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Butterflies in bags: permanent storage of Lepidoptera in glassine envelopes

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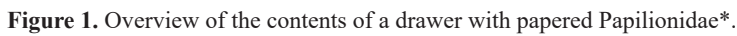
Abstract. In terms of amateurs and professionals studying and collecting insects, Lepidoptera represent one of the most popular groups. It is this popularity, in combination with wings being routinely spread during mounting, which results in Lepidoptera often taking up the largest number of drawers and space in entomological collections. As resources grow increasingly scarce in natural history museums, any process that results in more efficient use of resources is a welcome addition to collection management practices. Therefore, we propose an alternative method to process papered Lepidoptera: a workflow to digitize (imaging and data registration) papered specimens and to store them (semi)permanently, still unmounted, in glassine envelopes. The mounting of specimens will be limited to those for which it is considered essential. The entire workflow of digitization and repacking can be carried out by non-expert volunteers. By releasing data and images on the internet, taxonomic experts worldwide can assist with identifications. This method was tested for Papilionidae. Results suggest that the workflow and permanent storage in glassine envelopes described here can be applied to most groups of Lepidoptera.

Introduction

Butterflies and moths are amongst the organisms that have always been very popular with collectors and scientists. They also are among the most difficult invertebrates to prepare: it takes skill, time and proper tools to expertly set specimens. Hence, most collectors who are away from home do not prepare specimens straight away but store them temporarily, either pinned but not spread, or stored flat in boxes or opaque paper envelopes. Papering traditionally consists of placing specimens with the wings folded upwards in triangles of paper, or in small rectangular envelopes of glassine paper (Gibb 2015).

Over time, most natural history museums have amassed large collections of papered specimens, originating from private collectors and expeditions of these museums, and most of these have never been properly prepared. Due to lack of resources, many of these collections have been left undisturbed, often for decades, and curators are struggling how to make this material accessible for research.

In 2015 the number of papered Lepidoptera in the collection of Naturalis Biodiversity Center, Leiden, the Netherlands was estimated to consist of roughly 500,000 specimens, some being as old as 80 years. A substantial part of the backlog (>200,000 specimens) consisted of Lepidoptera collected by J.M.A. van Groenendael, a Dutch physician working in the former Dutch East Indies between 1931 and 1954 (de Boer 1998). Most butterflies and moths in the papered collection are placed in opaque envelopes (Fig. 1), which makes it impossible to check the contents without opening the

Figure 1. Overview of the contents of a drawer with papered Papilionidae*.

envelopes and risking damage. The papered Lepidoptera specimens have been partly sorted to some extent in the past. Still, the geographic and taxonomic information available on storage unit level (e.g. box or drawer), if present, is often no more specific than country and family. The individual envelopes, on the contrary, often contain more specific information on the collecting event (Fig. 2).

Though specimens in papered envelopes potentially contain a wealth of information for research, their current storage method seriously hinders study. In practice this means that such collections have been neglected for decades, with only an occasional search for interesting specimens. This involves a high risk for damage and sometimes results in damaged legs, antennae or even wings. Processing this number of Lepidoptera following current practice, namely pinning and spreading the specimens, would take up roughly 7500 drawers, not to mention the personnel it takes. As space, time and money are resources that have been growing increasingly scarce for natural history museums in the past decade (Dalton 2003; Pyke and Ehrlich 2010; Bradley et al. 2014; McLean et al. 2016), an alternative for making papered Lepidoptera collections accessible would be a welcome addition to current collection management practices.

For collections of Odonata (dragonflies and damselflies), rectangular 3×5 inch index cards and transparent envelopes of film were proposed some decades ago as a permanent storage solution for unmounted specimens (Beatty and Beatty1963). This method was to replace storing specimens in envelopes of cellophane and triangular folds of newspapers. This system has become common practice for the Odonata collections at Naturalis Biodiversity Center, using glassine envelopes (van Tol 2001). Glassine envelopes are semi-transparent, acid-free, do not build up static charge, are

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* Photographs were taken by the first author unless mentioned otherwise.

Figure 2. An old drawer filled with papered Lepidoptera. The drawer contains rough information (on stickers) about locality and collector, while the individual envelopes have more specific information on locality and date written on them. Photo by Luisa de Bruin.

inexpensive and easy to use. They have been widely applied for temporary storage of entomological specimens for about forty years now (Gray 1971; Gibb 2015; Winter 2000). At Naturalis they are also used to store leaves with insect damage, such as leafmines. The proven benefit of glassine envelopes leads to the assumption they could be appropriate for Lepidoptera as well, and that the use of cards in combination with glassine envelopes could be an incredibly convenient and space efficient alternative to conventional spreading and mounting.

In order to properly process the collection of 500,000 papered butterflies and moths at Naturalis, repacking should be combined with a digitization workflow. Additionally, to potentially reduce costs, non-expert volunteers should be able to carry out this workflow. A pilot project was carried out in 2016 to put this to the test, funded by the Van Groenendael-Krijger Foundation. During this project a workflow was developed to repack and digitize large collections of unmounted Lepidoptera. This is an approach that differs from the traditional practice of mounting Lepidoptera in a way that is less time, space and money consuming, while ensuring optimal accessibility to the collection, both physical and digital. In this paper a workflow for digitizing and permanently storing unmounted Lepidoptera is presented, that resulted from the 2016 pilot. The digitization and repacking of all papered Lepidoptera is currently (June 2018) still going on. It is estimated that another ten years are required to completely deal with the backlog of papered specimens. We will continuously seek to refine the workflow and storage method, and are happy to confer with prospective users.

Why mount butterflies and moths?

Throughout the 18th and 19th centuries, collectors devised different methods to preserve delicate butterflies and moths. The apothecary James Petiver (1663–1718), for example, preserved his butterflies dried, pressed and glued in books "after the same manner you do the Plants" (Petiver [1709?]),

but he also advised to push a pin through the thorax and pin them to one's hat, until the specimen could be placed into a box (Salmon 2001). The physician and botanist Sir Hans Sloane (1660–1773) also preserved butterflies pressed on paper sheets, sometimes in combination with plants, and sometimes he placed specimens between thin layers of mica, sealed with a 'passe-partout' (MacGregor 1994). Another example of this kind of storage can be found in the beautiful collection of butterflies of the Dutch bookseller Jacob Ehrlich (1787–1863). The specimens are preserved between glass plates and placed in cases of Mahogany wood made to resemble books (Zwakhals et al. 2015). Although Petiver already mentioned the use of pins in the early 1700s, this method seems to have become more widespread only after the mid-18th century. In 1753, David Hultman, in a work supervised by Carl Linnaeus, advised to spread legs, antennae and wings of insects and to preserve them dried and pinned, taking extra care not to damage the delicate butterfly scales (Hultman 1753). Linnaeus also kept his insects dried, spread and pinned (Mikkola and Honey 1993).

Regarding the methods to spread Lepidoptera, the artist Benjamin Wilkes (died c. 1749) was one of the first to write down instructions for setting butterflies and moths in his "Directions for Making a Collection" (Wilkes 1742; Salmon 2011). So, as early as the mid-18th century, many collectors preserved butterflies and moths air-dried, wings spread, and pinned in boxes or drawers lined with cork or other material. It is not only pleasant to look at, but it also facilitates the examination of the wings and body. The combination of pinning and spreading made the specimens easy to handle, it provided space for labels, and is the best way to study them from all sides. All the specimens from this earlier period, either pinned, pressed or placed between layers of glass, were spread in a 'natural' position, that is, with the leading edge of the forewings nearly perpendicular to the body and behind the head. Since the turn of the 19th century, Lepidoptera are always mounted so that the forewings are pushed forward far enough so that their hind margins form a nearly straight line and are perpendicular to the body's axis, which allows for better examination of the wings. This is ideal for detailed descriptions of external morphology and for imaging. Moreover, when the preparation of genitalia is needed, it is easy to break off the abdomen from pinned specimens without damaging the rest of the specimen. As a result, nearly all entomology and collection handbooks describe this now as the standard method of mounting butterflies and moths on boards, and describe the papering as the method for transport and temporary storage (Greene 1863; Greene and Farn 1880; Martin 1977; Schauf 2000).

More recently, new preservation and storage techniques have been developed for fresh or recently acquired material in order to facilitate morphological and molecular research, such as fluid preservation and freezing of the specimen bodies, while the wings are saved in clear plastic coin holders (see for example Cho et al. 2016 or Brower 2000). However, none of these methods have become as important as for other groups of invertebrates, so papering and pinning are still widely used to store Lepidoptera.

In order to maximize the use of storage space, curators of large collections often fill insect drawers as much as possible by letting the wings of the butterflies overlap, or in case of moths, by not spreading some of them. This has obvious disadvantages. In the case of overlapping wings, it results in overcrowded drawers, with greater risk of damage for the specimens. Unmounted moths are, on the other hand, difficult to examine. Pinning moths without spreading, that is, with their wings close to the abdomen, however, may be more efficient than papering.

For specimens belonging to easily identifiable species, often only collected to serve as a faunistic voucher, there is no direct need for mounting in order to obtain the necessary information.

This means that for long series of specimens with similar label data, the default storing method could be unmounted in envelopes in order to save space. Only when other kinds of research require a traditionally mounted specimen, it is necessary to select the specimens and spread them.

For DNA studies, papered specimens may yield even better results, as they have never been relaxed. Relaxing requires exposure to moisture and therefore may be detrimental to DNA quality (see for example Prosser et al. 2016). However, many papered collections have been in very wet climates for some time and as a consequence they may have already suffered some form of DNA degradation by either climatic conditions or the use of chemicals that prevented mould or insect damage (Zimmermann et al. 2008; Tin et al. 2014; Prosser et al. 2016).

Butterflies in bags – an innovative workflow

Here we describe a three-phase workflow developed to tackle the enormous collection of 500,000 papered Lepidoptera at Naturalis Biodiversity Center. Central to this workflow is the idea that Lepidoptera will be permanently stored in glassine envelopes, still unmounted, in contrast to mounting as the traditional method of storage. In the first phase of the workflow non-expert volunteers repack and digitize the papered Lepidoptera, while taxonomic experts take care of the identification in the second phase. The post-processing in the third phase is the responsibility of collection managers. Step by step we will treat the three sequential phases of this workflow: repacking and digitization, identification and post processing.

Material and methods

To test the designed workflow during the pilot in 2016, a selection of envelopes was made that comprised the papered Papilionidae (swallowtails) collected by J.M.A. van Groenendael in Java, Indonesia. This family of butterflies was chosen for several reasons. In the first place, it is one of the smallest families of butterflies, comprising around 180 species in the Oriental region (Parsons 1998). Secondly, because of their beauty and large size, swallowtails have been intensively collected for centuries and are well represented in museum collections. Finally, the specimens are relatively easy to identify by wing undersides, the only visible part in papered butterflies. On top of that, because of their large size, swallowtails are ideal to test the potential gain in space that can be achieved by bagging instead of mounting the specimens.

Deciding which specimens should be processed first is primarily dependent on ongoing research. In the absence of ongoing research, prioritizing the papered Lepidoptera is based on taxonomy, so the project only needs to depend on one expert at a time. Therefore, once the Papilionidae are fully processed, the rest of the papered Lepidoptera collection will be processed by family during the expected 12 year life span of the project.

To digitize and store specimen metadata for each swallowtail together with their image, the Collection Registration System (CRS) in use by Naturalis was chosen. CRS was developed during the FES Collection Digitization project (Heerlien et al. 2015) from 2010 to 2015 to store collection related data and support collection management activities. It now holds over 8 million specimen records at object level and 32 million specimens at species/storage unit level. Eventually the data and images in CRS are published on http://bioportal.naturalis.nl/, including the papered butterflies (example: http://bioportal.naturalis.nl/multimedia/ZMA.INS.1332352_1/)

Phase 1, which does not require in-depth knowledge, is carried out by volunteers in order to reduce costs. A team of volunteers was recruited sufficient to occupy three workstations five days a week. As most volunteers are available for one day per week the team includes some 20 persons to accommodate for illnesses and holidays.

The Workflow

Phase 1 – Repacking and digitization

Step 1: Pre-processing by project coordinator

1. All information available on storage unit level, such as collecting date and location or collection name, is registered in the Naturalis Collection Registration System (CRS) as first basic information for further individual registrations.
2. A stock of blank 3×5 inch index cards is printed, which contain a unique data matrix and registration number. These labels are printed on thick, 100% cotton, acid free ledger paper to ensure sustainable storage. The registration numbers are consecutive to improve usability. Labels are subsequently cut to perfectly fit the glassine envelopes. This provides support and protection for the specimen in the glassine envelope.

The result is a prepared drawer of papered Lepidoptera with a supply of unique 3×5 inch cards (Fig. 3) alongside a general supply of glassine envelopes.

Step 2: Handling by volunteers (Fig. 4)

1. The next index card as well as a random papered butterfly or moth is taken from the prepared drawer. The information written on its envelope, most commonly collecting locality and date, is registered into the CRS database.

Figure 3. A drawer with papered Lepidoptera that has been prepared by the project coordinator for the volunteers. Next to the drawer is a stock of acid free labels which will be adjoining the specimens.

Figure 4. A work station which is occupied by volunteer Herman Hillebrand. On the right is the main stock of Lepidoptera and the computer screen on the left shows the live image of the camera. Photo by Luisa de Bruin.

2. The specimen is taken out of its original envelope and is imaged together with the original envelope and the newly printed index card (Fig. 5), using a digital camera. Care is taken that the part of the original envelope containing the source data is imaged, so the quality of transcription can always be checked.
3. The label is inserted into a glassine envelope, after which the specimen carefully follows. The glassine envelopes are finally placed in numerical order by their registration code, in small cardboard boxes that fit in tailor made drawers (Fig. 6). The data matrix on the index card can be scanned through the glassine envelope. Each working station has its own series of numbers to avoid the error-prone rearranging of specimens between stations.

Step 3: Completion by the project coordinator

After volunteers have registered, imaged and stored the specimens in glassine envelopes, the project coordinator makes sure that the quality of the data is up to standard and that the digital and physical collections are properly organised. This includes a weekly quality control of the data in the generated specimen records, renaming and linking the produced images to the corresponding specimen records and ensuring the specimens and drawers are stored properly.

Figure 5. An example of the standardized photographs produced during this project. These are later sent to taxonomic experts for identification and can be used for automated image recognition. They will also be visible online on bioportal.naturalis.nl.

Figure 6. The final storage of the specimens. The specimens are visible through the semi-transparent glassine envelope, and are stored in the cardboard boxes within drawers where they are easily accessible by unique specimen number.

The entire process of phase 1 is schematically represented by a flowchart (Fig. 7). Both the description of the workflow above as the flowchart for phase 1 provide a very global overview of the sequence of actions and do not cover all exceptions to the process and specifics on movement of objects and information.

Phase 2 – identification

The images taken during step 2 of phase 1 are sent by the project coordinator to a taxonomic specialist in the respective Lepidoptera family. Using the images, the specialist identifies specimens to the lowest taxonomic level possible and registers the taxonomic information in an external file. During this process, the specialist can mark the specimen for mounting and/or DNA-sampling when deemed necessary, i.e. when identification using solely the ventral view of the wings is not possible or the specimen is very rare in collections. The project coordinator imports the specialist's identifications from the external file into the CRS database so they correspond with the specimens and accompanying records.

For the Naturalis collection of papered Lepidoptera, it is practically impossible to determine whether all specimens in a drawer selected for repacking and digitization belong to a certain family. The collection is only roughly sorted by family and the envelopes are usually opaque. Since
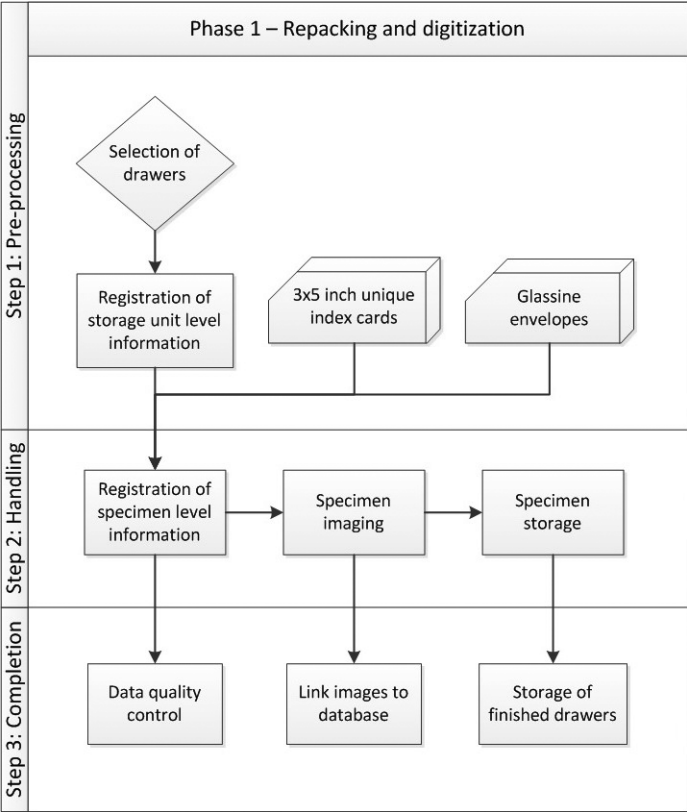

Figure 7. Flowchart of steps 1 through 3 during phase 1 of the workflow.

the volunteers are not expected to identify Lepidoptera, all specimens from prioritized drawers are processed even though they might belong to another family. In the case of the 2016 pilot, the Papilionidae expert identified the non-Papilionidae to at least family level so these can later be easily redirected to their respective expert.

At the time of writing, the project is experimenting with deep learning image recognition to reduce the time a specialist has to put in identifying specimens of common species (Hogeweg 2018; Schermer et al. 2018). This is done by using a validated set of identified and imaged collection specimens to train software, which develops a model of what the butterfly species look like. The software can then be used by volunteers to automatically identify common species, so that taxonomic specialists can focus on the rare and difficult species.

Phase 3 – post processing

The collection of Lepidoptera resulting from the first two phases, as described above, is ordered numerically by their registration code. On the other hand, the Naturalis collections of mounted Lepidoptera and papered Odonata are ordered taxonomically. A logical next step would be to reorder the butterflies in envelopes to fit this system. However, as the specimens are easily traced individually by their identification numbers, there is no direct necessity to reorder and handle all specimens again. If there is need for a taxonomically ordered collection of papered butterflies in the future, a re-curation workflow will be set up.

A more pressing issue at the moment is dealing with the specimens that are marked by the expert to be mounted or DNA sampled, for example, when identification was not possible using only the photograph. These specimens will be extracted from the papered collection and professionally mounted for further examination.

Results for curation

The described workflow yields a thoroughly curated collection, both physically and digitally. The physical collection of unmounted Lepidoptera in glassine envelopes is archived sustainably and is easily accessible due to being ordered numerically. The digital collection comprises a photograph, an identification and in most cases a collection date and locality information for each individual specimen. Notably, the new workflow is especially efficient when compared to the traditional practice of mounting. During the 2016 pilot the team of volunteers digitized a total of 16,440 specimens, mostly Papilionidae, none of which required mounting for further study. The gains in time, space and costs are discussed below. In the workflow here presented, the gains in resources are by and large dependent on the percentage that still requires mounting after digitization. Because Papilionidae in this respect are not representative for all Lepidoptera, several situations that depict varying levels of mounting requirements are included in the calculations as well.

Time efficiency

In Table 1 and Fig. 8, the time required for re-curating 16,440 specimens of Papilionidae is presented. The 'traditional practice' value is visualised as a dotted line in the graph because it is plotted only as a reference value for the new workflow. In the case of the 2016 pilot, 0% of the specimens

Table 1. Time required to handle and digitize 16,440 specimens of Papilionidae in four scenarios, compared to the traditional practice of direct mounting. Scenario 1 corresponds with the results of the 2016 pilot.

16,440 specimens of Papilionidae	Proposed method - scenario 1	Proposed method - scenario 2	Proposed method - scenario 3	Proposed method - scenario 4	Traditional practice - direct mounting
% that requires mounting	0	25	75	100	100
Handling time (days)					
a.　　envelopes (40 ex./day)	411	411	411	411	
b.　　mounting* (20 ex./day)	0	206	617	822	822
Total handling time (days)	411	617	1028	1233	822

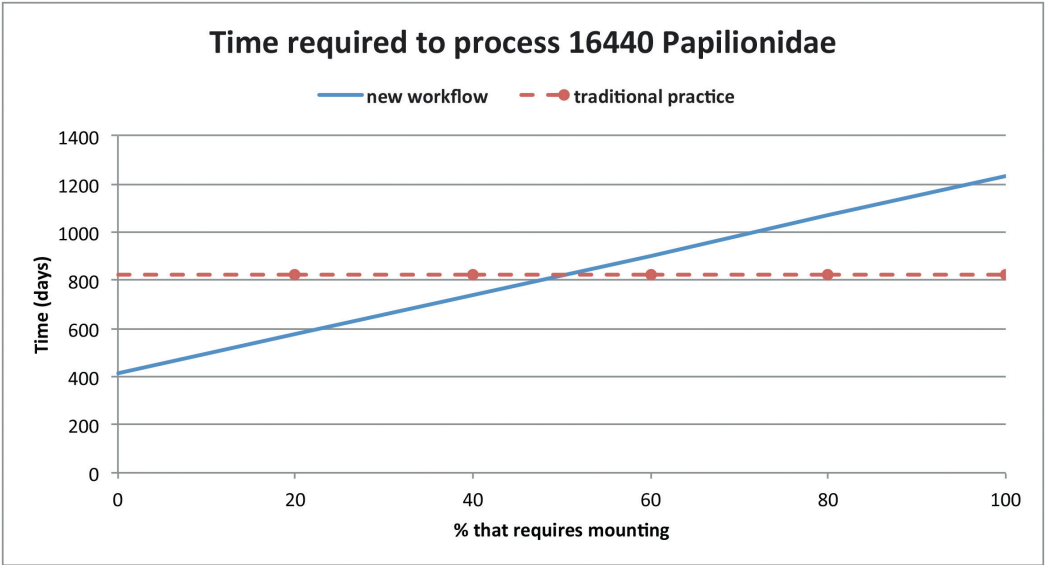*: includes mounting, labelling and digitizing, excludes relaxing time required for mounting.

Figure 8. Time required to process 16,440 Papilionidae. When more than 50% of the digitized Lepidoptera specimens require mounting after digitization, the new workflow will become time-inefficient.

needed mounting so the new workflow required only half the time of what the traditional practice would take. The percentage of specimens that requires spreading after digitization will be handled twice. Therefore, applying the proposed method will be too time-consuming if that percentage increases past 50%.

Space efficiency

In Table 2 and Fig. 9, the number of drawers required to store 16,440 specimens of Papilionidae is presented. The 'traditional practice' value is visualised as a dotted line in the graph because it is plotted only as a reference value for the new workflow. In the case of the 2016 pilot, 0% of the specimens needed mounting so the applied method required only one seventh of the number of drawers that traditional practice would take. Even if 99% of the specimens still require mounting after digitization, storing 1% of the specimens unmounted in glassine envelopes saves space.

Table 2. Drawers required to store 16,440 specimens of Papilionidae in four scenarios, compared to the traditional practice of direct mounting. Scenario 1 corresponds with the results of the 2016 pilot.

16,440 specimens of Papilionidae	Proposed method - scenario 1	Proposed method - scenario 2	Proposed method - scenario 3	Proposed method - scenario 4	Traditional practice - direct mounting
% that requires mounting	0	25	75	100	100
Number of drawers required					
a. envelopes (350 ex./drawer)	47	36	12	0	0
b. mounting (50 ex./drawer)	0	83	247	329	329
Total number of drawers required	47	119	259	329	329

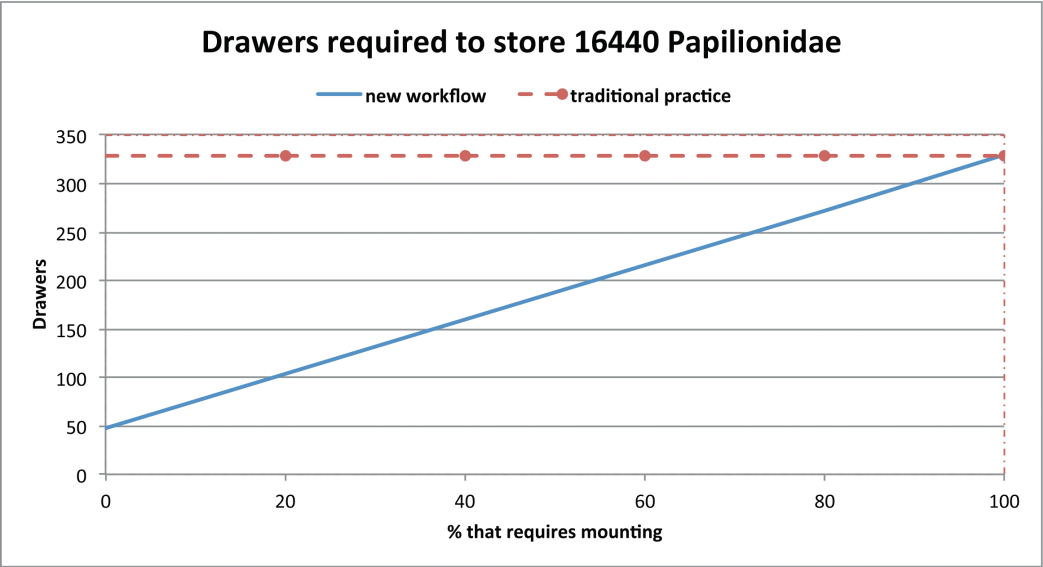**Figure 9.** Drawers required to store 16,440 Papilionidae. Storing unmounted Lepidoptera specimens in glassine envelopes is always space-efficient.

Cost efficiency

In Table 3 and Fig. 10, the cost required for re-curating 16,440 unmounted specimens of Papilionidae is presented. The 'traditional practice' value is visualised as a dotted line in the graph because it is plotted only as a reference value for the new workflow. In the case of the 2016 pilot, 0% of the specimens needed mounting so the new workflow required only a third of what traditional practice would take. Even though the amount saved decreases when the percentage that still requires mounting increases, the new workflow remains profitable up until the moment that 89% of all specimens require mounting after digitization.

Discussion

A new workflow is presented for processing papered Lepidoptera specimens as an alternative to mounting all individuals. This workflow entails digitizing the specimens and repacking them, still

Table 3. Costs required to handle and digitize 16,440 specimens of Papilionidae in four scenarios, compared to the traditional practice of mounting. Scenario 1 corresponds with the results of the 2016 pilot.

16,440 specimens of Papilionidae	Proposed method - scenario 1	Proposed method - scenario 2	Proposed method - scenario 3	Proposed method - scenario 4	Traditional practice - direct mounting
% that requires mounting	0	25	75	100	100
Material costs involved* (€)	6753	10277	16376	19426	18100

*: includes drawers, cardboard boxes, glassine envelopes and acid free ledger paper. A breakdown of costs per item can be found in the online Suppl. material 1.
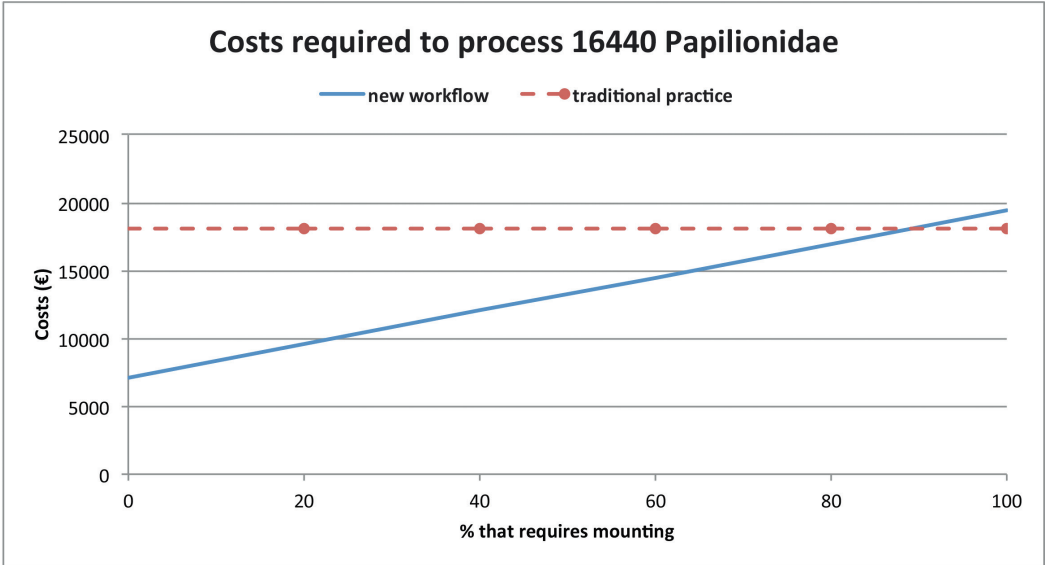

Figure 10. Cost (in euros) required to process 16,440 Papilionidae. When 89% of the papered Lepidoptera collection requires mounting after digitization, the new workflow will become cost-inefficient.

unmounted. Mounting is limited to those specimens that otherwise cannot be identified or are special or rare. Results indicate that the efficiency of this workflow depends on the number of specimens that still require mounting after processing. For Papilionidae, due to their size and relative ease of identification, saving resources when applying the new workflow is evident. For other groups, i.e. most moth families, this workflow most likely is less efficient when aiming at identifying all specimens to species level.

Nevertheless, even if identification of unmounted material is only possible to a higher taxonomic level (i.e. family or subfamily), applying this workflow is still advantageous. A large number of papered Lepidoptera will be individually processed and stored in an acid-free durable environment available for further study. Not only is a high level (family or subfamily) identification already an enormous improvement, the locality and date for each specimen becomes digitally available as well. This will facilitate research and improve selection of specimens to be mounted for further study. The photographs are disseminated online and sent to experts for identification.

When faced with the decision whether to apply this new method to process a collection of papered Lepidoptera or mount all specimens at once, being able to estimate the percentage that will require mounting is a welcome addition. So far this has proved to be quite difficult considering the historical nature of the collection with limited documentation and most envelopes being opaque. Perhaps knowledge about the collector might help in indicating what kind of Lepidoptera are to be expected, but in general the means of assessing beforehand the probable gains in time, space and cost requires further scrutiny.

Finally, future automated identification tools for unmounted Lepidoptera that recognize shape and colour patterns of the wings would perfectly fit into the workflow presented here (Schuettpelz et al. 2017; Hogeweg 2018; Schermer et al. 2018). For relatively difficult groups of Lepidoptera, this will allow for a rapid first identification and for easier groups these tools can be trained to identify specimens up to species level. We believe the workflow presented here demonstrates a promising way for processing and permanently storing unmounted Lepidoptera. It even holds the potential to be further developed and tailored to facilitate in-field registering and papering, resulting in specimens that upon arrival can be stored directly in the collection.

The original envelopes are not stored physically with the specimens, except for ones with exceptional historical information or where the source data is very hard to read and keeping the original envelope might provide beneficial. The method of digitization results in images and verbatim transcriptions of the source data on the original envelopes. This allows the source data to always be digitally accessible for inspection when there is doubt about the validity of the transcribed or interpreted data. A representative selection of the original envelopes is kept separately in our archive because of their historical value. Instead of disposing the rest of the original envelopes, alternative uses such as outreach are being explored.

Eventually a papered collection of taxonomically arranged Lepidoptera may form an important supplementary collection next to the traditional collection of mounted specimens. It is already common practice in some laboratories to keep voucher specimen used for tissue extraction for DNA in envelopes (N. Wahlberg, personal communication; Cho et al. 2016). By developing a way to store the papered Lepidoptera accessions, they can be incorporated in the main collection.

Acknowledgements

References

Beatty GH, Beatty AF (1963) Efficiency in caring for large Odonata collection. Proceedings of the North Central Branch of the Entomological Society of America 18: 149–153.

Boer AJ de (1998) J.M.A. Van Groenendael: a life between medicine and entomology in Indonesia. Van Groenendael-Krijger Foundation, Amsterdam, 45 pp.

Bradley RD, Bradley LC, Garner HJ, Baker RJ (2014) Assessing the value of natural history collections and addressing issues regarding long-term growth and care. BioScience 64(12): 1150–1158. https://doi.org/10.1093/biosci/biu166

Brower AVZ (2000) Phylogenetic relationships among the Nymphalidae (Lepidoptera) inferred from partial sequences of the wingless gene. Proceedings of the Royal Society of London, Series B, Biological Sciences 267: 1201–1211. https://doi.org/10.1098/rspb.2000.1129

Cho S, Epstein SW, Mitter K, Hamilton CA, Plotkin D, Mitter C, Kawahara AY (2016) Preserving and vouchering butterflies and moths for large-scale museum-based molecular research. PeerJ 4: e2160. https://doi.org/10.7717/peerj.2160

Dalton R (2003) Natural history collections in crisis as funding is slashed. Nature 423: 575. https://doi.org/10.1038/423575a

Gibb TJ (2015) Contemporary insect diagnostics: the art and science of practical entomology. Oxford: Academic Press.

Gray RE (1971) Papering Lepidoptera in glassine envelopes. Journal of the Lepidopterists' Society 25: 65–68. http://images.peabody.yale.edu/lepsoc/jls/1970s/1971/1971-25(1)65-Gray.pdf

Greene J (1863) The insect hunter's companion: being instructions for collecting and preserving butterflies and moths and comprising an essay on pupa digging. John van Voorst, London, 164 pp. https://archive.org/details/insecthuntersco00greegoog

Greene J, Farn AB (1880) The insect hunter's companion: being instructions for collecting and preserving butterflies, moths, beetles, bees, flies, &c. 3rd ed. Swan Sonnenschein & Allen, London (Natural history books), 145 pp.

Heerlien M, Leusen J van, Schnörr S, Jong-Kole S de, Hulsen K van (2015) The natural history production line: an industrial approach to the digitization of scientific collections. ACM Journal on Computing and Cultural Heritage 8(1): 3. https://doi.org/10.1145/2644822

Hogeweg L (2018) Reducing the taxonomist's burden through AI. EuropeanaTech conference, Abstract Booklet, Rotterdam (the Netherlands), May 2018: 18. https://pro.europeana.eu/files/Europeana_Professional/Event_documentation/Events/EuropeanaTech_2018/Speaker_PDF/Laurens-Hogeweg.pdf

Hultman D (1753) Instructio musei rerum naturalium, quam, consensus experient. Et nobiliss. Facult. Medic. Upsal. Sub Praesidio viri nobilissimi atque experimentissiimi Dn. Doct. Caroli Linnaei. L.M. Hojer – Upsala, 19 pp. https://hdl.handle.net/2027/hvd.32044106474109

Jong R de (2005) *Metamorpha sulpitia* (Lepidoptera, Nymphalidae) en de lotgevallen van oude collecties. Entomologische Berichten 65(4): 124–127.

Martin JEH (1977) Collecting, preparing, and preserving insects, mites, and spiders. Department of Agriculture. Publication, Ottawa, The Insects and arachnids of Canada 1: 182. http://esc-sec.ca/aafcmonographs/insects_and_arachnids_part_1_eng.pdf

MacGregor A (1994) Sir Hans Sloane: collector, scientist, antiquary, founding father of the British Museum. British Museum Press, London, 308 pp.

McLean BS, Bell, KC, Dunnum JL, Abrahamson B, Colella JP, Deardorff ER, Weber JA, Jones AK, Salazar-Miralles F, Cook JA (2016) Natural history collections-based research: progress, promise, and best practices, Journal of Mammalogy 97(1): 287–297. https://doi.org/10.1093/jmammal/gyv178

Mikkola K, Honey MR (1993) The Noctuoidea (Lepidoptera) described by Linnaeus. Zoological Journal of the Linnean Society 108: 103–169. https://doi.org/10.1111/j.1096-3642.1993.tb00292.x

Parsons M (1998) The butterflies of Papua New Guinea: their systematics and biology. Academic Press, London. xvi, 736 pp.

Petiver J (1709) Brief directions for the easie making, and preserving collections of all natural curiosities. 1-sided folio. London, printed for the author. https://www.biodiversitylibrary.org/item/173715#page/9/mode/1up

Pyke GH, Ehrlich PR (2010) Biological collections and ecological/environmental research: a review, some observations and a look to the future. Biological Reviews 85(2): 247–266. https://doi.org/10.1111/j.1469-185X.2009.00098.x

Prosser SWJ, deWaard JR, Miller SE, Hebert PDN (2016) DNA barcodes from century-old type specimens using next-generation sequencing. Molecular Ecology Resources 16: 487–497. https://doi.org/10.1111/1755-0998.12474

Salmon MA (2001) The Aurelian legacy: British butterflies and their collectors. University of California Press, Berkeley/Los Angeles, 432 pp.

Schauf ME (2000) Collecting and preserving insects and mites: techniques and tools. Systematic Entomology Laboratory, USDA, Washington DC, 68 pp.

Schermer M, Hogeweg L, Caspers M (2018) Using deep learning in collection management to reduce the taxonomist's workload. Biodiversity Information Science and Standards 2: e25917. https://doi.org/10.3897/biss.2.25917

Schuettpelz E, Frandsen P, Dikow R, Brown A, Orli S, Peters M, Metallo A, Funk V, Dorr L (2017) Applications of deep convolutional neural networks to digitized natural history collections. Biodiversity Data Journal 5: e21139. https://doi.org/10.3897/BDJ.5.e21139

Tin MM-Y, Economo EP, Mikheyev AS (2014) Sequencing degraded DNA from non-destructively sampled museum specimens for RAD-tagging and low-coverage shotgun phylogenetics. PLoS ONE 9: e96793. https://doi.org/10.1371/journal.pone.0096793

Tol J van (2001) The collection of Odonata of the National Museum of Natural History | Naturalis at Leiden. WDA International Symposium of Odonatology 2, Abstract Booklet, Gallivare (Sweden), July 2001: 20–21.

Wilkes B (1742) 'Supplement' to Twelve new designs of English butterflies [Directions for making a collection of moths and butterflies]. Included in the facsimile edition of Twelve new designs in Wilkinson RS (Ed.) Benjamin Wilkes: the British Aurelian, 1982. Faringdon, Classey.

Winter WD (2000) Basic techniques for observing and studying moths and butterflies. Memoirs of the Lepidopterists' Society 5, 444 pp.

Zimmermann J, Hajibabaei M, Blackburn DC, Hanken J, Cantin E, Posfai J, Evans TC (2008) DNA damage in preserved specimens and tissue samples: a molecular assessment. Frontiers in Zoology 5: 18. https://doi.org/10.1186/1742-9994-5-18

Zwakhals CJ, Sommeijer MJ, Heerebout GR (2015) De oudste vlindercollectie van Nederland. Entomologische Berichten 75(3): 105–109.

Supplementary material 1

Permanent storage of Lepidoptera in glassine envelopes

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Holcophora Staudinger, 1871, a senior synonym of *Aponoea* Walsingham, 1905, syn. n., (Lepidoptera, Gelechioidea, Gelechiidae): with *Holcophora inderskella* (Caradja, 1920), comb. n., transferred from *Blastobasis* Zeller, 1855 (Blastobasidae)

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http://zoobank.org/D9A5B421-8980-4807-B3E4-0AF0AA205C02

Abstract. *Blastobasis inderskella* Caradja, 1920, is transferred from Blastobasidae to Gelechiidae and placed in the Palaearctic genus *Holcophora* Staudinger, 1871. The genus *Aponoea* Walsingham, 1905, **syn. n.**, is newly synonymized with *Holcophora*, and three new combinations are proposed: *Holcophora inderskella* (Caradja, 1920), **comb. n.**, *H. obtusipalpis* (Walsingham, 1905), **comb. n.** and *H. pruinosella* (Chrétien, 1915), **comb. n.** Lectotypes are designated for *Holcophora statices* Staudinger, 1871, and *Aponoea obtusipalpis* Walsingham, 1905. *Holcophora inderskella* is redescribed with supportive images of diagnostic features. Its unique frontal process is described and illustrated and aspects of frontal processes in Gelechiidae are discussed.

Introduction

Blastobasis inderskella Caradja, 1920, is one of numerous micromoths almost never referred to again in the scientific literature after its description. During an examination of Caradja types by KS in 1987, it was discovered that *B. inderskella* was misplaced in Blastobasidae and should be transferred to the Gelechiidae. A manuscript transfer of *inderskella* to the Gelechiidae, but without assignment to a specific genus, was made by KS in the collection of the Natural History Museum, London, and specimens from Mongolia were associated with that name. The transfer from Blastobasidae to Gelechiidae was subsequently formalised by Sinev (2014: 81). We are now able to assign *inderskella* to the genus *Holcophora* Staudinger, 1871, with which we newly synonymize *Aponoea* Walsingham, 1905.

Materials and methods

Moths examined included type specimens as well as non-type specimens from Museum für Naturkunde, Berlin, Germany (MfN); Muséum Naţional d'Histoire Naturelle "Grigore Antipa", Bucharest, Romania (MGAB); Hungarian Natural History Museum, Budapest, Hungary (HNHM); Natural History Museum, London, United Kingdom (NHMUK); and Muséum national d'Histoire naturelle, Paris, France (MNHN). Kornerup and Wanschner (1978) was used as a colour standard for the description of the adult vestiture. Pinned specimens and their associated slide-mounted geni-

talia, and other features were examined with dissecting and compound microscopes, the head structure with a scanning electron microscope. In some instances heads were removed from a specimen, descaled and appropriately treated for SEM study. After examination they were re-attached to the thorax using water-soluble glue that would permit renewed detachment of the head should that become necessary. We use terms for the male and female genitalia following Hodges (1986) and Ponomarenko (2008, 2009). Abbreviations for male genitalia are as follows: ae = aedeagus; g = gnathos; t = tegumen; u = uncus; v = vinculum; vc = valva (costa); vs = valva (sacculus). Abbreviations for female genitalia are as follows: aa = apophyses anteriores; ap = apophyses posteriores; cb = corpus bursae; db = ductus bursae; ds = ductus seminalis; o = ostium; p = papillae anales; s = signum.

Results

Holcophora Staudinger, 1871

Holcophora Staudinger, 1871: 313. Type species: *Holcophora statices* Staudinger, 1871: 313, by monotypy. [Figs 1–4]
Aponoea Walsingham, 1905: 125. Type species: *Aponoea obtusipalpis* Walsingham, 1905: 125, by original designation and
     monotypy. **Syn. n.** [Figs 5–8]

Generic diagnosis. The two type species, *Holcophora statices* and *Aponoea obtusipalpis* have similarly patterned forewings (Figs 1, 5) and long labial palpi (the palpi are upturned in the former species and more or less porrect in the latter species). Although these characters were once considered important for generic recognition, the genitalia (Figs 2–4, 6–8) provide a more reliable suite of characters for setting generic limits. We propose that *Holcophora* Staudinger is a senior subjective synonym of *Aponoea* Walsingham because they share: a stout or shortened tegumen; bifurcate apices of the saccular part of the valvae; inwardly-curved and narrowed apical 2/3 of the costal part of the valvae; both saccular and costal parts of the valvae short, extending to or slightly exceeding the uncus; tegumen about .6 wider at widest point compared with width of tegumen at level of anterior arch; aedeagus shorter than saccular and costal parts of valvae; and signum with transverse mesial ridge.

     Hostplants. Plumbaginaceae.

     Distribution. Palaearctic.

     Remarks. The subfamily and tribal position of *Holcophora* is still somewhat uncertain. Meyrick placed *Holcophora* and *Aponoea*, separated only by *Rhynchopacha* Staudinger, 1871 (= *Athrips* Billberg, 1820), in his 'Group 7 (*Dichomeris* type)' (=Dichomeridinae), Karsholt et al. (1996: 120) in Gelechiinae (Chelariini). We follow Ponomarenko (2009: 195, English translation), who placed *Holcophora* in Gelechiinae (Gelechiini), based on specifcs of the male genitalia musculature.

Holcophora inderskella (Caradja, 1920), comb. n.
Figs. 9–17

Blastobasis inderskella Caradja, 1920: 122. Lectotype ♂ [damaged], KAZAKHSTAN: Oz Inder, ('Indersky Salzsee'),
     22.vi.1907 [(*Bartel*)], designated by Popescu-Gorj 1992: 156 (MGAB, Bucharest), (Fig 14). Paralectotype, ♀ [damaged], same label data as above, (Fig. 15).

Redescription [based on Mongolian specimens]. Adult: Head covered with narrow, convergent, mottled scales. Ocelli present. Proboscis present, basal part squamose, white. Outer surface of
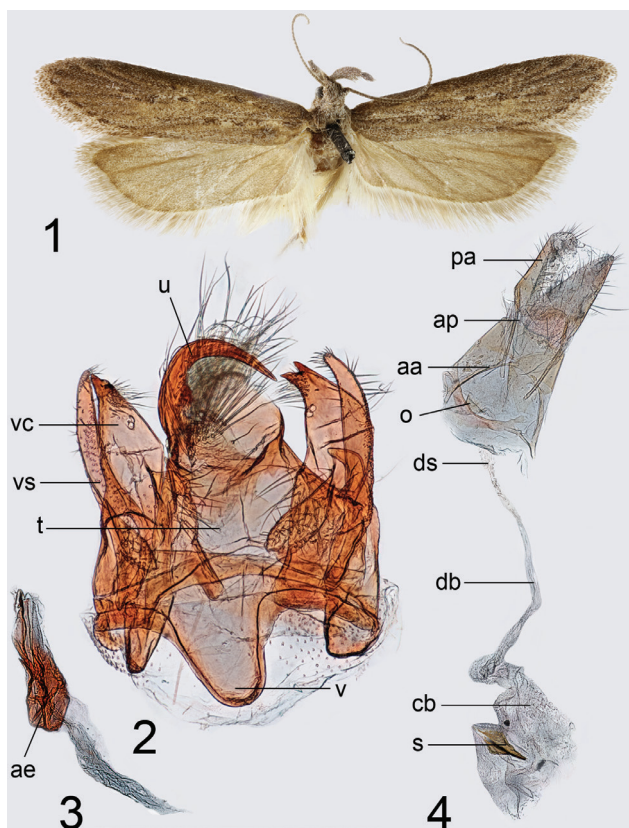
Figures 1–4. *Holcophora statices* Staudinger. **1.** Habitus. **2–3.** Male Genitalia, aedeagus and genital capsule, BM genitalia slide NHMUK0103114723. **4.** Female genitalia, BM genitalia slide NHMUK0110314724, (male and female specimens from Europe).

labial palpus gray intermixed with few pale gray scales tipped with white and pale gray scales, and white scales along apical margin of all palpomeres; inner surface similar but with white scales along dorsal part. Scape of antenna without pecten, pale gray intermixed with grayish brown scales, flagellum with alternating pale gray and brownish gray flagellomeres; male first flagellomere unmodified. Denuded head (Figs 9–13), with medio-posterior part of vertex raised, anteriorly tapered to form distinctive ridge ending at transfrontal sulcus, raised vertex area sparsely set with scale sockets in contrast to densely set surrounding area; frons beneath transfrontal sulcus with strongly protruding medial ridge bearing two or three processes of descending size, longest process at level of antennal sockets; medial ridge set in ovoid ring of strong teeth (each tooth originating from enlarged scale socket). Thorax: Tegula and mesoscutum white, intermixed with few white scales each tipped with brown. Legs with tibiae and femura with alternating white and brown banding on outer surface, mostly white intermixed with few brown scales on inner surface; tarsomeres mostly brown, with narrow white apical band. Forewing (Fig. 9): length 6.0–6.1 mm (n=2), white intermixed with brown, pale gray, and mottled scales; venational pattern demarcated by contrastingly darker scales on areas between veins than paler scales on areas above veins; two brown,
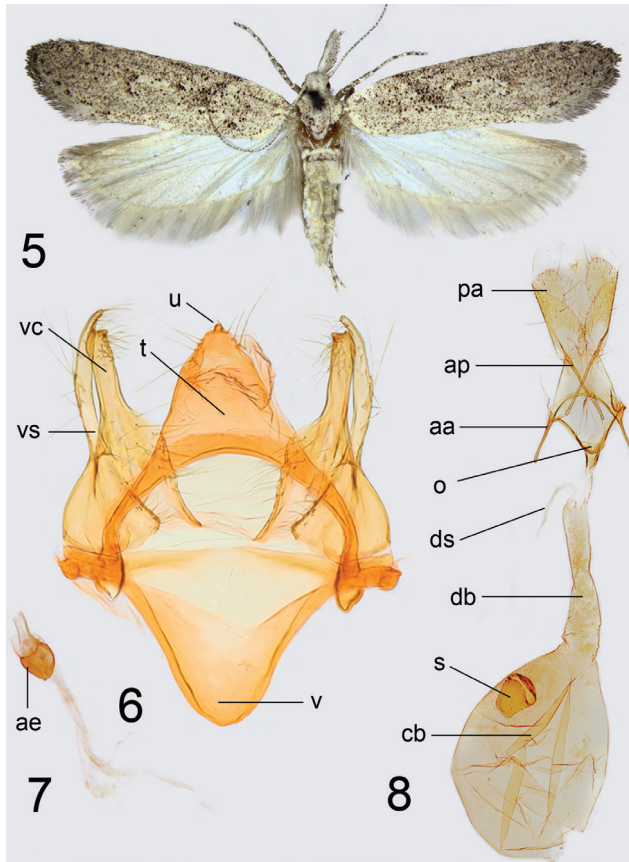
Figures 5–8. *Aponoea obtusipalpis* Walsingham. **5.** Habitus. **6–7.** Male genitalia, aedeagus and genital capsule, BM genitalia slide 25342. **8.** Female genitalia, BM genitalia slide 25343, Lectotype. (male and female specimens from North Africa).

oblique fasciae along base and near midcell; basal fascia bearing two brown scale tufts and midcell fascia bearing four brown scale tufts. Lower surface pale brownish orange, with white scales along costa; fringe white, tipped with brown. Hindwing: translucent pale grayish brown, slightly darkened towards apex; fringe pale grayish brown tipped with slightly darker banding of brown. Abdomen: All terga without transverse, irregular rows of spine-like setae (unlike Blastobasidae). Male genitalia (Figs 16–17): Uncus short, with acuminate apex. Gnathos elongate, with upwardly curved apical end, extending beyond uncus. Tegumen elongate. Left saccular part of valva with narrow shaft, broadly curved near middle, with setose cucullus; right saccular part of valva with apical half missing; costal part of valva about 2/3 length of tegumen, widened at base, gradually narrowing apically. Vinculum extending posterolaterally from wide base, curving abruptly, forming converging, opposable apices. Aedeagus downturned, somewhat widened basally, gradually narrowed towards acuminate apex, nearly as long and extending from a bulbous base. Female genitalia: not examined.

Hostplant(s). Unknown.

Distribution. Palaearctic: Kazakhstan, Mongolia.
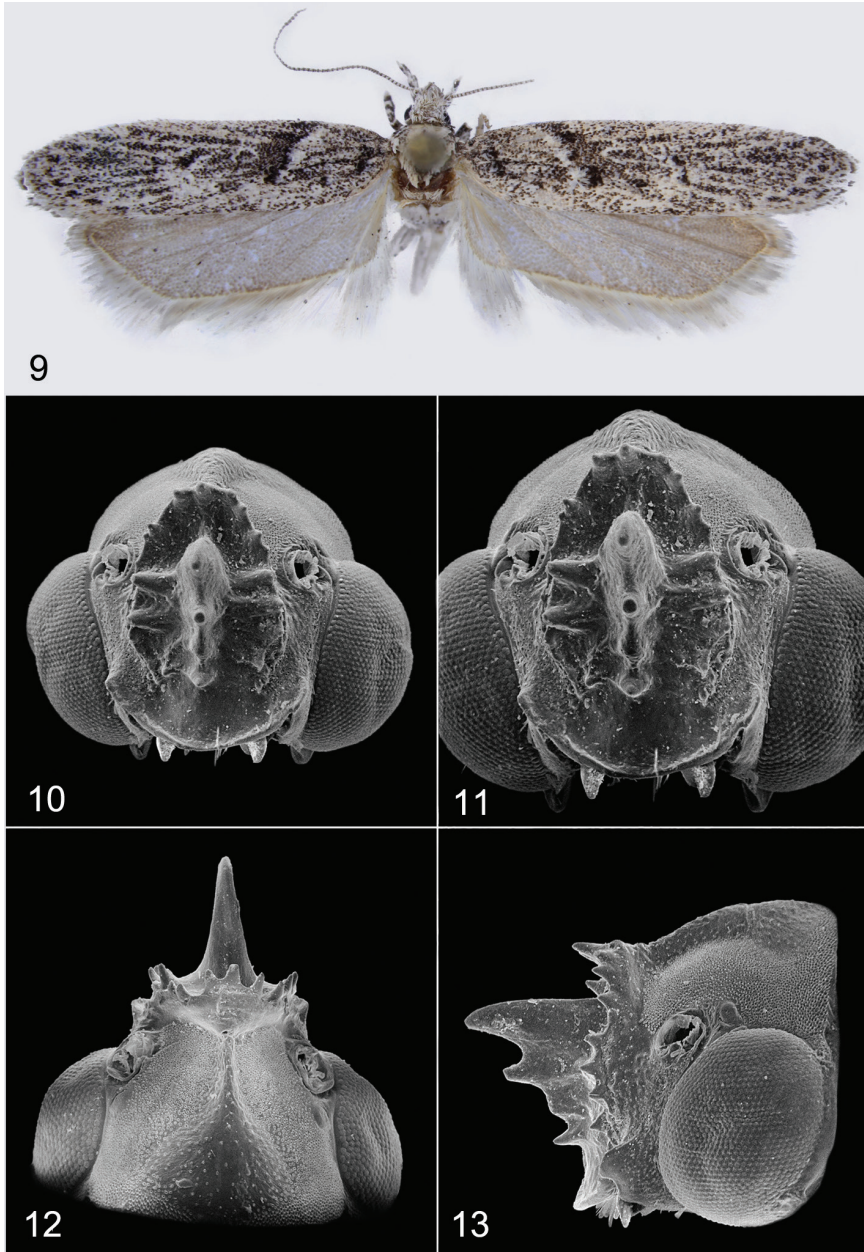
Figures 9–13. *Holcophora inderskella* (Caradja). **9.** Habitus. **10–11.** Denuded head showing frontal process (frontal view). **12.** Head showing vertex and frontal process (dorsal view). **13.** Head (lateral view). (male specimens from Mongolia).

Remarks. *B. inderskella* was described by Caradja from two fresh males from Oz Inder ('Indersky Salzsee'), about 48°30'N, 51°58'E, in Kazakhstan. The collector, the German lepidopterist and insect dealer Max Bartel, is not mentioned in the original description, nor is he recorded on the specimen labels, but can be inferred from Caradja (1910: 106).
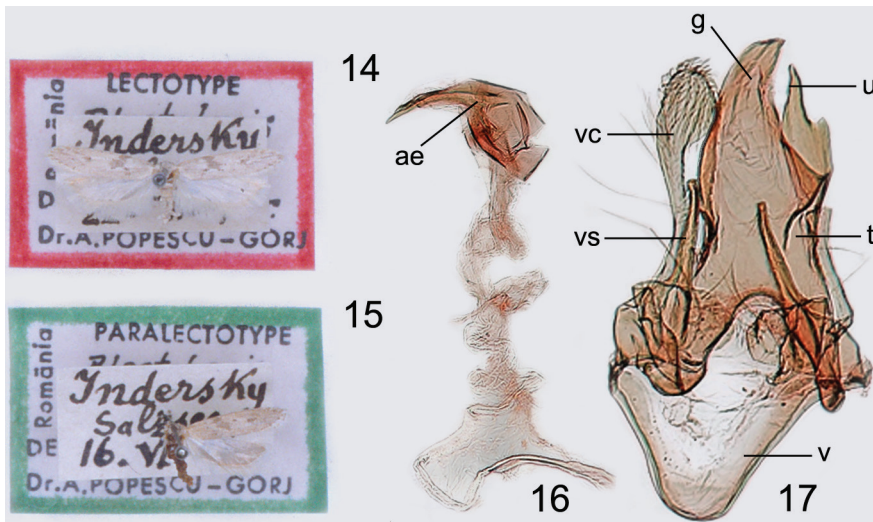
Figures 14–17. *Holcophora inderskella* (Caradja). **14.** Lectotype. **15.** Paralectotype. **16–17.** Aedeagus and genital capsule, BM genitalia slide 14883. (Male specimen from Mongolia).

Caradja gave no indication what characters he used to place this species in the Blastobasidae and we assume that it may have been superficial similarities in the forewing pattern. His proposal of the name *inderskella* is conditional. He introduced the species as '*Bl*. sp. (?*segnella* Z.)' and stated that, unless it were *Blastobasis* (now *Hypatopa*) *segnella* Zeller, a taxon unknown to him, it had to be a new species for which he proposed the name *B. inderskella*. Prince Aristide Caradja's collection survived, with little damage, the Second World War and the challenging post-war period on his estate at Grumăzești in north-eastern Romania before it was incorporated in the Muzeul Național de Istorie Naturală 'Grigore Antipa', Bucharest, Romania, where KS had the opportunity to study it in 1987. By that time all types had been extracted and placed in a separate type collection, lectotypes had been selected and a type catalogue, including formal designations of lectotypes, was subsequently published (Popescu-Gorj 1992).

Both type specimens of *B. inderskella* still exist in the collection but, as a result of psocid feeding before they reached the museum, are in poor condition: both lack the head and (most of) the abdomen, while the paralectotype also lacks the left-hand wings. On examination it was immediately obvious that *B. inderskella*, despite some rather superficial similarities to *Hypatopa segnella* (Zeller, 1873) in the forewing markings, was a species of Gelechiidae. Fresh specimens from Mongolia, collected by Z. Kaszab, Budapest, were tentatively identified by KS as *inderskella* and placed under that name in the NHMUK collection but without a generic assignment (further specimens exist in HNHM Budapest). We are confident that the generic placement in *Holcophora* is accurate, however, the specific identity should be re-assessed when fresh specimens from the type-locality (Kazakhstan) become available.

B. inderskella differs in the presence of a prominent frontal process from the other three species here included in *Holcophora*. We do not believe that character on its own justifies the separation of *inderskella* into its own genus as species with and without such modifications of the head structure coexist in otherwise undisputed genera of Gelechiidae (e.g. *Athrips* Billberg, *Ornativalva* Gozmány, *Prolita* Leraut, *Scrobipalpa* Janse).

Holcophora obtusipalpis (Walsingham, 1905), comb. n.

Aponoea obtusipalpis Walsingham, 1905: 125. Lectotype ♀, ALGERIA: Biskra, 7.iii.1903 (*Walsingham*, no. 96644). PRESENT DESIGNATION. (NHMUK, London).

Mesophleps cinerellus Turati, 1930: 80, pl. 2, fig. [unnumbered]. Lectotype ♂, LIBYA: Cyrenaica, Banghāzi ('Bengasi'), 21.iii.1922 (*Krüger*). (NHMUK, London), designated by Li and Sattler 2012: 57. Synonymized with *Aponoea obtusipalpis* Walsingham, 1905, by Li and Sattler 2012: 57.

Hostplant. *Limoniastrum guyonianum* Dirieu suspected (Walsingham 1905: 126, Chrétien 1917: 480) – Plumbaginaceae.

Distribution. Palaearctic: North Africa (Algeria, Libya), Spain.

Remarks. *Aponoea obtusipalpis* was described by Walsingham from 'Type ♀ (96644); ♂ (96648)'; the description was made from the female, which is here designated as the lectotype. No paratypes were specified in the original description although 18 specimens in all were mentioned and paratypes were labelled in Walsingham's collection.

Holcophora pruinosella (Chrétien, 1915), comb. n.

Aponaea [*sic*!] *pruinosella* Chrétien, 1915: 330. Syntypes, unspecified sex, TUNISIA: Gafsa, ex *Statice pruinosa*, v, vi., em. vii. (*Chrétien*). (MNHN, Paris).

Hostplant. *Limonium pruinosum* Chaz. ('*Statice pruinosa*') (Chrétien 1915: 330) – Plumbaginaceae.

Distribution. Palaearctic: North Africa (Tunisia).

Remarks. *Aponaea pruinosella* was described by Chrétien from an unspecified number of specimens from Gafsa, bred from larvae feeding in May and June on *Limonium pruinosum* (Plumbaginaceae), the adults emerging in July. Not examined.

Holcophora statices Staudinger, 1871

Holcophora statices Staudinger, 1871: 313. Lectotype ♂, RUSSIAN FEDERATION: S Russia, Volgograd, Krasnoarmeysk ('Sarepta') (*Christoph*), PRESENT DESIGNATION. (ZMHU, Berlin).

Hostplant(s). Unknown. *Limonium* sp. ('*Statice*') sp. suspected (Staudinger 1871: 313) – Plumbaginaceae.

Distribution. Palaearctic: Europe (France, Hungary, Romania, Ukraine, Russia), Kazakhstan, China.

Remarks. *Holcophora statices* was described by Staudinger from 15 specimens, both sexes, he had received from H. Christoph under the manuscript name *Ypsolophus statices*. As there was only one female amongst the 15 specimens Staudinger concluded that Christoph may not have bred the species but collected the adults from *Limonium* ('*Statice*') *Statice* flowers.

Head structures. Various modifications of the head structure, culminating in distinct frontal processes are widespread in Lepidoptera and have independently evolved in many families. If present they are equally developed in both sexes. Their function, although plausibly interpreted in a few instances as that of a cocoon cutter, is largely obscure. Sand grains found on the head around such processes suggest that in some species they may assist the adult in drilling through soil on eclosion from the pupa. In Gelechioidea frontal processes are known in Autostichidae (Symmocinae), Cosmopterigidae and Gelechiidae. In the last family they occur in several genera unrelated to each other, such as *Athrips* Billberg, 1820 (1 of 70+ spp., Palaearctic), *Catatinagma* Rebel, 1903 (1

of 5 spp., Palaearctic), *Caulastrocecis* Chrétien, 1931 (2 of 7 spp., Palaearctic), *Cerofrontia* Janse, 1951 (1 of 1 sp., Afrotropical), *Leistogenes* Meyrick, 1927 (1 of 1 sp., Neotropical), *Ornativalva* Gozmány, 1955 (about 15 of 60+ spp., Palaearctic), *Prolita* Leraut, 1993 (8 Nearctic, of 22 spp., Holarctic), *Scrobipalpa* Janse, 1951 (2 Palaearctic, of 300+ spp. worldwide) and others. Examples were illustrated by Hodges (1966, pl. 8, *Prolita*) and Sattler (1976, pls 6–12, *Ornativalva*). Such specializations of the head structure can provide useful characters for species recognition but they are not important at the generic level as in several genera (*Athrips*, *Ornativalva*, *Prolita*, *Scrobipalpa*) there exist species with processes next to such without. In Gelechiidae there seems to be a concentration of species with frontal processes in arid parts of the Palaearctic and Nearctic regions.

