Occurrence of *Olethreutes subtilana* (Falkovitsh, 1959) in Central Europe uncovered by DNA barcoding (Tortricidae: Olethreutinae)

Andreas H. Segerer [1], Alfred Haslberger [2] & Theo Grünewald [3]

[1] Zoological Collection of the State of Bavaria (Zoologische Staatssammlung München), Münchhausenstr. 21, 81247 München, Germany; Andreas.Segerer@zsm.mwn.de
[2] Waschau 14, 83317 Teisendorf, Germany; a.haslberger@bglmed.de
[3] Klötzlmüllerstr. 202, 84034 Landshut, Germany; dr_gruenewald@web.de

Abstract. We identified a total of 14 specimens of the olethreutine moth, *Olethreutes subtilana* (Falkovitsh, 1959) from various locations in southern and central Germany (Bavaria, Rhineland-Palatinate, Thuriniga). This species was previously known from European Russia through the eastern Palearctic; hence the findings represent the first records for Central Europe. Specimens were detected among large series of the widespread and common *O. arcuella* (Clerck, 1759); they had been overlooked and confused with the latter, as *O. arcuella*, with its very characteristic wing pattern, was believed to represent the only species of this group in Central and Western Europe. The first *O. subtilana* was only accidentally detected in the course of a genetic all-species survey of the Bavarian animals ("Barcoding Fauna Bavarica"). This paper compiles and illustrates the key characters allowing unambiguous identification, and describes the present state of knowledge of distribution, habitats and phenology in Germany. The impact of the finding is briefly discussed with respect to the potential of DNA barcoding, the value of scientific collecting, and the importance of sufficient sample sizes in faunistic surveys.

Zusammenfassung. Insgesamt 14 Exemplare des Wicklers *Olethreutes subtilana* (Falkovitsh, 1959) wurden von verschiedenen Fundorten in Mittel- und Süddeutschland nachgewiesen (Bayern, Rheinland-Pfalz, Thüringen); dies sind zugleich die ersten mitteleuropäischen Belege dieser nach bisheriger Kenntnis vom Nordwesten des europäischen Russlands bis Ostasien verbreiteten Art. Die Tiere befanden sich innerhalb großer Serien der verwandten, weit verbreiteten und häufigen *O. arcuella* (Clerck, 1759). Sie wurden bisher wahrscheinlich deswegen übersehen, weil man *O. arcuella* als die einzige in West- und Mitteleuropa vorkommende Art aus dieser Gruppe betrachtete, welche nach äußeren Merkmalen als unverwechselbar und eindeutig bestimmbar galt. Die Entdeckung des ersten Stückes erfolgte daher auch zufällig im Rahmen einer laufenden Studie zur genetischen Charakterisierung der bayerischen Tierarten ("Barcoding Fauna Bavarica"). Die vorliegende Arbeit fasst die wesentlichen Unterscheidungsmerkmale beider Arten zusammen und stellt den gegenwärtigen Stand des Wissens zu Verbreitung, Lebensraum und Phänologie von *O. subtilana* in Deutschland dar. Darüber hinaus werden einige übergeordnete Aspekte im Zusammenhang mit den Funden kurz diskutiert: das Potenzial der 'DNA Barcoding' Methode, die Bedeutung von wissenschaftlichen Aufsammlungen und von hinreichender Stichprobengröße.

Introduction

DNA barcoding is a modern molecular approach for efficient *ad hoc* re-identification of species by using a defined signature sequence (Hebert et al. 2003, 2004; Steinke & Brede 2006; Ratnasingham & Hebert 2007; Stoeckle & Hebert 2008). It is claimed that the sequence of the 658 bp long 'barcode region' at the 5' end of the mitochondrial cytochrome oxidase subunit I gene is a marker which allows unambiguous identification of animal species, though this topic remains controversial (e.g., Janzen 2004; Meyer & Paulay 2005; Will et al. 2005; DeSalle 2006; Rubinoff 2006; Rubinoff et al. 2006, Elias et al. 2007) and a number of exceptions to the rule have been reported (e.g., Hurst & Jiggins 2005; Whithwort et al. 2007; Wiemers & Fiedler 2007). Suitability of DNA barcoding for large-scale assessments of Lepidoptera was nevertheless shown by Hebert et al. (2010).

