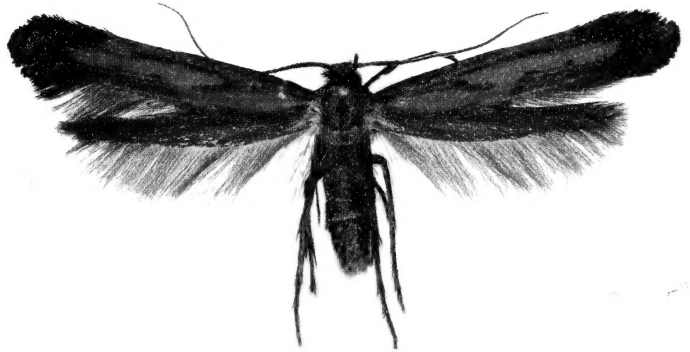




# NOTA

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## NOTA LEPIDOPTEROLOGICA

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# NOTA LEPIDOPTEROLOGICA

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## **Nota Lepidopterologica combines tradition and innovation through open access and advanced publishing model**

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The Societas Europaea Lepidopterologica (SEL) has joined forces with Pensoft as our new publisher to bring the Society's journal *Nota Lepidopterologica* on the way to open access and innovation. Our intentions were first announced at the SEL congress in Bulgaria in 2013 and now we are pleased to announce the first issue of the journal published in an advanced open access format at the brand new editorial platform of Pensoft (<http://nl.pensoft.net>).

Pensoft is well known as one of World's most innovative publishers focusing on all topics related to biodiversity. For years they have been revolutionizing publishing by integrating modern tools with traditional topics into high-quality products.

The scope of *Nota* has not changed and we continue to publish contributions to the study of mainly but not exclusively Palaearctic Lepidoptera, including taxonomy, morphology and anatomy, phylogenetics, biogeography, ecology, behaviour, conservation, as well as other aspects of lepidopterology.

The working interface has, however, changed and now the authors, subject editors, and reviewers work with Pensoft's online editorial management system from the first step of online submission of manuscripts, through the peer-review process, to the final step of proof checking. Pensoft's Editorial Secretary for *Nota Lepidopterologica*, Yordanka Banalieva, will be happy to provide technical support to authors, subject editors, and reviewers.

One of the many improvements is that the waiting time from acceptance of a paper to publication will now be much shorter. Each paper will be published online within weeks of acceptance on the new *Nota* website. Another change is that *Nota* is now an open-access journal and all published papers will be accessible to anyone in the world with an internet connection as soon as the paper is published online. The SEL is happy to be able to cover the open-access fee for all of its members for up to 25 pages per year when they are first authors of a paper. More details on this and all other aspects can be found on the new website.

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per year, one on June 15 and one on December 15, in which we will gather all the papers that were published online in the previous six months.

We hope you will enjoy the new editorial system and outlook of *Nota Lepidopterologica* and we welcome you to the first issue of *Nota* published by Pensoft. In this issue you can read about an unexpected discovery of *Pyrgus malvoides* in the Czech Republic, the revision of *Naarda* (Erebidae), a detailed examination of the Alpine populations of the butterfly *Erebia euryale*, an interesting new species of larentiine geometrids from the Kopet-Dagh Mountains, reduced mouth parts in coleophorids, the taxonomic status of two species of *Ochromolopis* (Epermeniidae), the description of the previously unknown female of *Ethmia cribravia* (Elachistidae), a remarkable new species of gelechiids from Turkmenistan, difficulties of identifying species of *Melitaea* based on their wing morphology, and about choreutids of Madeira.

# The first record of *Pyrgus malvoides* (Elwes & Edwards, 1897) in the Czech Republic (Lepidoptera, Hesperiiidae): an accidental introduction?

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**Abstract.** The Mediterranean skipper, *Pyrgus malvoides* (Elwes & Edwards 1897), is newly recorded for the Czech Republic, some 400 km away from its nearest known population. The specimen was collected in June 1993 in Hejtná, Bohemia and was discovered in the collection of Oldřich Jakeš during a revision of other *Pyrgus* species. The presence of this species elsewhere in the region could not be established and the best current explanation for this record is that it was an accidental introduction.

## Introduction

In recent decades, only a few new butterfly species have been recorded in the fauna of the Czech Republic. For example, in the last ten years *Pyrgus trebevicensis* (Warren, 1926) (Beneš et al. 2001; Hanč 2011) and *Spialia orbifer* (Hübner, 1823) (Šumpich 2011) (both Hesperiiidae) were recorded for the first time in the Czech Republic, and just two additional species were discovered during the last 20 years: *Leptidea reali* Reissinger, 1989 (Pieridae) and *Hyponephele lupinus* (Costa, 1836) (Nymphalidae); the first one very abundant and the latter already extinct, discovered as a museum specimen (Králiček and Povolný 1992; Laštůvka et al. 1995).

During a revision of skippers belonging to the genus *Pyrgus* (Hübner, 1819), an unusual specimen was found in the collection of Oldřich Jakeš with the collecting data: CZ – Bohemia, Hejtná, 1.vi.1993, Jakeš, O. lgt. (see Fig. 1). This locality is in the foothills of Šumava Mts. near the town of Sušice (49°17'13"N, 13°40'18"E); it is covered by supramontane xeric vegetation on limestone, and is part of the Pučanka Nature Reserve.

It was immediately noticed that this male specimen was unusually large and had different colouration from the common *Pyrgus malvae* (Linnaeus, 1758), which is known to occur in the region. The wingspan of this specimen is 16 mm, which is about three millimetres greater than the 11–13 mm wingspan described by Slamka (2004) for *P. malvae*, and it has a light brown colour (Figs 2, 3), which is atypical for *P. malvae* in the Czech Republic. After this discovery, the first author decided to examine more material from across the Czech Republic. The results

of this investigation, presented below, led us to conclude that this unusual specimen is actually *P. malvoides* (Elwes & Edwards 1897).

## Methods

The examined collections are listed below. All material is from the Czech Republic. The first author focused on the determination of different specimens of *P. malvae* from the region, studying 13 specimens from 6 localities near the foothills of Šumava Mts, and then the first author examined more material from other localities in the Czech Republic to attempt to find another specimen of *P. malvoides*. In addition to the examination of external characters, all genitalia were dissected and determined by the first author (det. V. Hula).

## Abbreviations

### Collections

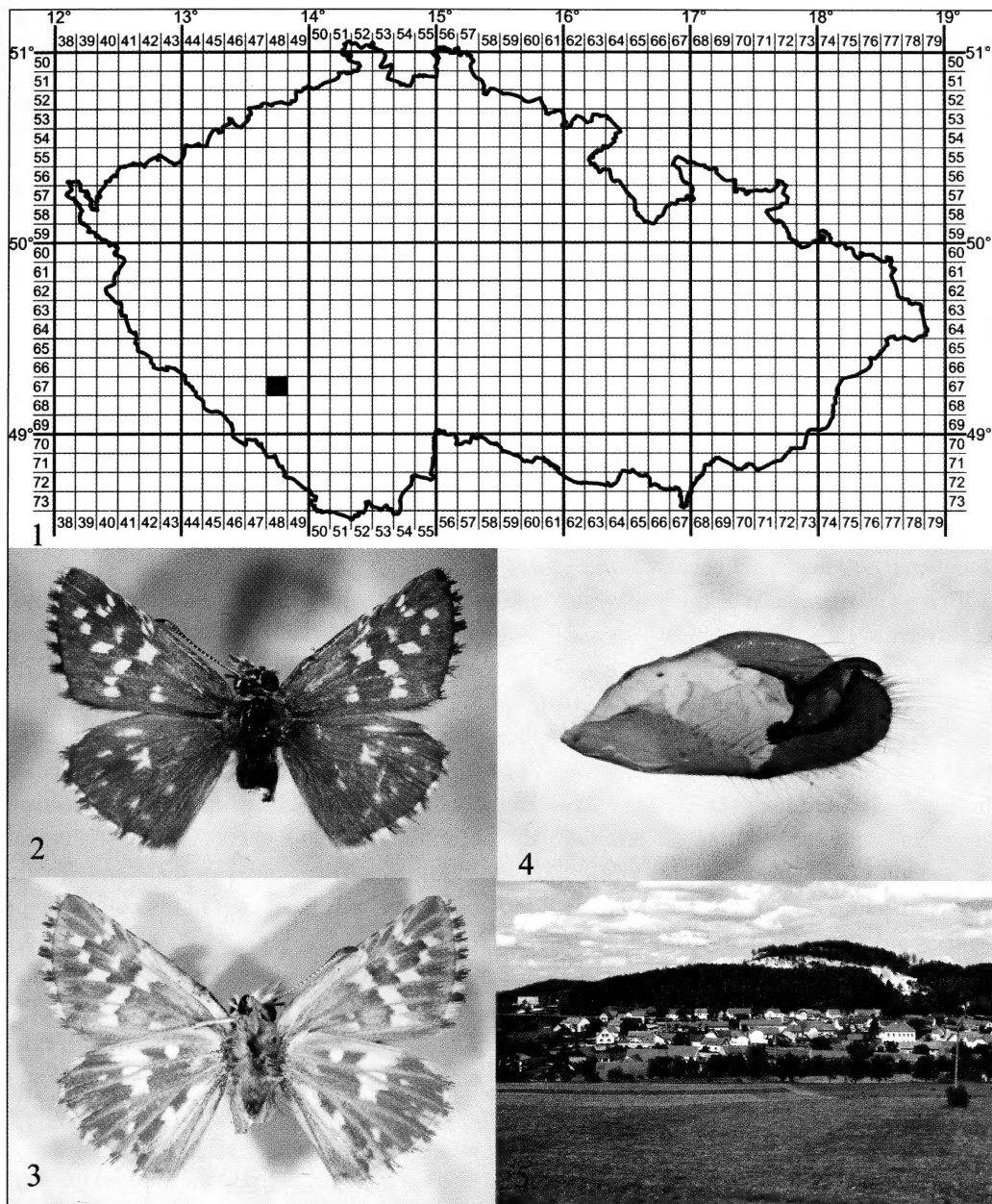
AP	Alois Pavličko, private collection, in Prachatice, Czech Republic
MM	Miroslav Mikát, private collection, in Hradec Králové, Czech Republic
MZMB	Moravian museum Brno, in Brno, Czech Republic
OJ	Oldřich Jakeš, private collection, in Brno, Czech Republic
PS	Pavel Skala, private collection, in Praha, Czech Republic
RMM	Regional museum Mikulov, in Mikulov, Czech Republic
VCMHK	East-bohemian museum Hradec Králové, in Hradec Králové, Czech Republic
VCMP	East-bohemian museum Pardubice, in Pardubice, Czech Republic
VH	Vladimír Hula, private collection, in Brno, Czech Republic
ZCMP	West-bohemian museum Pilsen, in Pilsen, Czech Republic
ZFF	Zdeněk Faltýnek Fric, Institute of Entomology, in České Budějovice, Czech Republic
ZN	Zdeněk Navrátil, private collection, in Brno, Czech Republic

### Other abbreviations

NPR	National nature reserve
PP	Nature monument
PR	Nature reserve

**Material examined. Bohemia:** Rakovník – Papírna, 4.vi.1991 (1 ex), leg., det. et coll. VH. PR Niva u Ždírců nad Doubravou, 28.v.2003 (2 ex), leg., det. et coll. VH. Bražec, 7.vi.2002 (1 ex), leg., det. et coll. VH. Kramolín - Podhora, 20.vi.2003 (1 ex), leg., det. et coll. VH. Plzeň, 28.iv.1957 (2 ex), 9.v.1946 (1 ex.), 25.iv.1966 (1 ex), 27.iv.1966 (1 ex.), leg. VI. Skala, coll. ZCMP; 23.iv.1960 (1 ex), leg. Fraj, coll. ZCMP; Dub. vrch, 9.v.1955 (1 ex), 14.iv.1946 (2 ex), leg. VI. Skala, coll. ZCMP. Dobřany, Břez. vrch, 20.v.1956 (1 ex), leg. VI. Skala, coll. ZCMP; 28.v.1956 (1 ex), leg. VI. Skala, coll. ZCMP. Chlístov, 6.vi.1954 (1 ex), leg. VI. Skala, coll. ZCMP; Jarov, 22.v.1925 (1 ex), coll. ZCMP; Vladař, 1908 (1 ex), coll. ZCMP; NPR Vyšenské kopce, 20.vi.2001 (1 ex), leg. et coll. ZFF; Hředle, 27.v.1995 (1 ex), leg. et coll. ZFF; Hlinky, 19.vi.1994 (1 ex), leg. J. Franz, coll. VH; Police nad Metují, 9.iv.1944 (1 ex), leg. G. Petr, coll. VCMHK; Srbsko, 21.v.1961 (1 ex), 9.v.1962 (1 ex) coll. VCMHK; Číhaná, 22.v.1971 (1 ex), 9.vi.1972 (1 ex), leg. J. Franz, coll. VH; Nové Kounice, 30.v.1992 (1 ex), leg. J. Franz, coll. VH; Rybníčná, 28.iv.1996 (1 ex), 29.v.1991 (1 ex), 31.v.1991 (1 ex), leg. J. Franz, coll. VH; Javorná, 3.vi.1972 (1 ex), leg. J. Franz, coll. VH; Píla, 27.vi.1991 (1 ex), leg. et coll. VH; Horní Tašovice, 31.v.1972 (1 ex), leg. Franz J., coll. VH; Teplička, 14.v.1992 (1 ex), leg. Franz J., coll. VH; Německý Chloumek, 9.vi.1972 (1 ex), leg. Franz J., coll. VH; Česká Třebová, 20.v.1964 (1 ex), leg. Široký L., coll. VCMHK; PP Bář, Hradčany, 1.v.2001 (1 ex), leg. et coll. MM; PP Na Plachtě, Hradec Králové, 15.v.2001 (1 ex), 25.v.2002 (1 ex), leg. et coll. MM; Vysoká and Labem, 8.v.1976 (1 ex), leg. et coll. MM; Slezia, Těšín, 18.v.1930 (1 ex), coll. VCMHK; Oblík, 2.v.1987 (1 ex), leg. Pacl, coll. VCMHK; Prachatice, 18.v.1982 (1 ex), 27.v.1978 (1 ex), 20.vi.1978 (1 ex), 26.iv.1981 (1 ex), 29.iii.1980 (1 ex), 10.vii.1977 (1 ex), leg. et coll. AP; Setuň, Prachatice, 24.v.1985





**Figures 1–5.** 1. Grid square map of the Czech Republic with marked square of record of *Pyrgus malvoides* (Elwes & Edwards, 1897). 2–3. The collected specimen *Pyrgus malvoides*, CZ – Bohemia, Hejná, 1.vi.1993, Jakeš, O. lgt. et coll., Hula, V. det. 4. Male genitalia (valva, lateral view) of collected specimen *P. malvoides*. 5. General view from the collecting locality of limestone quarry.

(1 ex), leg. et coll. AP; Irů Dvůr, Prachatice, 9.v.1986 (1 ex), leg. et coll. AP; Lípa, Prachatice, 10.vii.1977 (1 ex), leg. et coll. AP; Kunětická hora - Ráby, 9.v.1994 (1 ex), leg. J. Macourek, coll. VCMP; Střížovický vrch, Ústí nad Labem, 8.v.2002 (2 ex), leg. et coll. VH; PR Pučanka, Hejná, 1.iv.1993 (1 ex), leg. OJ, coll. VH; Klíčava, (1 ex), leg. Dr. VI. Vala, coll. RMM. **Moravia:** Filipovské údolí, 5.vi.1951 (1 ex), leg. Dr. VI. Vala, coll. RMM; Lideřovice, 11.v.1950

(2 ex), leg. Dr. VI. Vala, coll. RMM; Luhačovice, 20.v.1946 (1 ex), 13.v.1951 (1ex), leg. Dr. VI. Vala, coll. RMM; Uherské Hradiště, 9.v.1949 (1 ex), leg. Dr. VI. Vala, coll. RMM; Křižanovice, 13.v.1947 (1 ex), leg. Dr. VI. Vala, coll. RMM; Břeclav, 13.viii.1979 (1 ex), leg. E. Sandtner, coll. RMM; Valtice, 28.iv.1968 (1 ex), 10.v.1975 (1 ex), leg. E. Sandtner, coll. RMM; Milovice, 18.vii.1962 (1 ex), leg. Minařík, coll. RMM; NPR Malhotky, 25.vi.2004 (1 ex), leg., det. et coll. VH; Moravský Krumlov, 3.vi.1944 (1 ex), coll. MZMB; Senorady, 29.v.1935 (1 ex), leg. Lemberk, coll. MZMB; Habrůvka, 26.v.1982 (1 ex), leg. Kotlán and Vepřek, coll. MZMB; Hády, 1962-1982 (1 ex), leg. Kotlán and Vepřek, coll. MZMB; Macošská Stráň, 4.vi.1994 (1 ex), leg. et coll. PS; Brno - Medlánky, 30.iv.1994 (1 ex), leg. J. Macourek, coll. VCMP; 15.v.1983 (1 ex), leg. R. Čech, coll. ZN. Macošská Stráň, 20.vi.1995 (1 ex), leg. J. Macourek, coll. VCMP; Želešice, 30.v.1991 (1 ex), leg. J. Macourek, coll. VCMP; Perná, CHKO Pálava, 9.vi.2006 (1 ex), leg. et coll. OJ; Mohelno, 29.v.1977 (1 ex), leg. et coll. ZN; Kamenný vrch, Brno, 6.v.1979 (3 ex), leg. et coll. ZN; Soběšice, 11.v.1977 (1 ex), leg. et coll. ZN; Radějov, 25.iv.1948 (4 ex), 26.vi.1954 (1 ex), leg. Dr. VI. Vala, coll. RMM.

## Results and discussion

Twelve collections from 58 sites in the Czech Republic were studied. This included 88 specimens of *P. malvae* from a wide range of territories in the Czech Republic, and even one specimen of *P. malvae* from the same locality as the *P. malvoides* was found. A thorough search uncovered no other specimens of *P. malvoides*.

*Pyrgus malvoides* is a typical west-Mediterranean species with its distribution ending in Slovenia, Italy, westernmost Austria, Liechtenstein, Switzerland and France (Kaufmann 1955; Aistleiner 1996; Lepidopterologen Arbeitsgruppe 1997; Karsholt & Nieukerken 2011). Some authors consider the taxon *P. malvoides* only as a subspecies of *P. malvae* (see Tolman & Lewington 1997; Kudrna et al. 2011). These taxa are able to hybridize on the border of their distribution (Guillaumin 1962, 1971) and the hybrids show characters of both species (Albeti 1956). De Jong (1972, 1987) studied the whole complex of species in the *P. malvae* group and summarised the distribution of all species, specific characters and morphological phylogeny of the group. In this work clear morphological differences in male and female genitalia can be found. The specimen of *P. malvoides* that we discovered in the collection of Oldřich Jakeš has no marks of hybridisation (Fig. 4).

The origin of the specimen is unclear. All available material of *P. malvae* from the region (the foothills of Šumava Mts., the mainly limestone regions and some other places across the whole Czech Republic) was investigated. It is very unlikely that undiscovered populations exist because the butterfly fauna of the Czech Republic has been very well investigated (Beneš and Konvička 2002). It is also unlikely that *P. malvoides* had flown in from the nearest population, which is about 400 kilometres distant.

It is likely that this specimen of *P. malvoides* was accidentally introduced into the locality. There are several possibilities, but the most probable seems to be the introduction by mining techniques, as the locality is in the vicinity of a very large limestone quarry (Fig. 5) owned by the French company Lafarge. Nevertheless, it is an interesting record of this species in Central Europe, and illustrates the importance of paying close attention even to species as common as *Pyrgus malvae*.

## Acknowledgements

We are grateful to Oldřich Jakeš for providing the material of *P. malvae* and *P. malvoides* and all information about collecting. We are grateful to all who shared their *Pyrgus* collections. Thanks to anonymous referees for their comments. Many thanks to Marina Isaac for proofreading. This study was funded by the Internal Grant Agency of Agronomical Faculty (IGA TP6/2013 and TP 7/2014) and the Faculty of Forestry and Wood Technology (73/2013) of Mendel University.

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# Revision of the Palaearctic and Oriental species of the genus *Naarda* Walker, 1866 (Lepidoptera, Erebidae, Hypeninae). Part 3. Description of three new species from Asia

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**Abstract.** We revise the *Naarda egrettoides* species-group and describe and illustrate three new *Naarda* species: *N. ardeola* sp. n. (Thailand), *N. egrettoides* sp. n. (Thailand) and *N. pocstamasi* sp. n. (Vietnam).

## Introduction

The authors began revising the genus *Naarda* in 2000 with the study of the Noctuidae s.l. fauna of Taiwan. In the process, it was discovered that more species occur in Taiwan than was formerly indicated in the literature (Wileman 1915; Strand 1920; Heppner & Inoue 1992). Subsequent studies carried out on the extensive *Naarda* material from different parts of eastern and south-eastern Asia led to an unexpected increase of the known species richness of the genus, with the number of Asiatic species exceeding 80.

These results were first presented by BT (2010) at the IX<sup>th</sup> European Congress of Entomology (Lepidoptera taxonomy and biogeography section), announcing the publication of the undescribed species in the near future before the revision of the whole genus, including its African, Eurasiatic and Australian species, could be accomplished.

The first two parts of the relevant series of articles deal with the taxonomy and biogeography of the genus *Naarda* Walker, 1866 and contain an overview of this large and diverse group, including the general morphological characterisation of the main lineages and the descriptions of altogether 39 new species from eastern and south-eastern Asia (Tóth & Ronkay in press a, b).

The present paper contains the survey of a compact and in the male genitalia highly apomorphic species-group, the *Naarda egrettoides* lineage, including the description of three new species from the south-eastern border zone of the Palaearctic region.

## Material and methods

All but one specimen of the material examined belonging to the *Naarda egrettoides* lineage are deposited at the Hungarian Natural History Museum, Budapest (HNHM); a specimen of a currently unnamed species from eastern China is held by the Alexander Koenig Museum, Bonn (ZFMK).

Genitalia dissections were made using the standard method of preparation resulting in permanent microscopic slides. Genital structures were macerated in 10% KOH solution and lactic acid, stained with alcoholic solution of Eosine and mounted in Euparal. Eosine was preferred in contrast to Chlorazol Black because of the dominance of chitinous structures in the genitalia. The genital terminology follows the detailed explanation given in the first part of the series of papers (Tóth & Ronkay in press a).

### Abbreviations

BMNH	The Natural History Museum, London
HNHM	Hungarian Natural History Museum, Budapest
MNHN	Museum National d'Histoire Naturelle, Paris
RL	genitalia slide of László Ronkay
TB	genitalia slide of Balázs Tóth
ZFMK	Alexander Koenig Museum, Bonn – Zoologisches Forschungsinstitut und Museum Alexander Koenig

### Characterisation of the *Naarda egrettoides* species-group

The species-group is very compact and comprises three closely related species, which are easily separable from members of all other lineages of the genus by the features of the males (see below); the females are also characteristic but display no key features except for the colouration and the fine and less prominent dark markings.

**External morphology.** The diagnostic features are the long bipectinate male antenna, the very long, porrect labial palpi of both sexes, the rather large size, the long and relatively broad forewings with straight costa, and the weak, fine darker markings on a pale greyish or brownish ground colour. The rami of the antenna are at the maximum of its width 9–10 times longer than the axis of the antenna; the apical segments lack the rami. The palpi are ca. 5 times as long as the diameter of the eye, with the third segment comparatively longer, and more elongated than in most other members of the genus. The wingspan is 19–22 mm.

**Male genitalia.** The most prominent autapomorphy of the group is the structure of the uncus (see Figs 1, 3, 5), which appears as a head of a long-billed bird. The uncus has a large, rounded subbasal dorsal bulb bearing a small but acute frontal spine and a large bundle of long hairs standing apart (like a forelock) and long, straight, apically finely dilated and rounded distal section. Other group features of the clasping apparatus are the large, broad tegumen, the well-developed and strong transtilla and the variably broad, generally triangular valvae with which the entire structure resembles a flying bird (wader). Phallus short, thick and straight, vesica basally broad and inflated, ductus ejaculatorius directed forward in the axis of the phallus and vesica. Cornutus variably large, flattened and somewhat 'sponge cake'-like, situated subbasally at ventral side.

## Synopsis

*ardeola* sp. n. (Northern Thailand)

*pocstamasi* sp. n. (Northern Vietnam)

*egrettoides* sp. n. (Northern Thailand)

## Descriptions of the new species

### *Naarda ardeola* sp. n.

<http://zoobank.org/AB56DE34-4DA4-4BE4-87AD-DCD915EC5040>

Figs 1, 2, 7, 8

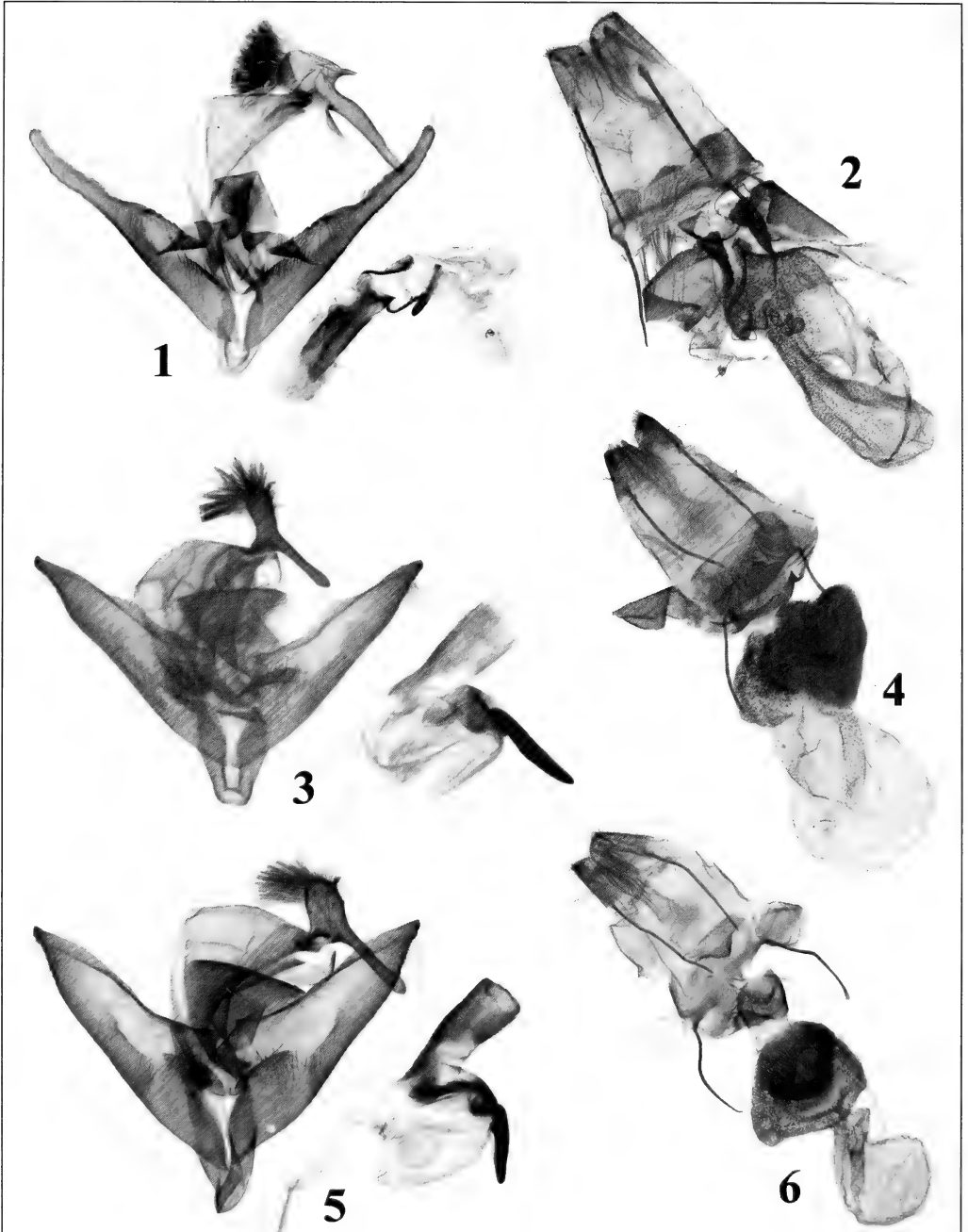
**Holotype.** ♂, ‘Thailand: Prov. Chiang Mai | between Chiang Dao and Kariang, 900 m | 99°48’E, 19°25’N, 26.x.2002 | leg. B. Herczig & G. Ronkay’ slide No. RL7898m (coll. HNHM).

**Paratypes.** Thailand: 2♀, data as holotype, slide No. TB398f; 1♀, ‘Prov. Chiang Mai | 1600m, between Fang and Nor Lae | 99°09’E, 20°02’N, 28.x.2002 | leg. B. Herczig & G. Ronkay’ (coll. HNHM); 1♀, ‘50 km NW Mae Hong-Son | by Shan, 800 m, 14.vii.2003 | leg. M. Fibiger’ slide No. TB424f (coll. HNHM).

**Description.** Wingspan 20–21 mm, length of forewing 10–11 mm. Antennae bipectinate in male but apical segments lack rami, filiform and ciliate in female; longest male rami at mid third, 9 times longer than diameter of flagellum, rami with cilia as long as diameter of flagellum; in female each segment with two cilia as long as diameter of flagellum. Labial palps similar in both sexes; their length 5 times diameter of eyes; 3rd segment relatively long and narrow, its tip pointed, light; 2nd segment broad, dorsal scales long, their length descending towards tip. Scale-hood of vertex broad, tapering, in male relatively long, apically slightly bifurcate, in female long, its tip rounded. Base of male forewing with scent-organ built up of long, hairy scales. Characteristic wing pattern features: forewing costa minutely concave in male; pattern similar in both sexes: ground colour light brown, subterminal line slightly paler, slightly sinuous, with some blackish dots at inner side; postmedial line slightly darker than ground colour, indistinct; reniform stigma big, oval, deep ochreous, hardly visible, with small blackish dot at bottom section; orbicular stigma also deep ochreous, indistinct. Hindwing slightly paler than forewing, with slightly more conspicuous fasciae.

Male genitalia (Fig. 1). Uncus relatively long, straight, tapering, its tip rounded, basal part containing globular bulb with short spine close to elongate part of uncus, and a hairy area oppositely. Scaphium very short, straight. Tegumen longer than vinculum. Transtilla enormously wide; its basal part is the broadest in the genus. Saccus not visible. Base of valva narrow; valva slightly tapering, its tip very narrow, rounded. Sacculus wide, short, distally fused with the homogeneous fused structure constructing the apical two-thirds of valva. Phallus elongate, straight, not tapering; carina with two narrow, curved processes; vesica oval, smooth, with long, broad diverticulum and strong, straight cornutus with rounded tip.

Female genitalia (Fig. 2). Ovipositor lobes slightly elongate, angular. Apophyses quite broad and long, apophyses posteriores 1.3 times longer than apophyses anteriores. Lamella antevaginalis short, angular. Sinus very short and wide. Sternum A7 with two strongly sclerotised narrow bands laterally from ostium, and a proximally located sclerotised triangular field. Ductus



**Figures 1–6.** Male and female genitalia of the species of the *Naarda egrettoidea* species-group. **1.** *Naarda ardeola* sp. n. (RL7898m); **2.** *Naarda ardeola* sp. n. (TB398f); **3.** *Naarda pocstamasi* sp. n. (RL10762m); **4.** *Naarda* sp. (TB443f); **5.** *Naarda egrettoidea* sp. n. (RL7899m); **6.** *Naarda egrettoidea* sp. n. (TB390f).



bursae short, membranous, colliculum small. Corpus bursae elongate, not tapering, with dense scobination except for a dorsal area.

**Diagnosis.** The external appearance of *N. ardeola* is nearly unique: it can only be confused with *N. pocstamasi* sp. n., although the ground colour of *N. ardeola* is somewhat more yellowish, and the transverse lines are less conspicuous than in the latter taxon. The male genitalia of *N. ardeola* differ from those of *N. pocstamasi* by the more angular basal bulge and the slightly broader elongate section of the uncus, the longer and considerably narrower valva, and the more simple structure of the vesica of phallus, with shorter and narrower cornutus. The very elongate valvae in the male genitalia are somewhat similar to those of *N. serra* Holloway, 2008, but the other characters are very different: the basal part of uncus is much broader, with a bulb which is absent in *N. serra*, the scaphium is much shorter, and the base of transtilla is much broader. The penicular processes, being typical of *N. serra*, are absent in *N. ardeola*. The apical half of the valva is made of different structures: in *N. ardeola* it is the complete fusion of costa, cucullus and harpe, while in *N. serra* this part is the free cucullus.

**Etymology.** The heron-shaped male genitalia inspired the specific name.

**Distribution.** The species is known from the mountains of northern Thailand.

### *Naarda pocstamasi* sp. n.

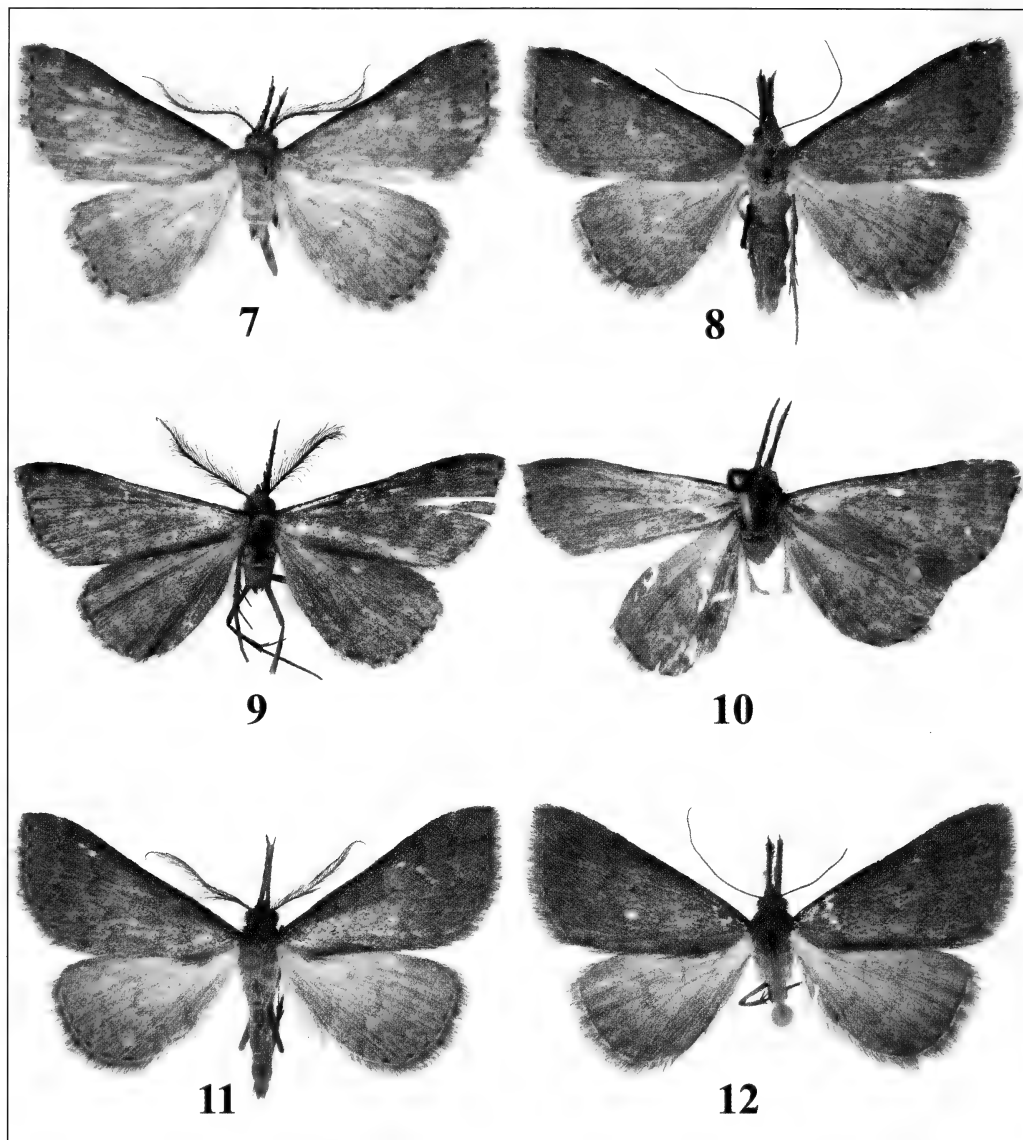
<http://zoobank.org/3364C604-943B-46B5-B4C7-4F604BA28DF2>

Figs 3, 9

**Holotype.** ♂, 'Vietnam, Prov. Lao Cai | Sa-pa, 1650 m | 23.ix.1963 | T. Pócs' 'lieu herb. second. | à la lumière' slide No. RL10762m (coll. HNHM).

**Description.** Wingspan 22 mm, length of forewing 11 mm. Antennae bipectinate (apical segments also), longest male rami at mid third, maximum 10 times longer than diameter of flagellum, rami with cilia ca 1.5 as long as diameter of flagellum. Labial palps slightly longer than 5 times diameter of eyes; 3rd segment quite long and narrow, its distal half lighter; 2nd segment relatively narrow, slightly tapering. Scale-hood of vertex broad and long, triangular and apically pointed. Fore- and midtibiae densely hairy. Characteristic wing pattern features: forewing costa not concave; ground colour light brownish grey (the specimen is somewhat worn), terminal line prominent, fragmented to blackish dots, subterminal, postmedial and antemedial lines present but indistinct, slightly sinuous; reniform stigma inconspicuous, medium-sized, broad, somewhat 8-shaped, with traces of dark edge, brownish ochreous with blackish dot at bottom third and traces of a brownish line in the axis; orbicular stigma rounded with indistinct blackish edge, its colouration like that of reniform, with some dark scales in its centre. Colouration and pattern of hindwing like that of forewing.