Frontal modifications are only obvious once the head is freed of scales, and even a prominent frontal process can otherwise remain hidden in the scale cover. On the denuded head the transfrontal sulcus, the border between frons and vertex, normally extends in an almost straight line between the antennal sockets and is clearly visible as a narrow band free of scale bases. If a discrete frontal process is developed it usually arises beneath the transfrontal sulcus which it tends to push dorsad. Various teeth and protuberances, when present, originate from enlarged scale bases, and the central process may be surrounded by such enlarged scale sockets which can be arranged into a distinctive arc. An additional arc of teeth sometimes arises above the transfrontal sulcus. The whole range of such developments is demonstrated in the genus *Ornativalva* Gozmány, 1955 (Sattler 1976: 92, pls 5–12), where the arrangement in *O. cornifrons* Sattler, 1976, is not unlike that in the unrelated *H. inderskella*.

Acknowledgements

References

Adamski D, Brown RL (1989) Morphology and systematics of North American Blastobasidae (Lepidoptera: Gelechioidea). Mississippi Agriculture and Forest Experiment Station Technical Bulletin No. 165. Mississippi Entomological Museum No. 1: 1–70. [figs 1–26]

Caradja A (1910) Beitrag zur Kenntnis über die geographische Verbreitung der Pyraliden des europäischen Faunengebietes nebst Beschreibung einiger neuer Formen. Deutsche Entomologische Zeitschrift Iris 24: 105–147. https://biodiversitylibrary.org/page/12688658

Caradja A (1920) Beitrag zur Kenntnis der geographischen Verbreitung der Mikrolepidopteren des palaearktischen Faunengebietes nebst Beschreibung neuer Formen. III. Teil. Deutsche Entomologische Zeitschrift Iris 34: 75–179. https://biodiversitylibrary.org/page/12720924

Chrétien P (1915) Contribution à la connaissance des Lépidoptères du nord de l'Afrique. Annales de la Société Entomologique de France 84: 289–374. https://biodiversitylibrary.org/page/8559267

Hodges RW (1966) Revision of Nearctic Gelechiidae, I. The *Lita* group (Lepidoptera: Gelechioidea). Proceedings of the United States National Museum 119(3547): 1–66. https://biodiversitylibrary.org/page/7760379

Hodges RW (1986) Fascicle 7.1, Gelechioidea: Gelechiidae (in part). In: Dominick RB, et. al. (Eds) The Moths of North America North of Mexico. Allen Press, Lawrence, Kansas 8: 1–195.

Karsholt O, Riedl T, Povolný D, Huemer P (1996) Gelechiidae. In: Karsholt O, Razowski J (Eds) The Lepidoptera of Europe. A distributional checklist. Apollo Books, Stenstrupia, 103–122.

Kaszab Z (1966) Ergebnisse der zoologischen Forschungen von Dr. Z. Kaszab in der Mongolei. 107. Liste der Fundorte der IV. Expedition. Folia entomologica hungarica (Series nova) 19: 569–620. [1 map]

Kornerup A, Wanschner JH (1978) Methuen Handbook of Colour (2nd edn). Methuen & Co. Ltd, London, 243 pp.

Lee S, Brown RL (2008) Revision of Holarctic Teleiodini (Lepidoptera: Gelechiidae). Zootaxa 1818: 1–55. [figs 1–66] http://www.mapress.com/zootaxa/2008/f/zt01818p055.pdf

Li H, Sattler K (2012) A taxonomic revision of the genus *Mesophleps* Hübner, 1825 (Lepidoptera: Gelechiidae). Zootaxa 3373: 1–82. [figs 1–150; Original wrapper of reprint erroneously dated 4 July 2011] http://www.mapress.com/zootaxa

Ponomarenko MG (2008) Functional morphology of the male genitalia in Gelechiidae (Lepidoptera) and its significance for phylogenetic analysis. Nota Lepidopterologica 31(2): 179–198.

Ponomarenko MG (2009) Functional morphology of the male genitalia in the gelechiid-moth genera *Holcophora* Stgr., *Ananarsia* Ams., and *Nothris* Hbn. (Lepidoptera, Gelechiidae: Gelechiini, Dichomeridini). Entomologicheskoe Obozrenie 88(1): 135–142. [In Russian, with English summary; figs 1–8; English translation: 2009, Entomological Review. Washington 89(2): 191–197]

Popescu-Gorj A (1992) Le catalogue des types de Lépidoptères gardés dans les collections du Muséum d'Histoire Naturelle "Grigore Antipa" (Bucarest) (Fam. Micropterigidae–Pterophoridae). Travaux du Muséum d'Histoire Naturelle 'Grigore Antipa' 32: 131–184. [figs 1–11]

Sattler K (1976) A taxonomic revision of the genus *Ornativalva* Gozmány, 1955 (Lepidoptera: Gelechiidae). Bulletin of the British Museum (Natural History) (Entomology) 34: 85–152. [pls 27] https://biodiversitylibrary.org/page/41004900

Sinev SY (2014) World catalogue of blastobasid moths (Lepidoptera, Blastobasidae). Russian Academy of Sciences, Zoological Institute, St Petersburg, 108 pp.

Staudinger O (1870–1871) Beschreibung neuer Lepidopteren des europäischen Faunengebiets. Berliner Entomologische Zeitschrift 14: 97–132, 193–208 [1870, April]; 273–330 [1871, January]. https://biodiversitylibrary.org/page/8340719

Turati E (1930) Novità di lepidotterologia in Cirenaica. III. Atti della Società Italiana di Scienze Naturali e dei Museo Civico di Storia Naturale in Milano 69: 46–92. [10 text-figs [unnumbered], pl. 2]

Walsingham Lord (1904–1911) Algerian Microlepidoptera. The Entomologist's Monthly Magazine 40 [1904]: 214–223, 265–273; 41 [1905]: 37–41, 124–128; 43 [1907]: 6–10, 55– 58, 125–129, 147–154, 187–195; 47 [1911]: 12–15, 187–191. https://biodiversitylibrary.org/page/8958392

Redescription of the alpine *Incurvaria stangei* Rebel, 1903 (Lepidoptera, Incurvariidae), reinstated from Prodoxidae

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http://zoobank.org/282B6590-CADE-4745-AA51-D64DDD4D7303

Abstract. *Incurvaria stangei* Rebel, 1903 revised combination, placed without justification in Prodoxidae, is redescribed and transferred back to the Incurvariidae. Male and female genitalia of this south-eastern alpine endemic species are illustrated for the first time. DNA barcode sequences as well as morphological traits support the revised family assignment.

Introduction

Incurvariidae and Prodoxidae are two out of formerly five (van Nieukerken et al. 2011), now six (Regier et al. 2015) known families of the superfamily Adeloidea Bruand, 1850. The type-species for Incurvariidae is *Incurvaria masculella* (Denis & Schiffermüller, 1775) and for Prodoxidae *Prodoxus decipiens* Riley, 1880 (Nye and Fletcher 1991). The monophyly of both families is well supported by a morphologically based phylogenetic analysis (Nielsen and Davis 1985) and by recently published molecular studies (Mutanen et al. 2010; Regier et al. 2015).

Incurvaria stangei was described by Rebel (1903) from a single female collected in the Italian Dolomites (Südtirol, Tschierspitze, 31.vii.1902) by G. Stange. Shortly after this discovery a single male was found in the area of Triglav mountain range, currently Slovenia (Rebel 1907). Despite its extraordinary and unmistakable external appearance, this remarkable alpine species of primitive moth remained neglected for more than eight decades. Only in 1988 P. Huemer discovered a specimen and published a re-description, although without examination of genitalic structures (Huemer 1991). Nevertheless, he still treated the species in the family Incurvariidae Spuler, 1898. In contrast, Wojtusiak (1996) in the European Lepidoptera catalogue listed the species under *Lampronia* Stephens, 1829 in the family Prodoxidae Riley, 1881 for the first time, without giving arguments for the new combination. Subsequent publications followed this new taxonomic concept (e.g. Deutsch 2012; Huemer 2013; Karsholt and Nieukerken 2004–2011). However, Kurz (2002–2012), after dissection and careful examination of a female, reconsidered the taxonomy and informally recombined the species in *Incurvaria* Haworth, 1828 (Incurvariidae). This work

is now supported after an extensive analysis of characters of the male and female genitalia and cytochrome *c* oxidase subunit I (COI-5P) DNA barcodes and we therefore now formally present this revised combination.

Material and methods

Our study is based on examination of ten specimens of *I. stangei* which are all deposited in the collections of the Tiroler Landesmuseum Ferdinandeum, Natural History Collections, Hall, Austria. Genitalia preparations followed standard techniques (Robinson 1976), with some modifications, especially the implementation of the so-called unrolling technique in male genitalia (Pitkin 1986). Although genitalia of both sexes are strongly sclerotized, male preparations were slightly stained with Mercurochrome and females with Chlorazol to highlight delicate structures. For photos, genitalia of both sexes were embedded in Euparal with ventral side up, male genitalia being unrolled. Additionally, male genitalia were photographed in their natural three-dimensional shape before spreading, both from lateral and dorsal aspect. Photos of adults were taken with a Canon EOS 5D Mark III and Canon lens MP-E 65 at 2:1, using a ring flash. Genitalia photos were taken with a microscope (Wild Heerbrugg) using a 10x objective and a 2.5x ocular. All photos were edited using the software HELICON FOCUS 4.80 and ADOBE PHOTOSHOP 6.0. For sequencing a single leg was removed, placed in a 96-well lysis plate and sent for analysis to the CCDB (Canadian Centre for DNA Barcoding, University of Guelph, Canada) where DNA extraction, PCR amplification, and sequencing were performed following standard high-throughput protocols (DeWaard et al. 2008). A Neighbor-Joining (NJ) tree of 26 European species of Incurvariidae and Prodoxidae plus a species of the type genus *Prodoxus* Riley, 1880 was constructed using MEGA 7 (Kumar et al. 2016). This tree is based on 132 barcode sequences which are available in the public dataset "DS-LEPIINCU Lepidoptera - Incurvarioidea" in the Barcode of Life Datasystems BOLD (Ratnasingham and Hebert 2007). Node confidences were assessed using 500 bootstrap replicates.

Abbreviations:
NHMW   Naturhistorisches Museum, Wien, Austria.
TLMF   Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria.

Results
Incurvaria stangei Rebel, 1903, revised combination

Lampronia stangei (Rebel, 1903) Wojtusiak 1996: page 29.

Type material. Holotype, female: Italy, South Tyrol, Tschierspitze, Felsgebiet nördlich des Grödner Jochs, 2500 m, 31.vii.1902, leg G. Stange, NHMW [not examined, see remarks].

Material examined. Austria: 1 male, East Tyrol, Lienzer Dolomiten, Karlsbaderhütte, 2300–2400 m, 02.viii.2013, leg. H. Deutsch, TLMF, barcode sample ID BC TLMF Lep 24036; 1 male, East Tyrol, Laserzgebirge, Lavanter Almtal, 1200 m, 27.vii.2012, leg. H. Deutsch, TLMF. Slovenia: 3 male, 1 female, Goriška, Julische Alpen, Triglav, Planika-Hütte, 2400 m, 05.viii.1991, leg. H. Habeler, TLMF; 1 male, Goriška, Julis-
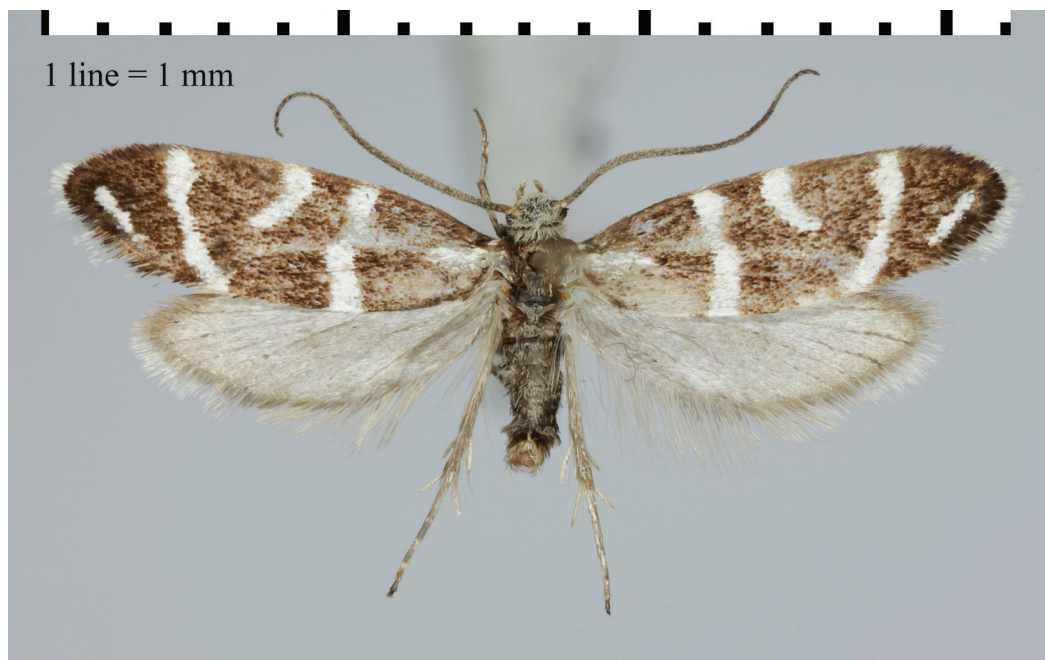
1 line = 1 mm

Figure 1. *Incurvaria stangei* Rebel, 1903, male, Austria, East Tyrol, Laserzgebirge, Lavanter Almtal, 1200 m, 27.vii.2012, leg. H. Deutsch.

che Alpen, Kamin, Podi, 2200–2300 m, 19.vii.1996, leg. H. Deutsch, TLMF. Italy: 1 male, Friuli-Venezia Giulia, Udine, Monte Sernio Massiv, Forcella Nuviernulis, 1700 m, 16.vii.1988, leg. P. Huemer, TLMF; 1 female, Lombardia, Bergamo, Alpi Orobie, Pizzo Arera, 2500 m, 20.vii.1992, leg. P. Huemer & G. Tarmann, TLMF; 1 male, Friuli-Venezia Giulia, Pordenone, Val Montanaia, linker Talhang/ Cimolais N, 2100 m, 23.vi.2017, leg. B. Wiesmair & P. Schattanek, TLMF, barcode sample ID BC TLMF Lep 24036.

Description. Adult (Figs 1, 2). Forewing length: male (n=8), 7.0–8.0 mm; female (n=2), 5.5–6.5 mm.

Head covered with yellowish scales, frons whitish; eyes hemispheric, black; labial palpi bright yellowish brown, apically whitish; antennae in both sexes delicately ciliated, brown, covered with small white scales in fresh specimens; thorax brownish black with golden shine; abdomen greybrown; ground colour of forewing upper side black-brown with a distinct white wing pattern consisting of: a short longitudinal basal streak, two fasciae developed as small narrow bands, inner fascia at 1/3 of the wing length runs from costal to dorsal margin (costal nearer to wing-base), outer fascia at 3/4 is arranged in the opposite direction, costal margin at 1/2 with a subtriangular spot, a short horizontal streak in the marginal area near the apex of the wing, fringes basal brownish black, distal white. Underside with the same pattern, but more blurred. Hindwings shiny grey, fringes basally dark grey, distal part brighter grey to white; legs dirty white to beige, tibiae and tarsi brownish beige, fore-tibia without epiphysis, hind-tibia with a pair of pre-apical spurs, with a long tuft of hair-like scales and a pair of long apical spurs.

DNA barcode. BOLD (BIN): BOLD:ADI9579, (Process ID):
LEAST678-17
LEAST151-17

Figure 2. *Incurvaria stangei* Rebel, 1903, female, Slovenia, Goriška, Julische Alpen, Triglav, Planika-Hütte, 2400 m, 05.viii.1991, leg. H. Habeler.
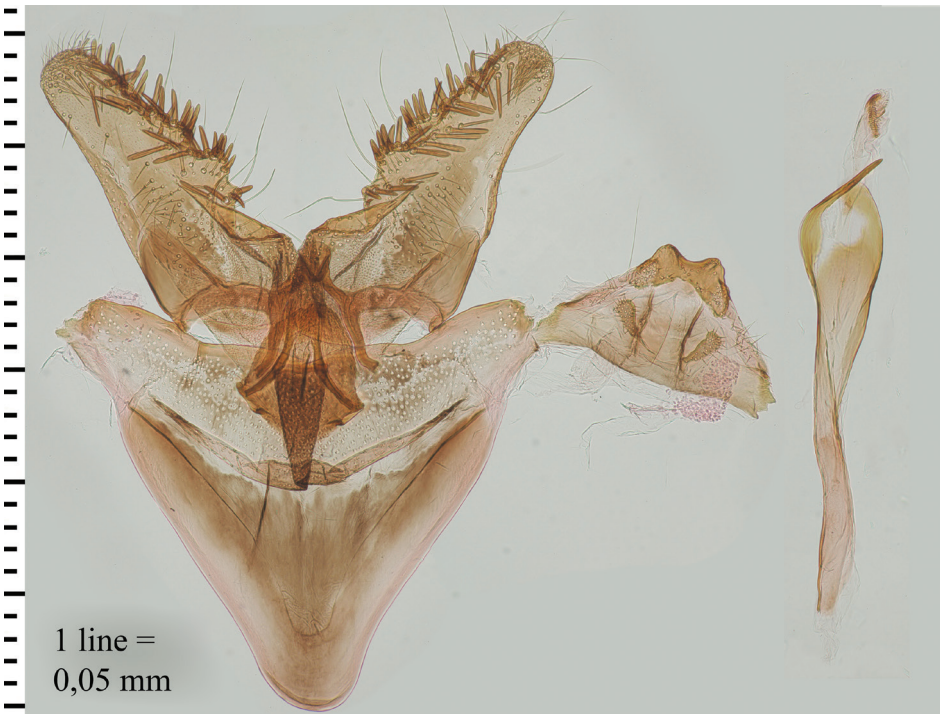

Figure 3. Unrolled male genitalia of *Incurvaria stangei* Rebel, 1903, Slovenia, Goriška, Julische Alpen, Triglav, Planika-Hütte, 2400 m, 05.viii.1991, leg. H. Habeler, Gen.Präp. m3331 P. Buchner.

Figure 4. left: Female abdominal segments, right: Female genitalia of *Incurvaria stangei* Rebel, 1903, Slovenia, Goriška, Julische Alpen, Triglav, Planika-Hütte, 2400 m, 05.viii.1991, leg. H. Habeler, Gen.Präp. w3349 P. Buchner.

Male genitalia (Fig. 3). Uncus and tegumen short, forming a trapezoid plate, posterior margin of uncus medially concave forming a double lobed structure; socius distinctly sclerotized, club-shaped, long, distally widened; transtilla with distinct medial knob and strongly sclerotized lateral arms; sub-medial anterior projections relatively long, finger-shaped and of equal width, ending in an edge; valva short and robust; costal margin slightly concave; cucullus hirsute, with a set of distinct spines along the margin, apex hirsute and rounded; vinculum V-shaped, broad and short, distal end broad and rounded.

Female genitalia (Fig. 4). Pre-genital segments. Sternum VII as well as tergum VII of rectangular shape. Posterior part of ovipositor triangular, tip rounded, lateral margins concave with two steps; apophyses posteriores and anteriores both relatively long, with blunt ends; ductus bursae as well as bursa copulatrix short, the latter bulky and without signa; ductus spermathecae with several convolutions.

Diagnosis. Due to the unique colouration and pattern of the forewings of both sexes, *I. stangei* can be easily separated from all other members of Adeloidea.

Bionomics. The biology of *I. stangei* is unknown; the host plant as well as primary stages are still undiscovered. Huemer (1991) assumed that adults are active in the early morning hours before

Figure 5. Habitat of *Incurvaria stangei* Rebel, 1903 in Friuli-Venezia Giulia, Pordenone.

sunrise, similar to some other alpine incurvariids such as e.g. *I. vetulella* (Zetterstedt, 1839). However, Deutsch (2012) also reported attraction to artificial light. The poorly known life habits likely explain the small number of observed individuals. The adults were found in the second half of July, mainly at the border zone between the subalpine shrubs and alpine meadows from about 1700 to 2500 m elevation (Huemer 1991) (Fig. 5). Deutsch (2012), however, found a single male at a lower elevation (1250 m), which is suspected to have been drifted from its natural habitat.

Distribution. The species is endemic to the south-eastern Alps: Julian Alps: Slovenia, Goriška (Carnelutti 1978; Habeler and Gomboc 2005; Govedič and Lesar 2010; Rebel 1907); Carnic Alps: Italy, Friuli-Venezia Giulia (Huemer 1991; unpublished record), Austria, East Tyrol (Deutsch 2012); Dolomites: Italy, South Tyrol (Rebel 1903); Orobian Alps: Italy, Lombardia (unpublished record).

Remarks. Despite of a personal search by PH, the holotype could not be traced at NHMW. The Rebel collection only contains a label of the species but without any evidence of the specimen, even a pinhole is absent.

Discussion

The family concept of Incurvariidae and Prodoxidae and consequently the correct combination of genera and species remained obscure through most of the 20th century. Even in some recent literature the European genera of Prodoxidae were lumped in the family Incurvariidae (Karsholt et al.
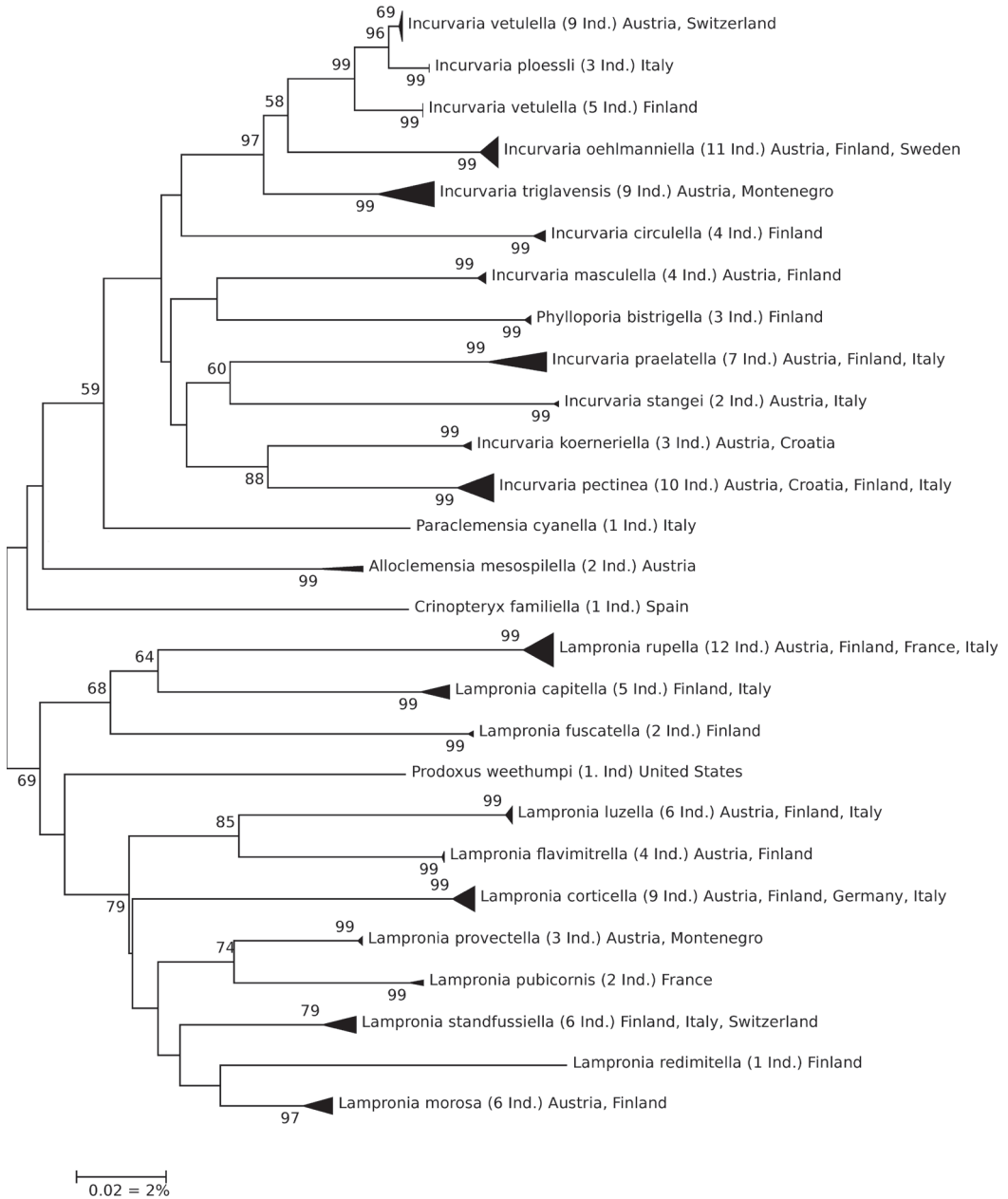
Figure 6. Neighbor-Joining tree based on DNA barcodes (658 bp fragment of the mt COI gene) of European Incurvariidae and Prodoxidae. The node support values in percentages are based on 500 bootstrap replicates.

1995). However, already Nielsen (1982, 1985) convincingly separated these families based on diagnostic characters, primarily of male and female genitalia morphology. Accordingly, males of Prodoxidae can be recognized by the presence of well-defined pectinifers of the valva, whereas in the female genitalia, Prodoxidae possess a pair of stellate signa in the corpus bursae and a posteri-

orly well-rounded sternum VII and a triangular tergum VII. Furthermore, the larvae of Prodoxidae – as far as known – are endophagous in herbs and shrubs, whereas nearly all larvae of Incurvariidae are free living in self-constructed cases.

The male genitalia of *I. stangei* lack the family characteristic pectinifers typical of Prodoxidae and the more flattened, scale-shaped spines of the valva are grouped together. These characteristics support the placement of *I. stangei* in Incurvariidae. Similarly the female genitalia of *I. stangei*, which completely lack stellate signa, perfectly fit into the concept of Incurvariidae as proposed by Nielsen (1982). Furthermore, sternum and tergum VII are both of rectangular shape, characteristic for Incurvariidae. In contrast, a posteriorly well-rounded sternum VII and a triangular tergum VII is considered diagnostic for Prodoxidae (Nielsen 1982).

A Neighbor Joining tree analysis was derived from DNA barcode sequences (COI) of selected European species of Incurvariidae and Prodoxidae with *I. stangei* nesting in Incurvariidae. Considering a mean interspecific distance of 8.5% (min. 1.2%, max. 13.7%) in Incurvariidae, *I. stangei* shows a remarkable distance (12%) to its closest species, *Paraclemensia acerifoliella* (Fitch, 1854), from North America, while it is over 12.3% to *Incurvaria praelatella* (Fig. 6).

Acknowledgments

References

Carnelutti J (1978) Živalski svet Triglava. Proteus 41(3): 83–87.

Deutsch H (2012) *Lampronia stangei* Rebel, 1903 (Lepidoptera: Prodoxidae): ein überraschender Fund in den Lienzer Dolomiten und Erstnachweis für Österreich. Zeitschrift der Arbeitsgemeinschaft Österreichischer Entomologen 64: 71–72.

DeWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA Barcodes: Analytical Protocols. In: Cristofre M (Ed.) Methods in Molecular Biology: Environmental Genetics. Humana Press Inc., Totowa, 275–293. https://doi.org/10.1007/978-1-59745-548-0_15

Govedič M, Lesar T (2010) Check list of Slovenian Microlepidoptera. Natura Sloveniae 12: 35–125.

Habeler H, Gomboc S (2005) Bemerkenswerte Schmetterlingsfunde aus Slowenien mit Erstnachweisen. Acta Entomologica Slovenica 13: 29–52.

Huemer P (1991) *Incurvaria stangei* Rebel, 1903, ein bemerkenswerter Kleinschmetterling aus den Südalpen (Lepidoptera, Incurvariidae). Berichte des naturwissenschaftlich-medizinischen Vereins in Innsbruck 78: 173–175.

Huemer P (2013) Die Schmetterlinge Österreichs (Lepidoptera). Systematische und faunistische Checkliste Studiohefte 12. Tiroler Landesmuseen-Betriebsges.m.b.H., Innsbruck, 304 pp.

Karsholt O, Nieukerken EJ van (Eds) (2004–2011) Lepidoptera, Moths. Fauna Europaea versions 1.1–2.4. http://www.faunaeur.org [Accessed 22.ix.2018]

Karsholt O, Nieukerken EJ van, Whitebread S, Zangheri S (1995) Lepidoptera Zeugloptera, Dacnonypha, Exoporia, Monotrysia (=Micropterigoidea, Eriocranioidea, Hepialoidea, Nepticuloidea, Incurvarioidea, Tischerioidea). In: Minelli A, Ruffo S, La Posta S (Eds) Checklist delle specie della fauna italiana 80. Calderini, Bologna, 1–12.

Kurz MA, Kurz ME (2000–2012) Naturkundliches Informationssystem. http://www.nkis.info/nkis/extaustaxonshow.cgi?uid=TaxOn&tax=339&lang=e [Accessed 10.ix. 2018]

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Mutanen M, Wahlberg K, Kaila L (2010) Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. Proceedings of the Royal Society B 277: 2839–2849. https://doi.org/10.1098/rspb.2010.0392

Nielsen ES (1982) Incurvariidae and Prodoxidae from the Himalayan area (Lepidoptera: Incurvarioidea). Insecta Matsumurana 26: 187–200.

Nielsen ES (1985) The monotrysian heteroneuran phylogeny puzzle: a possible solution. Proceedings of the 3rd Congress of Lepidopterology, Cambridge, 138–143.

Nielsen ES, Davis DR (1985) The first southern hemisphere prodoxid and the phylogeny of the Incurvarioidea (Lepidoptera). Systematic Entomology 10: 307–322. https://doi.org/10.1111/j.1365-3113.1985.tb00140.x

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

Nye IWB, Fletcher DS (1991) The generic names of the moths of the world. Vol. 6. Microlepidoptera. Natural History Museum Publications, London, 368 pp.

Pitkin L (1986) A technique for the preparation of complex male genitalia in Microlepidoptera. Entomologist's Gazette 37: 173–179.

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2006.01678.x

Rebel H (1903) Neue Microheteroceren aus Österreich-Ungarn. Verhandlungen der kaiserlich-königlichen zoologisch-botanischen Gesellschaft in Wien 53: 90–103.

Rebel H (1907) Lepidopteren aus dem Gebiete des Triglav und der Crna Prst in Krain. I. Nachtrag. Jahresberichte Wiener entomologischer Verein 17: 33–60.

Regier JC, Mitter C, Kirstensen NP, Davis DR, Nieukerken EJ van, Rota J, Simonsen TJ, Mitter KT, Kawahara AY, Yen S-H, Cumming MP, Zwick A (2015) A molecular phylogeny for the oldest (nonditrysian) lineages of extant Lepidoptera, with implications for classification, comparative morphology and life-history evolution. Systematic Entomology 40(4): 671–704. https://doi.org/10.1111/syen.12129

Robinson G (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. Entomologist's Gazette 27: 127–132.

Wojtusiak J (1996) Prodoxidae. In: Karsholt O, Razowski J (Eds) The Lepidoptera of Europe. A Distributional Checklist. Apollo Books, Stenstrup, 29–30.

Dichrorampha carpatalpina sp. n. (Lepidoptera, Tortricidae), a high mountain species of the Romanian Carpathians

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Abstract. *Dichrorampha carpatalpina* **sp. n.**, is described from the Southern Carpathians (Romania). It is closely related to *D. inconspiqua* (Danilevsky, 1948) and *D. podoliensis* (Toll, 1942). Adults, male and female genitalia, the habitat of the new species are described and figured and some details of the biology are given. The larva and its host-plant, *Achillea oxyloba schurii* (Sch. Bip.) Heimerl, are illustrated. The species inhabits the north-facing slopes of the highest regions of the Carpathians. During their flight period from the beginning of July to the beginning of August the moths are on wing in the morning and only in sunny weather.

Introduction

In the last few years important progress has been made in the knowledge of the high mountain *Dichrorampha* fauna of the Balkan Peninsula. A study of recently collected and historical material clarified the taxonomic status of *D. rilana* Drenowsky, 1909 and described *D. dinarica* Huemer, Zlatkov & Baixeras, 2012, a century-long misidentified species (Huemer et al. 2012). The previously unknown female of *D. rilana* was also described (Zlatkov 2013). At the same time *D. inconspiqua* (Danilevsky, 1948) was identified as new to the European fauna (Zlatkov and Budashkin 2010; Trematerra and Colacci 2017) and *D. pentheriana* (Rebel, 1917), a species previously known only from its type locality in Montenegro, was recorded from Bulgaria (Zlatkov 2016).

In the present paper we increase the knowledge of the high mountain *Dichrorampha* fauna of the Balkan Peninsula by describing a new species discovered in the subalpine and alpine regions of the Southern Carpathians, the high mountain chain forming the border between Central Europe and the Balkan Peninsula. The first males were collected nearly three decades ago, in 1989 in the Bucegi Mountains. In 1994 it was also discovered in the Făgăraş Mountains, when our first attempt to identify the species was unsuccessful because it was not in Kuznetsov (1978). The suspicion that it was an undescribed species arose when the second volume of the Tortricidae of Europe (Razowski 2003), including the genus *Dichrorampha*, was published. Our efforts to collect additional material were moderately successful initially, but consistent and persistent searches in the last few years resulted in numerous specimens being collected, and the discovery of the female, host-plant, larva and new sites. The specimens differ both externally and in the male and female genitalia from the known species of *Dichrorampha*, and are described below as a new species.

Material and methods

The examined material is dried, pinned and set. The genitalia preparation technique used by the authors is a simplified variant of the usual method, with unmounted preparations being stored in glycerine tubes. Only the preparation of genitalia slides that were made to be photographed followed standardized techniques (Robinson 1976). Photographs of the adults, larvae, host-plant and habitat were taken using Sony DSC–H2 and Sony DSC–W830 digital cameras. Photographs of the genitalia were taken using an Olympus SZX16 microscope with a motorized focus drive attached to an Olympus E520 digital camera. Images have been edited in ADOBE Photoshop.

Abbreviations

TLMF　　　Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria;

ZMUC　　　Zoological Museum, Natural History Museum of Denmark, Copenhagen, Denmark.

Results

Dichrorampha carpatalpina sp. n.

http://zoobank.org/4A7BD4E3-32AA-4DF2-BFD5-B600F8E38391

Figs 1–8, 12

Holotype. ♂. [România] Carpaţii Meridionali, Munţii Făgăraş, Căldarea Bâlea, 2100–2200 m, 14.vii.2016, legit & coll. S. & Z. Kovács (Miercurea Ciuc).

　　Paratypes. 112 ♂♂, 9 ♀♀, all collected by S. & Z. Kovács and if not otherwise mentioned are deposited in the authors' collection. [România, Carpaţii Meridionali, Munţii] Bucegi, Valea Jepilor, 1600 m, 8.vii.1989 (2 ♂♂); Idem, but 1900 m, 1.viii.1990 (♂); [România, Carpaţii Meridionali, Munţii] Bucegi, Caraiman, 2100 m, 1.viii.1990 (♂); Idem, but 2300 m, 22.vii.2006 (♂), genit. prep. no. 1731/♂/ Kovács (2015); Idem, but 2380 m, 9.vii.2013 (♂); Idem, but 2300 m, 8.vii.2015 (2 ♂♂); [România] Carpaţii Meridionali, Munţii Făgăraş, V[âr]f[ul]. Paltinu, 2350 m, 29.vii.1994 (3 ♂♂), genit. prep. no. 675/♂/ Kovács (1994); Idem, but 2400 m, 24.vii.2006 (♂); Idem, but 2200 m, 10.vii.2013 (5 ♂♂), 1 ♂ and slide no. 5288/♂/ O. Karsholt (2015), coll. ZMUC; Idem, but 2300 m, 10.vii.2013 (2 ♂♂); Idem, but 2300 m, 21.vii.2015 (3 ♂♂); Idem, but 2300 m, 14.vii.2016 (4 ♂♂); [România] Carpaţii Meridionali, Munţii Făgăraş, Căldarea Bâlea, 2100 m, 10.vii.2013 (♀), slide no. 5289/♀/ O. Karsholt (2015), coll. ZMUC; Idem, but 2100–2200 m, 21.vii.2015 (30 ♂♂, 2 ♀♀), genit. prep. no. 1732/♂/ Kovács (2015); Idem, but 2100–2200 m, 14.vii.2016 (9 ♂♂), 2 ♂♂ coll. TLMF; Idem, but 2100–2200 m, 18.vii.2016. ex pupa ([from samples of] *Achillea oxyloba* subsp. *schurii* (Sch. Bip.) Heimerl [with a shallow layer of soil] 14.vii.2016) (♂); Idem, but 2100–2200 m, 20.vii.2016 ex pupa ([from samples of] *Achillea oxyloba* subsp. *schurii* (Sch. Bip.) Heimerl [with a shallow layer of soil] 14.vii.2016) (2 ♀♀); Idem, but 2100–2200 m, 22.vii.2016 ex pupa ([from samples of] *Achillea oxyloba* subsp. *schurii* (Sch. Bip.) Heimerl [with a shallow layer of soil] 14.vii.2016) (♀); [România] Carpaţii Meridionali, Munţii Făgăraş, V[âr]f[ul]. Vânătoarea lui Buteanu, 2400–2500 m, 22.vii.2015 (18 ♂♂, 1 ♀), genit. prep. no. 1733/♂/ and 1735/♀/ Kovács (2015); [România] Carpaţii Meridionali, Munţii Făgăraş, Vârful Văiuga, 2300–2400 m, 22.vii.2015 (9 ♂♂); [România] Carpaţii Meridionali, [Munţii] Piatra Craiului, Brâna Caprelor, 1700 m, 9.vii.2015 (♂); Idem, but 1800 m, 23.vii.2015 (♂); [România] Carpaţii Meridionali, [Munţii] Piatra Craiului, Vârful Padina Popii, 2000 m, 9.vii.2015 (11 ♂♂, 1 ♀); Idem, but 23.vii.2015 (6 ♂♂, 1 ♀), genit. prep. no. 1734/♂/ Kovács (2015).

Figure 1. *Dichrorampha carpatalpina* sp. n., male, holotype, wingspan: 15.5 mm, Făgăraş Mountains, Căldarea Bâlea, 2100–2200 m, 14.vii.2016, photograph Z. Csata.

Figure 2. *Dichrorampha carpatalpina* sp. n., male, paratype, wingspan: 13.5 mm, same data as holotype, photograph Z. Csata.

Figure 3. *Dichrorampha carpatalpina* sp. n., female, paratype, wingspan: 13 mm, Făgăraş Mountains, Căldarea Bâlea, 2200 m, ex pupa, 20.vii.2016, photograph Z. Csata.

Description. Male (Figs 1–2). Head. Frons and vertex covered with brown scales. Ocellus present. Proboscis developed. Labial palpus about 2.5 times length of diameter of eye. First segment short, covered with short brown scales, second segment long, covered with long brown scales forming large trapezoidal brush, with pale yellow scales on central part of lateral surface. Segment 3 short and almost fully hidden by scalebrush of segment 2. Antennae filiform, brown.

Thorax. Dorsally covered with brown scales, similar to tegulae and ground colour of forewing. Wingspan of holotype 15.5 mm. Forewing with costal fold to about 1/3 of length of costa, apex slightly pointed, but with fringes appearing rounded. Brown ground colour of forewing extended at base and along dorsal margin. On distal 2/3 along costa brown ground colour mottled due to some mixed yellow scales. In subterminal, terminal and apical areas most brown scales replaced by yellow ones, remaining brown scales forming small patches along costa and few ill-defined, narrow and fragmented transverse lines. Large triangular dorsal patch greyish brown, mixed distally with small groups of yellow scales, and dorsally with brown scales. All other forewing markings which usually characterize species of the genus *Dichrorampha* namely costal strigulae, interspaces, metallic plumbeous striae and row of black dots along termen absent. Fringes light brown with brown basal line. Hindwing brown, fringes light brown with well defined brown basal line. Underside of thorax, wings and legs iridescent greyish brown.

Abdomen dorsally covered with brown scales, ventrally iridescent greyish brown. Male genitalia (Fig. 4). Uncus a small rounded apical prominence on arched tegumen. Gnathos weak, ribbon-like. Valva broad, basal cavity wide. Long sacculus rather straight and parallel with costa. Conspicuous caudal angle of sacculus nearly rectangular, ventral emargination deep and neck of valva short. Large and slightly pointed cucullus with broad ventral corner, distally covered with numerous long setae and short spiniform setae at ventro-distal edge. Juxta sub-triangular, anellus

Figure 4. *Dichrorampha carpatalpina* sp. n., male genitalia, paratype, Făgăraş Mountains, slide 5288 and photograph O. Karsholt.

Figures 5–7. *Dichrorampha carpatalpina* sp. n., male genitalia, paratypes, variation of end of phallus. **5.** Prep. genit. no. 5288, Făgăraş Mountains; **6.** Prep. genit. no. 1734, Piatra Craiului Mountains; **7.** Prep. genit. no. 1731, Bucegi Mountains.

without lobes. Phallus curved ventrally, moderately long, about 2/3 length of valva, without cornuti, distal half with large dorsal slit. Widened end of phallus with single triangular lateral tooth.

Variation. The wingspan varies between 12 and 16.5 mm, the colour of the yellow scales varies from yellowish grey to golden. The dorsal patch may be divided by one or two weak, brown, fragmented transverse lines. In worn specimens the mottling and dorsal patch become almost indistinguishable and so these moths look more or less uniformly brown. Male genitalia show small variation (n = 6) in the size and shape of the lateral tooth at the end of the phallus (Figs 5–6); one specimen has a small second tooth (Fig. 7).

Female (Fig. 3). Antennae narrower and shorter than male's. Forewing slightly broader at base, wingspan 13–15 mm. Brown ground colour more extensive, with fewer yellow scales in terminal

and apical areas compared with male. Female genitalia (Fig. 8). Papillae anales relatively large, apophyses anteriores almost twice as long as apophyses posteriores. Sterigma fused with seventh abdominal sternite, trapezoidal, with concave posterior margin, weakly convex lateral edges and almost straight anterior margin. Posterior part of ductus bursae forming large, strongly sclerotized tubular antrum, with its narrower anterior part not reaching anterior margin of seventh abdominal sternite. Anterior part of ductus bursae membranous, narrow, with distinct convex sclerite. Corpus bursae small, single signum a small rounded plate with a hook.

Diagnosis. The new species has the characters of the genus *Dichrorampha*: shape of wings, developed costal fold in male, reduction of vestiture of valva, neck of valva and caudal angle of sacculus clearly separate, distinct cucullus, phallus with large dorsal slit, sterigma fused with distal edge of seventh abdominal sternite, distinct convex sclerite of ductus bursae, and reduction to one signum (Razowski 2003).

Dichrorampha carpatalpina is unmistakable owing to its dominant brown colour and complete lack of most of the usual forewing pattern elements (strigulae, interspaces, metallic plumbeous striae, and row of black dots along termen). Only a faint greyish brown dorsal patch and the yellow mottled terminal and apical areas interrupt the uniform brown colour of the forewing. The male genitalia are also distinct. The long sacculus with nearly rectangular caudal angle, the robust cucullus, and the phallus widened terminally with 1(–2) lateral teeth are the main diagnostic features. The female genitalia are characterized by an evenly sclerotized antrum, which is shorter than the length of the seventh abdominal sternite, and a small corpus bursae.

Similar species. The genitalia resemble those of the species of the "section grunerianae" (Danilevsky and Kuznetsov 1968). Males of *Dichrorampha gruneriana* (Herrich-Schäffer, 1851), *D. melaniana* Nel & Varenne, 2016 and *D. infuscata* (Danilevsky, 1960) have a shorter sacculus, a longer neck of the valva, and a narrower ventral corner of the cucullus than *D. carpatalpina*. The poorly known *D. inconspiqua* is the most closely related species having a similarly shaped valva, but the longer and more pointed ventral corner of the cucullus and the phallus with a row of 5–6 terminal thorns clearly separate it from the newly described species. The valva of *D. podoliensis* (Toll, 1942) also has similar structure, but the phallus, which is considerably longer and bears a terminal row of small thorns, is strikingly different. The other members of the species-group, *D. nigrobrunneana* (Toll, 1942), *D. alpigenana* (Heinemann, 1863) and *D. tarmanni* Huemer, 2009 all have a shorter sacculus with a rounded caudal angle and a claw-like curved ventral corner of the cucullus. The antrum of the female of *D. carpatalpina* is shorter than the length of the seventh abdominal sternite, unlike in *D. nigrobrunneana*, *D. inconspiqua*, *D. alpigenana* and *D. melaniana*, in which the antrum is longer than the seventh abdominal sternite. The antrum of *D. tarmanni* is as long as that of the new species, but has a strong lateral fold and the shape of the seventh abdominal sternite also differs. *D. gruneriana* and *D. infuscata* possess a shorter antrum than the newly described species. The antrum of *D. podoliensis* has strong lateral folds, the ductus bursae is longer and the corpus bursae is larger than that of *D. carpatalpina* (Danilevsky and Kuznetsov 1968; Razowski 2003; Huemer 2009; Lepiforum 2016; Nel and Varenne 2016; Varenne and Nel 2017).

Systematic position. In view of the genitalia structure of both sexes, *Dichrorampha carpatalpina* should be placed near *D. inconspiqua* and their close relative *D. podoliensis*.

Habitat. *Dichrorampha carpatalpina* inhabits the highest mountains in the Romanian Carpathians. The moths are most abundant in the alpine zone from 2000 to 2500 m above sea level. Isolated
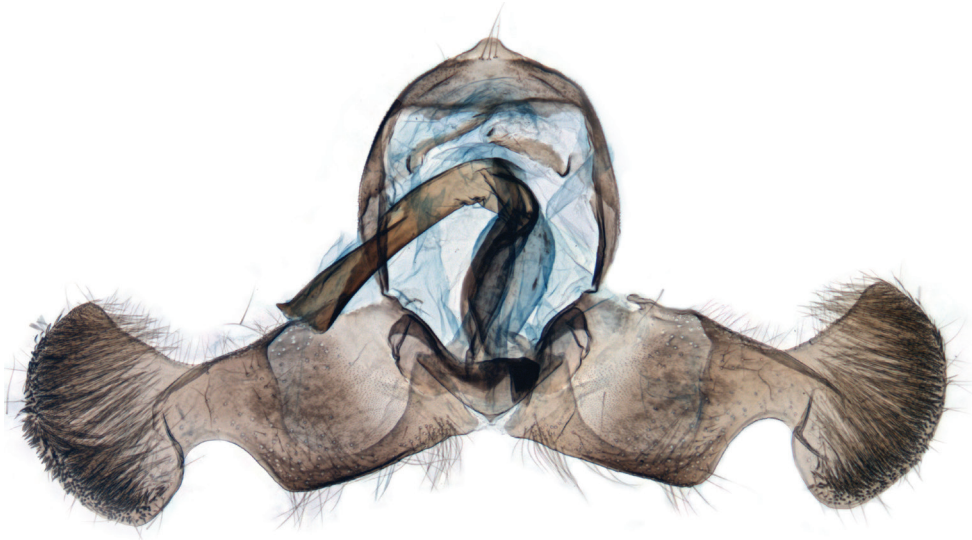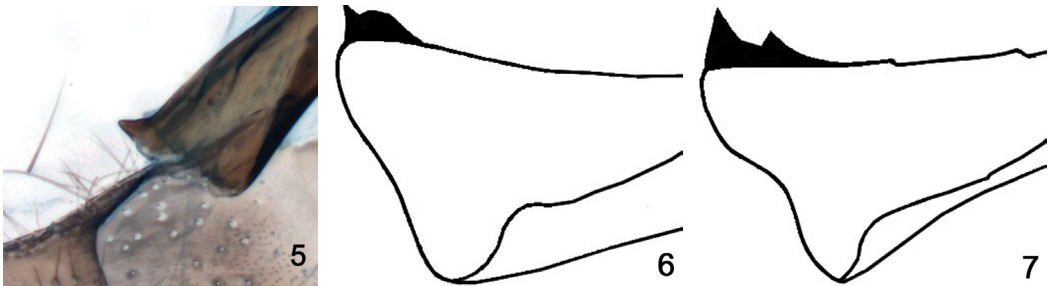
Figure 8. *Dichrorampha carpatalpina* sp. n., female genitalia, paratype, Făgăraş Mountains, slide 5289 and photograph O. Karsholt.

specimens were also observed in the subalpine zone between 1600 and 1900 m (5 males). The substrate is conglomerate in Bucegi, silicate in Făgăraş (Fig. 9) and limestone in Piatra Craiului Mountains (Fig. 10). The species prefers north-facing slopes with different inclinations, covered with sparse alpine vegetation (Fig. 11).

Phenology. Univoltine, adults are on the wing from the beginning of July to the beginning of August, highly depending on elevation, exposure of the sites and general weather conditions.

Figure 9. The habitat of *Dichrorampha carpatalpina* sp. n., Făgăraş Mountains, general view of north-facing slopes at 2200–2500 m, eastwards of Vânătoarea lui Buteanu Peak (north on left hand side).

Figure 10. The habitat of *Dichrorampha carpatalpina* sp. n., Piatra Craiului Mountains, north-west facing slopes at 2000–2100 m around Padina Popii Peak.

Figure 11. The habitat of *Dichrorampha carpatalpina* sp. n., Făgăraş Mountains, north-facing slope, Căldarea Bâlea, 2200 m.

At lower elevations, on north-east or north-west facing slopes they emerge earlier, from the beginning of July to mid July. At higher elevations and on north-facing sites adults emerge later, starting from mid July.

Biology. The early stages are still poorly known. Eggs are unknown. Larvae are yellowish white with a brown head (Fig. 12). They live in the soil, usually several individuals feeding on the roots of the host-plant. The latter, *Achillea oxyloba* (DC.) (Sch. Bip.) subsp. *schurii* (Sch. Bip.) Heimerl (Sârbu et al. 2013) (Fig. 13), was always common at the collecting sites. The place of pupation is unknown, but moths emerged within a few days of collecting samples of the host-plant with a shallow layer of soil. Pupation probably takes place in the spring after hibernation. Adults emerge in the morning, males a few days earlier than females. After the emergence of adults several differ-ent-sized larvae were found between the roots of the host-plant, which indicates that they hibernate more than once (a well-known life strategy in alpine Lepidoptera).

The moths fly only in sunshine as in cloudy weather we did not observe any kind of activity. In the morning, when north-facing slopes become sunny, males start to search for females. Their flight is not very fast, 10–20 cm above the ground, following a sinuous route, interrupted from time to time by short stops of only a few seconds on the dewy vegetation. Once disturbed their flight becomes faster and after 5–15 m they suddenly hide within the vegetation. During the afternoon the moths do not fly freely although in sunny weather they can be easily disturbed.

Females are less active than males. They rest on the vegetation and when disturbed they fly only a few metres before hiding again in the vegetation or between rocks. All the female specimens were collected during the morning or obtained from pupae. They were much rarer than males, the ratio

Figure 12. The larvae of *Dichrorampha carpatalpina* sp. n., 27.vii.2016, in laboratory (larvae collected: Făgăraş Mountains, Căldarea Bâlea, 2200 m, 14.vii.2016).

Figure 13. *Achillea oxyloba schurii* (Sch. Bip.) Heimerl, the host-plant of *Dichrorampha carpatalpina* sp. n., Făgăraş Mountains, Căldarea Bâlea, 2200 m.

being 1 female to 12 males collected. One morning a pair in copula was observed sitting on the low vegetation. Their mating was soon disturbed by other attracted males. *Dichrorampha carpatalpina* does not seem to be active by night as none was collected by light traps installed at sites where several specimens were observed during the same day.

Distribution. *Dichrorampha carpatalpina* seems to be widespread and locally common in the three main mountain ranges in the easten half of the Southern Carpathians: Bucegi, Făgăraş and Piatra Craiului. It may also occur in the western part, at least in the very similar Retezat and Parâng Mountains, where we made only one unsuccesful attempt to find it. The large distribution range of the host-plant, *Achillea oxyloba schurii*, along the whole Eastern and Southern Carpathians (Oprea 2005; Kliment et al. 2016) also suggests a wider distribution.

Etymology. The name „*carpatalpina*" alludes to the alpine range of the Southern Carpathians where the new *Dichrorampha* species was discovered. The epithet is used as an adjective.

References

Danilevsky AS, Kuznetsov VI (1968) Listovertki (Tortricidae). Triba plodozhorki (Laspeyresiini). Fauna SSSR, Nasekomye Cheshuekrylyle, 5(1). Nauka, Leningrad, 635 pp.

Huemer P (2009) *Dichrorampha tarmanni* sp. n. – ein Verwechslungsfall bei alpinen Schmetterlingen (Lepidoptera, Tortricidae). Wissenschaftliches Jahrbuch der Tiroler Landesmuseen 2: 110–119.

Huemer P, Zlatkov B, Baixeras J (2012) *Dichrorampha dinarica*, new species, a century of confusion in European lepidopterology (Lepidoptera: Tortricidae) resolved by combining morphology and DNA barcoding. Zootaxa 3389: 41–50.

Kliment J, Turis P, Janišová M (2016) Taxa of vascular plants endemic to the Carpathian Mts. Preslia 88: 19–76.

Kuznetsov VI (1978) Tortricidae. In: Medvedev GS (Ed.) Opredelitel' nasekomykh Evropeiskoy tchasti SSSR 4(1). Nauka, Leningrad, 193–680. [in Russian]

Lepiforum (2016) Lepiforum. http://www.lepiforum.de/lepiwiki.pl [accessed 27.03.2018]

Nel J, Varenne T (2016) Entomologie prospectrice: description de la femelle de *Nematopogon argentellus* G. & P. Leraut, 2014 et découverte de huit espèces nouvelles de microlépidoptères pour la France ou la science (Lepidoptera, Adelidae, Tineidae, Bucculatricidae, Gracillariidae, Yponomeutidae, Gelechiidae, Tortricidae). Revue de l'Association Roussillonnaise d'Entomologie 25(1): 28–40.

Oprea A (2005) Lista critică a plantelor vasculare din România. Editura Universităţii „Alexandru Ioan Cuza", Iaşi, 668 pp.

Razowski J (2003) Tortricidae (Lepidoptera) of Europe. Volume 2. Olethreutinae. Bratislava, 301 pp.

Robinson GS (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Micro-lepidoptera. Entomologist's Gazette 27: 127–132.

Sârbu I, Ştefan N, Oprea A (2013) Plante vasculare din România: determinator ilustrat de teren. Editura Victor B Victor, Bucureşti, 1320 pp.

Trematerra P, Colacci M (2017) Contribution to the faunal study of the Tortricidae of Pelion Mountains (Greece) with description of *Cydia magnesiae* Trematerra & Colacci, sp. n. (Lepidoptera: Tortricidae). SHILAP Revista de lepidopterología 45(179): 467– 480.

Varenne T, Nel J (2017) Descriptions de *Trifurcula mediocorsa* sp. n., *Eudarcia prealpina* sp. n., *Nemapogon inexpectata* sp. n., *Scrobipalpa fontanensis* sp. n. et du mâle de *Dichrorampha melaniana* Nel & Varenne, 2016 (Lepidoptera, Nepticulidae, Tineidae, Gelechiidae, Tortricidae). Revue de l'Association Roussillonnaise d'Entomologie 26(1): 46–55.

Zlatkov B (2013) Remarks on Tortricidae species with unknown and little known females. Nota Lepidopterologica 36(2): 191–197.

Zlatkov B (2016) Taxonomy of two montane *Dichrorampha* species from the Balkans and Caucasus. Nota Lepidopterologica 39(1): 13–20. https://doi.org/10.3897/nl.39.6760

Zlatkov B, Budashkin Y (2010) Tortricid moths new to the Bulgarian and European fauna (Lepidoptera: Tortricidae). The Entomologist's Record and Journal of Variation 122: 87–92.

The Eastern Palaearctic *Cosmopterix feminella* Sinev, 1988, introduced in Italy: taxonomy, biology and a new synonymy (Lepidoptera, Cosmopterigidae)

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http://zoobank.org/8BCC2D52-AE9F-430A-8C55-BFF440796885

Abstract. *Cosmopterix feminella* Sinev, 1988, previously known from the East Palearctic, Primorskiy Territory in Russia and Japan has been collected at light in Europe. In northern Italy 58 females were collected in two localities in the province Asti, two in Alessandria, three in Udine, and in one locality in Pordenone. *Cosmopterix feminella* is most likely parthenogenetic as only females are known. The caterpillars are leafminers on grasses. The species is redescribed and illustrated. DNA barcodes are provided and compared with other European species. *Cosmopterix feminae* Kuroko, 2015 is synonymised with *C. feminella*. The species was probably accidentally introduced into Italy.

Introduction

The genus *Cosmopterix* Hübner, 1825 (Gelechioidea: Cosmopterigidae) comprises small, sometimes very small, moths with a striking and often beautiful forewing pattern. The majority of the species can easily be recognized by the broad yellow to orange fascia on the forewing. This fascia is often bounded by tubercular golden or silver metallic fasciae or spots. The wings are very narrow and acutely pointed. The larvae are leafminers, usually oligophagous or monophagous and have been found in the following plant families: predominantly on Cyperaceae, Fabaceae and Poaceae, fewer species on Asteraceae, Cannabaceae, Convolvulaceae, Dipterocarpaceae, Hydrangeaceae, Lamiaceae, Theaceae and Urticaceae (Robinson et al. 2010; Kuroko 2015; Koster 2016). The genus occurs on all continents except Antarctica. The majority of the species have been found in tropical areas, while in temperate climates the number of species declines rapidly towards the poles. Europe (including Macaronesia) being well explored, has only 13 species recorded (Koster and Sinev 2003; Huemer and Koster 2006). Sinev (1997) mentioned 40 species in his review of the genus from the whole Palaearctic region, of which 12 species were also known from Europe.

Recently the number of species of the Palaearctic region further increased through the revision of the fauna of Japan by Kuroko (2015), who listed 49 species for this country, of which 25 were described as new. On the basis of these data, Sinev (2002) and Koster (2016), the total number of species worldwide is currently 344.

In Italy in June 2015 eight specimens of an unknown *Cosmopterix*-species were collected in a light trap in Cascine Bet, near Mombarone (Asti, Valmanera biotope) in the region Piemonte by O. Maioglio. An additional specimen of this unknown species was collected in a light trap by C. Cabella in September 2016 in Pertuso Le Strette, Cantalupo, also in the region Piemonte, roughly about 90 km east of the first locality. This material was brought to the attention of Giorgio Baldizzone who sent the material to the first author. Almost simultaneously, between August 2015 and September 2016, Helmut Deutsch collected six females in the region Friuli Venezia Giulia in three localities in the province Udine at light. It was tentatively identified as *C. crassicervicella* Chrétien, 1896 and sequenced in the framework of the barcoding campaign "Lepidoptera of the Alps". More material from the same and more localities became available in 2017 and 2018.

The collected specimens resembled *Cosmopterix attenuatella* (Walker, 1864) and *C. crassicervicella*, but lacked the orange brown or orange yellow markings on the dorsal side of the abdomen. After the examination of the female genitalia the species could be determined with certainty as *C. feminella* Sinev, 1988. DNA barcodes showed that the species from Piemonte and Friuli Venezia Giulia were conspecific.

Here we report the species new for Italy and Europe, and redescribe it in the format of the Microlepidoptera of Europe book series (Koster and Sinev 2003) and provide DNA barcodes.

Material and methods

Material. The material from the region Piemonte has been collected with Actinic light and a light trap. Similarly specimens from Friuli Venezia Giulia were all collected at light. Material is listed in Table 1, and more detailed in Suppl. material 1.

Morphology. Genitalia were dissected following the methodology presented by Brown (1997) and Robinson (1976). Chlorazol black was used to stain the unsclerotized parts of the female genitalia.

The morphological terminology follows Koster and Sinev (2003) and Koster (2016). In *Cosmopterix* the sterigma, formed from lamella antevaginalis and lamella postvaginalis, in the female genitalia is important for identification. It is often poorly sclerotized and can easily be distorted by too much pressure on the coverslip of the genitalia slide. Therefore the sterigma has been depicted here in both ventral and lateral position.

Illustrations. The photograph of the adult was taken with a Zeiss AxioCam digital camera attached to a motorized Zeiss SteREO Discovery.V12, using Carl Zeiss AxioVision software. The female genitalia were depicted in line drawings in ventral position, and the drawing of the enlarged sterigma are given separately; these drawings have been reduced to 70% of the original drawing size. Drawings of the genitalia were made with a compound microscope using the camera lucida method. For this purpose a strong light source (slide projector) was used for the illumination on the mirror of the microscope. A prism was placed on top of the microscope eyepiece to bend the projection 90° and project the subject on drawing paper. All outlines were drawn by pencil and later set in Indian ink. Scale bars alongside the drawing show the size of the genitalia in 0.1 mm.

The map (Fig. 5) was prepared with ArcGIS software.

DNA extraction and sequencing. DNA barcodes of two specimens of *C. feminella* (RMNH. INS.15509 and RMNH.INS.15510) were derived from extracts taken from abdomens following the procedures outlined by van Nieukerken et al. (2012a) and Doorenweerd et al. (2015), the abdomen and genitalia were taken from the lysis and prepared by JCK in the normal way. Legs of four other specimens were sent for analysis to the CCDB (Canadian Centre for DNA Barcoding, University of Guelph, Canada) where DNA extraction, PCR amplification, and sequencing were performed following standard high-throughput protocols (deWaard et al. 2008). The resulting COI sequences are combined with those from a representative set of other European *Cosmopterix* species from our own projects, or being publicly available, in the public BOLD dataset "Cosmopterix feminella in Europe [DS-COSMFEM]" [doi: 10.5883/DS-COSMFEM] and GenBank (Suppl. material 1). Unfortunately we have no barcode of the closely related *C. attenuatella* available from European samples, in BOLD there are only two incomplete barcodes from Galapagos and North America respectively, not yet in the public domain. The Neighbour Joining tree was prepared with the tree building tools on BOLD (Ratnasingham and Hebert 2007) and edited with Adobe Illustrator.

