in the forewing and M2 is absent in the hindwing. The tergal spines are moderate rather than dense. The male genitalia of *Thubana* are significantly different. The gnathos is strongly developed with a large downward mesial projection, the vinculum is U shaped and projects well beyond the base of the valva, the juxta has caudal projections and

the cornuti in the aedeagus are complex and not a single spike. In the female genitalia the ductus bursae is long and usually with sclerotisation or scobination and the corpus bursae has a signum.

6. *Halolaguna* (Type species *Halolaguna sublaxata* Gozmany, 1978, from China). This is one of the genera which appears in a molecular clade of Torodorinae with the present taxon (along with typical *Protolychnis* and *Sisyrodonta*, Fig. 1b, $pp = 0.99$, albeit without support for its sister taxon, and in a clade that has posterior support ($pp = 0.97$) in the linked rather than unlinked MrBayes analysis, see Suppl. material 3). Morphologically there is little similarity with this genus. In *Halolaguna* the apex of the forewing projects and the wing pattern, antennae and colour of the labial palps are different. M2 is also present in the hindwing. In the male genitalia the gnathos is developed with a strong downward mesial projection, the vinculum projects beyond the base of the valva, the saccus is developed, the juxta has caudal projections and the cornuti in the aedeagus are complex with minute spines. In the female genitalia the ductus bursae is long and the corpus bursae has a signum.

7. *Crocantnes* (Type species *Crocantnes prasinopis* Meyrick, 1886: 277, from Australasia) and *Aprosoesta* Turner, 1919 (type species *A. pancala* Turner, 1919). See above for discussion of *Crocantnes*. *Aprosoesta* is mentioned separately but only because of the relative proximity of the *A. pancala* sequences to those of the new taxon in Fig. 1b. *Aprosoesta* was previously treated as a junior synonym of *Crocantnes* but was re-established in Park 2015 on the basis of the similar maculations of the forewing and hindwing, and characters in the forewing venation and the third segment of the labial palps in the male. In terms of morphology, *Aprosoesta* is as divergent as *Crocantnes* from the new taxon (*A. 'pancala'* BOLD: AAO6447 is about 11.5% divergent by nucleotides).

The origin of the population of this taxon in Southern Spain is not clear. It could be a previously undiscovered but long resident population. Although the Microlepidoptera of Spain seem reasonably well studied, there must be many species still awaiting discovery. However, this is a distinctive taxon, evidently common where it occurs, which is not likely to be overlooked and the habitat in which it occurs is not uncommon in Southern Spain so there is no obvious reason why it would not occur elsewhere. A second possibility is that it is a previously unknown species originating from North Africa which is now spreading into Southern Europe (although it is clearly distinct morphologically and genetically from *Ceuthomadarus* from NW Africa). A number of species have been found around Tarifa which are taking this route, most likely as a result of climate change making conditions hotter and harsher in North Africa, causing resident species to expand northwards. DG has recorded in this area since 2017 but did not see this taxon until May 2020. Considering the increasing numbers of *X. stygioxanthus* at the locations around CIMA and the discovery of specimens at Huerta Grande, arrival from Africa may explain the Spanish population. A third possibility is that this taxon is an invasive species that has come, possibly from Australia, as a result of plantation of *Eucalyptus* spp. or other imported plants in the area. However, the taxon does not seem to be closely related to any known or previously DNA barcoded Australian genus of lecitocerid moth, and the DNA barcoding campaign on ANIC collections was more extensive than for any comparable tropical region.

Conclusions

It is noteworthy to detect a new subfamily for the European continent that is established in Spain. This interesting taxon from around Tarifa exhibits considerable morphological and genetic

divergence from hitherto known or DNA barcoded taxa (between about 10 and 13% to its nearest phylogenetic neighbours, and more than 9.2% from nearest hits as regards sequences not compromised by ambiguity codes). Its placement was not straightforward. It is clearly a lecitocerid both on the basis of its morphology and its DNA barcode, and it appears to fall by phylogenetic analysis within Torodorinae rather than Crocanthinae, despite its reduced gnathos. Our placement in a new genus is by elimination and should spur other attempts to find its closest relatives. The work we have carried out in seeking to place the taxon shows that existing data is insufficient to show that Torodorinae and Crocanthinae are separate clades, with an expanded phylogenomic dataset clearly needed, and that the morphological synapomorphies supporting Lecithoceridae, Torodorinae and Crocanthinae need further refinement. Torodorinae is a subfamily which is widespread palaeotropically and we are unable to narrow the origin of *X. stygioxanthus*. Further field and taxonomic studies of this taxon and other Lepidoptera in adjacent parts of North Africa and Southern Spain are likely in our view to yield interesting results in terms of taxonomic diversity, relationships of the currently established fauna, and changes in populations as a result of changing climate conditions.

Mandatory statements

The specimens examined for this study were collected in accordance with a permit issued by the Junta de Andalucía dated 11 April 2022. No specific funding was provided for this project and there are no conflicts of interest.

Acknowledgments

We would like to thank the management and staff of Fundacion Migres at the Centro Internacional de Migración de Aves (CIMA) for their warmth and hospitality and for all their help with this project and the Junta de Andalucía for issuance of the permit pursuant to which the study material was collected. Piotr Cuber (NHMUK) is acknowledged for laboratory work and providing details of methodology, and Ben Price (also NHMUK) is thanked for the bioinformatic processing, and access to the sequences. For their roles in the molecular work at NHMUK, Darren Choonea, Clementine Geeves, Silvia Salatino, Thomas J. Creedy, Claire Griffin, Inez Januszczak, Laura Sivess, Ian Barnes, and Raju Misra are also acknowledged. Ted Edwards, Paul Hebert, Akito Kawahara, Erik van Nieukerken, Kyu-Tek Park and Willi de Prins very kindly allowed us to use their unpublished DNA barcodes. The molecular work was supported via Wellcome Trust funding to the Wellcome Sanger Institute (206194) and the Darwin Tree of Life Discretionary Award (218328) to the DToL consortium. The Society for European Lepidopterology is also acknowledged for support. Our colleague Sir Anthony Galsworthy kindly read and commented on the manuscript and assisted us with the names of the new taxon. Shuxia Wang and Ole Karsholt are thanked for their reviews and insightful remarks, as well as the subject editor, Carlos Lopez-Vaamonde and editor, Théo Léger.

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Supplementary material 1

DNA sequences

Authors: David C. Lees

Data type: table (Excel spreadsheet)

Explanatory note: DNA sequences for terminals used in the trees (Fig. 1a, b; Suppl. materials 2, 3) with any changes in identification, BIN (or if not available distance to nearest BIN), Process Ids, GenBank Accession numbers for each of seven genes, prior publication/link to NCBI and/or permission to use.

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Link: <https://doi.org/10.3897/nl.46.101457.suppl1>

Supplementary material 2

50% majority rule consensus tree 1

Authors: David C. Lees

Data type: figure (tif image)

Explanatory note: 50% majority rule consensus tree for a partitioned analysis of the 51 taxa, 5408 bp dataset in MrBayes 3.1. Support values are posterior probabilities based on a subset of 7,500 trees out of 15,001 sampled every 1,000 generations per separate run, by which point the runs had converged to 1.000. All parameters (except branch length) were unlinked and a variable rate prior was applied across the seven partitions and the final log likelihood for the best state of each ‘cold’ run asymptoted at - 28054 or - 28059.

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Link: <https://doi.org/10.3897/nl.46.101457.suppl2>

Supplementary material 3

50% majority rule consensus tree 2

Authors: David C. Lees

Data type: figure (tif image)

Explanatory note: 50% majority rule consensus tree for a partitioned analysis of the 51 taxa, 5408 bp dataset in MrBayes 3.1. Support values are posterior probabilities based on a subset of 7,463 trees out of 15,000 sampled every 1,000 generations per separate run, by which point the runs had converged to 1.000. All parameters were linked (by default) and a variable rate prior was applied across the seven partitions and the final log likelihood for the best state of each ‘cold’ run asymptoted at -28634 or -28635.

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Link: <https://doi.org/10.3897/nl.46.101457.suppl3>

Two new species of the genus *Scrobipalpa* Janse, 1951 (Lepidoptera, Gelechiidae) from Ukraine

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<https://zoobank.org/76420E50-AF6C-4C79-BF76-624209EB2263>

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Subject Editor: David C. Lees.

Abstract. *Scrobipalpa arenicola* **sp. nov.** and *S. burkutica* **sp. nov.** are described from the Lower Dnipro Sands in the Kherson region of Ukraine. The differences in external characters and genitalia of the new species from their congeners are discussed. Photographs of the adults and genitalia of the new species are provided as well as DNA barcodes of *S. arenicola* **sp. nov.** and related species.

Introduction

Scrobipalpa Janse, 1951 with more than 300 Palaearctic (Povolný 2002; Bidzilya et al. 2019; Huemer and Karsholt 2020; Bidzilya et al. 2022), ten Nearctic (Lee et al. 2009), 36 Afrotropical (Bidzilya 2021) and five Australian species (Povolný 1977) is the most diverse genus of the tribe Gnorimoschemini and also one of the most diverse genera in the family Gelechiidae. As a result of a revision of European *Scrobipalpa* (Huemer and Karsholt 2010), 103 species had been recognized as occurring in Europe. Later, additional species have been described from Ukraine (Bidzilya and Budashkin 2011), France (Varenne and Nel 2013, 2017, 2018) and the Volga region of Russia (Anikin and Piskunov 2018). These records have been included in the list of European *Scrobipalpa* that now comprises 112 species (Huemer and Karsholt 2020). Most recently, two more species have been described from Spain (Huemer 2021) and the southern Urals of Russia (Bidzilya et al. 2022).

In 1999, Eugeny Rutjan (Kyiv, Ukraine) collected in the Tchernomorsky Nature Reserve (Kherson region of Ukraine) a series of males that could not be assigned to any known species of *Scrobipalpa*. In the account of *S. hyoscyamella* (Stainton, 1869) in their monograph on the European Gelechiidae Huemer and Karsholt (2010: 159) record that they examined a male from Ukraine with almost identical genitalia but with a rather different forewing colour and markings stating that it was uncertain whether it was a different species. They illustrated the genitalia (2010: 402, fig. 94a) as “*Scrobipalpa* cf. *hyoscyamella* (Stainton)”. In 2017, the present author collected three additional males of this species in another locality but in the same biotope. DNA barcodes (mtCOI gene) confirmed that these specimens represent an undescribed species of *Scrobipalpa*, whose description is given here. The second species was collected in sympatry with the first species. This species possesses a unique set of external and genitalia characters in both sexes that separate it clearly from all other *Scrobipalpa* species, and it is described here as a new species.