Male genitalia (Fig. 3). Uncus medium long, straight, its basal part a densely haired, elongate, apical part evenly narrow, its tip rounded. Tegumen as long as vinculum. Saccus broad-based, long, gradually tapering, its tip rounded. Juxta large, angular. Transtilla large, broad. Saccus not visible. Valva long, medium wide, gradually tapering, but dorsal edge slightly sinuous. Valval tip slightly rounded-truncate. Sacculus with very broad but short, densely haired basal half



**Figures 7–12.** Adults of the species of the *Naarda egrettoides* species-group. 7. *Naarda ardeola* sp. n. male; 8. *Naarda ardeola* sp. n. female; 9. *Naarda pocstamasi* sp. n. male; 10. *Naarda* sp. female; 11. *Naarda egrettoides* sp. n. male; 12. *Naarda egrettoides* sp. n. female.

and narrow, straight distal half connected to apical fused structure. Phallus very thick, evenly wide, straight; vesica globular, longitudinally striated with a large, straight, rounded, somewhat tongue-like cornutus.

**Diagnosis.** The external appearance of *N. pocstamasi* is similar to that of *N. egrettoides* sp. n., but the ground colour is lighter, and the transverse lines are more prominent in *N. pocstamasi*, making it easily recognisable. The male genitalia of this new species are very similar

to those of *N. egrettoides*, but the basal bulb of uncus is more elongate, the valva is somewhat narrower and more elongate, the costa is more sinuous, the phallus is shorter and thicker, and the cornutus is larger. In comparison with those of *N. kinabaluensis* Holloway, 2008, the uncus is much narrower and shorter, the scaphium is much shorter, the valva is somewhat narrower and its tip is pointed instead of being truncate.

**Etymology.** This new species is dedicated to its collector, a famous Hungarian botanist, Tamás Pócs.

**Distribution.** Northern Vietnam.

**Remarks.** This species appears to be the closest hitherto known relative of *N. egrettoides* sp. n. according to the male genitalia.

### *Naarda* sp. near *egrettoides*

Figs 4, 10

**Material examined.** ♀, 'China: Shanghai | Prov. Kiangsu | leg. Höne' slide No. TB443f (coll. ZFMK).

**Morphology.** Wingspan 21 mm, length of forewing 10 mm. Antennae lost except for a few basal segments, those being ciliate, without rami or crests, length of cilia half times diameter of flagellum. Labial palps longest in the genus: length slightly less than 6 times diameter of eyes; 3rd segment relatively long, narrow, its apical third light; 2nd segment broad, dorsal scales very short (or maybe worn). Scale-hood of vertex broad-based, relatively short, its tip pointed. Characteristic wing pattern features: costa minutely concave; the only one known specimen is worn and possibly faded, ground colour greyish brown; transverse lines indistinct; bottom half of postmedial line hardly visible, dark, narrow, probably strongly angled inwards below cell; reniform stigma hardly visible, quite small, oval, dark ochreous, with a big blackish dot at the bottom third; orbicular stigma also hardly visible, small, dark ochreous. Ground-colour of hindwing like that of forewing, with two fasciae.

Female genitalia (Fig. 4). Ovipositor lobes small, angular. Apophyses long; apophyses posteriores ca 1.5 times longer than apophyses anteriores. Lamella antevaginalis angular, sclerotised, sinus absent. Ductus bursae broad but short, membranous, its mouth to corpus bursae located at the dorsal side of corpus bursae. Corpus bursae elongate, pyriform, posterior 2/3 part being scobinate, becoming sparser anteriorly, posterior 1/3 part with a very broad but quite short, smooth appendix.

**Diagnosis.** The wing shape of *N. sp. nr. egrettoides* is similar to that of *N. egrettoides* and *N. ardeola*, but the reniform stigma seems to be narrower, and the orbicular is smaller than in the latter taxa. The female genitalia are somewhat similar to those of *N. barlowi* Holloway, 2008, especially in the configuration of the appendix bursae, which is broad and situated close to the posterior tip of corpus bursae. This species lacks the sinus and the colliculum of ductus bursae and the corpus bursae is only sparsely scobinate, in contrast to *N. barlowi*.

**Distribution.** South-eastern China (Prov. Jiangsu), at the transitional zone of the Palearctic and Oriental Regions.

**Remarks.** The long labial palps and the main structure of the female genitalia are similar to those of *N. egrettoides* and *N. ardeola*, but more material, and especially the knowledge of the

male genitalia, are essential to decide on the more exact taxonomic assignment of this individual. To make the situation even more difficult, the type locality has apparently been destroyed by development in the last seventy years.

### *Naarda egrettoides* sp. n.

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Figs 5, 6, 11, 12

**Holotype.** ♂, 'Thailand: Prov. Chiang Mai | 4 km S Kop Dong, 1800 m | 99°03'E, 19°52'N, 6.xi.2002 | leg. B. Herczig & G. Ronkay' slide No. RL7897m (coll. HNHM).

**Paratypes.** Thailand: 1♂, 3♀, 'Prov. Nan | Doi Phu Kha NP | between Pua and Bo Luang, 1350 m | 101°05'E, 19°12'N, 3.xi.2002 | leg. B. Herczig & G. Ronkay' slide No. RL7899m (coll. HNHM); 2♂, 2♀, 'Prov. Chiang Mai | 1600m, between Fang and Nor Lae | 99°09'E, 20°02'N, 28.x.2002 | leg. B. Herczig & G. Ronkay' slide No. TB390f (coll. HNHM).

**Description.** Wingspan 19–22 mm, length of forewing 10–11 mm. Antennae bipectinate in male but apical segments lacking rami, filiform and ciliate in female; longest male rami at mid third, 9 times longer than diameter of flagellum, rami with cilia as long as diameter of flagellum; in female each segment with four cilia on each side shorter than diameter of flagellum. Labial palps similar in both sexes; length 5 times diameter of eyes; 3rd segment quite long and narrow, its tip pointed, light; 2nd segment broad, dorsal scales long, in male length of scales descending towards tip, in female scale length uniform. Scale-hood of vertex broad-based and long in both sexes, in male its tip finely bifurcate, in female rounded. Characteristic wing pattern features: forewing costa not concave in male; sexes similar; ground colour greyish brown, subterminal and postmedial lines parallel, sinuous, inner part of them dark grey, outer part mouse-grey; medial and antemedial lines also present but indistinct; reniform stigma large, thick, oval, deep ochreous (honey-coloured) with blackish dot at bottom third; orbicular stigma longitudinally elongate, also honey-coloured, with indistinct blackish edge. Hindwing slightly paler than forewing, postmedial line more visible than subterminal line.

Male genitalia (Fig. 5). Uncus relatively long, straight, apically slightly dilated, its tip rounded, basal bulb oval, spine on bulb tiny, situated in front of the connection of bulb and tegumen. Scaphium very short, straight. Transtilla very broad, its base wide. Tegumen slightly longer than vinculum. Saccus not visible. Valva relatively broad-based, triangular, gradually tapering, its tip pointed. Dorsal edge of valva slightly concave. The fused structure present at distal half of valva. Sacculus with broad but short basal half and narrow, straight distal half connected to apical fused structure. Phallus thick, slightly curved, slightly tapering towards carina. Vesica globular, longitudinally striated, with strong, straight, broad and apically rounded, somewhat tongue-shaped cornutus (seen from edge on the figure).

Female genitalia (Fig. 6). Ovipositor lobes slightly elongate, relatively small, angular. Apophyses narrow, apophyses posteriores very slightly (1.1 times) longer than apophyses anteriores. Lamella antevaginalis short, angular. Sinus absent. Sternum A8 with small triangular plate arranged anteriorly. Ductus bursae broad, short, membranous. Corpus bursae composed from of two equal-sized globular halves connected by narrow region; posterior part densely

scobinate by tiny spines, anterior part smooth except for small, sparsely scobinate area near the connection to distal part.

**Diagnosis.** Based on their morphology, it appears that the sister-species of *N. egrettoides* is *N. pocstamasi*; their detailed comparison is given under the diagnosis of the latter. *N. egrettoides* differs externally from the other close relative, *N. ardeola*, by the darker, more greyish colouration. In the male genitalia, *N. egrettoides* and *N. pocstamasi* have, in comparison with *N. ardeola*, much broader valvae, more rounded basal bulge of uncus and much larger cornutus in the vesica. The male genitalia of *N. egrettoides* are somewhat similar to those of *N. ineffectalis* (Walker, 1858), but in the new species the tip of uncus is not concave while the base of uncus is much broader, the juxta is smaller, the valva is more elongate, the sacculus is broader, the cornutus of phallus is straighter and the vesica is larger and more globular than in *N. ineffectalis*.

**Etymology.** *Egrettoides* = egret-like; the shape of male genitalia is similar to a flying egret.

**Distribution.** The mountains of northern Thailand.

**Remarks.** The longitudinally elongate shape of the orbicular stigma is a rare feature in the genus.

## Acknowledgements

We are grateful to Dieter Stüning (ZFMK), Martin Honey (BMNH) and Joël Minet and Jerome Barbut (MNHN) for access to the material of their institutes and the loan of several *Naarda* specimens, which were essential to our work. The authors express their thanks to Béla Herczig, Gábor Ronkay, and the late Michael Fibiger for lending *Naarda* specimens for examination. The first author would like to express his thanks to the leaders of the Hungarian Natural History Museum for obtaining research authorisation for the examination of the *Naarda* material hosted at HNHM. He is indebted also to the staff members of the Lepidoptera Collection for their generous help during his research. The visits of László Ronkay to the BMNH and the MNHN were supported by the SyntheSys Project (Grant Nos GB-TAF-2656 and FR-TAF-562).

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# Reproductive isolation and intraspecific structure in Alpine populations of *Erebia euryale* (Esper, 1805) (Lepidoptera, Nymphalidae, Satyrinae)

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**Abstract.** The subspecies of *Erebia euryale* (Esper, 1805) have been split into three groups based on morphology, differing in male genital characters. Two of them, the *euryale* group and the *adyte* group, are known to be strongly, but not completely, reproductively isolated. There is genetic evidence that their separation preceded the differentiation of subspecies within the *euryale* group. No such data exist on the third group, the recently recognized *kunzi* group. In this study, the degree of reproductive isolation between the *kunzi* group and the other two groups is assessed. In three secondary contact zones, a series of *E. euryale* populations were sampled in a transect perpendicular to the dividing line. Morphological characteristics showed a clinal gradient along each transect. The steepest gradient was found between the *euryale* and *kunzi* groups. Morphologically detectable introgression did not exceed two kilometres. This is comparable to the situation described earlier in contact zones of the *euryale* and *adyte* groups. In the contact area of the *kunzi* and *adyte* groups, the character gradient slope is more gradual and the morphologically detectable introgression zone is at least five times wider. In contrast to this, contact between subspecies belonging to the same group leads to virtually unrestricted morphological intermingling. It is concluded that the *euryale* group is reproductively more strongly isolated from the other two groups than the *kunzi* group is from the *adyte* group, and that subspecies belonging to the same group are interfertile to a high degree. It is argued that loss of genetic compatibility by long term separation is the main cause of the reproductive isolation between groups, and that, consequently, the actual intraspecific structure of *E. euryale* results from at least two, probably three, temporally separated differentiation events.

## Introduction

*Erebia euryale* (Esper, 1805) is a butterfly species with a highly disjunctive distribution and considerable geographic variation. Both its genetic diversity and its distribution pattern have been mainly shaped by climatic fluctuations during the Pleistocene (Schmitt and Haubrich 2008), as was the case in most Palaearctic organisms (Hewitt 1996; Comes and Kadereit 1998; Hewitt 1999, 2000; Kropf et al. 2002; Tribsch and Schönswetter 2003; Schönswetter et al. 2005; Schmitt 2007, 2009). Climate induced range shifts, either latitudinal or altitudinal, repeatedly led to area fragmentation and to retraction or expulsion into glacial refugia. Long-term isolation in these refugia resulted in genetic divergence and, as a consequence, in morphologic differentiation and decreased reproductive compatibility. Each of these three aspects contributed to the actual knowledge of the intraspecific structure of *E. euryale*. Al-

lozyme data suggest a two-level intraspecific structure. Schmitt and Haubrich (2008) determined the genetic distances between eleven *E. euryale* populations. These clustered into four groups. The genetic distances between three of them were roughly equal, while the fourth one was more distant. The authors suggested that this group had split off earlier, and that the observed intraspecific genetic structure has resulted from two subsequent differentiation events. This nested structure was less obvious in a study of mitochondrial DNA (Vila et al. 2011). The morphological structure of *E. euryale* shows two differentiation levels as well. Cupedo (2010) analysed 72 populations, covering all described subspecies, and found them clustering into three groups. These groups differ in male genital anatomy. They are known as the *euryale*, *adyte* and *kunzi* group. The morphological and genetic structuring are concordant: the genetically most distant cluster belongs to the *adyte* group; the three more coherent clusters consistently represent different subspecies of the *euryale* group. The scarce existing data on reproductive isolation fit into this pattern. Representatives of the *euryale* group and the *adyte* group tend to remain separated in secondary contact zones (Rezbanyai-Reser 1991; Sonderegger 2005). Transitional zones are narrow, and hardly contain any hybrids. Some subspecies of the *euryale* group, on the other hand, build transitional zones of up to 40 km in width (Cupedo 2010), mainly consisting of morphological hybrids.

Altogether, genetics, morphology, as well as the degree of reproductive isolation, support the hypothesis of a two-level nested structure of *E. euryale*, at least as far as the *euryale* and *adyte* groups are concerned. Little is known, though, on the *kunzi* group. Morphologically, it has to be ranked in the first level of hierarchical differentiation because it differs considerably from both the *euryale* and *adyte* groups in male genital anatomy. At present, genetic data are lacking, and little is known about contact sites with the other two morphological groups (Cupedo 2010).

The aim of the present study is to assess the degree of reproductive isolation between the *kunzi* group and the other two groups, and to determine whether this is concordant with the morphological traits. For this purpose, three known contact zones, one with the *adyte* group and two with the *euryale* group, were intensively sampled. For each of these contact zones, three questions were addressed: (i) Are hybrid populations present in the contact zone? If so, (ii) what is their composition, and (iii) does their composition show a clinal character gradient across the contact zones? If the latter is found to be the case, the steepness of the cline will provide information regarding the strength of reproductive barriers between the groups. Finally, all available data on reproductive isolation in *E. euryale* are combined in order to establish whether or not they support the hypothesis that two intraspecific differentiation levels exist.

## Material and methods

### *The kunzi group*

The *kunzi* group occupies a restricted but well-defined part of the Italian Alps (Fig. 1). Its distribution area comprises (i) the entire pre-Alps between Lake Como and the Valcellina, (ii) the Bergamasque Alps, (iii) the Southern Rhaetian Alps (Ortler, Adamallo-Presanella, Brenta and Nonsberg Alps), and (iv) the Dolomites south of the Latemar-Focobon chain and west of the



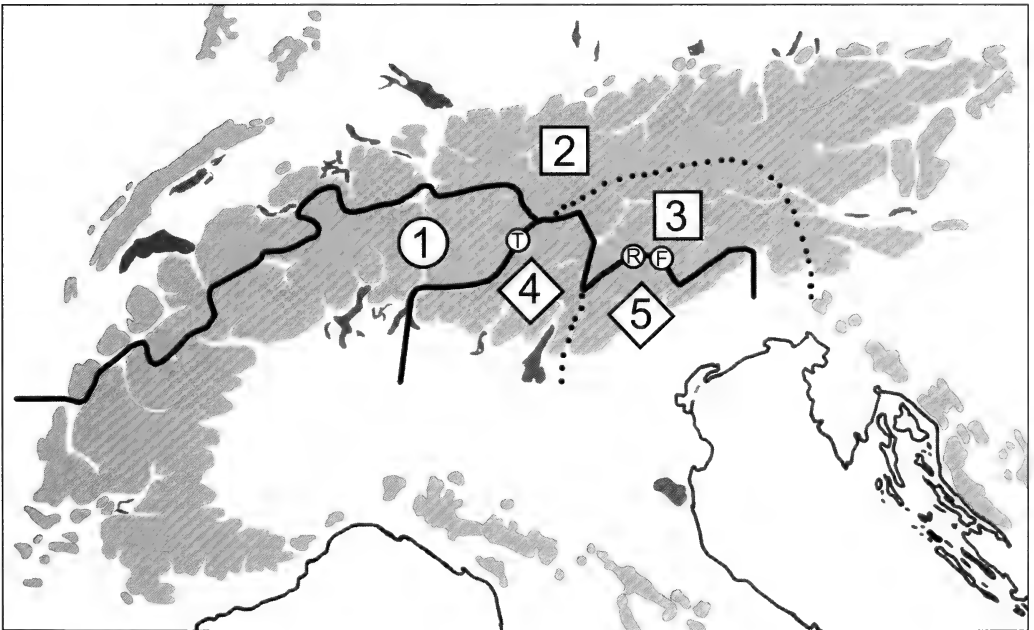
Cordevole river. This territory is almost entirely bordered by insurmountable river valleys and mountain chains.

### Contact sites

Secondary contact with populations of other groups requires natural interruptions in this chain of barriers. Four such “exchange windows” exist, three of which were investigated in this study. These are (i) the Falcade region and (ii) the Passo Rolle region, where the *kunzi* group (ssp. *kunzi*) is in contact with the *euryle* group (represented by ssp. *ocellaris*), and (iii) the Trafoi valley and the Sulden valley upstream of their confluence near Gomagoi, where the *kunzi* group (represented by ssp. *pseudoadyte*) meets the *adyte* group. The fourth exchange window, the upper Valtellina (Adda valley), was not sampled.

### Sampling

*E. euryale* has a two year life cycle. In the contact regions, samples were collected in 2009, 2011 and 2013, so the cohorts on the wing were the same in each collecting season. Samples in the Falcade region are labelled F1–F6 (Fig. 2), in the Passo Rolle region R1–R5 (Fig. 3), and in the Trafoi region T1–T5 (Fig. 4). In the Passo Rolle region, special attention was paid



**Figure 1.** The Alps, with geographic boundaries of the taxa of *E. euryale* discussed in this paper. Light grey: mountain areas above 1000 m, dark grey: lakes. Solid lines: group boundaries. Dotted lines: subspecies boundaries. Circle – the *adyte* group, with ssp. *adyte* (1); squares – the *euryle* group with ssp. *isarica* (2) and ssp. *ocellaris* (3); diamonds – the *kunzi* group, with ssp. *pseudoadyte* (4) and ssp. *kunzi* (5). T = Trafoi test region, R = Passo Rolle test region, F = Falcade test region. The intergradation zone *isarica* / *ocellaris* is included in the *ocellaris* area.

to Passo Colbricon. Here, a short transect was sampled and treated as a separate sample set: C1–C3 (Fig. 3).

Samples from the contact regions (called “test samples”) were compared to samples from populations outside the contact region (“reference samples”). Each reference sample consists of 150 individuals of one subspecies. These originate from five localities, scattered in the territory, but at a distance of at least 40 km from the exchange regions. In the *adyte* territory, no samples were taken west of Lago Maggiore, since ssp. *adyte* might not be genetically homogeneous in its entire distribution area (Schmitt and Haubrich 2008). For sampling locations and sampling sizes see Figs 2–4 and Table 1.

Female genitalia of different groups are indistinguishable. Female wing pattern enables a certain separation of the ssp. *kunzi* and *ocellaris*, but not of the ssp. *adyte* and *pseudoadyte*. Therefore this study is entirely based on male characters.

### Genital preparation

Male abdominal tips were macerated for 10 min in a 10% KOH solution at 100°C, the genital apparatus was extracted, dehydrated in ethanol (96%) for 10 min, and embedded in euparal.

### Variables

Individual males were characterised on the basis of four variables. Three of these are characteristics of the valve and one is derived from the wing pattern. Valve characteristics were measured on the right valve, as described and figured in Cupedo (2010): 1. shoulder index: the height/width ratio of the dorsal shoulder; 2. first tooth: the relative position of the most proximal tooth; 3. tooth length: the relative length of the longest tooth on the shoulder, as a percentage of the valve length; 4. the presence or absence of discriminating elements in the male wing pattern. A character is regarded discriminating if both its specificity and its positive predictive value are  $> 0.90$ , according to the extensive dataset in Cupedo (2010). Discriminating elements are the following (see Table 2): for ssp. *ocellaris* brown ringed ocelli on the hindwing underside; for ssp. *kunzi* white pupils in the ocelli on the forewing upperside, the absence of ocelli on the forewing upperside or on the hindwing upperside, no traces of the brown postdiscal band on the forewing upperside or on the forewing underside. In the case of ssp. *adyte* and ssp. *pseudoadyte*, none of the characters met these criteria. Character 3, on the other hand, does not discriminate between ssp. *kunzi* and ssp. *ocellaris*. Hence, in the contact zone *adyte-pseudoadyte*, characterization of individuals in test populations was based on variables 1, 2 and 3, in the contact zone *kunzi-ocellaris* on variables 1, 2 and 4.

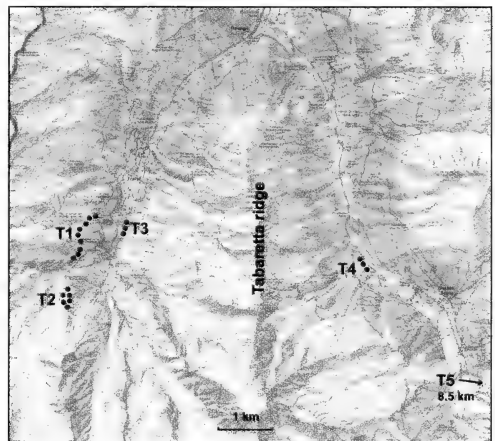
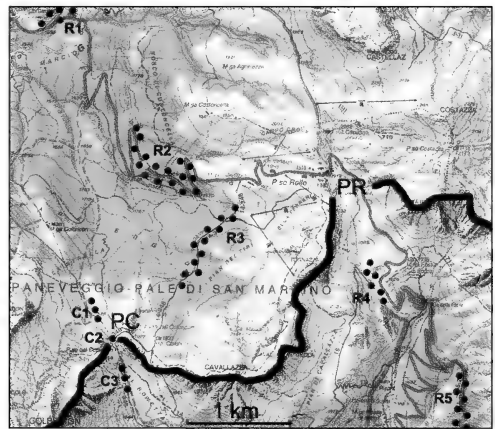
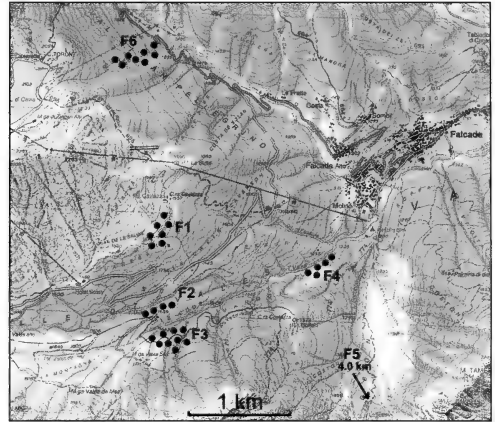
Measurements for shoulder index and first tooth were made using a Mitutoyo 176-902 measuring microscope (magnification 30-fold). Tooth length was measured from calibrated microphotographs on a monitor (final magnification 1000-fold). Variable 4 was assessed with +2 dioptre glasses.

## Scoring system and data analysis

**Characterizing individuals and samples.** A scoring system was developed by which each individual and each sample could be characterised. For each variable, the values of all individuals in both reference groups (e.g. *adyte* and *pseudoadyte*) were combined. The hereby obtained numerical range was split into seven categories, labelled -3 to +3. The centre of the zero category of the scale coincides with the intersection of the frequency distributions of the two reference groups. For each male, the value of each variable was converted into a score, equal to the category it falls into, thus ranging from -3 to +3. Each individual male was characterised by the sum of its scores for the three variables, potentially ranging from -9 (the most *adyte*-like individuals) to +9 (the most *pseudoadyte*-like ones). The scoring procedure was essentially the same in the analysis of the *kunzi* and *ocellaris* samples, except for the fact that variable 3 was replaced by variable 4, which has only three categories: -3 (*ocellaris*), +3 (*kunzi*) or zero (no discriminating wing character present). Each sample was characterised by the frequency distribution of its individual scores.

**Identifying transitional samples.** The frequency distributions of the scores in test samples were compared with those in the reference samples, using the Mann-Whitney U test. A test sample was considered transitional if it differed significantly ( $p < 0.05$ , two-sided) from both reference samples.

**Test for hybridization.** The question whether a transitional sample contains hybrids requires recognition of hybridization, not necessarily of hybrid individuals. Testing for hybridization was based on the assumption that hybrid butterflies are hardly ever equal to one of their parents: they exhibit either a combination of parental characters, or they have intermediate characters (Mayr 1963). In either



**Figures 2–4.** Topography of the test regions. 2. Falcade test region, 3. Passo Rolle test region and 4. Trafoi test region. Dotted: locations of test populations. Solid line in Figure 3: mountain chain. PR = Passo Rolle; PC = Passo Colbricon. Note the different scale in Figure 4. Reproduced from Tobacco maps 022 (Figures 2 and 3) and 08 (Figure 4).

**Table 1.** Sampling locations, sample codes and sample sizes of the sampled *E. euryale* populations. Code = sample code used in this paper; N = sample size

Sample location	Code	N	Sample location	Code	N
<i>spp. adyte</i>	<b>ad</b>	150	<i>Test region Falcade</i>		
Eggen am Simplon (CH)		30	Falcade-1	<b>F1</b>	60
Pontresina (CH)		26	Falcade-2	<b>F2</b>	35
Monte Tamaro (CH)		26	Falcade-3	<b>F3</b>	60
Langtauferertal (I)		30	Falcade-4	<b>F4</b>	48
Fusio (CH)		38	Valle di Gares	<b>F5</b>	14
<i>spp. pseudoadyte</i>	<b>ps</b>	150	Valle di Gares	<b>F5</b>	14
Val Malga, Adamello (I)		33	Passo San Pellegrino	<b>F6</b>	15
Monte Baldo (I)		30	<i>Test region Passo Rolle</i>		
Monte Tremalzo (I)		30	Paneveggio	<b>R1</b>	22
Monte Legnone (I)		27	Passo Rolle road, west	<b>R2</b>	29
Pradalago, Presanella (I)		30	Sentiero laghi di Colbricon	<b>R3</b>	44
<i>spp. kunzi</i>	<b>ku</b>	150	Path Rolle - Colbricon	<b>R3</b>	50
Monte Cavallo (I)		47	Passo Rolle road, east	<b>R4</b>	30
Vette Feltrine (I)		30	San Martino di Castrozza	<b>R5</b>	30
Cimonega (I)		30	Passo Colbricon (north)	<b>C1</b>	37
Col Visentin (I)		30	Passo Colbricon	<b>C2</b>	25
Monte Grappa (I)		13	Passo Colbricon (south)	<b>C3</b>	50
<i>spp. ocellaris</i>	<b>oc</b>	150	<i>Test region Trafoi</i>		
Geissler Gruppe (I)		30	Trafoi, Madatsch	<b>T1</b>	60
Sesto (I)		30	Trafoi, left bank	<b>T2</b>	60
Plöckenpass (I)		30	Trafoi, south of camping	<b>T3</b>	39
Passo Fedaia (I)		30	Sulden, south of Karnerbrücke	<b>T4</b>	60
Lienzer Dolomites (A)		30	Martelltal, Lify alm	<b>T5</b>	60

case, the scores tend to drift to zero, i.e. towards lower absolute values. To test for such shift, category labels were made absolute, and the values of identical categories were added (-9 and +9 became 9, and the numbers in the categories -9 and +9 were added in category 9, etc.). The cumulative graph of the values thus obtained (hereafter called the “absolute graph”) was compared to the graph of an imaginary cohabitational population without hybridization, created by combining both reference samples. If a test sample contains hybrids, its graph will show a left shift when compared to the reference graph. The magnitude of such a shift is an empirical estimation of the degree of hybridization in the transitional population. The procedure is illustrated in Fig. 5.

**Test for clinal variation.** In each test sample, the proportions of individuals with a negative score and with a positive score were calculated. This provides a good approximation of the proportions of parental characters in the population, without the need of individual identification, and regardless of the hybridization rate in the population. These proportions were used to detect geographic clines in the contact zones. Because of the small overlap of the distributions of scores in the *adyte* and *pseudoadyte* reference samples, 3.3% of the positive scores are incorrectly classified as *pseudoadyte*, and 5.3% of the negative scores are incorrectly classified as *adyte*. The more one of both types is predominant, the more this will affect the

**Table 2.** Specificity (sp) and positive predictive value (ppv) of characters discriminating between the subspecies *ocellaris* and *kunzi* of *E. euryale*. \* –Data underlying Table 4 in Cupedo (2010). Abbreviations: Hw = hindwing; Fw = forewing; Up = upperside; Un = underside; Oc = apical ocelli; B = brown postdiscal band.

spp	character	value		# true*	# false*	sp ppv	95% confidence interval
<i>ocellaris</i>	HwUnOc	with brown ring	pos	314	7	0.9857	0.9708-0.9940
			neg	54	484	0.9782	0.9556-0.9911
<i>kunzi</i>	FwUpOc	with white pupil	pos	183	3	0.9918	0.9763-0.9982
			neg	308	365	0.9839	0.9535-0.9965
<i>kunzi</i>	FwUpOc	absent	pos	78	7	0.9810	0.9612-0.9923
			neg	413	361	0.9176	0.8376-0.9661
<i>kunzi</i>	HwUpOc	absent	pos	323	30	0.9158	0.8857-0.9443
			neg	167	338	0.9150	0.8809-0.9419
<i>kunzi</i>	FwUpB	absent	pos	157	4	0.9891	0.9724-0.9970
			neg	334	364	0.9752	0.9375-0.9930
<i>kunzi</i>	FwUnB	absent	pos	115	0	1.0000	0.9899-1.0000
			neg	376	368	1.0000	0.9681-1.0000

*adyte* / *pseudoadyte* ratio. As a result, any clinal character gradient in the *adyte* / *pseudoadyte* contact zone will be slightly underestimated.

## Statistics

Statistical tests were performed with the SPSS 12.0 package. Specificity and positive predictive value of discriminating characters were calculated with MedCalc online statistical calculators for Windows, version 12.7.8.

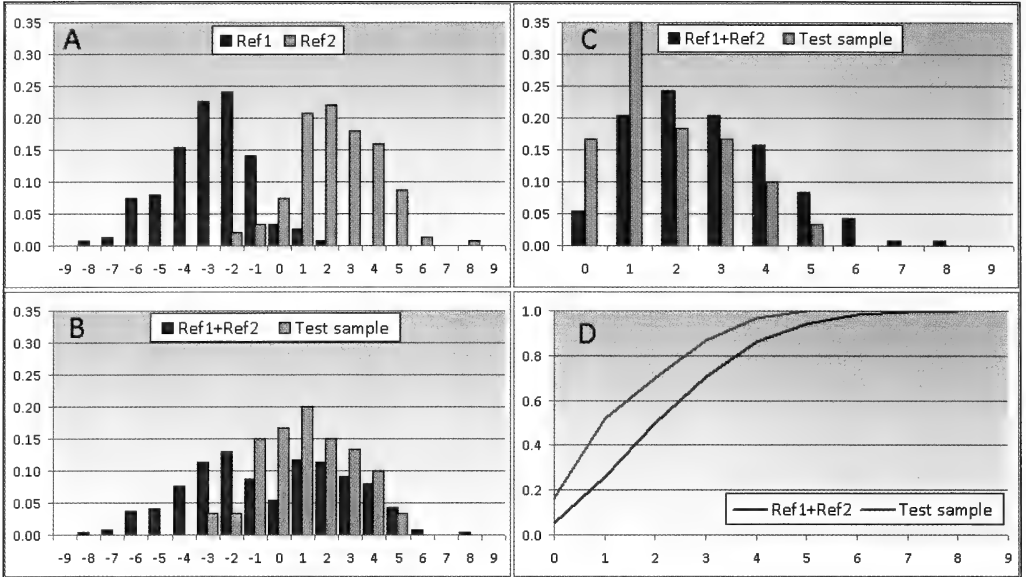
## Material deposition

Samples and genital preparations are deposited in the collection of the author.

## Results

### Identifying transitional samples

The distributions of individual scores per sample are shown in Table 3. The results of a statistic comparison of these distributions (Mann Whitney test), with each other and with the reference samples, for each of the contact zones, are presented in Fig. 6. In each contact zone there were (i) test samples that did not differ significantly either from each other or from one of the reference samples, but did differ from the second reference sample (grey in Fig. 6), and (ii) test samples that differed significantly from both reference samples and from the test samples mentioned under (i). The latter were considered transitional. These were: F2, F3 and F4 in the Falcade region; R3, C1 and C2 in the Passo Rolle region and T3 and T4 in the Trafoi region.



**Figure 5.** Illustration of the procedure for detection of hybrids. A: frequency distributions of scores in reference samples; B: frequency distribution of scores in the combined reference samples, and in a test sample; C: as B, X-axis categories are made absolute. D: as C, cumulative. X-axis: score (A and B) or absolute score (C and D); Y-axis: proportion of the sample.

### Test for hybridization in transitional samples

In Fig. 7, the absolute graphs of all samples are compared to the pooled reference samples. A left shift is obvious in all transitional samples and, to a much lower degree, in some samples that are not classified as transitional. This implies that intergroup mating produced adult hybrids in each of the studied contact zones.

### The composition of the transitional samples

The score distributions of the transitional samples in the hybrid zones *kunzi* / *ocellaris* are different from those in the hybrid zone *adyte* / *pseudoadyte* (Fig. 8). In the former, the ranges almost cover the combined ranges of both reference samples. F2, R3 and C2 show an explicit bimodal distribution; in F3, the distribution is strongly biased towards the *kunzi* phenotype. In the contact area *adyte* / *pseudoadyte*, on the other hand, the range of the individual scores covers no more (T3) or hardly more (T4) than the range of one of the reference samples. Typical *pseudoadyte* individuals are lacking in T3, and typical *adyte* are absent from T4. The distributions are unimodal, but both are skewed towards the hybrid end of the scale.

### Test for clinal variation in the transition zones

The fractions of negative and positive scores (Table 4) exhibited a clinal gradient in each of the contact zones. In the Falcade region, this is along the (F6-F1)-F2-F3-F5 line; in the Passo Rolle region along the R1-R2-R3-(R4-R5) line and along C1-C2-C3, and in the Trafoi test region

**Table 3.** Distributions of individual scores per sample, in reference samples and test samples of *E. euryale*.

		-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	N
Reference samples	ad		1	2	11	12	23	34	36	21	5	4	1								150
	ps								3	5	11	31	33	27	24	13	2		1		150
	oc		7	15	46	41	25	9	5	2											150
	ku											4	1	12	23	33	47	27	2	1	150
Falcade	F1		3	12	17	9	7	6	3	1	1		1								60
	F2		1	6	1	2	2	3	1			1	1	3	4	4	2	3	1		35
	F3				1			2		2	2	3	4	10	10	17	4	4	1		60
	F4			1	1	2	1	2	5	1	5	4	3	6	3	6	5	2	1		48
	F5												2	1	2	3	3	1	2		14
	F6		1	4	4	4	1				1										15
Passo	R1			2	6	8	2	3		1											22
Rolle	R2	1		2	8	8	2	3	2	2		1									29
	R3		1	4	8	5	1	6	10	1	2	2	1			5	3	1			50
	R4												1	4	6	9	5	3	2		30
	R5												3	2	6	9	5	5			30
	C1		1	5	3	5	2	5		1	4	2	1	1	2	3	1	1			37
	C2		1		2	3	1		1	1		1		4	2	3	4	2			25
	C3												1	3	10	16	12	6		2	50
Trafoi	T1			1	3	6	6	12	15	7	6	4									60
	T2				6	4	11	11	13	9	3	1	2								60
	T3					4	4	8	2	8	4	4	5								39
	T4							2	2	9	10	12	9	8	6	2					60
	T5								1	3	8	9	14	8	8	6	2	1			60

along (T1-T2)-T3-T4-T5. Samples in brackets do not differ significantly from each other ( $p < 0.05$ , two-sided).

## Discussion

### The composition of the hybrid samples

Transitional samples were present in each of the three contact zones, and in each of them hybridization took place, so the contact zones discussed in this paper explicitly are hybrid zones. There is, however, a noticeable variation in the score distributions of the test samples, both among and within contact areas. Field observations yield enough additional information to explain these differences.