Nota lepidopterologica, 24.01.2011, ISSN 0342-7536

In the course of a current all-species barcoding survey of the fauna of Bavaria, southern Germany (Haszprunar 2009; BFB 2010), we accidentally discovered the presence of the leaf roller *Olethreutes subtilana* (Falkovitsh, 1959) in Germany. This species, originally described from Siberia (Vaganovo, Kemerovskoy oblast), was hitherto known to occur from north-western and central Russia through Siberia, reaching the Amur and Primorye regions, the Kurils, Korea, China and Japan in the east (Kawabe 1982; Kuznetsov 1987; Park & Park 1988; Razowski 2003; Aarvik 2010; Dubatolov 2010). Though being readily identifiable by morphological characters, it has been overlooked in Central Europe and confused with the widely distributed and common species, *O. arcuella* (Clerck, 1759). This paper presents the first records of *O. subtilana* from Central Europe and discusses the impact of this finding under some general aspects.

Abbreviations

AH	Alfred Haslberger
AHS	Andreas H. Segerer
BC	Barcoding
BFB	Barcoding Fauna Bavarica
BOLD	Barcode of Life Data System
CCDB	Canadian Centre for DNA Barcoding
COI	Cytochrome oxidase subunit I
iBOL	international Barcode of Life project
RCAH	Research Collection of Alfred Haslberger, Teisendorf
RCTG	Research Collection of Theo Grünewald, Landshut
TG	Theo Grünewald
ZSM	Zoological Collection of the State of Bavaria, Munich

Methods

Dissection of terminalia was done following the standard protocol of Robinson (1976). Forewing length was measured under a stereo binocular from wing base to apex, excluding the fringes.

As a complement to traditional morphological observations, DNA barcodes were obtained by sampling dry legs from collected specimens and sequencing at the CCDB (Canada) using the standard high-throughput protocol as described in Ivanova et al. (2006); regularly updated protocols used at the CCDB can also be found at: http://www.dnabarcoding.ca/pa/ge/research/protocols. All barcoded specimens of the two species in question are listed below in the paragraph ‘Material’. Images and further details such as voucher hosting institution, GPS coordinates and trace files can be obtained from the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) in the public-access project FBLTO. Sequences were analyzed using BOLD analysis tools. The terms ‘sequence variation’ and ‘genetic distance’ refer to the analysis of the COI 5’ barcode fragment (full length 658 bp) with Kimura 2 Parameter. Genetic distances between species are given in % minimum pairwise distance, infraspecific variation in % maximum pairwise distance. The distribution map was created using the publicly accessible GPS Visualizer website (http://www.gpsvisualizer.com/).

Fig. 1. *Olethreutes subtilana* (left column) and *O. arcuella* (right column) from Germany, each column displaying a specimen of very small (top), typical (middle) and very large size (bottom). The specimen in the upper left was the first *O. subtilana* recognized in Germany (♂, Salzachau near Laufen, 2.6.2008; leg. et coll. A. Haslberger, barcode no. BC ZSM Lep 25167). The comparatively large size of the specimen in the lower left is untypical for *O. subtilana* (♀, Huberspitz, M.6.1993, leg. et coll. A. Speckmeier (in ZSM). Scale bar, 1 cm.