Material and methods

Male and female genitalia were dissected and prepared using standard methods (Huemer and Karsholt 2010). Male genitalia were spread using the unrolling technique described by Pitkin (1986) and Huemer (1988). Pinned specimens and details of external morphology were photographed with a Canon EOS 5DSR DSLR camera attached to a light box. Slide-mounted genitalia were photographed with a Canon EOS Rebel T5 DSLR camera attached to an Olympus U-CTR30-2 trinocular head mounted on a Carl Zeiss compound microscope. For each photographed specimen, sets of 10–20 images were taken at different focal planes and focus-stacked using Helicon Focus 6 with the final image edited further in Adobe Photoshop CS5.

Tissue samples from three specimens of *S. arenicola* sp. nov. were prepared to obtain DNA barcode sequences of a 658 bp segment of the mitochondrial COI gene (cytochrome c oxidase subunit 1). The tissues were successfully processed at the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) using the standard high-throughput protocol described in deWaard et al. (2008). In addition, respectively, three DNA barcode sequences of the two closest species from the Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007, Ratnasingham 2018) were used for analysis. All sequences were assigned Barcode Index Numbers (BINs), algorithm-based operational taxonomic units that provide a reasonably good proxy for species level (Ratnasingham and Hebert 2013). Further details including complete voucher data and images can be accessed in the public dataset “New species of *Scrobipalpa* from Ukraine - [DS-SCROUKRA]” (https://www.boldsystems.org/index.php/MAS_Management_DataConsole?codes=DS-SCROUKRA) in the Barcode of Life Data Systems BOLD (Ratnasingham and Hebert 2007).

Degrees of intra- and interspecific variation of DNA barcode fragments were calculated using the Kimura two-parameter model on the platform of BOLD systems v. 4.0. (<https://boldsystems.org>). A Neighbor-Joining tree was constructed using the Kimura two-parameter model in MEGA7 (Kumar et al. 2016).

The material examined including holotypes is deposited in Zoological Museum Kyiv Taras Shevchenko National University, Kyiv, Ukraine (ZMKU), some paratypes - in the Tiroler Landesmuseum Ferdinandeum, Hall in Tirol, Austria (TLMF) and the Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark (ZMUC).

The descriptive terminology of the genitalia structures follows Huemer and Karsholt (2010).

Results

Scrobipalpa arenicola sp. nov.

<https://zoobank.org/F8774C76-7A67-48DF-9FF3-A08FE3F28676>

Figs 2–5, 10–13

Scrobipalpa cf. *hyoscyamella* (Stainton) – Huemer and Karsholt 2010: 402, fig. 94a.

Material examined. Holotype: UKRAINE • ♂; Kherson reg., Holopristanskiy distr., Burkuty vill. env.; 21 Jun. 2017; O. Bidzilya leg; gen. slide 150/17, O. Bidzilya; TLMF Lep28327; BankIt2704426 gnl|uoguelph|PALEA032-20.COI-5P; OQ992194; ZMKU.

Paratypes: UKRAINE • 2 ♂; same data as for holotype; gen. slide 149/17, O. Bidzilya; TLMF Lep28328; TLMF Lep 28329; BankIt2704426 gn|uoguelph|PALEA034-20.COI-5P; OQ992195; ZMKU • 9 ♂, 1 ♀; Ukraine, Tchernomorskiy Nature Reserve, Ivano-Rybalchanskiy loc.; 28–30 Apr. 1999; E. Rutjan leg.; gen. slide 40/09♂, 89/10♀, 59/23♂, 143/23♂, O. Bidzilya; 01 1061♂, PH; TLMF; ZMKU; ZMUC.

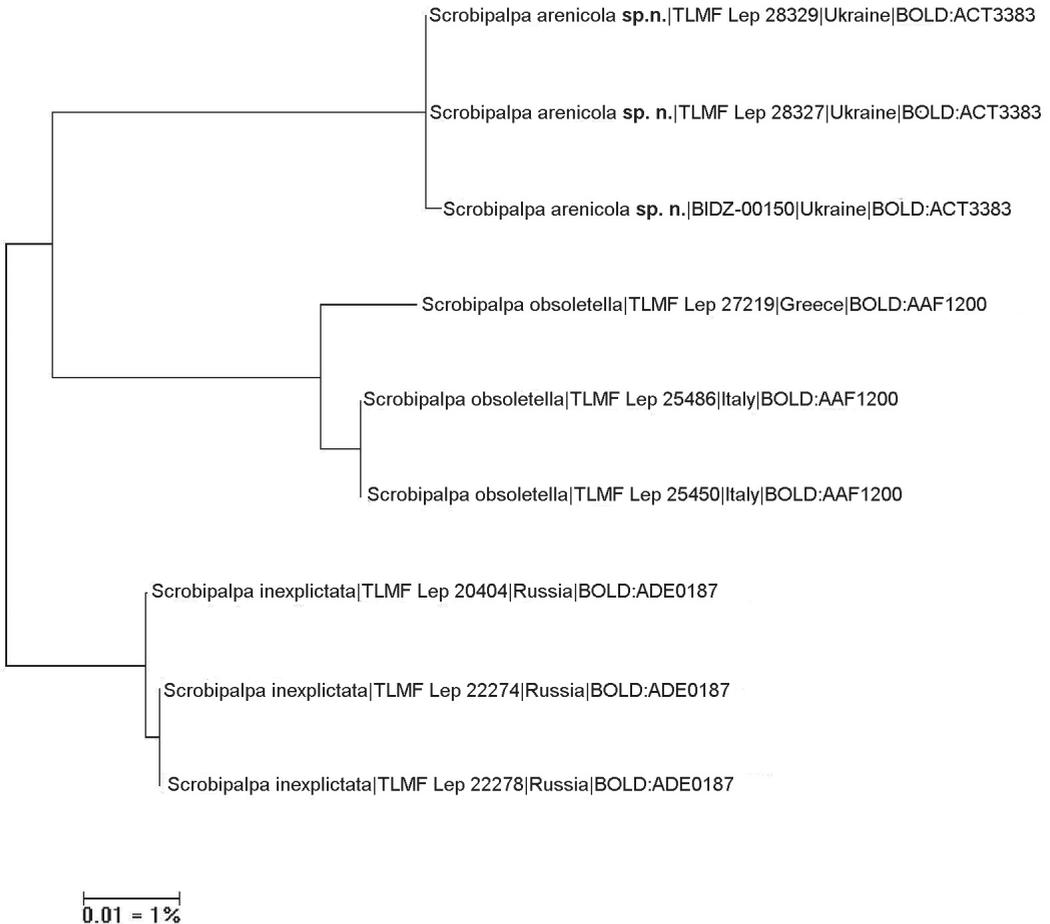
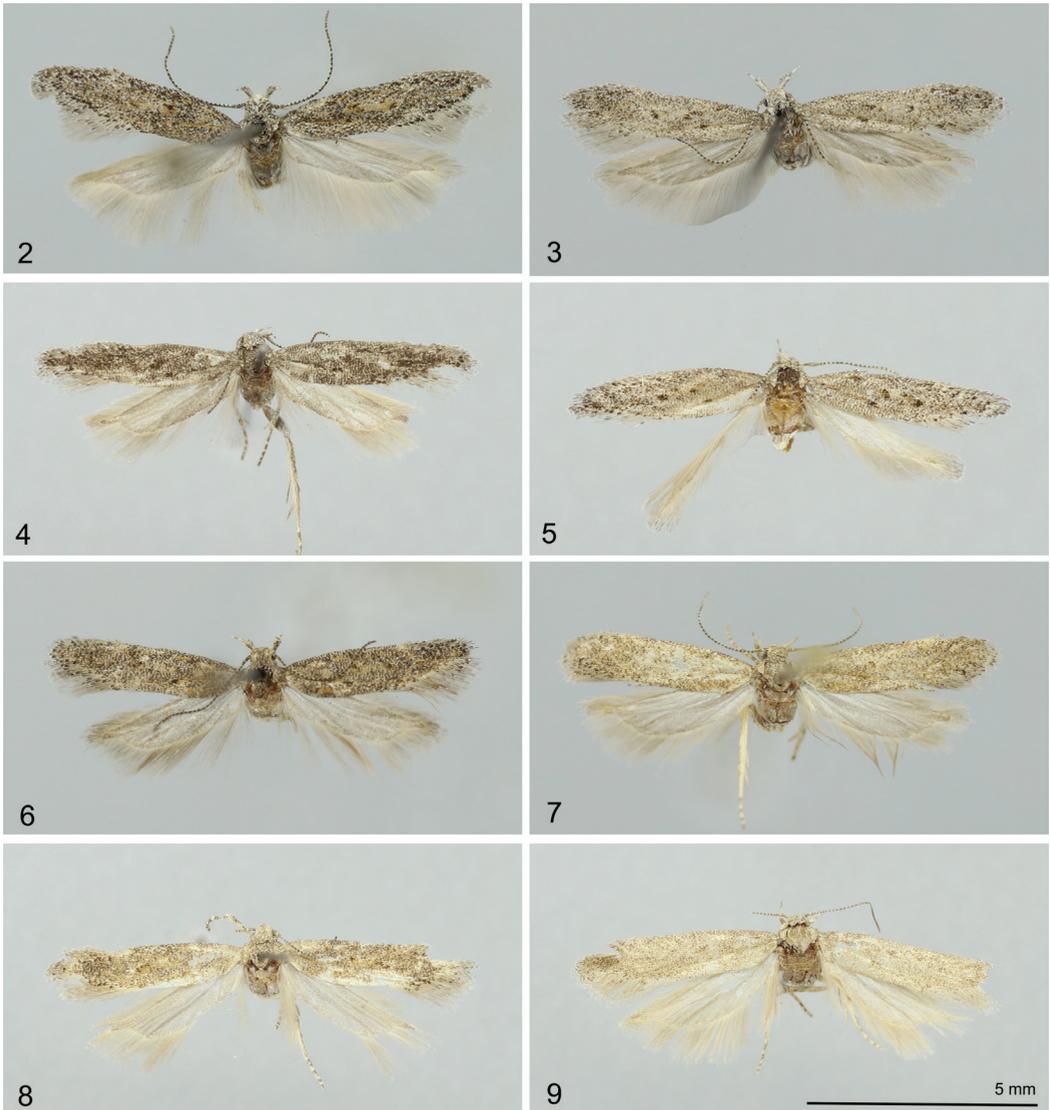


Figure 1. Neighbor-joining tree of *Scrobipalpa arenicola* sp. nov. and its closest species, *S. obsoletella* (Fischer von Röslerstamm, 1841) and *S. inexplicitata* Bidzilya, Huemer & Šumpich, 2022, in BOLD.