*1. The Falcade test region.* In the Falcade contact zone, the northern slopes of the Valle di Vales are inhabited by ssp. *ocellaris*, whereas ssp. *kunzi* occupies the southern slopes. Samples F1 and F3 were taken from high-density populations, living in clearings in a mixed fir-larix forest, with F1 at the upper tree limit on the northern slope (1870 m), and F3 in the lower part of the opposite slope (1670 m). In between, individuals of *E. euryale* were scarce, flying along roadsides and on hay meadows. A local concentration was only found at location

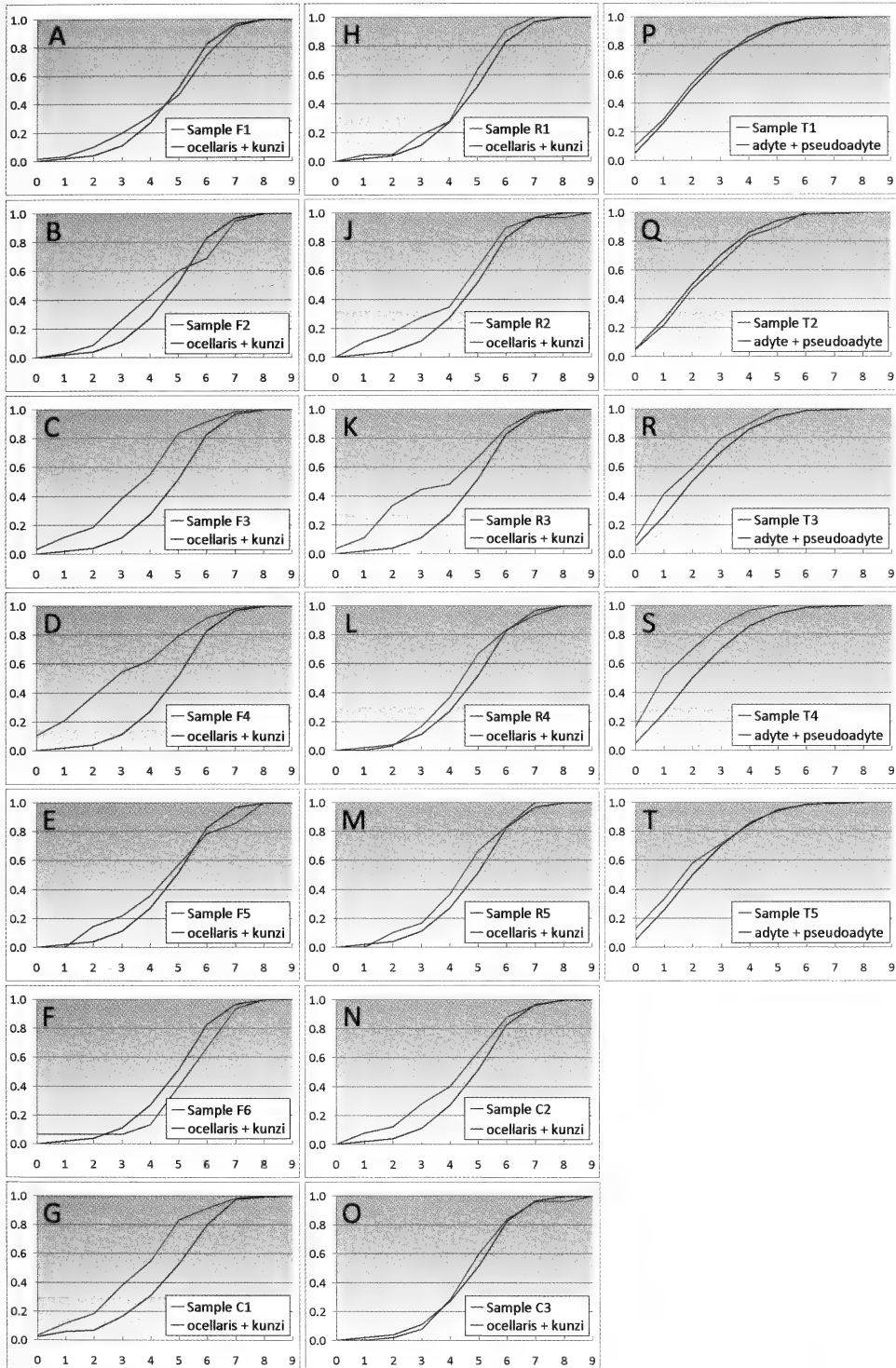
<b>A</b>	oc	F6	F1	F2	F3	F4	F5	ku	<b>B</b>	ad	T1	T2	T3	T4	T5	ps	
oc		ns	ns	***	***	***	***	***	ad		ns	ns	**	***	***	***	
F6			ns	**	***	***	***	***	T1			ns	*	***	***	***	
F1				**	***	***	***	***	T2				**	***	***	***	
F2					**	ns	**	***	T3					***	***	***	
F3						**	*	***	T4						**	***	
F4							**	***	T5							ns	
F5								ns	ps								
ku																	
<b>C</b>	oc	R1	R2	R3	R4	R5	ku	<b>D</b>	oc	C1	C2	C3	ku				
oc		ns	ns	***	***	***	***	oc		***	***	***	***				
R1			ns	**	***	***	***	C1			*	***	***				
R2				**	***	***	***	C2				**	***				
R3					***	***	***	C3					ns				
R4						ns	ns	ku									
R5							ns										
ku																	

**Figure 6.** Mann-Whitney's significance levels for pair wise comparison of the frequency distributions of the scores of all samples. A: Falcade test region; B: Trafoi test region; C: Passo Rolle test region; D: Passo Colbricon. For sample codes see Table 1. Legend for  $p$  values: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , n.s. not significant.

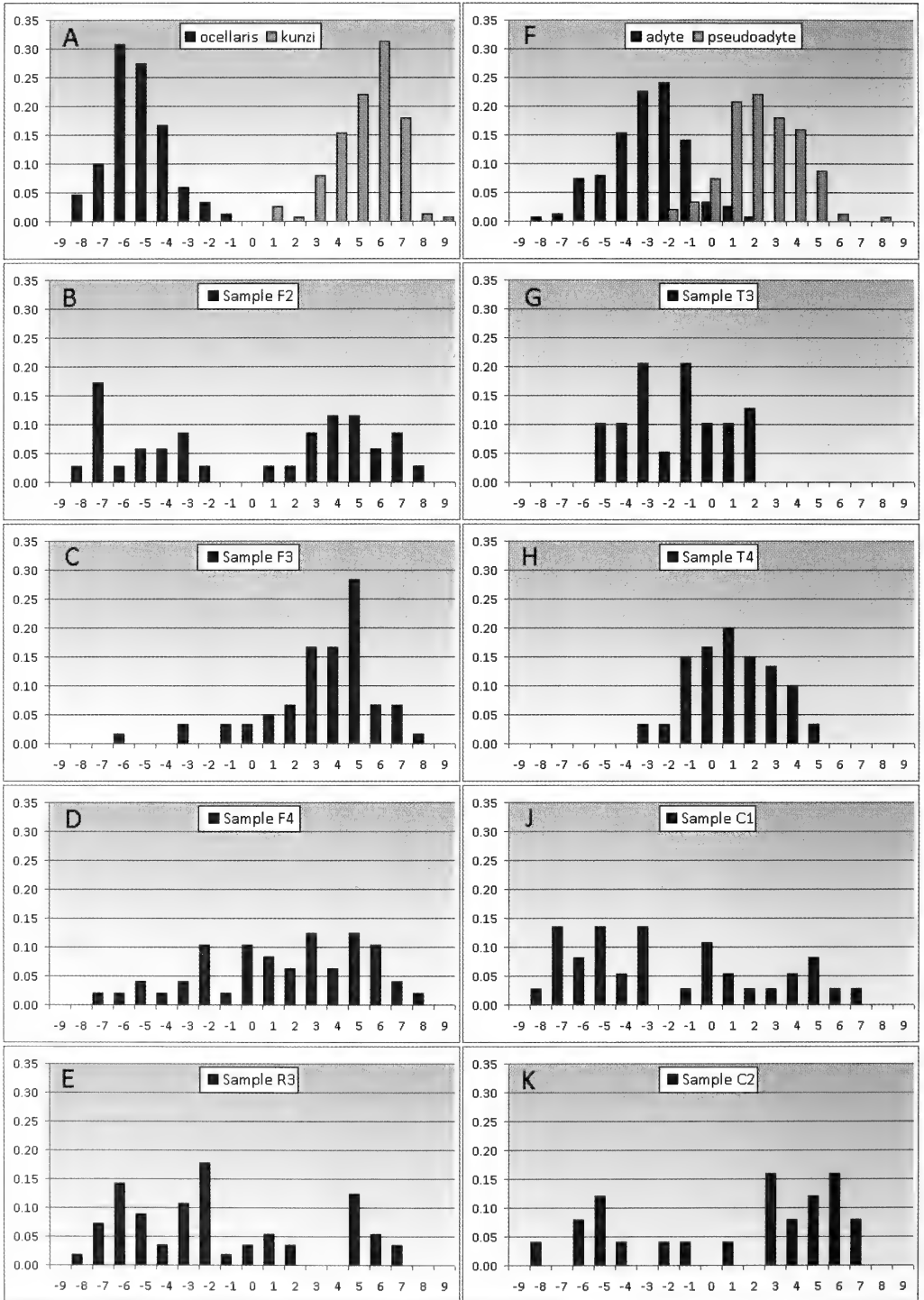
F2. The contact zone, which was sampled in 2009, was revisited in 2013. The situation at F1 and F3 was unaltered. In between, though, more individuals were present than in 2009, but no concentration was observed at site F2. This gives the impression of two stable, permanent populations (F1 and F3), from where individuals swarm out, annually, into the less suitable zone in between. This would explain the temporary character of F2, as well as its bimodal distribution. The score distribution of F1 does not differ significantly from the *ocellaris* reference sample, and F3 shows a *kunzi*-like distribution, which tails out on the left side, evidencing hybridization with *ocellaris*. Apparently, down slope roaming (F1 → F3) exceeds uphill movement in this locality.

One kilometre downstream, the valley floor (at 1250 m) was inhabited by the hybrid population F4. Due to the inaccessibility of the slopes here, no migration was actually observed, but the composition of the population strongly supports a regular influx from both sides. Given the





**Figure 7.** Test for the presence of hybrids in test samples of *E. euryale* in contact zones between groups. X-axis: absolute score; Y-axis: proportion of the sample. For sample codes see Table 1.



**Figure 8.** Frequency distribution of individual scores in reference samples (A, F) and in transitional test samples of *E. euryale*. X-axis: score; Y-axis: proportion of the sample. For sample codes see Table 1.

**Table 4.** Proportions of individuals with a negative and with a positive score, in each of the *E. euryale* samples.

Scores	Reference samples					Passo Rolle							
	ad	ps	oc	ku		R1	R2	R3	R4	R5	C1	C2	C3
<0	0.93	0.05	1.00	0.00		1.00	0.97	0.72	0.00	0.00	0.59	0.36	0.00
>0	0.03	0.87	0.00	1.00		0.00	0.03	0.24	1.00	1.00	0.30	0.64	1.00
Scores	Falcade						Trafoi						
	F1	F2	F3	F4	F5	F6		T1	T2	T3	T4	T5	
<0	0.97	0.46	0.08	0.27	0.00	0.93		0.83	0.90	0.67	0.22	0.07	
>0	0.02	0.54	0.88	0.63	1.00	0.00		0.07	0.05	0.23	0.62	0.80	

high population density in a favourable habitat, this population has to be considered a permanent and breeding population. This might explain why the hybrid ratio is the highest among the analysed hybrid populations.

2. *The Passo Rolle test region.* In the Passo Rolle region, it is the Latemar chain and its continuation, the Focobon chain, that separate *kunzi* from *ocellaris*. Two depressions in this chain, the Passo Rolle and the Passo Colbricon, are potential exchange windows. The largest one, Passo Rolle, is an ecologically devastated area, which offers no suitable habitat to *E. euryale*. A single specimen was observed. Exchange of individuals takes place over the much narrower Passo Colbricon, 2.5 km southwest of Passo Rolle. Here, *E. euryale* was present in relatively high density, on the pass and on both sides. In this continuous population, connecting the *ocellaris* area with the *kunzi* area, an extra set of three samples was taken from nearby sites. C2 was collected on the pass (within 20 metres around the pass mark, Fig. 9), C1 200 to 350 m to the North of the pass and C3 between 200 and 400 m from the pass at its southern slope. This southern sample did not differ significantly from the *kunzi* reference sample, nor was there any detectable hybridization (Table 3, Fig. 7). On the pass, the *ocellaris* portion was 36%, in the more northern C1 sample it was already 59%. This justifies three conclusions: the Colbricon pass, despite its small size, is an important exchange corridor between *kunzi* and *ocellaris*; dispersal is predominantly northward, as C1 and C2 contained a substantial portion of *kunzi*, but *ocellaris* was absent from C3; and maintenance of this sharp separation within a continuous population requires a strong reproductive isolation mechanism.

3. *The Trafoi test region.* In the Trafoi contact zone, *adyte* is widespread west of the Trafoi valley, and *pseudoadyte* occurs east of the Sulden valley. The Tabaretta chain of the Ortler Massif is inserted in between (Fig. 4). The hybrid populations T3 and T4 were found at its foot. The distance T3–T4 is 4.5 km in a straight line (across the Tabaretta ridge, 2800 m), and 8 km when measured along the 2000 m contour line (the most probable migration path). Both distances by far exceed the mean individual range of flight. Gene exchange therefore might take several generations, so parental individuals from T3 will normally not reach T4 and *vice versa*. However, the influx of hybrid phenotypes biases the score distributions of both populations towards the hybrid end of the scale (compare Figs 8G and 8H to Fig. 8F).



**Figure 9.** Passo Colbricon (1908 m), an important exchange corridor of *ssp. kunzi* and *ssp. ocellaris*. View to the south (31.vii.2013).

### Clinal gradients in hybrid zones

In each of the contact zones, a clinal gradient of characters is obvious (Table 4). Because morphologic and genetic clines have been shown to be coincident and concordant (Barton & Hewitt 1985; Collins et al. 1993; Dasmahapatra et al. 2002), the morphologic gradient can be considered to reflect genetic intergradation. Consequently, the rate of introgression can be estimated from the steepness of the morphological gradient.

*The kunzi group and the euryale group.* In the Falcade contact area, the fraction of *ocellaris* characters drops from 0.97 to 0.08 between F1 and F3, and the fraction of *kunzi* characters from 0.88 to 0.02 in the opposite direction (Table 4). That is a mean decline of 87.5% across one kilometre. In the Passo Rolle region, it is 97% across two kilometres (between R2 and R4), and on the Colbricon pass it is 65% over 400 m. These declines are extremely steep, especially when considering that the distances between adjacent samples in all cases are within the normal individual range of flight. In both contact areas, explicit hybrid samples exist (F2, R3, C1, C2), but the more peripheral samples are hardly affected. Morphologically detectable introgression fades out within two kilometres.

*The kunzi group and the adyte group.* In the Trafoi region, the mean decline is 42% over at least 4.5 km, more probably 8 km. Due to the different spacing of sampling, these data cannot directly be compared to those from the *kunzi / ocellaris* contact regions. Nonetheless, if either

in the Falcade or in the Passo Rolle region two populations had been analysed 4.5 km apart, hardly any morphological evidence of introgression was to be expected. In the Trafoi region, though, the decline over this distance is only 42%. This at least justifies the conclusion that introgression between the *kunzi* group and the *adyte* group is less inhibited by reproductive barriers than between the *kunzi* group and the *euryale* group.

*The euryale group and the adyte group.* Rezbanyai-Reser (1991) described three contact sites of the *euryale* group (ssp. *isarica*) and the *adyte* group (ssp. *adyte*) in Switzerland: Hasliberg (BE), Gitschen (UR) and Rophaien (UR). His observations are based on wing characters only, and quantitative data are lacking. In each of these localities, he found a transition zone less than 100 m in width. Intermediate individuals flew together with both parental forms, in the absence of any natural barrier. This is fully comparable to the situation in the Falcade and Passo Rolle regions. In one of his localities, Rezbanyai-Reser (1991) made the important observation that the situation remained unaltered over nine years. In other words, no progress of introgression was observed. Hybrid individuals seemed to be generated *de novo* in each generation. Sonderegger (2005) described two comparable situations in Switzerland, based on both wing pattern and valve characters. At Klosters (GR), a cohabitation zone of about 1 km was found. Only at one specific site in this range, intermediate individuals were found, flying together with both parental forms. At Monstein (GR), a cohabitation site without intermediates was observed. An entirely different situation was recorded in two other sites (Brusons, VS and Grindelwald, BE). Here, the ssp. *isarica* and ssp. *adyte* areas are spatially separated, and Sonderegger found an intermediate population in between. According to Mayr (1942), this is a recurrent phenomenon when hybrid populations are deprived of parental influx over a long period of time. Mayr (1942) states that these populations may achieve phenotypic stability by continuous selection against the most unbalanced hybrid genomes.

*Subspecies within groups.* Hybrid zones of two subspecies of *E. euryale* belonging to the same group are rare, since most of them have allopatric distributions. It is only in the Pyrenees and in the Alps that two subspecies of the same group (the *euryale* group) are in secondary contact. In both cases, one of the two subspecies is strongly melanistic, which enables easy identification of hybrid individuals by wing pattern. The hybrid zone in the Pyrenees is insufficiently documented, but it covers a considerable part of the Pyrenees (pers. obs.). The hybrid zone in the Alps (ssp. *isarica* and ssp. *ocellaris*) has been mapped (Cupedo 2010). All populations in the intergradation zone mainly consist of hybrid individuals; parental types are rare or absent. The hybrid zone attains its maximum width of about 40 km in the eastern Alps. More important than its actual width is the fact that, in contrast to the hybrid zones between groups, introgression between these intra-group subspecies proceeded until further dispersal was inhibited by natural barriers (mountain chains in the north and river valleys in the south). Obviously there is a discrepancy between the narrow but stable hybrid zones between groups, and the freely expanding hybrid zone between within-group subspecies.

### **The cause of the difference between hybrid zones**

More or less stable transition zones between genetically distinct populations have been described in a great variety of organisms (Mayr 1963; Barton & Hewitt 1985; Jiggins & Mallet 2000; Arnold 2006; Schmitt & Müller 2007; Schmitt et al. 2007). They range from some

hundreds of metres to some hundreds of kilometres. Barton and Hewitt (1985) showed that the width of hybrid zones strongly depends on the balance of two antagonistic factors: dispersal and selection. Immigration into the region of overlap tends to widen the transition zone but is continuously counteracted by selection. This selection may be either ecological, i.e. by differences in the environment or by different adaptations to the environment, or genetic, by selection against recombinant genotypes (Barton & Hewitt 1985; Arnold 2006). In the contact sites studied in this paper, ecological factors can be ruled out as selecting factors. The habitats at both sides of the intergradation zones are largely identical, and there are no indications of different ecological preferences among Alpine subspecies of *E. euryale*. Consequently, genetic selection, i.e. a decreased genomic compatibility of the populations in contact, is the most likely factor determining the width of the hybrid zones in *E. euryale*. This selection may be pre-zygotic, by assortative mating, or post-zygotic, by reduced hybrid fertility or viability. In the case of *E. euryale*, the occurrence of hybrids is a sign of random or at least incompletely assortative mating, and the low introgression rate is an indication of reduced hybrid fertility or viability.

Considering the width of the introgression zone, we should keep in mind that morphological markers are far less sensitive than genetic ones. Barton and Hewitt (1985) showed that gradients in secondary contact zones are s-shaped, and that introgression tails out in both directions. Morphologically, these tails will remain hidden because of a lack of resolution. In general, the width of the intergradation zone will be underestimated when based on morphology alone. Geiger and Rezbanyai (1982) have already demonstrated this phenomenon. They found a significantly lower genetic distance (Nei 1972) between *adyte* and the *isarica* population at Hasliberg (one of the Swiss contact sites) than between the same *adyte* population and a more remote, but morphologically identical *isarica* population ( $D=0.036$  and  $0.073$  respectively!). This demonstrates gene flow by introgression, which could not be detected morphologically.

### ***Differentiation levels***

This study of hybrid zones reveals that strong reproductive barriers exist between the *euryale* group and both the *adyte* group and the *kunzi* group. Our results suggest a less strong reproductive isolation of the *adyte* group and the *kunzi* group, but the different spacing of the test samples and the different characters used to discriminate between the groups impede an unambiguous numeric comparison of the results. Reproductive barriers between the subspecies *isarica* and *ocellaris*, both belonging to the *euryale* group, are so weak that they suggest random mating and a high hybrid viability. Consequently, at least two, maybe three, hierarchical levels of reproductive isolation exist between *E. euryale* populations. Since the degree of reproductive isolation is positively correlated with genetic distance, i.e. the duration of the interruption of gene flow (Coyne & Orr 1997; Jiggins & Mallet 2000), the results support the idea of at least two, maybe three, temporally separated differentiation events. In the latter case, disjunction of the *euryale* group and the *adyte-kunzi* precursor would have preceded the disjunction of the *adyte* group and the *kunzi* group. This scenario would be in agreement with the morphological structure, as the *adyte* group and the *kunzi* group are more similar to each other, both in male genital features and in wing pattern, than either is to the *euryale* group.

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## *Scotopteryx kurmanjiana*, a new species from the Kopet-Dagh Mountains (Lepidoptera, Geometridae, Larentiinae)

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**Abstract.** *Scotopteryx kurmanjiana* sp. n. is described from the Kopet-Dagh Mountains in Northeast Iran and South Turkmenistan. The new species is considered to be closely related to *Scotopteryx kuznetzovi* (Wardikian, 1957) described from Armenia. Detailed description of the external morphology and the genitalia characters of the male with six colour photographs and two illustrations of genitalia are provided.

### Introduction

*Scotopteryx* Hübner, 1825, is a widely distributed genus occurring from North Africa throughout Europe to the Pacific East Asia, South Africa and Southern America (Parsons et al. 1999; Scoble and Hausmann 2007). Traditionally it belongs to the tribe Xanthorhoini of the subfamily Larentiinae (Pierce 1914), but recently it was placed again into the tribe Scotopterygini Warren, 1895 (Viidalepp 2011; Schmidt 2013). The genus is one of the species-richest larentiine clades, as more than 70 species are known worldwide (Scoble & Hausmann 2007).

In the course of a recent taxonomic revision of the subfamily Larentiinae (Rajaei Sh. 2012) and after re-describing and publishing the new records of the poorly known *Scotopteryx kuznetzovi* (Wardikian, 1957) (Rajaei Sh. and Stüning 2012), two peculiar *Scotopteryx kuznetzovi*-like specimens have been discovered in the Zoological State Collection of Bavarian State and the private collection of Gyula M. László, collected in the Iranian and Turkmenian sides of the Kopet-Dagh Mountains. Although these two specimens are undeniably closely related to *S. kuznetzovi*, their wing pattern and the genitalic characters show remarkable differences in comparison to those of *S. kuznetzovi*. In addition, the Kopet-Dagh, where the two specimens were collected, is about 1000 km from the known range of *S. kuznetzovi*, which is distributed in South Armenia, West Iran and East Turkey, supporting the taxonomic separation of the examined specimens. These observations led the authors to a description of a new species *Scotopteryx kurmanjiana* sp. n. from the specimens from the Kopet-Dagh Mts as a potential sister species of *S. kuznetzovi*.

## Methods

Genitalia of the examined specimens were dissected following standard procedures (Robinson 1976). Permanent genitalia slides were studied and photographed with a digital stereo-microscope (ZEISS-SteREO: Discovery.V20) in ZFMK. The specimens examined in this study are deposited in the following collections (acronyms after Evenhuis and Samuelson 2007): ZFMK – Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; ZSM – Zoologische Staatssammlung München, Germany; HMIM – Hayk Mirzayans Insect Museum, Tehran, Iran; LG – number of genitalia slide made by Gy. M. László.

### *Scotopteryx kuznetzovi* (Wardikian, 1957)

Figs 3, 4, 6

**Material. Iran:** 2 ♂: Basmenj [NW Iran, SE Tabriz], 15.x. [19]74, [leg.] Damanabi, gen. preps slide Nos: 1063 & 1064/2010 H. Rajaei (coll. HMIM). 3 ♂, Prov. Azerbayejan, E-Garbi, 11 km S of Shoet, 1350m, 31.x-01.xi.2003, leg. P. Gyulai & A. Garai, slide No.: LG 2834; 1 ♂, Prov. Esfahan, 2750m, C-Zagros, Golestan Khuh, 10 km S of Khansar, 10-11.x.2001, leg. P. Gyulai & A. Garai, slide No.: LG 2339; 10 ♂, same data, but collected at 27-28.x.2003; 1 ♂, Prov. Esfahan, 7 km NW of Natanz, (to Kashan), Kuh-e-Karkas, 1500m, 18-19.x.2003, leg. P. Gyulai & A. Garai; 5 ♂, Prov. Esfahan, C-Zagros, 2600m, 2 km NE of Semirrom, 20-21.x.2003, leg. P. Gyulai & A. Garai, slide Nos: LG 2832, 2833 (coll. Gy. M. László). **Turkey:** 2 ♂, Ost Türkei, Van, 2700 m, Güzeldere Pass, 28.ix.1981, leg. P. Kuhna, gen. prep. slide No.: 3074, P. Kuhna; 1 ♂: Prov. Bitlis, Van Gölü, 19 km E of Ahlat, 1700 m, 42°34' E, 38°46' N, 18.x.1993, leg. Gy. Fábíán, B. Herczig, Gy. László and K. Szeőke, (coll. ZFMK). 4 ♂, Prov. Bitlis, Van Gölü, 19 km E of Ahlat, 1700 m, 42°34' E, 38°46' N, 18.x.1993, leg. Gy. Fábíán, B. Herczig, Gy. László and K. Szeőke, slide No. 394 Gy. M. László; 1 ♂, Prov. Dogubayazit, Ishak Pasha Serayi near Dogubayazit, 14–15.x.2003, leg. P. Gyulai & A. Garai (coll. Gy. M. László).

**Remarks.** A large series of *Scotopteryx kuznetzovi* specimens was examined in order to determine the individual variability of morphological characters within this species. As a supplementary result of this survey, *S. kuznetzovi* has been recorded from several new localities, extending our knowledge of its distribution.

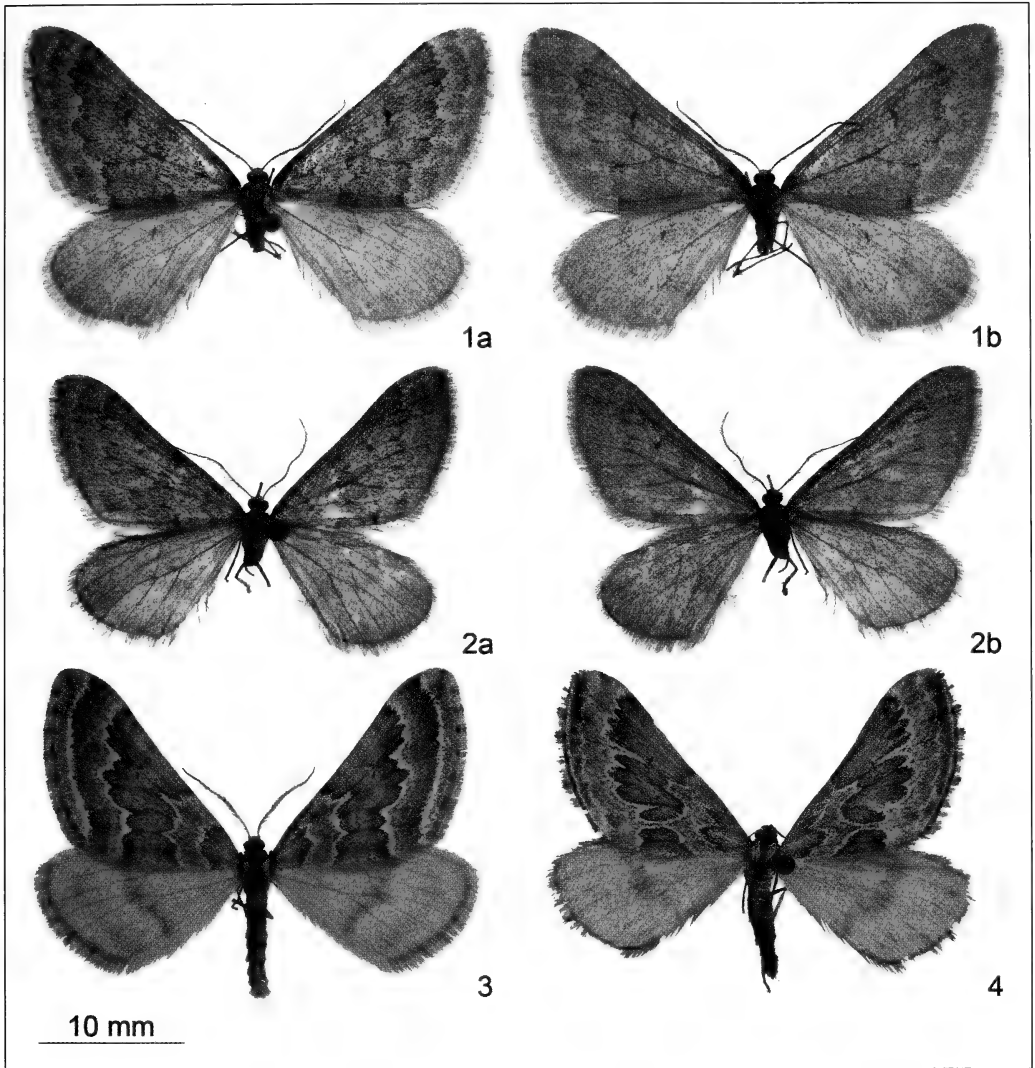
### *Scotopteryx kurmanjiana* sp. n.

<http://zoobank.org/ACDC8372-F2A6-445A-BCFF-B555558076E1>

Figs 1, 2, 5

**Material.** Holotype ♂, **Turkmenistan**, SW Kopetdagh, Garrygala env. 1994.xi.15-30, Leg. Miatleuski J., slide No.: LG 1747 (ZSM). Paratype: 1 ♂, **Iran**, Prov. Khorasan, Kopet-Dagh Mts, 80 km NE of Quchan [Quchan], 1900 m, 37°28' N, 58°34' E, 30.x.2000, leg. B. Benedek & Gy. Fábíán, slide No. LG 1746 (coll. Gy. M. László).

**Description. (Figs 1, 2).** Male: Wingspan 28–33 mm (Length of forewing: 16–18 mm). Antenna bipectinate from base to tip, except 2–3 distal segments, rami moderately long, black, dorsally unscaled, arising ventrally from the proximal end of the flagellum segments. Head, thorax and abdomen covered with mixed brown-white scales. Frons broad, slightly protruding, lower part smoothly covered with very small dark brown scales, upper margin covered with mix of whitish and pale brown scales; vertex with large white and pale brown scales. Chaeto-

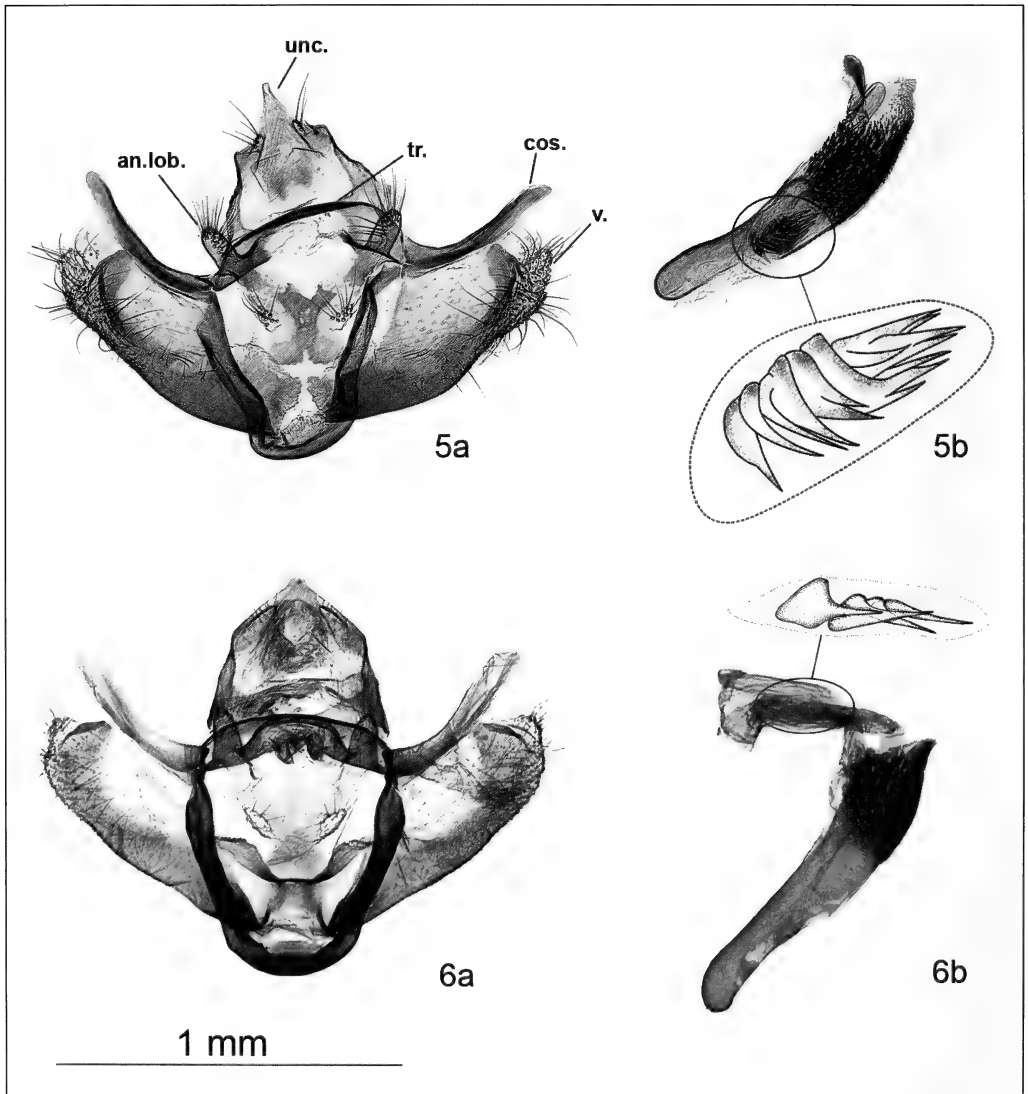


**Figures 1–4.** Wing pattern of adults (males). 1-2: *Scotopteryx kurmanjiana* sp. n. 1. Holotype; NE Quchan, Iran; 2. Paratype; Garrygala, S Turkmenistan; 3-4: *S. kuznetzovi*. 3. Guzeldere Pass, E. Turkey; 4. Basmendj, NW Iran; a: dorsal view, b: ventral view.

semata transversally extended. Palpi short and narrow, acute at tip, just reaching beyond the clypeus. Haustellum almost completely reduced. Index of spurs: 0-2-4. Forewings elongated, apex and tornus rounded; apical patch absent; ground colour pale greyish-brown; basal area and medial band slightly darker, edged with dark brown. Basal line indented. Antemedial line roundly curved in the middle, with two moderately acute incisions. Medial line shadow-like, poorly visible, median area uniformly pale greyish-brown, discal spot small, blackish, sharply defined. Postmedial line wavy, roundly curved outwards in the middle. Submarginal line blurred, poorly visible. Hindwings oval, elongated, cream-brown, crossline absent, discal spot very small, poorly visible. Fringes in both wings unicolorous and consisting of a row of shorter and darker basal scales and a row of longer and lighter terminal scales. Underside pale greyish

brown, generally paler than upperside, basal area and middle band darker, patterns of upper side partly visible. Underside of the hindwing even paler than that of the forewing. Abdomen long, narrow, light grey. Coremata absent.

**Male genitalia (Fig. 5).** Vinculum rather short and broad, gently rounded. Valva short, broad at the base, sacculus well sclerotized, slightly arched, having conspicuous, trapezoidal apical lobe. Distal margin of valva broadly rounded, setose; costal margin strongly sclerotized, slightly arcuate, with an apically rounded, finger-like apical process surpassing the distal edge of valva. Transtilla present, curved, band-like. Uncus broad at base, triangular with narrow, acute tip, curved ventrad. Anellus lobes conically elongated, distally rounded, setose. Juxta X-shaped.



**Figures 5–6.** Male genitalia. 5. *Scotopteryx kurmanjiana* sp. n., Holotype (Slide No.: LG 1747 M; S Turkmenistan); 6. *S. kuznetzovi* (Slide No.: 1063 H. Rajaei; NW Iran); a: genital armature, b: phallus. Abbreviations: an.lob. Anellus lobe; cos. Costa; tr. Transtilla; unc. Uncus; v. Valva.

Phallus tubular, slightly longer than valva, gently curved, distal half covered with the densely spined part of manica, apically with a well-developed finger-like process of carina; vesica bears a bunch (over 10) of broad-based, strongly sclerotized cornuti.

**Female.** Unknown.

**Diagnosis** (Figs 1–6). According to its morphological and genital features, *Scotopteryx kurmanjiana* appears to be closely related to *S. kuznetzovi* but is nevertheless easily distinguishable by several characters (for the distinctive features between *S. kuznetzovi* and other related *Scotopteryx* species see Rajaei Sh. and Stüning 2012). External features: the new species has less protruded frons in comparison with *S. kuznetzovi* and in general less shiny wing surface; the transverse lines are much less sharply defined, lacking the white highlight, which is very characteristic in *S. kuznetzovi*; the median area is much paler, less contrasting in *S. kurmanjiana*; the submarginal line is inconspicuous, more or less shadow-like, whereas it is sharply defined with whitish scales in *S. kuznetzovi*; the discal spots are present in both wings while these are absent in the hindwings of *S. kuznetzovi*. The apical patch of *S. kurmanjiana* is indistinct while a whitish triangular patch is present in *S. kuznetzovi*; the fringes are uniformly whitish-grey, while they are chequered with dark brown in *S. kuznetzovi*. Finally, the hindwing of *S. kurmanjiana* is unicolorous, without transverse line or band, while a well-defined dark-grey medial band is present in *S. kuznetzovi*.

The specific differences between the two species are well expressed in genitalia (see the Figs 5a and 6a): the new species has shorter and smaller apical lobe of sacculus, more sclerotized, much thinner costal margin of valva with conspicuously shorter and apically more tapering costal process, broader base of uncus, somewhat more strongly sclerotized and differently shaped juxta. The distal half of the phallus of the new species is covered with densely spined part of manica, while the spinose part of manica is only one third of the length of the phallus in *S. kuznetzovi*. Finally, the vesica of *S. kurmanjiana* is armed by more than 10 well-sclerotized cornuti, while the number of cornuti in *S. kuznetzovi* is fewer than five.

**Bionomy.** The known specimens of *S. kurmanjiana* were collected in the late autumn, similarly to *S. kuznetzovi*. The foodplant and the early stages are unknown.