Material. Olethreutes subtilana (Falkovitsh, 1959): 1♂, **Russia**, Tolmatschewo, Leningradsk. Obl.[ast], 14.vi.1961, leg. et det. Falkovitsh; ZSM (coll. Klimesch), slide M3667-AHS; 2♂, same data, not dissected; 1♀ dto., 12.vi.1961; slide M3668-AHS. 1♂, **Germany**, Thüringer Wald, 30.v.[no year specified, estimated ~1920], [collector unknown]; ZSM, slide M3677-AHS. 1♂, Oberbayern, Salzachau bei Laufen, 400m, 2.vi.2008, leg. A. Haslberger; RCAH, slide 111/2010 AH, BC ZSM Lep 25167, GenBank accession No. HM422134. 1♂, Emmerting/Alzau, 2.vi.1997, leg. L. Wihr; ZSM (coll. Wihr), slide M3680-AHS. 1♀, Hammer bei Siegsdorf, 26.vi.1994, leg. L. Wihr; ZSM (coll. Wihr), slide M3685-AHS. 1♂, Huberspitz, 800 m, M.vi.1993, leg. A. Speckmeier; ZSM (coll. Speckmeier), slide M3670-AHS; 1♀, same data, slide M3684-AHS; 3♀, same data, not dissected. 1♀, Pattenberg, 19.vi.1994, leg. L. Wihr; ZSM (coll. Wihr), slide M3688-AHS. 1♀, Pechschnait bei Traunstein, 1.vii.1994, leg. L. Wihr; ZSM (coll. Wihr), slide M3681-AHS. 1♀, Munich, Obermenzing, A.vi.1948, leg. H. Pfister; ZSM (coll. Pfister), slide M3671-AHS. 1♂, Dattinger Berg, 7.vi.2002, leg. T. Grünewald; RCTG, slide TG 01/2010-AHS. 1♀, Rhineland-Palatinate, Neustadt/Weinstraße, 31.v.1964, leg. U. Roesler senior; ZSM (coll. Roesler sen.), slide M3683-AHS. – Olethreutes arcuella (Clerck, 1759): 1♂, **Bulgaria**, Burgas, Primorsko, Arkutino Marshland Reserve, 25.v.–15.vi.1980, leg. Eichler; ZSM (coll. Eichler). 3♂, **Romania**, Südkarpathen, Transsylvanien, Cibinsgebirge, 25.v.1918, leg. Dannehl; ZSM, slide M3682-AHS. 1♀, **Slovakia**, Karpaten, Slowakischer Karst, Zádielska planina, 28.vi.1952, leg. Zouhar; ZSM (coll. Eichler), slide M3669-AHS. 1♂, Košický kraj, Turňa nad Bodvou, 30.v.1970, leg. Zouhar; ZSM, slide M3687-AHS. 1♂, **Czech Republic**, Mittelböhmen, [Praha-]Krč, 10.viii.1956, leg. Vorlíček; ZSM. 1♂, **Austria**, Steiermark, Liezen, Ardning, Pürgschachener Moos, 2.vii.1982, leg. J. Klimesch; ZSM (coll. Klimesch). 1♂, Niederösterreich, Leitzersdorf, Rohrwald, 19.v.1922, [collector unknown]; ZSM. 1♀, Niederösterreich, Leitzersdorf, Rohrwald, Waschberg, 31.v.1923, leg. Ortner; ZSM (coll. Klimesch), slide M3673-AHS. 1♂, Oberösterreich, St. Dionysen b. Traun, 29.v.1946, leg. J. Klimesch; ZSM (coll. Klimesch). 1♀, Land Salzburg, Muhr, 1200m, 15.vi.2004, leg. A. Haslberger, RCAH, slide 114/2010 AH. 1♀, Nordtirol, Landeck, Kauns, 1.–14.ix.1953, leg. Daniel; ZSM. 1♀, **Germany**, Mecklenburg-Vorpommern, Heringsdorf, Ahlbeck/Swinemünde, 5.vi.1941, [collector unknown]; ZSM. 1♂, Niedersachsen, Harz, Goslar-Grauhof, Grauhöfer Holz, 30.v.1915, leg. Bauer; ZSM, slide M3678-AHS. 1♀, Sachsen-Anhalt, Bitterfeld-Wolfen, Goitzsche, 1.vi.1914, [collector unknown]; ZSM. 1♂, Naumburg/Saale, Sperlingsholz, 17.v.1911, leg. Bauer; ZSM. 1♀, Thüringer Wald, [no data]; ZSM, slide M3686-AHS. 1♂, Pfalz, Neustadt/Weinstraße, 2.vii.1963, leg. U. Roesler senior; ZSM (coll. Roesler sen.). 