Diagnosis. Externally (Figs 2–5) *S. arenicola* sp. nov. is almost indistinguishable from *S. arenbergeri* Povolný, 1973 (Fig. 6) and *S. pauperella* (Heinemann, 1870) but the forewing is narrower, with a less distinct black pattern, and is smaller in wingspan (9.7–10.0 mm as opposed to 11.0 mm in *S. arenbergeri* and 12–15 mm in *S. pauperella*). *Scrobipalpa proclivella* (Fuchs, 1886) is also extremely similar to the new species but the black spots are usually elongate and the underside of the abdomen is paler cream (Gregersen and Karsholt 2022: 194). The male genitalia are identifiable by the trapezoidal uncus with straight or weakly emarginated posterior margin, short valva (not reaching top of uncus), long sacculus (1/3 length of valva) and a slender saccus. *Scrobipalpa*

karadaghi (Povolný, 2001) has similar valva, sacculus and vincular process (Fig. 15), but the new species clearly differs in the trapezoidal uncus and very slender saccus. The differences from *S. burkutica* sp. nov. are explained under the diagnosis of that species.

Description. Adult (Figs 2–5). Wingspan 9.7–10.0 mm. Head covered with grey brown-tipped scales, labial palpus recurved, segment 2 grey mottled with brown, outer and upper surface white, lower surface with groove beneath, segment 3 1/2 length and 1/2 width of segment 2, pointed acute,



Figures 2–9. *Scrobipalpa* spp., adults. **2–5.** *S. arenicola* sp. nov. **2.** HT, male (gen. slide 150/17, O. Bidzilya). **3.** PT, male (gen. slide 149/17, O. Bidzilya). **4.** PT, male (gen. slide 114/23, O. Bidzilya). **5.** PT, female (gen. slide 89/10, O. Bidzilya). **6.** *S. arenbergeri*, male. **7–9.** *S. burkutica* sp. nov. **7.** HT, female (gen. slide 141/23, O. Bidzilya). **8.** PT, female (gen. slide 67/07, O. Bidzilya). **9.** PT, male (gen. slide 9/11, O. Bidzilya).

greyish brown with diffuse medial ring and white apex; scape grey mixed with brown, flagellum grey ringed with brown; thorax and tegula concolorous with head; forewing covered with grey scales tipped with brown or black, a pair of black spots in middle of cell, black spot at cell corner and on fold, veins and fold weakly mottled with light brown, fringes grey brown-tipped; hindwing and fringes grey.

Variation. Specimens may appear lighter or darker depending on the development of black-tipped scales on the forewing; black markings are reduced in some specimens.

Male genitalia (Figs 10–13). Uncus trapezoidal, gradually narrowed posteriorly, posterior margin straight or weakly emarginate; distal sclerite of gnathos short, weakly curved; tegumen broad, with indistinct transition to uncus, anteromedial emargination broadly rounded, extending to 1/3 length of tegumen; valva slender, gradually curved, of even width except weakly inflated apex, extending to 1/2–2/3 length of uncus; sacculus gradually curved inwards, extending almost to 1/2 length of valva, twice as broad as adjacent part of valva, with pointed apex; vinculum twice as broad as long, posteromedial emargination deep, triangular, vincular process short, subtriangular, with distinct pointed tip, as broad at base as sacculus, extending to 1/2 length of sacculus; saccus broad at base, then slender, parallel-sided, apex obtuse, extending slightly beyond pedunculus; phallus nearly parallel-sided, weakly narrowed apically, apical arm slender, coecum strongly inflated, almost equal in length to phallic trunk.

Variation. Uncus varies in length, vincular processes slightly vary in width and length.

Female genitalia. Unknown (the genitalia slide 89/10 with the female genitalia was broken).

Molecular data. BIN, BOLD:ACT3383. The intraspecific average distance of the DNA barcode region is 0.12% (n = 3). The minimum distance to the nearest neighbor, *S. inexplicitata* Bidzilya, Huemer & Šumpich, 2022 (BIN, BOLD:ADE0187), is 5.77% (p-dist) (Fig. 1).

Biology. Adults have been recorded in late April and late June. The species inhabits dunes and sand steppes in the Lower Dnipro Sands in the Kherson region of Ukraine (Figs 18–21).

Distribution. Ukraine: Kherson region.

Etymology. The specific epithet is derived from the Latin words “*arena*” - sand, and “*colo*” - to inhabit, and indicates the restriction of the new species to sandy habitats.

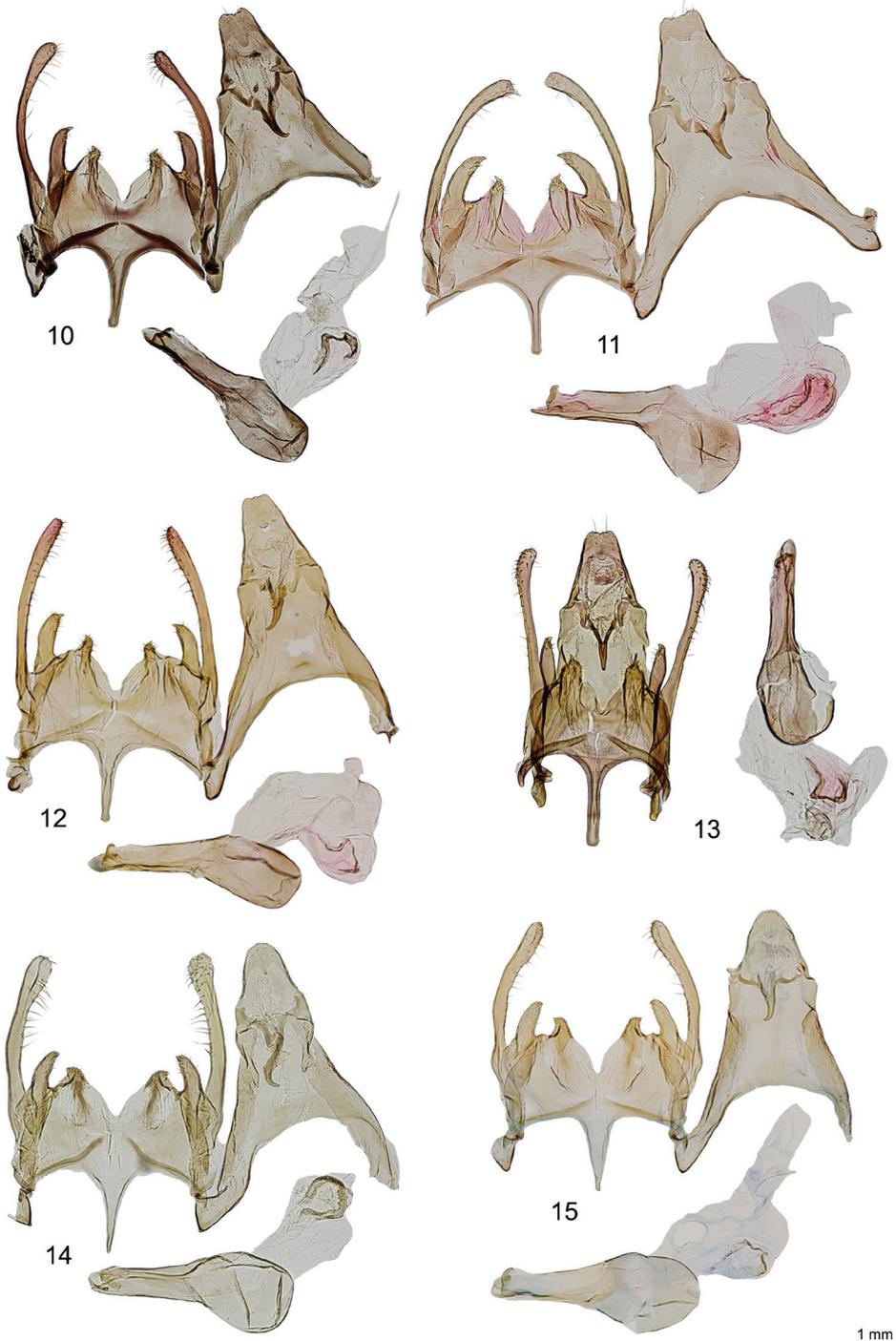
Scrobipalpa burkutica sp. nov.

<https://zoobank.org/FC38F514-2D2D-46C6-AB24-1A604B079EF7>

Material examined. *Holotype*: UKRAINE • ♀; Kherson reg., Holopristanskiy distr., Burkuty vill. env.; 21 Jun. 2017; O. Bidzilya leg.; gen. slide 141/23, O. Bidzilya; ZMKU.

Paratypes: UKRAINE • 1 ♂, 1 ♀; Tchernomorskiy Nature Reserve, Ivano-Rybalchanskiy loc.; 28–30 Apr. 1999; E. Rutjan leg.; gen. slide 67/07♀, 9/11♂, O. Bidzilya; ZMKU.

Diagnosis. *Scrobipalpa burkutica* sp. nov. is characterized by the uniformly greyish brown forewing with ochreous suffusion (Figs 7–9). *Scrobipalpa vasconiella* (Rössler, 1877) has a more contrasting forewing with a distinct black pattern. *Scrobipalpa amseli* Povolný, 1966 has the forewing with same ground colour, but differs in having black spots in the cell. The new species can be confused with unicolorous specimens of *S. nitentella* (Fuchs, 1902) with reduced markings (see Gregersen and Karsholt 2022, fig. 205 f). The male genitalia resemble those of *S. karadaghi* (Fig. 15) but in the latter the uncus is shorter and less produced, the valva is narrower at the base, the vincular process is slightly longer and wider, and the saccus is broader at the base. The male genitalia



Figures 10–15. *Scrobipalpa* spp., male genitalia. **10–13.** *S. arenicola* sp. nov. **10.** HT (gen. slide 150/17, O. Bidzilya). **11.** PT (gen. slide 01 1061, PH). **12.** PT (gen. slide 143/23, O. Bidzilya). **13.** PT (gen. slide 149/17, O. Bidzilya). **14.** *S. burkutica* sp. nov., PT (gen. slide 9/11, O. Bidzilya). **15.** *S. karadaghi* (gen. slide 7/19, O. Bidzilya).