**Distribution.** The species is known to occur on both sides of Kopet-Dagh Mountains in NE Iran and S Turkmenistan.

**Etymology.** The name of the species is dedicated to the Kurmanj people in northeastern Iran. This Kurdish tribe migrated from Kurdistan and settled in Khorasan-e Shomali, mainly in Quchan, Shirvan and Bojnurd.

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## Description of the reduced mouth parts of *Coleophora micronotella* Toll (Lepidoptera, Coleophoridae), with a new synonym

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<http://zoobank.org/103A6A77-76A0-4416-AEEF-1DD57AC3A019>

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**Abstract.** The reduced mouth parts of *Coleophora micronotella* Toll (Coleophoridae) are described and illustrated. The proboscis is lacking, but rudimentary maxillary and labial palpi are present. The mouth parts of *C. micronotella* are compared with those of other species of *Coleophora*, including some others with reduced mouth parts, and their possible adaptive value is discussed. *Ramidomia* Falkovitsh, 2005 and *Ramidiomia* Falkovitsh, 2005, a misspelling, are synonymized with *Coleophora* Hübner, 1822.

**Résumé.** La morphologie des pièces buccales très réduites de *Coleophora micronotella* Toll (Coleophoridae) est décrite et illustrée. Cette espèce présente des palpes maxillaires et labiaux rudimentaires, mais aucun proboscis. Les pièces buccales de *C. micronotella* sont comparées avec celles d'autres espèces de *Coleophora*, incluant quelques-unes ayant des pièces buccales réduites, et la possible valeur adaptative de la réduction des pièces buccales est discutée. *Ramidomia* Falkovitsh, 2005 et *Ramidiomia* Falkovitsh, 2005, à l'orthographe fautive, sont mis en synonymie avec *Coleophora* Hübner, 1822.

### Introduction

In Glossatan Lepidoptera, which comprise over 99.9% of the species of the order, the mouth parts typically consist of a labrum bearing a pair of lateral pilifers anteriorly, a haustellum (proboscis) formed by the modified galeae of the maxillae and a pair of maxillary palpi medially, and a pair of labial palpi posteriorly. Mandibles are fully formed only in the non-glossatan Micropterigidae and Heterobathmiidae. In some scattered groups of species of various families and in some larger groups, such as a good proportion of Lasiocampoidea and Bombycoidea, however, a reduction of the mouth parts can be observed. This phenomenon has not been dealt with comprehensively in the literature and we offer some observations in an attempt to explain it in one small species.

As shown in detail by Căpușe (1971), the members of Coleophoridae, although generally quite small, usually have well-developed mouth parts, especially in the form of prominent, recurved, tri-segmented labial palpi and a functional haustellum. Some species with reduced mouth parts are nevertheless known, such as *C. minipalpella* Baldizzone, 1998, described from Andalusia, Spain and known from only four specimens. Here we describe and illustrate the ves-

tigial mouth parts of *Coleophora micronotella* Toll, 1956, for which there are more specimens available for study.

In the original description, Toll (1956) mentioned the colour of the head and antennae, and indicated that the palps are missing in all of the nine specimens of *C. micronotella* that he had at hand, but said nothing about the lack of proboscis. Falkovitsh (2005) erected the monotypic genus *Ramidomia*, here synonymized with *Coleophora* Hübner, 1822 (along with *Ramidiomia* Falkovitsh, 2005, a misspelling) to accommodate *C. micronotella*. Along with Baldizzone et al. (2006) and others, supported by the phylogenetic analysis of Bauer et al. (2012), we find it more coherent to use one large genus *Coleophora* (1340+ described species) than a classification of *Coleophora* subdivided in numerous small genera that have been erected without comprehensive phylogenetic inference, as exemplified by Falkovitsh (2005). The latter author noted that *C. micronotella* has short palpi, with the third segment shorter than the second, but did not mention the lack of proboscis. Subsequent records of the species did not add any more morphological information (Baldizzone 1994; Baldizzone et al. 2006).

## Methods

The head of one specimen was mounted on slide in Euparal after maceration in about 15% KOH at 60°C in an oven for an hour, cleaning in 30% ethanol, staining in Orange G and chlorazol black, and dehydrating in baths of 95% ethanol and isopropanol for one hour each.

The photographs of the scaled head as well as that of the slide-mounted whole head were taken with a Leica MZ APO dissecting scope and AutoMontage, the close-ups of the mouthparts on slide with a Zeiss microscope and AutoMontage, and the scanning electron micrographs with a Zeiss DSM 940-A scanning electron microscope in the Muséum d'histoire naturelle de Genève (MHNG).

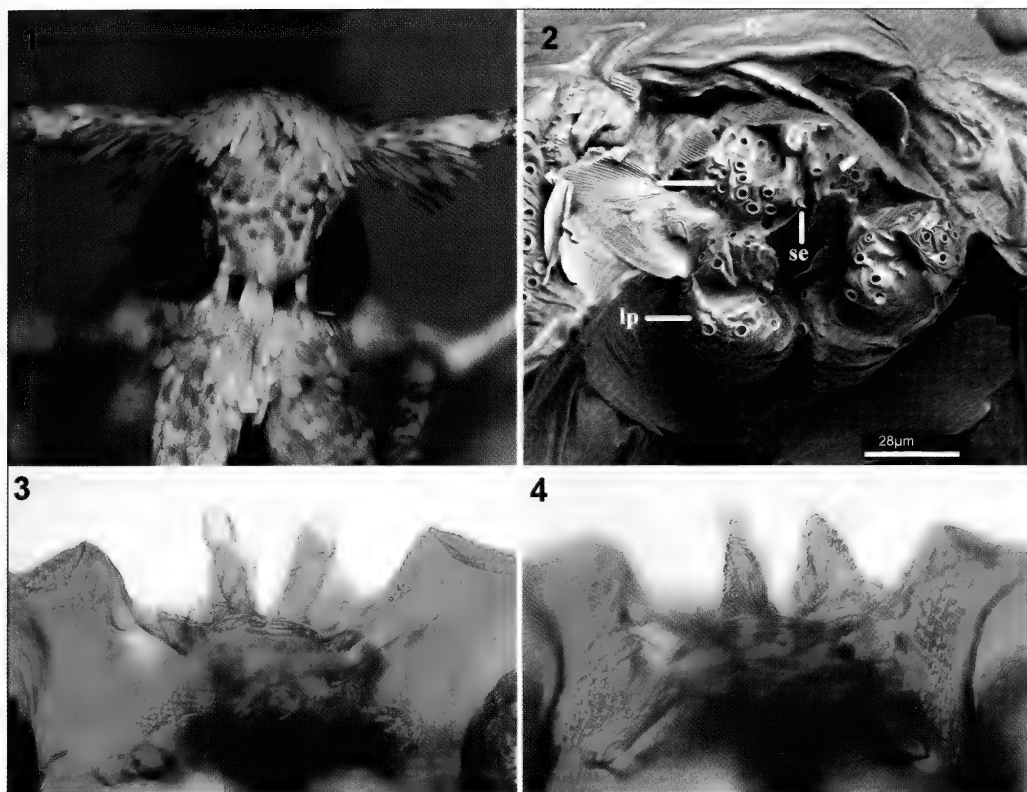
### *Coleophora micronotella* Toll, 1956

Figs 1–4

**Material.** 2 ♂ (one dissected, slide MHNG ENTO 5970), ♀ [Italy] 'SARDEGNA, Orien. | La Caletta | 2.VIII.81-lux | leg. Baldizzone'; 1 ♂ (head prepared for SEM), same data except '29.VII.81'; 2 ♂ (one dissected, slide MHNG ENTO 7501), 'SARDEGNA, Merid. | Porto Botte | 23.VII.81-lux | leg. Baldizzone', deposited in MHNG. 1 ♂ (dissected, slide MHNG ENTO 7503) [Tunisia] 'Mauretania | Tunesia-Sud | Oase Tozeur | 28.4.-11.5.1981 | leg. M. u. W. Glaser', deposited in MHNG. Additional material, undissected: 194 specimens from Sardinia (leg. Baldizzone); 30 from Tunisia (24 from Tozeur, 1921, leg. Dumont; 7 from Tozeur e Gabes, 1980, leg. Glaser); deposited in collection Baldizzone.

**Description of head.** Frontal aspect with appressed scales, with short, apparent scaled palpi, without proboscis. Ventral edge of fronto-clypeus slightly sinuate. Labrum not apparent. Maxillary palpi unsegmented, very short, with few scale sockets and setae, with apical sensillum styloconicum. Labial palpi slightly longer than maxillary palpi, reaching apices of subgenal apophyses, with two segments visible, apical segment about half as long as basal segment and lacking vom Rath's organ. With sac-like rounded structure laterally from labial palpi.





**Figures 1–4.** Head of *Coleophora micronotella* Toll. **1.** Frontal view. **2.** Scanning electron micrograph (fc: fronto-clypeus; lp: labial palp, partly collapsed; mp: maxillary palpus; se: sensillum styloconicum). **3.** Ventral view showing labial palpi. **4.** Dorsal view showing maxillary palpi.

**Notes.** The halophilous biotope of La Caletta, where three of the specimens of *C. micronotella* studied were collected, is about one kilometer away from the Mediterranean, on the sides of a small canal where tamarix (*Tamarix* sp., Tamaricaceae) grows. The specimens were collected with a black light in a salt marsh where *Salicornia* (Amaranthaceae) and other halophilous herbaceous plants were growing. Hundreds of specimens came to light, but could not come to rest on the sheet because of the presence of thousands of staphylinid beetles and flies. Falkovitsh (2005) recorded that the larva of *C. micronotella* feeds as a borer, without making a case, in ‘green branches (assimilating shoots)’ of *Halostachys caspica* C.A. Mey. and *Halocnemum strobilaceum* (Pallas) Bieb. (Amaranthaceae).

Described from Tozeur, Tunisia, the species is also found in Italy (mainland, Sardinia, Sicily) and Spain. It has also been reported from Iran (Baldizzone 1994), and Algeria, Kazakhstan, and Tadjikistan (Falkovitsh 2005). However, a specimen from the Iranian series mentioned in Baldizzone (1994) was re-examined and found to represent another, probably undescribed species. Its mouth parts are more developed than in *C. micronotella*, with the labial palpi about as long as 3/4 the widest diameter of the compound eye, but without apparent proboscis. Also, the male valva differs slightly in the distally larger and more evidently separated sacculus from the basally narrower valvula, and the phallus is narrow, needle-like, instead of spatulate.

### Other species of *Coleophora* with vestigial mouth parts

*Coleophora galligena* Falkovitsh, 1970, described from Uzbekistan, has vestigial palpi and no proboscis. *Coleophora daeva* Baldizzone, 1994, *C. minipalpella* Baldizzone, 1998, and *C. zagella* Falkovitsh, 1972, respectively described from Iran, Spain, and Mongolia, have short proboscis and palpi. These four species and *C. micronotella* can be associated with the 8<sup>th</sup> group of Toll (1953, 1962), based on their simple genitalia, but Toll (1956) only mentioned that *C. micronotella* is close to *C. plurifoliella* Chrétien, and never associated this species with any group subsequently. These species are associated with halophilous habitats and deserts and they were not treated by Bauer et al. (2012).

### Discussion

The sac-like rounded structures that protrude laterally from the labial palpi in *C. micronotella* may represent vestigial mandibles. Rudimentary mandibles were found in all species of *Coleophora* studied by Căpușe (1971), with variation in shape and size. Căpușe (1971) noted that the mandibles are usually sclerotized, but he found at least one species in which they were membranous (*C. asthenella* Constant). Căpușe (1971) did not study *C. micronotella*, but for comparison it may be noted that among the 100 species studied he mentioned that the maxillary palpi were missing in one only (*C. asthenella*). Although it is often difficult to count the number of maxillary palp segments precisely because the limits between segments may be not easily discernible, Căpușe found that the number of segments varied from one to five, the one-segmented species numbering 20, the 2-segmented 31, and the other cases in lower frequencies. Căpușe (1971) also found that *C. ciconiella* Zeller, a species with two maxillary palpomeres, has the apex of the maxillary palpi adorned with a sensillum styloconicum.

The labial palpi in the Lepidoptera groundplan are tri-segmented (Kristensen 2003). This is the condition found in *Coleophora*, but the overall length of the palpi, length of individual palpomeres, shape of terminal palpomere, and vestiture vary across species. Căpușe (1971) did not study any species with markedly reduced labial palpi. The lack of vom Rath's organ is unusual in Coleophoridae and presumed here to be linked with the reduction of the labial palpi.

Regarding the proboscis, Căpușe (1971) recorded no species in which it is lacking, but a few in which it is slightly shorter than the labial palpi, and membranous. No species with reduced mouth parts were found from the Western Hemisphere (J.-F. Landry, pers. comm. to G. Baldizzone), but several undescribed species from the Afrotropical region have reduced proboscis and palps.

Bauer et al. (2012) presented the first phylogeny of Coleophoridae based on a formal analysis of molecular characters; one hundred and two European species were analysed, but neither *C. micronotella*, any of the above-mentioned species with reduced mouth parts, nor any species of Toll's 8<sup>th</sup> group were included. Nevertheless, two of the species groups recovered, the *albella* and *alticolella* groups, have species feeding on Amaranthaceae, with the larvae of the *albella* group being internal miners in the first larval stages, until they become too large to be fully hidden in the nutritive tissue, after which they build a tubular silk case. This host plant choice and the internal feeding habits may point to an affinity of *C. micronotella* with the *albella* group.

Although the reduction or loss of the proboscis occurs in many glossatan lineages (Kristensen 2003: 54), the adaptations related to the absence of a functional proboscis are unclear, and no discussion of this phenomenon was found in the literature. Căpușe (1971: 23) believed that in species with reduced galeae, hence without a functional proboscis, the reduction is correlated with the digestive tract, without qualifying this correlation.

Several species of Saturniidae have reduced mouth parts and do not feed as adults (Taylor 1957), presumably to place most of the energy resources acquired during the development of the larva into the production of a larger quantity of eggs and/or into the development of a more efficient mate locating mechanism, hence the highly plumose male antennae. A shift in energy resource allocation may be the reason for reduced mouth parts in *Coleophora micronotella* and others, but it may also be that these species live in habitats where food sources (water, nectar) for adult Lepidoptera with low vagility are too scarce or poisonous, such as with a deleterious salt concentration, for example. The fact that *C. micronotella* and other species with reduced mouth parts live in halophilous or desert biotopes, and that the larva of *C. micronotella* is a borer (a strategy to avoid ingesting too much salt and/or to secure enough water, perhaps) lend support to this hypothesis. We believe that this phenomenon is akin to the loss of functional wings in some Lepidoptera species living on small, wind-swept islands (e.g., Davis & Mendel 2013) or the loss of functional eyes in cave-dwelling animals.

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# On the taxonomic status of *Ochromolopis ictella* (Hübner, 1813) and *O. zagulajevi* Budashkin & Sachkov, 1991 (Lepidoptera, Epermeniidae)

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<http://zoobank.org/D518C8D6-3E65-437E-AA4A-E9212D34C085>

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**Abstract.** A detailed study of specimens from several regions of the distribution of *Ochromolopis zagulajevi* Budashkin & Sachkov, 1991 and *O. ictella* (Hübner, 1813) shows that *O. ictella* and *O. zagulajevi* are parapatric species with overlapping distribution in the Balkan Peninsula. Details of morphological and molecular differences as well as a distribution map with locations of the examined specimens are given.

## Introduction

*Ochromolopis* Hübner, 1825 is one of the 11 described genera of Epermeniidae Spuler, 1910, a family currently comprising 188 species and distributed worldwide. It is the only family within the superfamily Epermenioidea Minet, 1983, which, according to Dugdale et al. (1998), shows indications of affinities with the alucitoid/pterophoroid assemblage, but the family placement has not received any support in recent molecular studies across all of Lepidoptera (Mutanen et al. 2010; Regier et al. 2013). The potential autapomorphies of Epermeniidae are the following: hind tibia with stiff bristles; forewing fringe with groups of lamellar scales; larval submentum with posterior protuberance; prothoracic prespiracular L group bisetose; pupa unspined, abdominal segments I–IV immovable, segment IX with characteristic paired lateral pits (Dugdale et al. 1998). The majority of species bear tufts or raised scales on the dorsum of forewings. Further characteristics are the loss of one of the apical forewing veins (M3 and Cu1 fused) and the widening of the ventral branch of anterior apophysae in females. The genus *Ochromolopis* currently comprises 11 species, with four of them distributed in the Palaearctic region, while the others have a Nearctic, Afrotropical and Oriental distribution.

We examined *Ochromolopis ictella* (Hübner, 1813) and *O. zagulajevi* Budashkin & Sachkov, 1991. The two species are closely related and not distinguishable superficially. Only the genital morphology shows clear differences. A more detailed study was made to determine the variability within the two taxa not only by using the traditional methods of morphological investigation but also by means of molecular methods (DNA Barcoding) by the second author.

## Material and methods

The examined material originates from numerous collections and was provided by museum curators as well as by private collectors. A list of examined material is given in the appendix.

### Morphological methods

Genitalia of both sexes were dissected in order to study morphological variability. Phallus and valvae were removed from the genitalia capsule (uncus-tegumen-vinculum with saccus) during dissection. The ring-shaped connection of tegumen-vinculum was not cut laterally but kept intact. Drawings (all at the same scale) were made from genitalia of the two taxa and their variation was compared.

### Molecular methods

For the molecular investigation of relationships between *Ochromolopis ictella* and *O. zagulajevi* we analysed the Barcode fragment of the mitochondrial COI gene. In order to obtain a high quantity of DNA, we performed the DNA extraction on the abdomen of dried specimens, followed by genital dissection from the macerated abdomen, as suggested by Knölke et al. (2005). DNA was extracted using the Macherey-Nagel NucleoSpin Tissue kit according to the manufacturer's suggestions. PCR amplification of an approximately 630 base pair (bp) fragment of the COI barcode sequence was done with the primer pair HybLCO/Nancy or in the case of fragmented DNA with the primer pairs HybLCO/K699 and Ron/Nancy, respectively (Folmer et al. 1994; Mitchell et al. 2005; Simon et al. 1994). The sequences were amplified with BIO-X-ACT Short DNA polymerase (Bioline). The PCR program for BIO-X-ACT Short DNA polymerase corresponds to the manufacturer's guidelines in conjunction with the respective primer annealing temperature of 48°C. The PCR products were checked for amplification success via gel electrophoresis on a 1% agarose gel, subsequent staining with GelRed, and final examination under UV light. The PCR products were cleaned with ExoSAP-IT (USB Corporation). The sequence PCR was performed with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). After final sodium acetate-ethanol clean-up of the samples, sequencing was carried out on a 3130 Genetic Analyzer (Applied Biosystems). A Mastercycler ep gradient S (Eppendorf) was used for PCR amplifications, ExoSAP-IT clean-up and sequence PCR.

Sequence alignment was carried out manually with PhyDE 0.9971 (Müller et al. 2008). Calculation of the genetic distances as well as of the dendrogram using the Neighbor-Joining (NJ) method (Saitou & Nei 1987; Studier & Keppler 1988) were performed under the uncorrected-p (uncorr-p) model (Srivathsan & Meier 2012) in PAUP\* 4.0b10 (Swofford 2002). *Ochromolopis kaszabi* Gaedike, 1973 was included in the analysis as an outgroup and for comparison of the genetic distances of the two investigated taxa to a more distantly related species.

## Distribution mapping

In order to examine the distributional pattern of *Ochromolopis ictella* and *O. zagulajevi*, collection localities were compiled from labels of studied specimens and from literature. Geographical coordinates of these collection localities were obtained via Google Earth, Version 5.2.1.1588 and subsequently plotted on a map using DIVA-GIS, Version 7.2.3 (Hijmans et al. 2004).

## Abbreviations

coll. Arenberger	Ernst Arenberger, Vienna, Austria
coll. Bengtsson	Bengt Å. Bengtsson, Färjestaden, Sweden
coll. Schmitz	Willibald Schmitz, Bergisch-Gladbach, Germany
ETHZ	Eidgenössische Technische Hochschule, Zürich, Switzerland
FMNH	Finnish Museum of Natural History, Helsinki, Finland
HNHM	Hungarian National History Museum, Budapest, Hungary
LMAD	Löbbecke Museum und Aquazoo, Düsseldorf, Germany
MNG	Museum der Natur, Gotha, Germany
MTD	Museum für Tierkunde, Senckenberg Naturhistorische Sammlungen Dresden, Germany
NHMB	Naturhistorisches Museum Basel, Switzerland
NHRS	Naturhistoriska Riksmuseet, Stockholm, Sweden
NMEG	Naturkundemuseum, Erfurt, Germany
NMPC	National Museum (Natural History), Prague, Czech Republic
NMW	Naturhistorisches Museum, Vienna, Austria
SDEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany
SMNK	Staatliches Museum für Naturkunde, Karlsruhe, Germany
SMNS	Staatliches Museum für Naturkunde, Stuttgart, Germany
TLMF	Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria
ZIN	Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia
ZMHB	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany
ZMUC	Zoological Museum, Copenhagen, Denmark
ZSM	Zoologische Staatssammlung, Munich, Germany

## Results

### Redescription of morphology

The two taxa do not differ in superficial appearance (see Fig. 1 for a specimen of *O. ictella*). Head, thorax, abdomen dark grey, shiny, forewing lead-grey, shiny, with two longitudinal golden-yellowish stripes: the first obliquely extending from base to dorsum shortly before half of wing, where first tuft of raised scales is situated, the second running parallel, initiating at 1/3 of cell, pointing straight towards outer margin and ending well before apex. The distal end of first stripe connected with the proximal end of the second stripe, forming a sustained Z. *O. ictella* and *O. zagulajevi* only differ in the shape of the genitalia (*ictella*: Figs 2–9, 19–21, 30; *zagulajevi*: Figs 10–18, 22–29, 31–32).

According to Budashkin & Sachkov (1991), *O. zagulajevi* has an area of numerous strongly sclerotized cornuti in the phallus within the posterior half of the vesica (Fig. 12). We found that,

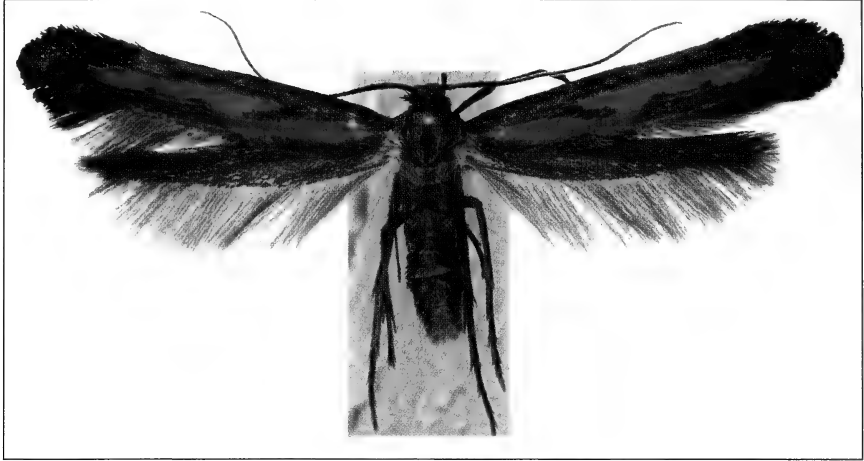
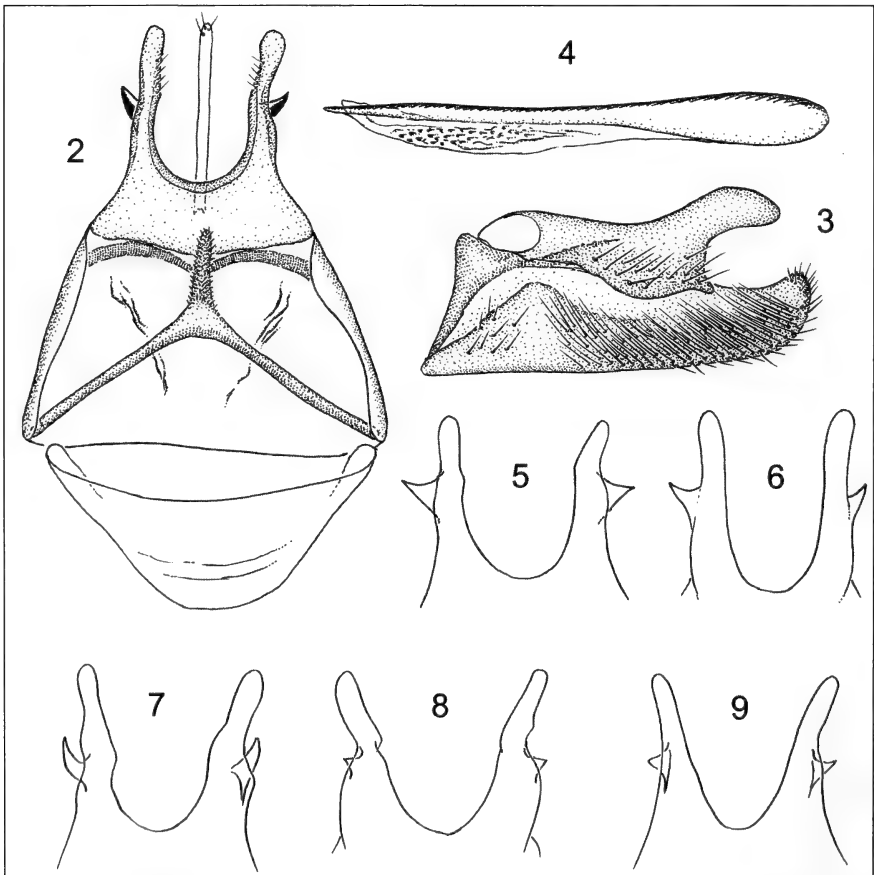
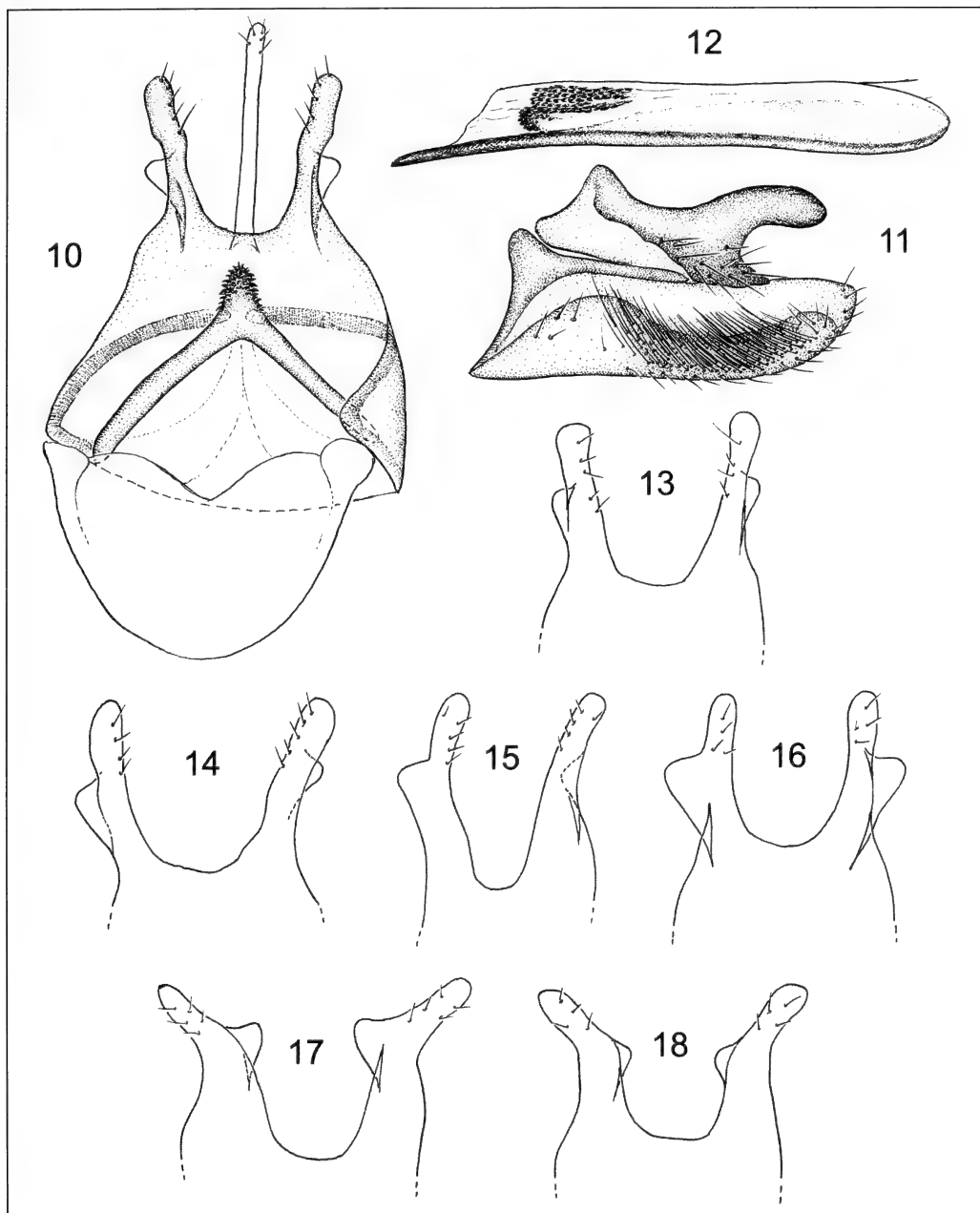


Figure 1. *Ochromolopis ictella*, imago.



Figures 2–9. Male genitalia of *Ochromolopis ictella*. 2–4. specimen from Zljeb, Montenegro: 2. uncus-tegumen-vinculum. 3. valva. 4. phallus. 5–9. variability in socii shape: 5. Xauen A'Faska, Mauretania. 6. Piedmont, Italy. 7. Neustadt, Germany. 8. Naumburg, Germany. 9. Vienna, Austria.

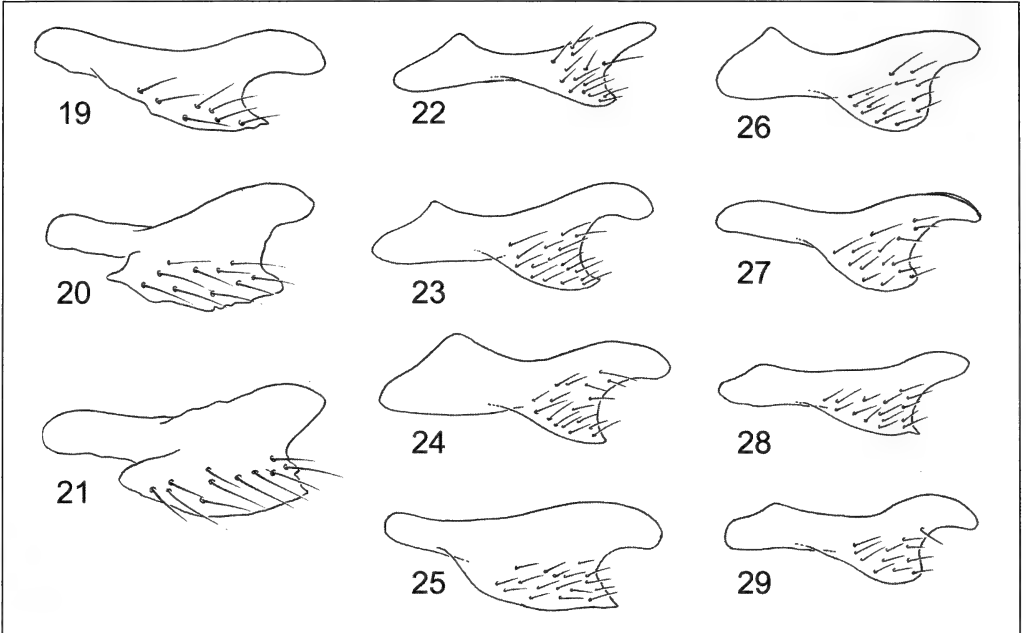




**Figures 10–18.** Male genitalia of *Ochromolopis zagulajevi*. 10–12. specimen from Crimea, Ukraine: 10. uncus-tegumen-vinculum. 11. valva. 12. phallus. 13–18. variability in socii shape: 13. Danubian delta, Romania. 14–15, 17. Kabardino-Balkarskij Nat. Res., Russia. 16. Crimea, Ukraine. 18. Djanik, Turkey.

due to the posteriad evertability of the vesica, the position of the cornuti may vary between different genital dissections. Characteristic for *zagulajevi* is the large quantity of cornuti.

In most cases the cornuti are in a more or less compact cluster, concentrated in the proximal fourth of the phallus, but in some cases the area of cornuti in the vesica covers the second- to



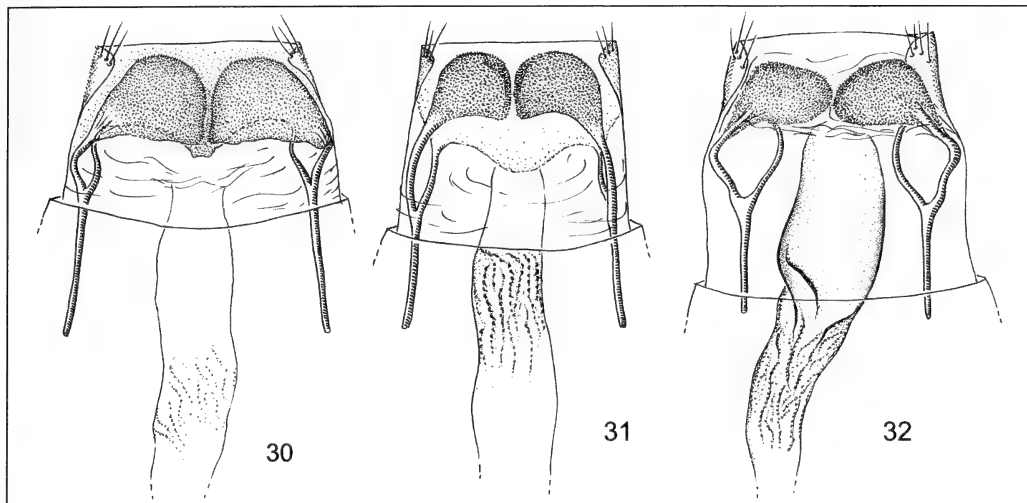
**Figures 19–29.** Variability in costa shape. 19–21. variability within *Ochromolopis ictella*: 19. Neustadt, Germany. 20. Vienna, Austria. 21. Naumburg, Germany. 22–29. variability within *O. zagulajevi*: 22–24. Kabardino-Balkarskij nat.res., Russia. 25. Northern Adriatic, Croatia. 26. Crimea, Ukraine. 27. Djanik, Turkey. 28. Gjalica Ljums, Albania. 29. Danubian delta, Romania.

third-fourth of the phallus length. The vesica of *ictella* also exhibits minute cornuti, but their number is mostly smaller, and they are not arranged as compactly as in *zagulajevi* (Fig. 4).

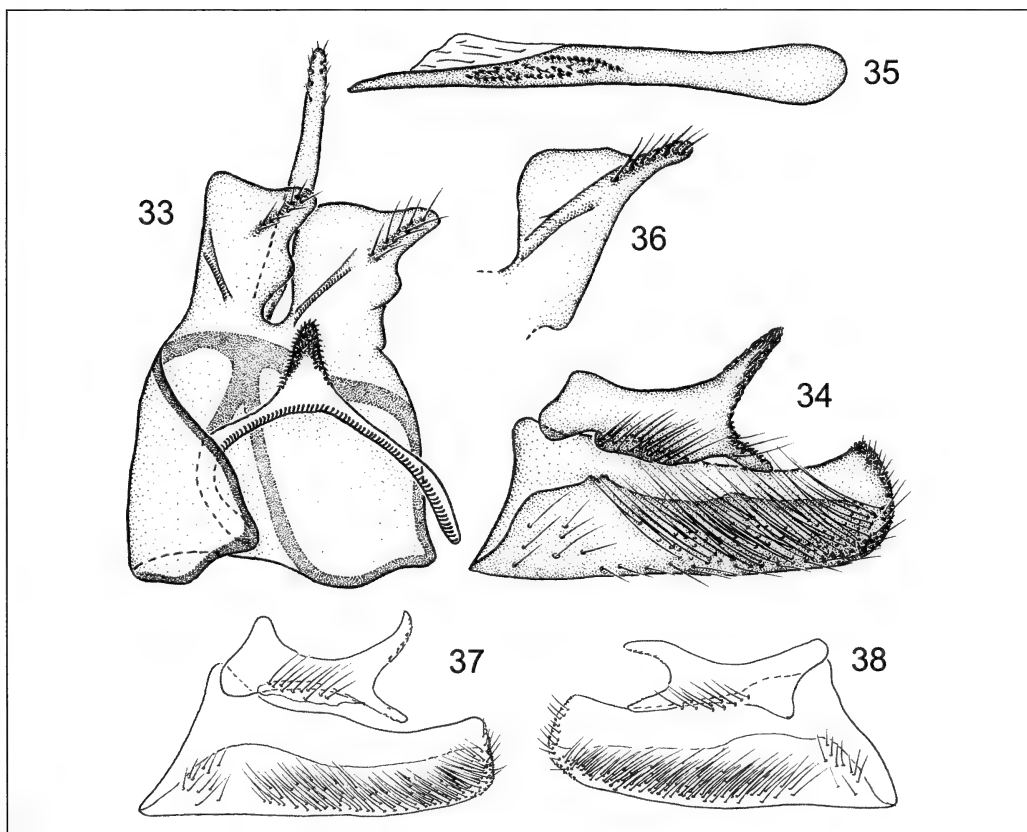
The shape of the narrow socii allows for an easier differentiation. In *zagulajevi* the socii have a dorsally attached lobe-shaped process at half of their length, which varies in size and shape (Figs 13–18). In contrast, in *ictella* this process is thorn- or hook-shaped and has a pointed tip, and is also variable in size and shape (Figs 5–9). The shape of the costal arm of the valva, which is nearly as long as the valva itself, is variable in both taxa. However, the costal arm appears to be more compact in *ictella* than in *zagulajevi*.