Results

Cosmopterix feminella Sinev, 1988

Figs 1–5

Cosmopterix feminella Sinev, 1988: 709. Holotype ♀, Russia: Primorskiy Kray, Khasanskiy district, 3 km southeast Andreyevka, 5.viii.1985, Sinev (Coll. Zoological Institute, Russian Academy of Sciences, St. Petersburg) [examined].
Cosmopterix feminae Kuroko, 2015: 48. Holotype ♀, Japan: Mt. Hikosan, Fukuoka Pref., 26.vii.1955, reared from *Digitaria violascens*, Kuroko. (Coll. Entomological Laboratory, Osaka Prefecture University). **Syn. n.** [not examined].

Material examined. Italy: 58♀. See Table 1 and Suppl. material 1.

Diagnosis. In Europe *C. feminella* resembles *C. crassicervicella* and *C. attenuatella*. The latter species occurs in Europe only in the Macaronesian Archipelago. It differs from both by the narrower forewings, which are ten times as long as wide compared to eight times in *C. feminella* and *C. crassicervicella*. In the forewing *C. feminella* can be distinguished from both other species by the absence of a white costal line in the basal area and by the absence of the apical protrusion of the orange-yellow fascia, and in the abdomen by the uniform ochreous-brown dorsal coloration. In *C. attenuatella* the abdomen is dorsally more or less spotted orange-brown and in *C. crassicervicella* the abdominal segments two to six are dorsally orange-yellow. The female genitalia of *C. feminella* can be recognised by the large central hump on the posterior edge of sternite VII, by the distally hood-shaped sterigma and by the small but prominent crescent-shaped signa.

Redescription. Female (Fig. 1). Forewing length 3.9–4.1 mm. Head: frons shining grey with greenish and reddish reflections; vertex and neck tufts shining dark brown with reddish gloss, medially and laterally narrowly lined white; collar shining dark brown with reddish gloss; labial palpus, first segment very short, grey, second segment four-fifths length of third, both segments dark brown, dorsally and ventrally lined white. Antenna: scape dorsally shining brown with reddish gloss and white anterior line, ventrally shining white; flagellum shining dark brown with short white line from base, changing in annulated white line to one-half, followed towards apex by four to five dark brown, one white, one dark brown, one white, four dark brown and three to four white segments at apex,

Table 1. Localities and material of *Cosmopterix feminella* in Italy. More details in Suppl. material 1.

# ♀	Region	Province	Locality	Collector	Date	DNA Barcodes
2	Friuli Venezia Giulia	Pordenone	Bachbett Torrente Cimoliana, Umg. Cimolais	Toni Mayr	24.vi.2017	
1	Friuli Venezia Giulia	Udine	Colloredo, Prati di Lavia	H. Deutsch	3.viii.2016	
3	Friuli Venezia Giulia	Udine	Confl. Torre-Natisone, Medeuzza	H. Deutsch	13.viii.2015	TLMF Lep 22239, 22240, 24198
2	Friuli Venezia Giulia	Udine	Interneppo, Mt. Festa	H. Deutsch	2.ix.2016	TLMF Lep 22241
2	Friuli Venezia Giulia	Udine	Risorgive di Flambro-Virco	H. Deutsch	4.viii.2016	
1	Piemonte	Alessandria	Cantalupo, Pertuso, Le Strette	C. Cabella	5.ix.2016	RMNH.INS.15510
1	Piemonte	Alessandria	Montechiaro d'Acqui, località Vaccamorta	G. Baldizzone	13.ix.2018	
1	Piemonte	Asti	fraz. Mombarone, Cascine Bet	O. Maioglio	11.vi.2015	
7	Piemonte	Asti	fraz. Mombarone, Cascine Bet	O. Maioglio	12.vi.2016	RMNH.INS.15509
15	Piemonte	Asti	fraz. Mombarone, Cascine Bet	G. Baldizzone	12.ix.2016	
1	Piemonte	Asti	fraz. Mombarone, Cascine Bet	G. Baldizzone	28.vii.2017	
8	Piemonte	Asti	fraz. Mombarone, Cascine Bet	G. Baldizzone	31.viii.2017	
4	Piemonte	Asti	fraz. Mombarone, Cascine Bet	O. Maioglio	14.ix.2018	
9	Piemonte	Asti	Valmanera, sotto Cascina Giardina	O. Maioglio	9.viii.2017	

sometimes apical segment grey to dark brown. Thorax and tegulae shining dark brown with reddish gloss, thorax with white medial line, often widening posteriorly, tegulae narrowly lined white inwardly. Legs: shining dark brown with reddish gloss; foreleg with white line on tibia and tarsal segments one to three and five; tibia of midleg with white oblique basal and medial lines and white apical ring, tarsal segments one and two with greyish white apical rings, tarsal segment five white; tibia of hindleg as midleg, tarsal segments one to four with narrow white apical rings, segment five dorsally white; spurs shining white, ventrally more grey. Forewing shining dark brown with reddish gloss; four white lines in basal area, subcostal from base to one-fourth and bending inwardly in distal half, short medial from one-fifth to just beyond subcostal, subdorsal underneath medial, about as long as medial or reaching slightly beyond it, very narrow dorsal from base to one-fifth, sometimes hardly visible; bright orange-yellow transverse fascia beyond mid length, narrowing in dorsal half; bordered at inner edge by broad tubercular golden metallic fascia, narrowing towards costa and with small subcostal patch of black scales on outside; bordered on outer edge by two tubercular golden metallic spots at costa and dorsum, dorsal about twice as large and nearer to base, costal and dorsal spots inwardly edged by few blackish scales; rather broad greyish white costal streak, inwardly edged dark grey; longitudinal silvery white spot in middle of apical area and shining white line in apical cilia; cilia dark brown around apex, paler towards dorsum. Hindwing shining brownish grey with reddish and greenish gloss; cilia dark greyish brown. Underside: forewing shining greyish brown, white costal and apical line distinctly visible; hindwing shining grey. Abdomen dorsally shining ochreous-brown with reddish golden gloss, laterally shining grey, ventrally shining grey, segments broadly banded white posteriorly; anal tuft dorsally ochreous-brown, ventrally blackish brown.

Male genitalia. Male unknown.

Female genitalia (Fig. 2a–c). Posterior edge of sternite VII deeply concave with very large convex hump in centre. Sterigma elongated, distally hood-shaped, ostium bursae rounded, basally V-shaped with rounded labiate extension. Ductus bursae narrow, slightly shorter to about as long as corpus bursae. Ductus seminalis from upper part of corpus bursae. Corpus bursae elongate, two small, crescent-shaped signa.

Figure 1. *Cosmopterix feminella*, female habitus, Asti, Cascine Bet, 12.vi.2016, RMNH.INS.15509. Scale bar: 2 mm. Photo J.C. Koster.

Biology. The biology has been described by Kuroko (2015) (as *C. feminae*), from which we cite the following. Host plants: *Digitaria ciliaris* (Retz.) Koeler and *D. violascens* Link (Poaceae). Larva (last instar): head blackish brown, body cylindrical, pale yellow, prothoracic shield and anal plate black, prothoracic legs blackish brown. The larva mines the leaves and makes an irregular blotch mine from near the base towards the apex of the leaf. The frass is ejected from a hole at the beginning of the mine. The larva changes leaves to make more than one mine. Pupation takes place outside the mine in a spindle-shaped whitish brown cocoon in a folded space on a leaf. In Japan (Kyushu: Mt. Hikosan) up to four generations per year occur. The larva of the last generation hibernates full grown and pupates in the middle of May the following year. The adults are on the wing in late May, in late July, from mid-August to mid-September and in late October. The European specimens of *C. feminella* were collected in the months June, August, September and October.

Distribution. Russia: Primorskiy Territory, Japan: Honshu and Kyushu, Italy: Piemonte and Friuli Venezia Giulia regions (Fig. 5).

DNA barcodes. We obtained completely identical DNA barcodes from six specimens of *C. feminella* from Italy, all belonging to the new Barcode Index Number BOLD:ADG7284, and with a distance of 5.8% to the nearest neighbour, an unidentified *Cosmopterix* species from Madagascar with BIN BOLD:ACT2622 (specimen BIOUG18998-F03). This specimen was collected with a Malaise trap and therefore its external morphology is poorly preserved (Lopez-Vaamonde et al. 2018). Unfortunately no barcodes are yet known from the Asian populations. The distance to barcodes of European species is larger, the smallest being 6.1% to *C. scribaiella* (Zeller, 1850) (Fig. 6).
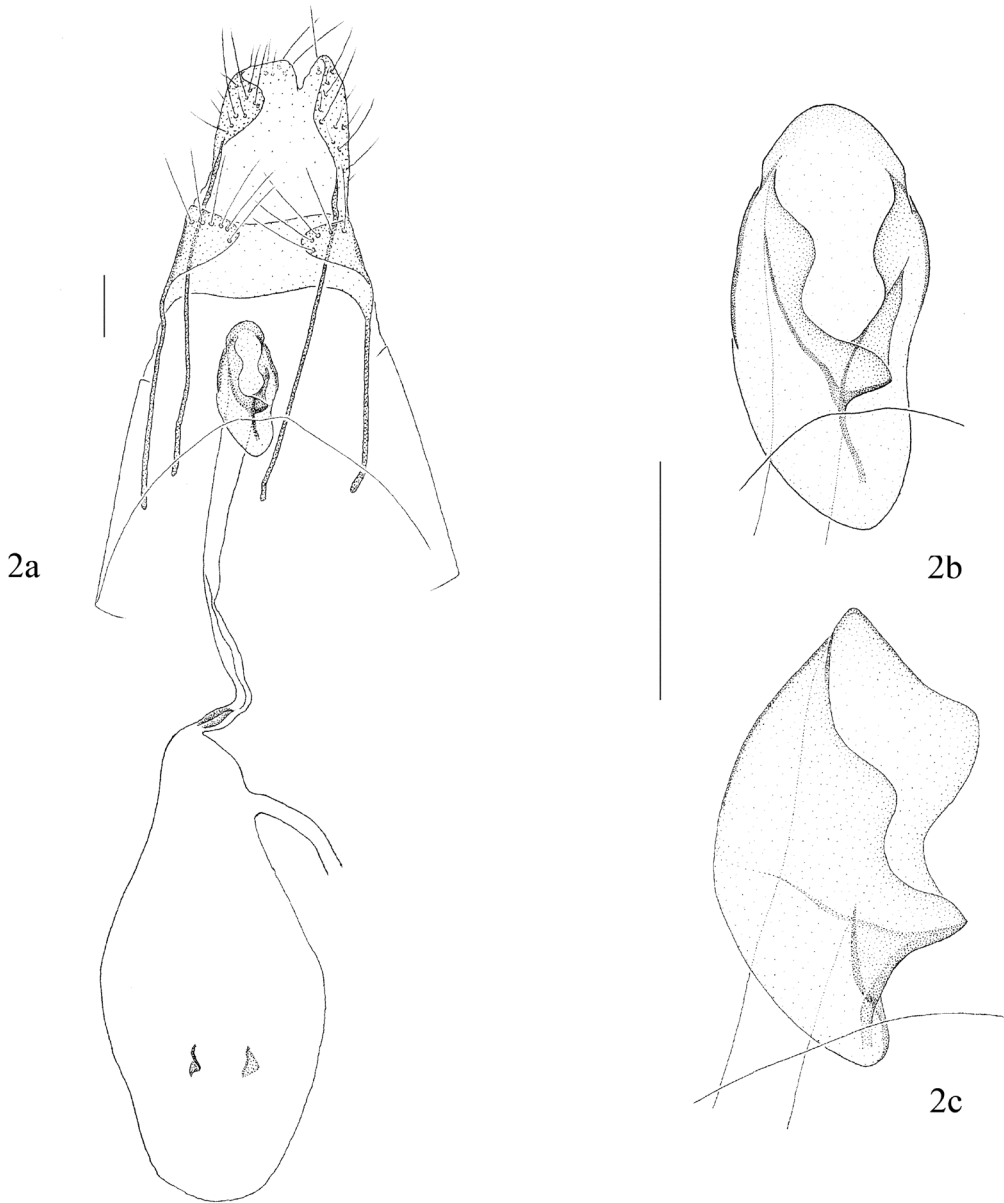
Figure 2. *Cosmopterix feminella*, female genitalia. **2a**. ventral aspect, genitalia slide JCK8673; **2b**. sterigma enlarged, in ventral view, genitalia slide JCK 8673; **2c**. sterigma enlarged in lateral view, genitalia slide JCK 8674. Scale bars: 0.1 mm. Drawings J.C. Koster.

Remarks. *Cosmopterix feminella* has been described on the basis of four females, caught between 25 July and 15 August in Primorskiy Kray, in the south-east of the Russian Far East.

In his study on the genus *Cosmopterix* of Japan, Kuroko (2015) described a new species, *C. feminae*, based on reared females only, and compared it with *C. feminella* from Russia, of which

Figures 3, 4. Collecting sites of *Cosmopterix feminella* in Italy. **3.** Asti, Cascine Bet. Photo G. Baldizzone; **4**. Udine, Medeuzza, Confl. Torrente Torre-Natisone. Photo H. Deutsch.
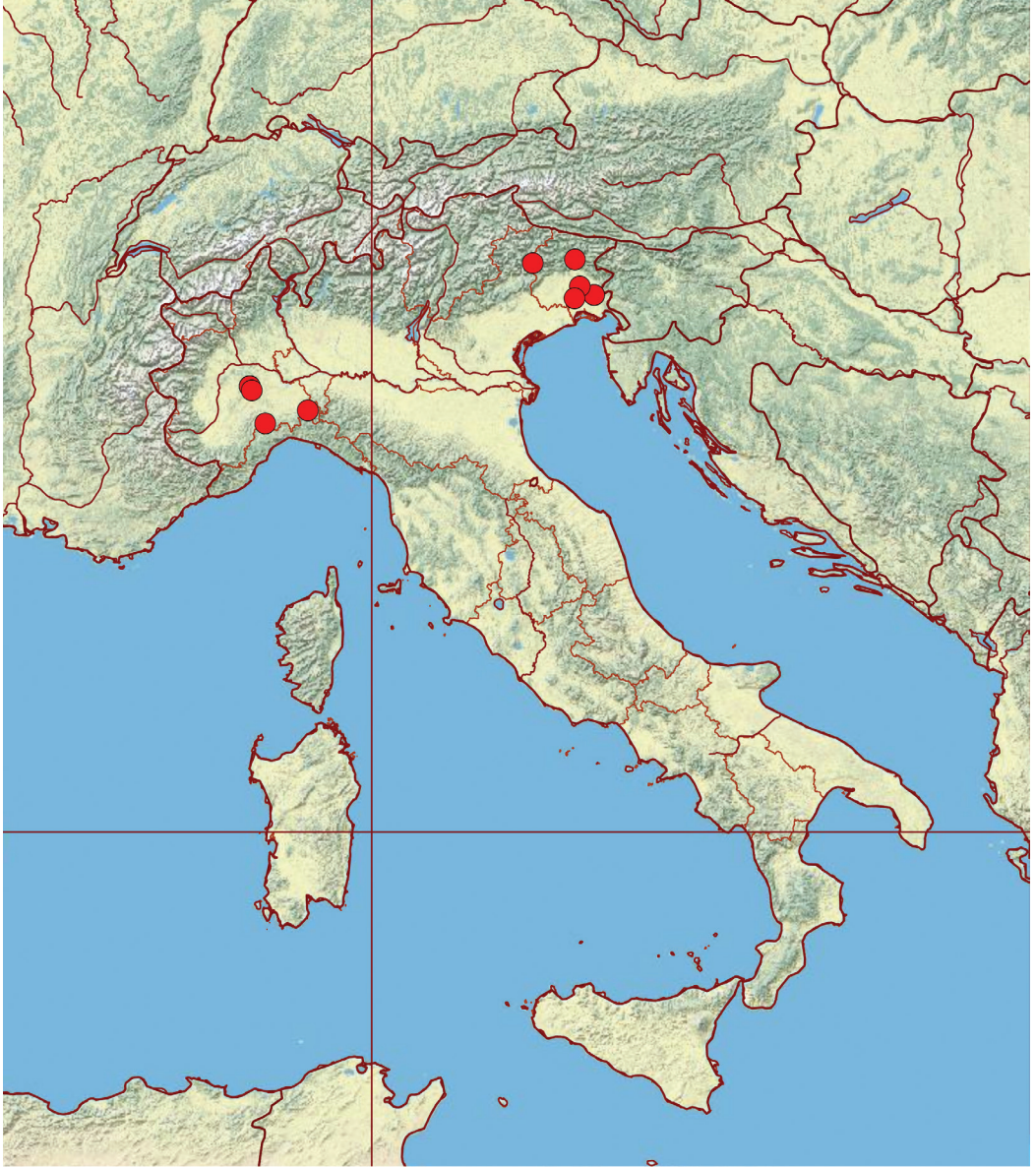
Figure 5. Map of collecting localities of *Cosmopterix feminella* in Italy.

also only females were known. His conclusion was that the Japanese species shows sufficient differences to describe it as a new species, based on the following features: the apical segment of the antenna is black in *C. feminae*; but white in *C. feminella*; in the forewing the subcostal line starts from the base of the wing in *C. feminae*, which is not the case in *C. feminella*; in the female genitalia the sterigma (lamella antevaginalis) lacks the tongue-shaped extension at the top as seen in *C. feminella*; the ductus bursae is about half the length of the corpus bursae in *C. feminae* instead of nearly the same length in *C. feminella*.

Figure 6. Neighbor Joining tree of DNA barcodes of European *Cosmopterix* species.

Cosmopterix feminae is here synonymized with *C. feminella* for the following reasons: In the European series from Italy the apical segment of the antenna is white in 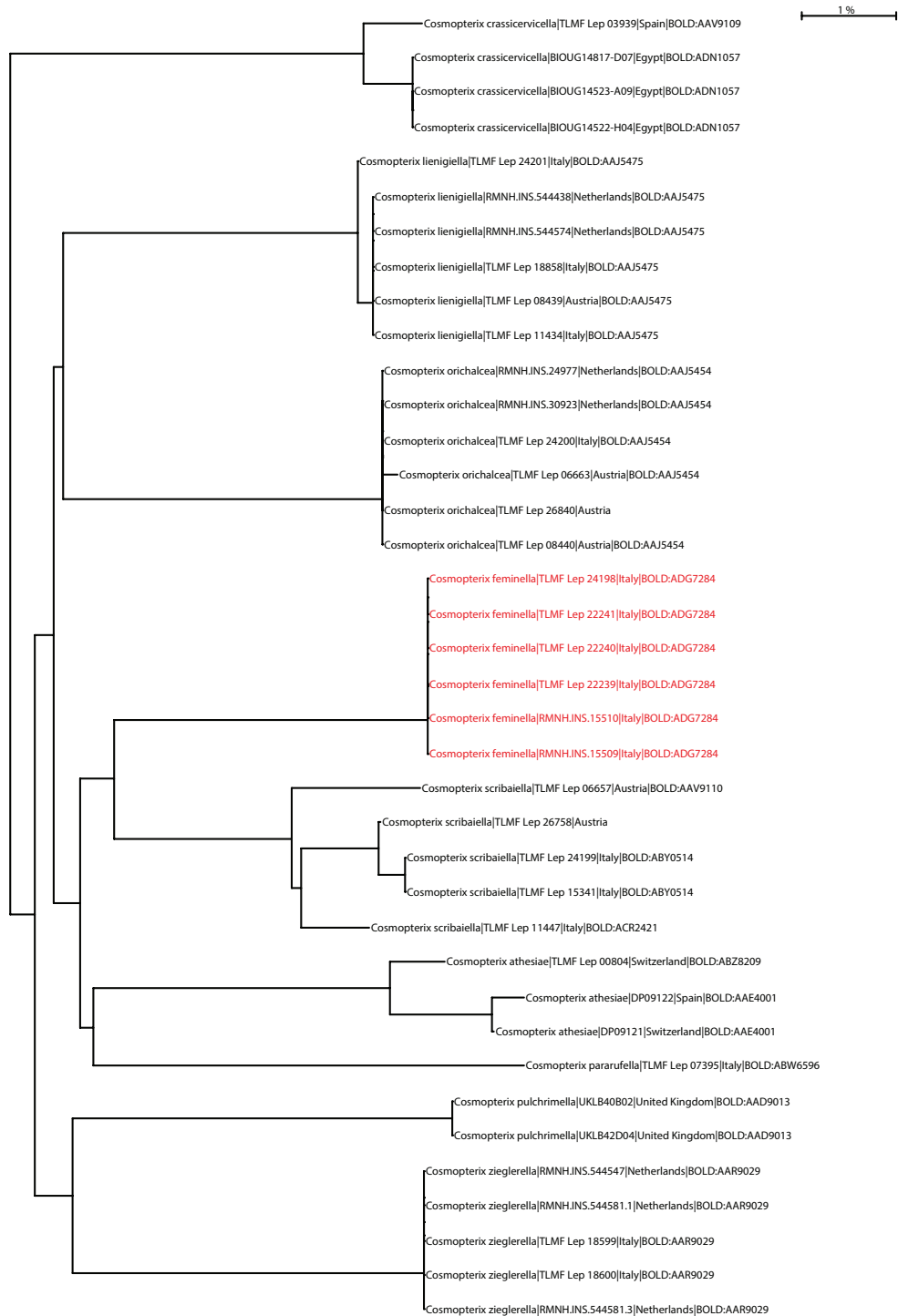12 specimens, but it is dark grey in two specimens and black in one specimen, thus this character is apparently not constant. According to the original description of *C. feminella* the subcostal line also starts from the base of the forewing. Sinev (1988) provided a drawing of the forewing in which the subcostal line does not reach the base of the wing. Actually it does start from the wing base, but the curve of the wing margin hides this when seen from above, as in the figure. Probably this has convinced Kuroko that this line starts beyond the base of the wing. As Sinev (1988) mentioned, the tongue-shaped extension at the top of the sterigma (lamella antevaginalis), belongs to the hood-shape form of the sterigma. When the sterigma is embedded on the genitalia slide in ventral position, the so-called tongue-shaped extension can be caused by pressure on the cover slip. Kuroko (2018) described the length of the ductus bursae as about half the length of the corpus bursae, however, in his drawing (Plate 21, fig. H) the length of the ductus bursae is only slightly shorter than the length of the corpus bursae and equal in length to the length of the ductus bursae compared to the corpus bursae in the drawing by Sinev (1988: fig. 2, below in centre). The above differences are thus considered insufficient to regard *C. feminae* as a separate species.

Discussion

The type series of both *C. feminella* from Russia and *C. feminae* from Japan, including reared material, consist of females only. On this basis Kuroko (2015) suggested the possibility of parthenogenesis. Also all specimens found in Italy are females. All this taken together suggests that it is very likely that we are dealing here with a parthenogenetic species, although confirmation from e.g. rearing experiments is required for more certainty.

The finding of so many specimens of a species otherwise only known from the eastern Palearctic, thousands of kilometres away, is probably best explained by accidental introduction. How this happened is difficult to trace, but it is likely aided by human transport, either imported with plant material from Asia, introduced unintentionally as a contaminant with foreign grass seed, or even as adults inside some means of transport. One piece of grass with a pupa might have been sufficient, as for introduction of a parthenogenetic species just one specimen could be enough. Globalization has led to an increasing number of introduced alien insects from many parts of the world (Roques et al. 2016). From all alien Lepidoptera known from Europe around 2009, most species originated from Asia (Lopez-Vaamonde et al. 2010), but for leafminers this number is relatively low, with best known example *Phyllonorycter issikii* (Kumata, 1963) on *Tilia* species, whereas most alien species originate from North America (Kirichenko et al. 2018). In several cases, Italy was the first country where these leafmining aliens were recorded, as in *Parectopa robiniella* Clemens, 1863, *Antispila oinophylla* van Nieukerken & Wagner, 2012 and *Coptodisca lucifluella* (Clemens, 1860) (Hellrigl 2006; Nieukerken et al. 2012b; Bernardo et al. 2015). Not all introduced species are able to establish populations, as was probably the case in the single record of the Eastern Asian fern feeder, found in Austria: *Cuprina fuscella* Sinev, 1988 (Stathmopodidae) (Wieser 2004). Possibly, *Cosmopterix feminella* is not the first alien *Cosmopterix* species in Europe, the widespread *C. pulchrimella* Chambers, 1875, feeding on *Parietaria* species (Urticaceae) is suspected to have been introduced into Europe from North America (Koster 2010). It was first found in Croatia in the 1930s (Koster 2010), and it is still spreading northward (Parsons and Sterling 2004; Ellis 2019).

For establishing populations, it is important that the species finds suitable hostplants. Both cited hostplants are known as introduced plants in Europe. The distribution of *Digitaria ciliaris* has been discussed in detail by Wilhalm (2010). The species has been described from China, but is nowadays widespread in Europe, especially in the south, but becomes scarcer towards the north. The species is not easy to separate from the native European *D. sanguinalis* (L.) Scop. *Digitaria ciliaris* can be found in northern Europe in harbour areas, dumps, waste places, gardens, roadsides, etc. In southern Europe it is also accompanying grass in lawns and plantations. *Digitaria violascens* is native in tropical Asia and probably also in tropical America from where the type was described (Verloove 2008). In Europe it has been found in France and Italy in different habitats. It is possibly introduced unintentionally as a contaminant with foreign grass seed. However, it is quite possible that *C. feminella* also feeds on other grass species, and the genus *Digitaria* has three native species in south-western Europe (Verloove 2008), giving ample opportunities for the moth to establish populations. A focussed search for leafmines is the next step, and it remains important to follow the further establishment and invasion of this species in Europe. As long as the species is attacking only the currently known food plants, which have also been introduced into Europe, it will probably not have any environmental or economic impact.

Acknowledgements

References

Bernardo U, Nieukerken EJ van, Sasso R, Gebiola M, Gualtieri L, Viggiani G (2015) Characterization, distribution, biology and impact on Italian walnut orchards of the invasive North-American leafminer *Coptodisca lucifluella* (Lepidoptera: Heliozelidae). Bulletin of Entomological Research 105: 210–224. https://doi.org/10.1017/S0007485314000947

Brown PA (1997) A review of techniques used in the preparation, curation and conservation of microscope slides at the Natural History Museum, London. Biology Curator 10: 1–34. http://www.natsca.org/article/455

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA Barcodes, analytical protocols. In: Cristofre Martin C (Ed.) Environmental genomics. Humana Press, Totowa, NJ, Environmental Genomics 410: 275–294. https://doi.org/10.1007/978-1-59745-548-0_15

Doorenweerd C, Nieukerken EJ van, Menken SBJ (2015) A global phylogeny of leafmining *Ectoedemia* moths (Lepidoptera: Nepticulidae): exploring host plant family shifts and allopatry as drivers of speciation PloS ONE 10: e0119586. https://doi.org/10.1371/journal.pone.0119586

Ellis WN (2019) Plant Parasites of Europe: leafminers, galls and fungi. http://bladmineerders.nl/

Hellrigl K (2006) Rasche Ausbreitung eingeschleppter Neobiota (Neozoen und Neophyten). Forest Observer 2/3: 349–388.

Huemer P, Koster JC (2006) *Cosmopterix athesiae* sp. n., a widespread new species from Europe and Africa (Lepidoptera: Cosmopterigidae, Cosmopteriginae). Veröffentlichungen des Tiroler Landesmuseums Ferdinandeum 86: 75–82. https://www.zobodat.at/pdf/VeroeffFerd_86_0075-0082.pdf

Kirichenko N, Augustin S, Kenis M (2018) Invasive leafminers on woody plants: a global review of pathways, impact, and management. Journal of Pest Science. https://doi.org/10.1007/s10340-018-1009-6

Koster JC (2016) The genus *Cosmopterix* Hübner of continental Sub-Saharan Africa (Lepidoptera: Gelechioidea: Cosmopterigidae). Tijdschrift voor Entomologie 158: 87–318. https://doi.org/10.1163/22119434-15802002

Koster JC, Sinev SY (2003) Momphidae, Batrachedridae, Stathmopodidae, Agonoxenidae, Cosmopterigidae, Chrysopeleiidae. Apollo Books, Stenstrup, Microlepidoptera of Europe 5: 387 pp.

Koster JC (2010) The genera *Cosmopterix* Huebner and *Pebobs* Hodges in the New World with special attention to the Neotropical fauna (Lepidoptera: Cosmopterigidae). Zoologische Mededelingen 84: 251–575. http://www.repository.naturalis.nl/document/193818

Kuroko H (2015) The genus *Cosmopterix* (Lepidoptera, Cosmopterigidae). Touka Shobo, Fukuoka, The Insects of Japan 5: 1–162.

Lopez-Vaamonde C, Agassiz D, Augustin S, De Prins J, De Prins W, Gomboc S, Ivinskis P, Karsholt O, Koutroumpas A, Koutroumpa F, Laštůvka Z, Marabuto E, Olivella E, Przybylowicz L, Roques A, Ryrholm N, Sefrova H, Sima P, Sims I, Sinev S, Skulev B, Tomov R, Zilli A, Lees D (2010) Alien terrestrial arthropods of Europe, chapter 11. Lepidoptera. BioRisk 4: 603–668. https://doi.org/10.3897/biorisk.4.50

Lopez-Vaamonde C, Sire L, Rasmussen B, Rougerie R, Wieser C, Allaoui AA, Minet J, deWaard JR, Decaëns T, Lees DC (2018) DNA barcodes reveal deeply neglected diversity and numerous invasions of micro-moths in Madagascar. Genome. https://doi.org/10.1139/gen-2018-0065

Nieukerken EJ van, Doorenweerd C, Stokvis FR, Groenenberg DSJ (2012a) DNA barcoding of the leaf-mining moth subgenus *Ectoedemia* s. str. (Lepidoptera: Nepticulidae) with COI and EF1- α: two are better than one in recognising cryptic species. Contributions to Zoology 81: 1–24. http://www.contributionstozoology.nl/vol81/nr01/a01.

Nieukerken EJ van, Wagner DL, Baldessari M, Mazzon L, Angeli G, Girolami V, Duso C, Doorenweerd C (2012b) *Antispila oinophylla* new species (Lepidoptera, Heliozelidae), a new North American grape-vine leafminer invading Italian vineyards: taxonomy, DNA barcodes and life cycle. ZooKeys 170: 29–77. https://doi.org/10.3897/zookeys.170.2617

Parsons M, Sterling P (2004) *Cosmopterix pulchrimella* Stt. now resident in mainland Britain. Atropos 23: 23–25.

Ratnasingham S, Hebert PDN (2007) BOLD: The Barcode of Life Data System [www.barcodinglife.org]. Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Robinson GS (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. Entomologist's Gazette 27: 127–132. http://idtools.org/id/leps/tortai/Robinson_1976.pdf

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernández LM (2010) HOSTS – a database of the world's Lepidopteran hostplants. Natural History Museum. http://www.nhm.ac.uk/our-science/data/hostplants/

Roques A, Auger-Rozenberg M-A, Blackburn TM, Garnas J, Pyšek P, Rabitsch W, Richardson DM, Wingfield MJ, Liebhold AM, Duncan RP (2016) Temporal and interspecific variation in rates of spread for insect species invading Europe during the last 200 years. Biological Invasions 18: 907–920. https://doi.org/10.1007/s10530-016-1080-y

Sinev SY (1988) Novye dannye po faune Uzkokrylykh Molej roda *Cosmopterix* (Lepidoptera, Cosmopterigidae) palearktiki. New data on the palaearctic fauna of the narrow-winged moths of the genus *Cosmopterix* (Lepidoptera, Cosmopterigidae). Zoologicheskii Zhurnal 67: 705–712. [In Russian]

Sinev SY (1997) Obzor Uzkokrylykh Molej roda *Cosmopterix* Hb. (Lepidoptera, Cosmopterigidae) Palearktiki. A review of the narrow-winged moths of the genus *Cosmopterix* Hb. (Lepidoptera, Cosmopterigidae) of Palaearctic Region. Entomologicheskoe Obozrenie 76: 813–829. [In Russian]

Sinev SY (2002) World catalogue of cosmopterigid moths (Lepidoptera: Cosmopterigidae). Katalog roskoshnykh uzkokrylykh molej (Lepidoptera: Cosmopterigidae) mirovoj fauny. Trudy Zoologicheskogo Instituta 293: 1–183.

Verloove F (2008) Studies within the genus *Digitaria* Haller (Poaceae, Panicoideae) in southwestern Europe. Candollea 63: 227–233. http://www.ville-ge.ch/cjb/publications/cando632/C632_227-233.pdf

Wieser C (2004) Die Schmetterlingsfauna der Auen im Oberen Drautal und weitere Erstnachweise für Kärnten und Österreich (Insecta/Lepidoptera). Carinthia II 194/114: 369–387. https://www.zobodat.at/pdf/CAR_194_114_0369–0387.pdf

Wilhalm T (2010) *Digitaria ciliaris* in Europe. Willdenowia 39: 247–259. https://doi.org/10.3372/wi.39.39203

Supplementary material 1

Specimen data *Cosmopterix feminella*

Authors: Sjaak J.C. Koster, G. Baldizzone, H. Deutsch, P. Huemer, E.J. van Nieukerken

Data type: Excel file

Explanation note: Specimen data, collection details, coordinates.

Link: https://doi.org/10.3897/nl.42.33962.suppl1

Ornamental plantings of *Arbutus unedo* L. facilitate colonisations by *Charaxes jasius* (Linnaeus, 1767) in Madrid province, central Spain

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http://zoobank.org/3BADDBC7-12FD-4F37-9D18-752A32BBFC32

Abstract. The distribution of butterfly species is limited by availability of larval host plants growing in suitable climatic conditions. The Two-tailed Pasha, *Charaxes jasius* (Linnaeus, 1767), is a Mediterranean butterfly with only sporadic historical records in Madrid, Spain's most central province, where the host plant is uncommon and winters are colder than in most parts of the butterfly's range. We show the first evidence of juvenile stages of the species in two towns of north-central Madrid and compile records of *C. jasius* from Madrid over the past four decades. Our results suggest that, in the absence of widespread host plants, *C. jasius* is using suburban ornamental plantings of its host plant to colonise a region which may be becoming more climatically suitable.

Introduction

The Two-tailed Pasha, *Charaxes jasius* (Linnaeus, 1767) (Lepidoptera: Nymphalidae: Charaxinae), has been reported from regions of mainly coastal Mediterranean or Atlantic climates (Kudrna et al. 2015; GBIF 2017). The species' distribution follows the native range of its main larval host plant, the Strawberry-tree (*Arbutus unedo* Linnaeus), that typically grows in coastal and inland areas with mild climates (Torres et al. 2002).

In the Iberian Peninsula, both the butterfly and its host plant occur mainly in areas of non-continental climate, with the exception of some occasional records of the adult butterfly in inland thermal refuges (Domínguez and Martínez 1993; García-Barros et al. 2004; Molina et al. 2011; García-Barros et al. 2013). In central Spain, *C. jasius* is reported to have settled in the southwestern extreme of Madrid province (Vicente Arranz and García-Carrillo 2009), corresponding to a small remnant population of Strawberry-trees (Domínguez and Martínez 1993; Baonza-Díaz 2001), closer to the stronghold of the butterfly, further to the west in the provinces of Cáceres and Toledo (García-Barros et al. 2004). Strawberry-trees are also grown ornamentally, and can be found throughout Madrid, thus presenting opportunities for *C. jasius* to expand its distribution to areas having more continental conditions.

This study was motivated by the unexpected sighting of an adult *C. jasius* in central Spain, in a north-central town of Madrid province, Colmenar Viejo, situated approximately 80 km from the species' southwestern stronghold (Vicente Arranz and García-Carrillo 2009). This led us to investigate whether the butterfly is breeding in the area, and if it is making use of ornamental Strawberry-trees

to expand from its southwestern settlement to more northern and central areas. To answer these questions, we carried out (i) a search and created a list of Strawberry-trees present in the town of Colmenar Viejo and in some neighbouring localities, looking for evidence of the butterfly's presence, and (ii) compiled a list of existing information on the distribution of the butterfly in Madrid province.

Material and methods

Evidence for colonisation of north and central Madrid

Following the sighting of an adult *C. jasius* in Colmenar Viejo on 12.ix.2017, an inventory of the ornamental Strawberry-trees present in this locality was carried out. The trees were then searched for the presence of *C. jasius* (eggs, larvae and/or adults). In addition, the neighbouring localities of Hoyo de Manzanares, Tres Cantos, Cantoblanco and San Agustín del Guadalix, situated west, south and east of Colmenar Viejo, were also searched. We selected these adjacent localities, as we expected that they would form part of the most likely approach of the butterfly from its southwestern settlement.

A total of 298 ornamental trees, found largely in urban parks and gardens across the five localities, were searched from mid-September to late October 2017, at the end of the summer generation of the butterfly. Seventy-one of the trees were located in Colmenar Viejo, while 201 trees were located in Tres Cantos, 7 in Cantoblanco, 4 in San Agustín del Guadalix and 15 in Hoyo de Manzanares.

Review of prior records

Information on the butterfly's distribution in Madrid province was reviewed using records from the following sources: Gómez Bustillo and Fernández-Rubio 1974; Martínez and Casado 1984; Simón 1986; Gómez de Aizpúrua 1987, 1997; Gómez de Aizpúrua et al. 1999, 2009; Vicente Arranz and García Carrillo 2009; Cobo 2013; García Carrillo et al. 2015. For each source, the locality name, geographic coordinates, observer name and development stage found (egg, larva and/or adult) were compiled into a table.

Results

Two butterflies, 25 eggs and one larva were found during the searches of the ornamental Strawberry-trees. Both butterflies, 21 eggs and the larva were found across the town of Colmenar Viejo, while the remaining four eggs were observed on three trees in Tres Cantos, south of Colmenar Viejo (Table 1). No evidence of the butterfly's presence was found in the three remaining localities, where only a small number of trees was located and searched (Table 1).

The review of prior distribution records spanned the past four decades, and revealed a number of sporadic observations which, like that at Colmenar Viejo, are located far from the butterfly's southwestern range (Table 2; Fig. 1). The majority of the records from the last 10 years were obtained in west and north-central localities of the province, closer to the Guadarrama mountain range, while the older records were generally found further south (Fig. 1). The two most northern records in the province constituted the two most recent sightings of the butterfly (Table 2; Fig. 1). The northernmost record, from Miraflores de la Sierra, was obtained approximately 15 km from one of the few relict populations of indigenous Strawberry-trees in the country (Molina et al. 2011; Bernal González 2012; García Carrillo et al. 2015), while the second-most northern record, from El Escorial, was obtained in an urbanization with numerous ornamental plantings (García Carrillo et al. 2015). The
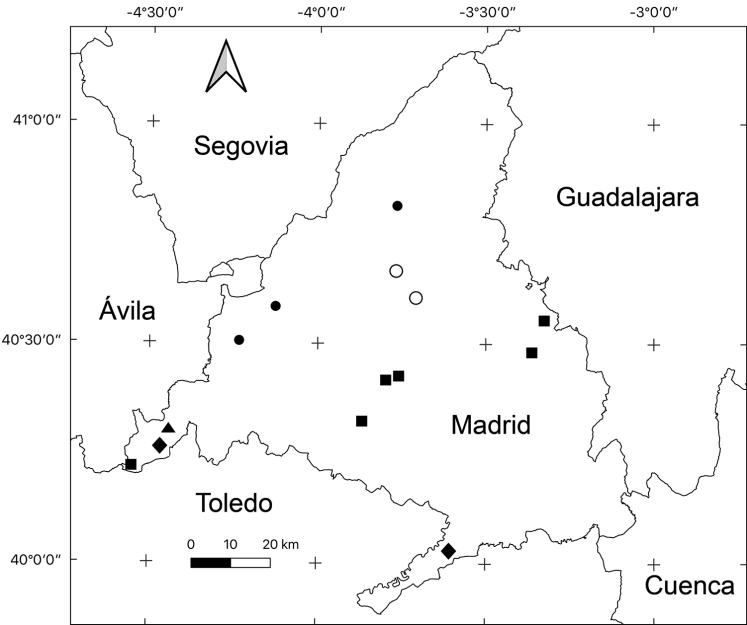
Figure 1. Map of the localities of Madrid province (central Spain) where *C. jasius* has been observed. Squares indicate records from 1980 to 1989, triangles from 1990 to 1999, diamonds from 2000 to 2009 and circles from 2010 to 2017 (see Table 2). White circles indicate both new records provided in this study (see Table 1).

Table 1. List of the localities in Madrid province where Strawberry-trees were examined for the presence of *C. jasius*. The geographical coordinates, number of trees searched and development stage found are also provided.

Locality	Geographical Coordinates	Number trees	Development stage
Colmenar Viejo	40°40'00.2"N, 3°46'17.5"W	–	1 adult
	40°39'57.9"N, 3°46'09.5"W	1	5 eggs, 1 larva
	40°39'49.7"N, 3°46'21.8"W	1	7 eggs
	40°39'11.5"N, 3°46'19.2"W	15	2 eggs
	40°39'40.0"N, 3°45'44.8"W	5	–
	40°39'59.5"N, 3°46'31.0"W	3	–
	40°40'06.5"N, 3°46'30.6"W	3	–
	40°40'05.0"N, 3°46'29.0"W	2	–
	40°39'31.7"N, 3°45'45.7"W	2	–
	40°40'04.4"N, 3°45'56.0"W	1	–
	40°39'48.1"N, 3°45'54.6"W	1	–
	40°40'10.1"N, 3°46'35.3"W	3	–
	40°39'15.1"N, 3°46'32.2"W	20	7 eggs
	40°40'07.6"N, 3°46'38.2"W	1	–
	40°39'36.0"N, 3°45'41.2"W	5	–
	40°39'55.0"N, 3°46'11.6"W	1	–
	40°39'41.7"N, 3°45'54.6"W	–	1 adult
	40°39'33.1"N, 3°45'24.7"W	7	–
Tres Cantos	40°36'18.3"N, 3°42'33.6"W	120	2 eggs
	40°35'44.0"N, 3°42'22.8"W	1	1 eggs
	40°36'30.9"N, 3°42'27.8"W	80	1 eggs
Cantoblanco	40°32'37.7"N, 3°41'42.8"W	4	–
	40°32'30.1"N, 3°41'25.9"W	3	–
San Agustín del Guadalix	40°40'53.3"N, 3°36'49.0"W	4	–
Hoyo de Manzanares	40°37'26.9"N, 3°54'16.9"W	15	–

Table 2. List of the localities in Madrid province where *C. jasius* has previously been observed. The geographic coordinates (Geo. coord.), 10×10 km MGRS grid references (MGRS), development stage (Dev. stage), date, observer name (Observ.) and source of each record are also provided.

Locality	Geo. coord	MGRS	Dev. stage	Date	Observ.	Source
Meco	40°33'16.1"N, 3°19'45.9"W	30TVK78	Adult	1974, 1999	J. Álvarez	Gómez Bustillo and Fernández-Rubio 1974; Gómez de Aizpúrua et al. 1999
Móstoles	40°19'25.0"N, 3°51'54.3"W	30TVK26	Adult	IX-1984	–	Martínez and Casado 1984
Sierra de la Higuera	40°13'03.2"N, 4°34'42.2"W	30TUK65	Adult	IX-1984	M.A. Martínez and F. Casado	Martínez and Casado 1984
Casa de Campo	40°25'20.7"N, 3°45'19.0"W	30TVK37	Adult	12-VI-1982	L.A. Rovenga	Simón 1986
Somosaguas	40°25'07.1"N, 3°47'48.5"W	30TVK37	Adult	IX-1984	F. Rodríguez	Simón 1986
Alcalá de Henares	40°28'57.5"N, 3°21'57.0"W	30TVK78	Adult	1987	C. Gómez et al.	Gómez de Aizpúrua 1987a
Cadalso de los Vidrios	40°18'07.3"N, 4°26'43.4"W	30TUK75	Adult	1997	C. Gómez de Aizpúrua	Gómez de Aizpúrua 1997
Aranjuez	40°01'50.9"N, 3°36'19.6"W	30TVK43	Adult	2009	C. Gómez de Aizpúrua	Gómez de Aizpúrua et al. 2009
Cenicientos	40°15'45.6"N, 4°27'57.0"W	30TUK65	Adult	2009	J.C. Vicente and A. García Carrillo	Vicente and García Carrillo 2009
Cadalso de los Vidrios	40°18'07.3"N, 4°26'43.4"W	30TUK75	Adult	2009	J.C. Vicente and A. García Carrillo	Vicente and García Carrillo 2009
Robledo de Chavela	40°30'16.9"N, 4°14'08.8"W	30TUK98	Larva	14-I-2012	A. Cobo	Cobo 2013
El Escorial	40°34'58.7"N, 4°07'41.0"W	30TVK09	Adult	1-VII-2015	R. de la Peña	García Carrillo et al. 2015
Miraflores de la Sierra	40°48'42.1"N, 3°45'58.1"W	30TVL32	Adult	21-VI-2015	K. Leahy	García Carrillo et al. 2015

majority of the records collected were observations of adult butterflies, although a larva had been detected in the west-central locality of Robledo de Chavela in 2013 (Cobo 2013) (Table 2; Fig. 1).

Discussion

Our findings of a small number of juvenile stages of *C. jasius* in two north-central localities of Madrid province suggest that a small breeding population occurs in the area. Furthermore, the existence of prior records in localities that are very distant from the butterfly's southwestern settlement, and where Strawberry-trees do not naturally grow, supports the possibility that the highly mobile *C. jasius* is using ornamental trees to colonise new areas.

Ornamental plantings of larval host plants have been found to play an important role in the expansion of other butterfly species, such as the Common Brimstone, *Gonepteryx rhamni* (Linnaeus, 1758), that has spread along plantings of Alder Buckthorn (*Frangula alnus* Mill.) on roadsides in North Wales (Gutiérrez and Thomas 2000). Breeding populations of the American Monarch, *Danaus plexippus* (Linnaeus, 1758), have been observed in the Azores islands following the introduction of the Swan Milkweed (*Gomphocarpus fruticosus* (L.) W.T. Aiton) for ornamental purposes (Neves et al. 2001). Similarly, Cycadians (genus *Eumaeus* Hübner, 1819) have been reported to follow and use ornamental cycads in Xalapa (Mexico) and Southeast Florida (USA) (Ramírez-Restrepo et al. 2017).

However, although ornamental Strawberry-trees have been present in the town of Colmenar Viejo for 30–40 years (Robert Wilson, pers. comm.), *C. jasius* has only now been recorded there.

This could be a result of the species not being surveyed in that locality before. However, extensive butterfly surveys have previously been conducted across north-central Madrid (Gómez de Aizpúrua 1987, 1997; Vicente Arranz and García Carrillo 2009), yielding only two records of *C. jasius* to date, both of which were adult individuals observed within the last five years (see Table 2). Given our additional observation of a small breeding population in an area where the winters are colder than in most parts of the butterfly's range, and the scarcity of north-central records until recent years, we believe that warming temperatures may be enabling the butterfly to colonise the ornamental trees and disperse under more continental conditions.

Conclusions

Our study extends previous findings on the importance of ornamental host plants for the expansion of butterfly species, showing that *C. jasius* may be using ornamental Strawberry-trees to colonise new areas in central Spain. We also postulate that the butterfly is able to progress under these continental conditions as the region becomes climatically more suitable. Nevertheless, additional sampling should be conducted in spring, to determine whether the butterfly is successfully overwintering in the area, together with an extensive network of surveys across the province, to understand if warming climate conditions are actually playing a role in the butterfly's expansion.

Acknowledgements

References

Baonza-Díaz J (2001) Influencia luso-estremadurense en la mitad oriental de la provincia de Madrid. Ecología 15: 101–109.

Bernal González R (2012) Nuevas citas de flora de óptimo luso-extremadurense en la Pedriza del Manzanares. Asociación Reforesta, Madrid, 40 pp.

Cobo A (2013) Nueva cita de *Charaxes jasius* (Linnaeus, 1767) en la Comunidad de Madrid (Lepidoptera: Nymphalidae). BV News Publicaciones Científicas 17: 18–21.

Domínguez F, Martínez F (1993) Acerca de la distribución española de *Arbutus unedo* L. (Ericaceae). Boletin Real Sociedad Española Historia Natural (Sección Biología) 89: 135–161.

García-Barros E, Munguira ML, Martín Cano J, Romo Benito H, García-Pereira P, Maravalhas ES (2004) Atlas de las mariposas diurnas de la Península Iberica e Islas Baleares. (Lepidoptera: Papilionoidea & Hesperioidea). Monografías de la Sociedad Entomológica Aragonesa 11: 1–228.

García-Barros E, Munguira ML, Stefanescu C, Vives A (2013) Lepidoptera Papilionoidea. In: Ramos MA, et al. (Eds) Fauna Iberica. Vol. 37. Museo Nacional de Ciencias Naturales. CSIC, Madrid, 1213 pp.

García Carrillo A, Leahy K, Vicente Arranz JC (2015) Primeros registros de *Callophrys avis* Chapman, 1909 y *Charaxes jasius* (Linnaeus, 1767) en la vertiente madrileña de la Sierra de Guadarrama (Madrid, España) (Lepidoptera: Papilionoidea). Boletín de la Sociedad Entomológica Aragonesa 57: 455–457.

GBIF (2017) *Charaxes jasius* Linnaeus, 1767 in GBIF Secretariat (2017). GBIF Backbone Taxonomy. Checklist Dataset. https://doi.org/10.15468/39omei [accessed via GBIF.org on 2017-xi-03]

Gómez Bustillo MR, Fernández-Rubio F (1974) Mariposas de la Península Ibérica II. Servicio de Publicaciones del Ministerio de Agricultura, Madrid, 258 pp.

Gómez de Aizpúrua C (1987) Atlas provisional lepidópteros de Madrid. Comunidad de Madrid. Consejería de Agricultura y Ganadería, Madrid, 101 pp.

Gómez de Aizpúrua C (1997) Mariposas diurnas de la Comunidad de Madrid. Consejería de Medio Ambiente y Desarrollo Regional, Madrid, 326 pp.

Gómez de Aizpúrua C, González Granados J, Viejo Montesinos JL (1999) Mariposas del sur de la Comunidad de Madrid. Riada. Estudios sobre Aranjuez 6. Ediciones Doce Calles, Aranjuez, 333 pp.

Gómez de Aizpúrua C, González Granados J, Viejo Montesinos JL (2009) Mariposas y sus biotopos – Lepidoptera (IV). Reserva natural El Regajal mar de Ontígola, memoria 2005–2008. Consejería de Medio Ambiente, Vivienda y Territorio de la Comunidad de Madrid, Madrid, 688 pp.

Gutiérrez D, Thomas CD (2000) Marginal range expansion in a host-limited butterfly species *Gonepteryx rhamni*. Ecological Entomology 25: 165–170. https://doi.org/10.1046/j.1365-2311.2000.00241.x

Kudrna O, Pennerstorfer J, Lux K (2015) Distribution atlas of European butterflies and skippers. Wissenschaftlicher Verlag Peks i.K., Schwanfeld, 632 pp.

Martínez MA, Casado F (1984) Confirmación de la presencia de *Ch. jasius* (L., 1776) en la provincia de Madrid y otras capturas de la zona centro. SHILAP Revista de Lepidopterología 12(48): 336.

Molina M, Pardo de Santayana M, Aceituno L, Morales R, Tardío J (2011) Fruit production of strawberry tree (*Arbutus unedo* L.) in two Spanish forests. Forestry 84: 419–429. https://doi.org/10.1093/forestry/cpr031

Neves VC, Fraga JC, Schäfer H, Vieira V, Bívar de Sousa A, Borges PA (2001) The occurrence of the Monarch butterfly, *Danaus plexippus* L. in the Azores, with a brief review of its biology. Arquipélago. Life and Marine Sciences 18A: 17–24.

Ramírez-Restrepo L, Koi S, MacGregor-Fors I (2017) Tales of urban conservation: *Eumaeus* butterflies and their threatened cycad hostplants. Urban Ecosystems 20: 375–378. https://doi.org/10.1007/s11252-016-0599-0

Simón A (1986) Los ropalóceros de la Casa de Campo. Boletín del grupo entomológico de Madrid 2: 39–82.

Torres JA, Valle F, Pinto C, García-Fuentes A, Salazar C, Cano E (2002) *Arbutus unedo* L. communities in Southern Iberian Peninsula mountains. Plant Ecology 160: 207–223. https://doi.org/10.1023/A:1015864821706

Vicente Arranz JC, García-Carrillo A (2009) Mariposas diurnas de la Comunidad de Madrid. Ediciones La Librería, Madrid, 424 pp.

Fruit-feeding in European cold season noctuid moths increases fecundity, but not longevity

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Abstract. Feeding on rotting fruits, rather than nectar, is linked to high adult life-expectancy in certain butterflies, notably tropical Nymphalidae. We experimentally tested whether cold-season central European noctuid moths may also derive longevity and fecundity benefits from feeding on fruits. Many cold-season noctuid moths avidly feed on such resources. We expected fitness benefits to be especially pronounced in moths which overwinter as adults, in relation to their unusually long and thus nutrient-demanding imaginal life (6–9 months). Field-caught female individuals representing four genera (*Allophyes* Tams, 1942; *Agrochola* Hübner, 1821; *Conistra* Hübner, 1821; *Eupsilia* Hübner, 1821) were offered sucrose solution, sucrose solution enriched with vitamins, or moisturized banana slices plus sucrose solution, respectively, under greenhouse conditions. These moths represented two life-cycle types (autumn species vs. adult hibernators). Life span differed between moth genera, but we did not observe any enhancement of life span through fruit-feeding. Rather, in some cases moths kept with access to banana slices experienced a minor reduction in life span, compared to moths fed sucrose solution only. We observed no benefits in terms of enhanced fecundity through fruit-feeding in autumn species. Among adult hibernators, in contrast, potential fecundity increased by over 50% in banana-fed females, when observed over their full lifetime. Yet, if kept in the lab only after completing their hibernation in the wild, fitness benefits no longer accrued to moths from supplementing their diet with fruits. We conclude that noctuids that hibernate as adults are indeed income breeders which potentially increase their fecundity by feeding on fruits.

Introduction

Regarding the sources of their reproductive investment, adult lepidopterans span the full range from capital to income breeders (Holm et al. 2016). While capital breeders derive all energy required during their adult stage from resources accumulated as larvae, in income breeders reproductive success also depends on nutrient intake of the imago. Carbohydrates and, more rarely so, amino acids taken up with nutritious liquids have been shown to increase longevity or fecundity in a range of butterfly and moth species (Bauerfeind and Fischer 2005; Mevi-Schütz and Erhardt 2005; Cahenzli and Erhardt 2012, 2013). Supplementing nectar carbohydrates with amino acids from pollen results in life-expectancies of 6–9 active months, but is confined to a small fraction of Neotropical *Heliconius* Kluk, 1780 butterfly species (Gilbert 1972; O'Brien et al. 2003). Tropical butterflies which feed on rotting fruit rather than floral nectar may achieve substantial fitness benefits from that mode of resource specialization (Bauerfeind and Fischer 2005; Beck 2007). Overall, fruit feeding in butterflies seems to be strongly linked to high longevity, with some tropical nymphalid butterflies (e.g. *Euphaedra medon* (Linnaeus, 1763)) surviving up to 293 days as adults without dormancy (Molleman et al. 2007).

We here set out to experimentally investigate whether fitness benefits in terms of life-expectancy or fecundity also accrue to moths which feed on rotting fruits. In Europe, few moth species are specialist fruit feeders, but many opportunistically utilize such resources whenever available (Ebert 1997–1998; Süßenbach and Fiedler 1999). In central Europe, the habit of feeding on rotting fruits is especially prevalent amongst Noctuidae species whose adult flight period covers the cold season, i.e. when floral nectar resources are scarce or totally lacking. The strong attraction to rotting fruits might even indicate that moth species using this type of resource gain fitness advantages over mere nectar feeding. Such benefits could be rooted in a more balanced complement of nutrients in fruits as opposed to nectar. In particular, fruits are expected to contain more vitamins or micro-nutrients than standard floral nectars that are notoriously dominated by a few mono- and disaccharides as nutrient compounds (González-Teuber and Heil 2009; Heil 2011).

From a bionomics perspective, central European cold season noctuid species can be divided into autumn species and adult hibernators. Autumn species emerge from the pupa between late August and October. They soon mate and lay their eggs until the onset of true winter weather (usually in November) when all adults die and only the eggs overwinter. Typical representatives are genera such as *Agrochola* Hübner, 1821 and *Allophyes* Tams, 1942. Adult hibernators (like *Eupsilia* Hübner, 1821 and *Conistra* Hübner, 1821) also emerge in autumn, but mating and egg-laying only take place in early spring, after hibernation. These moths intermittently resume flight activity during spells of mild weather in winter when remnants of rotting fruits may provide the only potential food resource. Adults of these hibernator species survive through to April or May, i.e. have life-expectancies of 6–9 months.

We sampled representatives of both life-cycle types from wild populations and subjected them to a feeding experiment in the laboratory. Specifically, we tested two hypotheses:

(a) When given access to a fruit resource of standardized quality in addition to pure carbohydrates, cold season noctuid moths survive for longer periods and lay more eggs than individuals offered sucrose solution only.
(b) Fitness benefits are more pronounced in noctuid species overwintering as adults which thus must survive longer, whereas benefits are smaller in more short-lived autumn species.

Material and methods

Moth sampling

Most moths were attracted using a bait mixture of red wine and sucrose (1:1 weight relation; e.g. Süßenbach and Fiedler 1999). Pieces of cotton cloth (approx. 5 × 10 cm in size) were soaked with this bait and exposed 1.3–1.7 m above ground on shrubs or trees at forest edges. Some additional moths used for experiments were attracted to a light tower (a gauze tower, 1.6 m high, diameter 0.7 m, equipped with two 15 W tubes: F15W/350BL-T8 UVA & F15W/BL B–T8, Sylvania). We sampled moths in the Nationalpark Donau-Auen, about 20 km east of Vienna, Austria (48°08'N, 16°41'E). The region is known to harbour a species-rich moth fauna typical for Central European lowland floodplain forests (Truxa and Fiedler 2012, 2016). There were 27 sampling evenings in the period from 25.ix.–15.xi.2005, and 6 evenings from 30.iii.–9.iv.2006. We exposed baits around sunset, and moths were taken at the baits over a period of 3–4 h per evening. Sampling only took place when ambient temperature was above 0 °C and there was no rain. Altogether, the following numbers of female moths entered into the experiments: 71 *Allophyes oxyacanthae* (Linnaeus, 1758); 14 *Agrochola circellaris* (Hufnagel, 1766); 3 *Agrochola macilenta* (Hübner, 1808);

63 *Eupsilia transversa* (Hufnagel, 1766); 76 *Conistra vaccinii* (Linnaeus, 1761); 6 *Conistra rubiginosa* (Scopoli, 1763); 1 *Conistra rubiginea* ([Denis & Schiffermüller], 1775); and 3 *Conistra erythrocephala* ([Denis & Schiffermüller], 1775).

Treatment in captivity

Upon capture, moths were put in glass vials (50 ml, lined with a piece of filter paper, with air holes in the lid), transferred into a cool and dark bag, kept in a refrigerator overnight and on the next day transported into the laboratory. On the morning after capture, each moth was identified to species and placed into an individually marked rearing jar (transparent 500 ml plastic cups covered with gauze on top). The bottom of each jar was lined with moist filter paper to maintain sufficient humidity. All moths had access to water ad libitum and to cut twigs of their larval host plants for egg laying. Jars were positioned in a greenhouse on the roof of the university building. Temperature varied substantially, with average temperature during day-time being 18 °C (maximum: 26 °C) and 12 °C (minimum: 7 °C) during the nights.

Moths were randomly assigned to one of three feeding treatments: (1) sucrose solution (150 g sucrose in 1000 ml distilled water); (2) sucrose solution of the same concentration, enriched with 7.5 g vitamin mixture (Vanderzant vitamin mix for insects no. 300100, Dyets Inc.; for composition see: http://dyets.com/vitamin-mixes); and (3) the same sucrose solution as in treatment (1) supplemented with fresh slices of banana. A 15 % sugar content (wet weight) in a nectar mimic is rather at the lower edge of profitability for nectarivorous Lepidoptera (e.g. May 1988; Rusterholz and Erhardt 1998) so that beneficial effects of supplementary nutrients could be expected to be particularly strong. Nutrient solutions were offered in 1 ml tubes, while banana slices were placed on top of the gauze lids. Banana was chosen since this fruit can be obtained in standardized quality throughout the year. Fruit slices were kept moist by spraying them with water daily. Sucrose solutions and banana slices were replaced every 2–6 days, as required. For analyses we only considered female individuals. We attempted to achieve a roughly even distribution of individuals across the three food treatments and life-cycle types.

Data collection and statistical analysis

Moths were inspected every 1–2 days, and it was noted if they were still alive. All eggs they had laid were removed and counted. Every individual was followed until its death. Dead moths were preserved in 70% ethanol and later dissected under a stereo microscope. Upon dissection, the number of mature oocytes was counted. The length of time between date of capture and death in captivity was taken as measure of the moth's physiological life span. We used the sum of eggs laid in captivity plus the mature oocytes remaining in the abdomen after death as a measure of potential fecundity. Longevity and fecundity data were log-transformed to improve fit to normality and homoscedasticity assumptions.

For analysis, we partitioned our data into three subsets: (1) autumn species; (2) adult hibernators that remained in the experiment throughout winter; and (3) adult hibernators that had only been collected in early spring, after hibernation in the wild. Autumn moths comprised representatives of two genera (*Allophyes* and *Agrochola*). Adult hibernators were *Eupsilia transversa* and representatives of the genus *Conistra*. We analysed our data by means of Gaussian Linear Models (GLMs), with food treatment and moth genus as categorical predictors. Capture date was included as a covariate to account for the fact that individuals sampled later in their flight season had spent some (unknown) fraction of their life span before being caught for the experiment. For the analysis of fecundity data, we included time spent in the lab as covariate, to account for its potential influence on the number of eggs produced during the

experiment. GLMs for longevity and fecundity were run separately for each of the three data partitions outlined above. Inspection of residuals revealed excellent fit of model assumptions in every case. Throughout the text, means of untransformed data are reported, together with their standard errors, for ease of comprehension. Analyses were performed with Statistica 8.0 (StatSoft Inc.).

Results

Longevity

In autumn moths, capture date had a clear effect on life-expectancy: the later individuals were caught in the season (standardized regression coefficient: $\beta = -0.255 \pm 0.081$), the shorter was their remaining life in captivity. *Allophyes oxyacanthae* were far more short-lived (11.0 ± 0.4 d; maximum: 21 d) than *Agrochola* moths (27.3 ± 4.6 d; maximum: 69 d; Table 1). Moths kept with access to banana slices in addition to sucrose solution lived a significantly shorter time than those in the two other food treatments, but this effect was confined to *Agrochola* moths, as indicated by the significant genus × food interaction term (Fig. 1a).