Figures 16, 17. *Scrobipalpa burkutica* sp. nov., female genitalia. **16.** HT (gen. slide 141/23, O. Bidzilya). **17.** PT (gen. slide 67/07, O. Bidzilya).

differ from those of *S. arenicola* sp. nov. in the rounded posterior margin of the uncus (straight or weakly emarginate in *S. arenicola* sp. nov.), broader valva, the sacculus more distinctly narrowed apically, the vincular process broader at base than the adjacent part of the valva (as broad as the adjacent portion of the valva in *S. arenicola* sp. nov.) and a basally broader saccus. The female genitalia are characteristic with papillae anales subrectangular in the basal half, sternum VIII twice as broad as long, with distinct sclerotised anterior edge and rounded lobes of ventromedial depression that are densely covered with reticulate network of sclerotised ridges that does not extend beyond the anterior margin of sternum VIII. *Scrobipalpa wiltshirei* Povolný, 1966 seems to be most similar in respect of the female genitalia, but differs in having a deeper anteromedial emargination between the lobes of the ventromedial depression, and subovate papillae anales.

Description. Adult (Figs 7–9). Wingspan 10–12 mm. Head covered by greyish brown scales with dark brown tips, labial palpus recurved, segment 2 light brown mottled with dark brown, inner and upper surface pale, with groove beneath, segment 3 light brown with broad pale medial ring and whitish tip, scape brown mixed with grey, flagellum brown ringed with grey thorax and tegula concolorous with head; forewing plainly coloured, covered with grey to ochreous-brown scales with dark brown tips, fold ochreous-brown with a very indistinct brown marking, fringes grey to light brown, tipped with dark brown; hindwing grey, fringes concolorous.

Variation. The forewing of the female paratype is distinctly mixed with dark brown and is smaller in size.

Male genitalia (Fig. 14). Uncus subtriangular, rounded apically, with indistinct transition to tegumen; distal sclerite of gnathos long, hook-shaped; tegumen subtriangular, anteromedial emargination broadly rounded, extending to 1/3 length of tegumen; valva moderately broad, of even width except slightly inflated apex, weakly curved, extending to top of uncus; sacculus gradually curved, apex pointed, about 1/3 length of valva; vinculum twice as broad as long, anteromedial emargination triangular, vincular processes subtriangular with outwardly curved tip, short, broad at base, extending to mid-length of sacculus; saccus subtriangular, widening posteriorly, pointed, extending slightly beyond pedunculus; phallus nearly parallel-sided, apical arm slender, coecum strongly inflated, slightly shorter than phallic trunk.

Female genitalia (Figs 16, 17). Papillae anales subrectangular in basal half, then narrowing on outer edge towards apex; apophysis posterioris three times as long as segment VIII and twice length of apophysis anterioris; segment VIII twice as broad as long, subrectangular, with distinct sclerotised anterior edge, lobes of subgenital plates broadly separated anteromedially and more closely connected posteromedially, with broad patch consisting of reticulate network of sclerotised ridges at base of apophysis anterioris; lobes of ventromedial depression broadly rounded, separated anteromedially by a short triangular incision, densely covered with reticulate sculpturing which overlaps laterally with medial portion of lobes of ventromedial depression; apophysis posterioris slightly longer than segment VIII, slender, acute; ductus bursae distinctly broadened anteriorly, gradual transition to corpus bursae, colliculum short, ring-shaped, corpus bursae ovate, signum with large subtriangular weakly serrated basal plate, distal hook slender, nearly straight.

Variation. Female paratype differs in the signum having a smaller basal plate and distinctly curved distal hook.

Molecular data. No DNA barcode available.

Biology. Adults have been recorded in late April and late June. The species inhabits dunes and sand steppes in the Lower Dnipro Sands in the Kherson region of Ukraine (Figs 18–21).

Distribution. Ukraine: Kherson region.



Figures 18–21. Dunes and sandy steppe in Oleshki sands in the Kherson region of Ukraine – habitat of *S. arenicola* sp. nov. and *S. burkutica* sp. nov.

Etymology. The specific epithet is derived from the Burkuty village in the Kherson region of Ukraine which is the type locality of the new species.

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Description of *Eretmocera hafeetensis* sp. nov. from UAE (Lepidoptera, Scythrididae)

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<https://zoobank.org/83BEC164-07F1-44C0-83AA-D78B2D94C3A5>

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Abstract. *Eretmocera hafeetensis* sp. nov., a moth in the family Scythrididae, is described and illustrated based on the examination of three male and 13 female specimens collected at the foot of Jebel Hafeet, an isolated 1,140 metres-high massif just south of Al Ain, after which the new species is named. The new species is distinct from congeners in details of its male and female genitalia and is also readily separated in the field from other *Eretmocera* species. The new addition brings the number of confirmed species in this genus represented in the UAE to three. The discovery of this species at Ain Al Waal, Jebel Hafeet highlights the conservation importance of this and similar nearby locations, which are beginning to reveal unique species among its already species-rich fauna at a time of huge human encroachment into the area.

Introduction

Moths in the family Scythrididae are often small-sized and somewhat cryptic in appearance. Although represented in most parts of the world, until late last century, only a small minority of the species described had been recorded in the Middle East (Bengtsson 2014).

Knowledge of Scythrididae species in Arabia is mostly derived from work in Oman and Yemen at around the turn of the 21st century, when Bengtsson (2002a, 2002b) described 45 new species and identified many others. Prior to that, some scythridids from Saudi Arabia had been recorded by Passerin d'Entrèves (1996).

In the UAE, knowledge of the fauna is less developed. Van Harten's literature review (van Harten 2005) – that was compiled prior to embarking on the first of six volumes in the Arthropod Fauna of the UAE series – documented only two species. As part of that project, van der Wolf (2008) brought to 12 the number of species of the family recorded from the UAE, six of them having been among those described from Oman and Yemen.

Two genera in Scythrididae are represented in the UAE, namely *Eretmocera* Zeller, 1952 and the more species-rich genus *Scythris* Hübner, 1825. The genus *Eretmocera* consists of rather small species with wingspan in general of 8–12 mm and are often recognisable in resting position by their dark brown upper wings with brightly coloured markings, and abdomen coloured with varying extents of orange, yellow and black. Many species have partly thickened antennae, especially in males. There are 50 species in *Eretmocera* worldwide (BÅB, private database). Most

species are found in the tropical zone, especially in Africa, but they also range to the Middle East, Asia and Australia.

Two species in the genus *Eretmocera* are known from the UAE, *E. impactella* (Walker 1864) and *E. bradleyi* (Amsel 1961), the former of which is found in small numbers on Jebel Hafeet. Two more, as yet unidentified species in the genus, have also been observed on the mountain with external markings quite distinct from each other, and different from *E. bradleyi*, *E. impactella* and *E. hafeetensis*.

The distinctively marked *E. hafeetensis* sp. nov. was first observed in 2010. Differences in its external appearance to other species in the region prompted discussion between the authors about its possible identity and led to specimens being collected for examination and dissection. Finding males among the specimens collected proved difficult, and indeed only females were present in the first two batches examined. The results bore confirmation that *E. hafeetensis* was indeed a new species, although it was not until later that males were found. A description of both male and female of the new species is provided here. The holotype is deposited in Natural History Museum, Abu Dhabi, UAE.



Figure 1. Type series of *Eretmocera hafeetensis* sp. nov. (pinned). Three males to the left (holotype at the top), the other specimens females.

Materials and methods

Specimens were collected, using sweep nets over various plants. Collected specimens were preserved and pinned, or they were sent as dry specimens to the laboratory in Sweden, where they were relaxed and mounted for dissection and examination.

Field photographs were taken using Nikon D850, D3 and Z9 camera bodies, with Nikkor 105 mm lenses and Nikon's R1 Wireless Close-Up Speedlight System. A stacked photograph (Fig. 6) was taken using Nikon D850 camera body using a 25mm f/2.8 Ultra-macro 2.5× -5.0× lens and a Cognisys Stackshot focus stacking rail. HELICON FOCUS 8.2.0 software was used for processing of the stacked image. Post processing was done using ADOBE PHOTOSHOP.

Genitalia slides (Figs 7a, b, 8a, b) were made according to the standard procedure described by Robinson (1976). The slides were photographed with Canon EOS 500D mounted on a compound microscope Euromex Arnheim EB No. 149508. The pictures were stacked with the software HELICON FOCUS Version 4.2.8 and then enhanced with ADOBE PHOTOSHOP Elements 2019.

Abbreviations

- BÅB** Private collection of Bengt Åke Bengtsson, Färjestaden, Sweden
NHMAD Natural History Museum Abu Dhabi, Abu Dhabi, UAE
HRCA Private Collection of Huw Roberts, Al Ain, UAE

Taxonomy

Eretmocera hafeetensis sp. nov.

<https://zoobank.org/E988D1E0-C559-4D99-9054-9D4227D75BE8>

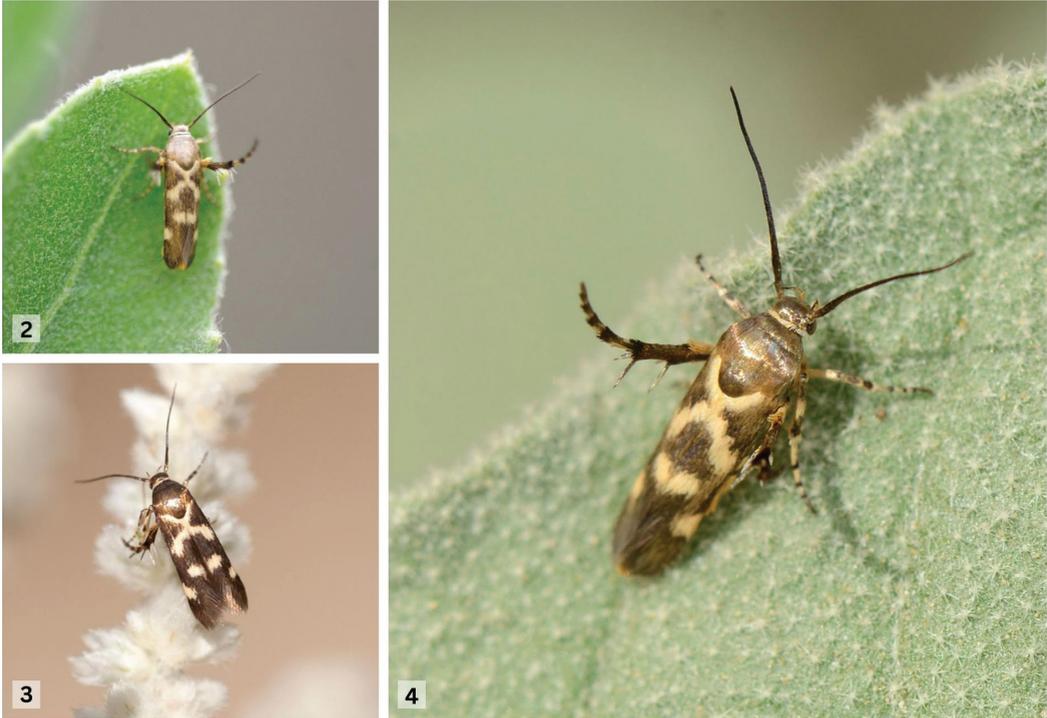
Type material. Holotype: • 1♂; UAE, Al Ain, Ain Al Waal; 24.067°N, 55.748°E; alt. ca 255 m; 15 May 2020; Huw Roberts leg. (at *Aerva javanica* / sweep net) – NHMAD.