The female genitalia of the two taxa also exhibit some structural differences: in *O. zagulajevi*, the posterior part of ductus bursae is more strongly sclerotized, but sometimes the posterior sclerotization of the ductus is not developed (compare Figs 31 and 32); the median part is wrinkled and covered with numerous minute semicircular sclerotizations. In contrast, *O. ictella* lacks strong sclerotization in the posterior ductus bursae and the wrinkles at the median part are weakly developed and have minute sclerotizations (Fig. 30).

In order to evaluate the significance of the investigated morphological characters for defining the taxonomic status of the two taxa it was deemed important to include a third taxon into the treatment, *Ochromolopis kaszabi* Gaedike, 1973. This species was described from Mongolia, and its currently known distribution ranges from Altai through Mongolia to Russian Far East and China. Superficially, *O. kaszabi* is not distinguishable from the above mentioned taxa, but it shows clear differences in the male genital structures: socii are broad, more or less parallel, nearly obliquely truncated, with a more or less pointed tip, depending on preparation



**Figures 30–32.** Female genitalia. 30. *Ochromolopis ictella* (Abruzzi, Italy). 31–32. variability within *O. zagulajevi*: 31. Crimea, Ukraine. 32. Greece.



**Figures 33–38.** Male genitalia of *Ochromolopis kaszabi* (Mongolia; according to Gaedike, 1973). 33. uncus-tegumen-vinculum. 34. valva. 35. phallus. 36. variable form of socius. 37–38. variability of valva (Hoengshan, China).

**Table 1.** DNA barcoding specimen information.

Taxon	DNA specimen voucher	Origin, date, collector	Sequence length	GenBank accession no.
<i>ictella</i>	MTD Lep1073	Italy, Piedmont, Valdieri, reserve, 850m 29.–30.vi.2008, leg. O. Karsholt	612 bp	KF511936
	ZSM Lep 27010	Germany, Bavaria, Oberpfalz, Nittendorf, 400m, 08.vi.1994, leg. A. Seegerer	658 bp	HM902062
	TLMF Lep 05228	Macedonia, Mavrovo NP, Korab, summit ridge, 2700m, 28.vii.2011, leg. P. Huemer & G. Tarmann	658 bp	KJ427720
	TLMF Lep 05229	Macedonia, Mavrovo NP, Korab, summit ridge, 2700m, 28.vii.2011, leg. P. Huemer & G. Tarmann	658 bp	KJ427721
<i>zagulajevi</i>	MTD Lep1071	Croatia, Istria, Belavići, Marčana, 08.–14.ix.2008, leg. W. Mey	612 bp	KF511934
	MTD Lep1072	Croatia, Istria, Belavići, Marčana, 08.–14.ix.2008, leg. W. Mey	612 bp	KF511935
	MTD Lep1074	Italy, Lucania, Mt. Pollino, 780m, 03.x.2010, leg. P. Skou	612 bp	KF511937
	MTD Lep1075	Italy, Lucania, Mt. Pollino, 780m, 03.x.2010, leg. P. Skou	612 bp	KF511938
	MTD Lep1076	SW Bulgaria, Pirin Sandanski, Ilindentsi, 500m, 28.iii.–04.iv.2011, leg. N. Savenkov	612 bp	KF511939
	MTD Lep1077	SW Bulgaria, Pirin Sandanski, Ilindentsi, 500m, 28.iii.–04.iv.2011, leg. N. Savenkov	612 bp	KF511940
	MTD Lep1078	SW Bulgaria, Pirin, Sandanski, Ploski, 250m, 17.–31.v.2010, leg. N. Savenkov	612 bp	KF511941
	MTD Lep1079	SW Bulgaria, Pirin Sandanski, Ilindentsi, 500m, 28.iii.–04.iv.2011, leg. N. Savenkov	612 bp	KF511942
MTD Lep1080	SW Bulgaria, Pirin, Sandanski, Ploski, 250m, 17.–31.v.2010, leg. N. Savenkov	591 bp	KF511943	
<i>kaszabi</i>	MTD Lep1081	Russia, Siberia, Chita, Ingoda river, 27.vii.1997, leg. I. Kostjuk	612 bp	KF511933

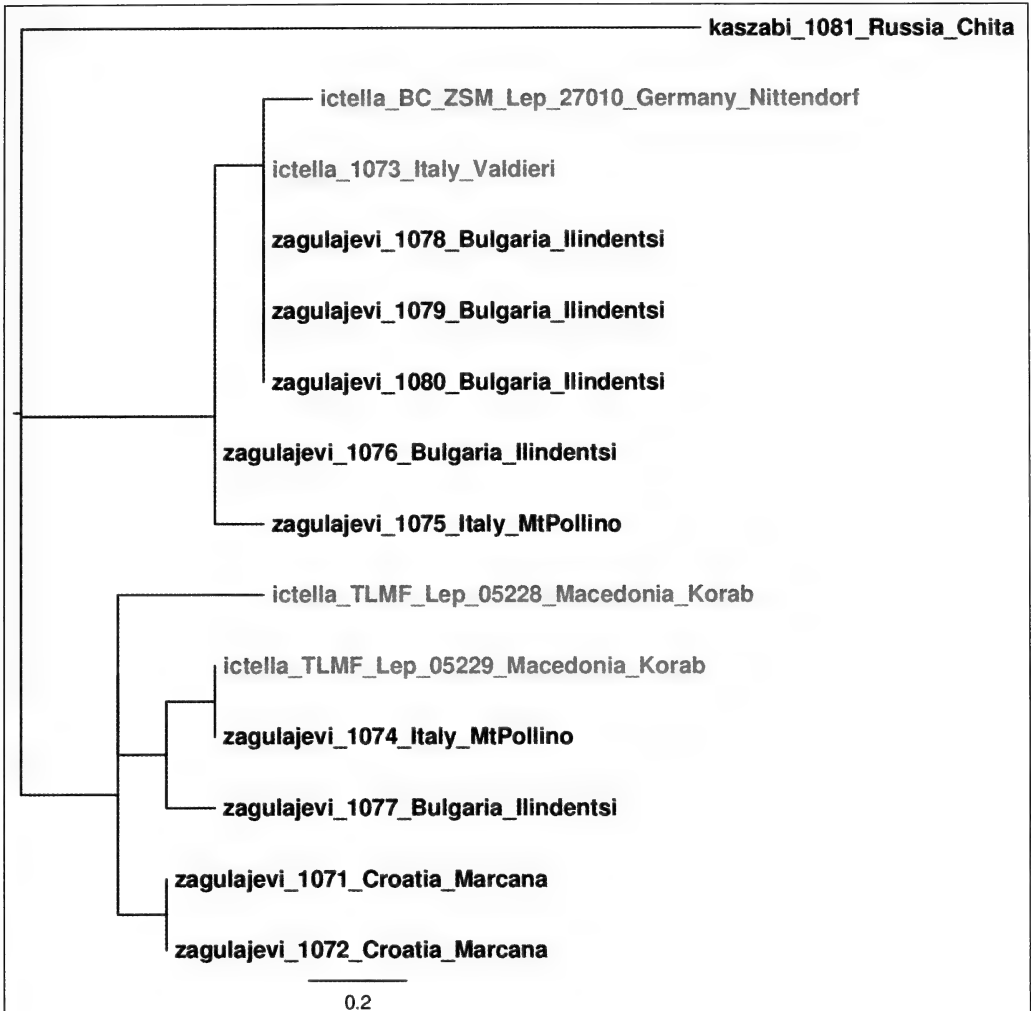
(see Figs 33 and 36). The costal arm of the valva is about two thirds of the valval length and has a forked distal end with a short ventral branch and a longer costal branch with pointed tip, directed more or less obliquely upwards, edges of the two branches with minute pointed thorns. The shape of the costal arm is variable (see Figs 37–38). Female genitalia (according to the description in Budashkin & Sachkov 1991: figs 3, 4) are characterised by the ductus bursae lacking strong sclerotization, but with strongly sclerotized sternal segments.

## Molecular results

The barcode sequences length was 591–658 bp (see Table 1). No indels have been observed in any of the sequences. All obtained barcode sequences have been submitted to GenBank (for accession numbers see Table 1).

In the NJ analysis we obtained two clusters comprising 6 and 7 samples, respectively (Fig. 39). The two clusters do not correspond to the two taxa. Rather, in one cluster there are two *Ochromolopis ictella* and five *O. zagulajevi* specimens, and in the other cluster there are two *O. ictella* and four *O. zagulajevi* specimens. In *O. zagulajevi* we observed two cases in which specimens from the same locality and period of collection are not found in the same Barcode cluster: 1) Lep1075 versus Lep1074 (Italy, Mt. Pollino) and 2) Lep1076 & Lep1079 versus Lep1077 (Bulgaria, Ilindentsi).

Intraspecific divergences within *ictella* range from 0.168% to 1.672% and in *zagulajevi* from 0% to 1.359% (see Table 2). Interspecific uncorr-p divergences between *ictella* and *zagulajevi*



**Figure 39.** NJ dendrogram, based on uncorrected-p distances; scale bar represents 2.0% uncorrected-p distance.

range from 0% to 1.528%. In contrast, *O. kaszabi* has a Barcode divergence of 2.922–3.100% with *ictella* and 2.451–3.045% with *zagulajevi*.

### Distribution map

The map of the investigated taxa (Fig. 40) shows that Budashkin & Sachkov's (1991) presumption in the original description of *O. zagulajevi* concerning the distribution was right. All examined specimens from Caucasus region, Crimea, Iran, Turkey, and Southern Europe (Greece, Bulgaria, Albania, S-Italy) belong to *O. zagulajevi*. On the contrary, all examined specimens from North Africa, southwestern, central and northern Europe (Spain, France, Switzerland, Denmark, Finland, Germany, Austria, N-Italy, Poland, Czech Republic, Slovakia, Hungary), and Montenegro (only one specimen studied) belong to *O. ictella*.

**Table 2.** Uncorrected-p sequence divergence matrix with divergence values as percentage.

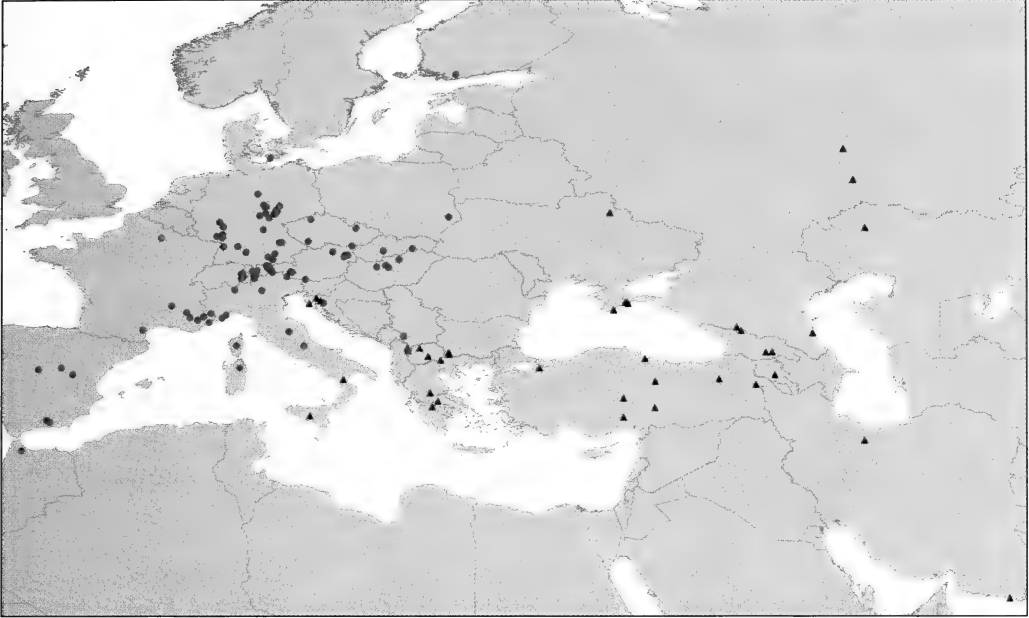
	1081 kaszabi	1073 ictella	27010 ictella	05228 ictella	05229 ictella	1071 zagul.	1072 zagul.	1074 zagul.	1075 zagul.	1076 zagul.	1077 zagul.	1078 zagul.	1079 zagul.
ictella MTD Lep1073	2.941												
ictella ZSM 27010	3.083	0.168											
ictella TLMF 05228	3.100	1.476	1.672										
ictella TLMF 05229	2.922	1.299	1.520	0.760									
zagulajevi MTD Lep1071	2.451	1.144	1.295	0.654	0.477								
zagulajevi MTD Lep1072	2.451	1.144	1.295	0.654	0.477	0.000							
zagulajevi MTD Lep1074	2.941	1.307	1.465	0.822	0.000	0.490	0.490						
zagulajevi MTD Lep1075	2.941	0.327	0.490	1.477	1.300	1.144	1.144	1.307					
zagulajevi MTD Lep1076	2.778	0.163	0.326	1.313	1.136	0.980	0.980	1.144	0.163				
zagulajevi MTD Lep1077	2.941	1.307	1.465	0.822	0.323	0.490	0.490	0.327	1.307	1.144			
zagulajevi MTD Lep1078	2.941	0.000	0.168	1.476	1.299	1.144	1.144	1.307	0.327	0.163	1.307		
zagulajevi MTD Lep1079	2.941	0.000	0.168	1.476	1.299	1.144	1.144	1.307	0.327	0.163	1.307	0.000	
zagulajevi MTD Lep1080	3.045	0.000	0.172	1.528	1.351	1.187	1.187	1.359	0.338	0.170	1.356	0.000	0.000

In Croatia and in Macedonia both taxa occur sympatrically. Additional sympatrical distribution might be present in Italy, where *O. ictella* is present from the northern part southwards to Umbria, and *O. zagulajevi* in the southern regions and Sicily. No material was available from Slovenia.

## Discussion

The comparison of genital morphology between the two species reveals broad concordance of the investigated structures. Only one differing feature was found between *O. ictella* and *O. zagulajevi*, namely the shape of the socii within male genitalia. The divergence in genital morphology is evidently larger between *O. kaszabi* and the *O. ictella-zagulajevi* complex than between *ictella* and *zagulajevi*.

The analysis of DNA Barcodes reveals that the range of interspecific Barcode divergence between *ictella* and *zagulajevi* (0–1.528%) is within the range of intraspecific divergence of 0.168–1.672% in *ictella* and 0–1.359% in *zagulajevi*. This suggests that it is possible that these two taxa might actually represent one somewhat variable species. However, the finding of one constant morphological difference in the male genitalia between *ictella* and *zagulajevi* and the sympatric occurrence of both taxa on the Balkan peninsula imply the validity of their species status.



**Figure 40.** Distribution map of *Ochromolopis ictella* (red circles) and *O. zagulajevi* (black triangles) in the West Palearctic.

Until further molecular work with much greater specimen sampling, focusing on establishing reasons behind the two DNA barcoding clusters (e.g., incomplete lineage sorting; Funk & Omland 2003), can provide more data on the status of the two species, we recommend no change in their taxonomic status. Future work should also aim to infer a more accurate distribution border between these two taxa in Europe, especially in the areas of Central Italy southwards, of the Balkan Peninsula and from Slovenia and Hungary eastwards.

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## Appendix

A list of examined specimens of *Ochromolopis ictella* (Hübner 1813), *O. zagulajevi* Budashkin & Sachkov, 1991 and *O. kaszabi* Gaedike, 1973 follows below. All specimens were determined by R. Gaedike or were part of the original description of *zagulajevi*. The list contains material examined from approximately 1965 to 2013. The localities in this list are recorded as they appear on the labels. The current depository of the material (if available) is provided using the abbreviations listed in the Abbreviations section.

### *Ochromolopis ictella* (Hübner, 1813)

**Mauretania**, Xauen, A'Faska: 1♂, 20.vi.1931, leg. Reisser (NMW). **Spain**, Granada: 1♂, 1♀, 12.iv., 22.vi., leg. Staudinger (ZMHB); Escorial: 1♂, vii.1924; Castellon, 3km S of Forcall: 1♀, 16.vi.1989, leg. et coll. Bengtsson; Guadalajara, 1km NW Trillo: 1♂, 21.vii.1988, leg. Fibiger (ZMUC); Cuenca, 5km SW Huelamo, by Rio Jucar: 1♀, 19.vii.1988, leg. Fibiger (ZMUC); Sieddar Nevada, Camino de la Veleta: 1♂, 1♀, 29.vii.1985, 3.vii.1986, leg. Traugott-Olsen (ZMUC). **France**, Bourgogne: 1♂, leg. Constant (ZSM); Mt. Panaglia, env. of Nizza: 1♂, 3.x.1964, leg. Glaser (SMNK); Aude, Villedeigne: 1♂, 9.vii.1961, leg. Burmann (SMNK); La Voulte-sur-Rhône: 2 specimens, leg. Dresney; env. of Digne: Les Mees: 1♂, 20.v.1977, leg. Bruer (ZSM); Basses Alpes, la Baume: 2 specimens, 26.vii.1973, leg. Groß (LMAD); Alpes mar., St. Bres: 1 specimen, 24.vii.1973, leg. Groß (LMAD); Provence, 4km N Eyquians: 1♂, 4.vii.1989, leg. et coll. Bengtsson; Corsica (Rungs, 1988). **Italy**, Piedmont, Valdieri: 1♂, 29.–30.vi.2008, leg. Karsholt (ZMUC); Abruzzi, Mte Sirente: 1♀, leg. Dannehl (ZSM); Liguria, Noli (Savona): 1♂, 21.–30.ix.1951, leg. Klimesch (ZSM); Liguria, Andora: 1♀, 6.ix.1965, leg. Klimesch (ZSM). **Montenegro**, Zljeb, Neumontenegro: 1♂, 1916, leg. Penther (NMW). **Macedonia**, NP Mavrovo, Korab, summit ridge, ca. 2700–2750m, 20°32'48"E, 41°47'20"N: 4♂, 28.vii.–1.viii.2011, leg. Huemer & Tarmann (TLMF). **Austria**, Wien: 4♂, leg. Mann (NMW); Lobau: 1♂, 1♀, viii.1916, leg. Predota (NMW); Mödling: 1♂ (NMW); Hochzire/Tirol: 3♂, vii.1927, viii.1927 (NMW); Umgebung Seefeld: 1♂, 1.vii.1922, leg. Bauer (ZSM); Innsbruck: 1♂, 14.vi.1938, leg. Burmann (SMNK); Lechtaler Alpen, 1700m: 1♂, 1♀, 5.–11.viii.1940, leg. Osthelder (ZSM); Pasterz: 1♂; Heiligenblut: 3♂, vii.1896; Dürnstein: 2♀, iv., vii., leg. Klimesch (ZSM); Brennersee, 1400m: 1♂, 14.vii.1968, leg. Burmann (SMNK); Stuben/Vorarlberg, 1500m: 1 specimen, 11.viii.1962, leg. Groß (LMAD); Schütt near Villach: 1 specimen, 22.vii.1972, leg. Groß (LMAD); Tirol, Tessenberg: 1♂, 12.–15.vii.1981, leg. Schnack (ZMUC). **Switzerland**, Kanton St. Gallen Vättnerberg: 1♂, 12.viii.1909, leg. Müller-Rutz (NHMB); Vättis: 1♂, 1♀, vii., leg. Müller-Rutz (NHMB); Kanton Graubünden, Endagin: Val Fuorn: 1♂, 19.vi.1905 (ETHZ); Ekschis, Safien: 1♂, 27.vi.1929, leg. Müller-Rutz (NHMB); Parpan: 1♂, 1♀, vi.1920, vii.1920, leg. Müller-Rutz (NHMB); Fetan: 1 specimen, 31.vii.1923, leg. Müller-Rutz (NHMB); Remüs: 1♂, 1♀, vii.1933, viii.1935, leg. Weber (ETHZ); Mathon: 2♂, 3., 5.viii.1929, leg. Weber (ETHZ); Salorino: 1♂, 1♀, 26.vii.1926, 13.vii.1927, leg. Weber (ETHZ). **Czech Republic**, Env. of Litomerice, Libochovany: 1 specimen, leg. Zimmermann (NMPC); Zalezly: 1 specimen, leg. Wihan; Lednice: 1 specimen, leg. Zimmermann (NMPC); Hrabasice: 1♂, v.1977 (ZMUC). **Slovakia**, Zadiel: 1 specimen, leg. Povólny; Cenko: 1 specimen, leg. Patočka; Banska Stiavnica: 1 specimen, leg. Patočka. **Hungary**, Budapest: 1♂, 18.v.1913, leg. Uhrík (HNHM); Csákvár: 1♂, 7.vii.1961, leg. Gozmány (HNHM); Puszta Peszér: 1♂, 10.vii.1929, leg. Osthelder (ZSM); Bagloyirtás, Mátra: 1♂, 12.vi.1951, leg. Gozmány (HNHM). **Germany**, Halle/Saale: 1 specimen, leg. Eichler (ZSM); Naumburg: 1 specimen, leg. Bauer (ZSM); Kyffhäuser: 5 specimens, leg. Hockermeyer / Lenthe / Beer / Petry / Soffner (NMEG; SDEI); Ochsenburg/Kyffhäuser: 1 specimen, leg. Sutter (SMNK); Bad Blankenburg: 1 specimen, leg. Steuer (ZMHB); Jena: 1 specimen, leg. Nikolaus (MNG); Inselsberg, Georgenthal: 1 specimen, leg. Lenthe (MNG); Alter Stolberg near Nordhausen: 1 specimen, leg. Petry (NMEG); env. of Erfurt: 1 specimen, leg. Beer (MNG); Löberschütz: 1 specimen, leg. Faulwetter; Tautenburg: 1 specimen, leg. Faulwetter; Flachsleite: 1 specimen, leg. Faulwetter; Gleisberg: 1 specimen, leg. Faulwetter; Totentäler, Kreis Nebra: 1 specimen, leg. Eichler (ZSM); Braunschweig: 1 specimen, leg.

Heinemann; Neustadt/Haardt: 1 specimen, leg. Eppelsheim (ZSM); Kaiserlautern: 1 specimen, leg. Heuser; Grünstadt: 1 specimen, leg. Wörz (SMNS); Hambach: 1 specimen, leg. Wörz (SMNS); Battenberg: 1 specimen, leg. Wörz (SMNS); Wiesbaden: 1 specimen, leg. Wörz (SMNS); Stuttgart: 1 specimen, leg. Wörz (SMNS); Schelklingen: 1 specimen, leg. Wörz (SMNS); Baden: 1 specimen, leg. Hering (ZMHB); Ascholding: 1 specimen, leg. Osthelder (ZSM); Steinebach: 1 specimen, leg. Osthelder (ZSM); Garching-Heide: 1 specimen, leg. Osthelder (ZSM); Garmisch: 1 specimen, leg. Osthelder (ZSM); Mittenwald: 1 specimen, leg. Osthelder (ZSM); Regensburg: 1 specimen (ZSM); Ruhpolding: 1 specimen (ZSM); Bamberg: 1 specimen, leg. Garthe (ZSM). **Poland**, Katy, distr. Zamosc: 1♂, 4.viii.1978, leg. Buszko (ZMUC). **Denmark**, Lolland, Rodbyhaven: 1 specimen, vii 2010, leg. Larsen (ZMUC). **Finland**, Karislojo: 1♂, 12.viii.1967, leg. et coll. Krogerus.

### *Ochromolopis zagulajevi* Budashkin & Sachkov, 1991

**Italy**, Lucania, Mt. Pollino, 39°50'N, 13°33'E: 4♂, 3.x.2010, 26.vii.2011, leg. Skou (ZMUC). **Croatia**, Fiume: 2♂, 1853 (NMW); Istria, Belavici, Marcana: 1♂, 6♀, 8.–14.ix.2008, leg. Mey (ZMHB; SDEI); Dalmatia, env. of Selce: 4♂, 8.–15.viii.1989, leg. Gestberger (SDEI). **Macedonia**, Stari Dojran: 1♀, 2.–10.vi.1956, leg. Klimesch (ZSM); Drenovo near Kavadar: 1♂, 10.–20.vi.1956, leg. Klimesch (ZSM). **Albania**, Kula Ljums [Kula e Lumes]: 1♂ (NMW); Korab: 1♂, 23.–31.vii.1918 (NMW); Gjalica Ljums [Mail i Gjalices]: 2♂, 17.–16.vi.1918 (NMW; SDEI); Sisevo near Üsküb: 1♂, 11.v.1918 (NMW). **Greece**, Peloponnese, Zachlorou near Kalavrita: 1♀, 1.–14.vii.1959, leg. Noack (LMAD); Karia: 1 specimen, 12.vii.1974, leg. et coll. Arenberger; Itea/Desfina: 2♂, 29.v.2006, leg. et coll. Schmitz. **Bulgaria**, Pirin, Sandanski: 9♂, 7♀, 17.–31.v.2010, 28.iii.–4.iv.2011, leg. Savenkov (coll. Roweck; SDEI). **Turkey**, Zeitoon: 1♂ (ZMHB); Sivas, darende Günpınar: 1♂, 18.x.1986, leg. Moberg & Hilman (ZMUC); env. of Ürgüp: 1 specimen, 24.vi.1969, leg. et coll. Arenberger; 50km N Tarsus: 1 specimen, 19.v.1969, leg. et coll. Arenberger; Yalova at Sea of Marmara: 1 specimen, 11.v.1969, leg. et coll. Arenberger; Djanik, E of Terme: 1♂, 6.v.1969, leg. Glaser (SMNK); Erzurum: 1♂, 17.ix.1993, leg. Fibiger (ZMUC); Agri, 23km W Dagubayazit: 1♂, 5.ix.1993, leg. Fibiger (ZMUC). **Ukraine**, Crimea, Dobroje Krasnolesje: 48♂, 5♀, 18.v.1983, 5.vii.1986, leg. Zagulajev (ZIN; SDEI); Crimea, Karadag: 1♂, 22.v.1984, leg. Zagulajev (ZIN). **Russia**, Caucasus, Kabardino-Balkarski nature reserve, 35km SE of Elbrus: 10♂, 2♀, 9.–13.vii.1990, leg. Jalava (FMNH; SDEI). **Georgia**, Tbilissi: 1♂, 30.v.–1.vi.1971, leg. Muče. **Armenia**, Geghard, 40km E of Erewan: 1♂, 26.–27.vii.1976, leg. Kasy & Vartian (NMW). **Iran**, Keredj, Elburs Mountains: 2♂, 1♀, 15.iv.1936, leg. Brandt (NHRS); Balochistan, Bendar Tschahabahar: 1♂, 1937, leg. Brandt (NHRS).

### *Ochromolopis kaszabi* Gaedike, 1973

**Russia**, SW-Altai, Kuragan valley, 15km S Katanda, 1200m: 6♂, 23.–25.vii.1983, Exp. K. Mikkola, H. Hippa & J. Jalava (FMNH; SDEI); Buryatia, 53°40'N, 109°00'E, Svyatov Nos pns. Monahovo, 460m: 2♂, 13.vii.1996, leg. J. Jalava & J. Kullberg (FMNH); Sibiria, Tschita, reka [=river] Ingoda: 1♂, 27.vii.1997, leg. I. Kostjuk (ZMHB); Primorskij kraj, Pogranitschnyj Rajon, Barabasch-Levada: 1♂, 5♀, 14.vii.1989, leg. S. Zinjov (ZIN); Chazanskij Rajon, Kedrovaja pad': 2♂, 2♀, 25.vii., 1., 2.viii.1988, leg. S. Zinjov; 1 specimen, 15.vii.1974, leg. Ermolajev (ZIN); Chazanskij Rajon, Rezanovka: 2♂, 2♀, 22., 23.viii.1982, leg. S. Zinjov (ZIN); Chazanskij Rajon, 3km SE Andrejevka: 5♂, 5♀, 21., 24.vii., 7., 11., 15.viii.1985, 12.viii.1984, leg. S. Zinjov (ZIN); Chazanskij Rajon, 7km N Zanadvorovka: 5♀, 14.viii.1984, leg. S. Zinjov (ZIN); Ussurijskij Rajon: 2♂, 4♀, 3., 12., 19.vii., 14., 19.viii.1982, leg. S. Zinjov (ZIN); Ussurijskij Rajon, surround of Ussurijsk: 3♂, 6♀, 28.vi., 7., 8., 28.vii., 3.ix.1983, leg. S. Zinjov (ZIN); Ussurijskij Rajon, 20km E of Ussurijsk: 5♂, 4♀, 20.viii.1980, 22.viii.1981, 9.vii.1984, 3., 13.vii.1985, leg. S. Zinjov (ZIN). **Mongolia**, Bulgan aimak, 7km NW of Somon, Chanzar-galant, 1350m: 16♂, 22.vii.1968, leg. Z. Kaszab (HNHM; SDEI).

## Description of the female of *Ethmia cribravia* Wang and Li 2004 (Lepidoptera, Elachistidae, Ethmiinae)

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**Abstract.** The previously unknown female of *Ethmia cribravia* Wang and Li, a species known from Yunnan, China, is described and illustrated with colour photographs of the habitus, as well as images of genitalia mounted on slides. The species is sexually dimorphic, with the male having prominent androconial scales on the hindwing.

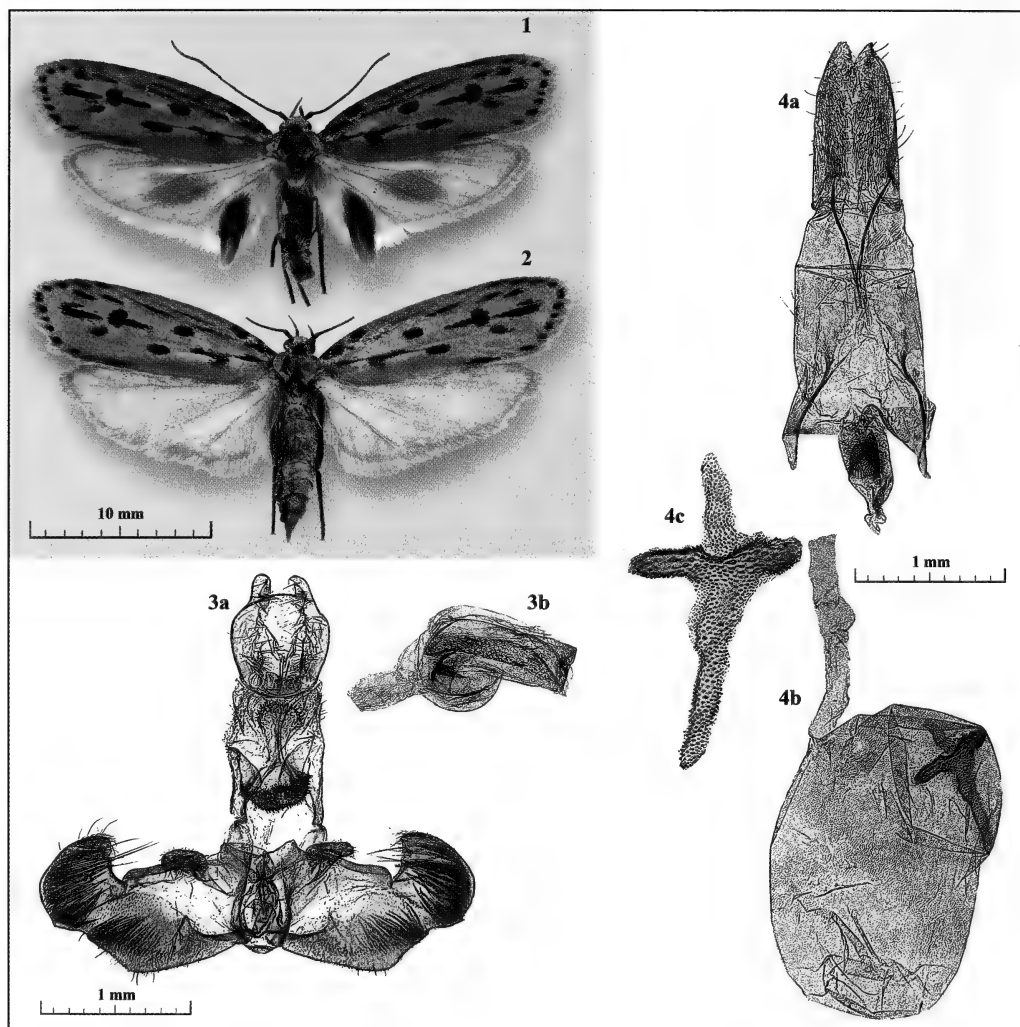
**Резюме.** В статье приводится описание ране неизвестной самки *Ethmia cribravia*. Впервые даны цветные иллюстрации бабочек, так же даны черно-белые фотографии генитальных структур самца и самки.

### Introduction

*Ethmia cribravia* was described by Wang and Li (2004) ten years ago, based on three male specimens from Yunnan Province of China. In the collection of the Hungarian Natural History Museum (Budapest) we found two male and two female specimens of this little-known species. *E. cribravia* is peculiar as it displays remarkable sexual dimorphism. The male has a tuft of long, piliform androconial scales on the anal margin of the hindwing (Fig. 1). A similar degree of sexual dimorphism is known from only one other *Ethmia* species – the male of the African species *Ethmia melanocrates* Meyrick, 1923 has similar androconial scales on the anal margin of the hindwing (Mey & Shovkoon 2014: fig. 14).

### Abbreviations

HNHM Hungarian Natural History Museum, Budapest.



**Figures 1–4.** *Ethmia cribravia*. **1.** Male, China, Yunnan Diqing Tibetan (HNHM). **2.** Female, China, Yunnan Diqing Tibetan (HNHM). **3.** Male genitalia: **a** general view and **b** phallus; Shovkoon praeparavit, prep. № 219 (HNHM). **4.** Female genitalia: **a** general view, **b** bursae, **c** signum; Shovkoon praeparavit, prep. № 220 (HNHM).

### *Ethmia cribravia* Wang and Li 2004

Figs 1–4

**Material.** 2♂, 2♀ China, Yunnan Diqing Tibetan Auf. Pref. Tiger Leaping Gotge, SE Slope at Sean's Guesthouse, 2000-2500 m N 27°16.113', E 100°10.233' 9-12.vi.2008, leg. B. Benedek. (HNHM).

**Description of female.** (Fig. 2) Length of forewing 29.5–30.5 mm. In general coloration and pattern of forewing essentially as male. Hindwing lacking the dark field in the centre and piliform androconial scales on anal margin of hindwing, typical of male.

Female genitalia (Fig. 4). Papillae anales elongated, setose. Eighth tergite medially with membranous incision. Posterior apophyses slender, as long as papillae anales; anterior apophyses wedge-shaped, distally pointed (Fig. 4a). Posterior part of antrum sclerotized with armed sclerotized thorns. Ductus bursae as long as abdomen, with 5–7 coils, bursa copulatrix spherical (Fig. 4b). Signum very large, cruciform, evenly covered with small teeth (Fig. 4c).

**Distribution.** The collecting site of the specimens examined by us is only 20 kilometers north-east of the type locality, both sites in Yunnan Province of China.

**Taxonomic notes.** The moth habitus and the structure of the male (Fig. 3) and female genitalia (Fig. 4) suggest a close relationship with *E. dehiscens* Meyrick, 1924, and the species is therefore placed in the *dehiscens* species-group *sensu* Sattler (1967).

The similarity in the appearance of the androconial scales of *E. cribravia* and the African species *E. melanocrates* is puzzling. The two species do not belong in the same species-group *sensu* Sattler (1967). Based on the morphology of the genitalia of *E. melanocrates* (Mey & Shovkoon 2014: figs 17, 18), this species belongs to its own independent species-group, the members of which are known only from Kenya, Namibia, and South Africa and are not present in the Palaearctic region.

Additionally, these two species differ in their external appearance. The costal half of the forewing of *E. melanocrates* is suffused with yellow scales, and the basal half with a complex pattern formed by dark and light fields; tiny black marginal dots are present; the cilia are pale grey, with black scales at the tip of the forewing; the hindwing is yellow with yellow cilia; and the abdomen and thorax are grey-yellow (Mey & Shovkoon 2014: figs 14, 15).

It is very interesting that, in spite of such differences in morphology and distribution, the androconial scales of these two species are similar to such an extent. In both species the piliform androconial scales are located on the anal margin of the hindwing starting from the  $An_1$  vein and they are as long as the width of the hindwing.

## Acknowledgements

We are very grateful to Dr. Zsolt Bálint (Budapest) for his help in studying museum collections of Hungarian Natural History Museum.

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## A remarkable new species of the genus *Catatinagma* Rebel, 1903 (Lepidoptera, Gelechiidae) from Turkmenistan

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Subject Editor: Lauri Kaila

**Abstract.** A new highly specialized *Catatinagma* Rebel, 1903 species is described from Turkmenistan. Both sexes have completely reduced hindwings and strongly reduced forewings. The adults are active in February, jumping amongst *Carex physodes* M. Bieb. and being associated with rodent burrows. The new species is similar to *Metanarsia trisignella* Bidzilya, 2008, in the male genitalia. Both species are placed here provisionally in *Catatinagma* Rebel, 1903, and their position within Apatetrini is briefly discussed. The adult and the genitalia of both sexes are illustrated, and the behaviour of the new species is described.

### Introduction

As a result of my study of material deposited in the Zoological Institute of the Russian Academy of Sciences (Russia, Sankt-Petersburg, ZIN), a very remarkable narrow-winged species of Gelechiidae with prominent frontal process from Repetek Nature Reserve (SE Turkmenistan) was discovered.