1♂, Bavaria, Mittelfranken, Lauf (Pegnitz 16.vi.1972, leg. H. Pröse; ZSM (coll. Pröse), slide M3676-AHS. 1♀, Oberpfalz, „Regensburg", no data, leg. E. Frank; ZSM (coll. Osthelder). 1♂, Oberpfalz, Etterzhausen, 17.vi.1965, leg. Kuchler; ZSM (coll. Fauna Bavarica). 1♀, Oberpfalz, Nittendorf, Tf 5.vi.2009, leg. A. Segerer; ZSM (coll. Segerer), BC ZSM Lep 25740, GenBank Accession No. GU707046. 1♀, Oberpfalz, Nittendorf, Tf 30.v.2009, leg. A. Segerer; ZSM (coll. Segerer), BC ZSM Lep 25481, GenBank accession No. GU706704. 1♀, Oberpfalz, Pfatter, Geisling, 16.vi.1941, leg. M. Sälzl jun; ZSM (coll. Pfister), slide M3666-AHS. 1♀, Oberpfalz, Regenstauf, Linglhof, Tf 27.vi.1995, leg. A. Segerer; ZSM (coll. Segerer), BC ZSM Lep 28613, GenBank accession No. HM391970. 1♀, Oberpfalz, Schwarzenbach, Parkstein-Hütten, Lf 26.vi.1999, leg. A. Segerer; ZSM (coll. Segerer), BC ZSM Lep 25249, GenBank accession No. GU706703. 1♂, Niederbayern, Kelheim, 9.vi.1918, leg. L. Osthelder; ZSM, slide M3674-AHS. 1♀, Niederbayern, Eugenbach, 30.v.–1.vi.1996, leg. T. Grünewald; RCTG, BC ZSM Lep 23174, GenBank accession No. HM391732. 1♂, dto., 28.v.1942, leg. M. Sälzl jun.; coll. Pfister, ZSM, slide M3665-AHS. 1♀, Oberbayern, Berg, Sibichhausen, 5.vi.1969, leg. Pröse; ZSM (coll. Pröse). 1♂, Oberbayern, Bernau/Chiemsee, 12.vi.1916, leg. Dorsch; ZSM (coll. Fauna Bavarica). 1♂, Oberbayern, Grafrath, 27.vi.1969, leg. A. Speckmeier; ZSM (coll. Speckmeier). 1♂, Oberbayern, Miesbach, Miesbach, 1.vi.1952, leg. Freund; ZSM (coll. Fauna Bavarica). 1♂, Oberbayern, Mittenwald, 3.vi.1923, leg. Bauer; ZSM (coll. Fauna Bavarica). 1♂, dto., Hoher Kranzberg, 27.vi.1922, leg. Bauer; ZSM (coll. Fauna Bavarica). 1♂, Oberbayern, Oberschleißheim, 26.v.1949, leg. Marx; ZSM (coll. Fauna Bavarica). 1♂, dto., 24.vii.1948, leg. Marx; ZSM (coll. Fauna Bavarica). 1♂, Oberbayern, Starnberg, 12.vi.1955, leg. Stadelhofer; ZSM (coll. Fauna Bavarica). 1♀, Oberbayern, Obing, Griessee, 11.vi.1947, leg. L. Osthelder; ZSM (coll. Fauna Bavarica). 1♂, Oberbayern, Sachsenkam, Kirchsee, 16.vi.1931, leg. L. Osthelder; ZSM (coll. Osthelder), slide M3672-AHS. 1♂, Oberbayern, Samerberg, Hochries, 2.vii.1993, leg. Heinsdorff; ZSM. 1♂, Oberbayern, Siegsdorf, Hammer, 15.vi.1953, leg. Wihr; ZSM (coll. Wihr). 1♂, Oberbayern, Marktlerforst/Überfuhr, 26.v.1997, leg. L. Wihr; ZSM (coll. Wihr), slide M3679-AHS. 1♀, Oberbayern, Salzachau bei Fridolfing/Nilling, 370m, 24.vi.2010, leg. A. Haslberger, RCAH, slide 113/2010 AH. 1♀, Oberbayern, Schneizlreuth, Baumgarten/Rötelbach, 800 m, 27.vi.2005, leg. A. Haslberger; RCAH, slide 112/2010 AH, BC ZSM Lep 23463, GenBank accession No. GU706567. 1♀, Oberbayern, Melleck, Steinbachtal, 580m, 1.vii.2010, leg. A. Haslberger, RCAH, slide 115/2010 AH. 1♂, **France**, Lorraine, Vosges, Haute Moselotte, Ventron, 13.vi.1940, leg. Fischer; ZSM (coll. Osthelder).