Paratypes: • 2♂, 5♀; UAE, Al Ain, Ain Al Waal; 24.067°N, 55.748°E; alt. ca 255 m; 15 May 2020; Huw Roberts leg. (at *Aerva javanica* / sweep net) – males with genitalia slides BÅB 2348X & 2349X. – NHMAD and HRCA. • 4♀; UAE, Al Ain, Ain Al Waal; 24.067°N, 55.748°E; alt. ca 255 m; end of May, 2014, Huw Roberts leg.; 78168–78171 [journal number in coll. BÅB]; genitalia on slides BÅB 2273X & 2274X. – In coll. BÅB, HRCA, and NHMAD. • 3♀; UAE, Al Ain, Ain Al Waal; 24.067°N, 55.748°E; alt. ca 255 m; 25 May 2020; Huw Roberts leg.; genitalia on slides BÅB 2333X. – In coll. HRCA and NHMAD. • 3♀; UAE, Al Ain, Ain Al Waal; 24.067°N, 55.748°E; alt. ca 255 m; 15 May 2020. 87698-70; Huw Roberts leg. – In. coll. HRCA and NHMAD.

Additional material. • 1♀; UAE, Al Ain, Ain Al Waal; 24.067°N, 55.748°E; alt. ca 255 m; 17 May 2022. Huw Roberts leg. – In. coll. HRCA.

Diagnosis. *Eretmocera hafeetensis* sp. nov. (Figs 2, 3, 4) is readily recognised in position of repose by the pale, X-shaped marking combined across the two forewings, most evidently in females. These markings are not observed in any other species in the genus. In contrast to most other species in *Eretmocera*, the antennae in the new species are only insignificantly thickened. The male genitalia are similar to those of e.g. *Eretmocera arabica* (Amsel 1961), but the structure of the gnathos differs in its diverging posterior prongs. They also resemble those of *E. bradleyi* (Amsel 1961), but the uncus is furnished with a row of sclerotized teeth. The female genitalia are characterised by the sclerotized structure in segment 8 with a pair of posteriorly directed extensions furnished with long bristles, and anteriorly displaying narrow, sclerotized “pockets”.

Description. Male (Fig. 5): Wingspan 9–10 mm. Head, collar, and thorax blackish brown with semi-metallic shine. Labial palp slender, up-curved, ivory; second and third (pointed) segment of equal length. Antenna black and slightly thickened in basal part (segment 1–6) and with indication



Figures 2–4. 2. *E. hafeetensis* sp. nov. in resting position on *Chrozophora oblongifolia* (Ain Al Waal, 27.v.2012). 3. *E. hafeetensis* sp. nov. in position of repose on *Aerva javanica*. (Ain Al Waal, 15.iv.2010). 4. *E. hafeetensis* sp. nov. in resting position on *Chrozophora oblongifolia* (Ain Al Waal, 21.v.2014).

of erect scales on segments 4–10; length 0.7 of forewing length. Forewing dark brown or blackish brown, markings yellow: at base a short oblique patch; at one fourth a longer patch directed outwards; near tornus a round spot; and near apex a dorsal round spot. Hindwing with pale yellow tinge, covered by dark brown scales, denser apically. Ventral side of forewing ochreous yellow with faint markings, mirroring the markings on the dorsal side. Ventral side of hindwing ochreous yellow, darkening apically. Fringes in both wings dark fuscous, in hindwing with faint cilia line and richer brown basally. Coxa yellow; femur yellow with many dark brown scales; tibia dark brown with a broad yellow ring in middle; tarsal segments dark brown with few pale scales basally. Abdomen rich yellow-orange, segment 2–4 (5) with a blackish brown ring, in some specimens almost covering the whole segment, segment 5–6 with some blackish scales in middle, last segment black with yellow hair scales in middle.

Female (Fig. 6): Size, colouration, and markings as in male but antenna simple without indication of erect scales.

Male genitalia (Fig. 7a, b): Uncus thick, thorn-shaped. Gnathos large, V-shaped in posterior half. Tegumen conical. Valva slender, claviform, in posterior third densely bristled. Phallus slender, slightly sigmoid, tapered to a point. Vinculum large, spatular. Sternum VIII trapezoid with posterior indentation. Tergum VIII trapezoid, posteriorly and anteriorly concave.

Female genitalia (Fig. 8a, b): See Diagnosis.

Etymology. The species is named after the mountain (Jebel Hafeet) on which it has been found, Jebel being the Arabic word for mountain.

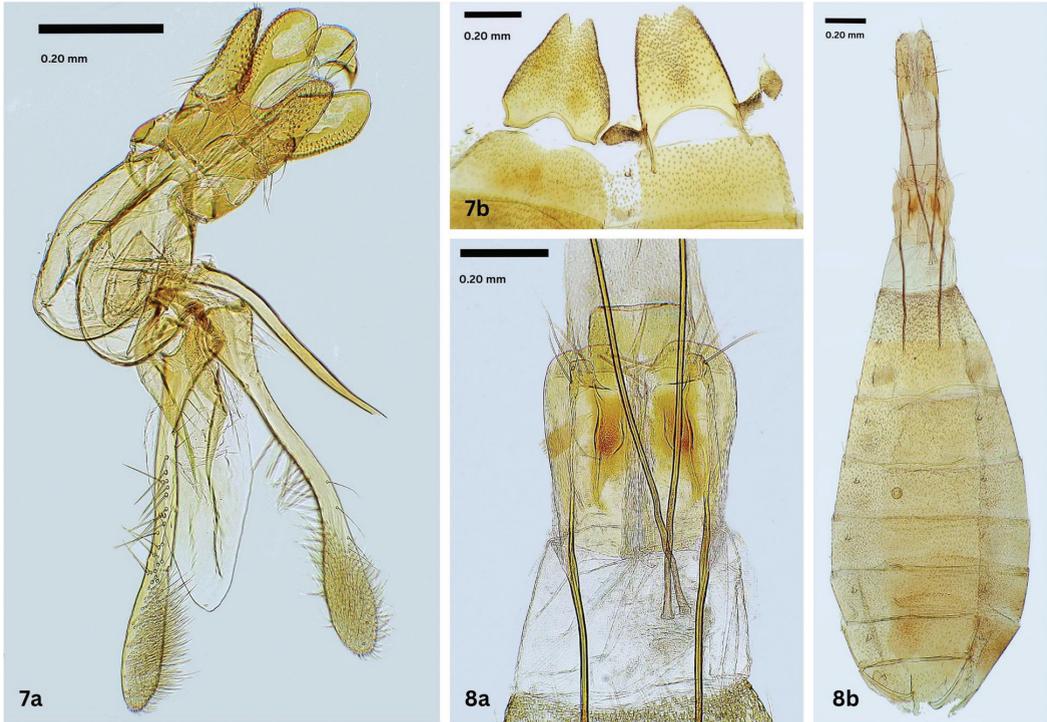


Figure 5. Holotype male of *Eretmocera hafeetensis* sp. nov.



Figure 6. Adult female of *E. hafeetensis* sp. nov. leg. HR, Ain Al Waal, 17.v.2022.

Distribution. The majority of specimens were found on the west flank of Jebel Hafeet, a mountain that straddles the border between UAE and Oman. This isolated anticlinal massif springs up dramatically to over 1,140 metres from a ‘pancake’-flat surrounding area just south of Al Ain, in



Figures 7, 8. **7a.** Male genitalia of *E. hafeetensis* sp. nov. (ventral view) (BÅB 2349X); **7b.** Male genitalia S8 (left) & T8 (right) of *E. hafeetensis* sp. nov. (ventral view) (BÅB 2348X). **8a.** Female genitalia S8 and the ostium region of *E. hafeetensis* sp. nov. (ventral view) (BÅB 2274X); **8b.** Female genitalia of *E. hafeetensis* sp. nov. (ventral view) (BÅB 2274X).

Abu Dhabi emirate. The type locality is given as 24.067°N, 55.748°E (Figs 9, 10). The species was found on plants at elevations of ca. 255–370 m. Two other locations for this species are included in its known distribution, Ain Al Fayda Ladies' and Children's Park (historical), at 24.092°N, 55.719°E (elevation 242 m) and Wadi Nahyan, at 24.096°N, 55.751°E (elevation 290 m).

The discovery was made in the context of an ongoing faunal study of an area measuring 900 meters by 700 metres, at Ain Al Waal (Figs 9, 10). The study area is sandwiched between the mountain and a raised road that provides a barrier between it and a recently built housing area. It is characterized by mature trees (*Ziziphus spina-christi* (L.) Desf., *Prosopis cineraria* (L.) Druce and the non-native, invasive species *Prosopis juliflora* (Sw.) DC.) in the lower lying central area, giving way to scrub land with smaller plants such as *Physorrhynchus chamaerapistrum* (Boiss.) Boiss and *Ochradenus arabicus* S. Chaudhary, Hillc. & A.G. Mill on higher ground. Several wadis feed into this area, including one that features a series of semi-permanent pools, that have in the past been supplemented to render them permanent (for the benefit of a small population of wild Arabian Tahr, *Arabitragus jayakari* (Thomas, 1894) on the mountain). Also, deep holes and caves punctuate the landscape in many locations.