As it turned out after a detailed examination, the species was an undescribed member of the subfamily Apatetrinae, tribe Apatetrini (Karsholt et al. 2013) but its generic assignment was unclear. A well-developed beak-shaped frontal process on the head and stenoptery in both sexes with fully reduced hindwing were recognized as external morphological specializations of the new species. The male genitalia of the new species resemble those of *Metanarsia trisignella* Bidz., but the female genitalia differ in the shape of the signum. Both species are placed provisionally in *Catatinagma* Rebel, 1903, until a more appropriate place can be found.

In addition to strongly modified wings, the new species has a very unusual behaviour. The adults hide in the burrows of rodents in cold weather. This unique life style and the rather remarkable morphology of this species justify its description in advance of a broader taxonomic revision of the Apatetrini.

***Catatinagma stenoptera* Bidzilya, sp. n.**

<http://zoobank.org/83160C15-A639-4756-A9C8-31C73BC82F9D>

**Material.** Holotype, ♂, Karakum desert, Repetek reserv[e], Carex, 3.ii.1983, Krivokhatsky (gen. slide 55/11) (ZIN). Paratypes: 1 ♂, same data as holotype (gen. prep. in glycerol); 1 ♂, same data as holotype, but 18.ii.1983, trap *Rhombomys*, night (ZIN-00002); 1 ♀, SAME data, but 15.ii.1983, trap *Rhombomys* (gen. slide 56/11) (ZIN-00005); 1 ♂, 2 ♀, Repetek, SE Karakum, Turkmenia, trap *Rhombomys*, 25.ii.1983 (ZIN-00004 ♂, ZIN-00003 ♀, ♀ gen. prep. in glycerol) (all ZIN).

**Description. Adult** (Figs 1–3). Wingspan 9.1–10.9 mm. Head smooth-scaled, whitish-brown, with prominent beak-shaped, pointed process, that sometimes bears additional small dorsal knob arising from middle; frons medially with depression resembling excavator bucket with three short tooth-shaped projections at bottom; labial palpus weakly up-curved, outer surface black with white basal and apical belts, inner surface white, segment 3 nearly straight, acute; segment 2 about 1.5 times width and nearly 2.5 times length of segment 3; proboscis reduced; antenna brown with very narrow whitish ring at base of each segment, pecten with numerous long white hair-like scales; forewing strongly narrowed after  $\frac{1}{4}$ , nearly filiform in distal half, light brown, mottled white along margins, cilia white; hindwing vestigial.

**Abdomen** (Figs 4–7). Male sternite VIII rectangular, without modification. Female tergite VII nearly twice length of other abdominal segments, rectangular, slightly broader than long, densely covered posteriorly with short hair-like scales, sternum VII rectangular without modification. Sternite I+II of both sexes sub-quadratical, with pair of distinct long venulae and well-developed apodemes, tergite I distinctly broader than long, strongly edged, anterolateral margin rounded, posterior margin straight.

**Male genitalia** (Fig. 8). Uncus reduced to trapezoidal lobe with inward folded edges; gnathos absent; tegumen narrow, considerably broader than long, posterior edge strongly sclerotized; cucullus digitate, moderately broad, apex rounded, densely haired; sacculus flat, about  $\frac{3}{4}$  length of cucullus and slightly broader, posterior margin straight with two or three small teeth; transtilla lobes well developed; vinculum narrow, band-shaped, terminating in short rounded saccus; phallus longer than cucullus, with tapered lateral processes, basal half sclerotized dorsally, distal half sclerotized mainly laterally, apex beak-shaped, base bifurcated.

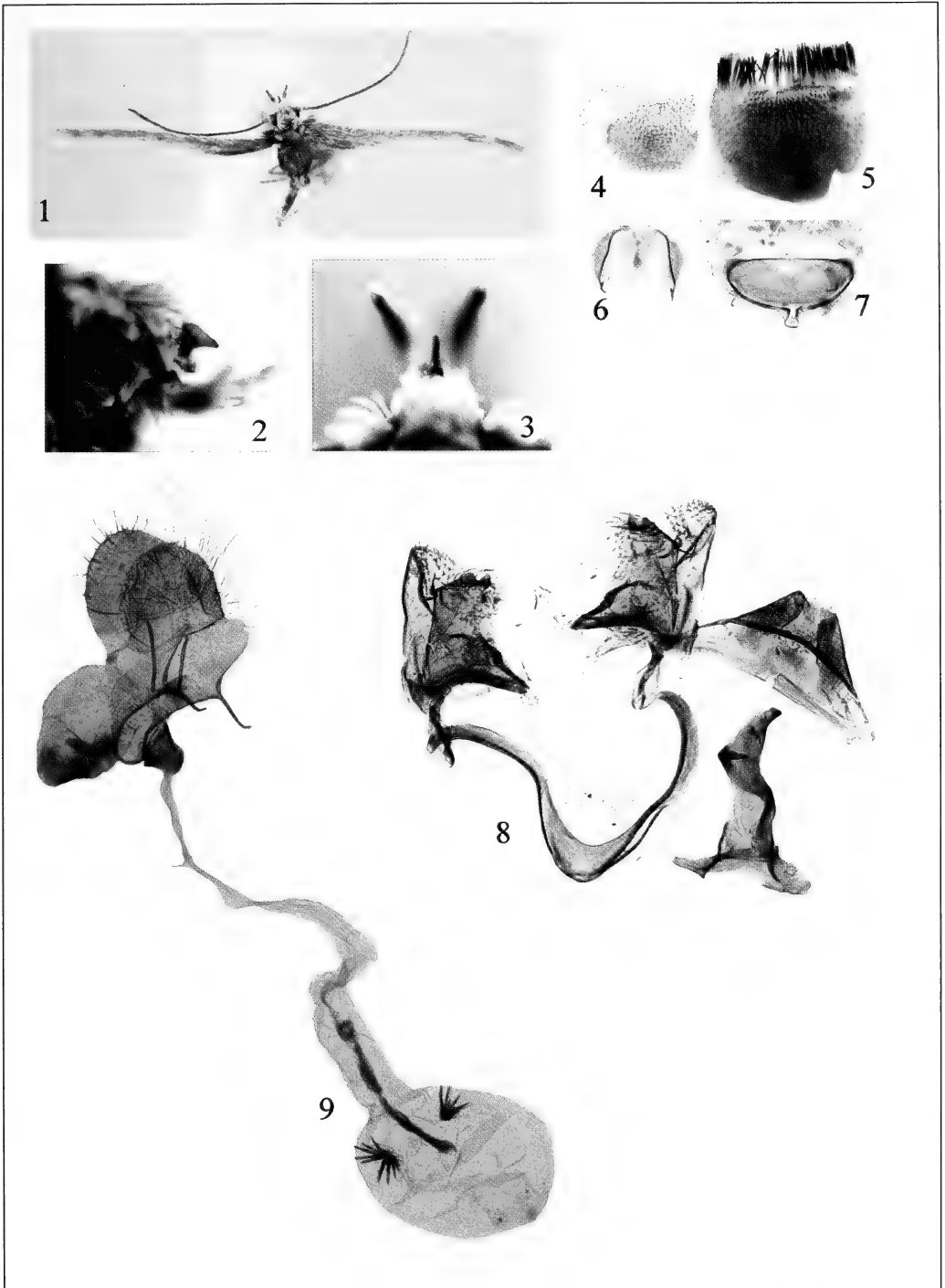
**Female genitalia** (Fig. 9). Papilla analis rounded, with straight basal edge, densely covered with short setae except for patch of long hair-like setae arising from dorsal margin; apophyses anteriores about one-half length of apophyses posteriores, straight, terminally curved; tergite and sternite VIII extremely narrow, strongly sclerotized, ribbon-shaped; lateral part of segment VIII evenly sclerotized, band-shaped; ostium rounded, opening near anterior edge of sternite VIII; antrum short, funnel-shaped; ductus bursae long, membranous, posterior half thin, anterior half moderately wide; corpus bursae globular; signum paired, with long spines arising from rounded plate.

**Diagnosis.** The new species is easily recognizable both externally and in the genitalia characters. For details see the Discussion.

**Distribution.** SE Turkmenistan (Repetek Nature Reserve).

**Derivation of name.** The specific name refers to the extremely narrowed forewing, the most characteristic feature of this species.





**Figs 1–9.** *Catatingama stenoptera* sp. n. 1. Adult, holotype. 2. Head, lateral view. 3. Head, view from above; 4. Male sternum VIII (gen. slide 55/11). 5. Female tergum VII (gen. slide 56/11). 6. Male sternum I+II (gen. slide 55/11). 7. Male tergum I (gen. slide 55/11). 8. Male genitalia (gen. slide 55/11). 9. Female genitalia (gen. slide 56/11).

**Biology.** The new species is hitherto only known from the Repetek Nature Reserve, SE Turkmenistan. The adults were collected from 3<sup>rd</sup> to 25<sup>th</sup> of February. According to field observations by Viktor A. Krivokhatsky, who collected the type-series, the adults were active during the warm days when they were seen jumping on the sand. They have also been collected by sweeping amongst *Carex physodes* M. Bieb. (Cyperaceae). This plant is most likely the host for this species, although the preimaginal stages have not yet been found. The adults have also been observed and collected in the burrows of the great gerbil (*Rhombomys opimus* (Lichtenstein, 1823)) and the long-clawed ground squirrel (*Spermophilopsis leptodactylus* (Lichtenstein, 1823)) (Mammalia, Rodentia, Muridae, Sciuridae). The moths go deeply into the burrows at night and when there is frost in the daytime.

## Discussion

**Wing reduction and biology.** Within the family Gelechiidae species with different degrees of wing reduction are known in eleven genera, but are most common in *Megacraspedus* Zeller, 1839, *Ephysteris* Meyrick, 1908, *Kiwaia* Philpott, 1930, and *Sattleria* Povolný, 1965 (Sattler 1991). The forewing transformation, for the majority of such Gelechiidae species, resulted in forewing reduction of length and width in the apical one-third. As a result the forewings become “lanceolated”: their basal half remains nearly of usual width, whereas the distal portion is considerably narrowed towards the apex. According to the classification proposed by Sattler (1991) such species must be called “brachypterous”, whereas the species described here has to be treated as “stenopterous”, having the forewing strongly reduced in width without any significant reduction in length. Brachyptery and other cases of wing reduction are mainly restricted to the female sex. *Megacraspedus dolosellus* (Zeller, 1839), *Eulamprotes libertinella* (Zeller, 1872) and all species of the genus *Sattleria* are some examples of brachypterous Palaearctic Gelechiidae. Wing reduction in both sexes is a rather rare phenomenon that is known in about 25 species from different families of Lepidoptera world-wide (Karsholt & Sattler 1998; Sattler 1991). In Gelechioidea real stenoptery is only known in the female of *Pleurota marginella* (Denis & Schiffermüller, 1775), Oecophoridae, and previously has not been observed within Gelechiidae, making *C. stenoptera* sp. n. the first case of stenoptery in both sexes in that family.

Wing reduction in both sexes characterizes the species that inhabit mainly small oceanic islands, costal localities (e.g. dunes) and high mountain area with continuous strong winds (Heppner 1991; Sattler 1991; Karsholt & Sattler 1998). *Ephysteris brachyptera* Karsholt & Sattler, 1998, from Madeira and at least two undescribed *Ephysteris* species from the Tuva Mountains (Karsholt & Sattler 1998) and Mongolia (Bidzilya *in press*) are examples of brachyptery in both sexes in Palaearctic Gelechiidae. Brachyptery in females is much more common and observed in addition to the above cases, in a large number of species with imagoes active during the cold season. *C. stenoptera* sp. n. undoubtedly falls into this group of “winter” moths. The adults were found in winter time in the Karakum desert. Among Turanian Gelechioidea species with winter activity of the imago are known only in Ethmiidae: *Dasyethmia hiemalis* Danilevsky, 1969, from the sands of south-eastern Kazakhstan (middle course of the Chu river) flies in the daytime in January. The males of this species are densely haired, the females are unknown but probably wing-reduced (Shovkoon 2010). *Cheimoptena pennigera*

Danilevsky, 1969 (Geometridae) is another winter moth described from the south-eastern Kazakhstan but also known from Repetek (Krivokhatsky 1985c). The female of this species is apterous, the males are capable of flight and densely haired, as an adaptation for activity under low temperatures.

Adults of *C. stenoptera* are flightless, they move by jumping. The jumping and/or running may be considered as a regular way of locomotion for many flightless species, that mainly occurs in habitats with sparse vegetation and exposed soil, although jumping is quite common in many fully winged species capable of flight (Sattler 1991). It is unknown whether jumping is the only way of locomotion in *C. stenoptera* or whether the adults can also walk or run. However, it is clear that *C. stenoptera* is a quite mobile species: both sexes were found deep in rodent burrows, where they hide at night and during frost also in the day time. The association of *C. stenoptera* with rodent burrows is the most interesting behavioural phenomenon of this species. The great gerbil and the long-clawed ground squirrel are quite common in the sand deserts of Turkmenistan. The first species is considered ecologically one of the most important mammals, whose activity (mainly digging holes) considerably affects the local landscape. Many invertebrate species are associated with the great gerbil and inhabit its burrows. According to observations in the Repetek Nature Reserve (Krivokhatsky 1985a) about 345 animal species, mainly insects, were found in the burrows of great gerbil and other rodents. The Coleoptera (121 species of which 54 are obligate residents), Hymenoptera (50 species / 5 obligate) and Hemiptera (29 species / 6 obligate) predominate amongst the insects. The Lepidoptera with 15 species occupy the fourth position. Only two species - *Calycobathra calligoni* Sinev, 1979, and *Asclenia decolorella* Sinev, 1980 (both Cosmopterigidae) - overwinter in the burrows of the great gerbil and are considered as permanent residents (Krivokhatsky 1985b). *C. stenoptera* is the third species strongly associated with the great gerbil's burrows. In contrast to the Momphidae species, the adults of *C. stenoptera* use rodent burrows as a refuge during the period of their activity in winter time, but not for overwintering in them.

The host plant of *C. stenoptera* is unknown. The adults have been observed amongst *Carex physodes*, but *Haloxylon* spp., *Kochia* spp. (Chenopodiaceae) and *Ephedra strobilacea* Bunge (Ephedraceae), which are common in the habitats of *C. stenoptera*, are also possible candidates for the host plant of this species.

The function of frontal processes in Gelechiidae remains unclear. It was suggested that species pupating under ground use the frontal process to bore through the soil after emerging from the pupa. However, as it was correctly noted by Sattler (1976), many species in the same habitats manage perfectly well without such modifications. Frontal modifications occur independently in several gelechiid genera from different subfamilies and tribes, such as *Ornativalva* Gozmány, 1955 (Anomologinae), *Athrips* Billberg, 1820 (Gelechiinae), *Caulastrocecis* Chrétien, 1931 (Apatetrinae) and others. In the Apatetrinae the frontal modifications are common and were observed in Palearctic *Catatinagma* as well as in the South African genera *Cerofrontia* Janse, 1951, and *Rhynoceros* Bidzilya & Mey, 2011. As most species with frontal modifications are restricted to arid areas, one may suspect some relationship of this modification to occurrence in arid habitats, but not as an adaptation to the cold season.

The Repetek Nature Reserve, where *C. stenoptera* has been found, represents the southern sand deserts of the Turanian province (Karakum desert). The area is characterized by an

arid continental climate with hot summers and cold winters. The mean annual temperature is +16.3°C, with the average summer temperature ranging +29–33°C (with a record high of +50.1°C); temperatures below freezing are usually observed from December to February (with a record low of -31°C). The average precipitation is 117 mm per year with a maximum in winter and spring (Zapovednik Repetek 1990). The climate of Repetek is in general similar to that of the type localities of *D. hiemalis* and *Ch. pennigera*, but the winter is milder. Although these three winter species occur under very similar climatic conditions, *C. stenoptera* has rather different morphological and biological adaptations for the cold season. The phenomenon of wing reduction in cold season moths was discussed in detail by Sattler in his review of the wing reduction in Lepidoptera (Sattler 1991).

Although our current knowledge of the distribution, habitat preferences, host plants and behaviour of *C. stenoptera* is rather limited it seems clear that the regular occurrence of *C. stenoptera* in the burrows of the great gerbil and the long-clawed ground squirrel is a permanent adaptation for surviving under a low temperature, at least in the Repetek.

## Systematics

The monotypic genus *Catatinagma* with the only included species *C. trivitellum* Rebel, 1903, was considered for a long time as a synonym of *Apatetris* Staudinger, 1879. Its status has been recently revised and it was recalled from synonymy, whilst another monotypic genus, *Coloptilia* Fletcher, 1940, has been synonymized with *Catatinagma* (Junnilainen & Nupponen 2010). According to Junnilainen & Nupponen (2010), the head with a strongly developed frontal process within a crater-like depression and a short, nearly straight labial palpus are considered as external characteristic features of *Catatinagma*. The male genitalia are characterized by a sacculus that is strongly separated from the valva, a reduced gnathos and membranous uncus. A paired brush-shaped signum is typical for the female genitalia of *Catatinagma* (Junnilainen & Nupponen 2010).

The genus *Catatinagma* in this new concept comprises three species: *C. trivitellum* Rebel, 1903, *C. kraterella* Junnilainen & Nupponen, 2010 and *C. conchylidella* (Hofmann, 1898) (type-species of *Coloptilia*). Whilst it is evident that the first two species are congeneric, the proximity of *C. conchylidella* to them, and consequently the synonymy of *Coloptilia* with *Catatinagma*, remains in doubt due to considerable differences between the male genitalia of *C. trivitellum* and *C. conchylidella* (see Junnilainen & Nupponen 2010: 6, pl. 2).

The male genitalia of *C. stenoptera* resemble those of *Metanarsia trisignella* Bidzilya, 2008, having a short digitate cucullus in combination with a short, apically serrated sacculus. The female genitalia of both species differ in the shape of the signum: triple whisk-shaped in *M. trisignella* and paired, covered with strong spines in *C. stenoptera*. *M. trisignella* was described in *Metanarsia* Staudinger, 1871, based on the close similarity of its male genitalia to the other members of this genus, although a reduced gnathos and triple whisk-shaped signum as well as a prominent frontal process are characteristic features of *Catatinagma*, but not *Metanarsia* (Bidzilya 2008). *C. trivitellum* is the second species in addition to *M. trisignella* which appears to be intermediate between *Metanarsia* and *Coloptilia* according to the current diagnosis of these genera. It should be noted that the male genitalia of both species are more like those of *C. conchylidella* than those of *C. trivitellum* and *C. kraterella*. Despite differ-

ences in the shape of the signum, both species seem to be closer relatives of each other than any other genera of Apatetrini. The recent attempts of finding an appropriate position for such “problematic” species within the tribe Apatetrini based on traditional morphological characters usually resulted either in their provisional association with collective genera of unclear definition (Sakamaki 2000; Nel & Varenne 2012) or in the establishment of new genera for the most exceptional cases (Bidzilya & Mey 2011). The possibility cannot be excluded that both species require a separate genus. However, it seems more appropriate at the present stage to place them in *Catatinagma* (*Catatinagma trisignella* (Bidzilya, 2008) **comb. n.**)), until a global revision of Apatetrini supported by DNA-studies can be provided.

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# Wing pattern morphology of three closely related *Melitaea* (Lepidoptera, Nymphalidae) species reveals highly inaccurate external morphology-based species identification

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**Abstract.** Wing morphology of the three closely related species of *Melitaea* – *M. athalia* (Rottemburg, 1775), *M. aurelia* (Nickerl, 1850) and *M. britomartis* Assmann, 1847 – co-occurring in the Balkans (SE Europe) was investigated in detail through visual inspection, morphometric analysis and multivariate statistical analysis. Results are compared to recent phylogenetic studies, searching for concordant patterns and discrepancies between the two approaches. The morphology of the genitalic structures is also compared with the results of the other two approaches. The main conclusions are as follows: (1) small albeit significant differences in wing morphology exist among the three species and (2) while the structure of male genitalia and phylogenetic position of the three species are concordant, they are (3) in discordance with the wing morphology. The present study represents another example where identification based on external morphology would lead to highly unreliable determinations, hence identification based on phylogenetic studies and/or genitalia is strongly recommended not only for the three studied species but also more broadly within the genus. Furthermore, we show that some of the characters generally used in the identification of these three *Melitaea* species should be avoided in future.

## Introduction

The genus *Melitaea* Fabricius, 1807 (Lepidoptera: Nymphalidae) as it is known today (Leneveu et al. 2009), comprises approximately 80 species, all restricted to the Palearctic region. A recent phylogenetic analysis showed that the genus can be divided into two clades ('*Melitaea* clade' and '*Didymaeformia* clade') with high branch support from both maximum likelihood and Bayesian analyses (Leneveu et al. 2009). Within the '*Melitaea* clade' five subclades have been recognized (*sensu* Leneveu et al. 2009): '*cinxia*', '*diamina*', '*arcesia*', '*minerva*' and '*athalia*' groups. The latter subclade comprises fourteen species, three of which are present in the area of north-western Balkans: *Melitaea athalia* (Rottemburg, 1775), *Melitaea aurelia* (Nickerl, 1850) and *Melitaea britomartis* Assmann, 1847. These three species belong to two different monophyletic groups within the subclade '*athalia*', one comprising *M. aurelia* and five other species, and the other *M. athalia*, *M. britomartis* and six other species (Leneveu et al. 2009). The split between the latter two monophyletic groups has been estimated to have

occurred about 11 million years ago (Serravallian period) while the subsequent speciation occurred during the Messinian Period (5.3–7.1 Mya) (Leneveu et al. 2009), when the continuous decrease of temperature coincided with the aridification of the climate in Eurasia and a subsequent expansion of grassland of C4 plants (e.g. Maki et al. 2003; Quade et al. 1995). Although the separate evolution of those species has been in progress for a long period of time, the clear genetic differentiation (Bátori et al. 2012a) is not reflected in their external morphology (Tolman & Lewington 2008).

The external morphology of the three species is highly similar, rendering the accurate identification based on external morphology questionable (Tolman & Lewington 2008). There are several morphological characters that have been proposed for species identification of *M. athalia*, *M. aurelia* and *M. britomartis*: the coloration of the marginal line of the hindwing underside; the spacing of the marginal, submarginal, postdiscal and discal line on the forewing upperside; and the colour of the submarginal spots on the hindwing underside (Tolman & Lewington 2008). The high variability of those characters, however, sometimes prevents proper identification (Tolman & Lewington 2008). The genitalia of these species are highly species specific, and have been known to be a good identification tool (Urbahn 1952; Paulavičiūtė & Tamutis 2009). Nevertheless, even when genitalia based identification is used, only males can be unambiguously recognized to a species rank, whereas this is not always possible in females (e.g. see Urbahn 1952).

*Melitaea athalia* is a trans-European species (except for south-western Europe, where *Melitaea celadussa* Fruhstorfer, 1910, a former subspecies of *M. athalia* occurs (Leneveu et al. 2009)). *M. aurelia* and *M. britomartis* are absent from the majority of SW Europe (including the Iberian and Apennine peninsulas). *M. britomartis* is absent also from most of Central Europe being present only in eastern parts of Europe. A part of its distribution, however, reaches the NW Balkans – Slovenia, Croatia and Bosnia and Herzegovina (Koren & Jugovic 2012) – where these three species live in sympatry. In this area the distribution of *M. britomartis* is separated from the center of its distribution (Tolman & Lewington 2008).

The ecology of the three species in the area of NW Balkans (Slovenia) is similar in terms of flight period, altitudinal distribution and habitat requirements including foodplant (Koren & Jugovic 2012; Verovnik et al. 2012). Since their habitat requirements and foodplants partly overlap, they often occur in the same habitat (Bátori et al. 2012a; Koren & Jugovic 2012).

Morphological traits can reflect either historical isolation and/or local adaptation despite recurrent gene flow (Alexandrino et al. 2005). Some taxa remain morphologically unidentifiable despite detailed morphometric analysis, hence a consideration of a large set of morphometric characters from different anatomical regions may greatly increase the chances of revealing taxonomic differences within seemingly cryptic or morphologically highly variable species (e.g. Jugovic et al. 2011).

We wanted to check the concordance in the amount of morphological and genetic differences among the three closely related species. For this purpose a morphometric approach with subsequent multivariate statistical analysis was used in the *M. athalia* complex for the first time. We evaluated the following hypotheses: (1) the genetic divergence of the three species is accompanied by corresponding morphological differences exceeding the intra-species variability and (2) the level of accompanying morphological differences is concordant with the level



of molecular distance between the taxa, meaning that *M. aurelia* should be the most distant in morphological space from the other two species.

## Materials and methods

### Samples and species identification

Samples of the three species of *Melitaea* were collected over the last 40 years all over the NW Balkans (see Koren & Jugovic 2012 for the list). Altogether, samples from 56 localities were collected, sample sizes ranging from 1 to 12 for males and 1 to 3 for females. The genitalia of each specimen were isolated using the standard procedure for genitalia isolation (see Koren & Jugovic 2012 for details). Specimens were attributed to a species according to the genitalia structure that is highly species-specific in males (Urbahn 1952; Paulavičiūtė & Tamutis 2009). Using these methods we were able to identify all of the collected males. A total of 42 males and 24 females of *M. athalia*, 29 males and 6 females of *M. aurelia* and 24 males and 4 females of *M. britomartis* were used in further morphometric analysis.

Separation of these three species is possible using male genitalia as follows (see also Urbahn 1952): 1. uncus absent -> *M. aurelia*; 2. uncus present, long and slender, its processus longer than wide -> *M. athalia*; 3. uncus present, short and robust, its processus almost as long as wide -> *M. britomartis*. In females there are slight differences among the species, but not all specimens can be reliably identified in this way (Urbahn 1952; Bátori et al. 2012a).

### Morphometric methods

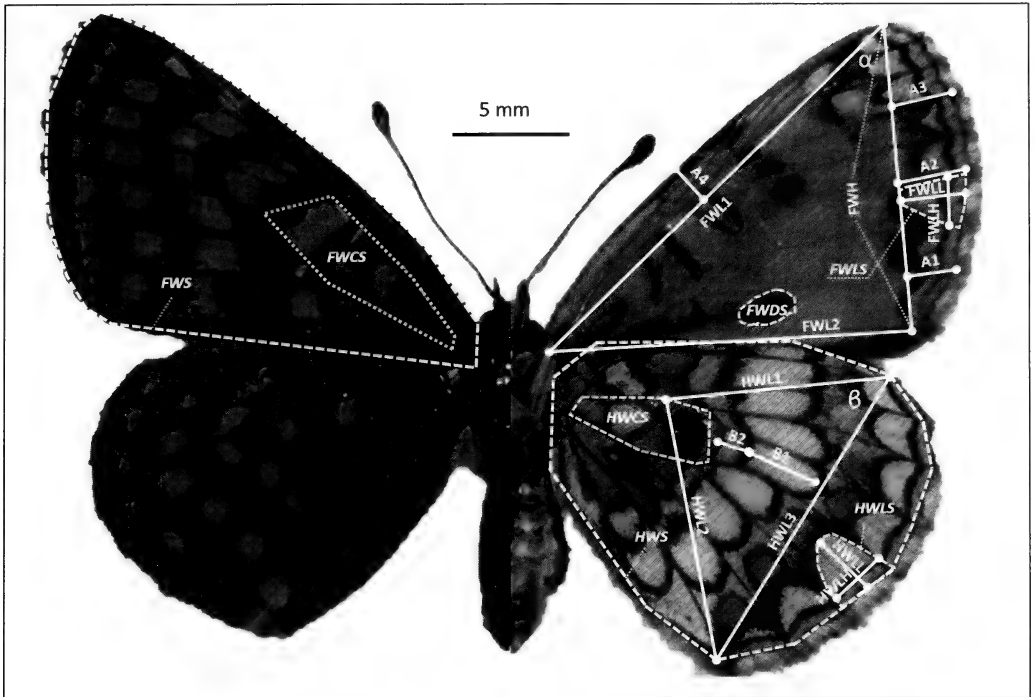
Specimens used in this study were mounted and photographed on a millimeter grid from the same angle (90°) using a DSLR camera (Canon 450D). Subsequently, 25 metric characters were measured from the photographs using freeware ImageJ (Abramoff et al. 2004), 14 on the forewing and 11 on the hindwing (Table 1, Fig. 1). The measurements included a wide range of morphometric characters (i.e. lengths, surfaces and angles between measured distances of different parts of both wings). Moreover, one categorical character, i.e. marginal line colour in contrast to lunular colour (for categories see Table 1 and Appendix A) was also recorded. In order to exclude the impact of the animal's size, all metric characters were transformed into 18 additional ratios (Table 1). The photographs of both wings were used for comparison and investigated in detail in order to find some other descriptive morphological species-specific characteristics and to describe intraspecific variability. Although no body asymmetry was detected, all characters were measured on the right side of an animal in order to exclude any possible influence of this phenomenon. The left side, however, was used in rare occasions when the right side was damaged.

### Statistical analysis

Since in our samples males were prevalent and not all females could be reliably identified to a species, females were excluded from subsequent statistical analyses. With this approach, we

**Table 1.** List of structures (with morphological characters, their explanation with measuring units) measured in three species of *Melitaea*.

No.	Character abbreviation	Description	Unit
<b>Forewing</b>			
1	<i>FWS</i>	forewing surface	mm <sup>2</sup>
2	<i>FWCS</i>	forewing cell surface	mm <sup>2</sup>
3	FWL1	forewing length 1	mm
4	FWL2	forewing length 2	mm
5	FWH	forewing height	mm
6	$\alpha$	angle at forewing apex	°
7	A1	distance A1	mm
8	A2	distance A2	mm
9	A3	distance A3	mm
10	A4	distance A4	mm
11	<i>FWDS</i>	forewing dot surface	mm <sup>2</sup>
12	<i>FWLS</i>	forewing lunule surface	mm <sup>2</sup>
13	FWLL	forewing lunule length	mm
14	FWLH	forewing lunule height	mm
<b>Hindwing</b>			
15	<i>HWS</i>	hindwing surface	mm <sup>2</sup>
16	<i>HWCS</i>	hindwing cell surface	mm <sup>2</sup>
17	HWL1	hindwing length 1	mm
18	HWL2	hindwing length 2	mm
19	HWL3	hindwing length 3	mm
20	$\beta$	angle at hindwing apex	°
21	B1	distance B1	mm
22	B2	distance B2	mm
23	<i>HWLS</i>	hindwing lunule surface	mm <sup>2</sup>
24	HWLL	hindwing lunule length	mm
25	HWLH	hindwing lunule height	mm
<b>Categorical character</b>			
26	Marginal line colour in contrast to lunular colour	(1=equal; 1.5=slightly darker; 2=darker; 3=much darker)	
<b>Ratios</b>			
27	<i>FWCS/FWS</i>		
28	FWL2/FWL1		
29	FWH/FWL1		
30	A2/A1		
31	A3/A1		
32	<i>FWDS/FWCS</i>		
33	<i>FWDS/FWS</i>		
34	FWLH/FWLL		
35	<i>FWLS/FWS</i>		
36	<i>FWLS/FWCS</i>		
37	$\beta / \alpha$		
38	<i>HWCS/HWS</i>		
39	HWL1/HWL2		
40	HWL3/HWL1		
41	B2/B1		
42	<i>HWLS/HWS</i>		
43	<i>HWLS/HWCS</i>		
44	HWLH/HWLL		



**Figure 1.** A photograph of *Melitaea* sp. with measured structures. Distances are denoted with full lines, surface measurements with dashed lines and angles with Greek alphabet. Left: upperside; right: underside. For explanation of characters' abbreviations see Table 1. For categorical character, see Appendix A.

also avoided the influence of possible sexual dimorphism. Only for the analysis of the marginal line colour were a few unambiguously identified females added to the sample (no sexual dimorphism was noticed in this character). We also had to remove from the analysis three aberrant individuals which lacked measuring points for some characters, hence they are commented on separately.

For each species, the Kolmogorov-Smirnov test (at  $p = 0.05$ ) and Normal Q-Q plots were used to examine the normality of the data distribution, and the homogeneity of variances was evaluated visually through scatterplots. The multivariate analysis of variance (MANOVA, at  $p = 0.001$ ) was used to test for significant differences between species. One-way analysis of variance (ANOVA) was used to assess the variation within a species for each character, significant variation in a character being accepted if the between species variation was significant at  $p < 0.001$ . Pearson's correlation coefficients ( $r$ ) were computed to evaluate the extent to which each character contributes unique information; only one character was chosen to represent a pair or a group of characters where  $|r| > 0.9$ . The Durbin-Watson test (at  $p = 0.05$  and  $p = 0.01$ ) was applied to test for possible spatial (latitude, longitude, altitude) and temporal (year of collection) autocorrelations of morphometric data (Savin & White 1977; Farebrother 1980) in each of the three species. In subsequent multivariate statistical analyses, only selected characters (according to the limitations listed above) were used.

Multivariate Principal Component Analysis (PCA) was used to identify the structure of our data i.e. to detect the possible influence of the species specific characteristics. Also, Discriminant Function Analysis (DFA) was carried out to examine possible separation of the three species. In DFA, the contribution of each species was weighted according to its sample size (number of specimens). Post-hoc Games-Howell and Bonferonni tests were performed to assess the rates of morphological divergences between pairs of species in details. The analyses were performed using Microsoft Excell (2010), SPSS 14.0 for Windows (2005) (Norusis, 2005) and Past (PAlaeontological STatistics) software (Hammer et al. 2001).

## Results

The multivariate analysis of variance (MANOVA) showed significant differences between species ( $p < 0.001$ ). Out of 43 morphometric characters, 12 characters (without a single ratio) were selected after Kolmogorov-Smirnov ( $p < 0.05$ ), ANOVA ( $p < 0.001$ ) and Pearson correlation ( $|r| < 0.9$ ) tests. No spatial or temporal autocorrelation was detected in these characters neither at  $p = 0.01$  nor at  $p = 0.05$  (Durbin-Watson test). Statistic description of selected 12 characters (mean, standard error, 12th percentile, 88th percentile and extreme values) is presented in Table 2. When the Pearson correlation test was set to  $|r| < 0.7$ , only two morphometric characters (forewing height [FWH] and distance A4) were left after step-wise exclusion of characters, meaning that correlations among most pairs of morphological characters were high.

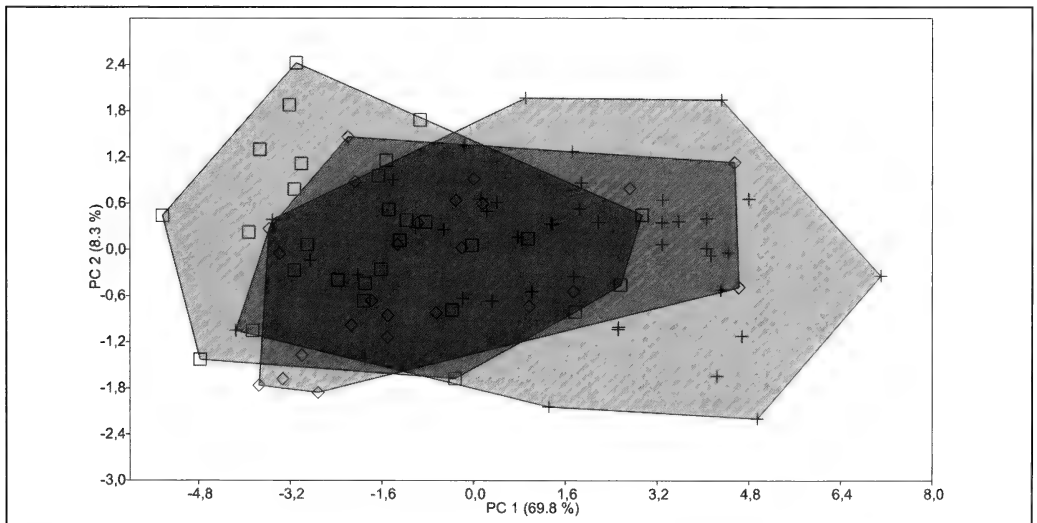
The principal result of the PCA (Fig. 2) run on 95 males from the NW Balkans, using 12 morphometric characters (Table 2), revealed a significant overlap of the three species along the first two principal components (PCs). PC 1 explained almost 70% of the total variance and PC2 explained an additional 8%. All characters are positively correlated to PC1, starting with the smallest *M. aurelia* and finishing with the largest of three species, *M. athalia*. Nevertheless, the overlap between the three species in the middle section along PC 1 is significant.

In the DFA (Fig. 3) run using the same morphometric characters as in the PCA, 95 males were analyzed to (1) provide adequate species-grouping according to external morphological characters and (2) detect intra-species variability of selected characters. Two Discriminant Functions (DFs) explain total variance, DF 1 explaining over 75% of the total variance. Species differ significantly only along DF 1 ( $p < 0.001$ ), and only *M. athalia* differs significantly from the other two species (Games-Howell Test,  $p < 0.001$ ). The characters most correlated with DF 1 (indicated by the standardized discriminant function coefficients:  $DC > 0.75$ , Table 3) are: forewing height (FWH), forewing lunule surface (FWLS), forewing lunule height (FWLH) and hindwing surface (HWS). For the differences among the three species in these four characters, see Fig. 4 and Table 2.