In adult hibernators overwintering in the lab, capture date was only weakly and not significantly associated with remaining life span ($\beta = -0.080 \pm 0.192$). *Eupsilia transversa* were not significantly more long-lived (135.4 ± 4.8 d; maximum: 156 d) than *Conistra* moths (128.9 ± 10.2 d; maximum 198 d), and adult food type had no significant influence on longevity (Table 1; Fig. 1b).

Finally, among adult hibernators that had overwintered in the wild, capture date was again strongly and negatively associated with remaining life span ($\beta = -0.275 \pm 0.095$). *Eupsilia transversa* females survived for almost the same period of time in the lab (19.1 ± 0.8; maximum: 32 d) as did *Conistra* moths (18.1 ± 0.5; maximum: 28 d). Adult food type significantly influenced

Table 1. Results of GLMs (*F* statistics and *p* values) on moth longevity (log-transformed), with genus and experimental food treatment as categorical factors, and date of capture as covariate. In the two adult hibernator genera, analyses were further partitioned between individuals that had hibernated in the lab, or were included into the experiment only after natural hibernation. df – degrees of freedom. Significant results ($p<0.05$) printed in bold. See Fig. 1 for means and confidence intervals.

	df	F	p
Autumn species			
Genus	1	40.97	**<0.0001**
Food type	2	4.54	**0.0135**
Genus × Food type	2	4.18	**0.0186**
Capture date	1	9.94	**0.0023**
Residual	82		
Adult hibernators, lab			
Genus	1	0.85	0.3627
Food type	2	0.23	0.7957
Genus × Food type	2	0.08	0.9231
Capture date	1	0.18	0.6779
Residual	30		
Adult hibernators, field			
Genus	1	0.01	0.9286
Food type	2	3.69	**0.0284**
Genus × Food type	2	0.49	0.6142
Capture date	1	8.32	**0.0048**
Residual	105		
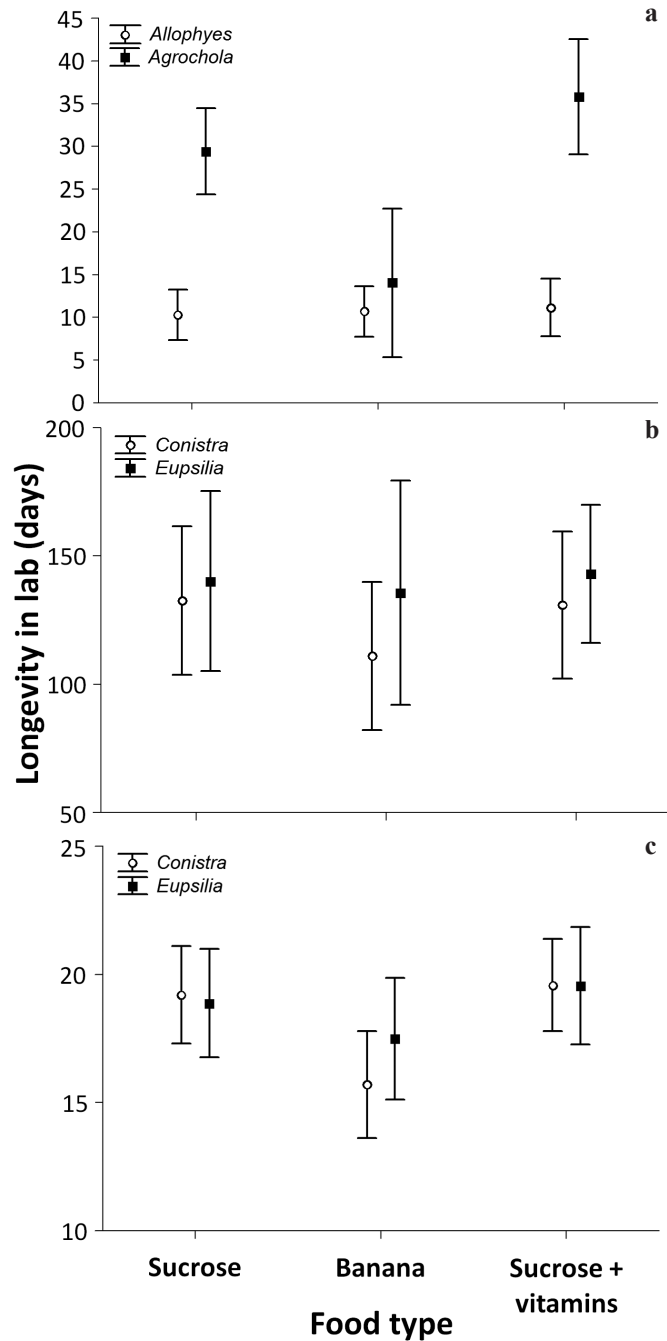
Figure 1. Longevity of cold season noctuid moth females in captivity, according to the three experimental food treatments. 'Banana' refers to moths that had access to fruit slices in addition to sucrose solution as adult food. Given are means (controlling for capture date) and 95% confidence intervals. **a** autumn moths **b** adult hibernators captured in autumn and overwintered in the lab **c** adult hibernators captured after hibernation in the wild.

longevity, with moths offered access to banana slices having lower average life spans after hibernation than those fed only sucrose, or sucrose plus vitamins, solution.

Potential fecundity. Among autumn moths, *A. oxyacanthae* had less than half of the reproductive potential (180.2 ± 10.6 eggs; maximum: 429) than that seen in *Agrochola* females (528.8 ± 54.3 eggs; maximum: 848; Fig. 2a). The longer moths survived in captivity, the more eggs they produced ($\beta = 0.531 \pm 0.091$). However, adult food did not modulate reproductive capacity, nor was there a differential response to food type between the two moth genera (Table 2). Inspection of their bursa copulatrix revealed that all these moths had been mated prior to collection, apart from four *A. oxyacanthae* individuals and two *Agrochola macilenta*. Overall, potential fecundity in the adult hibernator species was far higher. When hibernated in the lab, *Conistra* (730.4 ± 55.3 eggs; maximum: 1100) and *Eupsilia* (604.9 ± 79.5 eggs; maximum: 1427) had similar reproductive capacity, and their life span in the lab was not significantly associated with potential fecundity ($\beta = 0.1023 \pm 0.1495$). However, there was a strong effect of adult food: moths kept with access to banana slices in addition to sucrose solution produced on average over 50% more eggs or oocytes (985.9 ± 78.9) than those in the other two food treatments (with mean fecundity around 600 eggs; Fig. 2b). Except one *Eupsilia* female, none of these moths had been mated when entering into the experiment. When moths of the same life-cycle type were transferred to the lab only after hibernation in the wild, the pattern changed distinctly. Then, *Eupsilia* moths produced far more eggs (853.1 ± 41.7 eggs; maximum: 1569) than *Conistra* females (476.8 ± 17.6 eggs, maximum: 1141) and potential fecundity was again strongly and positively associated with life span in the lab ($\beta = 0.3040 \pm 0.0686$). However, access to banana slices supplementing the sucrose nectar mimic no longer had any detectable influence on fecundity (Table 2, Fig. 2c). All of the females that had hibernated in the wild were mated at the time of capture.

Table 2. Results of GLMs (*F* statistics and *p* values) on moth fecundity (log-transformed), with experimental food treatment and moth genus as categorical factors, and life-time in the lab (log-transformed) as covariate. Among adult hibernators analyses were further partitioned between individuals that had hibernated in the lab, or were included into the experiment only after natural hibernation. df – degrees of freedom. Significant results (*p*<0.05) printed in bold. See Fig. 2 for means and confidence intervals.

|	df	F	p
Autumn species			
Genus	1	11.71	**0.0010**
Food type	2	1.88	0.1599
Genus × Food type	2	0.46	0.6317
Longevity	1	33.91	**<0.0001**
Residual	81		
Adult hibernators, lab			
Genus	1	0.25	0.6217
Food type	2	5.02	**0.0134**
Genus × Food type	2	2.90	0.0711
Longevity	1	0.47	0.4991
Residual	29		
Adult hibernators, field			
Genus	1	89.76	**<0.0001**
Food type	2	0.96	0.3878
Genus × Food type	2	2.39	0.0967
Longevity	1	19.64	**<0.0001**
Residual	104		
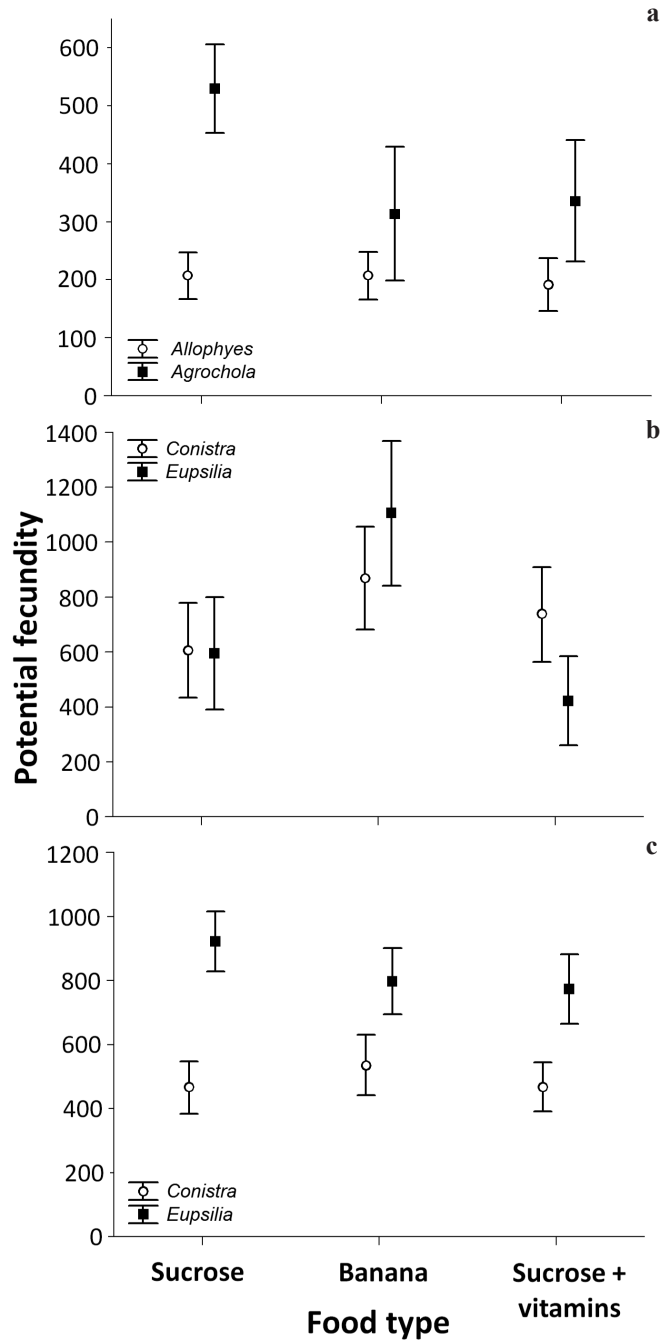
Figure 2. Potential fecundity of cold season noctuid moths in captivity, according to the three experimental food treatments. 'Banana' refers to moths that had access to fruit slices in addition to sucrose solution as adult food. Given are means (controlling for life expectancy in the lab) and 95% confidence intervals. **a** autumn moths **b** adult hibernators captured in autumn and overwintered in the lab **c** adult hibernators captured after hibernation in the wild.

Discussion

Fitness benefits. Concerning moth longevity our experiments revealed no benefits from feeding on fruits, relative to solely sucrose-feeding. This result was comparable across four genera of Central European cold season noctuid moths representing two different life-cycle types (adults only active in autumn; or adults active from autumn to spring with imaginal dormancy). In fact, where subtle food effects could be observed at all, these were in the opposite direction than what might have been expected: female *Agrochola* and *Conistra* kept with access to banana slices in addition to sucrose solution even lived slightly shorter in the laboratory than pure sucrose feeders. Enrichment of the nectar mimic with a vitamin mixture had no discernible effect on life span in any of the three experimental groups.

These results are surprising at a first glance, since other studies have established a clear link between longevity and fruit-feeding in butterflies (Molleman et al. 2007; Bauerfeind and Fischer 2005). Also in multi-species comparisons, the habit of feeding on rotting fruits was associated with unusually long adult life-spans (Molleman et al. 2007; Beck 2007; Beck and Fiedler 2009). Yet, such statistical contingencies across species do not necessarily indicate a causal relationship between high life-expectancy and fruit feeding within species.

Rather, our findings challenge the notion that fruit-feeding among adult Lepidoptera were per se associated with high longevity. This relationship appears to hold true for specialist fruit-feeders like the African satyrine butterfly *Bicyclus anynana* (Butler, 1879) (Bauerfeind et al. 2007; but see Molleman et al. 2008), and many other tropical genuine fruit-feeders. Here fruit-feeding as a specialist strategy has been selected for in combination with a suite of other life-history characters. In contrast, for nectarivores among the Lepidoptera which only visit fruits to opportunistically supplement their diet when needed, this resource use does not necessarily yield longevity benefits over the mere use of nectar.

We attribute this lack of positive response to the digestive physiology of essentially nectarivorous moths. Licking at banana slices will likely yield dissolved mono- and disaccharides as well as other dissolved micro-nutrients. Yet, these nutrients did apparently not translate into longevity benefits. Moreover, fruit slices undergo microbial decay under the relatively warm and humid conditions in our experiment (E.-M. McMannis and K. Fiedler, unpublished observations). Even though we replaced fruit slices frequently this decay may have been the reason for premature death in some banana-fed individuals, especially in *Agrochola* and *Conistra* moths.

In insects that specialize on rotting fruits, decomposition products, such as yeast amino acids, and the complex admixture of nutrients encountered in fruits are essential to achieve fitness benefits (Bauerfeind et al. 2007; Geister et al. 2008; Molleman et al. 2008; Beaulieu et al. 2017). To better locate their resources, specialist fruit-feeders also tend to be highly responsive to alcohols and other by-products of microbial decay of fruits (Ômura et al. 2000; Dierks and Fischer 2008; Tang et al. 2013). While noctuid moths may be responsive to complex odour blends emitted by food resources (e.g. Gregg et al. 2010), we are unaware of any studies on the perception of rotting fruit volatiles among these insects.

While we did not observe any increase of life span through access to fruit in addition to sucrose, clear positive effects of fruit-feeding on moth fecundity could be established. However, this increase in potential fecundity was confined to the two genera *Eupsilia* and *Conistra*. These unusually long-lived moths hibernate as adults and thus experience particularly strong demands for nutrient income beyond their larval stages. Indeed, benefits were very substantial, with an increase in potential fecundity of >50% as compared to individuals kept on an exclusive carbohydrate diet. These considerable

fitness benefits however, only became apparent in moths captured in autumn and subjected to the experiment over the whole winter into early spring, i.e. over a life time of 3–4, and occasionally more than 5, months. In representatives of the same moth species subjected to the food treatments just after hibernation in the wild, the beneficial effects of banana feeding totally vanished. We attribute this to the rather short remaining life span of these insects after hibernation (on average about 2.5 weeks in the laboratory). This time was probably insufficient to allow these insects to gain additional advantages in terms of egg maturation, beyond the fitness benefits accruing from sugar consumption alone. In line with this interpretation, we did not observe any fecundity benefits from providing banana slices in addition to carbohydrates in the autumn moths with their average adult life-span of just 2–3 weeks.

Except for one single *Eupsilia transversa* female, none of the adult hibernators sampled in autumn had already been mated when entering into the experiment. Hence, these moths lacked all nutrients they might possibly derive from the spermatophore donated by their mates (Torres-Vila et al. 2004). In contrast, all *Conistra* and *Eupsilia* females captured in early spring were mated when transferred into the laboratory. It is possible that the absence of spermatophores increased the fecundity effect of additional nutrients in the lab-hibernated females.

All moths studied in our experiment are opportunistic feeders during their adult stage. They drink floral nectar whenever available (see Ebert 1997–1998 for records of flower visits of our study species in SW Germany). Apparently, concentrated sucrose solution provides these moths with sufficient nutrients relevant to longevity and fecundity, except for those few species with extraordinarily long adult life-spans. Hence, the habit of avidly feeding on rotting fruits as seen in these cold-season moths does not offer them a genuine advantage over nectar feeding. Rather, our results suggest that this peculiar habit just helps cold-season moths to survive periods of very restricted availability of nectar flowers.

Differences between taxa in life-history strategies. The principal aim of our experimental study was to compare the two life-history types (autumn species vs. adult hibernators) and the two modes of adult hibernation (in the field vs. under experimental conditions). Yet, we also observed taxon-specific differences in life-history strategies. For example, even though adult flight periods and basic life-cycle characters are very similar between *Allophyes oxyacanthae* and the various Central European *Agrochola* species, females of the latter genus lived distinctly longer, and also developed more than twice as many eggs. These differences are opposite to what one might expect from variation in body size (*Allophyes* is larger, with fore wing length 17–20 mm, compared to 14–19 mm in *Agrochola*: Skou 1991). In our experiments, *Allophyes oxyacanthae* laid three times larger eggs (mean diameter ± 1 SD: 0.439 ± 0.079 mm, n = 1249) than *Agrochola circellaris* (0.133 ± 0.015 mm, n = 235; E. McMannis & K. Fiedler, pers. observ.). Hence, the overall investment in eggs was likely to be rather larger in *A. oxyacanthae* than among *Agrochola* moths. A possible explanation for the far higher egg number could be that early larval mortality is so high in *Agrochola* that a larger number of eggs are required to compensate for such losses. The mandatory tight matching between the timing of larval hatching and bud-break of host trees in spring could be important here.

In the adult hibernators *Conistra* and *Eupsilia* fecundity was overall far higher than in autumn moths. Differences in fecundity between these two genera were small in individuals kept over winter in the lab and only became distinct after hibernation in the wild. Eggs of *Conistra vaccinii* (0.279 ± 0.024 mm, n = 664) were considerably larger than in *Eupsilia transversa* (0.221 ± 0.029 mm, n = 905), which is the opposite pattern than with regard to body size (*Eupsilia transversa*: fore wing length 18.16 ± 0.64 mm, n = 61; *Conistra vaccinii*: 14.58 ± 0.53 mm, n = 74). These adult hibernators have much more time to develop eggs during their extended adult life span and, as shown above, they

indeed have the capacity to benefit from extra-nutrients obtained via fruit-feeding. High fecundity accompanied by small egg size indicates that they both are r-selected insects (see Davis et al. 2016), *Eupsilia* even more strongly so than *Conistra*. Larvae of *Eupsilia* and *Conistra* have a reputation to preying on insects like other spring caterpillars, including immatures of their own species (Schweitzer 1979; Pierce 1995; Ebert 1997–1998; see also Richardson et al. 2010). It may thus be desirable for a female to spread her offspring over many host plants, rather than laying multiple eggs at each site. As in *Agrochola*, a tight synchronization between larval hatching and budbreak is also of paramount importance in these adult-hibernating species. Hence, selection might have favoured investment into more, but smaller eggs in such species, if early larval mortality risks are particularly severe.

Methodological issues. Our data on life spans are underestimates of true potential physiological longevity, since we sampled moths from natural populations and hence their age and individual history at the onset of the experiment was unknown to us. However, this was true for all moths tested from all species and life-cycle types in the same manner. Moreover, we included date of capture as a co-variate to alleviate effects of pre-capture life-time as much as possible. Rearing all experimental insects from the egg stage under controlled conditions would of course have been optimal, but was out of scope for the present study due to the high demand regarding lab rearing. Overall, the high maximum longevity of all taxa observed (*Allophyes*: 22 d; *Agrochola* 69 d; *Conistra*: 198 d; *Eupsilia*: 159 d) indicates that laboratory conditions were suitable for the moth species concerned. An adult life span of close to 200 d may well represent a new record high for any temperate-zone noctuid moth that does not undergo a true diapause.

In the analyses presented above, we focussed on potential fecundity, i.e. the sum of eggs laid in captivity plus the mature oocytes remaining in a female's abdomen after its death. We feel this procedure to be justified since the mere number of eggs laid, viz. realized fecundity, can be misleading in experimental situations such as ours. For example, it was not always possible to offer high quality egg-laying substrates in such large amounts that females could have spread their eggs singly or in small groups, as they would do in nature. Hence, motivation to lay eggs may have been confounded by the circumstances applicable in captivity. Moreover, mating status and health of the moths may sometimes preclude that potential fecundity also translates into oviposition acts. However, analysis of realized fecundity yielded largely the same patterns as we present here with regard to potential fecundity (Zimmermann 2017).

In our experiments we offered banana as a standardized food. This is, of course, not a resource naturally available to any of our study species. We do not know whether decaying fruits that noctuid moths may encounter in autumn and winter in Central European woodlands, such as brambles, apples, plums or similar, may provide nutrients in different amounts than fresh banana slices. Hence, it remains to be seen if similar fecundity benefits might accrue in experiments employing more 'natural' food resources.

In summary, our experiments reveal that over a range of cold season moths, including two genera with unusually long adult life, there was no indication that facultative fruit-feeding, in comparison to nectar mimics, would enhance longevity. In contrast, beneficial effects on fecundity were pronounced in adult hibernators, which represent extreme cases of income breeders among the Lepidoptera. Autumn noctuid species, in contrast, turned out to be positioned more at the capital breeders' part of the continuum. It would be interesting to test if similar fecundity benefits can be found in other European moths that hibernate as adults. Among the Noctuidae, the genera *Lithophane* Hübner, 1821 and *Xylena* Ochsenheimer, 1816 are the most likely candidates to behave similarly as *Eupsilia* and *Conistra*.

Acknowledgements

References

Bauerfeind SS, Fischer K (2005) Effects of adult-derived carbohydrates, amino acids and micronutrients on female reproduction in a fruit-feeding butterfly. Journal of Insect Physiology 51: 545–554. https://doi.org/10.1016/j.jinsphys.2005.02.002

Bauerfeind SS, Fischer K, Hartstein S, Janowitz S, Martin-Creuzburg D (2007) Effects of adult nutrition on female reproduction in a fruit-feeding butterfly: the role of fruit decay and dietary lipids. Journal of Insect Physiology 53: 964–973. https://doi.org/10.1016/j.jinsphys.2007.01.013

Beaulieu M, Franke K, Fischer K (2017) Feeding on ripening and over-ripening fruit: interactions between sugar, ethanol and polyphenol contents in a tropical butterfly. Journal of Experimental Biology 220: 3127–3134. https://doi.org/10.1242/jeb.162008

Beck J (2007) The importance of amino acids in the adult diet of male tropical rainforest butterflies. Oecologia 151: 741–747. https://doi.org/10.1007/s00442-006-0613-y

Beck J, Fiedler K (2009) Adult life spans of butterflies (Lepidoptera: Papilionoidea + Hesperioidea): broad-scale contingencies with adult and larval traits in multi-species comparisons. Biological Journal of the Linnean Society 96: 166–184. https://doi.org/10.1111/j.1095-8312.2008.01102.x

Cahenzli F, Erhardt A (2012) Nectar sugars enhance fitness in male *Coenonympha pamphilus* butterflies by increasing longevity or realized reproduction. Oikos 121: 1417–1423. https://doi.org/10.1111/j.1600-0706.2012.20190.x

Cahenzli F, Erhardt A (2013) Nectar amino acids enhance reproduction in male butterflies. Oecologia 171: 197–205. https://doi.org/10.1007/s00442-012-2395-8

Davis RB, Javoiš J, Kaasik A, Õunap E, Tammaru T (2016) An ordination of life histories using morphological proxies: capital vs. income breeding in insects. Ecology 97: 2112–2124. https://doi.org/10.1002/ecy.1435

Dierks A, Fischer K (2008) Feeding responses and food preferences in the tropical, fruit-feeding butterfly, *Bicyclus anynana*. Journal of Insect Physiology 54: 1363–1370. https://doi.org/10.1016/j.jinsphys.2008.07.008

Ebert G (1997–1998) Die Schmetterlinge Baden-Württembergs. Vols. 5–7. E. Ulmer, Stuttgart.

Geister TL, Lorenz MW, Hoffmann KH, Fischer K (2008) Adult nutrition and butterfly fitness: effects of diet quality on reproductive output, egg composition, and egg hatching success. Frontiers in Zoology 5(10). https://doi.org/10.1186/1742-9994-5-10

Gilbert LE (1972) Pollen feeding and reproductive biology of *Heliconius* butterflies. Proceedings of the National Academy of Sciences of the United States of America 69: 1403–1407. https://doi.org/10.1073/pnas.69.6.1403

González-Teuber M, Heil M (2009) Nectar chemistry is tailored for both attraction of mutualists and protection from exploiters. Plant Signaling & Behavior 4: 809–813. https://doi.org/10.4161/psb.4.9.9393

Gregg PC, Del Socorro AP, Henderson GS (2010) Development of a synthetic plant volatile-based attracticide for female noctuid moths. II. Bioassays of synthetic plant volatiles as attractants for the adults of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Australian Journal of Entomology 49: 21–30. https://doi.org/10.1111/j.1440-6055.2009.00734.x

Heil M (2011) Nectar: generation, regulation and ecological functions. Trends in Plant Science 16: 191–200. https://doi.org/10.1016/j.tplants.2011.01.003

Holm S, Davis RB, Javoiš J, Õunap E, Kaasik A, Molleman F, Tammaru T (2016) A comparative perspective on longevity: the effect of body size dominates over ecology in moths. Journal of Evolutionary Biology 29: 2422–2435. https://doi.org/10.1111/jeb.12966

May PG (1988) Determinants of foraging profitability in two nectarivorous butterflies. Ecological Entomology 13: 171–184. https://doi.org/10.1111/j.1365-2311.1988.tb00345.x

Mevi-Schütz J, Erhardt A (2005) Amino acids in nectar enhance butterfly fecundity: a long-awaited link. The American Naturalist 165: 411–419. https://doi.org/10.1086/429150

Molleman F, Zwaan BJ, Brakefield PM, Carey JR (2007) Extraordinary long life spans in fruit-feeding butterflies can provide window on evolution of life span and aging. Experimental Gerontology 42: 472–482. https://doi.org/10.1016/j.exger.2007.01.008

Molleman F, Ding J, Wang JL, Brakefield PM, Carey JR, Zwaan BJ (2008) Amino acid sources in the adult diet do not affect life span and fecundity in the fruit-feeding butterfly *Bicyclus anynana*. Ecological Entomology 33: 429–438. https://doi.org/10.1111/j.1365-2311.2008.00986.x:

Pierce NE (1995) Predatory and parasitic Lepidoptera: carnivores living on plants. Journal of the Lepidopterist's Society 49: 412–453. http://images.peabody.yale.edu/lepsoc/jls/1990s/1995/1995-49(4)412-Pierce.pdf

O'Brien DM, Boggs CL, Fogel ML (2003) Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. Proceedings of the Royal Society of London B: Biological Sciences, 270: 2631–2636. https://doi.org/10.1098/rspb.2003.2552

Ômura H, Honda K, Hayashi N (2000) Identification of feeding attractants in oak sap for adults of two nymphalid butterflies, *Kaniska canace* and *Vanessa indica*. Physiological Entomology 25: 281–287. https://doi.org/10.1046/j.1365-3032.2000.00193.x

Richardson ML, Mitchell RF, Reagel PF, Hanks LM (2010) Causes and consequences of cannibalism in noncarnivorous insects. Annual Review of Entomology 55: 39–53. https://doi.org/10.1146/annurev-ento-112408-085314

Rusterholz HP, Erhardt A (1998) Effects of elevated CO_2 on flowering phenology and nectar production of nectar plants important for butterflies in calcareous grasslands. Oecologia 113: 341–349. https://doi.org/10.1007/s004420050385

Schweitzer DF (1979) Predatory behavior in *Lithophane querquera* and other spring caterpillars. Journal of the Lepidopterist's Society 33: 129–134. http://images.peabody.yale.edu/lepsoc/jls/1970s/1979/1979-33(2)129-Schweitzer.pdf

Süßenbach D, Fiedler K (1999) Noctuid moths attracted to fruit baits: testing models and methods of estimating species diversity. Nota lepidopterologica 22: 115–154.

Skou P (1991) Nordens ugler: Handbog over de i Danmark, Norge, Sverige, Finland og Island forekommende arter af Herminiidae og Noctuidae (Lepidoptera). Apollo Books, Stenstrup, 565 pp.

Tang Y, Zhou C, Chen X, Zheng H (2013) Foraging behavior of the dead leaf butterfly, *Kallima inachus*. Journal of Insect Science 13: 58. https://doi.org/10.1673/031.013.5801

Torres-Vila LM, Rodríguez-Molina MC, Jennions MD (2004) Polyandry and fecundity in the Lepidoptera: can methodological and conceptual approaches bias outcomes? Behavioral Ecology and Sociobiology 55: 315–324. https://doi.org/10.1007/s00265-003-0712-2

Truxa C, Fiedler K (2012) Down in the flood? How moth communities are shaped in temperate floodplain forests. Insect Conservation and Diversity 5: 389–397. https://doi.org/10.1111/j.1752-4598.2011.00177.x

Truxa C, Fiedler K (2016) Massive structural redundancies in species composition patterns of floodplain forest moths. Ecography 39: 253–260. https://doi.org/10.1111/ecog.01430

Zimmermann E-M (2017) Frugivorous Noctuoidea in the Danube flood plain east of Vienna – guild structure and reproductive biology. MSc thesis, University of Vienna, 69 pp. http://othes.univie.ac.at/49328/1/52242.pdf

Incurvaria pirinella sp. nov., a new species of the *vetulella* species-group (Lepidoptera, Incurvariidae) from Bulgaria, with release of DNA barcodes for European species of *Incurvaria*

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Abstract. *Incurvaria pirinella* Junnilainen, Kaitila & Mutanen, **sp. nov.** is described from Bulgaria based on specimens collected by netting and artificial light from several low-elevation localities in Bulgaria. The species is morphologically and genetically most similar to *I. triglavensis* Hauder, 1912. Differences between these two species are present in external appearance and genitalia of both sexes. Additionally, *I. pirinella* shows a distance of 4.74% to its nearest neighbour *I. triglavensis* in the standard DNA barcoding marker (COI-5P). We provide preliminary observations of phylogenetic affinities of European *Incurvaria* and briefly discuss habitat preferences of some species. All species have distinct barcodes with minimum K2P divergences between species averaging 7.05% (range 1.2–12.8%). A world checklist of *Incurvaria* Haworth, 1828 is provided and DNA barcodes for all European species are here released. Finally, we document morphological variation in male genitalia within *I. triglavensis* Hauder, 1912.

Introduction

During several entomological expeditions to the southern Pirin mountain range in Bulgaria, the authors JJ and JPK collected specimens of *Incurvaria* Haworth, 1828, which originally were believed to belong to *Incurvaria triglavensis* Hauder, 1912, described from the Triglav mountain in Slovenia. Later, however, we noticed constant differences in the external habitus between our specimens and those of *I. triglavensis* as illustrated by Huemer (1993). We also observed that the Bulgarian taxon occurs in low-elevation habitats with mainly Mediterranean vegetation, whereas other species of the *vetulella*-group prefer alpine habitats in Central and Southern Europe. Therefore, a tissue sample of the Bulgarian taxon was sequenced for the DNA barcode region of the mitochondrial COI gene, which turned out to differ clearly from all other European species of *Incurvaria*. Examination of the genitalia of Bulgarian specimens showed differences from *I. triglavensis* in both male and female genitalia. Therefore, we describe the taxon here as a new species. We also provide DNA barcodes for all European species of *Incurvaria*, as well as for three other European Incurvariidae.

Material and methods

Collecting. The specimens of *I. pirinella* sp. nov. were captured with insect nets during daytime, in the evening and early in the morning, as well as using artificial light late at night. Specimens were stored alive in glass vials and killed in a freezer, after which they were spread.

Morphological examination. Genitalia preparations were conducted following standard techniques (Robinson 1976). Although the sclerotised parts are well visible without staining, male genitalia were moderately stained with mercurochrome, because unstained parts become nearly transparent after a few years in Euparal. To make membranous parts of the female genitalia more visible, they were stained with chlorazol-black. The unrolling technique (Nielsen 1980) was applied for some male specimens to present different views and to make them comparable with earlier illustrations. Additionally, photos of free-floating genitalia were taken prior to final preparation to give an overview of its three-dimensional structure, as some diagnostic features are best viewed that way. Morphometrics of several genital features of *I. triglavensis* and *I. pirinella* sp. nov. are given in Suppl. material 1 (with two-directional T-tests applied when applicable). Numbers of measured samples (n) vary between species and sexes due to material limitations and in some instances also due to mounting artefacts resulting from three-dimensionality of genital structure and different preparation techniques. We therefore only included samples in the morphometric analyses that were comparable in terms of mounting and preparation technique.

Photographic documentation. Photographs of *I. triglavensis* specimens were taken with a Canon EOS 5D Mark III and Canon lens MP-E 65 at 2:1 using a ring flash. Genitalia photos were taken with a Wild Heerbrugg microscope using a 10× objective and a 2.5× ocular. Photos were edited using Helicon Focus 4.80 and Adobe Photoshop 6.0. For photography of free-floating genitalia, they were placed on a microscope slide with an excavation, filled with equal amounts of glycerol and water. A trace of anionic surfactant was added. Finally, the excavation was covered with a cover glass, leaving no air in the excavation. Photographs were taken with the same equipment as the embedded genitalia.

The camera system used for photos of adult *I. pirinella* was a Nikon D800 with Mikro Nikkor 105 mm 1:2.8 D objective and three flash heads. The camera was moved between shots with a Cognisys Stackshot focussing rail. Serene Stacker v. 1.04 and Adobe Lightroom 6.7 were used for processing the photos, and 36 shots were combined in each photo stack.

DNA barcoding. For the DNA analyses, tissue samples were sent in a lysis plate to the Canadian Centre for DNA barcoding, Ontario, Canada, through the Finnish Barcode of Life campaign (www.finbol.org). DNA extraction, amplification, and sequencing of the barcode region of the mitochondrial cytochrome oxidase I (COI) gene (658 base pairs at the 5' terminus) were carried out following protocols by deWaard et al. (2008). The taxonomic and collection data, voucher image, COI sequence and GenBank accession numbers are available for all specimens in the BOLD database (http://www.boldsystems.org) through the public dataset DS-INCURVEU (dx.doi.org/10.5883/DS-INCURVEU).

We compared the DNA barcodes of *I. pirinella* with all other European species of *Incurvaria*, each of which had been barcoded along with national DNA barcoding initiatives in Finland, Austria and Germany. These data were supplemented with DNA barcodes of three other European species of the family Incurvariidae: *Alloclemensia mesospilella* (Herrich-Schäffer, 1854), *Phylloporia bistrigella* (Haworth, 1828) and *Crinopteryx familiella* Peyerimhoff, 1871, the last

representing the subfamily Crinopteryginae (all other European species belong to the nominal subfamily). Therefore, only two European species of the family, *Paraclemensia cyanella* (Zeller, 1850) and *Vespina slovaciella* (Zagulajev & Tokár, 1990) are not included. Kimura 2-parameter (K2P) DNA barcode divergences were examined using the BOLD v. 4beta barcode gap analysis tool (Ratnasingham and Hebert 2007). A Neighbor-Joining (NJ) tree was constructed with Mega 7 (Kumar et al. 2016) under the Kimura 2-parameter nucleotide substitution model. A Maximum Likelihood (ML) analysis was conducted with Mega 7 under the GTR+G model for nucleotide substitutions. The node confidences were examined with 500 bootstrap replicates. Both trees were rooted on *Crinopteryx familiella*.

Terminology. The morphological terminology used here mainly follows Huemer (1993) and Davis (1998). In our comparisons of the newly described species with previously described species of the *vetulella*-group, we largely rely on the thorough revision by Huemer (1993).

Results

The genus *Incurvaria* Haworth, 1828 contains nine described species in Europe (van Nieukerken and Kozlov 2017). Four of these species were assigned to the *Incurvaria vetulella* species-group in a revision by Huemer (1993): *I. vetulella* (Zetterstedt, 1839), *I. circulella* (Zetterstedt, 1839), *I. triglavensis* Hauder, 1912, *I. ploessli* Huemer, 1993. *Incurvaria pirinella* is a new member of European Incurvariidae clearly belonging to the *vetulella* species-group.

To the best of our knowledge, only four other species of *Incurvaria* are known from the Holarctic region: *I. evocata* (Meyrick, 1924) described from India, (Nielsen 1982) *I. takeuchii* Issiki, 1957 and *I. alniella* (Issiki, 1957) described from Japan (Okamoto and Hirowatari 2004) and *I. similella* Schmitz, 1969 described from the Caucasus (Schmitz 1969). These species do not belong to the *I. vetulella* species-group. In North America, only *I. vetulella* and *I. circulella* are known (Pohl et.al. 2016, 2018).

Incurvaria muchei Soffner, 1969 from the Caucasus has been transferred to the genus *Alloclemensia* Nielsen, 1981 and considered as synonym of *A. devotella* (Rebel, 1893) (Nielsen 1981). The poorly known species *I. triangulifera* Tengström, 1869 has recently been considered to be a junior synonym of *Lampronia provectella* Heyden, 1865 (van Nieukerken and Kozlov 2017) and thus belongs to the family Prodoxidae. *Incurvaria kivatshella* Kutenkova, 1987, described from a single male specimen, was considered not to belong to the *vetulella*-group due to the absence of the distal projection of the phallus (Huemer 1993), but Kozlov (1996) synonymised it with *I. vetulella*. *Incurvaria brigantinella* Amsel, 1961 was described from Germany, Baden-Württemberg, Bodensee, based on a single type specimen, which is probably lost; it is therefore considered to a be nomen dubium and was not included in Fauna Europaea (van Nieukerken and Kozlov 2017). The original description including a black and white drawing of the holotype clearly shows that *I. brigantinella* does not belong to the *vetulella* species-group. It resembles *I. praelatella* in having rather similar forewing markings and distinctly white forewing fringe.

World checklist with type localities

Incurvaria Haworth, 1828
      =*Excurvaria* Kuprijanov, 1994
I. alniella (Issiki, 1957). Japan, Honshu.

I. circulella (Zetterstedt, 1839). Sweden, Lappmark, Lycksele.
   (original combination *Adela circulella* Zetterstedt, 1839)
I. evocata (Meyrick, 1924). India, Assam.
I. koerneriella (Zeller, 1839). Europe.
   (original combination *Tinea koerneriella* Zeller, 1839)
I. masculella (Denis & Schiffermüller, 1775). Austria, Vienna.
   (original combination *Tinea masculella* Denis & Schiffermüller, 1775)
I. oehlmanniella (Hübner, 1796). Europe.
   (original combination *Tinea oehlmanniella* Hübner, 1796)
I. pectinea Haworth, 1828. Great Britain.
   (original combination *Tinea zinckenii* Zeller, 1839)
I. pirinella sp. nov. SW Bulgaria, Blagoevgrad, Pirin mountain range.
I. ploessli Huemer, 1993. Italy, Alps Maritime.
I. praelatella (Denis & Schiffermüller, 1775). Austria, Vienna.
   (original combination *Tinea praelatella* Denis & Schiffermüller, 1775)
I. similella Schmitz, 1969. Caucasus.
I. takeuchii Issiki, 1957. Japan, Honshu.
I. triglavensis Hauder, 1912. Slovenia, Vosshütte.
I. vetulella (Zetterstedt, 1839). Norway, Nordland.
   (original combination *Adela vetulella* Zetterstedt, 1839)
   =*Incurvaria kivatshella* Kutenkova, 1987

Patterns of DNA barcode divergences of European Incurvariidae

Of five analysed specimens of *I. pirinella*, only one yielded a sequence (658 bp, full barcode fragment). Altogether 50 specimens of 13 species of European Incurvariidae, including all ten species of *Incurvaria*, were compared for their DNA barcodes. The species of the genera *Alloclemensia*, *Phylloporia* and *Crinopteryx* (each containing only one species in Europe) show unique DNA barcodes (K2P divergences to the closest species 12.95–13.82%). For *Incurvaria* minimum K2P divergence to the closest species averages 7.05% (range 1.2–12.82%). The maximum intraspecific variation averages 0.81% across species (range 0–3.46%; mean n=4.2; *I. pirinella* is not considered, as only one specimen was barcoded). The K2P distance between the genetically closest species exceeds 4.7% across all species except between *I. vetulella* and *I. ploessli*, which differ by a K2P divergence of 1.2% only. Moreover, *I. vetulella* appears paraphyletic with respect to *I. ploessli*, although the latter species forms a distinct barcode cluster.

Description of the new species

Incurvaria pirinella Junnilainen, Kaitila & Mutanen, sp. nov.
http://zoobank.org/DBDEC675-83A6-4AF6-A4C0-523D58C1E286

Type material. Holotype: ♂, Bulgaria, Blagoevgrad district, Struma river valley, Stara Kresna 275m a.s.l., 41.795N, 23.157E; 03.v.2013. J. Junnilainen leg. & coll. with red label "HOLOTYPE of *Incurvaria pirinella* Junnilainen, Kaitila & Mutanen". - Paratypes 21♂; 6♀: 4♂; 1♀ same locality and data as holotype, Genitalia prep. ♂ No: GPJJ201702, GPPB3334, 2♂ in glycerol, J. Junnilainen leg. & coll.; 7♂; 1♀ Bulgaria, Blagoevgrad district, southern Pirin 1200m a.s.l., 41.528N, 23.584E; 30.v.2006, Genitalia prep. ♂ No: GPJJ201701, 1 ♂ genitalia in glycerol, J. Junnilainen leg. & coll. , 1♂ in. Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria;

1♂ Bulgaria, Blagoevgrad district, southern Pirin 1300m a.s.l., 41.574N, 23.656E; 21–24.vi.2001 with green label DNA sample 24476 Lepid phyl., J. Junnilainen leg. & coll.; 4♂; 3♀ Bulgaria, Blagoevgrad district, Ilindenci road, meadows below barrier 900m a.s.l., 41.675N, 23.278E; 16.v.2012. J-P. Kaitila & Bo Wikström leg, coll. J-P. Kaitila, 1♂, 1♀ in Finnish Museum of Natural History, University of Helsinki, 1♀ in Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria. 2♀ Bulgaria, Blagoevgrad district, Slavianka, Goleshovo road, highest point 1000m a.s.l., 41.4034N, 23.389E; 25.v.2012, Genitalia prep. No: GPJJ201704. In coll. J-P. Kaitila; 5♂ Bulgaria, Blagoevgrad district, Ilindenci 900m a.s.l., 41.67N, 23.27E; 30.v.2012. Bo Wikström leg. & coll. Genitalia prep. No: GPJJ201701, GPJJ201702, GPJJ201704, GPPbf3327, GPPbf3328, GPPbm3329, GPPbm3330, GP-Pbm3334. All paratypes with red label "PARATYPE of *Incurvaria pirinella* Junnilainen, Kaitila & Mutanen".

Deposition of types. The holotype of *Incurvaria pirinella* is deposited in the research collection of J. Junnilainen. Paratypes are deposited in the Finnish Museum of Natural History, University of Helsinki, Tiroler Landesmuseum Ferdinandeum (Innsbruck, Austria) and in the research collections of J. Junnilainen, J-P. Kaitila & Bo Wikström. The holotype is available on loan by request through the Finnish Museum of Natural History, University of Helsinki or directly from the first author.

Etymology. The name of the new species is derived from the Pirin mountain range, where the new species is widely distributed.

Diagnosis. Considering similar forewing ground colour and markings, *Incurvaria pirinella* (Figs 3–5) is externally closest to *I. circulella* (Fig. 14), but is easy to separate from it by the dark grey-brown fringe and distinct white spot in the forewing fringe present in *I. circulella*. *Incurvaria pirinella* is also widely allopatric as *I. circulella* occurs only in northern latitudes in Europe, e.g. the northern part of Sweden and Finland.

Although *I. triglavensis* is rather variable externally, it is always easy to separate from *I. pirinella* based on its relatively narrow forewing shape, paler ground colour and differences in forewing markings (Figs 6–11); see details in Suppl. material 2.

Incurvaria pirinella is easy to separate externally from Scandinavian *I. vetulella* (Figs 12–13) by a white narrow dorsal spot situated 2/5 from base and extending more than halfway across the forewing in *I. pirinella*. North European *I. vetulella* also typically have a distinctly broader dorsal spot, sparser forewing scaling and usually a pale medial area in the fringe, which is always absent in *I. pirinella*.

Male genitalia of *I. pirinella* are most similar to those of *I. triglavensis*, but small constant differences are present. *I. pirinella* has a stouter valva and vinculum. The valva of *I. pirinella* is broader in middle and the margin of the sacculus is more bulged (Figs 21–22). The vinculum of *I. pirinella* is relatively short and broad with clearly concave lateral margins. In *I. triglavensis*, the apex of the valva is more elongated and the vinculum is clearly longer and more slender with straight or slightly concave lateral margins (Figs 1a–b, 17e, 19b, 20e). The lateral arms of the transtilla are more robust (Fig. 20a), and submedial anterior projections shorter in *I. pirinella* (Figs 1c, 20d). The length of the transtilla's medial knob with anterior submedial projection is 0.26–0.27 mm in *I. pirinella* and 0.29–0.33 mm in *I. triglavensis*. The juxta is longer (0.95–0.98 mm) in *I. pirinella* than in *I. triglavensis* (0.8–0.85 mm) (Fig. 16, Suppl. material 1). Also, the ratio between the length of the juxta versus the transtilla's medial knob with its anterior projections is different between the species, being 3.56–3.77 in *I. pirinella* and 2.76–2.83 in *I. triglavensis* (see Suppl. material 1). The shape and angle of the distal thorn/hook-shaped projection of the phallus show variation, that is at least partly due to the position of the phallus in genitalia preparations (Figs 15d1, 18a–b); thus, this does not provide a good characteristic. However, the ratio between the maximum length

Figure 1. *Incurvaria pirinella*, green and *I. triglavensis* magenta. Shape of male genitalia compared: **1a.** left valva; **1b.** vinculum; **1c.** knob of transtilla with anterior projections.
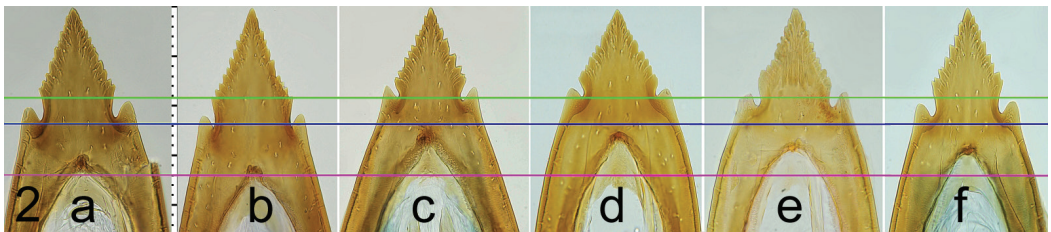

Figure 2. *Incurvaria pirinella* and **c-f.** *I. triglavensis* length of female oviscapt compared with coloured horizontal lines. Slide numbers **a.** GPPbf3327; **b.** GPPbf3328; **c.** GPPbf3324; **d.** GPPbf3325; **e.** GPPH-Inc17; **f.** GPPbf3335. Scale bar: 1 line = 0.01 mm.

Figures 3–5. *Incurvaria pirinella* habitus of adult. **3.** Male (Holotype). **4.** male (Paratype). Ilindenci road. Coll. Kaitila **5.** Female (Paratype). Slavianka. Coll. Kaitila.

of phallus versus the length of the distal projection seems to be different between the species as well (Figs 18a–b). In the examined samples, it was 3.34–3.78 for *I. pirinella* and 3.82–4.07 for *I. triglavensis*. Shape, size and ratio between length/width of abdominal segments T8 and S8 are different: See shape in Figs 23, 24, 25 and size and ratio measurement values in Suppl. material 1. Although *I. triglavensis* is a rather variable species, additional slightly different but sometimes

Figures 6–11. *Incurvaria triglavensis* habitus of adults. **6.** Male. Austria Osttirol-Defereggen Gebirge 2200m 2013.vii.22 H. Deutsch leg.; **7.** Male. Slovenia Julijske Alpe Kamin, Sudseite 1700m 19.vii.1997 H. Deutsch leg.; **8.** Male. Slowenien-Steiner Alpe 1500m 1992.viii.12 Habeler leg.; **9.** Female. Slovenia, Steiner A. Krvavec Veli zwoh 20.vii.1992 1950m Habeler leg.; **10.** Female. Austria Salzburg Nockgebiet Matchanshöhe 1900m 24.vi.1999 Habeler leg.; **11.** Female. Austria Steiermark Wöitzer Tauern Greimberg 1700m 26.vii.2001 Habeler leg. All in coll. TLMF.

Figures 12, 13. *Incurvaria vetulella* habitus of adults. **12.** Male. Finland EnL Enontekiö Tuolljehuhput 7676:8265, 13.-15.vii.2009 Marko Mutanen leg. & det.; **13.** Female. Finland EnL Enontekiö Saana 767:825, 16.vii.2012, Marko, Nestori & Anttoni Mutanen leg., coll. Marko & Tomi Mutanen.

overlapping features are found in other parts: details shown in Suppl. material 1. Genitalic structures of both sexes are overall more strongly sclerotised in *I. pirinella*.

In the female genitalia, differences between *I. triglavensis* and *I. pirinella* are found in size of the oviscapt, especially distance from tip to bottom of basal excavation. In the examined material, it was 0.12 mm in *I. pirinella* and 0.09–0.11 mm in *I. triglavensis* (Fig. 2). The length of the apophyses posteriores is 2.70 mm in *I. pirinella* and 2.48–2.55 mm in *I. triglavensis*. Segment VIII also shows differences, but this feature shows overlap and therefore is not useful for determination of a single specimen. Differences between membranous parts of the bursa are not included as diagnostic characteristics, because they may show variation due to the age of specimens and may be altered after copulation. For more details, see Suppl. material 1 for morphometrics of genitalia of *I. triglavensis* and *I. pirinella* and photos of female genitalia (Figs 26–28, 30) of both species.

Description. Male (Figs 3–4). Wingspan 16.5–17.0 mm. Labial palp covered with brown and ochre-yellow scales, most pronounced on inner surface, 2nd segment equal in length to diameter

Figure 14. *Incurvaria circulella* male, Finland KiL. Muonio 7540:8362, 4.vii.2014, Marko, Anttoni & Nestori Mutanen leg., coll. Marko & Tomi Mutanen.
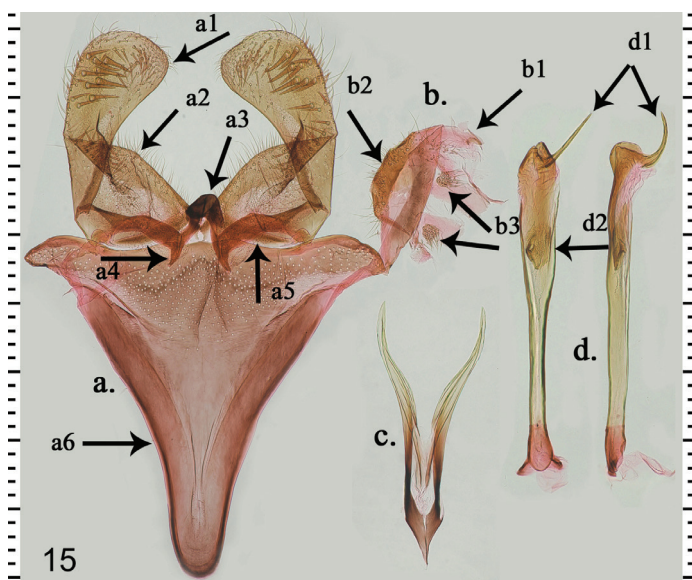

Figure 15. *Incurvaria pirinella* male genitalia unrolled: **a.** vinculum, transtilla and valvae; **a1.** apex of valva; **a2.** margin of sacculus; **a3.** medial knob of transtilla; **a4**. anterior submedial projection of transtilla; **a5.** strongly sclerotised lateral arm of transtilla; **a6.** strongly concave lateral margin of vinculum. **b**. Unrolled tegumen and uncus; **b1.** tegumen; **b2.** uncus; **b3.** socii. **c.** Juxta. **d.** Phallus from semilateral and lateral view; **d1.** hook-shaped distal projection of phallus; **d2.** sclerotised plate in vesica. Scale bar: 1 line = 0.05 mm.

of eye, pointed forward, 3rd segment half as long, turned upward. Antenna faintly ringed with dark brown and paler greyish-brown scales, slightly serrate, reaching three-quarters length of forewing. Head with yellow tufted scales. Thorax and tegula brown. Forewing relatively broad and roundish, ground colour grey-brown; two subtriangular yellowish-white dorsal spots, first narrower, 2/5 from base, extending over half width of forewing, second smaller, close to termen; a small yellowish-white triangular costal spot rather close to apex, sometimes a second usually smaller quadrangular yellowish-white spot close to the first one; fringe as ground colour. Hindwing and fringe fuscous. Abdomen and legs pale greyish-brown. Ventral side of forewing and fringe fuscous throughout except for distinct yellowish-white costal and dorsal markings.

Figure 16. *Incurvaria pirinella* (left) and *I. triglavensis* (right) juxta: **a.** tip of lateral arm; **b.** medial part of lateral arm; **c.** basal part. Scale bar: 1 line = 0.05 mm.
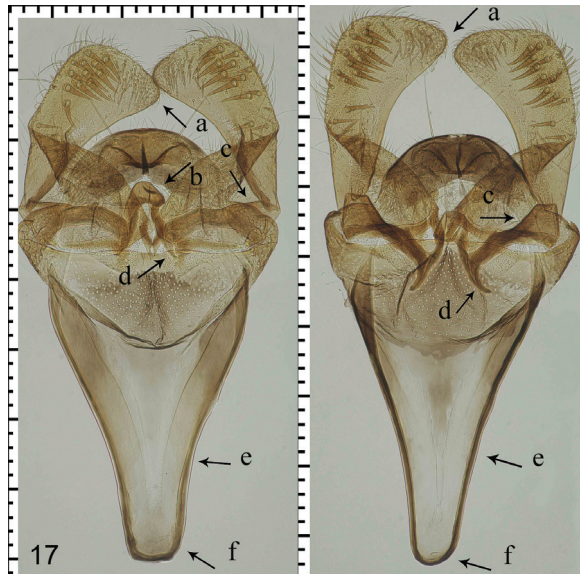

Figure 17. *Incurvaria pirinella* left and *I. triglavensis* right male genitalia compared: **a**. apex of valva; **b.** medial knob of transtilla; **c.** subrectangular plate-like structure in lateral end of transtilla arm; **d.** anterior submedial projection of transtilla; **e.** lateral margin of vinculum; **f.** apex of vinculum. Scale bar: 1 line = 0.05 mm.

Female (Fig. 5). Wingspan 16.0–16.5 mm. Differs from male in darker grey-brown ground colour of forewings; two subapical costal spots always present; female dorsal spots larger and extending even more than 2/3 across forewing towards costa, the second white dorsal spot at tornus extending across the wing and sometimes reaching inner costal spot forming a complete fascia. Antennae not serrate.

Male genitalia (Figs 1, 15, 23, 25). Seven specimens examined. Uncus (Fig. 15b2) and tegumen (Fig. 15b1) short, forming together a relatively broad subrectangular plate, posterior margin of uncus somewhat roundish, medially and sublaterally slightly concave. Socii (Fig. 15b3) distinctly
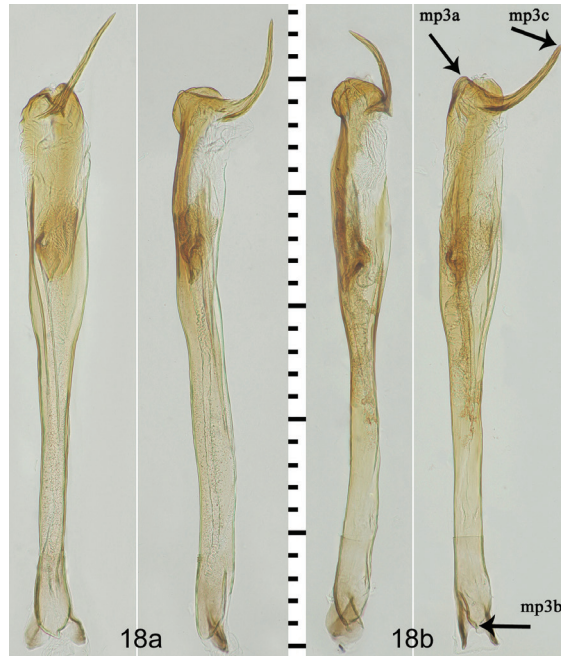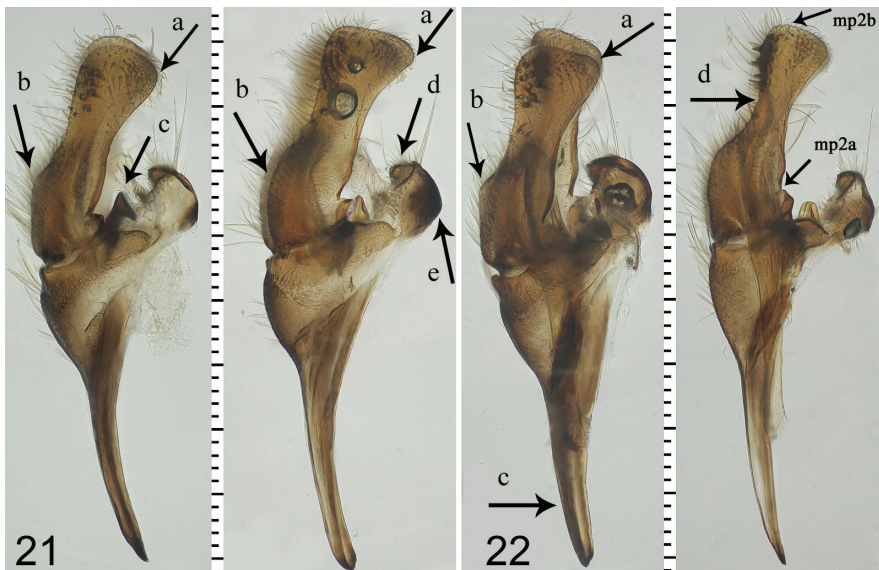
Figure 18. a. *Incurvaria pirinella* and **b.** *I. triglavensis* phallus from semilateral and lateral view. **mp3a-c.** indicate measurement points parts of phallus. Scale bar: 1 line = 0.05 mm.

sclerotised, round-shaped and hirsute. Transtilla (Figs 15a3–a5) a conspicuous structure; medial knob (Fig. 15a3) and its lateral arms (Fig. 15a5) comparatively robust and strongly sclerotised; arms ending laterally to relatively low and broad subrectangular plate-like structure (Fig. 17c); submedial anterior projections (Fig. 15a4) digitate and relatively short. Valva relatively short and robust; costal margin comparatively slightly concave; dorsal margin of sacculus (Fig. 15a2) hirsute and somewhat obtuse-angled at 2/3 from base (Figs 21b, 22b); cucullus (Fig. 15a1) hirsute, moderately roundish with group of strong setae subdorsally and second smaller group of strong setae subdorsally near sacculus; apex hirsute and not significantly elongated (Fig. 21a). Vinculum V-shaped and relatively stout; lateral margins strongly concave (Fig. 15a6); posterior half strongly tapering towards slender distal half; apex moderately broad and roundish. Juxta (Figs 15c, 16) bifurcate, moderately long (length 0.95–0.98 mm), tip of lateral projections relatively pointed (Fig. 16a), medial part relatively narrow (Fig. 16b), basal part arrowhead-shaped and moderately strongly sclerotised (Fig 16c). Phallus (Fig. 15d) relatively long (length without distal hook 1.21–1.27 mm); distally hook-shaped, also a relatively long (0.32–0.38 mm) strongly sclerotised projection (Figs 15d1, 18a-b), basal part broad plate-like, forming about 90° angle with hooked distal part; vesica with moderately short and broad irregular sclerotised plate at base (Fig. 15d2). Abdominal segments S8 and T8 (Figs 23, 25) as subrectangular plates. S8: narrow posterior margin slightly concave; wide anterior margin roundish; lateral margins slightly convex. T8: short and broad; posterior margin concave; anterior and lateral margins convex.

Female genitalia (Figs 2, 26–28, 30). Three specimens examined. Tip of oviscapt triangular, lateral margins concave, 9–11 small distinct teeth on both sides; basal notch somewhat U or V-shaped; lateral projection pointed and relatively short (Fig. 29), shape and size of lateral

Figures 19, 20. Male genitalia from ventral view. **19.** *Incurvaria pirinella* left and *I. triglavensis* right: **a.** margin of uncus; **b.** lateral margin of vinculum. **20.** Male genitalia free-floating ventral view. *I. pirinella* left and *I. triglavensis* right: **a**. robust and strongly sclerotised lateral arms of transtilla; **b.** apex of valva; **c.** medial part of valva; **d.** medial knob of transtilla together with it anterior submedial projections; **e.** lateral margins of vinculum; **f.** apex of vinculum; **mp1a-b.** are measurement points length of vinculum. Scale bar: 1 line = 0.05 mm. Second smaller scale bar parallel to transtilla knob: 1 line = 0.01 mm.