The lack of disturbance in this area over time has undoubtedly helped it to evolve a rich biodiversity. The city's expansion from being an oasis town with population of around 1,500 in 1950 to the fourth largest city in the UAE with a population of around 630,000 in 2022 (Worldpopula-



Figure 9. Type locality of *Eretmocera hafeetensis*: Ain Al Waal, Al Ain, UAE (24.067°N, 55.748°E).



Figure 10. Coverage area of Ain Al Waal study. Map data 2019 (C) Google.

tionreview.com 2022) has until recent years concentrated in areas at least 10 km to the north. Even since 2014, as thousands of houses were being built nearby, security, via a border police presence and a security gate operated by the construction site's general contractor, kept the Ain Al Waal area free of human disturbance.

Climate, habitat and biology. The climate of the region is characterized by high temperatures and low rainfall. In summer, the mean temperature is 36.4 °C (Climate Data 2022), although daytime temperatures often exceed 50 °C. With an average of 17.7 °C, January is the coldest month. Rainfall is erratic, although in most years, there is some rain in January and February, which encourages the growth and proliferation of spring flowering plants.

From late April to June, in most years of average winter rainfall, the new species was found in good numbers, especially on *Aerva javanica*. It was also found once during November. Although the larvae and host plants were not identified during the study, adults were collected on the following plants:

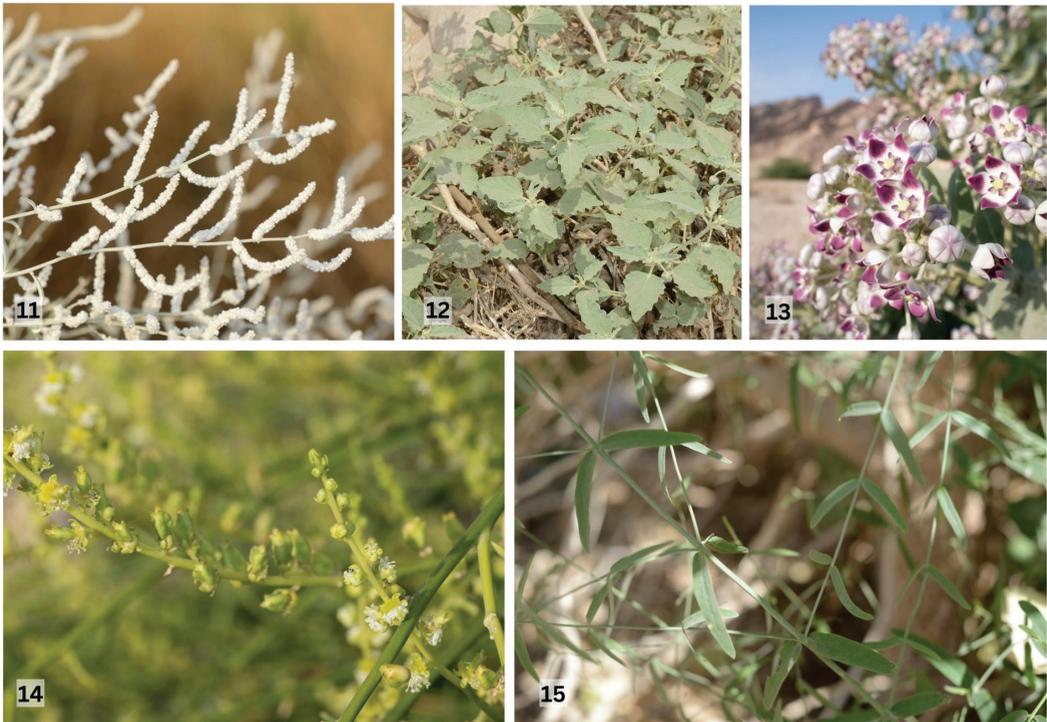
Aerva javanica (Burm.f.) Shult. (Amaranthaceae) 15.iv.2010, 28.v.2012, 8.ii.2019, 19.v.2020, 21.v.2020, 28.v.2020 & 11.xi.2022 (Figs 3, 11)

Chrozophora oblongifolia (Delile) A.Juss. ex Spreng (Euphorbiaceae), 30.iv.2013 & 21.v.2014 (Figs 2, 4, 12)

Calotropis procera (Aiton) W.T.Aiton (Apocynaceae) 1.vi.2019 & 5.vi.2020 (Fig. 13)

Ochradenus aucheri (Boiss) (in Resedaceae) 21.iii.2022, 4.iii.3022, 8.iv.2022 & 17.iv.2022 (Fig. 14)

Salvadora persica L. (Salvadoraceae) at a nearby park, Ayn Al Fayda 3.v.2010 (Fig. 15)



Figures 11–15. Plants on which *Eretmocera hafeetensis* was found. **11.** *Aerva javanica* (Burm.f.) Shult. (Ain Al Waal, 20.xi.2014). **12.** *Chrozophora oblongifolia* (Delile) A.Juss. ex Spreng (Ain Al Waal, 22.v.2014). **13.** *Calotropis procera* (Aiton) W.T.Aiton (Ain Al Waal, 14.iv.2021). **14.** *Ochradenus aucheri* (Boiss) (Ain Al Waal, 7.iv.2023). **15.** *Salvadora persica* L. (Ain Al Waal, 16.vi.2014).

Discussion

Given that nearly all the specimens of this new species have been found at Ain Al Waal, its future prospects would appear to be closely tied up with that of the site itself. However, despite benefitting from an undisturbed environment so far, it is not guaranteed that the area will remain in its present state. While two layers of security have kept the area free of disturbance in recent years,

the encroachment of a human residential area to within metres of the site is likely to be a constant threat in the coming years.

Away from the type locality, there are a few possible habitats for *E. hafeetensis* nearby. Despite many searches on the eastern side of the mountain, and to a species-rich wadi that intersects the mountain from the north (Wadi Tarabat), surprisingly, the species has not been found at those other locations. Ain Al Waal is different in some ways (e.g., has a steeper face, more permeable and fractured composition and hundreds of deep holes and caves), but those other parts of the mountain share much of the fauna and flora occurring at Ain Al Waal, so it is speculated that there is a good chance that the species may be found there as well with a more concerted search.

The other location where this species has been recorded is Ain Al Fayda's Ladies' and Children's Park, 4 km further west, where a few of this species were observed in 2014. That location is no longer irrigated, and it is earmarked for development. That area is flat, and not a mountainside habitat, but the plant at which it was seen, *Salvadora persica*, is commonly planted and irrigated at roadsides. The new moth species may have dispersed away from the mountain, and this particular record does at least show that the species is not confined strictly to mountainside habitats.

Whether the new species might occur further away in the Hajar Mountain range in the northern Emirates (or Oman) is open to question. Between the two mountainous areas, there are differences climatically (the Hajars with more rainfall and cooler), geographically (at least 40 km separation), geologically (predominantly igneous rocks in the Hajars rather than tertiary sedimentary rock in Jebel Hafeet: Kirkham 2003) and in terms of the fauna and flora found in those areas. A large collecting effort by numerous entomologists over several years in recent times (van Harten 2008–2017) failed to discover this species among 12 scythridid species that they found during collections of tens of thousands of arthropod specimens, mostly in the Hajar range of mountains of the UAE.

The discovery of new species at Ain Al Waal will hopefully help to raise awareness of this site. A list of 800 terrestrial fauna species (Roberts unpublished) will show that it is an area that has a rich biodiversity.

Given that *Eretmocera hafeetensis* sp. nov. is not guaranteed to occur away from the mountain, hopes of its long-term survival are pinned on the mountain's new status as a National Park, which it received in 2017. This may lead to greater monitoring of this and other mountainside sites. That may be necessary to protect this new species and hundreds of others that are only now starting to be revealed.

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First pyraloid (Insecta, Lepidoptera) caterpillar from Dominican amber

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Abstract. Only three fossils in the Pyraloidea (Insecta, Lepidoptera) have been confirmed to date, two adults and one larva. The first confirmed larva, in the subfamily Pyraustinae (Crambidae), was described from Baltic amber. Recently, another pyraloid larva from Dominican amber has come to our attention. We describe this second confirmed larval fossil as *Penestola wichardi* Solis, Léger & Neumann, **sp. nov.**, based on larval morphological characters, such as setal patterns and the shape of their sclerotized bases or pinacula, and place it in the subfamily Spilomelinae (Crambidae).

Introduction

Lepidoptera are often said to be extremely rare in the fossil record, including their preservation in amber (Grimaldi and Engel 2005; Sohn et al. 2012). This general observation also applies to Dominican amber, where, among the Amphiesmenoptera, the Lepidoptera have thus far been recorded only sporadically (Penney 2010); in contrast, the Trichoptera are much more common, with 34 species (Wichard 2007; Wichard and Neumann 2021). Dominican amber is a fossil resin that was produced by an extinct *Hymenaea* L. (Fabaceae) tree species and is late Early Miocene to early Middle Miocene in age (20–15 Ma) (Iturralde-Vinent and MacPhee 1996, 2019). This amber is derived mainly from outcrops in the mountains north and northeast of Santiago, Dominican Republic. Weitschat and Wichard (2002) observed that in Baltic amber many lepidopteran inclusions involve caterpillars that most likely fed on the resin produced by the host plant and were embedded in resin flowing or dropping down the tree. The same process can be observed today in extant caterpillars (Fig. 1).

Heikkilä et al. (2018) reassessed the eleven known fossil specimens listed in Sohn et al. (2012) purported to belong to the superfamily Pyraloidea and concluded that only three could be confidently placed in this superfamily based on morphological characters (the fourth fossil from Mizunami amber was not located). Of the three, two were adults, *Eopyralis morsae* Simonsen, 2018, a compression fossil, and *Glendotricha olgae* Kusnezov, 1941. The latter was an inclusion in copal, not amber as had been reported. The third fossil, *Baltianania yantarnia* Solis, 2018, was the only known pyraloid larva in amber (Baltic). Recently, another pyraloid larval fossil in amber



Figure 1. A caterpillar in the act of getting trapped in the resin of a spruce tree near Berlin.

(Dominican) was brought to our attention. We describe this second known fossil crambid larva based on morphological characters such as setal patterns and the shape of the pinacula.