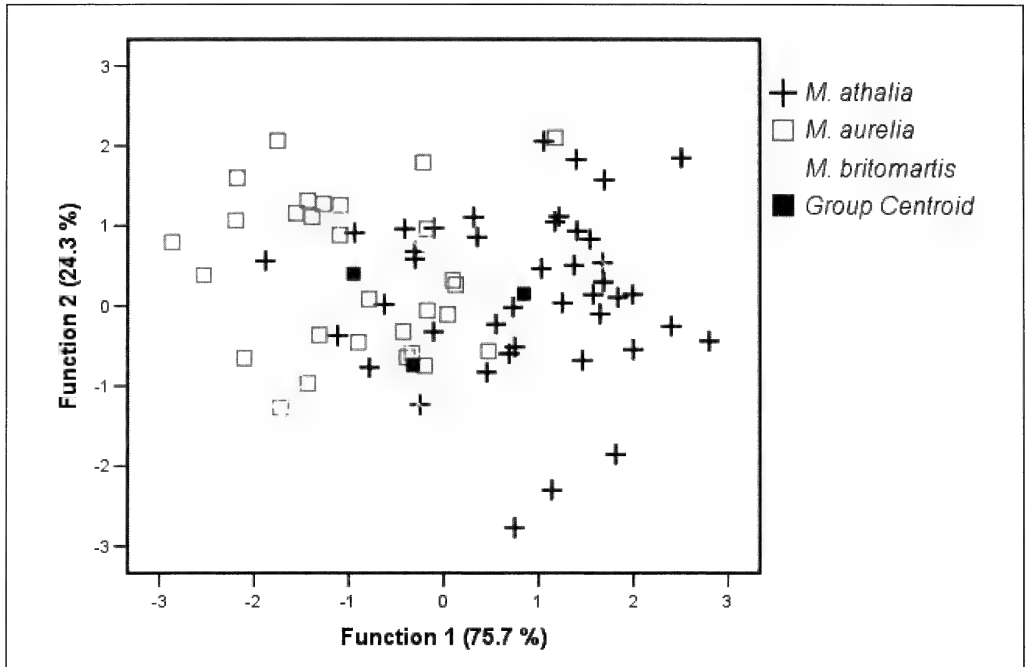
Although the DFA aims to find the differences between the *a priori* defined groups, the misclassification rates were high, especially when cross-validation process was employed (Fig. 5). Considering both DFs, only 67.4% specimens were correctly classified, and in the cross-validation procedure, the percentage dropped down to only 52.6% of correctly classified specimens. We then repeated DFA with the same twelve morphometric characters, adding the coloration of the marginal line in contrast to the lunular colour on the underside of the hindwing, not

**Table 2.** Statistical description of twelve metric characters in males of three species of the genus *Melitaea* that were used in subsequent Principal component and Discriminant Function analyses. Upper row: average  $\pm$  std. error; lower row: (min), 12<sup>th</sup> percentile–88<sup>th</sup> percentile (max).

		<i>M. athalia</i>	<i>M. aurelia</i>	<i>M. britomartis</i>
forewing height	FWH	11.62 $\pm$ 0.15	10.25 $\pm$ 0.16	10.73 $\pm$ 0.17
		(9.27) 10.50–12.79 (13.56)	(8.93) 9.15–11.46 (12.23)	(9.19) 9.63–11.51 (12.78)
forewing lunule surface	FWLS	3.82 $\pm$ 0.10	2.82 $\pm$ 0.10	3.09 $\pm$ 0.18
		(2.01) 3.07–4.79 (4.98)	(1.82) 1.95–3.55 (4.21)	(1.74) 2.19–4.29 (5.28)
forewing lunule height	FWLH	1.69 $\pm$ 0.03	1.45 $\pm$ 0.03	1.56 $\pm$ 0.03
		(1.21) 1.50–1.99 (2.09)	(1.11) 1.24–1.61 (1.66)	(1.33) 1.36–1.76 (1.79)
hindwing surface	HWS	138.70 $\pm$ 4.60	109.55 $\pm$ 20.50	120.20 $\pm$ 4.40
		(96.40) 106.64–171.47 (181.71)	(81.09) 89.78–144.80 (153.48)	(93.94) 103.09–161.06 (170.21)
distance B1	B1	3.86 $\pm$ 0.09	3.21 $\pm$ 0.08	3.50 $\pm$ 0.09
		(2.76) 3.09–4.67 (5.10)	(2.39) 2.63–3.76 (4.06)	(2.63) 3.02–4.09 (4.57)
hindwing cell surface	HWCS	9.23 $\pm$ 0.24	7.61 $\pm$ 0.26	7.94 $\pm$ 0.26
		(6.39) 7.35–11.42 (12.66)	(5.17) 5.75–9.42 (10.85)	(6.30) 6.60–9.44 (12.16)
hindwing length 1	HWL1	8.53 $\pm$ 0.14	7.54 $\pm$ 0.18	7.90 $\pm$ 0.16
		(6.48) 7.52–9.88 (10.15)	(5.58) 6.45–8.78 (10.20)	(6.44) 7.05–8.96 (9.40)
hindwing lunule surface	HWLS	4.29 $\pm$ 0.15	3.39 $\pm$ 0.14	3.58 $\pm$ 0.20
		(2.52) 3.14–5.32 (6.75)	(2.08) 2.65–4.57 (5.00)	(2.44) 2.53–4.82 (6.26)
hindwing lunule length	HWLL	2.48 $\pm$ 0.05	2.27 $\pm$ 0.05	2.20 $\pm$ 0.06
		(1.73) 2.02–2.85 (3.24)	(1.68) 1.95–2.62 (2.79)	(1.73) 1.81–2.49 (2.82)
hindwing lunule height	HWLH	1.95 $\pm$ 0.04	1.71 $\pm$ 0.03	1.80 $\pm$ 0.05
		(1.44) 1.69–2.34 (2.70)	(1.28) 1.48–1.93 (2.16)	(1.46) 1.55–2.08 (2.33)
distance A4	A4	1.84 $\pm$ 0.05	1.59 $\pm$ 0.05	1.59 $\pm$ 0.05
		(1.09) 1.39–2.14 (2.49)	(1.11) 1.32–1.92 (2.18)	(1.13) 1.30–1.83 (2.01)
forewing lunule length	FWLL	2.46 $\pm$ 0.05	2.13 $\pm$ 0.05	2.19 $\pm$ 0.09
		(1.63) 1.98–2.80 (3.04)	(1.52) 1.83–2.40 (2.56)	(1.56) 1.59–2.75 (3.32)



**Figure 2.** Two-dimensional plot (PC 1 vs. PC 2) generated from Principal Component Analysis (PCA) run on 95 males of three *Melitaea* species using twelve metric characters (see Table 2). Legend: crosses: *M. athalia*; diamonds: *M. britomartis*; squares: *M. aurelia*.



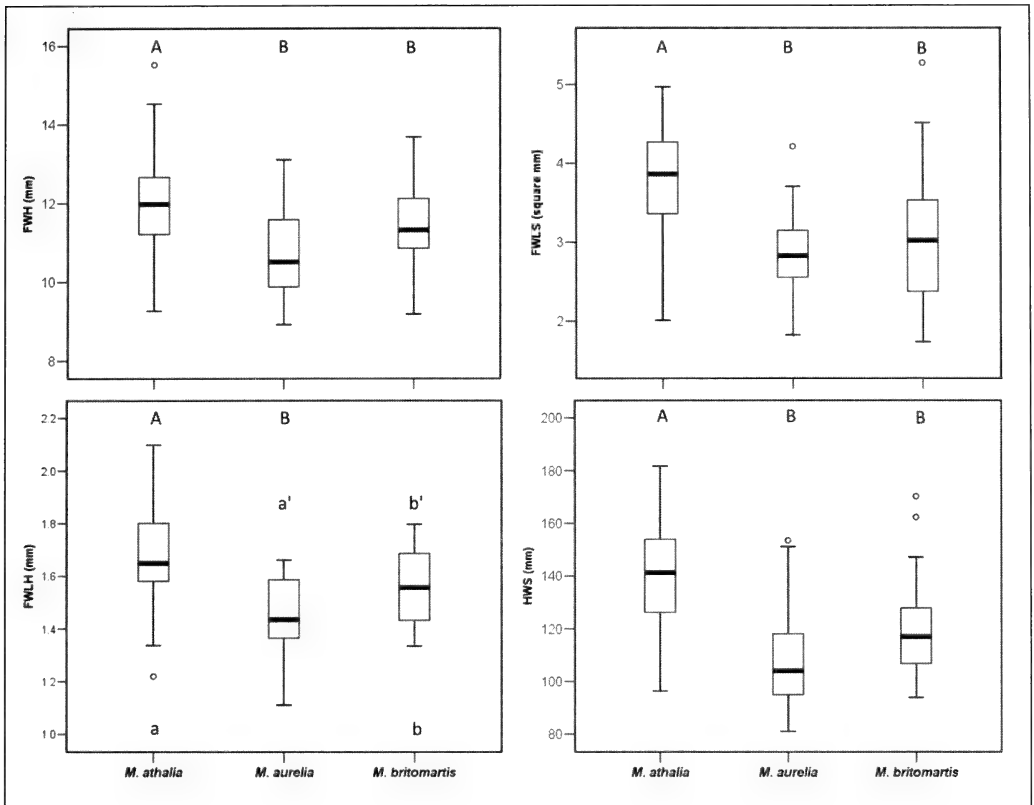
**Figure 3.** Plot of the scores of 95 males of three species of *Melitaea* on both discriminant functions, using 12 morphometric characters. Centroids are larger and darker than symbols denoting individual specimens.

**Table 3.** Contributions of 12 metric characters (MC) to discrimination along both discriminant functions (DF). Discriminant function analysis run on 95 males of three species of *Melitaea*. Asterisk (\*) denotes the highest absolute correlation between the character and DF.

MC	(symbol)	DF 1	DF 2
forewing height	(FWH)	0.851*	-0.002
forewing lunule surface	(FWLS)	0.810*	0.110
forewing lunule height	(FWLH)	0.791*	-0.129
hindwing surface	(HWS)	0.787*	-0.022
distance B1	(B1)	0.680*	-0.120
hindwing cell surface	(HWCS)	0.651*	0.158
hindwing length 1	(HWL1)	0.624*	-0.013
hindwing lunule surface	(HWLS)	0.575*	0.128
forewing lunule length	(FWLL)	0.571*	0.147
hindwing lunule height	(HWLH)	0.562*	-0.032
distance A4	(A4)	0.533*	0.285
hindwing lunule length	(HWLL)	0.455	0.476*

considering that this character was deviating from normal distribution (Kolmogorov-Smirnov test, all data pooled:  $Z = 2.642$ ;  $p < 0.001$ ). However, this did not improve the classification significantly (original grouping: 70.5%; cross-validation procedure: 55.8%).

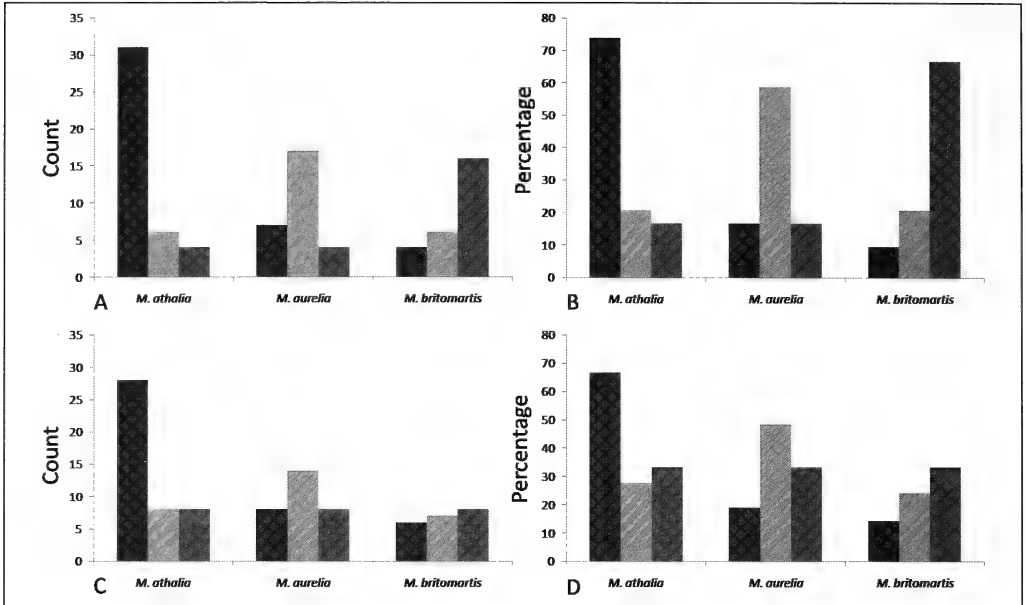
In addition to the characters used in the DFA, two characters frequently mentioned in the literature as diagnostic for the recognition of (some of) *Melitaea* species should be mentioned.



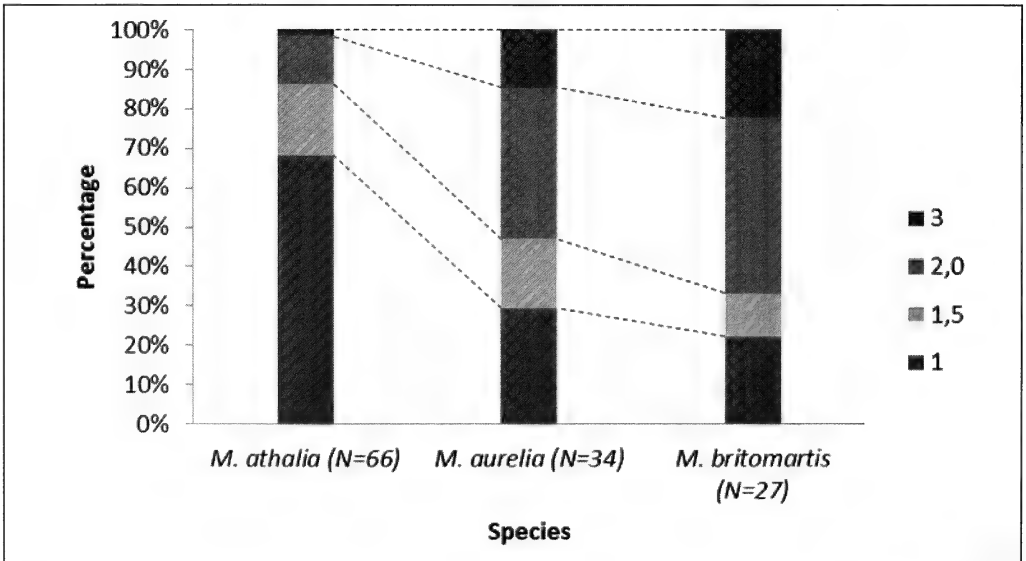
**Figure 4.** Variation in four morphometric characters in *M. athalia*, *M. aurelia* and *M. britomartis*: forewing height (FWH), forewing lunule surface (FWLS), forewing lunule height (FWLH) and hindwing surface (HWS). Statistically significant differences between pairs of species (Bonferroni test) are shown with capital ( $p < 0.001$ ) and small ( $0.001 < p < 0.05$ ) letters. When only a trend in differences between species is shown ( $0.05 < p < 0.1$ ), small letters with apostrophe are used.

For the characters like the spacing of the marginal, submarginal, postdiscal and discal line on the forewing upperside, the colour of the submarginal spots on the hindwing underside, the coloration of hairs on the palps as well as that of the marginal line in contrast to the lunular colour on the underside of the hindwing, no consistency with species attribution was found in our examination. For the latter, slight differences were found between the three species; however, species could not be identified with certainty using the lunular coloration on the hindwing underside due to the high intraspecific variability of this character. The coloration of the marginal line is in general equal to the coloration of the lunules in *M. athalia*, much darker in *M. britomartis* and only slightly darker in *M. aurelia*. Nevertheless, all of these categories were noticed in each species (Fig. 6, Appendix B).

The wing pattern and coloration show high intra- and interspecific variability. While in some species dark melanistic forms are common (e.g. in *Melitaea britomartis* ssp. *michieli* from Slovenian Karst and surroundings (Carnelutti 1992)), other peculiar forms also had been found. In some of these, the pattern deviated from normal form so much so that in these animals some



**Figure 5.** DFA classification results of 95 males of *Melitaea athalia* (first column), *M. aurelia* (second column) and *M. britomartis* (third column); A, B – original; C, D – cross-validation procedure.



**Figure 6.** Colour categories of marginal line colour in contrast to lunular colour on underside of hindwing of three *Melitaea* species (1=equal; 1.5=slightly darker; 2=darker; 3=much darker).

metric characters could not be measured. We found three such *M. athalia* specimens (i.e. with extremely reduced markings) from Kamniški Vrh and Smrekovec (both in Slovenia) and Vugrovec (Croatia).



## Discussion

The genitalia structures (Urbahn 1952) and the phylogeny based on three genes (mitochondrial gene cytochrome oxidase subunit I, and two nuclear genes, elongation factor-1a, and wingless; Leneveu et al. 2009) of the three *Melitaea* species are in agreement, clearly showing that *M. aurelia* is more distant from the other two species. In our analysis this is proved by a genitalia based species identification of males without any questionable cases. However, the phylogenetic relationships and the differences in the genitalia morphology are not reflected in the external morphology of the three species' wing patterns.

All analysis conducted in this study indicates the important differences among the three species in the size of most of the measured characters, however, only in average values. Due to high variability of these characters, the clinal variation among species has been noticed (see the results of multivariate analyses). According to literature (e.g. Tolman & Lewington 2008), *M. athalia* is indeed the largest of the three species, with forewing height (FWH, Table 2, Fig. 4) being the most obvious character reflecting the difference in size from our study. Without exception, all measured characters had the smallest average values in *M. aurelia* on one side of the clinal variation and the largest in *M. athalia* on the other. High correlations among most of the characters show the stability of their wing shape, which can therefore be represented by a subset of all the characters. The wing shape stability is also supported by the exclusion of all ratios (that partly exclude the size impact and describe the wing shape) in ANOVA tests. In order to minimize the number of highly correlated characters, only one character was chosen from a pair or group of highly correlated characters. This goal was only partially achieved as further stepwise removal of characters (with Pearson correlation coefficients  $0.7 < |r| < 0.9$ ) would result in only two weakly correlated remaining characters ( $|r| < 0.7$ ) and prevent the implementation of the multivariate analyses. Although the differences are small, *M. athalia* is the most distant from the other two in a powerful discriminant function analysis. All except one character that were included in the multivariate statistical analyses significantly separate *M. athalia* from the other two, and only slight differences in just one (out of 43) metric character (forewing lunule height, FWLH; ANOVA:  $0.05 < p < 0.1$ ) between *M. aurelia* and *M. britomartis* were shown. Although the differences are small, this further supports the discrepancies between the external morphology and phylogenetic results (sensu Leneveu et al. 2009). Considering the phylogenetic position of the three species, *M. athalia* should resemble *M. britomartis* more than *M. aurelia* but in our analysis the latter two were clustered more closely together. According to the phylogenetic data, the first split within the 'athalia' group that separated the clade containing *M. aurelia* from the clade containing the two other species occurred in the Tortorian period (approximately 11 MYA) and the later split from which also *M. athalia* and *M. britomartis* emerged happened during Messinian period (approximately 7.1 – 5.3 MYA; Leneveu et al. 2009). Hence, the three lineages that emerged – *M. athalia*, *M. aurelia* and *M. britomartis* – underwent at least 5 million years of separate evolution. Despite such a long time of separation, no obvious external morphological differences have evolved. This is somewhat surprising when compared to the situation in some of their close relatives. For example, clear differences in external morphology have emerged in some species of *Melitaea*, although they have become separate evolutionary lineages more recently than *M. athalia*, *M. aurelia* and *M. britomartis* (e.g. *M.*

*asteria*–*M. aurelia*, also within the same ‘*athalia*’ group; Tolman & Lewington 2008; Leneveu et al. 2009).

The allozyme polymorphism of these three species was studied in samples from the Carpathian basin, and revealed that *M. britomartis* and *M. aurelia* are more closely related, while *M. athalia* appears to be a further relative (Bátori et al. 2012a). This result contradicts the phylogenetic analysis of Leneveu et al. (2009). The latter analysis, however, finds support also in the genitalia structures as follows: in males of *M. athalia* and *M. britomartis*, the spines on the uncus are well developed, whereas they are completely absent in *M. aurelia* (Urbahn 1952). It should not be a surprise that the level of differentiation in genitalic structures corresponds to the phylogenetic position of the species since sexual differentiation usually represents one of the most rapid and obvious taxon-specific events during speciation in many animal groups, invertebrates in particular (Mayr & Ashlock 1991). In addition, the genitalic structures are in comparison to the external morphology less subjected to environmental factors (Cesaroni et al. 1994; Dapporto et al. 2011). For example, *M. celadussa*, recently elevated to a species level by Leneveu et al. (2009) from a subspecies rank of *M. athalia*, has not yet been reliably recognized on the basis of wing pattern and coloration, whereas some differences from *M. athalia* were found in male genitalia alone (e.g. see Higgins & Riley 1978; Leneveu et al. 2009). As long as external differences are not found, this species pair could be treated as cryptic (*sensu* Hawksworth 2010: “populations which are phylogenetically distinct, but distinguished by molecular or other features that are either not evident macroscopically or generally overlooked”).

In contrast to the allozyme study, the phylogenetic analysis used as a framework for the explanation of our results (Leneveu et al. 2009) (1) included a vast majority of known species of *Melitaea* (whereas in Bátori et al. 2012a only *M. athalia*, *M. aurelia* and *M. britomartis* were included) and (2) three different genes provided support for the same topology within the ‘*athalia*’ group discussed herein. Hence, no serious consideration was given to the study of Bátori et al. (2012a).

No external characters were proven to be reliable for species delimitation even though the coloration of the marginal line of the hindwing underside shows the trend towards the correct identification. Although the majority of specimens correspond to the character states given in literature for a particular species, all three presumably species-specific categories of this character (Tolman & Lewington 2008) used in our analysis were present in each of the three species. Moreover, the percentage of misidentifications when this is the only character used would be very high (see Fig. 6). Other external characters suggested in literature (see Introduction) show even higher rates of variability, and no species-specific correspondence was found. Even when the twelve (out of 43, see Tables 1 and 2) most powerful characters are simultaneously used (see results of the DFA, Fig. 2) the misidentification rate reaches almost 50% in the cross-validation procedure (Fig. 5), rendering the appropriate identification of these species highly unreliable. To further support the high morphological variability within a single *Melitaea* species, as an example we should mention a comparison of populations of *M. athalia* from the Caucasian basin (Bátori et al. 2012b), where small, albeit significant differences in wing morphology were found among some of them (all contributing to a high intraspecies variability). Although partly reflecting the molecular differences between

the populations, these differences cannot be assigned to a genetic background alone. They rather may through selection also be a result of an adaptation to local conditions (Bátori et al. 2012b), such as food availability, climate and microhabitat selection. This phenomenon is not uncommon since high levels of phenotypic plasticity are important for a response that ensures the survival of a population exposed to unstable environmental factors (Shapiro 1976; Brakefield & French 1999). High species plasticity together with accommodation to local conditions can in practice result in description of many subspecies and morphological forms within *M. athalia* as well as within some other *Melitaea* species. Nonetheless, the differences among populations in reality might have resulted from locally specific selection pressure (e.g. see Tolman & Lewington 2008). On the other hand, the three species exploit similar (and partly overlapping) resources (e.g. see Verovnik et al. 2012; Koren and Jugovic 2012), which is probably the reason for the high similarity among them. It is important to note that the regional pattern of differentiation in *M. athalia* from the Caucasian basin was less expressed in genitalia than in wing characters (Bátori et al. 2012b).

The high variability of these species is further shown in their qualitative characters, for example in wing pattern and coloration. In *M. athalia* three out of more than 120 sampled specimens had a very peculiar coloration, with no visible marginal lines. Albeit unrecognized, we believe that differences in (some of) local conditions clearly show the importance of environmental factors for the wing morphology. This further demonstrates the importance of genitalia or phylogeny based identification in the herein investigated *Melitaea* species. Moreover, the latter two approaches should have a significant advantage also for the identification of (some) other species of the genus that should undergo thorough revision in the future (cf. Leneveu et al. 2009).

## Conclusions

The morphometric analysis of three closely related *Melitaea* species (*M. athalia*, *M. aurelia* and *M. britomartis*) revealed the following: (1) small albeit significant differences in wing morphology exist among the three species; (2) only characters describing the size of the species with no characters describing their wing shapes (ratios) were statistically important for the separation of the species; (3) whereas the structure of the male genitalia and the phylogenetic position of the three species are concordant, the sequence of phylogenetic splits is not reflected in the rate of external morphological differences among them; (4) our study represents another example where external morphology based identification would lead to highly unreliable determinations, hence a use of genitalia based identifications is strongly recommended.

## Acknowledgements

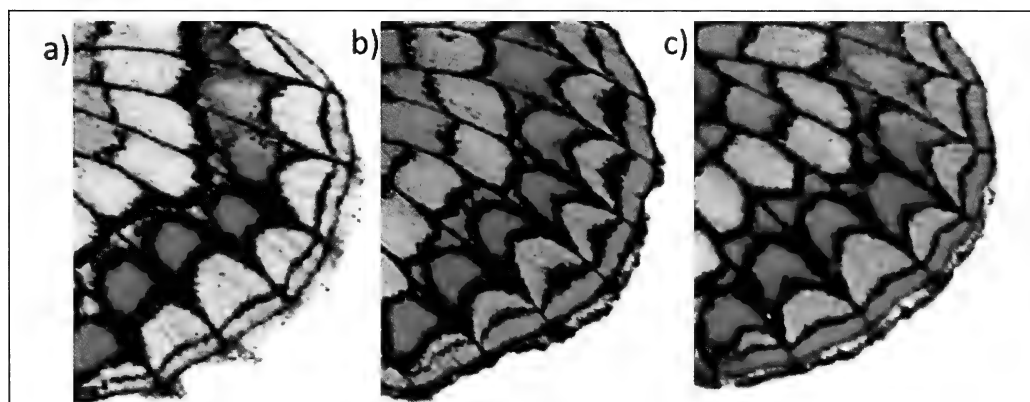
We are grateful to Ivan Jugovic (Kranj, Slovenia) for data from his collection, useful discussion and help in identification. We also thank Barbara Zakšek (Voličina, Slovenia) for providing us with the literature on genitalia preparation, and Elena Varljen Bužan (Koper, Slovenia) for valuable comments regarding the molecular background. We are thankful to Roman Luštrik (Ljubljana, Slovenia) for valuable help with statistical analysis. The referees and the editor contributed greatly to the improvement of the manuscript.

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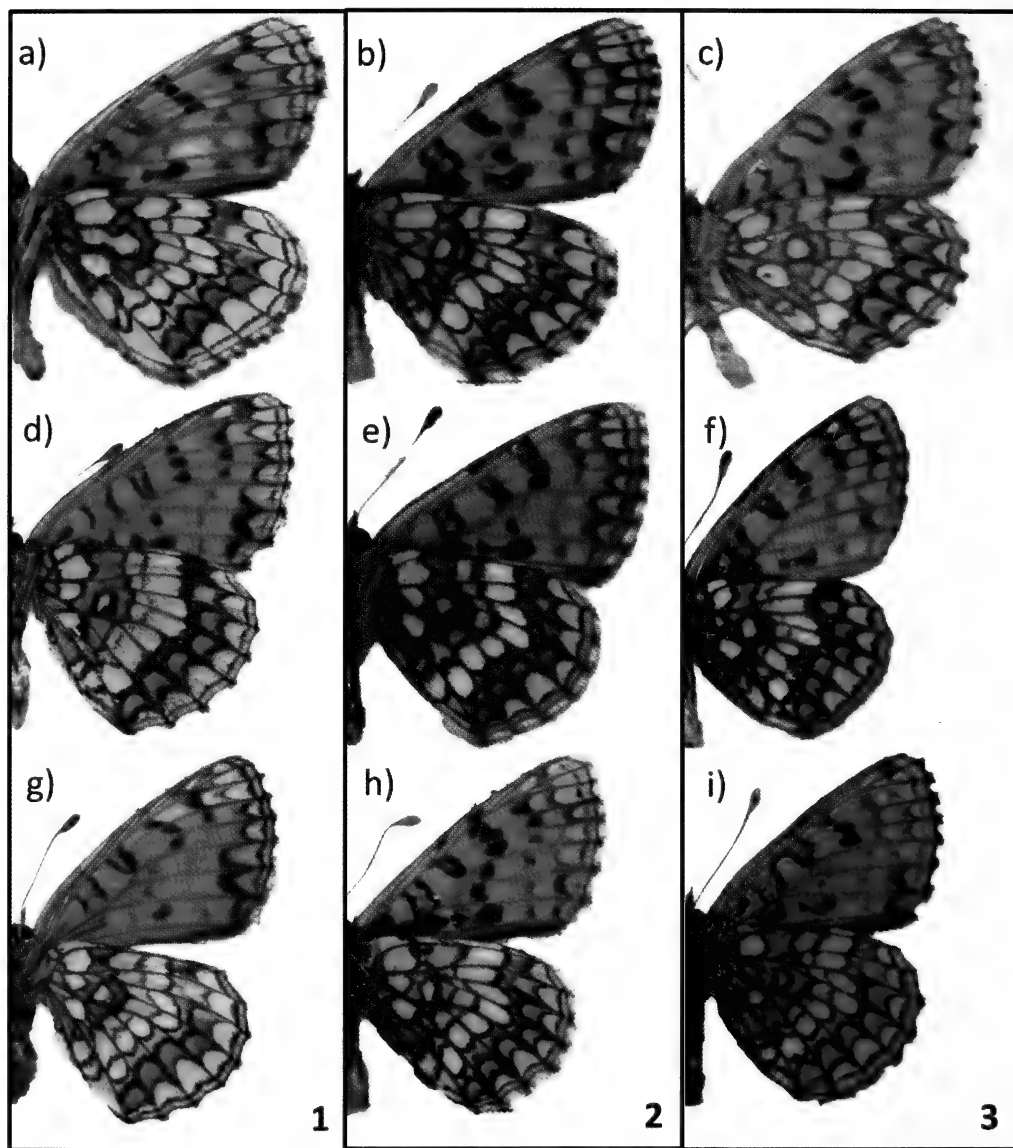
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## Appendices



**Appendix A.** Three categories of marginal line colour in contrast to lunular colour: 1=equal (a); 2=darker (b); 3=much darker (c) were used. In some cases when decision between categories 1 and 2 was hard (i.e. when the two authors disagree on whether the category should be assigned to category 1 or 2), value 1.5 was used.



**Appendix B.** Representatives of three categories of marginal line colour in contrast to lunular colour (categories 1, 2, 3) in *Melitaea athalia* (a–c), *M. aurelia* (d–f) and *M. britomartis* (g–i).

# Choreutidae of Madeira: review of the known species and description of the male of *Anthophila threnodes* (Walsingham, 1910) (Lepidoptera)

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<http://zoobank.org/9CD3F560-D46D-4E63-A309-E74D061799E7>

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**Abstract.** We review and illustrate the four species of Choreutidae recorded from Madeira – *Anthophila threnodes* (Walsingham), *A. fabriciana* (Linnaeus), *Choreutis nemorana* (Hübner), and *Tebenna micalis* (Mann) – and describe and illustrate for the first time the male of *A. threnodes*, as well as the biology of this Madeiran endemic. We provide brief notes on each of the species and give short diagnoses for correctly identifying them. Finally, we discuss previous misidentifications of Madeiran choreutids and the occurrence of choreutids on other oceanic islands.

## Introduction

The Lepidoptera fauna of the Madeira Islands consists of only 331 species (Aguiar & Karsholt 2008). This is mainly due to the isolated position of these islands in the Atlantic Ocean, and only to a lesser extent to insufficient collecting efforts. The Macrolepidoptera fauna, and especially the butterflies (Papilionoidea), are considered to be well known, with only a few and mostly invasive species being added in recent years. Among the Microlepidoptera, new discoveries still occur regularly, and a number of taxonomic problems still await resolution.

Madeiran lepidopterology began with Thomas Vernon Wollaston who, for health reasons, stayed in Madeira for long periods between 1847 and 1855 and also made later visits to the island. His results, including descriptions of many new species, were published by himself (Wollaston 1858) and Stainton (1859). Subsequent important contributions to the Lepidoptera fauna of the Madeira Islands were made by Walsingham (1894) and Rebel (1917, 1940). Easier access to the islands by airplane and the use of modern equipment (e.g., mercury vapour lamps) during the second half of the 20<sup>th</sup> century resulted in an increasing number of specimens available for study. Results of these efforts were summarized in the recent catalogue by Aguiar & Karsholt (2006), and data from that work were used for the checklists by Aguiar & Karsholt (2008) and Karsholt & Nieukerken (2011). A brief introduction to lepidopterology in Madeira was published by Karsholt (2000).

Below we treat the small family Choreutidae. Currently, 413 species of choreutids are described, and most of them are found in the tropics (Rota unpublished database). They are usually diurnal and often brightly coloured. So far four species have been found in Madeira. We describe and illustrate the unknown male and the biology of the endemic *Anthophila threnodes* (Walsingham), and provide images and brief notes on the other three species: *Anthophila fabriciana* (Linnaeus), *Choreutis nemorana* (Hübner), and *Tebenna micalis* (Mann). At the end we discuss earlier misidentifications of Madeiran choreutids.

## Methods

Genitalia dissections and terminology follow Rota (2008a). Plant names are from the Plant List website (2010). Photographs of adults were taken using Leica Application Suit MZ 16A and Zerene Helicon Stacker program for stacking subsequent images at ZMUC. Photographs of genitalia slides were taken using an Olympus SZX16 microscope with motorized focus drive attached to an Olympus E520 digital camera and they were then combined by using the programs Deep Focus 3.1 and Quick Photo Camera 2.3 at the Zoological Museum of the University of Turku. All images were improved in Adobe Photoshop CS3.

We conducted standard DNA extraction from abdomens of two specimens of *Anthophila threnodes* (voucher codes noted below) and attempted standard PCR amplification of the DNA barcode (*cytochrome oxidase subunit I*) using primers LCO and HCO (Folmer *et al.* 1994). When this failed, we used a primer internal to the DNA barcode (K699; Mitchell *et al.* 2005) in combination with LCO and successfully amplified 326 base pairs from one of the specimens (DNA voucher An\_th2; see details under material examined). As this fragment was very short, we attempted to obtain more sequence data and after some trials with the commonly amplified nuclear genes in Lepidoptera (Wahlberg and Wheat 2008), we were finally successful in obtaining two fragments of the nuclear gene GAPDH using newly designed primers by Niklas Wahlberg (GAP1F/GAP2R and GAP3F/GAP4R; primer sequences in Table 1), resulting in a total of 580 base pairs of GAPDH. We carried out a phylogenetic analysis of the concatenated sequences from *A. threnodes* together with the sequences of four other species of *Anthophila*, and another choreutid, *Prochoreutis inflatella*, as an outgroup (Appendix), using MrBayes v.3.2 (Ronquist *et al.* 2012) running on the CIPRES server (Miller *et al.* 2010). The data were analysed unpartitioned, with two concurrent runs each with one cold and three heated chains for one million generations, and 25% of trees were discarded as burnin. Convergence was assessed by ensuring that standard deviation of split frequencies was well below 0.05 (it was 0.001 at the end of the analysis), that PSRF values were all very close to 1.000 (they ranged 1.000–1.005), and by visual inspection of plots of log likelihood and all parameters in Tracer v.1.5 (Rambaut and Drummond 2007). Sequence length and GenBank accession number for each species are listed in the Appendix.

Data from the type material are cited literally whereas data from the other material are given in a standardized format.



**Table 1.** Primers used for PCR amplification of the nuclear gene GAPDH.

Primer pairs and sequence
<b>GAP1F</b> (AARGCTGGRGCTGAATATGT) / <b>GAP2R</b> (TAACTTTGCCACAGCYTT)
<b>GAP3F</b> (GTGCCCARCARAACATCAT) / <b>GAP4R</b> (CGGCTGGAGTARCCATAYTC)

### Abbreviations

AMFA	Collection of Antonio M. F. Aguiar, Funchal, Madeira.
BMNH	The Natural History Museum, London, U.K.
ICLAM	Insect Collection Laboratório de Qualidade Agrícola, Madeira, Portugal
SIP	Collection of Leo Sippola, Pirkkala, Finland
ZMUC	Zoological Museum, University of Copenhagen, Denmark

### *Anthophila threnodes* (Walsingham, 1910)

[http://species-id.net/wiki/Anthophila\\_threnodes](http://species-id.net/wiki/Anthophila_threnodes)

Figs 1, 2, 9–18

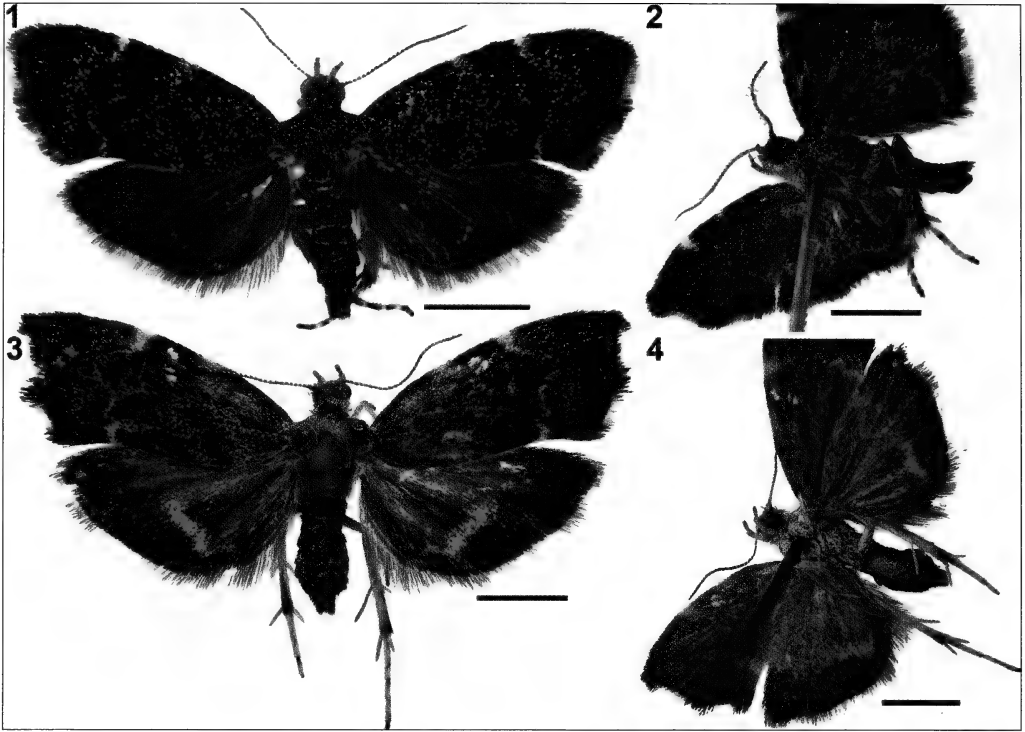
*Hemerophila threnodes* Walsingham, 1910: 257.