Figures 21, 22. Male genitalia from lateral view. **21.** *Incurvaria pirinella* left and *I. triglavensis* right: **a.** apex of valva; **b.** sacculus; **c.** medial knob of transtilla; **d.** uncus; **e.** tegumen). **22.** Male genitalia free-floating lateral view. *I. pirinella* left and *I. triglavensis* right: **a.** apex of valva; **b.** sacculus; **c.** vinculum; **d.** medial part of valva; **mp2a-b.** are measurement points length of valva. Scale bar: 1 line = 0.05 mm.
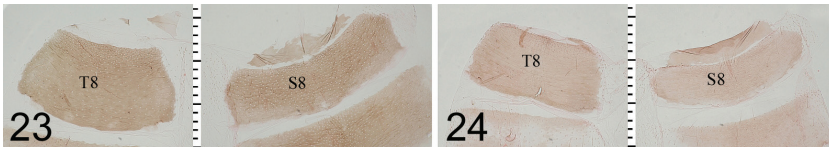
Figures 23, 24. Male abdominal tergites T8 and S8. **23.** *Incurvaria pirinella*; **24.** *I. triglavensis*. Scale bar: 1 line = 0.05 mm.
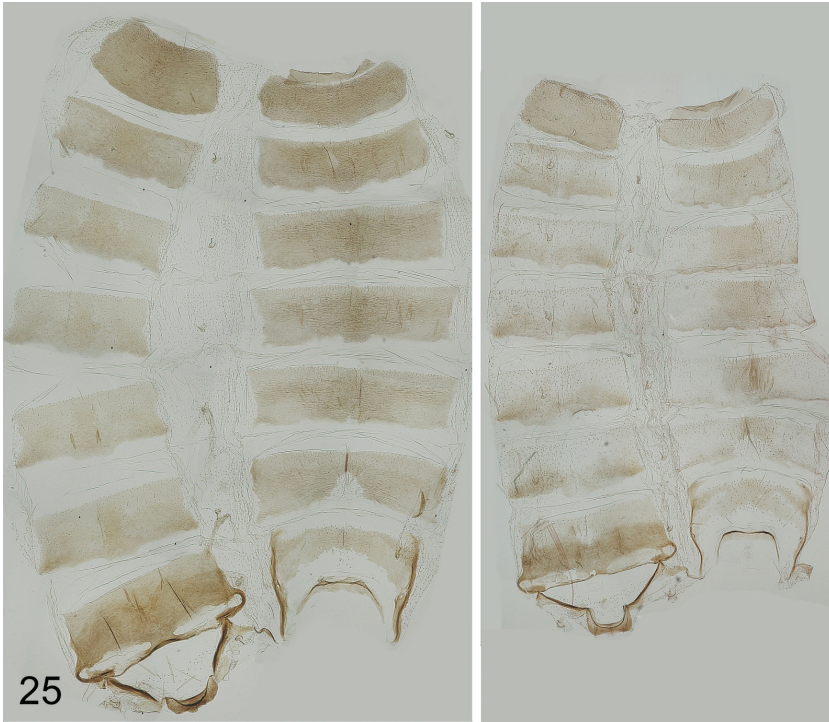

Figure 25. Male abdominal segments of *Incurvaria pirinella* (left) and *I. triglavensis* (right). Male abdominal segments.

margin teeth and basal notch variable. Apophyses relatively long (Figs 26–27, 30). Segment VIII a strongly sclerotised subrectangular plate (Figs 26a, 27); posterior 2/5 strongly tapering towards posterior end, anterior margin rounded. Vestibulum a subrectangular membranous sack (Fig. 28a), about twice as long as wide, posterior 1/3 angular, medially somewhat narrower, anterior half with rounded expansions on both lateral margins (expansions not clearly visible in photo because they are turned behind vestibulum), anteriorly membranous (Fig. 28b) funnel-shaped structure probably belonging to ductus bursa, 1/6 the length of remaining part of ductus bursae (Fig. 28c). Structure of ductus bursae complex, posterior 2/5 narrow tube-like, slightly widening anteriorly, anterior 3/5 broad sack-shaped, anterior end strongly tapered towards corpus bursae. Spermatheca spiral-shaped. Corpus bursae (Fig. 28d) a roundish sack, signa absent. Abdominal segments S8 and T8 relatively large subrectangular plates (Fig. 31). S8: posterior margin roundish and hirsute;
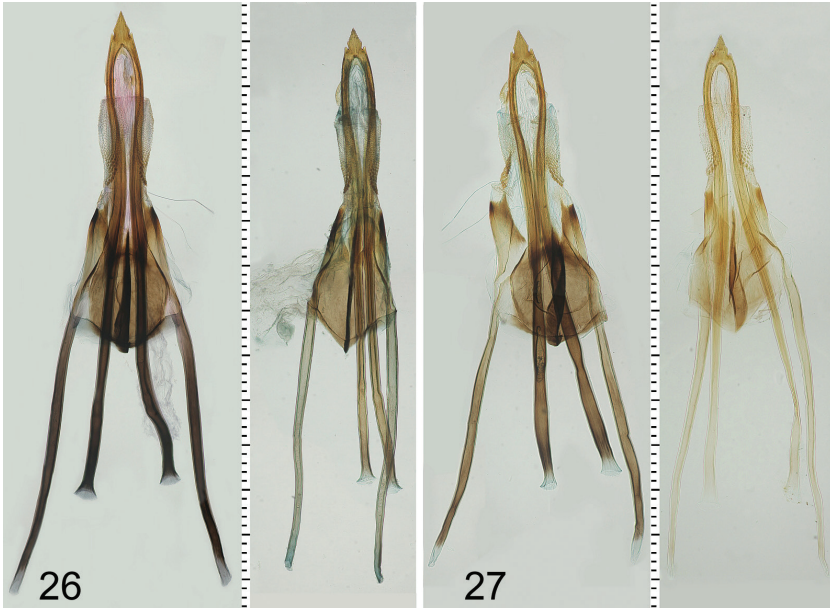
Figures 26, 27. 26. *Incurvaria pirinella* (left) and *I. triglavensis* (right). **27.** *I. pirinella* (left) and *I. triglavensis* (right). Female genitalia from ventral view. Scale bar: 1 line = 0.05 mm.
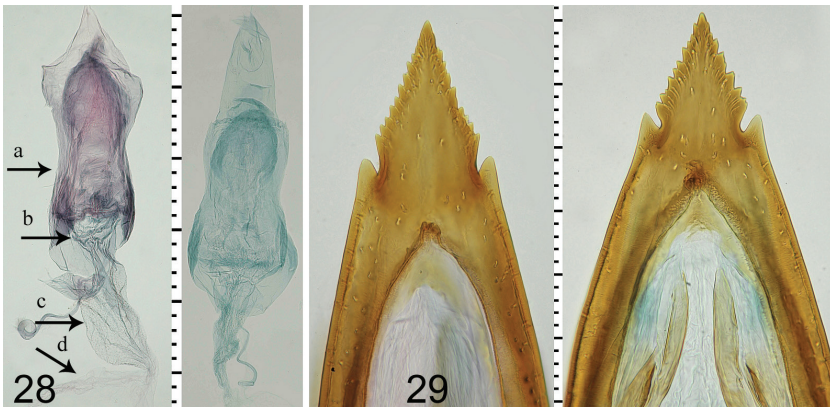
Figures 28, 29. 28. *Incurvaria pirinella* (left) and *I. triglavensis* (right) female bursae: **a.** vestibulum; **b.** membranous funnel-shaped structure in posterior end of ductus bursa; **c.** ductus bursae; **d.** corpus bursae. Scale bar: 1 line = 0.05 mm. **29.** *I. pirinella* (left) and *I. triglavensis* (right). Oviscapt of female genitalia. Scale bar: 1 line = 0.01 mm.

anterior margin slightly concave; lateral margins almost straight. T8: posterior margin concave; broader anterior margin straight; lateral margins somewhat roundish.

Molecular diagnostics (Fig. 33). Barcode Index Number (BIN, see Ratnasingham and Hebert 2013): BOLD:ACW2589. The nearest neighbour of *I. pirinella* in our dataset is *I. triglavensis* (BOLD:AAI8097) with a K2P minimum divergence of 4.74%. The next closest species are *I. vetulella* (BOLD:AAD4279)
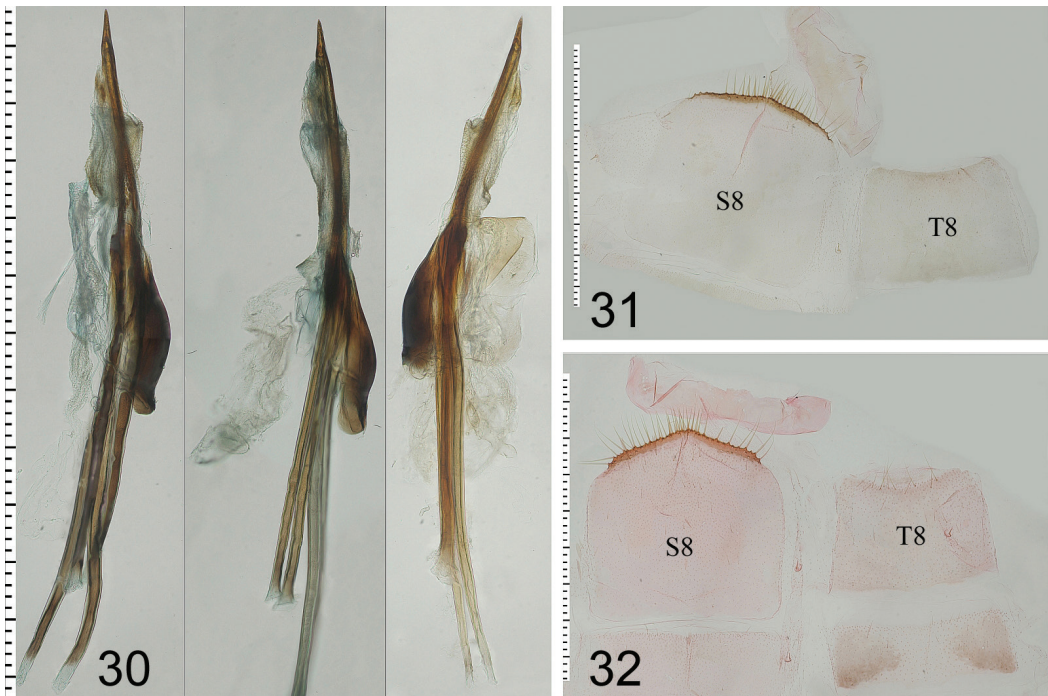
Figures 30–32. 30. a. *Incurvaria pirinella*; **b.** and **c.** *I. triglavensis*. Female genitalia free-floating from lateral view. Scale bar: 1 line = 0.05 mm. **31.** *Incurvaria pirinella*; **32.** *I. triglavensis*. Female abdominal tergites S8 and T8. Scale bar: 1 line = 0.05 mm.

with 7.06%, *I. ploessli* (BOLD:AAP5641) with 7.07% and *I. oehlmanniella* (BOLD:AAD1334) with 8.45% minimum divergences, respectively.

Variation. The external habitus of both sexes is rather constant. Females are darker overall than males. One male has a second yellowish white costal spot near forewing apex. Sometimes the tornal spot and inner subapical costal spot form a complete fascia in the female forewing. Genitalic structures of both sexes are only slightly variable, whereas those of *I. triglavensis* show significant variation both externally and in genitalic structures of both sexes.

Distribution. Known from four different localities in the south-west corner of Bulgaria around the Struma river valley and its adjacent regions, which all belong to the Blagoevgrad district and the Pirin mountain range. The elevational range is wide: 200–1200 m, at least.

Biology. Many specimens were captured with insect nets during daytime, and females especially were disturbed in the evening from shrubs such as *Rosa* L. Males were observed swarming early in the morning, but they were also caught with artificial light late at night, which is an unusual collecting method for the species of the *vetulella*-group in the Alps (P. Huemer pers. comm.) and northern Europe (own observations, and although summer nights are light in the North, *I. circulella* comes readily to light). *Incurvaria pirinella* is an early species, flying at lower elevations in early May and at higher elevations in late May and June. The biology of the early stages
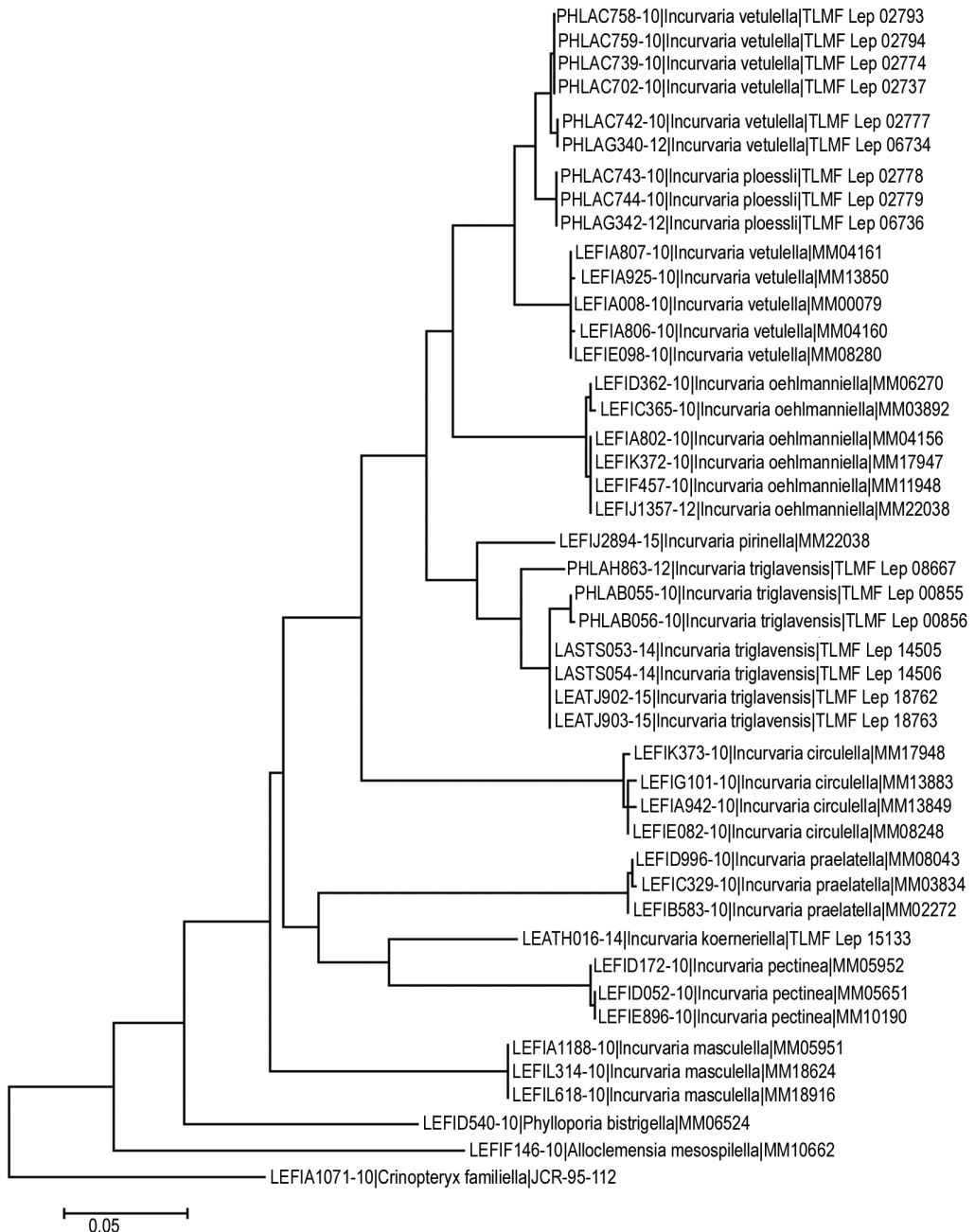
Figure 33. Maximum likelihood tree as based on DNA barcodes (658 bp fragment of the mt COI gene) of European *Incurvaria* species and three members of other genera of Incurvariidae. The node support values are based on 500 bootstrap replicates. The tree was rooted on *Crinopteryx familiella* (Crinopteryginae).

Figure 34. Habitat of *Incurvaria pirinella* Slavianka, Southern Pirin 1000 m a.s.l.

remains unknown. The food-plant of *I. vetulella* is reported to be *Vaccinium* L. (Ericaceae), especially *V. myrtillus* L. (Klimesch 1961, Wojtusiak 1976), but the life history details for the other members of the *vetulella*-group remain largely unknown. It is suspected that the other European members of the group are also associated with *Vaccinium* (Huemer 1993). However, no species of *Vaccinium* or *Erica* L. are present, at least not in most localities where we have found *I. pirinella*, suggesting that they are not its food-plants. Specimens of *I. pirinella* have been collected from completely different habitats from the other species of the *vetulella*-group, which in the Alps are always found in alpine areas usually above timberline and especially from north-facing slopes. In Scandinavia, *I. vetulella* occurs in alpine and subalpine habitats, but also boreal coniferous forest and boggy habitats. *Incurvaria pirinella* seems to prefer open xerothermic south-facing slopes with rich deciduous forest edges of *Quercus* L., and *Fagus* L. (Fig. 34), and a variety of shrubs, including *Crataegus* L., *Corylus* L., and *Rosa*, sometimes also *Cornus* L., *Malus* Mill., *Pyrus* L., and *Chamaecytisus* Link. Along with *I. pirinella*, *I. masculella*, well known as an early flying species, was collected in numbers.
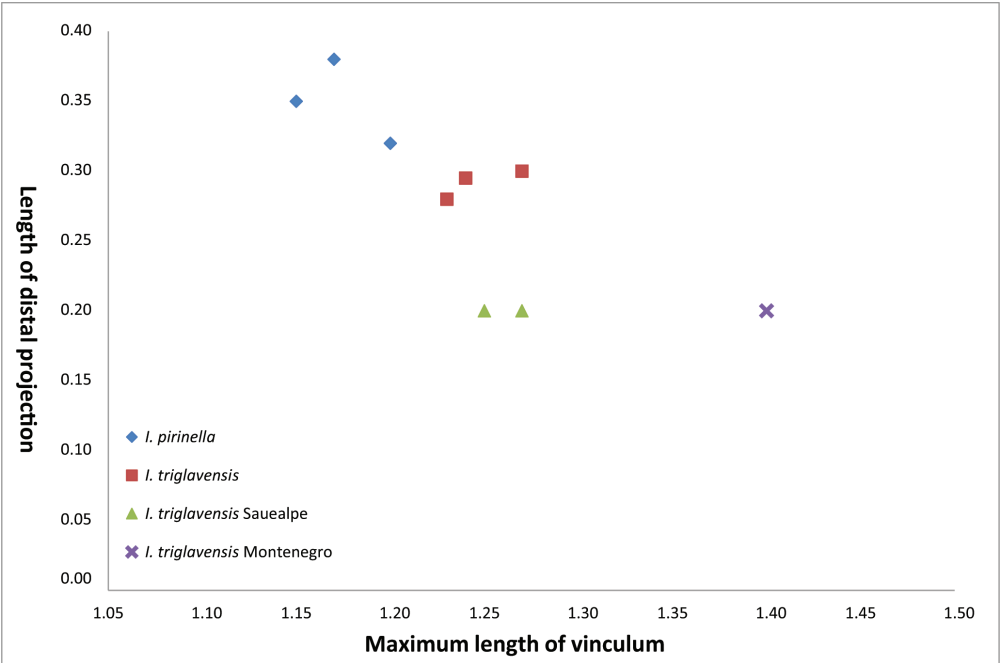
Discussion

To our knowledge, detailed illustrations of female genitalia of *Incurvaria* have not been published before. Only the oviscapt plate was illustrated in earlier publications on the *Incurvaria vetulella* group
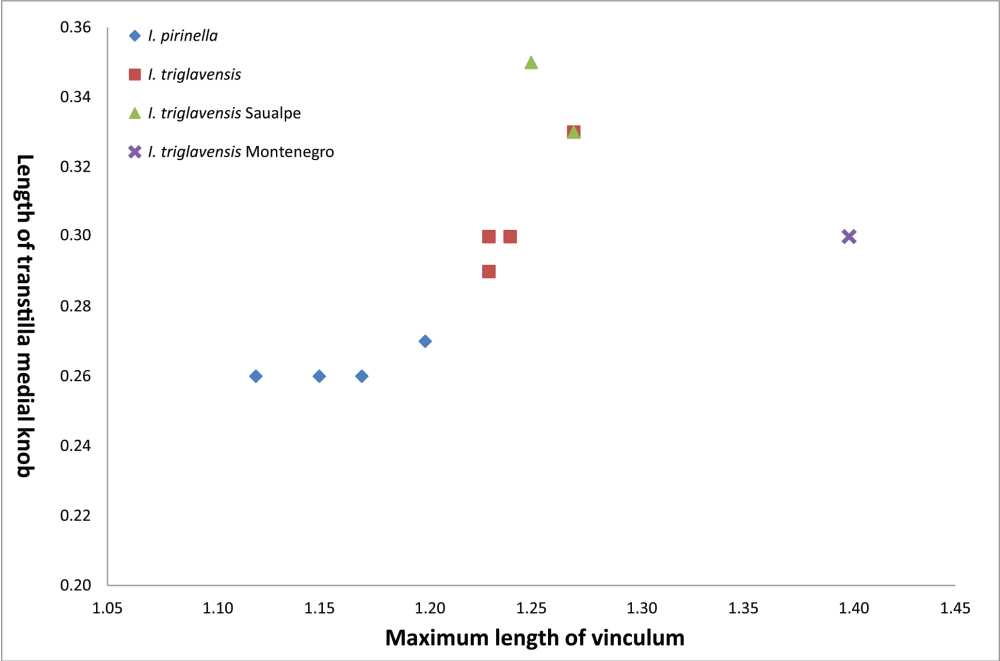
Figures 35, 36. Morphometrics of *Incurvaria pirinella* and *I. triglavensis* with genetically different populations from Saualpe and Montenegro of the latter species included. **35.** Length of vinculum versus transtilla medial knob with anterior projections. **36.** Length of vinculum versus distal projection of phallus. The measurements are in millimetres.

(Laasonen et. al. 1981, Huemer 1993). The size and the shape of the oviscapt appear useful new characteristics, but the number and shape of lateral teeth of oviscapt seems to vary quite a lot even within species. We found also significant differences in the length of apophyses posteriores and minor differences in the shape and length of segment VIII and the shape and size of abdominal plates T8 and S8.

DNA barcodes of *I. vetulella* fall into two clearly separate clusters within Europe, with *I. ploessli* being nested within these clusters, rendering *I. vetulella* paraphyletic. The first group contains samples from North European countries and the second samples from the Alps. We suspect that the *I. vetulella* population in the Alps actually represents an undescribed taxon, as *I. vetulella* shows an overall 3.46% intraspecific divergence in DNA barcodes in Europe. Genomic-based studies on whether these clusters represent a different species are underway (Huemer and Mutanen in prep.). In Central Europe, the *vetulella*-group contains also *I. triglavensis* and *I. ploessli*. We consider *I. pirinella* as a new member of this species group. Each of the Central European species appears to have a rather restricted distribution. Although *I. triglavensis* has also been mentioned from Balkan countries such as Bosnia and Herzegovina (Huemer 1993), further material is needed to clarify the status of the Balkan specimens, because the single barcoded specimen from Montenegro deviates significantly from other barcoded samples of *I. triglavensis*. Moreover, a new DNA barcode cluster was recently found from the Saualpen in Austria by Huemer (personal communication). These new findings indicate that the *I. vetulella*-group should be revised again.

Originally, genetically deviant specimens of *I. triglavensis* from the Alps and Montenegro were included in the morphometric analyses. While some morphological differences between them and other *I. triglavensis* as well as *I. pirinella* were observed, we excluded them from the final analyses (see Figs 35–36) as this was beyond the scope of our study. The illustrated *I. triglavensis* samples were verified not to include specimens of this putatively undescribed species.

In northern Europe, the superficially similar *I. circulella* occurs sympatrically with *I. vetulella*. Despite superficial resemblance of these species, Huemer (1993) considered that *I. circulella* does not belong to the *vetulella*-group. This is supported by the maximum Likelihood analysis of DNA barcodes, as *I. circulella* appears also genetically relatively distant to the species of the *vetulella*-group. It forms a sister lineage to the *vetulella*-group + *I. oehlmanniella*. Based on the DNA barcodes, *I. oehlmanniella* is nested within the *vetulella*-group (Fig. 33). However, a phylogenetic analysis based on DNA barcodes alone may be misleading due to a single locus and due to the small number of characters, and further studies, preferably including nuclear markers, are needed. Interestingly, Bengtsson et al. (2008) consider *I. circulella* as likely to feed on *Betula* spp., which we find unlikely as this species is often abundant in spruce "taiga" forests with plenty of *Vaccinium myrtillus* but no or little *Betula* L. (Betulaceae). Misleading habitat and food plant association may be the reason why Bengtsson et al. (2008) consider *I. circulella* a rare species. In Finnish Lapland it is common, especially in spruce forest habitats ("taiga" forests), but does not occur or is rare in the subalpine and alpine habitats where *I. vetulella* is often abundant. The males of both *I. circulella* and *I. vetulella* swarm most actively late at night and in the early morning.

In our Maximum likelihood analysis (Fig. 33), *Incurvaria* appears to be monophyletic, although with poor support as the distances between the species are generally large. Also, several of its basal branches are short and groupings are overall poorly supported. The *vetulella*-group together with *I. oehlmanniella* is well supported.

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References

Bengtsson BÅ, Johansson R, Palmqvist G (2008) Fjärilar: Käkmalar-Säckspinnare. Lepidoptera: Micropterigidae-Psychidae. Nationalnyckeln till Sveriges flora och fauna DE, ArtDatabanken, SLU, Uppsala, 1–13.

Davis DR (1998) The monotrysian Heteroneura. In: Kristensen NP (Ed.) Lepidoptera, moths and butterflies, Vol. 1 – Evolution, systematics and biogeography – Handbuch der Zoologie/Handbook of Zoology 4(35). Walter de Gruyter, Berlin and New York, 65–90. https://doi.org/10.1515/9783110804744.65

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA Barcodes: analytical protocols. In: Cristofre M (Ed.) Methods in molecular biology: Environmental Genetics. Humana Press Inc., Totowa, 275–293. https://doi.org/10.1007/978-1-59745-548-0_15

Huemer P (1993) Review of the *Incurvaria vetulella* species-group in the Alps (Lepidoptera: Incurvariidae). Entomologica Scandinavica 24: 109–120. https://doi.org/10.1163/187631293X00091

Klimesch J (1961) Ordnung Lepidoptera. 1. Teil. Pyralidina, Tortricina, Tineina, Eriocraniina und Micropterygina. In: Franz H (ed), Die Nordost-Alpen im Spiegel ihrer Landtierwelt 2: 481–789.

Kozlov MV (1996) Incurvariidae and Prodoxidae (Lepidoptera) from Siberia and the Russian Far East, with descriptions of two new species. Entomologica Fennica 7: 55–62.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Laasonen E, Jalava J, Nielsen ES (1981) The identity of *Incurvaria vetulella* (Zetterstedt, 1839) and *I. circulella* (Zetterstedt, 1839), two distinct species (Lepidoptera: Incurvariidae). Entomologica Scandinavica 12: 57–64. https://doi.org/10.1163/187631281X00373

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

Nielsen ES (1981) A taxonomic revision of the species of *Alloclemensia* n.gen. (Lepidoptera: Incurvariidae s. str.). Entomologica Scandinavica 12(3): 271–294. https://doi.org/10.1163/187631281794709764

Nielsen ES (1982) Incurvariidae and Prodoxidae from the Himalayan area (Lepidoptera: Gracillariidae). Insecta matsumurana 26: 187–200. http://hdl.handle.net/2115/9819

Nieukerken EJ van, Kozlov M (2017) Fauna Europaea: Lepidoptera, Incurvariidae. In: Nieukerken EJ van, Karsholt O (Eds) Lepidoptera, Fauna Europaea version 2.6. http://www.fauna-eu.org

Okamoto H, Hirowatari T (2004) Distributional records and biological notes on Japanese species of the family Incurvariidae (Lepidoptera). Lepidoptera Science 55: 173–195. https://doi.org/10.18984/lepid.55.3_173

Pohl GR, Patterson B, Pelham JP (2016) Annotated taxonomic checklist of the Lepidoptera of North America, North of Mexico. ResearchGate, 766 pp. https://doi.org/10.13140/RG.2.1.2186.3287

Pohl GR, Landry J-F, Schmidt BC, Lafontaine JD, Troubridge JT, Macaulay AD, Nieukerken EJ van, DeWaard JR, Dombroskie JJ, Klymko J, Nazari V, Stead K (2018) Annotated checklist of the moths and butterflies (Lepidoptera) of Canada and Alaska. Pensoft serie faunistica 118: 1–580. https://ebooks.pensoft.net/books/13080

Ratnasingham, S, Hebert, PDN (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2006.01678.x

Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLoS ONE 8(8): e66213. https://doi.org/10.1371/journal.pone.0066213

Robinson G (1976) The preparation of slides of Lepidoptera genitalia with special reference to the Microlepidoptera. Entomologist's Gazette 27: 127–132.

Schmitz P (1969) Eine neue Incurvaria-Art aus dem Kaukasus. Deutsche Entomologische Zeitschrift 16: 383–387. https://doi.org/10.1002/mmnd.19690160408

Wojtusiak J (1976) Heliozelidae, Incurvariidae . Klucze Do Oznaczania Owadów Polski 27: 1–60.

Supplementary material 1

Morphometrics of genitalia of *I. triglavensis* and *I. pirinella* sp. nov.
Authors: Jari Junnilainen, Peter Buchner, Jari-Pekka Kaitila, Marko Mutanen
Data type: measurements
Explanation note: The measurements are in millimetres.
Link: https://doi.org/10.3897/nl.42.13026.suppl1

Supplementary material 2

External characteristics of the European *Incurvaria vetulella* species-group
Authors: Jari Junnilainen, Peter Buchner, Jari-Pekka Kaitila, Marko Mutanen
Data type: species data
Link: https://doi.org/10.3897/nl.42.13026.suppl2

Stability in Lepidoptera names is not served by reversal to gender agreement: a response to Wiemers et al. (2018)

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In a recent paper in ZooKeys, Wiemers et al. (2018) provided an updated list of European butterfly names. In this list the authors follow gender agreement for species names, when interpreted as adjectival in derivation, in contrast to the common practice among most lepidopterists. Here we comment on this aspect of the paper, and voice our concern that this reversal does not benefit the stability of Lepidoptera names and is, indeed, inimical to their stability.

Modern zoological science needs the communities of taxonomists and users to agree on the names that are used to communicate information about the taxa we study and cherish. In this age,

such collegiate acceptance is more important than ever, given that the number of users of scientific names has increased enormously. Agreement is particularly important when considering the numerous online databases, observation sites, Wikipedia, etc. Several global and local initiatives over the last several decades have begun to compile authoritative lists of taxonomic names to serve the community and build towards a greater stability, including Species 2000 / Catalogue of Life (Roskov et al. 2018; Roskov et al. 2019), Global Biodiversity Information Facility (GBIF Secretariat 2019) and Fauna Europaea (de Jong et al. 2014; Fauna Europaea 2017).

Unfortunately, the current (and likely future) funding situation for most of these projects is poor, to say the least, and populating these databases relies heavily on a diminishing number of taxonomists, who rarely receive recognition for their work. The Fauna Europaea database, which is of special importance as Europe's main zoological taxonomic index, has suffered particularly, being an EU-supported project that was only funded by the European Commission between 2000 and 2004. Subsequently, updating was carried out at the Zoological Museum of Amsterdam (de Jong et al. 2014), first under the umbrella of the PESI project (PESI 2011; de Jong et al. 2015), then later without funding, until the Amsterdam museum was merged with Naturalis Biodiversity Center in Leiden in 2011. Since then, the Fauna Europaea database has been run by the Museum für Naturkunde, Leibniz-Institute for Research on Evolution and Biodiversity, Berlin, Germany. Recently, however, updating has come to a stand-still, very much to the frustration both of taxonomists who wish to update their lists and of users who need an up-to-date and authoritative nomenclature.

Given these circumstances, we enthusiastically applaud the initiative that several specialists of European butterflies have taken separately to publish an update for butterflies (superfamily Papilionoidea) in an open access journal, to produce a new list for the use of the scientific community (Wiemers et al. 2018).

Gender agreement or not

The Preamble of the International Code of Zoological Nomenclature (*the Code*) states: *The objects of the Code are to promote stability and universality in the scientific names of animals and to ensure that the name of each taxon is unique and distinct* (International Commission on Zoological Nomenclature 1999).

It is generally accepted (though perhaps not always welcomed) that names of taxa change as a result of new taxonomic and phylogenetic research. Such changes are an increasingly frequent occurrence, especially when new data sources (such as nucleotide sequences, from DNA barcodes to entire genomes) are brought to bear on taxonomic, evolutionary and phylogenetic problems. We fully support such changes when they are implemented as a result of rigorous scientific study and have been accepted by the scientific community. Other changes, by contrast, are nomenclatural in nature and based on the application of rules from the international codes of nomenclature. Some follow from research into original descriptions, types, etc., often depending on new interpretations of old literature, while others are purely linguistic, and these usually cause the most controversy. In this paper, we discuss one of these issues – gender agreement – which, arguably, jeopardizes the very aim of name stability in Linnaean nomenclature.

The major problem that this new list of European butterflies (Wiemers et al. 2018) creates might seem trivial, but in its wider context threatens the stability of thousands of names of moths and butterflies globally, namely, the question as to whether a species name (or epitheton), when

interpreted as adjectival in derivation, should agree in grammatical gender with the genus name or not. Although such agreement is still formally required by *the Code*, most lepidopterists ceased following the rule many years ago (e.g. Scoble 1999). The justification for ignoring the rule was described in detail and defended by Sommerer (2002), who argued strongly that the original spelling (orthography) be used. This recommendation was adopted at the General Meeting of the Societas Europaea Lepidopterologica (SEL) in a resolution proposed at the 13th European Congress of Lepidopterology in Korsør (Denmark) on June 4, 2002 (the text of which immediately follows the paper by Sommerer 2002). Welter-Schultes (2012: 92) in his nomenclature textbook published by GBIF, also described "the Lepidoptera model", where gender agreement never gained a strong position, and suggests (page 93): *In such a situation it can only be recommended to maintain the names in the incorrect forms as they are, and not to change them.*

Since 2002, but also earlier, almost all major Lepidoptera databases and catalogues have followed this principle of original orthography (summarised in Table 1). The managers of Fauna Europaea initially opposed the position (Sommerer 2002), but finally adopted it as a general principle for Lepidoptera in the first update and published it as such in the broad description of the project (de Jong et al. 2014). Apart from the catalogues listed in Table 1, major modern handbook series, such as "Microlepidoptera of Europe", "Noctuidae Europaeae", "Geometrid Moths of Europe", "The Moths and Butterflies of Great Britain and Ireland", "Moths of Borneo", "Heterocera Sumatrana", "Moths of Thailand" and "Monographs of Australian Lepidoptera", all adhere to the use of original spellings. We could only find some six recent national catalogues and two global catalogues that follow the *Code* strictly (Table 1), in addition to several checklists where a mixture of correct gender agreement and original spelling was applied, a further indication that adhering to this principle is not easy, even for those familiar with the complexities of Latin and Greek grammar.

The stance taken by so many lepidopterists has been adopted by some other zoologists. For example, Welter-Schultes (2012), although providing rules on how to apply gender agreement or avoid its necessity, concluded (page 87) "A gender is nothing useful". In ornithology, too, a discussion is taking place as to whether gender agreement is of any use (Schodde and Bock 2016; 2017). To quote from the first of these papers (page 167): *The second point is the sheer mind-numbing, time-consuming complexity of determining gender for species-group names in zoological nomenclature. This is also the single greatest source of regulation-driven change in the spelling of species-group names, often disruptively so.*

These are precisely the reasons why the vast majority of lepidopterists long ago decided to use original spellings. To apply gender agreement correctly, not only is it necessary to determine the correct gender of the generic name, which is far from straightforward in many cases, but often it is even more difficult to ascertain whether the species epithet is an adjective or participle, which both can be declined, or a (composite) noun. The number of errors caused by this latter problem is huge, which is understandable both because most modern taxonomists have no training in Latin or Greek, and because many names can be easily interpreted in different ways. Such difficulties are particularly prominent in Lepidoptera, given that many artificial names sometimes are not Latin adjectives but rather composite nouns, e.g. those ending in the suffixes -ella, -ellus, -ellum (Huemer 1988). The name *Erebia aethiopellus* (Hoffmansegg, 1806), among the names discussed here, belongs also in this category (Table 2). Certainly, in the current age of diminishing resources for taxonomy (both financial and human), and given the great need for taxonomy at a time of catastrophic biodiversity loss, it is indefensible to spend time on these kinds of disputes instead of

Table 1. Survey of recent national, regional and global taxonomic catalogues of Lepidoptera, with indication of whether gender agreement is observed or not. When authors have explained their position on this matter, the page number for this explanation or statement is provided.

Area	Reference	Page of statement	Gender agreement?
Local catalogues			
Europe			
Europe	Karsholt and Razowski (1996)	12	No
Europe	Karsholt and van Nieukerken (2017)	see de Jong et al. (2014: 7)	No
Europe	Lepiforum e.V. (2008–2019)	Glossar	No
Austria	Huemer (2013)	–	No
Belgium	De Prins (2016)	8	No
Czech Republic	Laštůvka and Liška (2011)	–	No
Denmark	Karsholt and Stadel Nielsen (2013)	5	No
Estonia	Jürivete and Õunap (2008)	–	Yes[1]
Finland	Kullberg et al. (2002)	–	Yes[1]
France	Leraut (1997)	22, 48, etc.	No
Germany	Gaedike et al. (2017)	–	No
Hungary (micromoths)	Pastorális and Buschmann (2018)	–	No
Latvia	Savenkov and Šulcs (2010)	–	No
Lithuania	Ivinskis (2004)	–	No
Netherlands	Kuchlein and de Vos (1999)	6	No
Nordic-Baltic Europe	Aarvik et al. (2017)	3	No
Norway	Aarvik et al. (2000)	–	No
Poland	Buszko and Nowacki (2017)	–	No
Portugal	Corley (2015)	53	No
Romania	Rákosy et al. (2003)	–	Mixed
Russia	Sinev (2008)	–	No
Serbia (micromoths)	Jakšić (2016)	–	No
Slovakia	Pastorális et al. (2013)	–	No
Slovenia	Lesar and Govedic (2010)	–	No
Spain	Vives Moreno (2014)	–	Mixed
Sweden	Bengtsson et al. (2016)	7	Yes[1]
Switzerland	SwissLepTeam (2010)	–	No
United Kingdom	Agassiz et al. (2013)	2	No
Other continents			
North America	Pohl et al. (2016)	19	No
Canada	Pohl et al. (2018)	23	No
Afrotropics	De Prins and De Prins (2018b)	–	No
Southern Africa	Vári et al. (2002)	–	Mixed
Australia	Nielsen et al. (1996)	2	No
New Zealand	Dugdale (1988)	–	Yes
Borneo	Holloway (2011)	4	No
China	Hua (2005)	–	Mixed
Japan	Kishida (2011a, b), Hirowatari et al. (2013), Nasu et al. (2013)	–	Mixed
Russia Far East	Lelej (2016)	–	No
Taiwan	Heppner and Inoue (1992)	xx	Mixed[2]
Neotropics	Heppner (1984)	xv	No

Area	Reference	Page of statement	Gender agreement?
Global catalogues			
Global LepIndex	Beccaloni et al. (2005)	–	No
Catalogue of Life	Roskov et al. (2018); Roskov et al. (2019)	–	No
Blastobasidae	Sinev (2014)	–	No
Bombycoidea	Kitching et al. (2018)	4	No
Coleophoridae	Baldizzone et al. (2006)	–	N/A[3]
Cosmopterigidae	Sinev (2002)	–	No
Cossidae	Yakovlev (2011)	–	Yes
Geometridae	Scoble (1999)	xxii	No
Gracillariidae	De Prins and De Prins (2018a)	–	No
Hepialidae	Nielsen et al. (2000)	827	No
Nepticuloidea	van Nieukerken et al. (2016)	90	No
Noctuoidea	Poole (1989)	–	No
Notodontidae	Schintlmeister (2013)	10	No
Papilionidae, Pieridae	Häuser et al. (2012)	–	No
Psychidae	Sobczyk (2011)	14	No
Pterophoridae	Gielis (2003)	6	No
Pyraloidea	Nuss et al. (2003–2017)	introduction	No
Scythrididae	Passerin d'Entreves and Roggero (2007)	–	N/A[3]
Sesiidae	Pühringer and Kallies (2004, 2017)	–	Yes
Stathmopodidae	Sinev (2015)	–	No
Tineidae	Robinson (2019)	–	No
Tortricidae	Gilligan et al. (2018)	–	No
Yponomeutoidea	Lewis and Sohn (2015)	12	No

Notes:

1 Lists of Estonia, Finland and Sweden, three of the four European lists with gender agreement, are superseded by the Nordic-Baltic List with original spelling.

2 Introduction states to use original spelling, but in fact it is a mixture.

3 In this family all generic names are feminine, gender agreement is not relevant.

Table 2. Recommended correct names - with original spelling of epithet - of 14 European butterfly species for which names in Wiemers et al. (2018) were reversed through applying gender agreement.

Agriades pyrenaica (Boisduval, 1840)
Carcharodus tripolina (Verity, 1925)
Colias croceus (Geoffroy, 1785)
Cupido decolorata (Staudinger, 1886)
Erebia aethiopellus (Hoffmansegg, 1806)
Erebia alberganus (Prunner, 1798)
Erebia montanus (Prunner, 1798)
Erebia stirius (Godart, 1824)
Hipparchia azorinus (Strecker, 1899)
Hyponephele lupinus (Costa, 1836)
Kretania hespericus (Rambur, 1839)
Lycaena dimorphus (Staudinger, 1881)
Lycaena ottomanus (Lefèbvre, 1831)
Tarucus balkanica (Freyer, 1844)

doing real taxonomy, as it requires delving into ancient Latin and Greek linguistics and grammar that few specialists understand. In contrast, adopting original spellings means they have only to be checked once, which is a relatively easy process nowadays, especially as most databases include original names anyway, and as most old publications have become easily accessible through initiatives such as the Biodiversity Heritage Library (2005–2019).

Although we strongly adhere to the objects of the *Code* as expressed in our quote of its Preamble above, we consider that Article 34.2, which deals with gender agreement between genus and species names, contradicts that part of the Preamble.

Conclusion

Given that the overwhelming majority of authors of publications on Lepidoptera use original spelling rather than gender agreement, we conclude that Wiemers et al. (2018) did a disservice to nomenclatural stability as well as to the users of butterfly names, who are generally unaware of the intricacies of zoological nomenclature. Clearly, Wiemers et al. (2018: 15) were conscious of the practice adopted by most lepidopterists and the reasons for it as shown in this quote:

This includes the controversial article 34.2, which mandates that »the ending of a Latin or Latinised adjectival or participial species-group name must agree in gender with the generic name with which it is at any time combined«. Due to its linguistic complexity, this rule has led to many wrong or ambiguous decisions and causes additional instability of nomenclature each time a species name is transferred to another genus. Therefore a majority of lepidopterists, including the group editors of Fauna Europaea, have decided to ignore this rule and use the original spelling instead (de Jong et al. 2014).

Yet, curiously, Wiemers et al. (2018) gave no reasons for not following the "original spelling" convention that has become common practice among their lepidopterist colleagues. The published list (Wiemers et al. 2018) was presented as an update for Fauna Europaea, which, as noted above, has expressly adopted original spellings for Lepidoptera. Two of us (OK, EvN), as Group coordinators for Lepidoptera in Fauna Europaea, made critical comments on the draft list, and objected to the use of gender agreement at the time (e-mails late 2016).

We strongly recommend that users of the list ignore the changes to the 14 names affected, which are published by Wiemers et al. (2018) in table 7, page 7, and recommend that the names in the right hand column be used instead. We provide in this response these names for convenience (Table 2), and recommend most strongly that these original spellings are adopted by all users for purpose of nomenclatural stability.

Continuation of the current situation, in which workers on a particular group of animals elect to ignore a rule of the Code, will undoubtedly lead to new discussions in future. Thus, we also plead for a consistent change in the next version of the Code to allow for greater stability in the endings of names in general and for Lepidoptera in particular. Suggestions for abandoning gender agreement have been proposed before. One option, perhaps, could be to make a special dispensation to exempt Lepidoptera from the practice of following gender agreement given that this has led to relative stability of global Lepidoptera names (Welter-Schultes 2012: 92 and personal communication). However, we are aware that such special pleading could easily open the floodgates to numerous bespoke claims in other groups in other areas of nomenclature, leading to increased instability. The simplest and most stabilizing way forward would clearly be to do away with gender agreement altogether.

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References

Aarvik L, Berggren K, Hansen LO (2000) Catalogus Lepidopterorum Norvegiae. Zoologisk Museum, Oslo, 192 pp.

Aarvik L, Bengtsson BÅ, Elven H, Ivinskis P, Jürivete U, Karsholt O, Mutanen M, Savenkov N (2017) Nordic-Baltic Checklist of Lepidoptera. Norwegian Journal of Entomology. Supplement 3: 1–236. http://www.entomologi.no/journals/nje/Suppl/Aarvik_et_al_2017_Nordic-Baltic_Checklist_of_Lepidiptera.pdf.

Agassiz DJL, Beavan SD, Heckford RJ (Eds) (2013) A checklist of the Lepidoptera of the British Isles. Handbooks for the Identification of British Insects, Royal Entomological Society of London, London, iv + 206 pp.

Baldizzone G, Wolf H van der, Landry J-F (2006) Coleophoridae, Coleophorinae (Lepidoptera). World Catalogue of Insects 8: 1–215.

Beccaloni G, Scoble M, Robinson G, Pitkin B (2005) The Global Lepidoptera names index. Natural History Museum. http://www.nhm.ac.uk/our-science/data/lepindex/index.html

Bengtsson BÅ, Gustafsson B, Palmqvist G (2016) Katalog över svenska fjärilar. Catalogue of the Lepidoptera of Sweden. Entomologiska Föreningen, Stockholm, 328 pp.

Biodiversity Heritage Library (2005–2019) BHL, Biodiversity Heritage Library. Inspiring discovery through free access to biodiversity knowledge. https://www.biodiversitylibrary.org/

Buszko J, Nowacki J (2017) A distributional checklist of the Lepidoptera of Poland. Polish Entomological Monographs 13: 1–222.

Corley MFV (2015) Lepidoptera of Continental Portugal: a fully revised list. Corley, Faringdon, vi + 282 pp.

De Prins, J, De Prins, W (2018a) Global Taxonomic Database of Gracillariidae (Lepidoptera). Belgian Biodiversity Platform. http://www.gracillariidae.net/

De Prins, J, De Prins, W (2018b) Afromoths, online database of Afrotropical moth species (Lepidoptera). Belgian Biodiversity Platform. http://www.afromoths.net/

De Prins, W (2016) Catalogus van de Belgische Lepidoptera. Catalogue of the Lepidoptera of Belgium. Entomobrochure 9: 1–279. http://www.phegea.org/Documents/CatalogueBelgianLepidoptera_2016.pdf.

Dugdale, JS (1988) Lepidoptera: annotated catalogue, and keys to family-group taxa. Fauna of New Zealand 14: 1–262. http://www.biotaxa.org/fnz/article/view/1754

Fauna Europaea (2017) Fauna Europaea, all European Animal Species online, version 2017.06. Fauna Europaea. https://fauna-eu.org/

Gaedike, R, Nuss, M, Steiner, A, Trusch, R (2017) Verzeichnis der Schmetterlinge Deutschlands (Lepidoptera). 2. überarbeitete Auflage. Entomologische Nachrichten und Berichte Beiheft 21: 1–362.

GBIF Secretariat (2019) Global Biodiversity Information Facility, Free and open access to biodiversity data. GBIF. http://www.gbif.org/

Gielis C (2003) Pterophoroidea & Alucitoidea (Lepidoptera). Apollo Books, Stenstrup, World Catalogue of Insects 4: 1–198.

Gilligan TM, Baixeras J, Brown JW (2018) T@rts, Online World Catalogue of the Tortricidae (Version 4.0). Tortricid.net. http://www.tortricid.net/catalogue.asp

Häuser CL, Holstein J, Steiner A (2012) The Global Butterfly Information System. Museum für Naturkunde. http://www.globis.insects-online.de

Heppner JB (Ed.) (1984) Checklist 1: Micropterigoidea-Immoidea. Atlas of Neotropical Lepidoptera 1, W. Junk, The Hague etc., xxvii + 112 pp.

Heppner JB, Inoue H (1992) Lepidoptera of Taiwan. Volume 1. Part 2: checklist. Scientific Publishers Inc. Gainesville, Florida, xlix + 276 pp.

Hirowatari T, Nasu Y, Sakamaki Y, Kishida Y (Eds) (2013) Nihon-san garui hyojun zukan. The standard of moths in Japan 3. Zygaenidae, Sesiidae, Limacodidae, etc. Gakken Kyoiku Shuppan, Tokyo, 359 pp.

Holloway JD (2011) The Moths of Borneo: Families Phaudidae, Himantopteridae and Zygaenidae; revised and annotated checklist. Malayan Nature Journal 63: 1–548.

Hua Li-Zheng (2005) List of Chinese Insects, Vol. 3. Sun Yat-sen University, Guangzhou, 595 pp.

Huemer P (1988) A taxonomic revision of *Caryocolum* (Lepidoptera: Gelechiidae). Bulletin of the British Museum (Natural History). Entomology 57: 439–571. https://www.biodiversitylibrary.org/part/78676

Huemer P (2013) Die Schmetterlinge Österreichs (Lepidoptera). Systematische und faunistische Checkliste. Tiroler Landesmuseum Ferdinandeum, Innsbruck, StudioHefte 12: 1–304 pp.

International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature. Fourth edition. Adopted by the International Union of Biological Sciences. The International Trust for Zoological Nomenclature, London, xxix + 306 pp. http://iczn.org/code.

Ivinskis P (2004) Lepidoptera of Lithuania. Annotated catalogue. Lietuvos drugiai (Lepidoptera). Katalogas. Vilniaus Universiteto Ekologijos Institutas, Vilnius, 379 pp.

Jakšić P (2016) Tentative check list of Serbian Microlepidoptera. Ecologica Montenegrina 7: 33–258. https://www.biotaxa.org/em/article/view/21598/20703

Jong Y de, Verbeek M, Michelsen V, Bjørn PdP, Los W, Steeman F, Bailly N, Basire C, Chylarecki P, Stloukal E, Hagedorn G, Wetzel FT, Glöckler F, Kroupa A, Korb G, Hoffmann A, Häuser C, Kohlbecker A, Müller A, Güntsch A, Stoev P, Penev L (2014) Fauna Europaea – all European animal species on the web. Biodiversity Data Journal 2: e4034. https://doi.org/10.3897/BDJ.2.e4034

Jong Y de, Kouwenberg J, Boumans L, Hussey C, Hyam R, Nicolson N, Kirk P, Paton A, Michel E, Guiry MD, Boegh PS, Pedersen HÆ, Enghoff H, Raab-Straube Ev, Güntsch A, Geoffroy M, Müller A, Kohlbecker A, Berendsohn W, Appeltans W, Arvanitidis C, Vanhoorne B, Declerck J, Vandepitte L, Hernandez F, Nash R, Costello MJ, Ouvrard D, Bezard-Falgas P, Bourgoin T, Wetzel FT, Glöckler F, Korb G, Ring C, Hagedorn G, Häuser C, Aktaç N, Asan A, Ardelean A, Borges PAV, Dhora D, Khachatryan H, Malicky M, Ibrahimov S, Tuzikov A, De Wever A, Moncheva S, Spassov N, Chobot K, Popov A, Boršić I, Sfenthourakis S, Kõljalg U, Uotila P, Olivier G, Dauvin J-C, Tarkhnishvili D, Chaladze G, Tuerkay M, Legakis A, Peregovits L, Gudmundsson G, Ólafsson E, Lysaght L, Galil BS, Raimondo FM, Domina G, Stoch F, Minelli A, Spungis V, Budrys E, Olenin S, Turpel A, Walisch T, Krpach V, Gambin MT, Ungureanu L, Karaman G, Kleukers RMJC, Stur E, Aagaard K, Valland N, Moen TL, Bogdanowicz W, Tykarski P, Węsławski JM, Kędra M, Martins AM de Frias, Abreu AD, Silva R, Medvedev S, Ryss A, Šimić S, Marhold K, Stloukal E, Tome D, Ramos MA, Valdés B, Pina F, Kullander S, Telenius A, Gonseth Y, Tschudin P, Sergeyeva O, Vladymyrov V, Rizun VB, Raper C, Lear D, Stoev P, Penev L, Rubio AC, Backeljau T, Saarenmaa H, Ulenberg S (2015) PESI - a taxonomic backbone for Europe. Biodiversity Data Journal 3: e5848. https://doi.org/10.3897/BDJ.3.e5848

Jürivete U, Õunap E (2008) Eesti Liblikad, katalog. Estonian Lepidoptera, catalogue. Estonian Lepidopterists' Society, Tallinn, 175 pp.

Karsholt O, Razowski J (Eds) (1996) The Lepidoptera of Europe. A distributional checklist. Apollo Books, Stenstrup, 380 pp. + CD-ROM.

Karsholt O, Nieukerken EJ van (Eds) (2017) Fauna Europaea: Lepidoptera. Fauna Europaea version 2017.06. Fauna Europaea. https://fauna-eu.org/

Karsholt O, Stadel Nielsen P (2013) Revideret fortegnelse over Danmarks Sommerfugle. Revised checklist of the Lepidoptera of Denmark. Lepidopterologisk Forening, København, 120 pp.

Kishida Y (Ed.) (2011a) Nihon-san garui hyojun zukan. The standard of moths in Japan 1. Callidulidae, Epicopeiidae, Drepanidae, Uraniidae, Geometridae, Lasiocampidae, Bombycidae, Saturniidae, Sphingidae. Gakken Kyoiku Shuppan, Tokyo, 352 pp.

Kishida Y (Ed) (2011b) Nihon-san garui hyojun zukan. The standard of moths in Japan 2. Notodontidae, Lymantriidae, Arctiidae, Aganaidae, Micronoctuidae, Nolidae, Noctuidae. Gakken Kyoiku Shuppan, Tokyo, 416 pp.

Kitching IJ, Rougerie R, Zwick A, Hamilton CA, St Laurent RA, Naumann S, Ballesteros Mejia L, Kawahara AY (2018) A global checklist of the Bombycoidea (Insecta: Lepidoptera). Biodiversity Data Journal 6: e22236. https://doi.org/10.3897/BDJ.6.e22236

Kuchlein JH, Vos R de (1999) Geannoteerde naamlijst van de Nederlandse vlinders [Annotated checklist of the Dutch Lepidoptera]. Backhuys, Leiden, 302 pp.

Kullberg J, Albrecht A, Kaila L, Varis V (2002) Checklist of Finnish Lepidoptera. Suomen perhosten luettelo. Sahlbergia 6: 45–190.

Laštůvka Z, Liška J (2011) Komentovaný seznam motýlů České republiky. Annotated checklist of moths and butterflies of the Czech Republic (Insecta: Lepidoptera). Biocont Laboratory, Brno, 146 pp. https://www.researchgate.net/publication/310774240_Annotated_checklist_of_moths_and_butterflies_of_the_Czech_Republic

Lelej AS (Ed.) (2016) Annotirovannyy Katalog Nasekomykh Dal'nego Vostoka Rossii. Tom. II, Lepidoptera - Cheshuekrylye. Annotated catalogue of the insects of Russian Far East. Volume II. Lepidoptera. Dal'nauka, Vladivostok, 812 pp.

Lepiforum e.V. (2008–2019) Bestimmungshilfe für die in Europa nachgewiesenen Schmetterlingsarten. Lepiforum e.V. http://www.lepiforum.de/lepiwiki.pl?Schmetterlingsfamilien_Europa

Leraut P (1997) Liste systématique et synonymique des Lépidoptères de France, Belgique et Corse (deuxième édition). Supplément à Alexanor, Paris, 526 pp.

Lesar T, Govedic M (2010) Check list of Slovenian Microlepidoptera. Natura Sloveniae 12: 35–125. http://web.bf.uni-lj.si/bi/NATURA-SLOVENIAE/pdf/NatSlo_12_1_3.pdf

Lewis JA, Sohn J-C (2015) Lepidoptera: Yponomeutoidea I (Argyresthiidae, Attevidae, Praydidae, Scythropiidae, and Yponomeutidae). Brill, Leiden, World Catalogue of Insects 12: xxiv + 253 pp.

Nasu Y, Hirowatari T, Kishida Y (Eds) (2013) Nihon-san garui hyojun zukan. The standard of moths in Japan 4. Tortricidae, Pyralidae, Gracillariidae etc. Gakken Kyoiku Shuppan, Tokyo, 552 pp.

Nielsen ES, Edwards ED, Rangsi TV (Eds) (1996) Checklist of the Lepidoptera of Australia. CSIRO, Canberra, xiv + 529 pp.

Nielsen ES, Robinson GS, Wagner DL (2000) Ghost-moths of the world: a global inventory and bibliography of the Exoporia (Mnesarchaeoidea and Hepialoidea) (Lepidoptera). Journal of Natural History 34: 823–878. https://doi.org/10.1080/002229300299282

Nieukerken, EJ van, Doorenweerd C, Hoare RJB, Davis DR (2016) Revised classification and catalogue of global Nepticulidae and Opostegidae (Lepidoptera: Nepticuloidea). ZooKeys 628: 65–246. https://doi.org/10.3897/zookeys.628.9799

Nuss M, Landry B, Mally R, Vegliante F, Tränkner A, Bauer F, Hayden J, Segerer A, Schouten R, Li H, Trofimova T, Solis MA, De Prins J, Speidel W (2003–2017) GlobiZ: Global Information System on Pyraloidea. Senckenberg Collection of Natural History, Museum of Zoology, Dresden (Germany). http://www.pyraloidea.org/

Passerin d'Entreves P, Roggero A (2007) Gelechioidea: Scythrididae. Lepidopterorum catalogus (new series) 3: i–xiv, 1–85.

Pastorális G, Buschmann F (2018) A Magyarországon előforduló molylepke-fajok névjegyzéke, 2018. Checklist of the Hungarian micro-moths, 2018 (Lepidoptera). microlepidoptera.hu 14: 77–258. https://doi.org/10.24386/Microlep.2018.14.77

Pastorális G, Kalivoda H, Panigaj L (2013) Zoznam motýľov (Lepidoptera) zistených na Slovensku. Checklist of Lepidoptera recorded in Slovakia. Folia faunistica Slovaca 18: 101–232. http://www.ffs.sk/18-2013/15-pastoralis-et-al

PESI (2011) A Pan-European Species directories Infrastructure. http://www.eu-nomen.eu/pesi/

Pohl GR, Patterson B, Pelham JP (2016) Annotated taxonomic checklist of the Lepidoptera of North America, North of Mexico. Working paper published online by the authors at ResearchGate.net, 766 pp. https://www.researchgate.net/publication/302570819_Annotated_taxonomic_checklist_of_the_Lepidoptera_of_North_America_North_of_Mexico

Pohl GR, Landry J-F, Schmidt BC, Lafontaine JD, Troubridge JT, Macaulay AD, Nieukerken EJ van, De-Waard JR, Dombroskie JJ, Klymko J, Nazari V, Stead K (2018) Annotated checklist of the moths and butterflies (Lepidoptera) of Canada and Alaska. Pensoft Publishers, Sofia, 580 pp. https://ebooks.pensoft.net/book/13218/annotated-checklist-of-the-moths-and-butterflies-lepidoptera-of-canada-and-alaska

Poole RW (1989) Noctuidae, 3 volumes. Lepidopterorum catalogus (new series), xii + 1314 pp.

Pühringer F, Kallies A (2004) Provisional checklist of the Sesiidae of the world (Lepidoptera: Ditrysia). Mitteilungen der Entomologischen Arbeitsgemeinschaft Salzkammergut 4: 1–85. http://www.sesiidae.net/literature/Mitteilungen_2004_001-085.pdf

Pühringer F, Kallies A (2017) Checklist of the Sesiidae of the world (Lepidoptera: Ditrysia). http://www.sesiidae.net/Checklst.htm

Rákosy L, Goia M, Kovács Z (2003) Catalogul Lepidopterelor României. Verzeichnis der Schmetterlinge Rumäniens. Societatea Lepidopterologica Romana, Cluj-Napoca, 446 pp.

Robinson GS (2019) Global taxonomic database of Tineidae (Lepidoptera), version 9.0. Species 2000 & ITIS Catalogue of Life 2016. http://www.catalogueoflife.org/col/details/database/id/31

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E van, Zarucchi J, Penev L (2018) Species 2000 & ITIS Catalogue of Life, 2018 Annual Checklist. http://www.catalogueoflife.org/annual-checklist/2018

Roskov Y, Ower G, Orrell T, Nicolson D, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, Nieukerken E van, Zarucchi J, Penev L (2019) Species 2000 & ITIS Catalogue of Life, 20th February 2019. http://www.catalogueoflife.org/col.

Savenkov N, Šulcs I (2010) Latvian Lepidoptera Catalogue. Estonian Lepidopterists' Society, 176 pp.

Schintlmeister A (2013) Notodontidae & Oenosandridae (Lepidoptera). Brill, Leiden, World Catalogue of Insects 11: 608 pp. https://doi.org/10.1163/9789004259188

Schodde R, Bock WJ (2016) Conflict resolution of grammar and gender for avian species-group names under Article 31.2.2 of the ICZN Code: is gender agreement worth it? Zootaxa 4127: 161–170. https://doi.org/10.11646/zootaxa.4127.1.9

Schodde R, Bock WJ (2017) Interpreting Article 31.2.2 of the Code, *Tanagra bresilia* Linnaeus, and gender agreement—a response to Dickinson et al. (2017) towards more positive outcomes. Bulletin of the British Ornithologists' Club 37: 145–150. https://doi.org/10.25226/bboc.v137i2.2017.a10

Scoble MJ (Ed.) (1999) Geometrid moths of the world : a catalogue : (Lepidoptera, Geometridae), 2 volumes. CSIRO & Apollo Books, Collingwood, VIC, Stenstrup, Denmark. https://doi.org/10.1071/9780643101050

Sinev SY (2002) World catalogue of cosmopterigid moths (Lepidoptera: Cosmopterigidae). Katalog roskoshnykh uzkokrylykh molej (Lepidoptera: Cosmopterigidae) mirovoj fauny. Trudy Zoologicheskogo Instituta 293: 1–183.

Sinev SY (Ed.) (2008) Katalog cheshuekrylykh (Lepidoptera) Rossii. Catalogue of the Lepidoptera of Russia. KMK Scientific Press, St. Petersburg, Moscow, 421 pp.

Sinev SY (2014) World catalogue of blastobasid moths (Lepidoptera: Blastobasidae). Katalog serykh molej (Lepidoptera: Blastobasidae) mirovoj fauny. ZIN-RAS, Sankt-Peterburg, 108 pp.

Sinev SY (2015) World catalogue of bright-legged moths (Lepidoptera: Stathmopodidae). Katalog pestro-
nogikh molej (Lepidoptera: Stathmopodidae) mirovoj fauny. ZIN-RAS, Sankt-Peterburg, 84 pp.

Sobczyk T (2011) Psychidae (Lepidoptera). Brill, Leiden, World Catalogue of Insects 10: 1–467. http://book-
sandjournals.brillonline.com/content/books/9789004261044

Sommerer M (2002) To agree or not to agree - the question of gender agreement in the International
Code of Zoological Nomenclature. Nota lepidopterologica 25: 191–204. http://biodiversitylibrary.org/
page/41371966.

SwissLepTeam (2010) Die Schmetterlinge (Lepidoptera) der Schweiz: eine kommentierte, systematisch-fau-
nistich Liste. Centre Suisse de Cartographie de la Faune (CSCF) & Schweizerische Entomologische Ge-
sellschaft, Neuchâtel, Fauna Helvetica 25: 350 pp.

Vári L, Kroon DM, Krüger M (2002) Classification and checklist of the species of Lepidoptera recorded in
Southern Africa. Simple Solutions, Chatswood, Australia, xxi, 384 pp.