Material and methods

The holotype is a single finding and consists of an excellently preserved larva in Dominican amber. The specimen was provided by Prof. Wilfried Wichard (Bonn), who purchased it in 1974 from a commercial amber trading company (Ámbar del Caribe). It is now located in the amber collection of the Museum für Naturkunde Berlin (MfN), with the inventory number MB.I 11433 (former Wichard amber collection). Digital photographs of the holotype were taken at the MfN using Canon EOS 80D digital camera (Canon, Tokyo, Japan) mounted on a Carl Zeiss AxioScope A5 compound microscope (Carl Zeiss, Oberkochen, Germany), with incident and transmitted light simultaneously. The image of the *Penestola bufalis* (Guenée, 1854) specimen used for comparison was taken

with the Visionary Digital imaging system at the National Museum of Natural History, Washington, D.C. (NMNH) (Fig. 2). Drawings of morphological details (such as setae) were produced using Adobe Illustrator CS6. The amber piece was cut and polished to enhance views of the lateral side.

Images of the fossil larval were compared with the following extant larval taxa at the NMNH: *Pyrausta* Schrank, 1802, and *Achyra* Guenée, 1849 in the closely related Pyraustinae, and genera in several tribes of the Spilomelinae (Mally et al. 2019), including *Diaphania* Hübner, 1818, *Teras-tia* Guenée, 1854 (Margaroniini), *Herpetogramma* Lederer, 1863 (Herpetogrammatini), *Spoladea* Guenée, 1854 (Hymeniini), *Desmia* Westwood, 1832, *Nomophila* Hübner, 1825 (Nomophilini), *Udea* Guenée, 1845 (Udeini), *Penestola* Möschler, 1890, and *Duponchelia* Zeller, 1867 (Steniini). The following larval specimens of *Penestola bufalis* were examined: U.S.A., Florida, Key Largo, 17-I-1974, J.B. Heppner (1 larva), identified by D. M. Weisman 1985 [USNMENT01363016]; U.S.A., Florida, Alachua Co., Gainesville, ex. colony reared on soy-wheat germ diet, 2-VII-2022, J. E. Hayden (7 larvae), identified by J. E. Hayden 2023 [USNMENT01895254].

Morphological terminology for larval characters follows Hasenfuss (1960), Hinton (1946), and Neunzig (1987).

Acronyms and abbreviation of depositories

| | |
|-------------|--|
| A | Abdominal segment; |
| D | Dorsal seta; |
| L | Lateral seta; |
| MfN | Museum für Naturkunde, Berlin, Germany; |
| NMNH | National Museum of Natural History, Washington, D.C., United States; |
| SD | Subdorsal seta; |
| SV | Subventral seta; |
| T | Thoracic segment; |
| V | Ventral seta; |
| XD | Prothoracic seta. |

Taxonomic account

Class: Insecta Linnaeus, 1758

Order: Lepidoptera Linnaeus, 1758

Superfamily: Pyraloidea Latreille, 1809

Family Crambidae Latreille, 1810

Subfamily Spilomelinae, Guenée, 1854

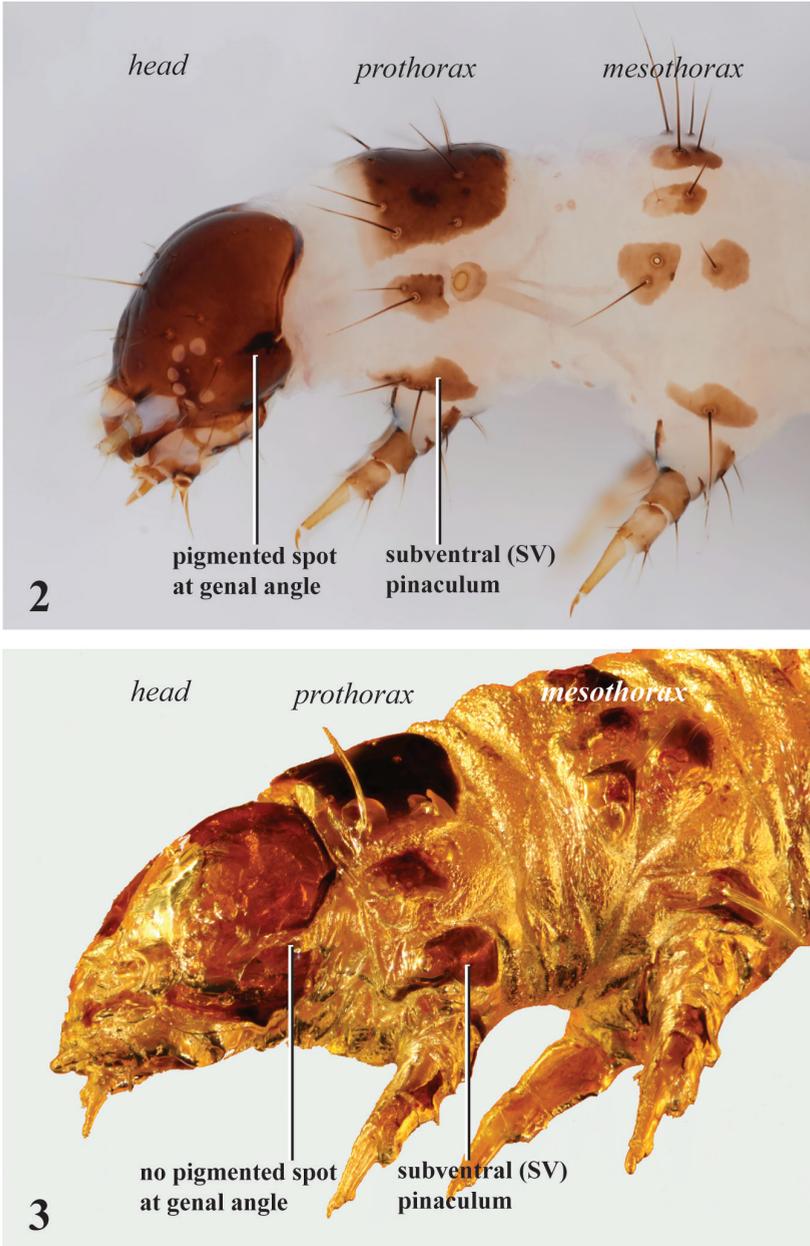
Genus: *Penestola* Möschler, 1890

***Penestola wichardi* Solis, Léger & Neumann, sp. nov.**

<https://zoobank.org/102BCE60-30D2-49AE-8D80-DBEFBAC51ED2>

Figs 3–5

Type material. *Holotype*. The holotype is a larva preserved in Dominican amber of mid Miocene age. It is located in the amber collection of the MfN, inventory number: MB.I 11433 (former Wichard amber collection) (Figs 3, 4, 6).



Figures 2, 3. Lateral view of larval head and thoracic segments. **2.** *Penestola bufalis* (Guenée, 1854) (NMNH); **3.** *Penestola wichardi* Solis, Léger, and Neumann, sp. nov., holotype (MfN).

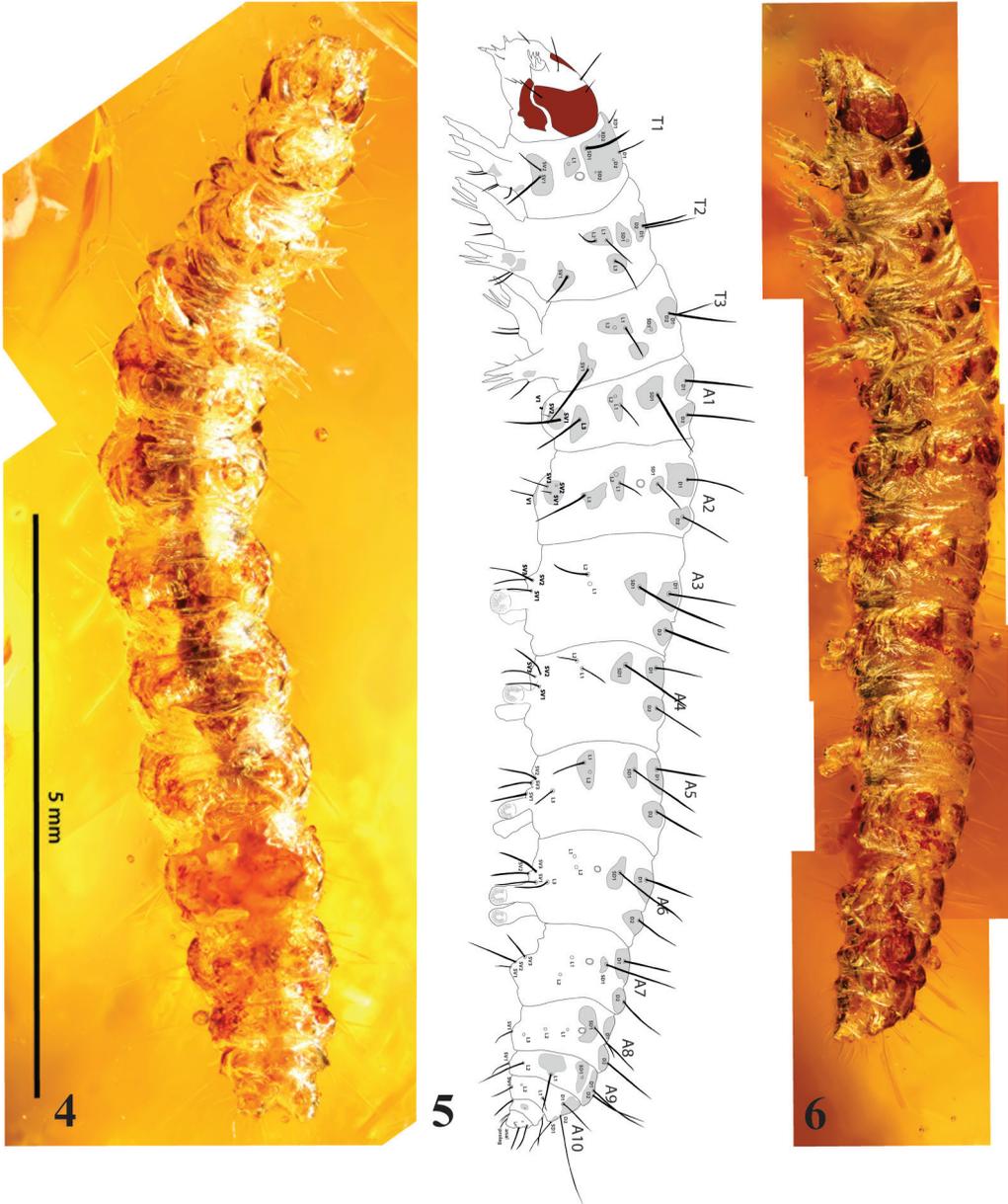
Condition. Excellently (completely) preserved caterpillar (length 9.7 mm) exposing lateral, ventral, and dorsal views. Size of amber piece after preparation: 3.7 cm.