**Material examined.** Holotype. 1♀, ‘B.M. Genitalia Slide 20115 A. Diak’ | ‘Type’ | Madeira, V. 1886, Leech, 62296’ | ‘Walsingham Collection 1910-427’ | ‘*Hemerophila threnodes* Wals., ♀, Ent. Monthly Mag. 46, p. 257 (1910). Type ♀ (1/1) descr. 62296’ (BMNH). 1♂, 1♀, ‘Madeira: 1858. Wollaston. BM 1858-21.’ | ‘*Simaethis fabriciana* L. teste Stn. Ann-Mag. N. H. (3. s) III. 210, no 4 (1859), Topotype Madeira.’ | ‘V’ [May] (BMNH); 1♂, Funchal, 20–31.iii.1995, leg. L. Sippola, genitalia slide Rota JR2013-05, DNA voucher for extraction An\_th2 (ZMUC); 1♂, Cabo Girão, Cruz da Caldeira, 8.vii.1999, leg. A. M. F. Aguiar, genitalia slide Rota JR2013-04, DNA voucher for extraction An\_th1 (AMFA); 1♀, Vereda da Entrosa, Arco de São Jorge, 225 m, 12.ii.2000, leg. A. M. F. Aguiar (AMFA); 3♂, 2♀, same data but la. 14.ii.2001, *Urtica* sp., leg. A. M. F. Aguiar & J. Jesus (AMFA, ICLAM); 2♂, 3♀, same data but 200 m, la. 29.xi.2001, *Urtica* sp., leg. A. M. F. Aguiar & O. Karsholt, genitalia slides Hendriksen 5240, Karsholt 5236 (AMFA, ZMUC); 2♀, same data but, 15.v.2003, leg. A. M. F. Aguiar & J. Jesus (AMFA); 1♀, AM 5089, Levada Grande, Boaventura, 285m, 4.iii.2000, larva on *Urtica membranacea*, leg. A. M. F. Aguiar (AMFA); 1♀, Monte, 20.iii.2002, leg. L. Sippola (SIP).

**Remarks.** *Hemerophila threnodes* was originally described from one female collected in Madeira by J. H. Leech in May 1886. No exact locality was stated. Based on the material collected more recently, we provide a description of the male. We also illustrate female genitalia.

**Description.** *A. threnodes* is characterized by its dark, almost black wings and a scattering of light bluish scales on the thoracic dorsum and forewing upperside (Figs 1, 2). The forewings have a cream-white spot at two-thirds of costa and another such spot at four-fifths of the dorsum; fringes are black and cream-white beyond black fringe-line. Hindwings are uniform dark brown.

Male genitalia (Fig. 9). Tegumen triangular with small uncus extending from its apex. Papillae anales present as elongate, somewhat elliptical patches with long hairs. Gnathos well developed as a long, somewhat curved, pointed hook. Vinculum ventrally rounded with a small, triangular saccus (obscured in Fig. 9a, but visible in slide JR2013-04, which is not illustrated). Valva broad, somewhat oval, with a pointed costal process and an unsclerotized triangular, rounded extension distally; distally and ventrally covered with hairs. Juxta as a hood-like plate (in Fig. 9b attached to phallus). Phallus shorter than valva, slightly sigmoidal, with a sharp spine at one-third from apex (Fig. 9c).



**Figures 1–4.** Adults in dorsal and lateral view. 1–2. *Anthophila threnodes*; 3–4. *Anthophila fabriciana* (scale bar = 2 mm).

Female genitalia (Fig. 10). Apophysis posterioris slender, slightly broader at base; ca. 1.5 times as long as anterioris and much less thick. Apophysis anterioris greatly enlarged in the middle, tapering basally and even more so distally. Ostium on segment 7. Ductus bursae very gradually widening into corpus, with a slight twist of about one to two revolutions. Corpus bursae oval, small, with a signum as small patch of dentations (Fig. 10b).

**Host plant.** *Urtica membranacea* Poir. ex Savigny and probably other *Urtica* spp. (Urticaceae).

**Remarks.** Larvae have been found in March, May, and November, and adults have been collected in February, March, May, and July, indicating at least two broods. The adult flies during the day. It occurs in open landscapes at low altitudes.

**Immature stages and biology** (Figs 11–18). Larva is off-white with dark brown spots (Figs 14, 15) and it spins a thin web on or around the young leaves on which it feeds (Figs 12, 13). The pupa is reddish brown (Fig. 16), and in addition to the single rows of dorsal spines on abdominal segments A2–7 (Figs 17, 18) it also has dorsal lacunae – small round holes in a row immediately posterior to the spines.

**Diagnosis.** *A. threnodes* is characterized by its blackish wings, and should be relatively easy to differentiate from the other Lepidoptera in Madeira. It resembles *A. fabriciana* but the wings of that species are much lighter greyish brown. Male genitalia are very similar to those of *A. fabriciana*, but they differ in having a much shorter spine on the phallus. Female genitalia are similar to those of other species of *Anthophila*, but they differ in only having a hint of spiraliza-

tion in the basal third of the ductus bursae, unlike those of e.g. *A. fabriciana* (L.), *A. abhasica* Danilevsky, and *A. armata* Danilevsky, where the spiralization is apparent. We cannot find differences between the larvae of *A. threnodes* and *A. fabriciana* in their general appearance (the differentiation between the two would likely be possible based on the ultrastructure as seen with the scanning electron microscope, but this has not been done).

### *Anthophila fabriciana* (Linnaeus, 1767)

Figs 3, 4

*Phalaena (Tortrix) fabriciana* Linnaeus, 1767: 880

**Material examined.** 1♀, Vereda da Entrosa, Arco de São Jorge, 225 m, 14.ii.2001, leg. A. M. F. Aguiar (ICLAM).

**Host plant.** *Urtica* sp. (Urticaceae).

**Remarks.** This is a new record for Madeira. The single specimen was collected at the same place as several *A. threnodes*. We examined photographs of this specimen deposited in the BMNH and are of the opinion that it is correctly identified.

**Diagnosis.** *A. fabriciana* resembles *A. threnodes*, but differs by its greyish brown wings. It has the cream-white spots apically at costa and dorsum connected by a light zigzag-line, and has a white streak near the margin in the hindwings (Figs 3, 4).

### *Choreutis nemorana* (Hübner, 1899)

[http://species-id.net/wiki/Choreutis\\_nemorana](http://species-id.net/wiki/Choreutis_nemorana)

Figs 5, 6, 19, 20

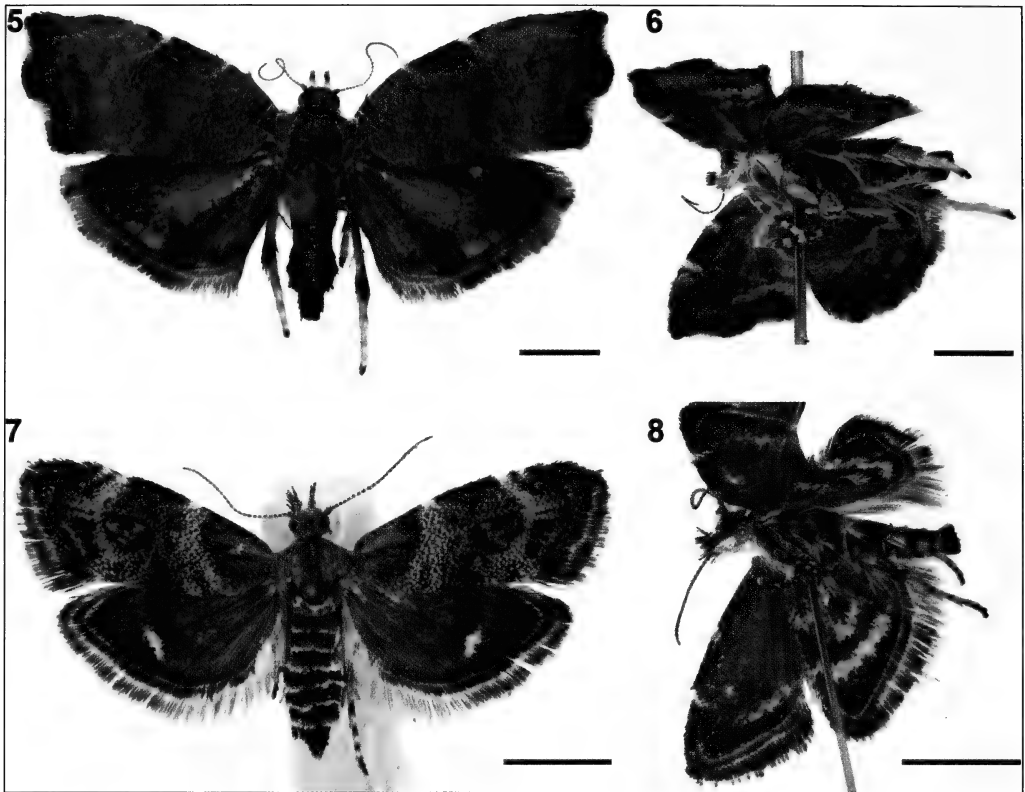
*Tortrix nemorana* Hübner [1799]: pl. 1, fig. 3.

**Material examined.** Serra d'Água, Pousada dos Vinhaticos, 660 m, 10♂, 13♀, 12–13.ix.1975, leg. O. Lomholdt & N. L. Wolff; São Vicente, sea level, 1♀, 16.vi.1993; 5♂, 3♀, same data, but larva on *Ficus carica*, leg. O. Karsholt (ZMUC); São Vicente, Ribeira do Inferno, 79m, 3♂, 12.ix.1996, leg. F. Aguiar & J. Jesus (AMFA, ICLAM); Ponta do Pargo, Porto do Pesqueiro, 311 m, 1♂, 1♀, 23.iv.1994, leg. A. M. F. Aguiar (AMFA); Fajã da Nogueira, 600–1000 m, 1♀, 8.x.1994, leg. O. Karsholt (ZMUC); Curral das Freiras, 850 m, 1♂, 20.ix.1997, leg. O. Karsholt (ZMUC); same data, but 597 m, 3♂, 2♀, la. 16.iv.1998, *Ficus carica*, leg. F. Aguiar & J. Jesus; 3♂, 1♀, same data, but 7.v.1998 (ICLAM); Estreito de Câmara de Lobos, Levada do Norte, Garachico, 1♂, 1♀, 538 m, 26.vii.2001, J. Jesus (AMFA, ICLAM).

**Host plant.** *Ficus carica* L. (Moraceae).

**Remarks.** This species is common wherever there are *Ficus carica* trees. Larvae, hiding under a thin web, skeletonise mainly young leaves (Figs 19, 20). They have been found in June, and adults have been collected in April–July and September–November, at altitudes from sea level to 1000 m. *C. nemorana* is only a minor pest on figs in Madeira. The adult flies during day. Male and female genitalia were illustrated by Diakonoff (1986): pl. 66, fig. 81 (male), and pl. 142, fig. 81 (female).

**Diagnosis.** The almost square, brown forewings, and the black and yellow hindwings make *C. nemorana* unmistakable among Madeiran Lepidoptera (Figs 5, 6).



Figures 5–8. Adults in dorsal and lateral view. 5–6. *Choreutis nemorana*; 7–8. *Tebenna micalis* (scale bar = 2 mm).

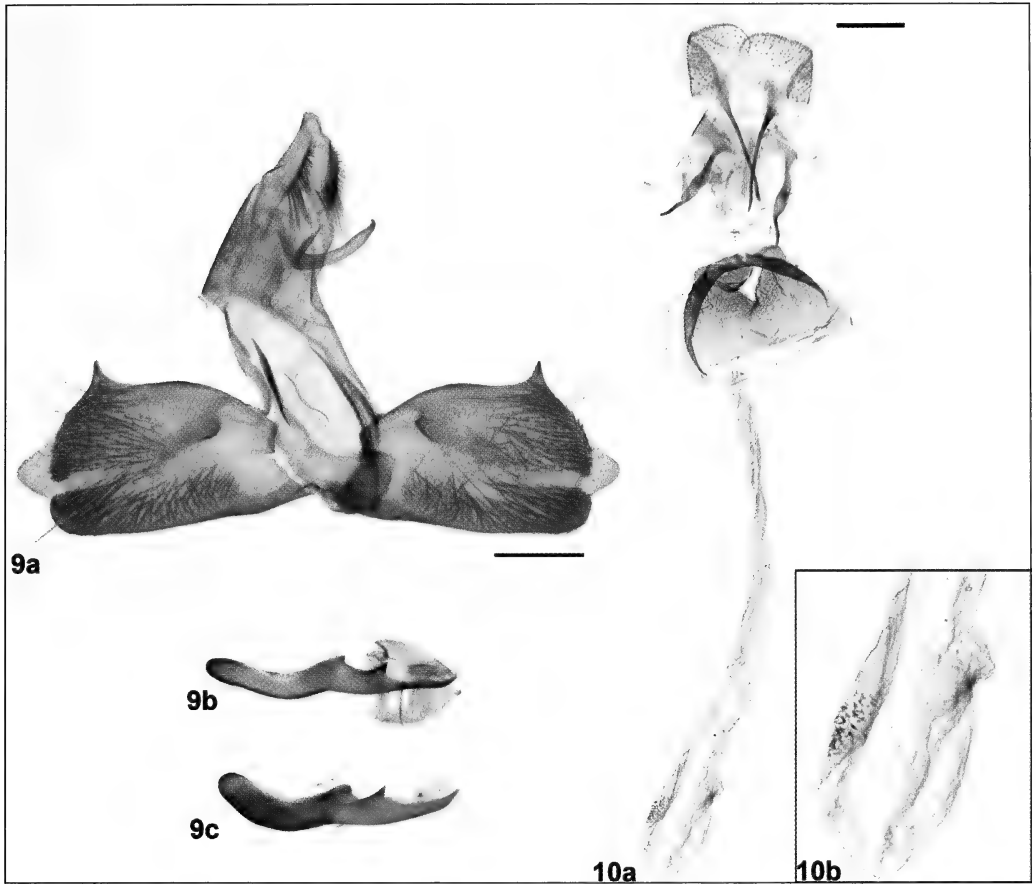
### *Tebenna micalis* (Mann, 1857)

[http://species-id.net/wiki/Tebenna\\_micalis](http://species-id.net/wiki/Tebenna_micalis)

Figs 7, 8

*Choreutis micalis* Mann, 1857: 181.

**Material examined.** Funchal, Lido, 1♂, 20.viii.1974, leg. E. Traugott-Olsen, genitalia slide Wolff 4298; same locality, but 50 m, 1♀, 18.x.1997, leg. O. Karsholt (ZMUC); Fajã da Nogueira, 1000 m, 4♂, 2♀, 23.viii.1974, leg. E. Traugott-Olsen, genitalia slide Wolff 4294, Rasmussen 4849; same locality, but 600–1000 m, 2♂, 8.x.1994, leg. O. Karsholt, genitalia slide Hendriksen 5765; same locality, but 700 m, 3♂, 2♀, 18.ix.1997, leg. O. Karsholt; same locality, but 1♀, 500 m, 25.xi.2001, leg. O. Karsholt (ZMUC); same locality, but 1♀, 864 m, la. 14.vii.1998, *Helichrysum foetidum*, leg. F. Aguiar & J. Jesus (ICLAM); Caniço, 2♂, 12–18.ix.1977, leg. O. Lomholdt & N. L. Wolff (ZMUC); Ponta de São Lourenço, sea level, 9♂, 4♀, 24–27.vi.1993, 1♀, 11.vii.1993, 1♂, 17.ix.1997, leg. O. Karsholt (ZMUC); Machico, sea level, 1♂, 27.vi.1993, leg. O. Karsholt (ZMUC); Achadas da Cruz, 725 m, 3♂, 2♀, 8.vii.1993, leg. O. Karsholt (ZMUC); Porto Moniz, sea level, 1♀, 9.x.1994, leg. O. Karsholt (ZMUC); Santo da Serra, 700 m, 8♂, 2♀, 26.x.1994, larva on *Helichrysum foetidum*, leg. O. Karsholt, genitalia slide Hendriksen 5766 (ZMUC); 1♂, Estreito de Câmara de Lobos, Jardim da Serra, 1130 m, 22.x.1998, leg. F. Aguiar & J. Jesus (ICLAM); 1♀, Porto da Cruz, Chão das Feiteiras, 1251 m, 12.xi.1998, leg. A. M. F. Aguiar (AMFA); 1♂, 5♀, Santana, Pico, Posto Agrario, 411 m, la. 12.xi.1998, *Arctium minus*; same data, but 1♀ 1.vi.1999, leg. F. Aguiar & J. Jesus (ICLAM); 1♂, Boaventura, Vereda da Entrosa, 130 m, 12.ii.2000; 5♂, 3♀, same data, but 175 m, la. 14.ii.2001,

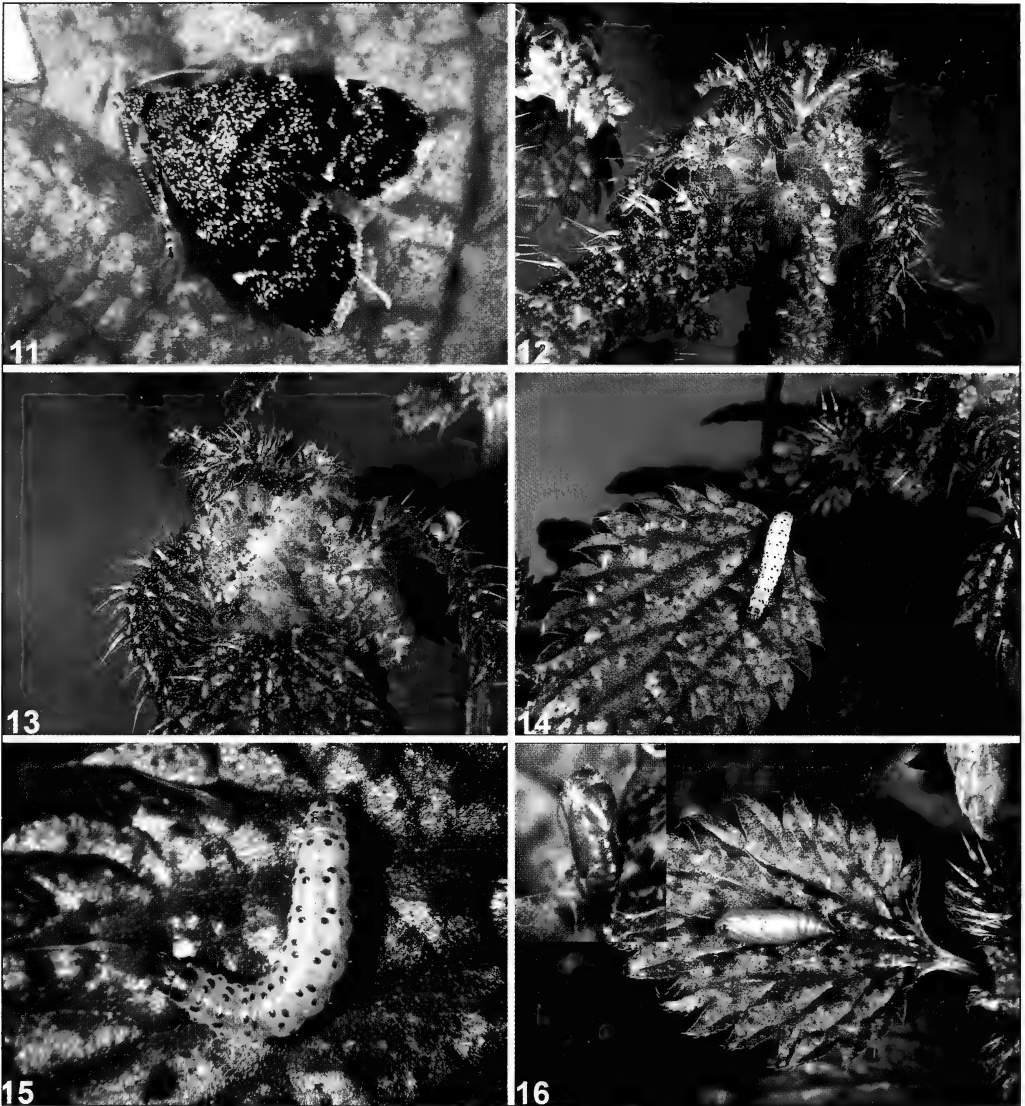


**Figures 9–10.** *Anthophila threnodes*: male genitalia (9a), phallus (9b, c) (9a and 9b from slide Karsholt 5236, ZMUC; 9c from slide JR2013-04, ZMUC), female genitalia (10a), inset showing magnified corpus bursae (10b) (scale bar = 0.2 mm).

*Helichrysum melaleucum*, leg. F. Aguiar & J. Jesus (AMFA, ICLAM); 1♂, 1♂, Estreito de Câmara de Lobos, Levada do Norte, Garachico, 538 m, 26.vii.2001 leg. J. Jesus (ICLAM); 1♀, Machico, Funduras, 605 m, 12.vi.2003, leg. J. Jesus (ICLAM); 1♀, Santana, Achada do Gramacho/ Cais de São Jorge, 267 m, 21.vii.2011, leg. J. Jesus (ICLAM); 1♂, Camacha, Levada dos Tornos, direction Camacha – Monte, 788 m, *Cirsium vulgare*, la. 13.vi.2013, leg. S. Fontinha (ICLAM).

**Host plants.** *Arctium minus* (Hill.) Bernh., *Cirsium vulgare* (Savi) Ten., *Helichrysum foetidum* (L.) Cass. and *Helichrysum melaleucum* Rchb. (Compositae).

**Remarks.** Larvae have been found in October, November, February, June, and July, and adults have been collected in all months from June to November, at altitudes from sea level to 1250 m. The adult flies during the day and comes to light. This is a widespread species. In addition to being present on Madeira, it occurs on the Canaries, throughout the Mediterranean, from central and eastern Europe throughout southern Asia to China and Japan, as well as possibly on Marianne Islands, Java, and New Zealand (Diakonoff 1986). It is the only choreutid occurring in the Azores Islands, where it inhabits all larger islands (Karsholt & Vieira 2005).

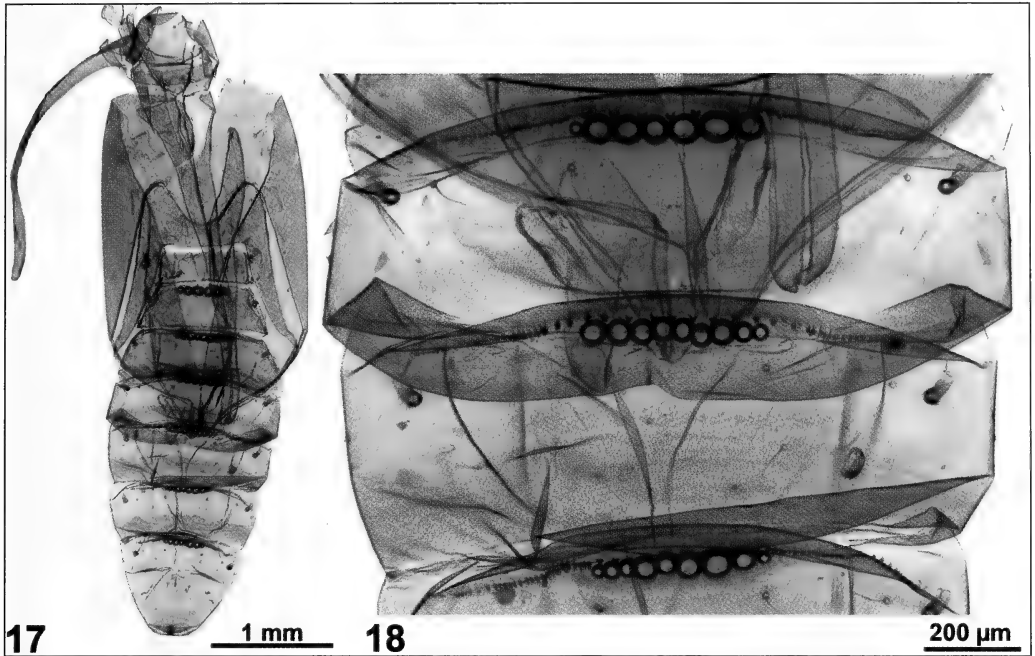


**Figures 11–16.** *Anthophila threnodes*: 11. Adult on its host plant; 12–13. Larval webbing tying young leaves; 14–15. Larva; 16. Pupa and an empty pupal shell in the inset.

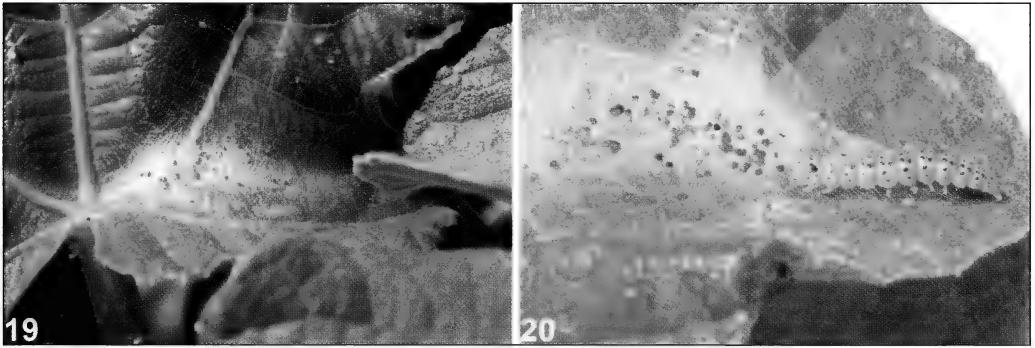
Male and female genitalia were illustrated by Diakonoff (1986): pls. 46, 47, figs 54-1 and 54-2 (male), pl. 127, fig. 54 (female).

**Diagnosis.** With its sub-triangular forewings with black and metallic markings adult *T. micalis* is unique among Madeiran Lepidoptera (Figs 7, 8). It can be separated from the similar *T. bjerkandrella* (Thunberg, 1784) by the presence of a subapical black and metallic spot in the forewing in *T. micalis*; such a spot is missing in *T. bjerkandrella* (Diakonoff 1986).

**Molecular results.** The successfully amplified *A. threnodes* COI haplotype is unique and it differs from the *A. alpinella* haplotype in 14 bases, and from the *A. fabriciana* haplotype in 12 bases. In the resulting phylogenetic tree, *Anthophila threnodes* and *A. fabriciana* are sister



Figures 17–18. *Anthophila threnodes* pupa (17) with the close-up of dorsal spines and lacunae (18).

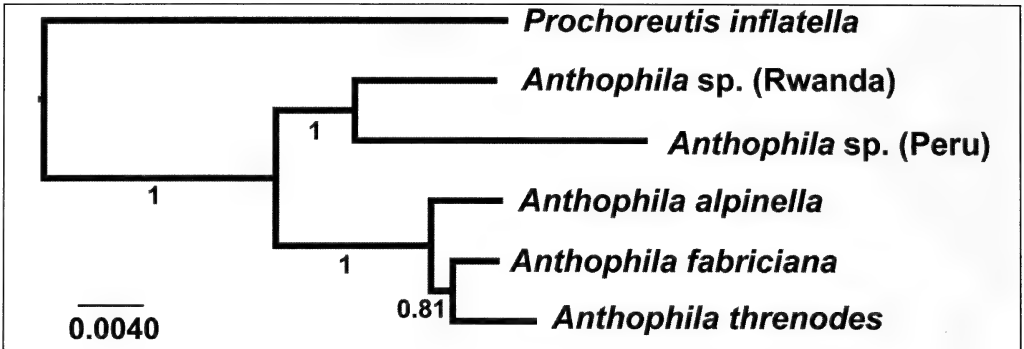


Figures 19–20. *Choreutis nemorana*: larva under its webbing on the host plant *Ficus carica* (19) and larva exiting its web-shelter after being disturbed (20).

species, but without statistical support (PP or posterior probability = 0.81). Together, they are strongly supported as being the sister group to the North American species *Anthophila alpinella* (PP=1).

## Discussion

One of the aims of this paper was to provide the necessary information for correctly identifying Madeiran choreutids because misidentifications have been common in the past. For example, *A. threnodes* was misidentified as *A. fabriciana* by Stainton (1859) (as ‘*Simaëthis Fabriciana* Linnaeus’) and subsequent authors, and it remained as such on the list of Madeiran Lepidoptera



**Figure 21.** Bayesian tree showing the relationships among species of *Anthophila*. The numbers below branches are posterior probabilities.

until 2006 (Aguiar & Karsholt 2006). The specimen of *A. fabriciana* listed above is the only specimen of that species known from Madeira, while all the other ones previously identified as *A. fabriciana* are actually *A. threnodes*. Although *A. fabriciana* is a common species in mainland Europe, in addition to being recorded only once from Madeira, it has also been found only once in the Canary Islands, “based on a single specimen (“61978”), taken in April 1884 [in Tenerife], by the late Mr. J. H. Leech” (Walsingham 1908). The Lepidoptera fauna of the Canary Islands (and especially Tenerife) is relatively well studied, and it is surprising that *A. fabriciana* had not been found again in the islands, suggesting that the species failed to establish itself there, which is in agreement with Rebel’s opinion (1911) that the single specimen might have resulted from an accidental importation.

Furthermore, *Tebenna bjer kandrella* (Thunberg), a species similar to *T. micalis*, has been noted as found in Madeira several times (e.g., by Walsingham 1894, 1908; Rebel 1911). However, all Madeiran specimens examined by us belong to the latter species, and the presence of *bjer kandrella* in Madeira requires confirmation. It is likely that records of *bjer kandrella* from other Macaronesian archipelagos also refer to *T. micalis* (Aguiar & Karsholt 2006).

The biology of the immature stages of *A. threnodes* is typical for the whole family. Spinning a thin web on or around the young leaves on which the larva feeds is known from a number of other genera (Rota 2005, Rota 2008b). Likewise, the morphology of the pupa is also shared with several other genera. For example, the dorsal lacunae (Figs 17, 18) that are found in the pupa of *A. threnodes* are also known to occur in the other species of *Anthophila* (Patočka 1999), as well as in *Asterivora* Dugdale (Dugdale 1979), *Caloreas* Heppner (Keifer 1937), *Hemero-phila* Hübner (Rota unpublished), *Rhobonda* Walker (Rota 2005), *Prochoreutis* Heppner, and *Tebenna* Billberg (Patočka 1999). Their function remains unknown.

Finally, we discuss other choreutids that are known to occur on oceanic islands. For example, *Brenthia leptocosma* Meyrick is currently known only from Mauritius (Williams 1951). Then there is a species of *Choreutis* that appears to be a pest on *Ficus* sp. (Moraceae) on the Hawaiian islands (W. Nagamine, pers. comm.), and another species, *Niveas kone* Rota, is known from the Solomon Islands and Papua New Guinea (Rota and Miller 2013). All of this suggests that choreutids disperse fairly well, but most likely through passive wind dispersal because they are small moths without strong flight capabilities.



As *A. threnodes* is endemic to Madeira, it would be interesting to firmly establish its sister species so as to try to ascertain the origin of this species. We attempted to do so using molecular data, but were highly limited by the small amount of sequence that we obtained from our material and therefore our finding that *A. fabriciana* is the sister species of *A. threnodes* can only be taken as a preliminary result although this also appears to be supported by the close similarity of the two species in their external appearance, as well as their genitalia. Ideally, molecular work employing phylogeographic methods, conducted on freshly collected material of a large number of specimens of *A. alpinella*, *A. fabriciana*, and *A. threnodes*, as well as other potential close relatives of *A. threnodes*, would provide an answer with more certainty than we can do at this point.

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We are grateful to Kevin Tuck, The Natural History Museum, London, U.K. for access to the collections and for information and photographs of specimens; to Jurate De Prins, Royal Museum for Central Africa, Belgium; and Leo Sippola, Pirkkala, Finland for loan of specimens. We greatly appreciate the input on an earlier version of the manuscript provided by Martin Corley and Marko Mutanen. JR was funded by the Finnish Kone Foundation while doing this research. OK's work on the Lepidoptera of Madeira project was supported by a grant from the Carlsberg Foundation (Denmark).

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## Appendix

Specimen information, sequence length, and GenBank accession numbers.

Species	voucher code	museum (ID No.)	CAD	COI-begin	COI-end	EF1a-begin	EF1a-end	GAPDH	IDH	MDH	RpS5	wingless
<i>Prochoreutis inflatella</i>	Pr_in_CT_1	-	850 bp	593 bp	756 bp	506 bp	675 bp	691 bp	-	321 bp	590 bp	400 bp
<i>Anthophila threnodes</i>	An_th2	ZMUC	-	326 bp	HQ533107	HQ541504	HQ541504	580 bp	-	-	-	-
<i>Anthophila fabriciana</i>	An_f_BE_2	-	412 bp	584 bp	774 bp	517 bp	675 bp	661 bp	402 bp	405 bp	596 bp	365 bp
<i>Anthophila alpinella</i>	An_a_CA_2	-	412 bp	588 bp	774 bp	495 bp	645 bp	690 bp	697 bp	406 bp	596 bp	400 bp
<i>Anthophila</i> sp. (Peru)	An_sp651	ZMUC	-	326 bp	HQ533053	HQ541450	HQ541450	298 bp	-	-	-	-
<i>Anthophila</i> sp. (Rwanda)	An_JDPI_5570	RNCA (ENT 5570)	414 bp	620 bp	453 bp	177 bp	313 bp	691 bp	315 bp	-	550 bp	342 bp
			KJ844057	KJ844051	KJ844051	KJ844058	KJ844058	KJ844054	KJ844056	-	KJ844060	KJ844061

ZMUC: Zoological Museum, University of Copenhagen

RNCA: Royal Museum for Central Africa, Tervuren, Belgium



## Book review: **I Lepidotteri del Parco Naturale delle Capanne di Marcarolo**

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<http://zoobank.org/59A5A3BD-242F-4749-85C5-9376144BDB70>

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**Giorgio Baldizzone, Carlo Cabella, Faustino Fiori & Pier Giuseppe Varalda, 2013. I Lepidotteri del Parco Naturale delle Capanne di Marcarolo. *Memorie dell'Associazione Naturalistica Piemontese*, vol. 12: 1-316, pls 1-32. ISBN: 978-88-902859-5-0**

Can be obtained from the first author ([giorgiobaldizzone@tin.it](mailto:giorgiobaldizzone@tin.it)). Price: see below.

The nature park Capanne di Marcarolo is situated in north-western Italy north-west of Genova on the border between Piedmont and Liguria, viz. in the transition zone between the Alps and the Apennines. It is a so-called 'regional park' and has an area of about 82 km<sup>2</sup>. It ranges from 335 m to 1772 m (Monte delle Figne) in altitude and includes a number of vegetation types. Much of the original forest has been cut in former times and partially replaced by pines. The park has different types of wetlands including micro-peatbogs. One can get a good impression about the park from a video on YouTube (<http://www.youtube.com/watch?v=uPKTKO131HU>).

This publication presents the result of the study of the Lepidoptera fauna of Capanne di Marcarolo during the years 2002 to 2012, and it is the first book that treats all species of Lepidoptera (micro and macro) of a protected area in Italy. The research has served to draw up an action plan for the management and conservation of the Lepidoptera of the protected area and has resulted in the approval of the plan by the regional government of Piedmont. It is written in Italian, but as the main part of the book is a systematic review of the collected specimens the language should for most foreign readers of the book not be a serious problem (for writing this review I had good help from Google translate).

The book starts with a summary (also in English), and continues with a preface, an introduction to the study area (3 pp.), climate (5 pp.) and flora and vegetation (3 pp.) (these three chapters are written by specialists other than the authors). Then follows a general chapter on the entomological fauna of Capanne di Marcarolo (6 pp.) and a chapter with acknowledgements.

The main part of the book (pp. 34-284) presents the results of the study. Each of the 1464 (sic!) recorded species is presented with full name (with author and year of description). The studied material with exact dates and localities is listed. For each species both the general distribution and the distribution in Italy are summarized, with references to literature, as well as host

plants and other biological details. For a few species there are more detailed remarks (like the description of the hitherto unknown female genitalia of *Homoeosoma incognitellum* (Roesler)).

Even though a lot of progress has been made in the study of the Lepidoptera fauna of Mediterranean countries during the last decades our knowledge of some families of Microlepidoptera is still imperfect, and hardly any person is able to recognize all species of Lepidoptera occurring in an area like Capanne di Marcarolo. The authors have solved this problem by allying themselves with a number of taxonomic specialists of different families of Microlepidoptera. Thereby (probably) all collected specimens have been identified probably as well as possible at the present time.

The systematic part is followed by chapters with a discussion and conclusion (4 pp.), an extensive reference list (8 pp.) and indexes to Lepidoptera and plant names. The book is completed by a map of the area and 32 colour plates with photographs of aspects of climate, geological features, plant species, characteristic biotopes and, especially, selected species of Lepidoptera occurring in the studied area.

The book is published as a volume of *Memorie dell'Associazione Naturalistica Piemontese* and founded by *Fondazione Cassa di Risparmio di Torino*. It is not literally for sale, but can be obtained from its first author by giving a donation of at least 25€ plus shipping costs. The donation will be used for research and publications dedicated to the knowledge of the Lepidoptera fauna of Italia habitats included in the Natura 2000 network. This is an interesting kind of crowdsourcing, and both the idea and the book are herewith warmly recommended.

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# SOCIETAS EUROPAEA LEPIDOPTEROLOGICA e.V.

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