Vives Moreno A (2014) Catálogo sistemático y sinonímico de los Lepidoptera de la Península Ibérica. de
Ceuta, de Melilla y de las Islas Azores, Baleares, Canarias, Madeira y Salvajes (Insecta: Lepidoptera).
SHILAP, Madrid, Suplemento de SHILAP Revista de lepidopterología: 1184 pp.

Welter-Schultes FW (2012) Guidelines for the capture and management of digital zoological names infor-
mation. 2013–03 ed. Global Biodiversity Information Facility, Copenhagen, 121 pp. http://www.gbif.org/
document/80625

Wiemers M, Balletto E, Dincă V, Fric ZF, Lamas G, Lukhtanov V, Munguira ML, Swaay CAM van, Vila R,
Vliegenthart A, Wahlberg N, Verovnik R (2018) An updated checklist of the European Butterflies (Lepi-
doptera, Papilionoidea). ZooKeys 811: 9–45. https://doi.org/10.3897/zookeys.811.28712

Yakovlev RV (2011) Catalogue of the Family Cossidae of the Old World. Neue Entomologische Nachrichten
66: 1–129. https://www.zobodat.at/pdf/Neue-Entomologische-Nachrichten_66_0001-0129.pdf

Two new species of *Spiniphallellus* Bidzilya & Karsholt, 2008 (Lepidoptera, Gelechiidae) from Afghanistan and Iran

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Abstract. *Spiniphallelus eberti* **sp. nov.** (Iran) and *Spiniphallellus naumanni* **sp. nov.** (Afghanistan) are described. The position of the genus within the subfamily Anomaloginae is briefly discussed, as is the degree of development of the gnathos in the male genitalia of two species within the same genus. A key to all *Spiniphallellus* species is given, and adults and male and female genitalia of the new species are illustrated.

Introduction

The genus *Spiniphallellus* was established for three species of Gelechiidae, respectively from the deserts of Kazakhstan, Uzbekistan and Turkmenistan (*S. desertus* Bidzilya & Karsholt, 2008), mountains of Kazakhstan (*S. stonisi* Bidzilya & Karsholt, 2008) and mountains of Turkey (*S. fuscescens* Bidzilya & Karsholt, 2008). Recently an additional new species, *S. chrysotosella* Junnilainen, 2016, was described from Bulgaria, Turkey and Georgia. The first three species are externally very similar, but can easily be separated by their genitalia. *S. chrysotosella* looks externally quite distinct from the other species both by its wingspan and wing pattern, but its genitalia match well the configuration for the genus, being most similar to those of *S. fuscescens*.

The host plant is known only for *S. desertus*, whose larva feeds on *Rheum* sp. (Polygonaceae) in Kazakhstan (Falkovitsh and Bidzilya 2009). The adults of *S. chrysotosella* were observed around *Jasminum fruticans* L. (Oleaceae) in all three localities where this species was recorded (Junnilainen 2016).

As a result of studying collected material in the Staatliches Museum für Naturkunde in Karlsruhe, five plain coloured, rather narrow-winged greyish black specimens of Gelechiidae were discovered amongst material from Afghanistan and Iran. Their assignment to the genus *Spiniphallellus* was proved by the study of the genitalia. It turned out that they represent two different species, which do not match any known species in the genus. Their description is given below.

Material and methods

Male and female genitalia were dissected and prepared using standard methods. Pinned specimens were photographed with an Olympus E-410 digital camera attached to an Olympus SZX12 micro-

scope. Slide-mounted genitalia were photographed with a Canon EOS 600D digital camera mounted on an Olympus U-CTR30-2 combined with a Carl Zeiss microscope. Sets of 4–7 images were taken of each specimen and montaged using Helicon Focus 6 and edited in Adobe Photoshop CS5. The descriptive terminology of the genitalia structures generally follows Bidzilya and Karsholt (2008) and Huemer and Karsholt (2010).

The type material is deposited in the Staatliches Museum für Naturkunde, Karlsruhe, Germany (SMNK).

Results

Key to *Spiniphallellus* species based on external characters

(*S. fuscescens* and *S. naumanni* can hardly be separated without examination of the genitalia)

1	Forewing with shining golden spots, wingspan 9–9.5 mm	*S. chrysotosella*
–	Forewing without golden spots, wingspan 14–18 mm	**2**
2	Hindwing at ¾ length distinctly narrower than at base	*S. eberti*
–	Hindwing at ¾ length about as wide as at base	**3**
3	Forewing light grey, brown irroration indistinct	*S. desertus*
–	Forewing dark greyish black, brown irroration well developed	**4**
4	Black stigmata in the forewing distinct	*S. stonisi*
–	Black stigmata in the forewing indistinct	*S. fuscescens*, *S. naumanni*

Key to *Spiniphallellus* species based on male genitalia

1	Gnathos present	*S. eberti*
–	Gnathos absent	**2**
2	Uncus plate arrow-shaped	*S. stonisi*
–	Uncus plate of another shape	**3**
3	Valva 7 times as long as broad	*S. chrysotosella*
–	Valva 1.5–3.5 times as long as broad	**4**
4	Distal part of phallus weakly s-shaped, lateral process absent	*S. naumanni*
–	Distal portion of phallus straight, lateral process present	**5**
5	Valva 3.5 times as long as broad, transtilla lobe slender, digitate	*S. fuscescens*
–	Valva 2 times as long as broad, transtilla lobe broad, rounded	*S. desertus*

Key to *Spiniphallellus* species based on female genitalia

(females of *S. stonisi*, *S. chrysotosella* and *S. naumanni* are unknown)

1	Antrum distinct, funnel-shaped	*S. desertus*
–	Antrum indistinct, rounded or tubular	**2**
2	Antrum rounded, medial sclerites of sternum VIII indistinct, anterior margin weakly sclerotized	*S. fuscescens*
–	Antrum tubular, medial sclerites of sternum VIII distinct, anterior margin strongly sclerotized	*S. eberti*

Description of new species

Spiniphallellus eberti sp. nov.

http://zoobank.org/26EBA944-A295-4D13-B40B-31E8C64A4A8F

Type material. Holotype ♂, W Iran, Kordestan, Strasse Baneh-Marivan, 86 km SE Baneh, 1950 m, 5.vii.1975 (Ebert & Falkner) (genitalia slide 64/17, O. Bidzilya) (SMNK). Paratypes: 2 ♂, 1 ♀, same data as for holotype (genitalia slide 55/17♂; 60/17♀, O. Bidzilya) (SMNK).

Diagnosis. The new species is characterized superficially by a greyish brown forewing with black markings. It can be separated from its congeners by the hindwing which is distally more narrowed. The male genitalia are unique in having a short and broad valva with a lateral process and well developed distal triangular sclerite of the gnathos. The female genitalia are defined by the presence of distinct medial sclerites on sternum VIII, strongly sclerotized anterior margin of segment VIII and a long anterior apophysis. *S. fuscescens* differs in the weaker sclerotized anterior margin of sternum VIII, the shorter posterior apophysis, less distinct medial sclerites and a rounded rather than tubular antrum.

Description. *Adult* (Figs 1–3). Wingspan 15–17 mm. Head, thorax and tegulae covered with grey scales with light brown tips, labial palpus greyish brown, segment 2 twice as broad and slightly longer than segment 3, lower surface with short brush of modified scales, apex and upper surface light grey, scape grey with pale apex, flagellum ringed black and grey; forewing narrow, pale greyish brown, with indistinct black spots at base and in middle, sub-costal vein mottled with grey, light grey sub-apical transverse fascia at ¾ wing length, cilia grey. Hindwing covered with grey, brown-tipped scales, medial third pale grey, distinctly narrowed from base to ¾ length of wing.

Variation. The female is more unicolorous brown, and the grey pattern on the subcostal vein and sub-apical fascia are not developed.

Male genitalia (Figs 5, 6). Uncus broadly rounded, posterior margin with long setae; distal sclerite of gnathos short, triangular, strongly edged; tegumen broader than long in middle, anteromedial emargination trapezoidal, about 1/3 length of tegumen; valva about 1.5 times as long as broad, strongly sclerotized, with distinct lateral process, posterior margin weakly serrated and thickened, densely setose, extending to the top of uncus; transtilla lobes reduced; vinculum 2.5 times as broad as long, posterior margin broadly emarginated with narrow drop-shaped medial incision; saccus twice as broad as long, narrowed at base, anterior margin broadly rounded. Caecum as long and twice as wide as phallus, rounded, distal part of phallus gradually narrowing towards rounded apex, lateromedial process thorn-shaped.

Female genitalia (Fig. 7). Papilla analis sub-ovate, densely covered with short setae; posterior apophysis as long as the length of ductus bursae; anterior apophysis twice as long as segment VIII; sternum VIII sub-rectangular, slightly broader than long, anterior margin strongly sclerotized, paired narrow ribbon-like gradually curved sclerite extending from posterolateral corner of sternum VIII to sub-rhomboid ostium; antrum short, tubular, as broad as ductus bursae, strongly sclerotized laterally; ductus bursae long, nearly of equal width; corpus bursae sub-oval, elongated; signum a sub-oval plate with serrated margins and transverse medial ridge, near the entrance of corpus bursae.

Figures 1–4. Adults of *Spiniphallellus* spp. **1–3.** *S. eberti* sp. nov. **1.** Holotype (genitalia slide 64/17, O. Bidzilya). **2.** Paratype, ♂ (genitalia slide 55/17, O. Bidzilya). **3.** Paratype, ♀ (genitalia slide 60/17, O. Bidzilya). **4.** *S. naumanni* sp. nov., holotype (genitalia slide 46/17, O. Bidzilya).

Biology. Host plant unknown. Adults have been collected in early July at an elevation of about 2000 m.

Distribution. Iran.

Etymology. The new species is named after one of its collectors, Günter Ebert, who collected a huge amount of material of Lepidoptera, including Gelechiidae, in Iran and Afghanistan.

Spiniphallellus naumanni sp. nov.
http://zoobank.org/18845647-6057-43B3-AEB4-E3BC9D25C5F7

Type material. Holotype ♂, NO Afghanistan, Wakhan-Tal, 3450 m, Darrah-e-Shaur, 25.vii.1971, UV-Li.[ght], coll. Nr. 263 (Ebert & Naumann) (genitalia slide 46/17, O. Bidzilya) (SMNK).

Diagnosis. The new species can hardly be recognized externally without examination of the genitalia. The male genitalia are characterized by a rounded valva densely covered with short, strong setae, very short and broad saccus and phallus with narrow weakly s-curved distal portion and reduced lateral process.

distal sclerite of gnathos

lateral process of valva
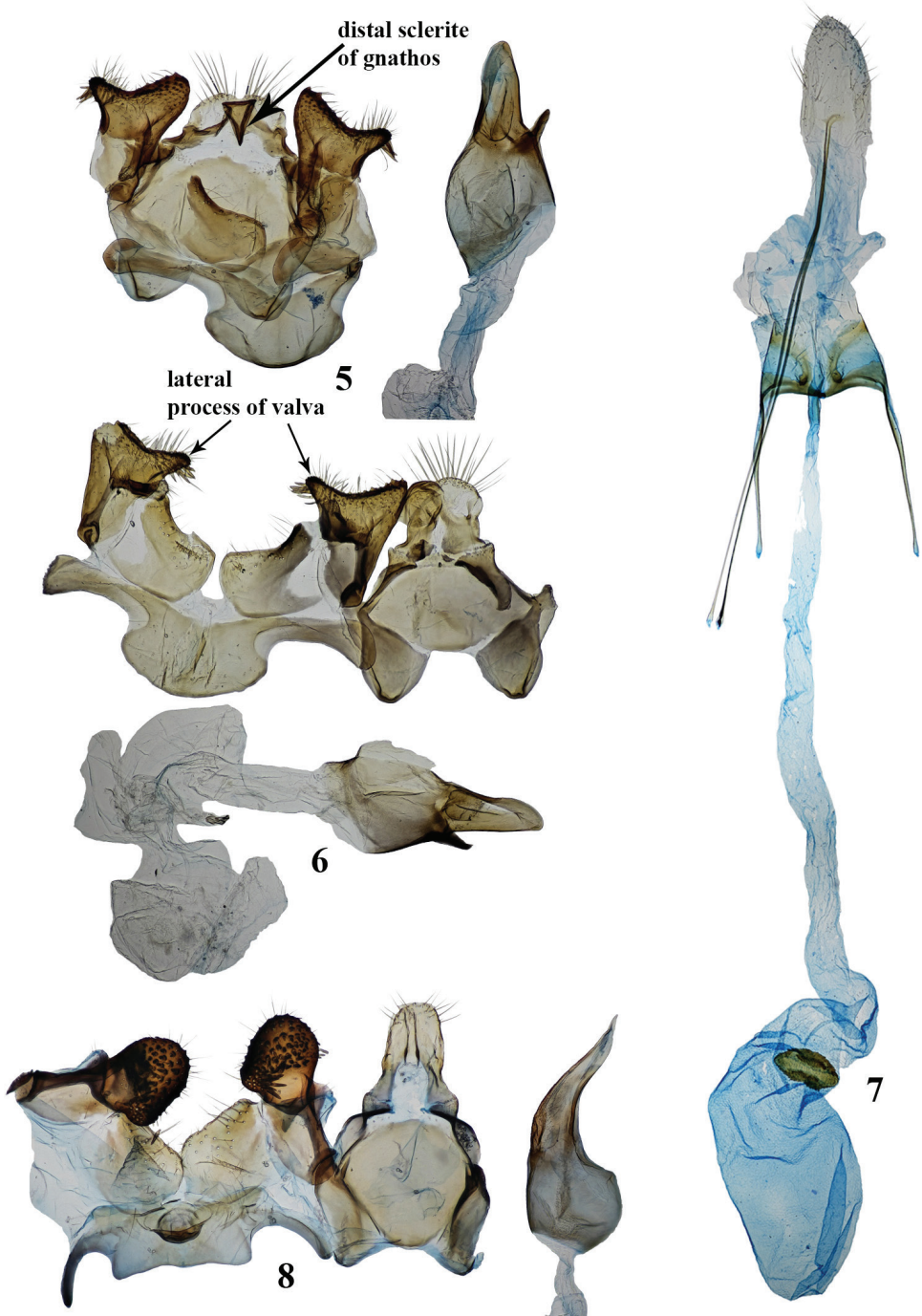
Figures 5–8. Genitalia of *Spiniphallellus*. **5–7.** *Spiniphallellus eberti* sp. nov. **5, 6.** Male genitalia. **5.** Holotype (genitalia slide 64/17, O. Bidzilya). **6.** Paratype, unrolled (genitalia slide 55/17, O. Bidzilya). **7.** Female genitalia (genitalia slide 60/17, O. Bidzilya). **8.** *S. naumanni* sp. nov., male genitalia, holotype, unrolled (genitalia slide 46/17, O. Bidzilya).

Description. *Adult* (Fig. 4). Wingspan 15 mm. Head, thorax, tegulae and labial palpus black, segment 2 twice as broad and slightly longer than segment 3; forewing narrow, plain greyish-brown, with diffuse light brown costal spot at ¾ wing length, cilia grey. Hindwing light grey.

Male genitalia (Fig. 8). Uncus three times as long as broad, posterior margin weakly rounded, covered with long setae; gnathos reduced; tegumen as broad as long in middle, anteromedial emargination very short; valva rounded, extending to about the tip of uncus, anterolaterally covered with strong setae; transtilla lobes reduced; vinculum 2.5 times broader than long, posterior margin broadly emarginated with very narrow medial incision; saccus four times as broad as long. Caecum rounded, distal part of phallus twice as long as caecum, weakly s-curved and gradually narrowed towards pointed apex, without lateral process.

Female genitalia. Unknown.

Biology. Host plant unknown. The holotype was collected in late July at an elevation of about 3500 m.

Distribution. Afghanistan.

Etymology. The new species is named in the honour of one of its collectors, the late Clas M. Naumann, a famous German lepidopterist.

Note. The holotype is rather greasy, a situation often seen in other specimens of *Spiniphallellus* (Bidzilya and Karsholt 2008). One can argue that a new species should not be based on a single, greasy holotype. Even if the holotype had been in perfect condition it would probably have added little to the diagnosis of this species. As mentioned above most *Spiniphallellus* species are externally similar, with the diagnostic characters being found in the structures of the genitalia. The male genitalia of *S. naumanni* sp. nov. show some distinct characters which adds to our knowledge of the diversity of the genus. A further argument for describing this species is that it is very unlikely that additional material will become available in the foreseeable future, if the distribution of *S. naumanni* is restricted to the high mountains of Afghanistan.

Discussion

The genus *Spiniphallellus* was placed in Anomologinae based on the general similarity of the male genitalia characters, such as sternum VIII and tergum VIII separate, tendency to reduction of gnathos and short valvae covered with hairs. Within the subfamily the genus was provisionally associated with a group of genera related to *Monochroa* Heinemann, 1870, namely *Eulamprotes* Bradley, 1971, *Metzneria* Zeller, 1839, *Ptocheuusa* Heinemann, 1870 and *Isophrictis* Meyrick, 1917 (Bidzilya and Karsholt 2008). However, it was noted that the phallus without cornuti and a well developed uncus of *Spiniphallellus* are not characteristic for the above group of genera. The discovery of a distinct distal sclerite of the gnathos in *S. eberti* sp. nov. indicates that *Spiniphallellus* is less related to *Monochroa* and other related genera than was initially argued. The position of the genus within Anomologinae remains rather unclear and may be clarified in the context of a global revision of this subfamily with the application of data obtained from the DNA-studies. Only a DNA barcode for *S. chrysotosella* (cluster number BOLD:ACW1628) is yet available whose placement is uninformative.

In the original description of *Spiniphallellus* it is stated that the gnathos of the male genitalia is absent. However, a gnathos is at least to some extent present in all species of the genus, but in different stages of reduction. This is true for *S. naumanni*, which has a reduced distal sclerite

of gnathos, whereas the male genitalia of *S. eberti* have a short, triangular, strongly edged distal sclerite of the gnathos. In most Lepidoptera families the presence or absence of a gnathos would be considered as a character important at genus level, but several genera of Gelechiidae (especially within the Anomologinae and the Litini) show a tendency to reduction of the gnathos and sometimes also the uncus. Based on other characters *S. eberti* fits well into *Spiniphallellus*. The species of *Spiniphallellus* vary also in the degree of development of transtilla lobes. This character is represented by slender or broad medially projecting processes in *S. desertus*, *S. fuscescens* and *S. chrysotosella*. The transtilla lobes are reduced in *S. stonisi* and both species described here.

Acknowledgements

References

Bidzilya O, Karsholt O (2008) New data on Anomologini from Palaearctic Asia (Gelechiidae). Nota Lepidopterologica 31(2): 199–213.

Falkovitsh MI, Bidzilya OV (2009) A list of gelechiid-moths (Lepidoptera, Gelechiidae) of the southern Kyzylkum. Proceedings of Zoological Museum Kyiv Taras Shevchenko National University 5: 65–98. [In Russian with English abstract]

Huemer P, Karsholt O (2010) Gelechiidae II (Gelechiinae: Gnorimoschemini). In: Huemer P, Karsholt O, Nuss M (Eds) Microlepidoptera of Europe, 6. Apollo Books, Stenstrup, 1–586.

Junnilainen J (2016) A new species of the genus *Spiniphallellus* Bidzilya & Karsholt, 2008 (Lepidoptera, Gelechiidae, Anomologini). Nota Lepidopterologica 39(1): 79–83. https://doi.org/10.3897/nl.39.8382

In memoriam: Campbell Robert Smith (1951–2019)

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http://zoobank.org/12F077C3-38C6-4FC4-8141-1B43E1B06FE5

Figure 1. Campbell with *Graphium policenes*, ca 2000, courtesy NHM.

Campbell Smith was employed as an entomologist at London's *Natural History Museum* for 35 years, during which time he worked on various catalogues, curated parts of the museum collections, and contributed to the output of several research groups. In 2009 he took early retirement to look after his widowed father. His sudden and unexpected death on 11th March 2019 came as a shock to former museum staff and various entomologists around the world, acknowledging his loss with sorrow mixed with good memories of this most amiable colleague and friend.

Campbell was born at Stoke Newington, North London, on 28th November 1951, the only child of Arthur William and Olga Lilian Smith. Olga (née Harris) and Arthur were both from Hackney, and had married there in 1951. Arthur, who worked most of his life before retirement as a Postal & Telegraph Officer (as had his father before him), served in the Army during WWII. By the time he was demobbed, he had become involved with ENSA (Entertainments National Service Association), and was later encouraged by famed trombone player Don Lusher to enter the dance band world as a singer. Arthur declined (apparently because 'he couldn't read the dots'), but maintained

Figure 2. The young Campbell, courtesy Malcolm and Sheila Catlin.

a strong interest in music – something that definitely 'rubbed off' on Campbell. Very sadly his mother died in 1981, at a relatively young age, but not before the family had moved to Leigh-on-Sea, an Essex town on the banks of the Thames estuary that now forms part of the borough of Southend-on-Sea.

Figure 3. In the early 1970s, courtesy Malcolm and Sheila Catlin.

During this time Campbell developed not only his interest in music, but also an enduring fascination with the natural world, including birds and fossils. Olga, who had been a clerical officer in a bank, was very studious herself, and did much to encourage Campbell's academic interests. He completed his secondary education at Southend High School for Boys in 1970. Thereafter he enrolled as an undergraduate at University College London, from October 1970 to June 1973, and was awarded a BSc in Zoology that August.

A year later, after a short spell as a Southend bus conductor, Campbell was recruited to the Entomology Department of the Natural History Museum on 27th August 1974, as an Assistant Scientific Officer. Assigned to Coleoptera, he first worked in a team headed by Bob Pope, helping with curation of the museum's collection of longhorn beetles (Cerambycidae). By 1977 he was assisting Richard Thompson with a biometric study of the green weevil *Phyllobius pyri* species complex, including fieldwork close to home at Two Tree Island nature reserve. Three years later he was working with Peter Hammond on an assessment of beetles as colonists.

Following promotion to Scientific Officer in 1982, Campbell was assigned for a time to the group fulfilling Berry Nye's vision of a comprehensive catalogue of the generic names of moths of the world. His contributions to this project are acknowledged in Fletcher and Nye 1984 (*The Generic Names of Moths of the World* volume 5, Pyraloidea: p. xv), and Nye and Fletcher 1991 (*op. cit.* volume 6, Microlepidoptera: p. xxix).

By 1984, however, Campbell had joined the museum's Butterfly Section, working alongside Phil Ackery. The cataloguing skills he had acquired found a natural home in a project to complete a comprehensive synonymic list for all butterflies found in the Afrotropical Region, based on a manuscript by Bob Carcasson (former Director of the National Museum of Kenya). This culminated in an 800-page work published under Campbell's name as one of three editors (Ackery et al. 1995). The first publication under his own name, on the type material and a bibliography of the celebrated amateur butterfly specialist Lionel George Higgins, appeared in 1988. Campbell also turned his hand to curation of parts of the museum's huge butterfly collection, including European, South American and later African species.

Figure 4. Campbell 1984, old Entomology block, NHM; photo courtesy Prof Kyoichiro Ueda.

Campbell's move to the butterfly group, with its strong interest in biology and cladistics, gave him more opportunities for attending conferences – and on just one occasion, overseas fieldwork. Conference trips included feisty Willi Hennig Society meetings in London (1984) and Paris (1992), convivial biology of butterflies symposia in Stockholm (1994: see Smith and Pope 1995), Crested Butte (1998) and Leeuwenhorst, Netherlands (2002), and, at the invitation of Prof Osamu Yata, a memorable visit to Fukuoka, Japan, in December 2004, to give a presentation on *Graphium*. For

Paris, Campbell acted as unofficial travel organiser for the London contingent, which Prof Brian Gardiner recalls was greatly appreciated. Other visits included Stuttgart to work with Christoph Häuser and Axel Steiner on 'Butterfly Taxonomy on the Internet' (2003), and Oxford, Frankfurt, Brussels, Tervuren, Paris, Berlin, Karslruhe, Stuttgart and München to work on African collections of Papilionidae. There was also a brief excursion to the Wissenschaftskolleg zu Berlin in spring 1994, as part of the NHM Biogeography and Conservation Laboratory's contribution to an international project on priority area analysis for the conservation of biodiversity.

Figure 5. With the bronze bull Goshingyu that guards the shrine of Michizane Suagawara (845–903), God of Learning, Dazaifu City, Fukuoka, Japan, 13.xii.2004. It is said you become wise if you touch the head of Goshingyu. Courtesy Prof. Osamu Yata.

His one attempt to undertake fieldwork in the tropics – Korup National Park, Cameroon, 1989, in the company of David Lees, did not end well. Campbell contracted malaria and, on his return, had to spend a while recovering in the old Rochford Hospital, a few miles from the family home at Leigh. Even so, while at Korup, together with Andrew Rawlins, he assisted David in valuable work compiling a Korup butterfly checklist, and on the life history of the enigmatic butterfly *Pseudopontia* (Pieridae).

As already noted, from 1990–2004 Campbell also worked in support of the Biogeography and Conservation Lab., including research with myself on the systematics of swallowtail butterflies – intended at the time as a long-term focal group for conservation planning, following up from the 1985 IUCN Red Data book by Mark Collins and Mike Morris. This ambitious aim was, sadly, never to be realised – although an idea of what was in mind can be found in Smith and Vane-Wright (2001) with its analytical section on biogeography, endemism and conservation evaluation. Campbell's research on swallowtails was otherwise largely of a revisional nature, concerned with species limits, synonymy and distribution, but the output did include the description of one new species, *Graphium abri* Smith & Vane-Wright, 2001, from the Central African Republic, as well as several

cladistic analyses based on detailed morphological data. He also made a significant contribution to the checklist of butterflies of Sulawesi, published in 2003 under the authorship of Rienk de Jong and myself (Zoologische Verhandelingen 343, 267 pp.).

From 2005 onward, after my retirement from the Natural History Museum in 2004, and throughout the construction of the Darwin Centre 2 building at South Kensington, all Museum lepidopterists were relocated to the museum's outstation at Wandsworth, SW London. During this period, as well as finishing up some significant joint publications on the swallowtails, Campbell collaborated with Ian Kitching and Malcolm Scoble as part of the CATE team, 'Creating a Taxonomic E-science', a three-year project funded by the National Environment Research Council. His last day of museum service is recorded as 31st January 2009. After retirement he did some voluntary work on the NHM 'LepIndex' online database, and the *Sphingidae Taxonomic Inventory*, a scratchpad project into which the original CATE hawkmoth project had been converted.

Figure 6. Campbell (far left) with CATE team, Royal Botanic Gardens Kew, 2008, courtesy Ian Kitching.

From an early age Campbell had taken a considerable interest in the natural world and, evidently inspired by the writings of Gerald Durrell, the need for its conservation. Wildlife societies that he supported included Fauna & Flora International, World Land Trust, Essex Wildlife Trust, Butterfly Conservation, the Zoological Society of London, and the Royal Society for the Protection of Birds. In the course of his academic work he also became a member of several learned societies, notably the Willi Hennig Society, Royal Entomological Society, and Linnean Society of London. Probably influenced by Colin Patterson, Brian Gardiner and Chris Humphries, 'The Linn', which he joined in 1989, was his favourite, and not long before his unexpected death he planned to start attending meetings again. In 1988 he was also elected Executive Secretary of the Prolegs Club – a shadowy, light-hearted, after-hours occasional association of lepidopterists, reflecting his ready social engagement with colleagues.

Intensely interested in music of many genres (an extraordinary late-night session in Fukuoka with two Japanese didgeridoo players comes to mind!), but especially 'classical', Campbell was a

subscriber to *The Gramophone*. While in Berlin in 1994 he took an unexpected opportunity to meet members of the Berlin Philharmonic, making instant friendships with several of the musicians that were to endure for years to come.

Campbell took early retirement in 2009, at the age of 57, to care for his long widowed father, by then in his late 80s. Campbell evidently had a very strong bond with Arthur, and for years they had taken holidays together, notably several continental train tours – which also fitted well with some of Campbell's other interests, including good food, wine, beer, railways and ships. They also travelled together to sites where Arthur saw action during WWII, as part of a publicly funded 'Heroes Return' programme.

During 2017 Arthur agreed to be moved to a nearby care home, as Campbell felt no longer able to cope. He died, aged 97, in March 2018. In an email Campbell wrote "Though I miss him and will continue to do so, I am not grieving; in the end, his death was a release from extreme discomfort. Instead, I have a sense of relief. Dad has been the prime focus of my thoughts and action. Now I have to make a new life for myself. I hope I'll be able to renew contact with old friends and colleagues."

Sadly this never really happened, largely due to a broken ankle that he suffered a few months before Arthur's death. Campbell's recovery seemed slow; I met with him in London one lunchtime during July 2018, and it was clear that walking was still difficult, and he had lost confidence in being able to get about. But he was making progress and, having survived Christmas that year (always a challenge when on your own), in January he was clearly looking forward again, even contemplating reviving some unfinished research. So his sudden end (due to a stroke), almost exactly a year after his father's passing, was a considerable shock to his numerous friends in Southend and Leigh, as well as myself and his many former colleagues.

At times outspoken (his left-wing views engendered a healthy distrust of management, and 'bosses' in particular!), Campbell was nonetheless a popular member of Natural History Museum staff, often remembered for his remarkable knowledge of classical music (museum quiz nights), railways and real ale. Professionally, he was a skilled museum entomologist, insightful researcher, and excellent cataloguer. Well regarded by those who knew him, his passing has been marked with sadness by many butterfly specialists around the world. The African lycaenid *Eresiomera campbelli* Collins & Larsen, 1998, was named in his honour.

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Bibliography: publications by Campbell Robert Smith

1988   Lionel George Higgins – a bibliography and type catalogue. *Entomologist's Gazette* 39(1): 17–37.

1991   Phylogenetic relationships of three African swallowtail butterflies, *Papilio dardanus, P. phorcas* and *P. constantinus*: new data from hybrids (Lepidoptera: Papilionidae). *Systematic Entomology* 16: 257–273. [Joint: Clarke Sir C, Gordon IJ, Vane-Wright RI & Smith CR]

1991   Phylogenetic relationships of three African swallowtail butterflies, *Papilio dardanus, P. phorcas* and *P. constantinus*: a cladistic analysis (Lepidoptera: Papilionidae). *Systematic Entomology* 16: 275–291. [Joint: Vane-Wright RI & Smith CR]

1992   Occurrence and significance of natural hybrids between *Papilio dardanus* and *P. phorcas* (Lepidoptera: Papilionidae). *Systematic Entomology* 17: 269–272. [Joint: Vane-Wright RI & Smith CR]

1994   *Papilio saharae* Oberthür, 1879, specifically distinct from *Papilio machaon* Linnaeus, 1758 (Lepidoptera: Papilionidae). *Entomologist's Gazette* 45(4): 223–249. [Joint: Pittaway AR, Larsen TB, Clarke Sir CA, Smith CR, Crnjar R & Clarke FMM]

1994   Systematic assessment of diversity by summation. *In* Forey PL, Humphries CJ & Vane-Wright RI (eds), *Systematics and Conservation Evaluation*. Oxford University Press, Oxford, pp. 309–326. [Joint: Vane-Wright RI, Smith CR & Kitching IJ]

1995   Are butterflies the "New Birds"? Report on the meeting: Butterfly Ecology and Evolution, held at Stockholm University, Sweden, 10th-12th October, 1994. *Antenna*, London 19(1): 28–30. [Joint: Smith CR & Pope JE]

1995   *Carcasson's African butterflies: an Annotated Catalogue of the Papilionoidea and Hesperioidea of the Afrotropical Region*. CSIRO, East Melbourne, pp. xi + 803 pp. [Joint: Ackery PR, Smith CR & Vane-Wright RI (eds)]

1995   Biodiversity, systematics, and conservation: a case study of swallowtail butterflies (Lepidoptera: Papilionidae). *Verhandlungen der Deutschen Zoologischen Gesellschaft* 88: 148. [Joint: Häuser CL, Smith CR & Vane-Wright RI]

1999   Bionomics of African kite swallowtails, with a request for information. *Metamorphosis* 10(2): 81–83. [Joint: Vane-Wright RI & Smith CR]

2001   A review of the Afrotropical species of the genus *Graphium* (Lepidoptera: Rhopalocera: Papilionidae). *Bulletin of The Natural History Museum London* (Entomology) 70(2): 503–718. [Joint: Smith CR & Vane-Wright RI]

2001   [POSTER] Citrus swallowtails – from the East, or out of Africa? Poster abstracts, *4th International Congress on the Biology of Butterflies*, Leeuwenhorst, The Netherlands 23–27 March 2002, pp. 54–55. [Joint: Smith CR & Vane-Wright RI]

2002   [ELECTRONIC PUBLICATION] Distribution of Afrotropical kite swallowtails. http://www.nhm.ac.uk/research-curation/research/projects/afrotropical-kite/ [Link dead, and data, though supposedly archived, now inaccessible] [Joint: Smith CR & Vane-Wright RI]

2003   *Graphium* butterflies of the Afrotropical Region [in Japanese]. *The Nature and Insects,* New Science Co., Tokyo 38(7): 7–12.

2004   Independent gene phylogenies and morphology demonstrate a Malagasy origin for a wideranging group of swallowtail butterflies. *Evolution* 58(12): 2763–2782. [Joint: Zakharov EV, Smith CR, Lees DC, Cameron A, Vane-Wright RI & Sperling FAH]

2004   [ABSTRACT] Why are we studying Oriental *Graphium*? *In* Yata, O. (Ed.) *Abstracts of International Symposium "Network construction for the establishment of insect inventory in Tropic Asia (TAIIV)"*, *Kyushu University, 11–12 December 2004,* p. 7. Kyushu University, Fukuoka, Japan.

2005   [ELECTRONIC PUBLICATION] *Papilionidae – revised GloBIS/GART species checklist (2nd draft)*. [Online - http://www.insects-online.de/frames/papilio.htm] [Joint: Häuser C, de Jong R, Lamas G, Robbins RK, Smith CR & Vane-Wright RI]

2005   Why we are studying Oriental *Graphium* (Lepidoptera: Papilionidae) at the Natural History Museum, London. *In* Yata O (ed.) *A Report on Insect Inventory Project in Tropic Asia (TAIIV)* "Network construction for the establishment of insect inventory in Tropic Asia (TAIIV)". Kyushu University, Fukuoka, Japan, pp. 49–56.

2007   [POSTER] Getting involved with CATE. XVth European Congress of Lepidopterology, Erkner, Germany, September 2007. [Joint: Smith CR, Scoble MJ, Kitching IJ, Clark B, Godfray HCJ, Mayo S, Blagoderov V, Haigh A, Jackson M, James S, Reynolds L & Young R]

2007   [POSTER AND ABSTRACT] Getting involved with CATE. TAIIV meeting, Kyushu University, Japan, December 2007. *In* Abe, Y. & Yata, O. (eds) *Program of International Symposium "Network construction for the establishment of insect inventory in Tropical Asia (TAIIV)", Kyushu University, 1–2 December 2007,* pp. 81–82 [p. 82 in Japanese]. Kyushu University, Fukuoka, Japan. [Joint: Smith CR, Scoble MJ, Kitching IJ, Clark B, Godfray HCJ, Mayo S, Blagoderov V, Haigh A, Jackson M, James S, Reynolds L & Young R]

2008   Classification, nomenclature and identification of lime swallowtail butterflies: a post-cladistic analysis (Lepidoptera: Papilionidae). *Systematics and Biodiversity* 6(2): 175–203; http://www.journals.cup.org/abstract_S1477200008002703. [Joint: Smith CR & Vane-Wright RI]

2008   On the status of *Papilio sjoestedti* Aurivillius, 1908, the "Kilimanjaro Swallowtail" (Lepidoptera: Papilionidae). *Journal of Natural History* 42(19–20): 1349–1359. [Joint: Smith CR, Liseki S & Vane-Wright RI]

2008   Introducing CATE – a model for moving taxonomy to the web. *In* Yata O (ed.) *The 2nd Report on Insect Inventory Project in Tropic Asia (TAIIV)* "Network construction for the establishment of insect inventory in Tropic Asia (TAIIV)". Kyushu University, Fukuoka, Japan, pp. 137–144. [Joint: Smith CR, Godfray HCJ, Scoble MJ, Clark BR, Kitching IJ, Mayo SJ, Blagoderov V, Haigh AL, Jackson M, Sadler S, Lay LN & Young RPW]

A new species of *Crocidosema* Zeller (Lepidoptera, Tortricidae) from the Andes of northern Chile

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http://zoobank.org/18830D74-3935-4277-9EA7-78C30A8FED48

Abstract. *Crocidosema nitsugai* **sp. nov.** (Lepidoptera, Tortricidae, Olethreutinae, Eucosmini) is described and illustrated from the Andes of northern Chile based on adults obtained from larvae collected on leaves, flowers and unripe fruits and seeds of *Lupinus oreophilus* Phil. (Fabaceae). This represents the first record of insect herbivory on this Chilean endemic perennial herb. The genitalia morphology of *C. nitsugai* suggests a close evolutionary relationship with two congenerics from high elevation environments of the Ecuadorian and Peruvian Andes.

Introduction

Crocidosema Zeller, 1847 (Lepidoptera, Tortricidae, Olethreutinae, Eucosmini) is a mainly Neotropical genus with 69 described species, 45 of which have their type locality in South America (Gilligan et al. 2018). Many of the South American representatives were described in the last fifteen years based on the study of specimens collected relatively recently using light traps in different localities of Brazil, Colombia, Ecuador, Peru and Venezuela (Razowski and Wojtusiak 2006a, b, 2008a, b, 2009, 2010, 2011, 2013; Razowski and Becker 2014, 2017). *Crocidosema insulana* Aurivillius, 1922, is the only species of the genus native to Chile, originally described from Masatierra, Juan Fernandez Islands, and subsequently recorded from most of the mainland part of the country (Razowski and Pelz 2010). *Crocidosema aporema* Walsingham, 1914, an important pest of several legume crops described from Costa Rica and currently widespread in Central and South America (Gilligan and Epstein 2014), is also recorded from Chile in the agricultural literature (Artigas 1994).

The northernmost part of Chile has a considerable diversity of arid environments along a wide elevational gradient from the lowlands of the Atacama Desert to the highlands of the Andes (Luebert and Pliscoff 2006). Within this elevational gradient, records of native species of Tortricidae are mainly restricted to habitats below 1000 m elevation (Clarke 1987; Brito and Vargas 2018), with the exception of one species that reaches about 2000 m in the transverse valleys of the Atacama Desert (Vargas-Ortiz and Vargas 2018). However, the high elevation habitats of this region harbor a distinctive native flora (Gatica-Castro et al. 2015), some of whose species support populations of host-specific Lepidoptera (Vargas 2014; Ramírez-Fischer et al. 2016), suggesting that the current absence of records of Tortricidae in this area is likely a sampling artifact. In support of this suggestion, adults of this family were recently obtained from larvae collected on *Lupinus oreophilus* Phil.

(Fabaceae), a perennial herb endemic to the elevational belt between 2700 and 4400 m in the Andes of northern Chile (Orrego et al. 2013). The Near Threatened status of populations of the shrub was recently proposed (Gatica-Castro et al. 2015). Subsequent examination of the morphology of the micromoths revealed that they represent a previously unknown species of *Crocidosema*, whose description is provided below.

Material and methods

The sampling was undertaken near Putre (18°12'58"S; 69°33'38"W), Parinacota Province, at 3670 m elevation on the western slopes of the Andes of northern Chile. The site has a tropical xeric bioclimate with seasonal rains mainly concentrated between December and March (Luebert and Pliscoff 2006). This rainfall regime allows the development of a seasonal vegetation growth that reaches higher coverage shortly after the rains (Muñoz and Bonacic 2006). Larvae were collected on leaves, flowers and fruits of *L. oreophilus* in March 2019 following the summer rainfall. The collected larvae and the respective plant organs were placed in plastic vials with paper towel at the bottom and brought to the laboratory, where the vials were periodically cleaned and fresh leaves, flowers and fruits were provided until the larvae finished feeding and pupated. After pupation the vials were regularly observed until the emergence of adults, which were mounted. The abdomen of the adults was removed, cleared in hot 10% KOH for a few minutes, stained with Eosin Y and Chlorazol black and slide-mounted with Euparal. Images were captured with Sony CyberShot DSC-HX200V and Micropublisher 3.3 RTV-QImaging digital cameras attached to a Leica M125 stereomicroscope and an Olympus BX51 optical microscope, respectively.

Abbreviations of institutional collections

MNNC　Museo Nacional de Historia Natural de Santiago, Santiago, Chile
IDEA　　Colección Entomológica de la Universidad de Tarapacá, Arica, Chile

Results

Crocidosema nitsugai sp. nov.

http://zoobank.org/ABAD0B64-6794-4666-AE38-6471B431407A

Figs 1–5

Type material. HOLOTYPE, male, CHILE: Putre, Parinacota, Chile, emerged April 2019, H.A. Vargas coll., ex-larva *Lupinus oreophilus*, March 2019, genitalia slide HAV-1278 (MNNC).

Paratypes, CHILE. One male, two females, same data as holotype, genitalia slides HAV-1261, 1270, 1271 (MNNC); four males, one female, same data as holotype, genitalia slides HAV-1259, 1275, 1276, 1277, 1279 (IDEA).

Diagnosis. The genitalia of *C. nitsugai* are remarkably similar to those of *C. marcapatae* (Razowski & Wojtusiak, 2010), described under *Epinotia* Hubner, [1825] from Cusco, Peru. However, the female genitalia of *C. nitsugai* have a parallel-sided antrum with the dorsal wall posteriorly
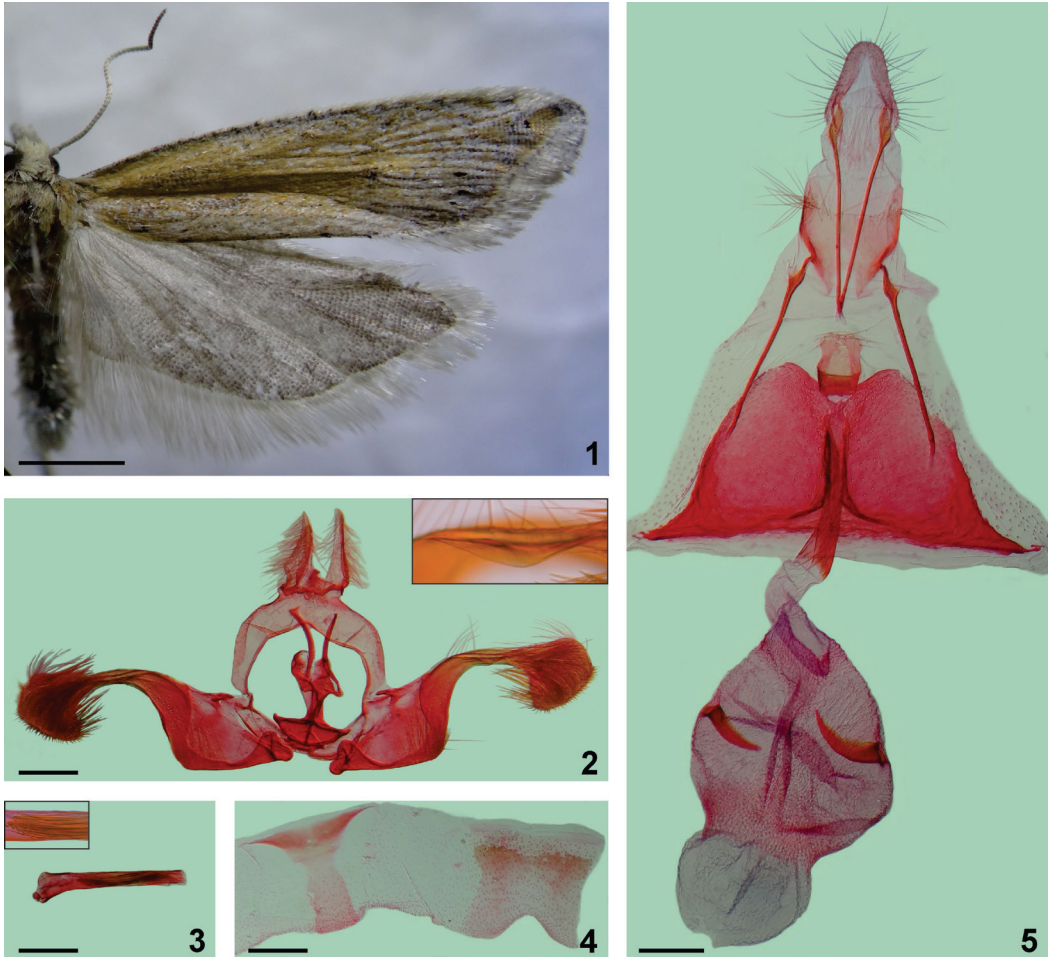
Figures 1–5. Adult stage of *Crocidosema nitsugai* sp. nov. **1.** Holotype male in dorsal view. **2.** Male genitalia in ventral view, phallus removed; upper right rectangle showing longitudinal carina on the neck of the right valva. **3.** Phallus in lateral view; upper left rectangle showing base of cornuti. **4.** Tergum (left) and sternum (right) of male abdominal segment VIII. **5.** Female genitalia in ventral view. Scale bars: 2 mm (**1**), 0.3 mm (**2-4**), 0.25 mm (**5**).

projected and the posterior margin of sternum VII widely notched at the middle. In contrast, those of *C. marcapatae* have a cup-shaped antrum without posterior projection of dorsal wall and posterior margin of sternum VII almost straight. In the male genitalia of *C. nitsugai* the neck of the valva is almost uniform in height throughout its length with a narrow longitudinal carina, and dorsal and ventral lobes of the cucullus are similar in size. In contrast, in *C. marcapatae* the neck of the valva is broadened on basal half and lacks carina, and the dorsal lobe of the cucullus is conspicuously smaller than the ventral lobe. The female genitalia of *C. nitsugai* also resemble those of *C. pusula* Razowski & Becker, 2014, described from Carchi, Ecuador. However, the parallel-sided antrum, cingulum longer than the larger signum and two longitudinal stripes along the middle of sternum VII of *C. nitsugai* contrast with the cup-shaped antrum, cingulum slightly shorter than the smaller

signum and absence of longitudinal stripes on the sternum VII of *C. pusula*. The male of *C. pusula* is unknown, impeding comparisons with *C. nitsugai*.

Description. Male. (Figs 1–5) Forewing length 8.5–9.5 mm.

Head. Vertex and frons mainly whitish gray with a few scattered grayish brown scales. Labial palpus mainly grayish brown with a few scattered whitish gray scales. Antenna whitish gray.

Thorax. Mainly yellowish brown dorsally with a few scattered dark gray scales, whitish gray latero-ventrally; patagium grayish brown. Foreleg with anterior face grayish brown, posterior face whitish gray. Midleg similar to foreleg in coloration, tibial spurs whitish gray. Hindleg whitish gray, including tibial spurs. Forewing mainly yellowish brown with abundant whitish gray and grayish brown scales intermixed outside the discal cell, a few scattered dark gray scales near external margin; fringe grayish brown. Hindwing mainly grayish brown, scattered whitish gray scales, fringe whitish gray.

Abdomen. Grayish brown. Tergum VIII (Fig. 4) somewhat T-shaped; anterior margin straight; lateral margin widely excavated on anterior half; posterior margin about 1/3 the width of anterior margin. Sternum VIII somewhat square-shaped, posterior margin widely excavated in the middle.

Male genitalia (Figs 2, 3). Tegumen with anterior and posterior margins mainly parallel. Uncus cylindrical, slightly sclerotized, broadened basally, apex rounded, covered with hair-like setae. Socius slightly longer than uncus, narrow, tapering apically. Juxta semicircular, dorsal margin straight. Henion (sclerite between anellus and gnathos) narrow, elongated, well sclerotized, slightly longer than socius. Valvae symmetrical, wide incision on ventral margin; base of valva triangular; sacculus narrow; neck of valva almost uniform in height throughout its length, a narrow longitudinal carina on medial face; cucullus densely covered with hair-like scales, dorsal and ventral lobes similar in size. Phallus cylindrical, slightly longer than sacculus, slightly broadened basally; vesica with several spine-shaped cornuti.

Female. Similar to male in maculation and size.

Female genitalia (Fig. 5). Papillae analis narrow, elongated, slightly sclerotized, with hair-like setae. Posterior apophysis spine-shaped, slightly broadened basally, about twice length of papillae analis. Anterior apophysis similar to posterior apophysis in shape and length. Tergum VIII with U-shaped notch on anterior margin. Sternum VII with anterior margin straight; lateral margin widely excavated close anterior margin; posterior margin bilobed, widely notched in middle; two mainly parallel longitudinal stripes along the middle diverging close the anterior margin. Antrum parallel-sided, dorsal wall posteriorly projected. Ductus bursae slightly curved, membranous basally and apically; a well-developed cingulum with apex exceeding anterior margin of sternum VII. Corpus bursae membranous, pear-shaped, about 1.5 times length of ductus bursae; two slightly curved saw-like signa laterally.

Geographic distribution. *Crocidosema nitsugai* is known only from the type locality, in the surroundings of Putre, Parinacota Province, at 3670 m elevation on the Andes of northern Chile (Fig. 6).

Host plant. The only host plant currently known for *C. nitsugai* is the perennial herb *Lupinus oreophilus* Phil. (Figs 7–9), upon which the larvae feed on leaves, flowers and unripe fruits and seeds.

Etymology. The specific epithet is dedicated to the memory of the great Paraguayan guitarist and composer Agustín Pío Barrios, also known as Nitsuga Mangoré, as an acknowledgement to his amazing musical contribution.

Figures 6–9. Habitat and host plant of *Crocidosema nitsugai* sp. nov. **6.** Habitat of *C. nitsugai* in the type locality, near Putre, Parinacota Province, at 3670 m elevation on the Andes northern Chile. **7.** The host plant *Lupinus oreophilus*. **8.** Inflorescence of *L. oreophilus*. **9.** Fruits of *L. oreophilus*.

Discussion

Although many species of *Crocidosema* have been described recently from the Neotropics, the discovery of *C. nitsugai* during a short field trip following the summer rainfall in the type locality highlights the need for additional sampling in different Neotropical environments to more accurately characterize the taxonomic diversity of this genus. Surveys of native plants appear to be

especially valuable, because these provide the first insights on the host range of each newly found species (e.g. Brito and Vargas 2018). Host plants have been recorded for only nine species of *Crocidosema*, six of which feed on plants of only one family, whereas three feed on plants of more than one family. At one extreme is the widespread *C. plebejana* Zeller, 1847, whose larvae have been collected on representatives of nine families (Brown et al. 2008). Fabaceae has been recorded as host for four species of *Crocidosema* (Brown et al. 2008), among which only the Neotropical pest *C. aporema* feeds on *Lupinus* (Callohuari et al. 2018).

Crocidosema nitsugai represents the first record of insect herbivory on *L. oreophilus*. Previous studies indicated consumption of *L. oreophilus* by two herbivorous rodents in high elevation environments of the Andes of northern Chile (Cortés et al. 2002). The host plant range of *C. nitsugai* appears to be narrow, as it was absent in surveys undertaken on about ten plants of each of the additional native species of Fabaceae found in the study site: *Adesmia verrucosa* Meyen, *A. spinosissima* Vogel, *Dalea pennellii* (J.F. Macbr.) J.F. Macbr. var. *chilensis* Barneby and *Senna birostris* (Dombey ex Vogel) H.S. Irwin & Barneby var. *arequipensis* (Meyen ex Vogel) H.S. Irwin & Barneby. However, surveys for *C. nitsugai* should be expanded to additional Fabaceae of northern Chile outside the study site, where three other species of *Lupinus* have been recorded (Gatica-Castro et al. 2015). In addition, as Near Threatened status was recently proposed for *L. oreophilus* (Gatica-Castro et al. 2015), further studies are needed to understand better the effect of herbivory by larvae of *C. nitsugai* on populations of this plant.

The Andes uplift has been recognized as an important event in the diversification of several Neotropical animal groups, including Lepidoptera (Massardo et al. 2015; De-Silva et al. 2016). Unfortunately, despite a few recent additions (Vargas and Mundaca 2016; Vargas 2018), the taxonomy and natural history of the micromoths of the Andes of northern Chile have been little studied, impeding further evolutionary studies with these organisms. Indeed, *C. nitsugai* is the first species of Tortricidae described from these arid high elevation environments. Its genitalia are remarkably different of those of *C. insulana*, the only other representative of the genus native to Chile (Razowski and Pelz 2010), suggesting that they are only distantly related. The close morphological resemblance of the genitalia of *C. nitsugai* with those of other two highland species, *C. marcapatae* (Cusco, Peru, 3600 m) and *C. pusula* (Carchi, Ecuador, 2200 m), suggest that a group of closely related *Crocidosema* diversified along the still underexplored high elevation environments of the Andes, an evolutionary scenario that should be assessed in further studies.

Acknowledgements

References

Artigas JN (1994) Entomología Económica. Insectos de interés agrícola, forestal, médico y veterinario. Ediciones Universidad de Concepción, Concepción, Chile. 943 pp.

Brito R, Vargas HA (2018) A new leaf-tying *Episimus* Walsingham (Lepidoptera: Tortricidae) feeding on the vulnerable tree *Haplorhus peruviana* (Anacardiaceae) in the Atacama Desert of northern Chile. Studies on Neotropical Fauna and Environment 53: 113–119. https://doi.org/10.1080/01650521.2017.1413824

Brown JW, Robinson G, Powell JA (2008) Food plant database of the leafrollers of the world (Lepidoptera: Tortricidae) (Version 1.0). http://www.tortricid.net/foodplants.asp [accessed 25 Jun 2019]

Callohuari YT, Vergara CE, Jiménez JE (2018) Insect pests associated with Andean lupin (*Lupinus mutabilis* Sweet) and their parasitoids in Peruvian central coast (Lima, La Molina). Peruvian Journal of Agronomy 2: 27–33. https://doi.org/10.21704/pja.v2i2.1199

Clarke JFG (1987) Two new *Cryptophlebia* Walsingham from Chile. (Lepidoptera: Tortricidae). Acta Entomológica Chilena 14: 7–12.

Cortés A, Rau JR, Miranda E, Jiménez JE (2002) Hábitos alimenticios de *Lagidium viscacia* y *Abrocoma cinerea*: roedores sintópicos en ambientes altoandinos del norte de Chile. Revista Chilena de Historia Natural 75: 583–593. https://doi.org/10.4067/S0716-078X2002000300009

De-Silva DL, Elias M, Willmott K, Mallet J, Day JJ (2016) Diversification of clearwing butterflies with the rise of the Andes. Journal of Biogeography 43: 44–58. https://doi.org/10.1111/jbi.12611

Gatica-Castro A, Marticorena A, Rojas G, Arancio G, Squeo FA (2015) Estado de conservación de la flora nativa de las regiones de Arica-Parinacota y de Tarapacá, Chile. Gayana Botánica 72: 305–339. https://doi.org/10.4067/S0717-66432015000200013

Gilligan TM, Baixeras J, Brown JW (2018) T@RTS: Online World Catalogue of the Tortricidae (Ver. 4.0). http://www.tortricid.net/catalogue.asp [accessed 28 May 2019]

Gilligan TM, Epstein ME (2014) TortAI, Tortricids of Agricultural Importance. http://idtools.org/id/leps/tortai/index.html [accessed 24 Jun 2019]

Luebert F, Pliscoff P (2006) Sinopsis bioclimática y vegetacional de Chile. Editorial Universitaria, Santiago. 316 pp.

Massardo D, Fornel R, Kronforst M, Gonçalves GL, Moreira GRP (2015) Diversification of the silverspot butterflies (Nymphalidae) in the Neotropics inferred from multi-locus DNA sequences. Molecular Phylogenetics and Evolution 82: 156–165. https://doi.org/10.1016/j.ympev.2014.09.018

Muñoz AE, Bonacic C (2006) Variación estacional de la flora y vegetación en la precordillera andina de la comuna de Putre (I Región de Tarapacá, Chile) durante el período 2002–2003. Gayana Botánica 63: 75–92. https://doi.org/10.4067/S0717-66432006000100003

Orrego F, Watson J, Flores AR, Rojas G (2013) Flores del Norte Grande. Jack Stern y CL, Museo Nacional de Historia Natural y SMC, Santiago. 165 pp.

Ramírez-Fischer FJ, Benyamini D, Vargas HA (2016) An endangered hemiparasitic shrub is the only host plant of the little-known Neotropical hairstreak *Strymon flavaria* (Lepidoptera: Lycaenidae) in the arid Andes. Journal of Insect Conservation 20: 923–928. https://doi.org/10.1007/s10841-016-9919-1

Razowski J, Becker VO (2014) Systematics and faunistics of Neotropical Eucosmini, 2: *Epinotia* Hübner, *Catastega* Clemens, and *Crocidosema* Zeller (Lepidoptera: Tortricidae). Polish Journal of Entomology 83: 23–59. https://doi.org/10.2478/pjen-2014-0003

Razowski J, Becker VO (2017) Systematics and faunistics of Neotropical Eucosmini, 3: *Epinotia*-group of genera (Lepidoptera: Tortricidae). Polish Journal of Entomology 86: 303–345. https://doi.org/10.1515/pjen-2017-0018

Razowski J, Pelz V (2010) Tortricidae from Chile (Lepidoptera: Tortricidae). SHILAP Revista de Lepidopterología 38: 5–55.

Razowski J, Wojtusiak J (2006a) Tortricidae (Lepidoptera) in the valley of Río Gualaceo, East Cordillera in Ecuador, with descriptions of new taxa. Acta Zoologica Cracoviensia 49B: 17–53. https://doi.org/10.3409/000000006783995346

Razowski J, Wojtusiak J (2006b) Tortricidae from Venezuela (Lepidoptera: Tortricidae). SHILAP Revista de Lepidopterología 34: 35–79.

Razowski J, Wojtusiak J (2008a) Tortricidae from the Mountains of Ecuador. Part III: Western Cordillera (Insecta: Lepidoptera). Genus 19: 497–575.

Razowski J, Wojtusiak J (2008b) Tortricidae (Lepidoptera) from the mountains of Ecuador. Part 1: Southern Highlands. Acta Zoologica Cracoviensia 51B: 7–41. https://doi.org/10.3409/azc.51b_1-2.7-41

Razowski J, Wojtusiak J (2009) Tortricidae (Lepidoptera) from the Mountains of Ecuador and remarks on their geographical distribution. Part IV: Eastern Cordillera. Acta Zoologica Cracoviensia 51B: 119–187. https://doi.org/10.3409/azc.52b_1-2.119-187

Razowski J, Wojtusiak J (2010) Tortricidae (Lepidoptera) from Peru. Acta Zoologica Cracoviensia 53B: 73–159. https://doi.org/10.3409/azc.53b_1-2.73-159

Razowski J, Wojtusiak J (2011) Tortricidae (Lepidoptera) from Colombia. Acta Zoologica Cracoviensia 54B: 103–128. https://doi.org/10.3409/azc.54b_1-2.103-128

Razowski J, Wojtusiak J (2013) Accessions to the fauna of Neotropical Tortricidae (Lepidoptera). Acta Zoologica Cracoviensia 56: 9–40. https://doi.org/10.3409/azc.56_1.09

Vargas HA (2014) Lycaenid caterpillars (Lepidoptera, Lycaenidae) eating flowers of *Dalea pennellii* var. *chilensis* (Fabaceae) in the northern Chilean Andes. Revista Brasileira de Entomologia 58: 309–312. https://doi.org/10.1590/S0085-56262014000300015

Vargas HA (2018) A new species of *Ypsolopha* Latreille (Lepidoptera, Ypsolophidae) from the Andes of northern Chile. Nota Lepidopterologica 41: 199–205. https://doi.org/10.3897/nl.41.28570

Vargas HA, Mundaca EA (2016) First high-altitude record of *Bucculatrix mirnae* Vargas and Moreira (Lepidoptera, Bucculatricidae) on a newly documented host plant: the importance of host plant distribution for conservation on the western slopes of the Andes mountains of northern Chile. Revista Brasileira de Entomologia 60: 356–358. https://doi.org/10.1016/j.rbe.2016.06.007

Vargas-Ortiz M, Vargas HA (2018) A new species of *Strepsicrates* Meyrick (Lepidoptera: Tortricidae) from the Atacama Desert of northern Chile previously misidentified as *S. smithiana* Walsingham. Zootaxa 4370: 569–579. https://doi.org/10.11646/zootaxa.4370.5.8

DNA barcoding of Zygaenidae (Lepidoptera): results and perspectives

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Abstract. The present study provides a DNA barcode library for the world Zygaenidae (Lepidoptera). This study reports 1031 sequence data of the COI gene DNA barcodes for more than 240 species in four of the five subfamilies of the family Zygaenidae. This is about 20% of the world Zygaenidae species. Our results demonstrate the specificity of the COI gene sequences at the species level in most of the studied Zygaenidae and agree with already established taxonomic opinions. The study confirms the effectiveness of DNA barcoding as a tool for determination of most Zygaenidae species. However, some of the results are contradictory. Some cases of shared barcodes have been found, as well as cases of deep intraspecific sequence divergence in species that are well separated by morphological and biological characters. These cases are discussed in detail. Overall, when combined with morphological and biochemical data, as well as biological and ecological observations, DNA barcoding results can be a useful support for taxonomic decisions.

Introduction

Zygaenidae Latreille, 1809, is a family of Lepidoptera well known for the biochemical properties of its species, capable of synthesizing hydrogen cyanide used as a defensive mechanism. Zygaenids, commonly known as burnet, forester and smoky moths, are typically day-flying insects. The family encompasses about 1200 species distributed worldwide, of which several are known as pests. Many species have restricted distributions and represent very sensitive ecological indicators, often used, along with butterflies, as an important umbrella group for ecological evaluations (Nazarov and Efetov 1993; Schmitt 2003; Tarmann 2009).