Diagnosis. The holotype of *Penestola wichardi* differs by the absence of a pigmented spot at the genal angle of the head that occurs in *Penestola bufalis* (Figs 2, 3).

Generic placement. *Penestola wichardi* shares with the larva of the extant *Penestola bufalis* a setal number and a placement on pinacula for setae that can be observed. Most obviously, the holotype shares with *P. bufalis*, on the lateral view of the thoracic segments, a distinctive shape of the SV pinacula, a line thin anteriorly and broader posteriorly after the SV seta (Figs 2, 3). On the abdominal segments, the D1 pinacula is round, the D2 pinacula is elongate, and the D2 seta is situated near the lateral edge of pinacula. On the abdominal segments on A1 they share 3 SV setae. Most especially distinctive is the shape of the SV pinacula, a line thin anteriorly and broader posteriorly after the SV seta (this character does not occur in this shape in other spilomeline genera examined). This character also occurs in another taxon in the tribe Steniini, *Duponchelia fovealis* Zeller, 1847, but this species was originally distributed in Europe, and is an exotic species introduced this century into the Western Hemisphere. We should note that the morphological description is not complete due to inability to observe some features in the fossil, for example, the dorsal pinacula on abdominal segments A7–A9, the exact crochet pattern on the proleg, and ventral setal patterns (see Hayden and Burnette 2022 for comparison to *D. fovealis*).

Description. Body: (Figs 3–6) Elongate, cylindrical with a distinct head, thorax, and abdomen. **Head:** Hypognathous, height and width subequal, sclerotized light brown; posterior margin of gena dark brown, pigmented spot at genal angle absent; epicranial suture, frontoclypeus, labrum, and mandible not visible (specifically, the frontoclypeal area of the head capsule is missing, probably damaged, and appears white in color rather than light brown); stemmata 1–5 visible, 1–2 dorsal, 3–5 posterior to antennal base, stemma 6 only slightly visible. Hypopharyngeal complex with aciculate spinneret, prementum only slightly visible. Maxillary palpus and antenna visible. Chaetotaxy difficult to see with the exception of the presence of P1 and the socket of P2 on the frontal area of the head. **Thorax:** T1 dorsally with black pinacula; SD1, XD1 and D1 present; XD2, D2 and SD2 sockets present (seta missing); sockets of L1, L2 present on a sclerotized pinaculum, extending slightly below spiracle; sockets SV1, SV2 present on sclerotized pinaculum, less wide anteriorly. Ventrally T1 with sockets of V1 visible on a single triangular pinaculum. T2 with D1, D2 present on the same pinaculum, circular to subrectangular; SD1 and SD2 sockets present; L1 and L3 present; L2 present on a separate pinaculum; SV1 present, less wide anteriorly. T3 with D1, D2 present on separate pinacula; SD1 present; SD2 socket present; L1 present, L2 socket visible; SV1 present, shorter than in T1 and T2 and less-wide anteriorly. Ventrally T2 and T3 with V1 setae on separate pinacula (shape variable due to preservation distortion). **Abdomen:** A1 with D1 and D2 present on separate pinacula; D1 at same level as D2; SD1 present; L1 present, L2 pore present, L3 not visible; SV setae present, one long, conspicuous seta and one thin, short seta; one long V seta. A2 with D1, D2 present; SD1 socket visible; L1 present, L2 socket visible; 3 SV setae present, one V seta. A3–A6 with D1, D2; SD1 present, clearly visible; L1, L2 present, L3 present on A5, A6; SV1, SV2, SV3 setae visible at base of proleg. Prolegs present. Crochets in a biordinal mesal penellipse. A7 with D1, D2 (setae on separate pinacula), SD1 present; L1, L2 pores present; SV1, SV2, SV3 setae visible. A8 with D1 and D2 on separate pinacula, although D1 does not appear to be directly on the pinacula which could be an artefact; SD1 present; sockets L1, L2 present; 1 SV seta. A9 with D1 present, possibly D2 also present, but difficult to see; SD1 pore visible; L1, L2 setae present; 1 SV seta. A10 with D1, D2 setae present; SD1 present; L1, L2 setae present; SV1 present.

Remarks. The putative placement of this fossil in the Crambidae is based on a unisetose (sometimes bisetose) L group on A9, crochets in an incomplete circle (penellipse), and, most specifically, the lack of a pinaculum ring at the base of SD1 on A8 or any other segments (Hasenfuss 1960;



Figures 4–6. *Penestola wichardi* Solis, Léger, and Neumann, sp. nov. holotype, preserved in Dominican amber. **4.** Ventral view; **5.** Setal chart based on lateral view; **6.** Lateral view.

Neunzig 1987) which is present in the Pyralidae. Although Allyson (1984) was unable to find specific larval characters to define the subfamilies Pyraustinae and Spilomelinae, she noted that many Spilomelinae have the pinacula with setae D and SD fused (this larva does not have D and SD fused as in, for example, *Nomophila*, *Udea*, *Desmia*), and the pinaculum bearing SD1 reduced in abdominal segments 2 and 7, a character that is exhibited by this fossil larva.

The extant *Penestola bufalis* species (Fig. 2), which the fossil caterpillar resembles, is distributed throughout the Western Hemisphere, from Florida and Mexico south to northern South America and islands in the Caribbean, including the Dominican Republic, where its habitat consists of coastal mangrove swamps and shorelines. The Miocene forest biome producing Dominican amber has also been interpreted as a coastal (periodically flooded) swamp forest, as indicated by the occurrence of marine biota such as boring bivalves of the family Pholadidae (Mayoral et al. 2020).

Etymology. It is named in honor of the palaeoentomologist Wilfried Wichard (Bonn) who donated the specimen.

Discussion

Setae and pinacula, or small flat, usually sclerotized areas bearing setae that vary in size, shape, sclerotization, and placement on the larval body, have proven to be taxonomically stable and are used in larval descriptions and classification (Hinton 1946; Stehr 1987). Additionally, significant characters of the head also include setae, tonofibrillary platelets (place where muscles attach to the head), and/or simple sclerotization of various structures such as mandibles or the clypeus (Allyson 1984). In this paper, we define the fossil species and separate it from the extant *P. bufalis* based on the lack of a pigmented or sclerotized spot at the genal angle of the head (Fig. 2). Spots of various sizes and degree of sclerotization can occur in the genal angle of the head, or more commonly, a spot does not occur in the genal angle. In the Spilomelinae, there is an excellent example of the use of the presence and absence of the genal spot to separate larvae of two economically important species that feed on the potato or nightshade family. The Pickleworm *Diaphania nitidalis* (Stoll) has a dark spot at the genal angle, and the Melonworm *Diaphania hyalinata* (L.), does not exhibit the spot (see description and figs 26.218 a–e and 25.219 a–c in Neunzig 1987, and fig. 104 in Solis 1999). The lack of this spot is not an artifact of preservation in the fossil described here, although it appears to be slightly damaged in this area in the image (Fig. 3). Only the caudal edge of the head is sclerotized dark brown, and the spot is lacking on the genal angle on both sides of the caterpillar head.

Fossils in the Pyraloidea are rare, and the fossil described here represents only the second confirmed pyraloid caterpillar. The Order Lepidoptera is one of the larger, successful orders of Insecta, yet the fossil record is comparatively scant in comparison to other insect orders (e.g., Labandeira and Sepkoski 1993; Grimaldi and Engel 2005; Kristensen et al. 2007; Sohn et al. 2015). A comprehensive survey found 4,593 lepidopteran fossil specimens in the literature, of which only eleven fossils were purported to belong to the superfamily Pyraloidea (Sohn et al. 2012, 2015). When Heikkilä et al. (2018) reassessed these fossils, only three could be confirmed to belong to the Pyraloidea, one of which was a caterpillar in Baltic amber (a new genus and species were described for this fossil). And although it is not common to describe extant species based on larvae, we describe this second fossil caterpillar as new to distinguish it from the larva of its extant congener, *Penestola bufalis*. Our current discovery of another caterpillar in amber provides optimism that there are other undiscovered lepidopteran larvae in collections worldwide that have yet to be recognized or critically examined by lepidopterists.

Sohn et al. (2015) also noted that over 78% of lepidopteran fossils remain unidentified. Identification of fossil larvae requires comparison to extant taxa based on the details of morphology. Most of the species of lepidopteran groups such as Noctuoidea or Pyraloidea with larger, free-living larvae have less distinctive overall morphologies, and are usually described as naked or worm-like by

non-specialists, without reference to setal or pinacular morphologies in comparison to extant taxa, as was done in this paper and in Heikkilä *et al.* (2018). A recent paper on lepidopteran caterpillars in Dominican and Mexican amber (Haug *et al.* 2022) illustrates the obstacles in determining the identity of fossil larvae. They reported 19 caterpillar specimens in the literature, with some identified to the family level, but these were larvae whose general morphologies were obvious, e.g., Geometridae, or inchworm caterpillars.

Fischer (2021) stated that it is common practice not to compare fossils with extant taxa because it is often difficult to study internal morphological characters or to use molecular approaches. Additionally, in extant Pyraloidea, the larval stage has only been described for a small proportion of genera. But where pyraloid larval diagnostic morphologies have been examined and compared (e.g., Allyson 1984; Neunzig 1987; Hasenfuss 1960), morphological diagnostic characters are clearly expressed at the genus level. Although we place the caterpillar putatively in the genus *Penestola* and describe it as a new species, it could possibly belong to another genus within the subfamily Spilomelinae whose larval stage has yet to be discovered. There is insufficient knowledge about the morphologies of modern lepidopteran caterpillars, and we emphasize that more research in this field would undoubtedly improve identification of larval lepidopteran fossils.

The sister taxa Pyraustinae and Spilomelinae are the two largest subfamilies in the Crambidae (Mally *et al.* 2019). The Spilomelinae is the most speciose extant subfamily with over 4,126 valid species and the Pyraustinae less so with over 1,284 valid species (Nuss *et al.* 2003–2023). The superfamily Pyraloidea is estimated to have appeared in the Late Cretaceous, about 93 million years ago (Walhberg *et al.* 2013), and in a recent analysis, 91.6 to 77.6 million years ago (Kawahara *et al.* 2023). The two recently described caterpillars, the pyraustine from Baltic amber *Baltianania yantarnia* Solis, about 48–34 million years old and the spilomeline caterpillar described here from Dominican amber, *Penestola wichardi* Solis, Léger & Neumann, sp. nov., about 20–15 million years old, could be used to fine-tune diversification events and their genesis, as more accurately identified pyraloid fossils are discovered.

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