The taxonomy of these moths is generally well established; it is based on the comparison of morphological characters (habitus and genitalia morphology), but noticeably also in a large part on the integration of biological and ecological characters rarely available (or only available to a lesser extent) in other families of moths. A global taxonomic system was proposed by Alberti (1954, 1958–1959) in a comprehensive revision of the world's fauna. This system has been improved during the past 60 years (Naumann and Tremewan 1984; Tarmann 1984, 2004; Efetov and Tarmann 1999, 2012; Hofmann and Tremewan 1996; Yen 2003), based on an exceptional variety of characters such as larval morphology (the chaetotaxy of larvae (Efetov and Hayashi 2008), microstructures of the integument (Efetov and Tarmann 2017a)), head morphology (including biometry), characters in the structure of the antennae, wings, legs, scales, abdomen (e.g. coremata, lateral 'glands'), special habits of larvae (e.g. leaf mining, boring or free feeding), cocoon construction, special calling and mating habits (Efetov 1996a, 1998a, 1998b, 1999; Efetov and Tarmann 2013b, 2017b; Efetov et al. 2011b; Efetov and Knyazev 2014; Knyazev et al. 2015), pheromones (Subchev et al. 1998, 2013, 2016; Efetov et al. 2010b, 2014b, 2014c, 2015b, 2016, 2018; Razov et al. 2017; Cengiz Can et al. 2018), mimicry, the examination of the karyotypes (Efetov et al. 2004, 2015a), protein electrophoresis results, biochemical analyses combined with the toxicity of the Zygaenidae and the study of antigen properties of haemolymph proteins (monoclonal immunosystematics) (Efetov 2005). This refined system, however, still retains some unsolved questions, especially with respect to phylogenetic relationships. The evolution of the family has been partly investigated over the past decade through the use of molecules, morphology or a combination of both (Efetov 2006, 2012b; Efetov and Savchuk 2009; Efetov et al. 2011a, 2010b, 2014b, 2014c, 2015b, 2016; Subchev et al. 2013, 2016; Efetov and Tarmann 2013b; Mollet 2015), although genetic studies at intraspecific and interspecific levels have been generally limited to species of economic importance (Schmitt and Seitz 2004). The distinction of closely related species often relies on the use of very precise sets of characters and can be highly challenging for non-experts, precluding a broader use of these insects as ecological indicators.

The family Zygaenidae has a worldwide distribution and is divided into five subfamilies: Inouelinae Efetov and Tarmann, 2017 (Oriental), Procridinae Boisduval, 1828 (Holarctic, Afrotropical, Oriental, Australian, Neotropical), Chalcosiinae Walker, 1865 (Palaearctic, Oriental), Callizygaeninae Alberti, 1954 (Oriental), and Zygaeninae Latreille, 1809 (Palaearctic, Oriental, Afrotropical) (Alberti 1954, 1958–1959; Tarmann 1984, 1994, 2004; Efetov and Tarmann 2012, 2017a; Hofmann and Tremewan 1996; Efetov et al. 2014a; Yen 2003).

Several molecular phylogenetic studies of Zygaenidae were published by O. Niehuis et al. (2006a, 2006b, 2007). Selected morphological and biological characters were investigated together with RNA secondary structure variations (Niehuis et al. 2006a) or by the analysis of several mitochondrial and nuclear markers (Niehuis et al. 2006b, 2007). These studies are mainly restricted to species of the subfamily Zygaeninae (genus *Zygaena* Fabricius, 1775) (Niehuis et al. 2006a, 2007). Only a few specimens of Procridinae, Callizygaeninae and Chalcosiinae were included (Niehuis et al. 2006b) while Inouelinae are completely absent.

Our project "DNA barcoding of Zygaenidae moths" (ZYGMO) started in 2009 (Efetov et al. 2010) using the COI gene fragment proposed by Hebert et al. (2003a, 2003b) as a standard DNA barcode (Ratnasingham and Hebert 2007) with the goal to initiate a library of DNA barcode sequences for Zygaenidae species as a new tool for species identification in this family. Moreover, it was expected to confirm known species-complexes and possibly find new ones, as well as so

far overlooked cryptic diversity. Each Zygaenidae barcode record in the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org) (Ratnasingham and Hebert 2007) is accompanied by specimen images, detailed and geo-referenced collection data, complete taxonomic information and voucher repository data. These activities were undertaken in close cooperation between the Crimean Federal University (Crimea), the Tiroler Landesmuseen, Ferdinandeum (Austria) and the Biodiversity Institute of Ontario at the University of Guelph (Canada) under the framework of the "International Barcode of Life" (iBOL) project. There is another, smaller scale project focused on barcoding Zygaenidae and Papilionoidea, but this recent effort was geographically restricted to Switzerland (Litman et al. 2018).

In this paper, we conduct a critical analysis of our Zygaenidae DNA barcoding results. The current taxonomic system for studied species is discussed in light of the relevant results from our sequence analysis combined with traditionally used characters. Some principal remarks on barcoding and taxonomy as well as contradictory results and examples requiring special attention are listed and discussed below.

Materials and methods

Specimen sampling for DNA barcoding

Zygaenidae specimens from all biogeographical regions of the world were provided by scientists from different countries: K. A. Efetov (Russia), G. M. Tarmann (Austria), B. Mollet, E. Drouet and J.-M. Desse (France), O. G. Gorbunov and A. N. Zamesov (Russia), I. G. Pljushtch (Ukraine), Th. Keil (Germany), and others (see acknowledgements). The analysed species are listed in Suppl. material 1: Table S1.

DNA barcodes were obtained by sampling legs from dry specimens or specimens preserved in 96% ethanol in the following institutions: the Crimean Federal University (Crimea), Tiroler Landesmuseen, Ferdinandeum (Austria), Research collection of Bernard Mollet (France), Research collection of Thomas Keil (Germany), Research collection of Eric Drouet (France), Research collection of Jean-Marie Desse (France), Schmalhausen Institute of Zoology (Ukraine), Severtsov Institute of Ecology and Evolution of Russian Academy of Sciences (Russia). Sampling was usually restricted to a few specimens per species, with species coverage as the primary objective, although species with broad distribution ranges were sampled as much as possible from different and distant geographical origins.

All specimens were identified by K. A. Efetov and G. M. Tarmann, and genitalia dissections were carried out when necessary. Taxonomy and nomenclature are based on the most recent publications on the family (Tarmann 2004; Efetov and Tarmann 2012, 2017a; Hofmann and Tremewan 2010).

DNA Analysis

DNA extraction, PCR amplification and DNA sequencing were performed at the Canadian Centre for DNA Barcoding following standard high-throughput protocols (Ivanova et al. 2006; deWaard et al. 2008). All obtained DNA extracts are now stored in Canada.

Data Analysis

All obtained data were processed by the analytical tools available in BOLD 3.0. Sequence divergences were calculated using the Kimura 2 Parameter (K2P) model; the distances to the nearest

neighbour were retrieved using the Barcode Gap analysis. Specimens of 171 species of Procridinae, 24 Chalcosiinae, 1 Callizygaeninae, and 32 Zygaeninae with COI sequence length of more than 550 bp were used for tree construction. The Neighbour Joining ID-Tree was constructed in BOLD under the K2P-model and submitted alignment (see Suppl. material 2).

Data Availability

All sequences analyzed in the paper are available from the BOLD Systems database under the BOLD process ID numbers. BOLD process ID numbers and GenBank accession numbers are listed in Suppl. material 3.

Results

We obtained 1031 COI gene sequences for more than 240 described and undescribed species of Zygaenidae. The library comprises 975 public records from 60 countries, 247 BIN clusters. Complete specimen records, including images, specimen data and voucher information, GPS coordinates, applied primers, sequence and trace files, can be accessed in BOLD public dataset for ZYGMO (http://www.boldsystems.org/index.php/Public_SearchTerms).

Critical analysis of DNA barcode results in the light of traditionally applied species names demonstrates the specificity of sequences of this fragment of the COI gene at species level in the majority of the studied taxa (sequences longer than 550 bp). The mean intraspecific K2P divergence (within species) is 1.36%, interspecific (within genus) 7.44% and intergeneric (within family) 13.91%. So far, many species of Zygaenidae (especially Procridinae) could only be determined by examination of the genitalia structures. We found examples where the study of the COI gene sequence revealed misidentifications for specimens previously determined without dissection. Re-examination of these specimens based on the genitalia structure confirmed the barcoding result.

Several new Zygaenidae species were described during the last years by taking into account ZYGMO molecular data (Efetov 2012a; Efetov and Tarmann 2013a, 2014a, 2014b, 2016a, 2016b; Tarmann and Drouet 2015). In the discussion, as an example, we present in detail the application of COI gene investigation for the identification of *Adscita* (*Procriterna*) *pligori* Efetov, 2012.

Some cases of deep intraspecific sequence divergence as well as low interspecific divergence and shared barcodes within morphologically clearly distinguished species were found. At present, a significant number of the studied Zygaenidae species (nearly 15%) has shown deep intraspecific divergence – of more than 3%. Most of these cases are detected in Procridinae species, for example, in the subgenus *Jordanita* Verity, 1946, of the genus *Jordanita.* The maximum intraspecific distance in *Jordanita* (*Jordanita*) *graeca* (Jordan, 1907) is 5.72%, and in *Jordanita* (*Jordanita*) *chloros* (Hübner, 1813) it is 6.08%. On the other hand, the range of interspecific distance in this subgenus is very low, viz. 0.30–0.61% with barcode-sharing in morphologically well-separated species. Hence, the species of the subgenus *Jordanita* cannot be separated solely on the basis of DNA barcode data. Possible reasons for these results are discussed below.

An interesting result is that *Zygaenoprocris khorassana* (Alberti, 1939) is a distinct species from *Z. chalcochlora* Hampson, 1900, contrary to Efetov and Tarmann (1994), who had synonymized the two, and in support of Efetov and Tarmann (2012), who had reinstated *Z. khorassana* as a valid species.

Not long ago two new *Zygaenoprocris* species of the *chalcochlora*-group (*Z.* (*Z.*) *efetovi* Mollet & Tarmann, 2007, and *Z.* (*Z.*) *hofmanni* Mollet & Tarmann, 2007) were described, based on a different habitus to that of *Z.* (*Z.*) *chalcochlora* (the absence of shiny metallic scales) and slight differences in genitalia structures including larger papillae anales and shorter apophyses posteriores. The results of this study support these as distinct species.

Our DNA studies support high values of interspecific distances between species of the subgenus *Zygaenoprocris* with the mean distance being equal to 7.27%. Moreover, DNA barcode data show that *Z.* (*Z.*) *chalcochlora* possibly represents a species complex. The populations of *Z. khorassana* (with shiny metallic scales) in northern Iran are isolated from the populations of *Z. chalcochlora* in Pakistan (including the type locality of *Z. chalcochlora*) and Afghanistan (all specimens with small papillae anales and long apophyses posteriores). This information suggests that the above-mentioned characters of the papillae anales are much more important than we thought earlier.

The distance between specimens within one species sometimes exceeds the mean interspecific distance (1.36%). For example, we showed that all studied specimens of *Zygaenoprocris* (*Molletia*) *duskei* (Grum-Grshimailo, 1902) form one lineage on the ID Tree with the distance to nearest neighbour – *Z.* (*M.*) *taftana* (Alberti, 1939) – 4.74%, and to *Z.* (*M.*) *persepolis* (Alberti, 1938) – 5.72%. The distance between different specimens of *Z.* (*M.*) *duskei kliri* Keil, 2002, (Esfahan, Markazi) is 0.17%, the distance between specimens of the *Z.* (*M.*) *duskei kermana* (Alberti, 1967) (Kerman, Hormozgan) is 1.08%, while the distance between specimens of *Z.* (*M.*) *duskei duskei* (Grum-Grshimailo, 1902) (Sistan-e Baluchestan) is 3.93%.

It was known that the genus *Illiberis* (*sensu lato*) is a polyphyletic unit (Efetov 1996b, 1997, 1998a, 2010). The taxonomic status of some species-groups of this genus was revised (Efetov and Tarmann 2012). For example, the subgenera *Hedina* Alberti, 1954, and *Zama* Herrich-Schäffer, 1855, were raised to the generic level (Efetov and Tarmann 2012). This decision is supported by our DNA results (Fig. 1).

Some interesting findings were also made in the subfamily Zygaeninae. Since 2014 more than 80 COI sequences of specimens belonging to the *minos-purpuralis* complex from different localities have been studied. The COI results showed that the distances between *Zygaena* (*Mesembrynus*) *minos persica* Burgeff, 1926, from Iran and other barcode-sharing *minos-purpuralis* specimens are significant: 4.92% and 4.75% to the nearest *Z.* (*M.*) *minos* ([Denis and Schiffermüller], 1775) and *Z.* (*M.*) *purpuralis* (Brünnich, 1763) respectively. This is 2.5 times the distance of both *Z.* (*M.*) *minos* and *Z.* (*M.*) *purpuralis* to their nearest neighbour *Z.* (*M.*) *erythrus* (Hübner, 1806), which is 1.86%. The deep COI gene divergence of *Z.* (*M.*) *minos persica* supports the opinion that it may be a separate species (Nahirnić and Tarmann 2014).

Some other cases of barcode sharing (e.g. *Chalcosia pectinicornis* (Linnaeus, 1758) / *Ch. phalaenaria* (Guérin-Méneville, 1843); *Zygaena* (*Mesembrynus*) *tamara* Christoph, 1889 / *Z.* (*M.*) *cuvieri* Boisduval, 1828) and deep divergences (e. g. *Zygaenoprocris* (*Molletia*) *persepolis* (Alberti, 1938), *Jordanita* (*Roccia*) *paupera* (Christoph, 1887), some species of the subgenera *Tremewania* Efetov & Tarmann, 1999, and *Jordanita* of the genus *Jordanita*) were detected. Deep intraspecific divergences were also seen in *Myrtartona rufiventris* (Walker, 1854), *Illiberis* (*Alterasvenia*) *ochracea* Leech, 1898, *Zygaenoprocris* (*Zygaenoprocris*) *chalcochlora*, *Triprocris cyanea* Barnes & McDunnough, 1910, *Gynautocera papilionaria* Guérin-Méneville, 1831,
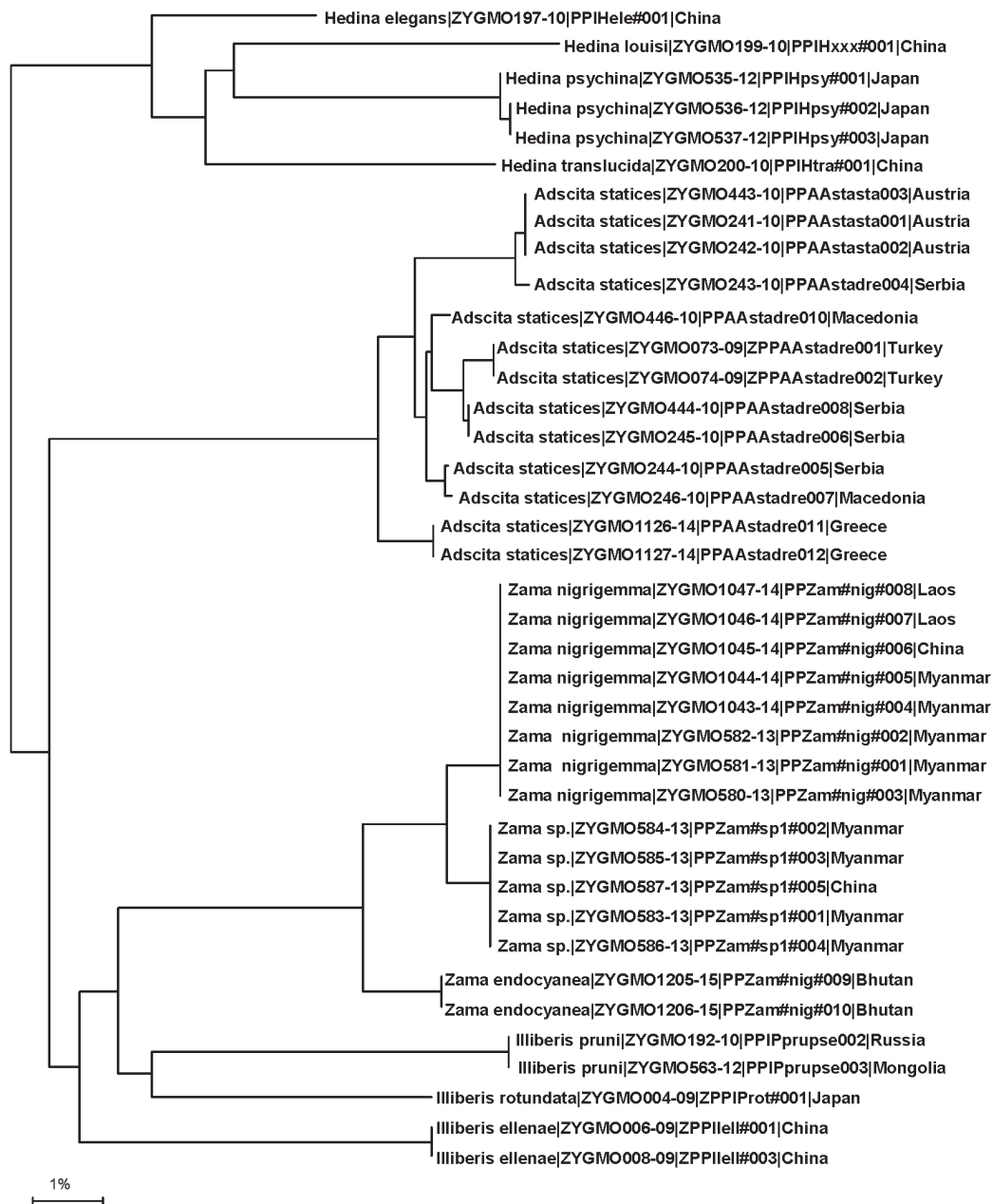
Figure 1. BOLD TaxonID Tree for some representatives of the genera *Zama*, *Illiberis*, *Hedina*, and *Adscita* (K2P, COI-5P, length > 550).

Zygaena (*Agrumenia*) *cocandica* Erschoff, 1874, *Z.* (*Zygaena*) *filipendulae* (Linnaeus, 1758), *Z.* (*Z.*) *lonicerae* (Scheven, 1777). In these cases futher investigations including appropriate genetic markers are necessary.

Discussion

Species-subspecies resolution

We conclude that based on the DNA barcode data only it is not possible to decide whether a population is represented by subspecies or species. For example, a comparison of the COI 658-bp barcode region of *Zygaena* (*Zygaena*) *transalpina transalpina* (Esper, 1780) (Trentino-Alto Adige) and *Z.* (*Z.*) *transalpina xanthographa* Germar, 1836 (Basilicata) from Italy demonstrates their genetic isolation. According to DNA data the studied specimens of *Z.* (*Z.*) *transalpina transalpina* (the maximum pairwise distance between specimens of this subspecies is 0.77%) are separated from the lineage of *Z.* (*Z.*) *transalpina xanthographa* specimens (the maximum pairwise distance between specimens of this subspecies is 0.76%). The range of pairwise distances between specimens of these two subspecies is 1.07–1.85%. This corresponds with values at intraspecific level.

Deep intraspecific divergence

Deep intraspecific as well as deep intrageneric divergence can sometimes be attributed to imperfections within the existing classification. Often in putative species complexes only one properly described species exists and sometimes the nominal genus may represent a genus complex. The above-mentioned situation in the genus *Zygaenoprocris* had been solved only in part after barcoding of specimens of known and newly described species and some taxonomic changes. According to Gap analysis at the current time in the genus *Zygaenoprocris* the nearest neighbour distance range is 2.47–7.55% (mean – 3.99%). The maximum intraspecific divergence in the studied specimens is represented by *Z.* (*Z.*) *chalcochlora* – 6.23%. At least several isolated populations known from central and southern Iran apparently belong to this *Zygaenoprocris chalcochlora* species complex; their taxonomic status must be clarified following further investigations.

The deep intraspecific divergence mentioned above in *J.* (*J.*) *graeca* and *J.* (*J.*) *chloros* represents a completely different situation. The COI sequence divergence in other species of the subgenus *Jordanita* is not so deep: the maximum intraspecific distance in *Jordanita* (*Jordanita*) *globulariae* (Hübner, 1793) is 1.86%, in *Jordanita* (*Jordanita*) *tenuicornis* (Zeller, 1847) it is 1.58%. As we obtained only singleton barcodes of *Jordanita* (*Jordanita*) *vartianae* (Malicky, 1961) and of *Jordanita* (*Jordanita*) *syriaca* (Alberti, 1937) it is not possible to discuss intraspecific distances for these two species. Further molecular genetic investigations are required to clarify this situation and should include appropriate genetic markers. It should be noted that all species of the subgenus *Jordanita* are well separated on the base of good differences in morphology of preimaginal stages and adults (including genitalia structure).

Disjunct mountain populations of the same species can show remarkably large divergence in COI gene sequences. A good example is provided by specimens of *Adscita* (*Procriterna*) *subdolosa* (Staudinger, 1887) from different mountain ranges in Central Asia with a maximum intraspecific distance of 4.24%.

Cases of low interspecific divergence

More than 25% of the studied species have a distance to the nearest neighbour of 2% or less (and for nearly 20% of species the distance is less than 1.0%). One of the possible explanations could be that these groups of species are evolutionarily young.

A complicated situation has been observed in the Australian genus *Pollanisus* Walker, 1854. According to DNA data, species of this genus have very low interspecific distances. However, the peculiarities of biology confirm high species diversity within the genus. These results show clearly that systematic revisions always have to be based on several sets of characters (molecular, morphological and biological).

Geographical factors and genetic divergences

During COI data analysis geographical factors can be taken into account. A good example is provided by the study of different populations of *Jordanita* (*Solaniterna*) *subsolana* (Staudinger, 1862). When comparing specimens from southern Italy, Macedonia, Turkey, Armenia, Crimea and Ukraine we found barcode similarity between Crimean, Turkish, southern Italian and Macedonian populations, while the Armenian and Ukrainian populations form an isolated group. This may be a result of populations from different geographical regions invading their present habitats at different times from different refugia.

Geographical isolation can cause large differences in COI sequences. The African species *Adscita* (*Adscita*) *mauretanica* (Naufock, 1932) is situated separately on the barcode ID tree and is isolated from all other species of the genus *Adscita* Retzius, 1783 (inhabiting Europe and Asia) with which it shares morphological and biological characters. According to Gap analysis only for specimens of the genus *Jordanita* and *A.* (*A.*) *mauretanica* the nearest neighbour for the latter is *J.* (*Roccia*) *budensis* (Speyer & Speyer, 1858), with a distance of 8.15%, while the maximum nearest neighbour distance within the genus *Jordanita* is 6.27%. Gap analysis of specimens of the genus *Adscita* only shows that the distance from *A.* (*A.*) *mauretanica* to its nearest neighbour *A.* (*Tarmannita*) *mannii* (Lederer, 1853) is 7.58%.

Another example is also found in the genus *Adscita*. Specimens of *Adscita* (*Adscita*) *geryon* (Hübner, 1813) from the Balkans and southern Italy show a greater distance from all other *A.* (*A.*) *geryon* populations of Europe (including Crimea) than Crimean *A.* (*A.*) *geryon* to Crimean *A.* (*A.*) *albanica* (Naufock, 1926) (a species that is morphologically and biologically well separated from *A.* (*A.*) *geryon*). The similarity of COI sequences between Crimean specimens of these two species allows us to consider the possibility of horizontal gene transfer.

DNA barcoding and the discovery of new species

DNA barcoding as an additional tool can support description of new species. For example, on two different dates (27.vi.2009 and 8.vii.2009) four specimens of *Adscita* (*Procriterna*) (three males, one female) were collected in two different localities in Afghanistan. The males have differences in the genitalia structure (the number of cornuti varied from three to five). The questions were: (1) Do the males and the female belong to one species? (2) Do males with a different number of cornuti collected in different localities and on different days belong to one species? DNA investigation was undertaken to show whether these specimens are conspecific. The DNA barcoding results clearly showed 100% similarity of COI sequences of male and the female specimens from both localities and also good differences (4.91%) from the nearest *Adscita* (*Procriterna*) species, viz. *Adscita* (*Procriterna*) *subdolosa* (Staudinger, 1887). This result allowed us to conclude that the female was conspecific with the males. This female also shows significant morphologic differences in genitalia from *A.* (*P.*) *subdolosa*. After having obtained these results, all the above-mentioned specimens have been included in the type series of *Adscita* (*Procriterna*) *pligori* Efetov, 2012 (Efetov 2012a).

Conclusions

Patterns of DNA barcode variation were examined in more than 240 species of the family Zygaenidae within the project "DNA barcoding of Zygaenidae moths" (ZYGMO), resulting in a DNA identification library available for most of the studied species. However, despite our efforts the major part of the Zygaenidae fauna is waiting to be DNA barcoded. As using DNA barcodes has proved itself as a quick and economical method for biodiversity investigations in this family, it is worthwhile to continue this effort.

Nevertheless, a significant number of cases of deep intraspecific sequence divergence as well as low interspecific divergence and shared barcodes within morphologically clearly separable Zygaenidae species were found. Further investigations within problematic groups should be undertaken, focused on the study of additional molecular markers.

The analysis of COI sequences provides additional data for a careful re-consideration of previously made decisions in Zygaenidae biogeography, systematics and taxonomy. Moreover, our results show that DNA barcoding results should always be discussed in combination with other data, viz. morphological, biological, ecological, biochemical etc.

References

Alberti B (1954) Über die stammesgeschichtliche Gliederung der Zygaenidae nebst Revision einiger Gruppen (Insecta, Lepidoptera). Mitteilungen aus dem Zoologischen Museum der Humboldt-Universität Berlin 30: 115–480. https://doi.org/10.1002/mmnz.19540300202

Alberti B (1958–1959) Über den stammesgeschichtlichen Aufbau der Gattung *Zygaena* F. und ihrer Vorstufen (Insecta, Lepidoptera). Mitteilungen aus dem Zoologischen Museum der Humboldt-Universität Berlin 34: 245–396 (1958); 203–242 (1959). https://doi.org/10.1002/mmnz.4830350111

Cengiz Can F, Efetov KA, Kaya K, Kucherenko EE, Okyar Z, Tarmann GM (2018) Zygaenidae (Lepidoptera) of Thrace Region of Turkey. Nota Lepidopterologica 41(1): 23–36. https://doi.org/10.3897/nl.41.21065

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA barcodes: analytical protocols. In: Martin C (Ed.) Methods in molecular biology: environmental genetics. Totowa, NJ: Humana Press, 275–293. https://doi.org/10.1007/978-1-59745-548-0_15

Efetov KA (1996a) The description of the female of *Adscita* (*Zygaenoprocris*) *rjabovi* (Alberti, 1938) (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 47(1): 31–35.

Efetov KA (1996b) The description of the female of *Illiberis* (*Alterasvenia*) *yuennanensis* Alberti, 1951 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 47(2): 111–113.

Efetov KA (1997) Three new species of the genus *Illiberis* Walker, 1854, from Taiwan and Vietnam (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 48(4): 231–244.

Efetov KA (1998a) A revision of the genus *Goe* Hampson, [1893] (Lepidoptera: Zygaenidae, Procridinae), with descriptions of two new species. Entomologist's Gazette 49(1): 49–62.

Efetov KA (1998b) *Jordanita* (*Jordanita*) *fazekasi* sp. n. from southern Hungary (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 49(3): 183–187.

Efetov KA (1999) *Inouela* gen. n. from Japan and Taiwan (Lepidoptera: Zygaenidae, Chalcosiinae). Entomologist's Gazette 50(2): 91–95.

Efetov KA (2005) The Zygaenidae (Lepidoptera) of the Crimea and other regions of Eurasia. CSMU Press, Simferopol, 420 pp.

Efetov KA (2006) Nine new species of the genus *Chrysartona* Swinhoe, 1892 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 57(1): 23–50.

Efetov KA (2010) *Illiberis* (*Hedina*) *louisi* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) from China. Entomologist's Gazette 61(4): 235–241.

Efetov KA (2012a) *Adscita* (*Procriterna*) *pligori* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) from Afghanistan. Entomologist's Gazette 63(2): 99–105.

Efetov KA (2012b) On the type series of *Zygaena* (*Agrumenia*) *ferganae* Sheljuzhko, 1941 (Lepidoptera: Zygaenidae, Zygaeninae). Entomologist's Gazette 63(4): 241–245.

Efetov KA, Can F, Toshova TB, Subchev M (2010b) New sex attractant for *Jordanita anatolica* (Naufock) (Lepidoptera: Zygaenidae: Procridinae). Acta Zoologica Bulgarica 62(2): 315–319.

Efetov KA, Hayashi E (2008) On the chaetotaxy of the first instar larva of *Artona martini* Efetov, 1997 (Lepidoptera: Zygaenidae, Procridinae, Artonini). Entomologist's Gazette 59(2): 101–104.

Efetov KA, Hofmann A, Tarmann GM (2014b) Application of two molecular approaches (use of sex attractants and DNA barcoding) allowed to rediscover *Zygaenoprocris eberti* (Alberti, 1968) (Lepidoptera, Zygaenidae, Procridinae), hitherto known only from the female holotype. Nota Lepidopterologica 37(2): 151–160. https://doi.org/10.3897/nl.37.7871

Efetov KA, Hofmann A, Tarmann GM, Tremewan WG (2014a) Taxonomic comments on the treatment of the Zygaenidae (Lepidoptera) in volume 3 of *Moths of Europe*, Zygaenids, Pyralids 1 and Brachodids (2012). Nota Lepidopterologica 37(2): 123–133. https://doi.org/10.3897/nl.37.7940

Efetov KA, Kirsanova AV, Lazareva ZS, Parshkova EV, Tarmann GM, Rougerie R (2010a) Early results in DNA barcoding of Zygaenidae (Lepidoptera). In: Can F (Ed.) XII International Symposium on Zygaenidae. Hatay: Mustafa Kemal University, 7–8.

Efetov KA, Knyazev SA (2014) New records of *Jordanita* (*Roccia*) *volgensis* (Möschler, 1862) (Lepidoptera: Zygaenidae, Procridinae) from Siberia (Russia) and Ukraine. Entomologist's Gazette 65(3): 175–178.

Efetov KA, Koshio C, Kucherenko EE (2018) A new synthetic sex attractant for males of *Illiberis* (*Primilliberis*) *pruni* Dyar, 1905 (Lepidoptera: Zygaenidae, Procridinae). SHILAP Revista de Lepidopterología. 46(182): 263–270.

Efetov KA, Kucherenko EE, Parshkova EV, Tarmann GM (2016) 2-butyl 2-dodecenoate, a new sex attractant for *Jordanita* (*Tremewania*) *notata* (Zeller, 1847) and some other Procridinae species (Lepidoptera: Zygaenidae). SHILAP Revista de Lepidopterología 44(175): 519–527.

Efetov KA, Parshkova EV, Baevsky MY, Poddubov AI (2014c) Sec-butyl ester of dodecenoate: synthesis and attractive properties. The Ukrainian Biochemical Journal 86(6): 175–182. https://doi.org/10.15407/ubj86.06.175

Efetov KA, Parshkova EV, Koshio C (2004) The karyotype of *Illiberis* (*Primilliberis*) *rotundata* Jordan, [1907] (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 55(3): 167–170.

Efetov KA, Parshkova EV, Tarasova LG, Tarmann GM (2015a) The karyotypes of Procridinae (Lepidoptera: Zygaenidae), with the first record of the karyotype of *Pollanisus commoni* Tarmann, 2004, a representative of the tribe Artonini. Entomologist's Gazette 66(2): 121–125.

Efetov KA, Savchuk VV (2009) The first record of *Jordanita* (*Roccia*) *volgensis* (Möschler, 1862) (Lepidoptera: Zygaenidae, Procridinae) from the Crimea. Entomologist's Gazette 60(3): 155–158.

Efetov KA, Subchev MA, Toshova TB, Kiselev VM (2011a) Attraction of *Zygaenoprocris taftana* (Alberti, 1939) and *Jordanita horni* (Alberti, 1937) (Lepidoptera: Zygaenidae, Procridinae) by synthetic sex pheromones in Armenia. Entomologist's Gazette 62(2): 113–121.

Efetov KA, Tarmann GM (1994) A revision of *Zygaenoprocris* Hampson, 1900, a subgenus of *Adscita* Retzius, 1783 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 45(2): 81–105.

Efetov KA, Tarmann GM (1999) On the systematic position of *Procris fusca* Leech, [1889] (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 50(3): 163–168.

Efetov KA, Tarmann GM (2012) A Checklist of the Palaearctic Procridinae (Lepidoptera: Zygaenidae). CSMU Press, Simferopol and Innsbruck, 108 pp.

Efetov KA, Tarmann GM (2013a) *Illiberis* (*Alterasvenia*) *cernyi* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) from northern Thailand. Entomologist's Gazette 64(1): 33–39.

Efetov KA, Tarmann GM (2013b) *Chrysartona* (*Chrystarmanna*) *mineti* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) from northern Vietnam. Entomologist's Gazette 64(3): 197–206.

Efetov KA, Tarmann GM (2014a) *Illiberis* (*Alterasvenia*) *banmauka* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) from China and Myanmar. Entomologist's Gazette 65(1): 62–70.

Efetov KA, Tarmann GM (2014b) A new European species, *Adscita dujardini* sp. nov. (Lepidoptera: Zygaenidae, Procridinae) confirmed by DNA analysis. Entomologist's Gazette 65(3): 179–200.

Efetov KA, Tarmann GM (2016b) A new *Illiberis* species: *I.* (*Alterasvenia*) *kislovskyi* (Lepidoptera: Zygaenidae, Procridinae) from Myanmar. Entomologist's Gazette 67(2): 137–142.

Efetov KA, Tarmann GM (2016a) *Pseudophacusa multidentata* Efetov & Tarmann, a new genus and species of Procridini from Myanmar, China and Laos (Lepidoptera: Zygaenidae, Procridinae). SHILAP Revista de Lepidopterología 44(173): 81–89. http://www.redalyc.org/articulo.oa?id=45545991011

Efetov KA, Tarmann GM (2017a) The hypothetical ground plan of the Zygaenidae, with a review of the possible autapomorphies of the Procridinae and the description of the Inouelinae subfam. nov. Journal of the Lepidopterists' Society 71(1): 20–49. https://doi.org/10.18473/lepi.v71i1.a5

Efetov KA, Tarmann GM (2017b) *Thibetana keili* Efetov & Tarmann, a new species of the genus *Thibetana* Efetov & Tarmann, 1995, from Tibet (Lepidoptera: Zygaenidae, Procridinae, Artonini). SHILAP Revista de Lepidopterología 45(180): 581–587. http://www.redalyc.org/articulo.oa?id=45553890006

Efetov KA, Tarmann GM, Toshova TB, Subchev MA (2015b) Enantiomers of 2-butyl 7*Z*-dodecenoate are sex attractants for males of *Adscita mannii* (Lederer, 1853), *A. geryon* (Hübner, 1813), and *Jordanita notata* (Zeller, 1847) (Lepidoptera: Zygaenidae, Procridinae) in Italy. Nota Lepidopterologica 38(2): 161–169. https://doi.org/10.3897/nl.38.6312

Efetov KA, Tarmann GM, Tremewan WG (2011b) *Zygaena nevadensis* Rambur, 1858 (Lepidoptera: Zygaenidae, Zygaeninae) newly recorded from the southern tip of the Penisola Appenninica (Apennine Peninsula), Italy. Entomologist's Gazette 62(2): 123–129.

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

Hebert PDN, Ratnasingham S, deWaard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. Series B, Biological Sciences 270: 596–599. https://doi.org/10.1098/rsbl.2003.0025

Hofmann A, Tremewan WG (1996) A systematic catalogue of the Zygaeninae (Lepidoptera: Zygaenidae). Harley Books, Colchester, 251 pp.

Hofmann A, Tremewan WG (2010) A revised check-list of the genus *Zygaena* Fabricius, 1775 (Lepidoptera: Zygaenidae, Zygaeninae), based on the biospecies concept. Entomologist's Gazette 61: 119–131.

Ivanova NV, deWaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. Molecular Ecology Notes 6: 998–1002. https://doi.org/10.1111/j.1471-8286.2006.01428.x

Knyazev SA, Efetov KA, Ponomaryov KB (2015) Zygaenidae (Lepidoptera) from Omsk Region. Zoologicheskiy Zhurnal 94 (11): 1297–1302.

Litman J, Chittaro Y, Birrer S, Praz C, Wermeille E, Fluri M, Schmid S, Wyler S, Gonseth Y (2018) A DNA barcode reference library for Swiss butterflies and forester moths as a tool for species identification, systematics and conservation. PLoS ONE 13(12): e0208639. https://doi.org/10.1371/journal.pone.0208639

Mollet B (2015) Two new species of the genus *Illiberis* from China (Lepidoptera Zygaenidae Procridinae). Antenor 2 (2): 224–231.

Nahirnić A, Tarmann GM (2014) The *Zygaena purpuralis* complex. A new approach to solve a complicated problem. XIV International Symposium on Zygaenidae. Tobermory Isle of Mull, Scotland, 13–14.

Naumann CM, Tremewan WG (1984) Das Biospecies-Konzept in seiner Anwendung auf die Gattung *Zygaena* Fabricius, 1775 (Insecta, Lepidoptera, Zygaenidae). Spixiana 7: 161–193.

Nazarov VV, Efetov KA (1993) On the role of Zygaenidae (Lepidoptera) in pollination of *Anacamptis pyramidalis* (Orchidaceae). Zoologichesky Zhurnal 72(10): 54–67.

Niehuis O, Naumann CM, Misof B (2006a) Phylogenetic analysis of Zygaenoidea small-subunit rRNA structural variation implies initial oligophagy on cyanogenic host plants in larvae of the moth genus *Zygaena* (Insecta: Lepidoptera). Zoological Journal of the Linnean Society 147 (3): 367–381. https://doi.org/10.1111/j.1096-3642.2006.00222.x

Niehuis O, Yen SH, Naumann CM, Misof B (2006b) Higher phylogeny of zygaenid moths (Insecta: Lepidoptera) inferred from nuclear and mitochondrial sequence data and the evolution of larval cuticular cavities for chemical defence. Molecular Phylogenetics and Evolution 39: 812–829. https://doi.org/10.1016/j.ympev.2006.01.007

Niehuis O, Hofmann A, Naumann CM, Misof B (2007) Evolutionary history of the burnet moth genus *Zygaena* Fabricius, 1775 (Lepidoptera: Zygaenidae) inferred from nuclear and mitochondrial sequence data: phylogeny, host-plant association, wing pattern evolution and historical biogeography. The Linnean Society of London, Biological Journal of the Linnean Society 92: 501–520. https://doi.org/10.1111/j.1095-8312.2007.00858.x

Ratnasingham S, Hebert PDN (2007) The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471–8286.2007.01678.x

Razov J, Efetov KA, Franin K, Toshova TB, Subchev M (2017) The application of sex pheromone traps for recording the Procridinae fauna (Lepidoptera: Zygaenidae) in Croatia. Entomologist's Gazette 68: 48–53.

Schmitt T (2003) Influence of forest and grassland management on the diversity and conservation of butterflies and burnet moths (Lepidoptera, Papilionoidea, Hesperiidae, Zygaenidae). Animal Biodiversity and Conservation 26(2): 51–67.

Schmitt T, Seitz A (2004) Low diversity but high differentiation: the population genetics of *Aglaope infausta* (Zygaenidae: Lepidoptera). Journal of Biogeography 31(1): 137–144. https://doi.org/10.1046/j.0305-0270.2003.01003.x

Subchev MA, Efetov KA, Toshova TB, Koshio C (2016) Sex pheromones as isolating mechanisms in two closely related *Illiberis* species – *I.* (*Primilliberis*) *rotundata* Jordan, 1907, and *I.* (*P.*) *pruni* Dyar, 1905 (Lepidoptera: Zygaenidae, Procridinae). Entomologist's Gazette 67(1): 51–57.

Subchev M, Harizanov A, Francke W, Franke S, Plass E, Reckziegel A, Schröder F, Pickett JA, Wadhams LJ, Woodcock CM (1998) Sex pheromone of female vine bud moth, *Theresimima ampellophaga* comprises (2*S*)-butyl (7*Z*)-tetradecenoate. Journal of Chemical Ecology 24(7): 1141–1151; Journal of Chemical Ecology 25(5): 1203; erratum, i.e. corrected to (2*R*)-butyl (7*Z*)-tetradecenoate. https://doi.org/10.1023/A:1022438717287 [erratum 1999]

Subchev M, Koshio C, Toshova T, Efetov KA, Francke W (2013) (2*R*)-butyl (7*Z*)-dodecenoate, a main sex pheromone component of *Illiberis* (*Primilliberis*) *pruni* Dyar (Lepidoptera: Zygaenidae: Procridinae)? Acta Zoologica Bulgarica 65: 391–396.

Tarmann G (1984) Generische Revision der amerikanischen Zygaenidae mit Beschreibung neuer Gattungen und Arten (Insecta: Lepidoptera). Entomofauna (s2, v1, s2, v2)1: 176 p. 2: 153 p.

Tarmann GM (1994) A preliminary review of the classification of the zygaenid subfamily Procridinae (Lepidoptera). Nota Lepidopterologica. Suppl. 5: 115–123.

Tarmann GM (2004) Zygaenid moths of Australia: A revision of the Australian Zygaenidae (Procridinae: Artonini). CSIRO Publishing, Collingwood, 248 p. https://doi.org/10.1071/9780643092198

Tarmann GM (2009) Die Vinschger Trockenrasen - ein Zustandsbericht auf Basis der Bioindikatoren Tagfalter und Widderchen (Lepidoptera: Rhopalocera, Zygaenidae). Wissenschaftliches Jahrbuch der Tiroler Landesmuseen 2: 306–350.

Tarmann GM, Drouet E (2015) Zygaenidae from French Guiana, with a key to the narrow-winged Zygaenidae genera of the New World. Journal of the Lepidopterists' Society 69(3): 209–235. https://doi.org/10.18473/lepi.69i3.a8

Yen SH (2003) Phylogeny and systematics of the major lineages of Chalcosiinae s.l. (Zygaenidae s.l.): preliminary observations on morphological characters. In: Efetov KA, Tremewan WG, Tarmann GM (Eds) Proceedings of the 7th International Symposium on Zygaenidae (Lepidoptera). CSMU Press, Simferopol, 293–348.

Supplementary material 1

Table S1. List of analyzed species, sample size

Authors: Konstantin A. Efetov, Anna V. Kirsanova, Zoya S. Lazareva, Ekaterina V. Parshkova, Gerhard M. Tarmann, Rodolphe Rougerie, Paul D. N. Hebert

Data type: species data

Explanation note: List of analyzed species, sample size (mfl = maximum fragment length (number of base pairs), n = number of analyzed sequences) and origin of mentioned material.

Link: https://doi.org/10.3897/nl.42.33190.suppl1

Supplementary material 2

BOLD TaxonID Tree: DNA barcoding of Zygaenidae moths [ZYGMO]

Authors: Konstantin A. Efetov, Anna V. Kirsanova, Zoya S. Lazareva, Ekaterina V. Parshkova, Gerhard M. Tarmann, Rodolphe Rougerie, Paul D. N. Hebert

Data type: species data

Link: https://doi.org/10.3897/nl.42.33190.suppl2

Supplementary material 3

List of specimens-ID (from BOLD database) and GenBank accession numbers of the analyzed Zygaenidae vouchers

Authors: Konstantin A. Efetov, Anna V. Kirsanova, Zoya S. Lazareva, Ekaterina V. Parshkova, Gerhard M. Tarmann, Rodolphe Rougerie, Paul D. N. Hebert

Data type: species data

Link: https://doi.org/10.3897/nl.42.33190.suppl3

Two new native larval host plants of *Hyles annei* (Guérin-Méneville, 1839) (Lepidoptera, Sphingidae) in the Atacama Desert of northern Chile following exceptional summer rainfall

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http://zoobank.org/26D14CCA-E628-45ED-9343-3B1E7B53F4E5

Abstract. Adults of the little-known Neotropical hawkmoth, *Hyles annei* (Guérin-Méneville, 1839) (Lepidoptera: Sphingidae: Macroglossinae: Macroglossini), were reared from larvae collected on *Allionia incarnata* L. (Nyctaginaceae) and *Fagonia chilensis* Hook. & Arn. (Zygophyllaceae) at about 1900 m elevation in the Atacama Desert of northern Chile. The two plants were growing after exceptionally high summer rainfall as part of a blooming desert event. These findings provide a clear example of the ability of this hawkmoth to use ephemeral and unpredictable resources in a hyperarid environment.

Introduction

The Atacama is the most arid desert in the world (Clarke 2006). As a result, many ephemeral herbaceous plants and some shrubs living there can display unusual and explosive development and flowering in years when there is exceptionally high accumulated rainfall, a biological phenomenon known as a "blooming desert" (Chávez et al. 2019). In the Atacama, this amazing event mostly occurs in the southern regions following the autumn-winter rainfall. In contrast, only one blooming desert has been reported from the northern Atacama, which started with the summer rainfall in February 2012 (Chávez et al. 2019). Consequently, blooming desert is currently poorly characterized and understood in the northern Atacama. However, some phytophagous insects would be expected to be able to use the abundant plant resources that suddenly become available in these events.

The Neotropical hawkmoth, *Hyles annei* (Guérin-Méneville, 1839) (Lepidoptera: Sphingidae: Macroglossinae: Macroglossini), is known from Argentina, Bolivia, Chile and Peru (Ureta and Donoso 1956; Haxaire and Herbin 1999; Cock and Boos 2006; Moré et al. 2014), where it has been found in a wide range of habitats from sea level to more than 2000 m elevation (Hundsdoerfer et al. 2009; Juárez and González 2016). It is a polyphagous species (Hundsdoerfer et al. 2017); however, its native host plant range has not been studied in detail in most of its geographic distribution. In Chile, Butler (1882) recorded "*Oxybaptrus parviflorus*", probably involving one of the eight species of *Mirabilis* Riv. ex L. (Nyctaginaceae) of the central part of this country (see Rodríguez et al. 2018), and González et al. (1973) recorded the exotic cultivated grape (*Vitis vinifera* L., Vitaceae), upon which the larvae may be voracious herbivores, and because of which *H. annei* is locally

known as the "monroy de la vid" (grape hawkmoth) (Artigas 1994). Chilean agricultural literature frequently refers to *H. annei* as a minor pest of some other exotic crops, and a few other sources (mainly internet pages) suggest the use of some native plants. However, all these records require confirmation, because they are based on recording only the presence of larvae on plants without subsequent rearing to obtain adults for identification. Detailed and accurate knowledge of the native host plant range of *H. annei* is necessary to understand its biology in natural habitats. This is an especially interesting issue in the hyperarid environments that this hawkmoth inhabits in northern Chile, where food availability can be remarkably unpredictable. The aim of this contribution is to provide the first confirmed records of native host plants of *H. annei* in northern Chile based on fieldwork undertaken in a blooming desert in northern Atacama.

Observations

In March 2019, hawkmoth larvae were found feeding on *Allionia incarnata* L. (Nyctaginaceae) and *Fagonia chilensis* Hook. & Arn. (Zygophyllaceae) at about 1900 m elevation in the lowest part of the Cardones Ravine, km 63 of 11-CH highway, Arica Province, northern Chile (Figs 1–5). The site is near the western limit of the narrow area in which desert blooming occurs in the northern Atacama, where the local rainfall is complemented by abundant ephemeral surface watercourses whose flow is derived from rainfall on the western slopes of the Andes. A few larvae were collected from each plant and brought into the laboratory in plastic vials to rear to adult to identify the species. Additional leaves of the respective plants were provided daily to the larvae until they finished feeding and pupated. The plastic vials were observed daily until adult emergence. Voucher specimens are deposited in the "Colección Entomológica de la Universidad de Tarapacá" (IDEA), Arica, Chile.

Seven adults were obtained, two females and two males from larvae on *A. incarnata* and three males on *F. chilensis*, all of which were identified as *H. annei* (Figs 6–8) based on comparison with figures provided by Kitching (2019). These results provide a clear example of the ability of this hawkmoth to use ephemeral and unpredictable resources, which is a great advantage for inhabiting the hyperarid environments of the Atacama Desert.

Discussion

Moré et al. (2014) mentioned Polygonaceae and Vitaceae as host plants of *H. annei*, although they indicated that each of these records was based on a single observation. Hundsdoerfer et al. (2019) reared *H. annei* larvae on *Chamerion angustifolium* (L.) Holub (Onagraceae) and on European natural hybrids of the genus *Epilobium* L. in captivity, and experimentally found the species retained a limited capacity to metabolise spurge (*Euphorbia* L., Euphorbiaceae) toxins. Representatives of Nyctaginaceae are consumed by larvae of several hawkmoth species (Robinson et al. 2010), including *H. livornicoides* (Lucas, 1892) in Australia (*Boerhavia diffusa*; Moulds 1981). Furthermore, *A. incarnata* is a host plant of *H. lineata* (Fabricius, 1775) (Felger et al. 2003) and *H. euphorbiarum* (Guérin-Méneville & Percheron, 1835) (Cates 1981). Although feeding on Nyctaginaceae by larvae of *H. annei* was already recorded (Butler 1882; Moss 1912), this is the first confirmed record of *A. incarnata* as a host of this hawkmoth. Species of Zygophyllaceae have been recorded as host plants of three species of *Hyles* Hübner, (1819) (Robinson et al. 2010; Hundsdoerfer et al. 2017); however, this is the first confirmed record of a representative of the Zygophyllaceae as a host plant of *H. annei*. As *A. incarnata* is distributed from the United States to Argentina and Chile

Figures 1–5. The study site following summer rainfall in 2019 and newly recorded host plants of *Hyles annei*. **1.** The lowest part of Cardones Ravine, Arica Province, northern Atacama Desert of Chile. **2.** *Fagonia chilensis*. **3.** Flowers of *F. chilensis*. **4.** *Allionia incarnata*. **5.** Flower of *A. incarnata*.

(Turner 1994), it could be used by *H. annei* throughout its range. In contrast, the geographic range of *F. chilensis* is restricted to northern Chile and southern Peru (Beier 2005), which covers only a small part of the range of *H. annei*. Interestingly, *H. livornicoides* from Australia has a very similar food plant family spectrum, feeding naturally on Nyctaginaceae and Zygophyllaceae, as well as the wine grape crop family Vitaceae (*Vitis vinifera*; all in Moulds 1981). Both these *Hyles* species belong to the first five species to branch off in the phylogeny of *Hyles* (Hundsdoerfer et al. 2017).
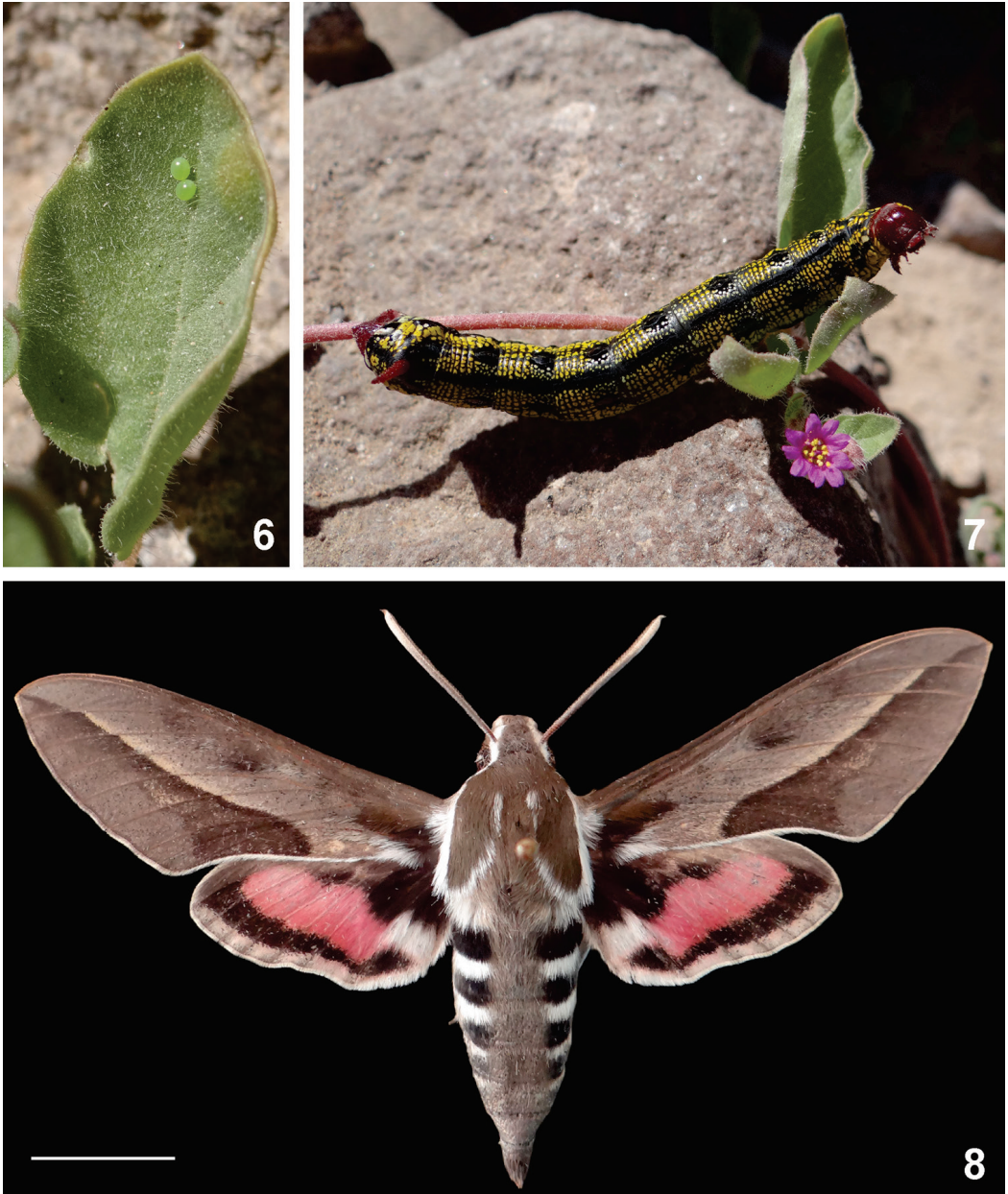
Figures 6–8. *Hyles annei* on *Allionia incarnata* in the lowest part of Cardones Ravine, Arica Province, northern Atacama Desert of Chile. **6.** Two eggs of *H. annei* on a leaf of *A. incarnata*. **7.** Last instar larva on *A. incarnata*. **8.** Male adult reared in the laboratory from a larva collected on *A. incarnata*. Scale bar: 10 mm.

In the present study, eggs and larvae of *H. annei* were found on all plants of *A. incarnata* (n = 15) and *F. chilensis* (n = 7) examined in the field. In contrast, at least three plants of native species of Amaranthaceae, Asteraceae, Brassicaceae, Fabaceae, Portulacaceae and Solanaceae were carefully examined at the study site but no eggs or larvae of *H. annei* were found. Thus, despite

the ability of *H. annei* to feed on plants of different families (Moré et al. 2014; Hundsdoerfer et al. 2019), the pattern found in northern Atacama suggests a preference for some plant species, an aspect of the species' biology that deserves further attention.

The first author (HAV) collected adults of *H. annei* at an elevation of about 200 m in the Azapa Valley, Arica Province, in October 2011. These adults were placed into a cage with tomato *Solanum lycopersicum* L. (Solanaceae) leaves to obtain eggs, because the labels of two adults from the same locality deposited in IDEA indicated they had been reared from this plant. The females deposited eggs. However, the larvae were unable to eat leaves of tomato or other native Solanaceae. They ate leaves of *V. vinifera*. In addition, HAV observed two larvae of Sphingidae, probably of *H. annei*, feeding on *Mirabilis* (Nyctaginaceae) in the Azapa Valley in December 2011. However, he did not collect these larvae to obtain adults to identify the species.

Many hawkmoths are powerful fliers, and some are migratory (Cary 1951). Angulo and Antezana (2001) reported a large number of female and male adults of *H. annei* flying at night over the Pacific Ocean about 10 km SW of "Lengua de Vaca", Tongoy, Coquimbo Region, and suggested that these were part of a pre-reproductive migration. Although further studies to confirm migratory behaviour in *H. annei* have been not undertaken, its ability to feed on ephemeral plants would be a great advantage to colonize new areas after migration, especially in extremely arid environments, such as those found in northern Chile.

Further field and laboratory studies are certainly needed to characterize the host range of *H. annei* in detail. These studies should be complemented with phylogeographic analyses using molecular markers (e.g., Mende et al. 2016; Cardoso et al. 2018), with samples from throughout the geographic distribution of *H. annei*, to understand better the ecology and evolution of this still little-known Neotropical hawkmoth.

Acknowledgements

References

Angulo AO, Antezana T (2001) Vuelo de mariposas nocturnas frente a la costa de Chile (Lepidoptera: Sphingidae). Revista de Biología Tropical 49(3–4): 1265–1266.

Artigas JN (1994) Entomología Económica. Insectos de interés agrícola, forestal, médico y veterinario. Ediciones Universidad de Concepción, Concepción, 943 pp.

Beier BA (2005) A revision of the desert shrub *Fagonia* (Zygophyllaceae). Systematics and Biodiversity 3(3): 221–263. https://doi.org/10.1017/S1477200005001684

Butler AG (1882) Heterocerous Lepidoptera collected in Chili by Thomas Edmonds, esq. Part I. Sphinges and Bombyces. Transactions of the Entomological Society of London 15: 1–30. https://doi.org/10.1111/j.1365-2311.1882.tb01566.x

Cardoso LW, Silva-Brandão KL, Duarte M (2018) *Adhemarius eurysthenes* (Felder, 1874) (Lepidoptera, Sphingidae) in the Atlantic Rain Forest: A phylogeographic perspective. Zoologischer Anzeiger 277: 231–241. https://doi.org/10.1016/j.jcz.2018.10.005

Cary MM (1951) Distribution of Sphingidae (Lepidoptera: Heterocera) in the Antillean-Caribbean Region. Transactions of the American Entomological Society 77(2): 63–129.

Cates RG (1981) Host plant predictability and the feeding patterns of monophagous, oligophagous, and po-lyphagous insect herbivores. Oecologia 48(3): 319–326. https://doi.org/10.1007/BF00346488

Chávez RO, Moreira-Muñoz A, Galleguillos M, Olea M, Aguayo J, Latín A, Aguilera-Betti I, Muñoz AA, Manríquez H (2019) GIMMS NDVI time series reveal the extent, duration, and intensity of "blooming desert" events in the hyper-arid Atacama Desert, Northern Chile. International Journal of Applied Earth Observation and Geoinformation 76: 193–203. https://doi.org/10.1016/j.jag.2018.11.013

Clarke JDA (2006) Antiquity of aridity in the Chilean Atacama Desert. Geomorphology 73(1–2): 101–114. https://doi.org/10.1016/j.geomorph.2005.06.008

Cock MJW, Boos JO (2006) Observations on Sphingidae (Lepidoptera) from Talara, North coastal Peru. Revista Peruana de Entomología 45: 75–78.

Felger RS, Turner DS, Wilson MF (2003) Flora and vegetation of the Mohawk Dunes, Arizona. Sida 20(3): 1155–1187.

González RH, Arretz VP, Campos LE (1973) Catálogo de las plagas agrícolas de Chile. Publicación de Ciencias Agrícolas, Universidad de Chile, 68 pp.

Haxaire J, Herbin D (1999) Les Lépidoptères Sphingidae de Bolivie. 1 ère Partie: sous-famille des Sphinginae Latreille. Revue de l'Association Roussillonnaise d'Entomologie 8(3): 69–79.

Hundsdoerfer AK, Rubinoff D, Attié M, Wink M, Kitching IJ (2009) A revised molecular phylogeny of the globally distributed hawkmoth genus *Hyles* (Lepidoptera: Sphingidae), based on mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution 52(3): 852–865. https://doi.org/10.1016/j.ympev.2009.05.023

Hundsdoerfer AK, Päckert M, Kehlmaier C, Strutzenberger P, Kitching IJ (2017) Museum archives revisited: central Asiatic hawkmoths reveal exceptionally high late Pliocene species diversification (Lepidoptera, Sphingidae). Zoologica Scripta 46(5): 552–570. https://doi.org/10.1111/zsc.12235

Hundsdoerfer AK, Buchwalder K, O'Neill MA, Dobler S (2019) Chemical ecology traits in an adaptive radiation: TPA-sensitivity and detoxification in *Hyles* and *Hippotion* (Sphingidae, Lepidoptera) larvae. Chemoecology 29(1): 35–47. https://doi.org/10.1007/s00049-018-0274-4

Juárez G, González U (2016) Los Sphingidae Latreille, 1802 (Lepidoptera: Bombycoidea) de la Región Piura (Perú). Arquivos Entomolóxicos 16: 61–66.

Kitching IJ (2019) Sphingidae Taxonomic Inventory. http://sphingidae.myspecies.info/ [accessed 22 May 2019]

Mende MB, Bartel M, Hundsdoerfer AK (2016) A comprehensive phylogeography of the *Hyles euphorbiae* complex (Lepidoptera: Sphingidae) indicates a 'glacial refuge belt'. Scientific Reports 6: 29527. https://doi.org/10.1038/srep29527

Moré M, Kitching IJ, Cocucci AA (2014) Sphingidae. In: Roig-Juñent S, Claps LE, Morrone JJ (Eds) Biodiversidad de Artrópodos Argentinos volumen 4. INSUE-UNT Ediciones, San Miguel de Tucumán, 281–295.

Moss AM (1912) On the Sphingidae of Peru. Transactions of the Zoological Society of London 20: 73–134. https://doi.org/10.1111/j.1469-7998.1912.tb07830.x

Moulds MS (1981) Larval food plants of hawk moths (Lepidoptera: Sphingidae) affecting commercial crops in Australia. General and Applied Entomology 13: 69–80.

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernández LM (2010) HOSTS – A Database of the World's Lepidopteran Hostplants. Natural History Museum, London. http://www.nhm.ac.uk/hosts [Accessed: 27 May 2019]

Rodríguez R, Marticorena C, Alarcón D, Baeza C, Cavieres L, Finot VL, Fuentes N, Kiessling A, Mihoc M, Pauchard A, Ruiz E, Sanchez P, Marticorena A (2018) Catálogo de las plantas vasculares de Chile. Gayana Botánica 75(1): 1–430. https://doi.org/10.4067/S0717-66432018000100001

Turner BL (1994) Revisionary study of the genus *Allionia* (Nyctaginaceae). Phytologia 77(1): 45–55. https://doi.org/10.5962/bhl.part.4444

Ureta E, Donoso R (1956) Revisión de la familia Sphingidae (Lep. Het.), en Chile. Boletín del Museo Nacional de Historia Natural 26: 237–256.

Elevational record of *Vanessa carye* (Hübner 1812) (Lepidoptera Nymphalidae) in the northern Chilean Altiplano Highlands

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http://zoobank.org/B86C7885-380F-44C9-B61C-332983032C0F

Abstract. *Vanessa carye* (Hübner, [1812]) has been reported to have a wide latitudinal range from Venezuela to the south of Chile (Patagonia). Populations are established at 3500 m in Putre region of Chile, with occasional observations around 4500 m. This article reports a new elevational record of *V. carye* above 5200 m located at the Sora Pata Lake, northeast of Caquena, in the highlands of the Chilean altiplano. This finding is the highest population ever reported for this migratory butterfly and one of the highest in the genus *Vanessa*.

Introduction

The cosmopolitan butterfly genus *Vanessa* Fabricius, 1807 (Lepidoptera: Nymphalidae) is a small genus that comprises approximately 20 species present in all the continents except Antarctica. There are six species (*V. cardui*, *V. virginiensis*, *V. atalanta*, *V. indica*, *V. carye* and *V. dimorphica*) within this genus that show impressively large ranges spanning several thousand kilometers (Wahlberg and Rubinoff 2011). These species comprise a well-known migratory group of butterflies, exemplified by the Painted Lady, *V. cardui* and its particularly long migratory flights. Every year *V. cardui* crosses the Sahara Desert twice between Europe and the Afrotropics, a flightpath of at least 4000 km (Talavera and Vila 2016). In addition to such long flight paths, they seem to navigate rather extreme climates. For example, some individuals of *V. cardui* have been recorded in extremely cold localities, even close to the Arctic Circle (e.g. on islands such as Iceland and Svalbard, or in Norway) or towards the Antarctic (e.g. on the island of Marion, 46°55'S) In Chile two butterfly species, *Vanessa terpsichore* Philipi, 1859 and *Vanessa carye* (Hübner, [1812]), have the widest geographical ranges of species in the genus occurring there (Fig.1). The distributional range of the former is much more restricted, ranging from Coquimbo to Magallanes in Chile, compared to the latter. As a result of this limited range, *V. terpsichore* is considered to be a poor disperser within the genus (Wahlberg and Rubinoff 2011). By contrast, *V. carye* ranges from Venezuela to Patagonia and is suspected to be a long-distance migrant. It has been stated that due to its capacity to breed on natural and ornamental Malvaceae among other plants, *V. carye* is one of the most frequently
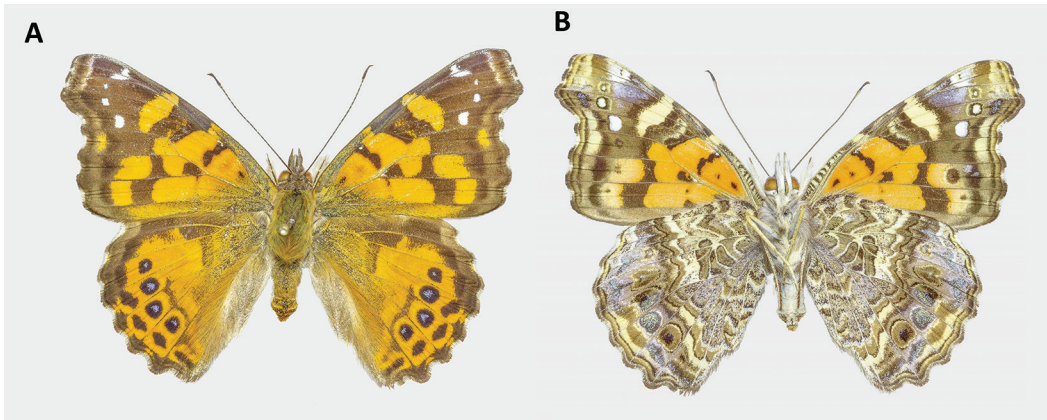
Figure 1. Dorsal and ventral view of *Vanessa carye* (Hübner, [1812]).

observed species in human modified landscapes in South America (Vargas 2013; Herrera 1987). Until now the highest elevation for *V. carye* was recorded at 4850 m in the Atacama region. This observation was made in conjunction with the description of the species pattern of long flight periods through the highest elevation areas in the Altiplano regions (Despland et al. 2012). Within the genus *Vanessa*, *V. cardui* has been one of the most studied migratory species, and it has been found that there is a strong correlation between its flight height and the presence of winds from the north of Africa between 1500 m to 3350 m (Shields 1992, Stefanescu et al. 2007). Likewise, there have been observations of migratory routes above 5118 m in the Himalayas and 5033 m. in Tibet (Shields 1992), but for *V. cardui* there are few published records of its elevational range (G. Talavera, *com. pers*.). Nevertheless, in South America, there is little available information regarding the full elevational range of *V. carye*, data that could provide important clues to understand the migratory routes and the pattern of climate adaptation of the species. Here, we report the first record for a population of *Vanessa carye* established at 5200 m Records of thermal or climatic endurance limits in Lepidoptera and other organisms are not merely of factual interest. They may be significant to help explaining how some species attain vast geographic ranges and provide subjects for new models of investigation about other biological traits. However, even in a highly vagile genus like *Vanessa*, it is interesting that some species are highly restricted in distribution and evolutionary diversification can still occur (Talavera and Vila 2016; Wahlberg and Rubinoff 2011).

Material and methods

In April 2018, *Vanessa carye* butterflies were observed flying near Casiri lake (Fig. 2) at 4838 m (18.068°S, 69.0743°W) in the northern Altiplano close to Caquena village (Fig. 3). Peña and Ugarte (1996) report for Chile that the hostplants for *V. carye* are mostly Malvaceae and Urticaceae, nevertheless, other families are reported to be hostplants of *V. carye* in South America by the NHM hosts database including Geraniaceae and Asteraceae that could be potential hosts of Chilean populations (Robinson et al. 2010). During a second expedition in May 2019, the route of the butterflies was followed a few kilometers north up to Sora Pata lake at around 5200 m (18.0635°S,
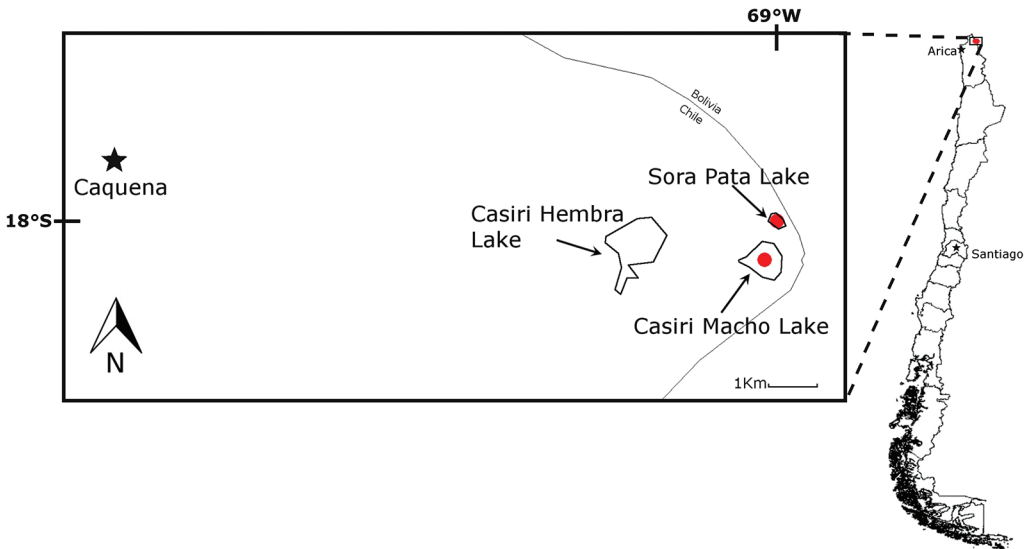
Figure 2. Map of the Northern Chile, Casiri Lake and Sora Pata Lake close to the border with Bolivia at 5200 m.

Figure 3. Casiri Lake, 4838 m 18.068°S, 69.0743°W (Northern Chile).

69.0662°W) (Fig. 4). An established population of *V. carye* was found flying and visiting a small Asteraceae, *Senecio serratifolius* (Meyen & Walp.) surrounding the lake. They were also observed to fly further up the mountain, presumably to hide and protect themselves from the wind.

Figure 4. Sora Pata Lake, 5200 m 18.0636°S, 69.0662°W (Northern Chile).

Results

This is the first *Vanessa* species ever reported in the high Andean Altiplano, flying at about 5200 m around Sora Pata Lake (Fig. 4). Furthermore, our observations indicate that this species may hide at a higher elevation at night, up to approximately 6000 m. Although ovipositing females were not observed among the *S. serratifolius*, signs of larval damage characteristic of *Vanessa* on these plants suggest an established population in the area. The Sora Pata lake is uniquely surrounded by mountains, providing this place with a unique microhabitat protected from the strongest winds at around 5200 m. An additional apparently significant observation was that individuals of *V. carye* were abundant and fresh (i.e., without broken wings) in 2019 in the month of May (when winds are particularly strong). Due to this abundance, 17 specimens could be collected for future molecular analysis.

Discussion

The genus *Vanessa* includes very well documented migratory butterflies, like the Painted Lady (*V. cardui*) which migrates from the Arctic Circle and crosses the Sahara Desert in Africa (Talavera and Vila 2016), and the Red Admiral (*V. atalanta*) which exhibit a seasonal migration throughout Europe and North Africa (Stefanescu 2008). This elevational record for *V. carye* establishes a starting point to analyse the general pattern of migration of this species, particularly in South America, where it has currently practically not been studied, in contrast to the breadth of knowledge already about *V. cardui*.

   In Chile *V. carye* shares its territory with other species of its genus; in the mid north of Chile with *V. terpsichore*, and in the altiplano with *V. braziliensis* (Moore, 1883). *V. braziliensis* was discovered to be expanding its territory in the north of Chile within the last six years, particularly

around Socoroma and towns nearby (Vargas 2013). During the 2019 Altiplano expedition, the team found *V. carye* flying together with *V. braziliensis* along a steep elevational gradient near Chapiquiña village at approximately 3340 m. In Chile, the Altiplano corresponds to a narrow strip in the northeast corner of the country (from approximately 17°30'S to 24°S) and is limited in the west by the eastern Andes. With a mean elevation of approximately 5000 m, this area can be characterized by a dry, cold climate, which almost exclusively allows the development of steppe vegetation. Despite this extreme environment, specimens of other butterfly families were found (Lycaenidae, Pieridae and Hesperiidae).

V. carye together with its European sister species *V. cardui* (Abbasi and Marcus 2015; Wahlberg and Rubinoff 2011), can be considered as "super butterflies" with the ability to survive in extreme environmental conditions. *V. carye* is considered to be a highly vagile species covering a large distribution in South America, including some oceanic islands like Easter Island, Juan Fernandez and the Tuamotu Archipelago (Field 1971, Wahlberg and Rubinoff 2011, Vargas 2013). As in other members of the genus, the expansive elevational range of *V. carye* is one of the most important facets of the ecological pattern of migration of one of the most outstandingly dispersive butterflies of the Andes and indeed South America.

Acknowledgements

References

Abbasi R, Marcus JM (2015) Color pattern evolution in *Vanessa* butterflies (Nymphalidae: Nymphalini): non-eyespot characters. Evolution & Development 17: 63–81. https://doi.org/10.1111/ede.12109

Brattström O, Kjellén, N, Alerstam T, Åkesson S (2008) Effects of wind and weather on red admiral, *Vanessa atalanta*, migration at a coastal site in southern Sweden. Animal Behaviour 76: 335–344. https://doi.org/10.1016/j.anbehav.2008.02.011

Despland E, Humire R, Martín SS (2012) Species richness and phenology of butterflies along an altitude gradient in the desert of northern Chile. Arctic, Antarctic, and Alpine Research 44: 423–431. https://doi.org/10.1657/1938-4246-44.4.423

Field WD (1971) Butterflies of the genus *Vanessa* and the resurrected genera *Bassaris* and *Cynthia* (Lepidoptera: Nymphalidae). Smithsonian Contributions to Zoology 84: 1–105. https://doi.org/10.5479/si.00810282.84

Herrera J (1987) Biología de *Cynthia carye* (Hübner, 1812), especie críptica de *C. annabella* Field, 1971 (Lepidoptera: Nymphalidae). Acta Entomológica Chilena 14: 65–116.

Peña L, Ugarte A (1996) Las Mariposas de Chile. Editorial Universitaria, Santiago.

Robinson GS, Ackery PR, Kitching IJ, Beccaloni GW, Hernández LM (2010) HOSTS – A Database of the World's Lepidopteran Hostplants. Natural History Museum, London. http://www.nhm.ac.uk/hosts [Accessed: 12 August 2019]

Stefanescu C (2001) The nature of migration in the red admiral butterfly *Vanessa atalanta*: evidence from the population ecology in its southern range. Ecological Entomology 26: 525–536. https://doi.org/10.1046/j.1365-2311.2001.00347.x

Stefanescu C, Alarcón, M, Àvila A (2007) Migration of the painted lady butterfly, *Vanessa cardui*, to north-eastern Spain is aided by African wind currents. Journal of Animal Ecology 76: 888–898. https://doi.org/10.1111/j.1365-2656.2007.01262.x

Talavera G, Vila R (2016) Discovery of mass migration and breeding of the painted lady butterfly *Vanessa cardui* in the Sub-Sahara: the Europe–Africa migration revisited. Biological Journal of the Linnean Society 120(2): 274–285. https://doi.org/10.1111/bij.12873

Wahlberg N, Rubinoff D (2011) Vagility across *Vanessa* (Lepidoptera: Nymphalidae): mobility in butterfly species does not inhibit the formation and persistence of isolated taxa. Systematic Entomology 36: 362–370. https://doi.org/10.1111/j.1365-3113.2010.00566.x

Vargas HA (2013) First record of *Vanessa braziliensis* (Moore) (Lepidoptera: Nymphalidae) in Chile. Gayana 77(2): 171–173. https://doi.org/10.4067/S0717-65382013000200009

Genetic confirmation of *Aricia artaxerxes* (Fabricius, 1793) (Lepidoptera, Lycaenidae) in the Czech Republic, its conservation significance and biogeographic context

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http://zoobank.org/5EB7C3A3-5BBE-468B-82DC-6D7D1676A679

Abstract. We report here the first molecular evidence for the occurrence of *Aricia artaxerxes* (Fabricius, 1793) (Lepidoptera: Lycaenidae) in the Czech Republic. In Central Europe, this species may co-occur with its more common sibling, *Aricia agestis* (Denis & Schiffermüller, 1775). We sequenced the cytochrome c oxidase subunit 1 of darkly-coloured, putative *A. artaxerxes* specimens in the Czech Republic. We confirmed *A. artaxerxes* only from a limestone area in South Bohemia (Vyšenské kopce National Nature Reserve), which is probably the only locality of the species in the Czech Republic. This area is located at ca. 550 m A.S.L., showing that the elevation overlap with *A. agestis* could be high in Central Europe. Other surveyed individuals were confirmed as *A. agestis*, with a minimum p-distance of 1.98% between the two species. The South Bohemian area of occurrence is probably highly isolated (approx. 190 km) from localities of the species in neighbouring countries, highlighting the conservation importance of the *A. artaxerxes* population and of the insular calcareous areas in the Šumava Mountains foothills. We used database sequences of *A. artaxerxes* to place the Czech population into a wider phylogeographic context. The Czech population is monomorphic, consisting of a single haplotype, which is present from Scandinavia through Germany to Central Asia.

Introduction

Aricia artaxerxes (Fabricius, 1793) is a Palaearctic species of lycaenid butterfly, occurring in Europe together with several cryptic siblings: the widely distributed *Aricia agestis* (Denis & Schiffermüller, 1775), the West Mediterranean *A. cramera* (Eschscholtz, 1821) and *A. montensis* Verity, 1928. *Aricia agestis* and *A. artaxerxes*, which are sympatric in Central Europe, differ in larval and pupal morphology, and rearing was traditionally used to distinguish them (Kames 1976; Lepidopterologen-Arbeitsgruppe 1987; Warecki 2010). Several studies combining morphology and molecular markers (Aagaard et al. 2002; Dincă et al. 2011; Sañudo-Restrepo et al. 2013) concluded that these taxa indeed represent true species and can be distinguished by allozyme profiles or the cytochrome c oxidase subunit 1 (COI, i.e., the standard DNA barcode for animals), but not by wing pattern or genital morphology. More specifically, adults from Scotland (nominotypical *A. artaxerxes artaxerxes* Fabricius, 1793) usually bear a white discoidal dot on the upper side of each brown fore wing. However, adults from mainland Europe (*A. artaxerxes allous* (Hübner, 1819), and other areas, cf. Sañudo-Restrepo et al. 2013) lack that trait, differing from adult *A. agestis* only

by a darker overall colouration, due to smaller or obscured orange spots on the upper sides of the fore wings, but also smaller spots on the hind wings (Tolman and Lewington 2008, p. 130).

Ecologically and biogeographically, the majority of *A. artaxerxes* records appear concentrated in calcareous short-turf grasslands at high latitudes or elevations (Lepidopterologen-Arbeitsgruppe 1987; Asher et al. 2001; Aagaard et al. 2002; Sañudo-Restrepo et al. 2013; Pecsenye et al. 2014), where the species has a single annual generation from June to August. Its confirmed larval host plants across the range include *Helianthemum* Mill. spp. (Cistaceae; *H. nummularium* (L.) Mill. in Britain: Asher et al. 2001) and large-flowered *Geranium* L. spp. (Geraniaceae; *G. sanguineum* L. in Poland: Warecki 2010; see also Tuzov 2000; Gorbunov and Kosterin 2003). The distribution of *A. agestis*, in contrast, includes most of the European continent except for the highest latitudes, and extends to the Tian Shan in Asia. It has two or three annual generations in the lowlands of Central Europe, and three or even more in more southerly regions (Hesselbarth et al. 1995). Its host plant range overlaps with *A. artaxerxes*, as it includes both many Geraniaceae (e.g., *Geranium dissectum* L., *G. pusillum* Burm. fil., *G. pratense* L. and *G. molle* L., and *Erodium* L'Her. ex Aiton spp.) and *Helianthemum* spp. in natural conditions (Thomas et al. 2001; Buckley et al. 2012). Reflecting the broad ecological range of the host plants used, *A. agestis* inhabits a broad range of biotopes, from xeric grasslands to mesic and even alluvial meadows (Bury 2016). In European mid-latitudes, the single generation of *A. artaxerxes* coincides with the gap between spring and summer generations of *A. agestis* (Beneš et al. 2002). *Aricia artaxerxes* is usually associated with higher elevations than *A. agestis* in Central and Southern Europe, but their occurrence could overlap at intermediate elevations, causing problems with identification in the potential contact zones (cf. Dincă et al. 2011).

Until the late 20th century, the two species were not distinguished in the lowlands of Central Europe, including in the Czech Republic. The first records of *A. artaxerxes* for the country are from 1964 (Králíček et al. 1970) and 1971 (Králíček and Gottwald 1980), from the warm region of South-Eastern Moravia (foothills of the White Carpathians Mts). These and later records were mainly identified based on the combination of flight period and a darker colouration than the more common *A. agestis*. The distribution atlas of Czech butterflies (Beneš et al. 2002) considered only the records of *A. artaxerxes* in a single atlas grid square (Vyšenské Kopce National Nature Reserve [= NNR] near Český Krumlov, South Bohemia) as recent and relatively reliable. The Czech Republic Butterflies and Moths Recording Database (Institute of Entomology, Biology Centre of the Czech Academy of Sciences) together with the Information System of Nature Conservation (Nature Conservation Agency of the Czech Republic) register possible, unreliable records of *A. artaxerxes* from 38 grid squares (as opposed to 427 for *A. agestis* – 63% of the country´s area) (Fig. 1). The national Red list (Hejda et al. 2017) considers *A. artaxerxes* as critically endangered and *A. agestis* as of least concern.

The recent increase in the use of molecular markers for species identification, the emergence of national barcoding programmes (e.g., Dincă et al. 2011; Hausmann et al. 2011; Litman et al. 2018) and butterfly recording in European countries have significantly improved our knowledge of *A. artaxerxes* distribution. Besides the previously known distribution at high elevations, the species was reliably confirmed from relatively low elevations of Baden-Württemberg (at ca. 850 m A.S.L.), Saxony-Anhalt (ca. 430 m A.S.L.), and Thuringia (ca. 370 m A.S.L.) (Hausmann et al. 2011; and specimens used in Mutanen et al. 2016). It is also reported from central-northern Poland (Buszko and Maslowski 2008; Sielezniew and Dziekanska 2010), northern Hungary (800–850 m A.S.L.) (Pecsenye et al. 2014) and central-eastern Slovakia (600–800 m A.S.L.) (Reiprich and
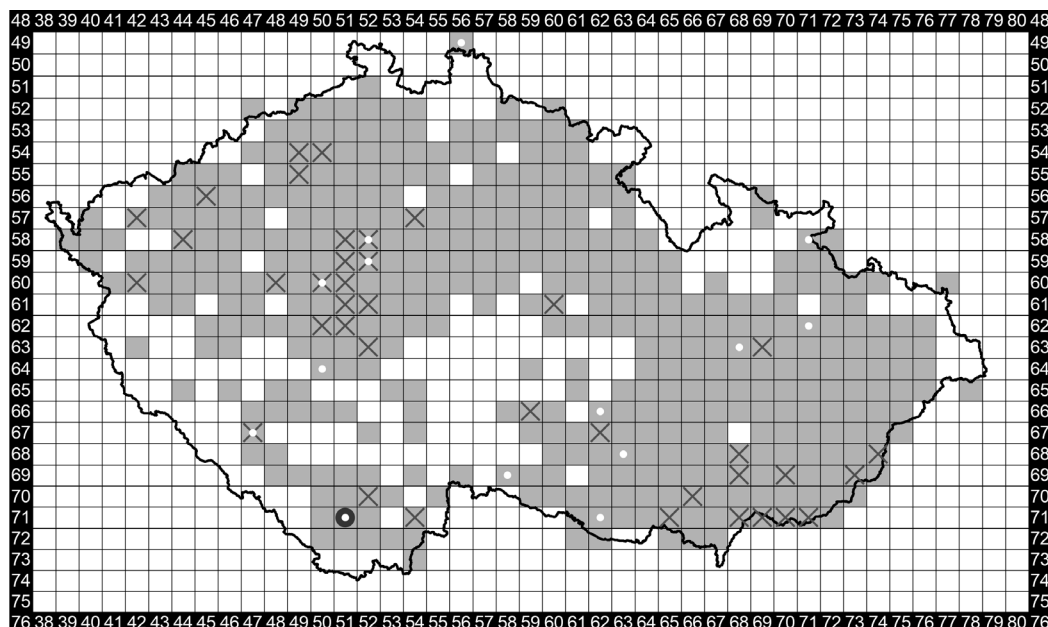
Figure 1. Distribution map of *Aricia artaxerxes* and *A. agestis* in the Czech Republic. The map was created based on 8464 records both historical and recent (until 2018) of these two species from The Czech Republic Butterflies and Moths Recording Database (Institute of Entomology, Biology Centre of the Czech Academy of Sciences) and from the Information System of Nature Conservation (Nature Conservation Agency of the Czech Republic). Grey squares: *A. agestis*, crosses: probably darkly-coloured *A. agestis* (putative *A. artaxerxes* records), white points: barcoded individuals, black circle: confirmed *A. artaxerxes*.

Okáli 1988–1989; Pecsenye et al. 2014; L. Víťaz, personal communication), but as far as we know, these populations have not been studied using molecular sequencing.

In this contribution, we use DNA barcodes to clarify the status of *A. artaxerxes* in the Czech Republic. Specifically, we barcoded material from the putative *A. artaxerxes* population near Český Krumlov, and individuals with missing or reduced orange spots on upper sides of the wings (hereinafter "dark") from several localities across the country with past *A. artaxerxes* reports, and a selection of typical *A. agestis* (with developed orange spots, hereinafter "light"). In addition to evaluating the current status of *A. artaxerxes* in the country, we place it into a wider biogeographic context and discuss its conservation significance.

Material and methods

We tested twelve individuals from the putative *Aricia artaxerxes* population from Vyšenské kopce NNR, South Bohemia (univoltine, dark, occurring between spring and summer broods of sympatric *A. agestis*; but also including reared individuals without reduced orange spots originating from dark females), five dark or intermediate individuals from other localities, and nine light individuals with developed orange spots (Table 1, Fig. 2). To place these individuals into a broader context, we further mined a total of 113 sequences from GenBank: *A. artaxerxes* (N=94), *A. agestis* (N=9), *A. anteros* (N=3), *A. cramera* (N=3) and *A. montensis* (N=4) (Suppl. materials 1, 3).

Table 1. Sampling sites of *Aricia artaxerxes* and *A. agestis* from the Czech Republic used for DNA-based identification.

Species	Voucher	Colouration	Locality	GenBank codes	Elevation [m A.S.L.]	Grid square
A. artaxerxes	6 specimens ZF-LY-001622 JB00138	dark	Vyšenské kopce National Nature Reserve, South Bohemia	MN107398 –	550	7151
	ZF-LY-001774 to 1776, 1778			MN107403		
	AB24-1 to 6	light	Vyšenské kopce National Nature Reserve, South Bohemia – reared	MN107404 – MN107409	550	7151
A. agestis	JB00137	dark	Čepičná Nature Reserve, South Bohemia	MN107393	500	6747
	ZF-LY-001779	dark	Koněprusy, Čertovy schody quarry, Central Bohemia	MN107390	400	6050
	ZF-LY-001785	dark	Martinice, Velké Meziříčí, Vysočina region	MN107392	470	6662
	ArAg2	dark	Čimické údolí Nature Monument, Prague	MN107394	250	5852
	ArAg1	dark	Prokopské údolí Nature Reserve, Prague	MN107395	250	5952
	JB00124	light	Nerestský lom Nature Monument, South Bohemia	MN107396	470	6450
	ZF-LY-001784	light	Černousy, Frýdlant, Liberec region	MN107391	250	4956
	AB30-23	light	Nová Ves, Litovel, Olomouc region	MN107384	300	6368
	AB30-28	light	Mohelenská hadcová step National Nature Reserve, Vysočina region	MN107385	350	6863
	AB44-8	light	Hraniční hill, Město Albrechtice, Moravian-Silesian region	MN107386	500	5871
	AB44-9	light	Město Libavá, Olomouc region	MN107387	550	6271
	AB44-10	light	Chomýž, Krnov, Moravian-Silesian region	MN107388	350	5871
	AB44-11	light	Toužínské stráně Nature Monument, South Bohemia	MN107389	470	6958
	KA-2698	light	Havranické vřesoviště, Podyjí National Park, South Moravia	MN107397	330	7162

DNA was extracted from the legs using the Genomic DNA Mini Kit – Tissue (Geneaid) following the manufacturer's protocols. Using the Polymerase Chain Reaction (PCR), we amplified the mitochondrial gene cytochrome c oxidase subunit 1 gene (barcode/COI). We used the primer pair LCO/Nancy; or LCO/K699 and RON/HCO in case of fragmented DNA (primers: Monteiro and Pierce 2001; Wahlberg and Wheat 2008). We prepared the PCR mixture in 12.5 µl volume (6.25 µl Bioline 2× MyTaq HS Red Mix, 4 µl PCR H2O, 0.625 + 0.625 µl primers; 1 µl DNA). The thermal cycling profile was 95 °C for 5 min; then 40 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s; and final extension 72 °C for 10 min. PCR products were cleaned with enzymes FastAP and ExoI (Thermofisher) and sequenced in one direction in Macrogen Inc. on ABI3730XL DNA analysers. Sequences were checked visually and aligned in GENEIOUS v. 8.0.5 (Kearse et al. 2012). Obtained sequences were submitted to the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/; accession codes MN107385–MN107409, Suppl. material 1).
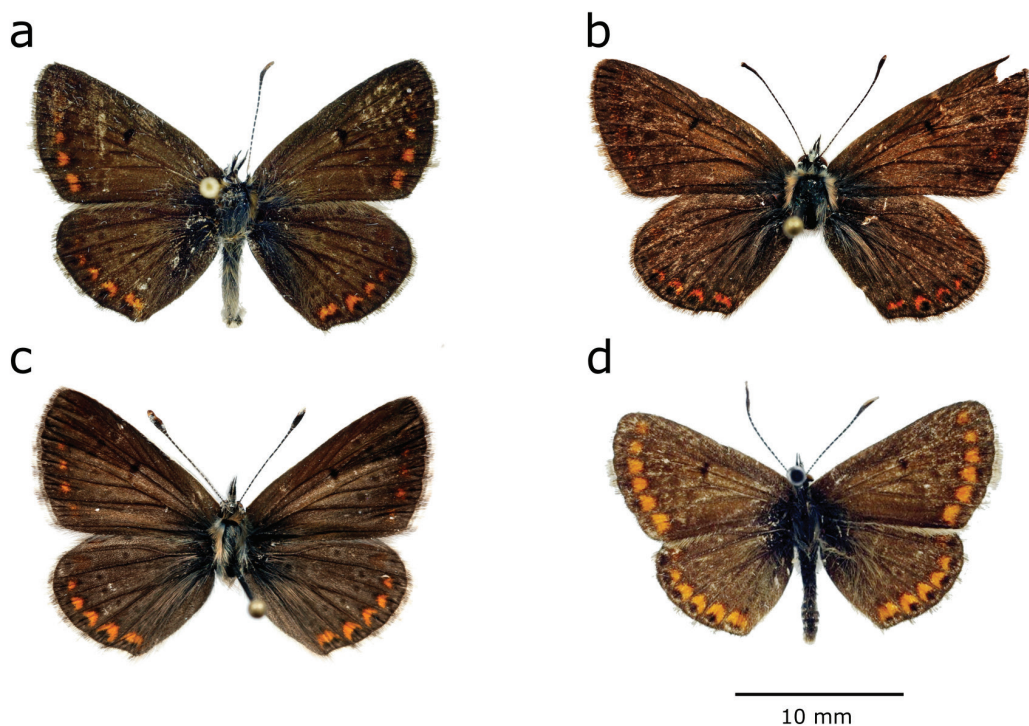
Figure 2. Examples of the sequenced specimens of *Aricia artaxerxes* and *A. agestis.* **a.** *Aricia artaxerxes*, (ZF-LY-001623, MN107399, Vyšenské kopce National Nature Reserve). **b, c.** *Aricia agestis*, darkly coloured individuals (**b.** JB00137, MN107393, Čepičná Nature Reserve. **c.** ZF-LY-001785, MN107392, Martinice, Velké Meziříčí). **d.** *Aricia agestis*, a typical, lightly coloured individual with developed orange spots (AB44-11, MN107389, Toužínské stráně Nature Monument).

The BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with GenBank reference database assigned the sequences to either *A. artaxerxes* or *A. agestis*. MEGA7 (Kumar et al. 2016) was used for computing p-distances (i.e., proportion of nucleotide site differences) among sequences and groups of sequences (species). We mined 94 sequences of *A. artaxerxes* from GenBank (Suppl. materials 1, 3), which we used, together with the twelve sequences of Czech *A. artaxerxes*, to construct the TCS statistical parsimony haplotype network (Clement et al. 2000) in the program POPART (Leigh and Bryant 2015). Using one sample of each *A. artaxerxes* haplotype from GenBank, together with nine randomly chosen samples of European *A. agestis* and representatives of *A. anteros*, *A. cramera* and *A. montensis* (Suppl. materials 1, 2), we reconstructed the Maximum Likelihood tree of all tested specimens using the IQTREE web server (Nguyen et al. 2015; Trifinopoulos et al. 2016) with ultrafast bootstrap (Minh et al. 2013).

Results

All darkly coloured as well as the reared light putative *Aricia artaxerxes* individuals from Vyšenské Kopce NNR reserve (N=12) were unequivocally *A. artaxerxes* according to both BLAST and Maximum Likelihood analysis (Fig. 3). All other tested individuals, both darkly- and lightly-coloured,
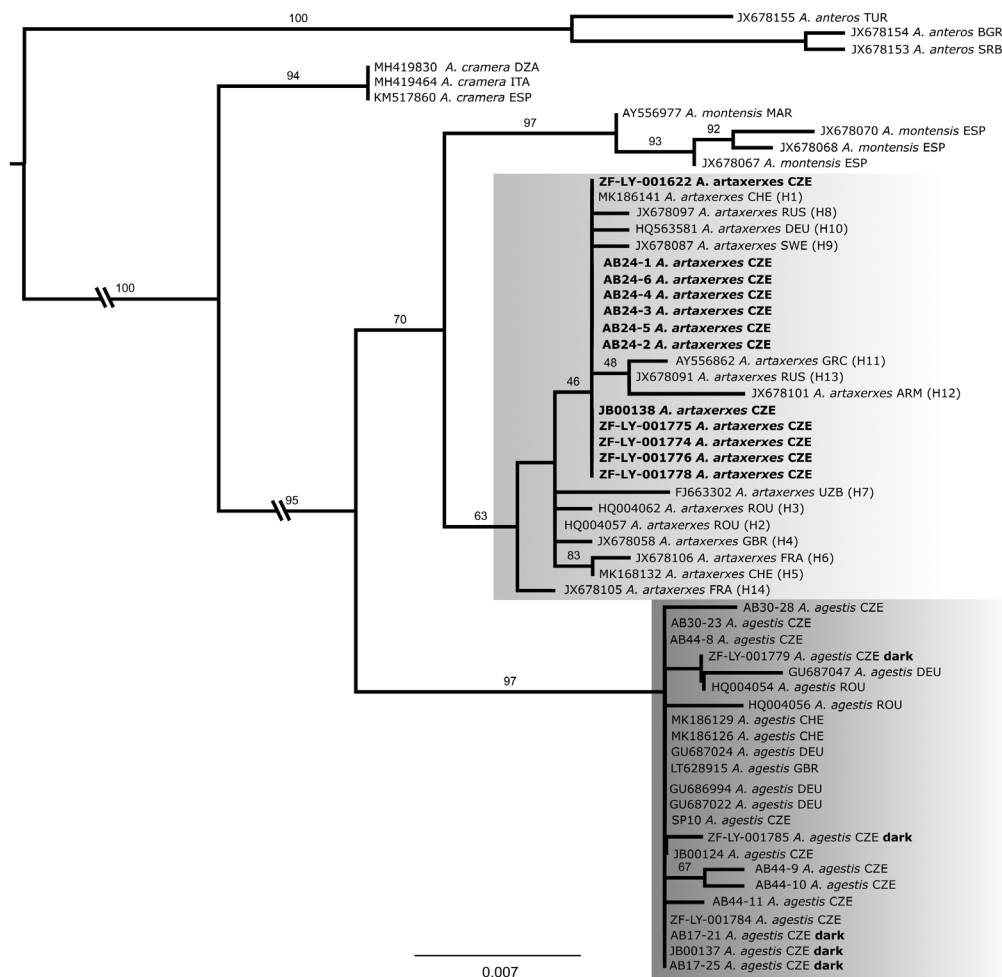
Figure 3. Maximum Likelihood tree of the Czech Republic *Aricia* spp. butterflies. The tree is based on cytochrome c oxidase subunit I (DNA barcode) sequences obtained for this study (Czech material) and retrieved from GenBank. The sample AY556977 from Morocco is labelled as *Aricia artaxerxes* in GenBank, This specimen was renamed as *A. montensis*, which occurs in North Africa (Sañudo-Restrepo et al. 2013). Branch labels show bootstrap values.

were *A. agestis*. The minimum p-distance between Czech *A. artaxerxes* and *A. agestis* was 1.98% and the mean p-distance was 2.10%.

The Czech *Aricia artaxerxes* population is monomorphic, consisting of a single COI haplotype (Fig. 4). Comparing the published barcodes of *A. artaxerxes*, this haplotype (H1) is widely distributed in the Palaearctic (Central Asia, Western Russia, Crimea, Central Germany, both Eastern and Western Alps and European North), and its derived haplotypes (Haplogroup A) are present in Greece, Baden-Württemberg, and reach the Far East. The other widespread haplotype (H2) and its derived haplotypes have a more southerly distribution – in the Balkans, Alps, and reach the United Kingdom. The highest haplotype diversity is in the Western Alps where five different haplotypes are present in twelve samples. However, as reported previously (Sañudo-Restrepo et al. 2013), the overall p-distance is low within this species (mean = 0.20%, max = 1.10%).
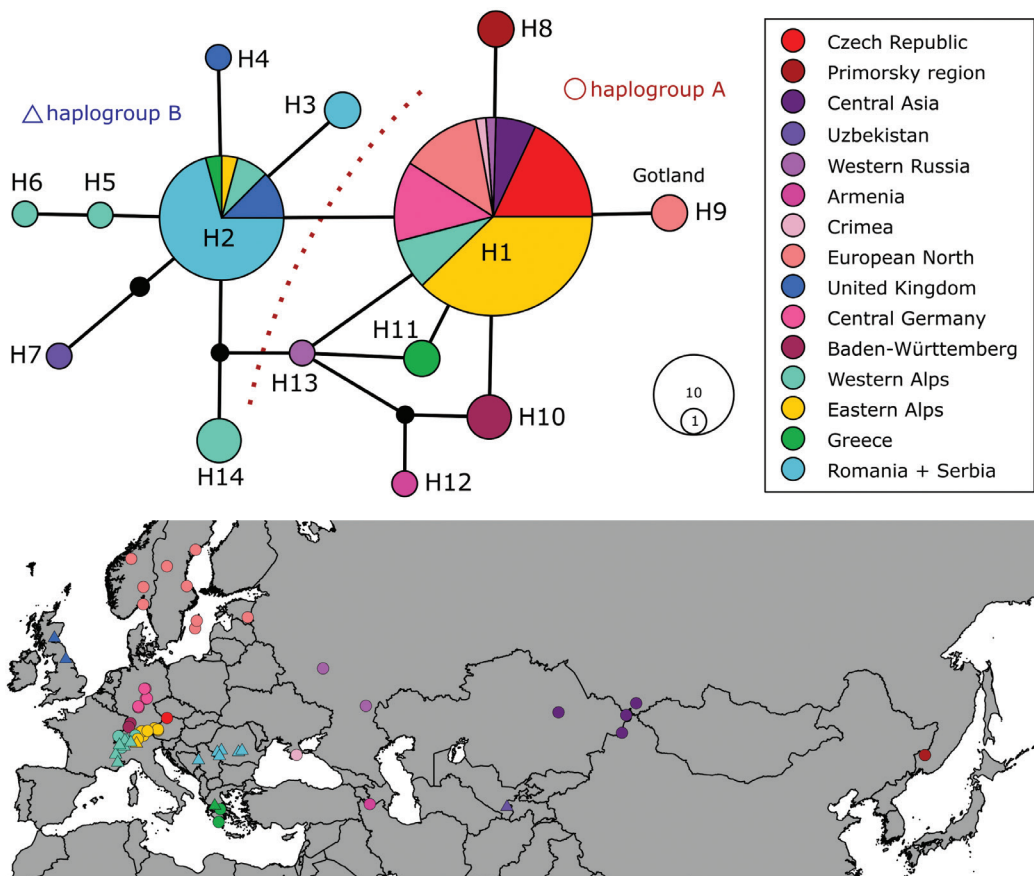
Figure 4. TCS haplotype network of *Aricia artaxerxes*. The network is based on cytochrome c oxidase subunit 1 (DNA barcode) sequences obtained for this study (Czech material) and retrieved from GenBank. Circles represent individual haplotypes, black dots represent putative haplotypes (not found among samples), and each connecting line represents one mutation.

Discussion

Using DNA barcoding, we confirmed that the putative *Aricia artaxerxes* population from Vyšenské Kopce NNR, Czech Republic, indeed belongs to this species, whereas other darkly-coloured samples from the country belong to *Aricia agestis* (Fig. 2). The fact that the reared individuals of *A. artaxerxes* resemble *A. agestis* suggests a phenotypic plasticity also in this species. Moreover, the Czech population is situated at a relatively low elevation (ca. 550 m A.S.L.). Relying on wing morphology or elevational records thus can lead to misidentifications between these two species. Our results highlight the need for DNA-based identification of these two species within their overlapping range (cf. Dincă et al. 2011).

Besides providing confirmation of *A. artaxerxes* distribution, our results indicate that its Czech population is isolated by ca. 190 km from the nearest genetically confirmed population of the species in Europe, possesses an extremely narrow distribution, and rightly deserves its critically endangered status (cf. Hejda et al. 2017).

This finding raises the national and even Central European importance of the Vyšenské Kopce NNR (area: 66.7 ha), plus two reserves within its closest environs (Výří vrch Nature Monument 12.9 ha, and Cvičák Nature Monument, 61.4 ha), for butterfly conservation. These three reserves, parts of the Blanský Les Landscape Protected Area, protect a system of (sub)xerophilous grasslands, shrublands and sparse woodlands on metamorphous limestones, a rare phenomenon within the prevailingly base-poor, cold and damp north-eastern foothills of the Šumava Mts. These calcareous localities have been rescued from successional overgrowth by restoration activities, partly depending on volunteers, since the 1970s onwards (Albrecht et al. 2003); the Cvičák Nature Monument was for a time used by the military (cf. Čížek et al. 2013). The current conservation management of these sites is exemplary, aiming at retaining a dynamic mosaic of coppiced woodlands, scrub and grasslands maintained by mowing and goat and cattle grazing of varying intensity. The reserves host a regionally outstanding butterfly diversity (Hanč 2005, 2011; Slámová et al. 2013). Within this system, *A. artaxerxes* inhabits short-sward, open-turf and flower-rich sites with dense growth of *Helianthemum grandiflorum obscurum* (most likely the local larval host), which cover an area not exceeding 20 ha in total (Fig. 5a).

The entire area belongs to a wider system of calcareous islets adjoining the Šumava Mts. in an approximately NW-SE direction, and supporting multiple range-restricted relict xerophilous plants and animals (Fig. 5b). These include species with southern distribution (e.g., the bush-cricket *Leptophyes bosci* Fieber, 1853 (Holuša et al. 2013)); south-western elements believed to have colonised Czech territory via south-western mountain passes (e.g., the lycaenid butterfly *Pseudophilotes baton* (Bergsträsser, 1779) (Konvička et al. 2008)); and alpine floral elements that likely descended to the Danube lowlands in colder periods of the Pleistocene (e.g., *Verbascum chaixii* subs. *austriacum* (R. et Sch.) Hayek (cf. Holub and Skalický 1959; Kaplan 2012)). The population of *A. artaxerxes* appears as another example of this "perialpine" element, a relict of a time when the currently high-elevation species descended to northern foothills of the Alps (Schmitt 2017). Our *Aricia* sp. samples included material from yet another calcareous islet of the Šumava Mts. foothills (Čepičná, see Table 1). Although the presence of *A. artaxerxes* has not yet been confirmed there, the possibility of its occurrence there or in other localities makes further surveys desirable.

On the other hand, we are sceptical regarding the earlier (1970s–1980s) records of *A. artaxerxes* from eastern parts of the Czech Republic (south-eastern Moravia). The records were not followed by subsequent observations, despite intensive recording in the region (e.g., Bělín and Gottwald 2001; Konvička et al. 2008; Spitzer and Beneš 2010; Uřičář et al. 2016). We have shown here that even *A. agestis* occasionally forms darker variants (Fig. 2). In addition, no *A. artaxerxes* records from adjoining Western Slovakia are known to Slovakian colleagues; the closest known populations of putative *A. artaxerxes* are from Central Slovakia (near Východná and Važec), more than 100 km distant from the East Moravian localities (L. Víťaz, personal communication).

In the wider geographical context, the overall COI genetic diversity within *A. artaxerxes* is low when compared to its sibling *A. agestis* (Sañudo-Restrepo et al. 2013; Vodă et al. 2015).

The mitochondrial haplotype network (Fig. 4) does not show pronounced genetic structure within *A. artaxerxes* and the following interpretation needs to be taken with caution. The network suggests the possible existence of two haplogroups, differing by a single mutation only, distributed in wide longitudinal belts across the Palaearctic. The first (haplogroup A) is distributed from the Far East to Scandinavia, including also the Eastern Alps and the German and Czech populations. The second (haplogroup B) has a more southerly distribution, in Romania and the Western Alps, but also
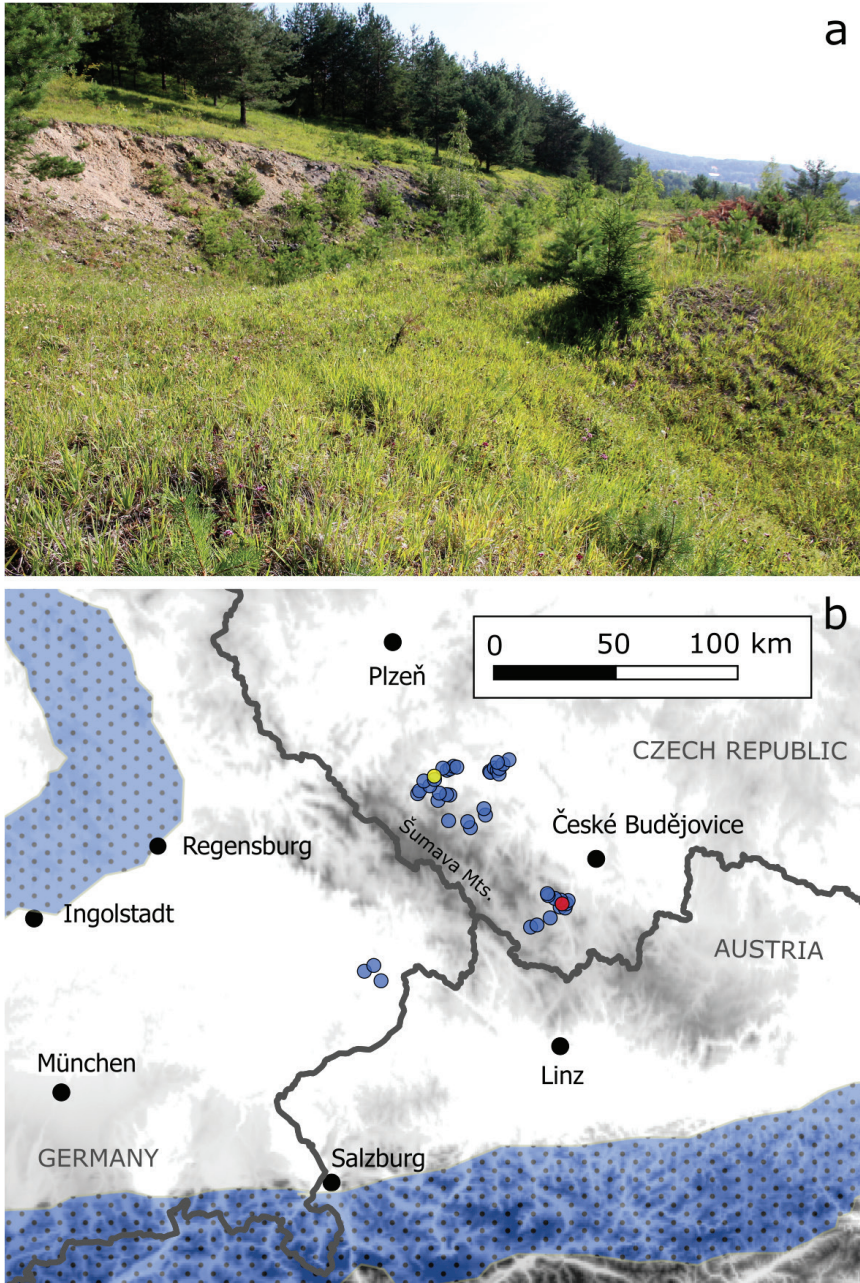
Figure 5. Calcareous localities adjoining the Šumava Mts. chain. **a.** Habitat of the Czech Republic population of *Aricia artaxerxes*, short-sward calcareous grassland near Český Krumlov, South Bohemia. **b.** Calcareous localities adjoining the Šumava Mts. chain in the wider geographic context. Although numerous, all the localities represent small and relatively isolated patches within predominately acidic bedrock, and the majority of their summed area is forested. The closest contiguous limestone areas are the Northern Alps, situated to the south, and the Franconian Jura, situated to the west. Blue field and coloured dots: calcareous areas. Yellow dot: Čepičná Nature Reserve, Red dot: Vyšenské kopce National Nature Reserve.

in northern Great Britain. Both haplogroups are present in the Western Alps, where several unique haplotypes also exist, and in Greece. The presence of unique haplotypes suggests that the species survived the glacial maxima in these topographically diverse regions, possibly on mountain foothills (cf. Schmitt 2017). Moreover, these areas could also represent a secondary contact zone for the two haplogroups. Samples from the Italian Peninsula, which are still lacking in the dataset, could shed more light on this problem. The Palaearctic-wide distribution of haplogroup A could either point to a rapid postglacial expansion from a refugium, or to a more continuous distribution during glacials, as detected also for other continental species (Marešová et al. 2019; Bartoňová et al. 2018). We incline towards the second option, as postglacial dispersal from a restricted refugium to the wide belt stretching from the Alps to the Altai Mts. seems unlikely. Unique haplotypes were detected in still more southerly areas (H7 – Uzbekistan, H11– Greece, H12 – Armenia), but none was separated from the most frequent haplotypes (H1, H2) by more than three mutations. Their existence might indicate further structuring of populations in these areas, but more data are needed to ascertain this.

In any case, *Aricia artaxerxes* constitutes a boreomontane species with the European part of its range divided into a northern area where it frequents short-sward grasslands with the warmest microclimates, and a more southerly area where it inhabits localities of a similar character, but usually situated at high elevations. Adjoining the southern mountains, there exist isolated relict sites in relatively low elevations, potentially threatened by successional changes. This situation is remarkably similar to other butterfly species occurring both at high elevation and on piedmont grasslands, whose piedmont sites have been disappearing due to modern land use changes, whereas the high-elevation populations still hold on (e.g., *Lasiommata petropolitana* (Fabricius, 1787) (Spitzer et al. 2018) or *Parnassius apollo* (Linnaeus, 1758) (Todisco et al. 2010; Habel et al. 2012)).

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References

Aagaard K, Hindar K., Pullin AS, James CH, Hammarstedt O, Balstad T, Hanssen O (2002) Phylogenetic relationships in brown argus butterflies (Lepidoptera: Lycaenidae: *Aricia*) from northwestern Europe. Biological Journal of the Linnean Society 75(1): 27–37. https://doi.org/10.1046/j.1095-8312.2002.00004.x

Albrecht J (2003) Českobudějovicko. In: Mackovčin P, Sedláček M (Eds) Chráněná území ČR VIII. Agentura Ochrany Přírody a Krajiny ČR and EkoCentrum Brno, Praha, 808 pp.

Asher J, Warren M, Fox R, Harding P, Jeffcoate G, Jeffcoate S (2001) The Millennium Atlas of Butterflies in Britain and Ireland. Oxford University Press, New York, 456 pp.

Bartoňová A, Konvička M, Korb S, Kramp K, Schmitt T, Faltýnek Fric Z (2018) Range dynamics of Palaearctic steppe species under glacial cycles: the phylogeography of *Proterebia afra* (Lepidoptera: Nymphalidae: Satyrinae). Biological Journal of the Linnean Society 125(4) 867–884. https://doi.org/10.1093/biolinnean/bly136

Bělín V, Gottwald A (2001) Motýli Bílých a Bielych Karpat. Přírodovědný klub v Uherském Hradišti, Uherské Hradiště, 153 pp.

Beneš J, Konvička M, Dvořák J, Fric Z, Havelda Z, Pavlíčko A, Vrabec V, Weidenhoffer Z (2002) Motýli České republiky: Rozšíření a ochrana I, II. SOM, Praha, 857 pp.

Buckley J, Butlin RK, Bridle JR (2011) Evidence for evolutionary change associated with the recent range expansion of the British butterfly, *Aricia agestis*, in response to climate change. Molecular Ecology 21(2): 267–280. https://doi.org/10.1111/j.1365-294X.2011.05388.x

Bury J (2016) New data on *Aricia agestis* (Lepidoptera: Lycaenidae), its life history and occurrence in the Podkarpacie region of Poland. Fragmenta Faunistica 59(1): 29–37. https://doi.org/10.3161/00159301 FF2016.59.1.029

Buszko J, Maslowski J (2008) Motyle dzienne Polski (Lepidoptera: Hesperioidea, Papilionoidea). Koliber, Kraków, 274 pp.

Čížek O, Vrba P, Beneš J, Hrázský Z, Koptík J, Kučera T, Marhoul P, Zámečník J, Konvička M (2013) Conservation potential of abandoned military areas matches that of established reserves: plants and butterflies in the Czech Republic. PLOS ONE 8(1): e53124. https://doi.org/10.1371/journal.pone.0053124

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9(10): 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Dincă V, Zakharov EV, Hebert PDN, Vila R (2011) Complete DNA barcode reference library for a country's butterfly fauna reveals high performance for temperate Europe. Proceedings of the Royal Society B: Biological Sciences 278(1704): 347–355. https://doi.org/10.1098/rspb.2010.1089

Gorbunov P, Kosterin O (2003) The butterflies of north Asia in nature (Hesperioidea and Papilionoidea) (Asian part of Russia), Volume 1. Publishers Koreshkov, Krylov, Moscow, 391 pp.

Habel JC, Reuter M, Drees C, Pfaender J (2012) Does isolation affect phenotypic variability and fluctuating asymmetry in the endangered Red Apollo? Journal of Insect Conservation 16(4): 571–579. https://doi.org/10.1007/s10841-011-9442-3

Hanč Z (2005) Denní motýli (Lepidoptera: Papilionoidea, Hesperioidea) Národní přírodní rezervace Vyšenské kopce. Klapalekiana 41: 33–42.

Hanč Z (2011) Denní motýli a vřetenušky (Lepidoptera, Papilionoidea a Zygaenidae) Chráněné krajinné oblasti Blanský les. Sborník Jihočeského Muzea v Českých Budějovicích, Přírodní vědy 51: 101–117.

Hanč Z, Beneš J, Faltýnek Fric Z, Pavlíčko A, Zapletal M (2019) Atlas denních motýlů a vřetenušek jižních Čech. Jihočeský kraj, České Budějovice, 437 pp.

Hausmann A, Haszprunar G, Segerer AH, Speidel W, Behounek G, Hebert PDN (2011) Now DNA-barcoded: the butterflies and larger moths of Germany. Spixiana 34(1): 47–58.

Hejda R, Farkač J, Chobot K (2017) Červený seznam ohrožených druhů České republiky. Bezobratlí. Agentura ochrany přírody a krajiny České republiky, Praha, 611 pp.

Hesselbarth G, Oorschot van H, Wagener S (1995) Die Tagfalter der Türkei unter Berücksichtigung der angrenzenden Länder. Goecke & Evers, Keltern, 1354 pp.

Holub J, Skalický V (1959) Floristicko-fytogeografické poznámky ke květeně jihovýchodní části Šumavy a přilehlé části Předšumaví. Preslia 31: 395–412.

Holuša J, Kočárek P, Vlk R, Marhoul P (2013) Annotated checklist of the grasshoppers and crickets (Orthoptera) of the Czech Republic. Zootaxa 3616(5): 437–460. https://doi.org/10.11646/zootaxa.3616.5.2

Kames P (1976) Die Aufklärung des Differenzierungsgrades und der Phylogenese der beiden *Aricia*-Arten *agestis* Den. et Schiff. und *artaxerxes* Fabr. (allous G.-Hb.) mit Hilfe von Eizuchten und Kreuzungsversuchen (Lep., Lycaenidae). Mitteilungen der entomologischen Gesellschaft Basel, N. F. 26: 7–13, 29–64.

Kaplan Z (2012) Flóra a fytogeografie České republiky. Preslia 84: 505–573.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: an integrated and extend-

able desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Konvička M, Dvořák L, Hanč Z, Pavlíčko A, Fric Z (2008) The Baton blue (*Pseudophilotes baton*) (Lepidoptera: Lycaenidae) in south-western Bohemia: iron curtain, military ranges and endangered butterfly. Silva Gabreta 14: 187–198.

Králíček M, Gottwald A (1980) Motýli Jihovýchodní Moravy I. Muzeum J.A. Komenského Uherské Hradiště, 112 pp.

Králíček M, Marek J, Povolný D (1970) Významné a nové nálezy Lepidopter z Moravy a Slovenska. Ochrana fauny (Bratislava) 4: 1–8.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. Methods in Ecology and Evolution 6(9): 1110–1116. https://doi.org/10.1111/2041-210X.12410

Lepidopterologen-Arbeitsgruppe (1987) Schmetterlinge und ihre Lebensräume, Band 1. Pro Natura – Schweizerischer Bund für Naturschutz, Basel, 516 pp.

Litman J, Chittaro Y, Birrer S, Praz C, Wermeille E, Fluri M, Stalling T, Schmid S, Wyler S, Gonseth Y (2018) A DNA barcode reference library for Swiss butterflies and forester moths as a tool for species identification, systematics and conservation. PLOS ONE 13(12): e0208639. https://doi.org/10.1371/journal.pone.0208639

Marešová J, Habel JC, Neve G, Sielezniew M, Bartoňová A, Kostro-Ambroziak A, Faltýnek Fric Z (2019) Cross-continental phylogeography of two Holarctic Nymphalid butterflies, *Boloria eunomia* and *Boloria selene*. PLOS ONE 14(3): e0214483. https://doi.org/10.1371/journal.pone.0214483

Minh BQ, Nguyen MAT, Haeseler von A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi.org/10.1093/molbev/mst024

Monteiro A, Pierce NE (2001) Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1alpha gene sequences. Molecular Phylogenetics and Evolution 18(2): 264–281. https://doi.org/10.1006/mpev.2000.0872

Mutanen M, Kivelä SM, Vos RA, Doorenweerd C, Ratnasingham S, Hausmann A, Huemer P, Dincă V, van Nieukerken EJ, Lopez-Vaamonde C, Vila R, Aarvik L, Decaëns T, Efetov KA, Hebert PDN, Johnsen A, Karsholt O, Pentinsaari M, Rougerie R, Segerer A, Tarmann G, Zahiri R, Godfray HCJ (2016) Species-level para- and polyphyly in DNA Barcode gene trees: strong operational bias in European Lepidoptera. Systematic Biology 65(6): 1024–1040. https://doi.org/10.1093/sysbio/syw044

Nguyen L-T, Schmidt HA, Haeseler von A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Pecsenye K, Rácz R, Bereczki J, Bátori E, Varga Z (2014) Loss of genetic variation in declining populations of *Aricia artaxerxes* in Northern Hungary. Journal of Insect Conservation 18(2): 233–243. https://doi.org/10.1007/s10841-014-9634-8

Reiprich A, Okáli I (1988–1989) Dodatky k Prodromu Lepidopter Slovenska. Biologické práce SAV, Bratislava, zv. 1 (1988), 135 pp., zv. 2 (1989), 106 pp., zv. 3 (1989), 139 pp.

Sañudo-Restrepo CP, Dincă V, Talavera G, Vila R (2013) Biogeography and systematics of *Aricia* butterflies (Lepidoptera, Lycaenidae). Molecular Phylogenetics and Evolution 66(1): 369–379. https://doi.org/10.1016/j.ympev.2012.10.010

Schmitt T (2017) Molecular biogeography of the high mountain systems of Europe: an overview. In: Catalan J, Ninot JM, Aniz MM (Eds) High Mountain Conservation in a Changing World. Springer International Publishing, Cham, 63–74. https://doi.org/10.1007/978-3-319-55982-7_3

Sielezniew M, Dziekańska I (2010) Motyle dzienne. Multico, Warszawa, 336 pp.

Slámová I, Klečka J, Konvička M (2013) Woodland and grassland mosaic from a butterfly perspective: habitat use by *Erebia aethiops* (Lepidoptera: Satyridae). Insect Conservation and Diversity 6(3): 243–254. https://doi.org/10.1111/j.1752-4598.2012.00212.x

Spitzer L, Beneš J (2010) Nové a významné nálezy denních motýlù a vřetenuškovitých (Lepidoptera) na Valašsku (okres Vsetín, Česká republika). Acta Carpathica Occidentalis 1: 19–39.

Spitzer L, Beneš J, Konvička M (2018) Extinction of *Lasiommata petropolitana* (Fabricius, 1787) (Lepidoptera: Nymphalidae) in the Czech Republic: a case of habitat loss at a range margin. Acta Musei Silesiae, Scientiae Naturales 66(3): 271–279. https://doi.org/10.1515/cszma-2017-0030

Thomas CD, Bodsworth EJ, Wilson RJ, Simmons AD, Davies ZG, Musche M, Conradt L (2001) Ecological and evolutionary processes at expanding range margins. Nature 411(6837): 577–581. https://doi.org/10.1038/35079066

Todisco V, Gratton P, Cesaroni D, Sbordoni V (2010) Phylogeography of *Parnassius apollo*: hints on taxonomy and conservation of a vulnerable glacial butterfly invader. Biological Journal of the Linnean Society 101(1): 169–183. https://doi.org/10.1111/j.1095-8312.2010.01476.x

Tolman T, Lewington R (2008) Collins butterfly guide: the most complete field guide to the butterflies of Britain and Europe. Collins, London, 384 pp.

Trifinopoulos J, Nguyen L-T, Haeseler von A, Minh BQ (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(W1): W232–5. https://doi.org/10.1093/nar/gkw256

Tuzov VK (2000) Guide to the butterflies of Russia and adjacent territories (Lepidoptera, Rhopalocera), Volume 2: Libytheidae, Danaidae, Nymphalidae, Riodinidae, Lycaenidae. Pensoft, Sofia-Moscow, 581 pp.

Uřičář J, Jongepierová I, Vondřejc TE (2016) Zásady péče o významné druhy motýlů Bílých Karpat. ZO ČSOP Bílé Karpaty, Veselí nad Moravou, 56 pp.

Vodă R, Dapporto L, Dincă V, Vila R (2015) Why do cryptic species tend not to co-occur? A case study on two cryptic pairs of butterflies. PLOS ONE 10(2): e0117802. https://doi.org/10.1371/journal.pone.0117802

Wahlberg N, Wheat CW (2008) Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of lepidoptera. Systematic Biology 57(2): 231–242. https://doi.org/10.1080/10635150802033006

Warecki A (2010) Motyle dzienne Polski. Atlas bionomii. Koliber, Nowy Sacz, 319 pp.

Supplementary material 1

The samples of *Aricia* spp. used for species identification in the Czech Republic and TCS haplotype network through its distributional range, including GenBank samples
Authors: Alena Sucháčková Bartoňová, Jiří Beneš, Zdeněk Faltýnek Fric, Martin Konvička
Data type: species data

Link: https://doi.org/10.3897/nl.42.38853.suppl1

Supplementary material 2

NEXUS alignment of *Aricia* spp. cytochrome c oxidase subunit I sequences used for Maximum Likelihood analysis, covering samples from the Czech Republic and samples from GenBank
Authors: Alena Sucháčková Bartoňová, Jiří Beneš, Zdeněk Faltýnek Fric, Martin Konvička
Data type: molecular data

Link: https://doi.org/10.3897/nl.42.38853.suppl2

Supplementary material 3

NEXUS alignment of *Aricia artaxerxes* cytochrome c oxidase subunit I sequences used for haplotype network analysis, covering samples from the Czech Republic and samples from GenBank
Authors: Alena Sucháčková Bartoňová, Jiří Beneš, Zdeněk Faltýnek Fric, Martin Konvička
Data type: molecular data

Link: https://doi.org/10.3897/nl.42.38853.suppl3