Results

Discovery of *Olethreutes subtilana* in Germany. *Olethreutes arcuella* (Clerck, 1759) – the type species of *Olethreutes* Hübner, 1822 – is a well-known leaf roller occurring in wood- and scrubland of almost any European country (Razowski 2003; Aarvik 2010). In the second half of the 20th century, two species of similar appearance were described from Russia, but thought to represent eastern palearctic vicariants not present in Central Europe: *O. subtilana* (Falkovitsh, 1959) and *O. captiosana* (Falkovitsh, 1960) (see also Aarvik 2010).

The discovery of *O. subtilana* in Germany just happened by chance in the course of the genetic BFB survey of Bavarian animals. Although the BFB database of *O. arcuella* had been already completed, we decided to include a further specimen which stood out from a series of *O. arcuella* by its exceptionally small size. To our great surprise, its COI 5' partial sequence ('barcode') turned out to be markedly different from that of

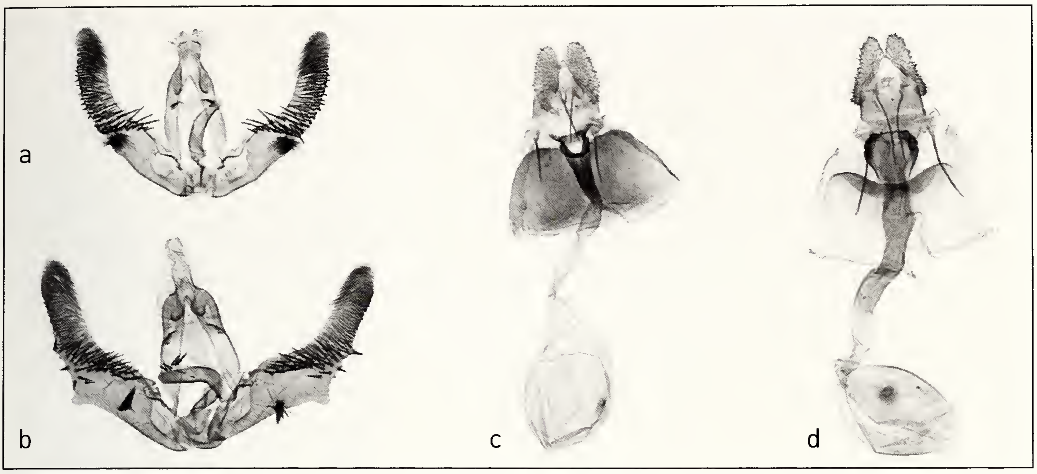

Fig. 2. Terminalia of *O. subtilana* and *O. arcuella*. **a:** *O. subtilana*, male, prep. M3677-AHS. **b:** *O. arcuella*, male, prep. M3672-AHS. **c:** *O. subtilana*, female, prep. M3671-AHS. **d:** *O. arcuella*, female, prep. M3666-AHS. Figures are not given to the same scale.

O. arcuella (6.8 % genetic distance), suggesting that it could belong to a distinct species of *Olethreutes*. For comparison, the mean distance of species within Olethreutinae genera is 8.9%, the mean variation within species 0.6% ($n = 455$; preliminary, original data from the BFB project, queried from BOLD as of June 30, 2010).

Subsequent dissection of the specimen in question showed the species to be *O. subtilana*. Morphological follow-up screening of our collections unveiled the presence of even more specimens of *O. subtilana* from further places in southern and central Germany (see chapter 'Material' above, and fig. 3), hitherto unrecognized within large series of *O. arcuella*. To date, we have been able to trace 14 specimens of *O. subtilana* among a total of ~250 Central, Western and Eastern European specimens of '*O. arcuella*' present in our collections.

Differential diagnosis. Though strongly resembling *O. arcuella* in its external markings, the wingspan of *O. subtilana* is conspicuously small. (The name '*subtilana*' was therefore very appropriately chosen). Of 18 specimens examined thus far, all but one were distinctly smaller than even the very smallest *O. arcuella*; only one exceptionally large specimen had a forewing length in the range of very small *O. arcuella* (6.8 mm) (Tab. 1, Fig. 1 – lower left).

Thus, size is a good marker for selecting specimens worth studying in further detail, and the very smallest ones of a series should be dissected to confirm the identity. The terminalia of both species are highly distinctive in both sexes, allowing unambiguous identification. As the original description of Falkovitsh (1959) may not be familiar to all microlepidopterists, and the figures of the adults in Razowski (2003) are unfortunately drawn to the same size (i.e., to different scales), we give a compilation of the essential differences of both species below in order to facilitate identification (Tab. 1, Figs 1–2). Alternatively, the species can be easily recognized by DNA barcoding (for access to reference data, see chapter 'Methods').

Tab. 1. Morphological key characters suitable for identification of *O. subtilana* and *O. arcuella*. * Only the smallest 45 out of a series of ~250 specimens were measured.

character	***O. subtilana***	***O. arcuella***
Length of forewing (Fig. 1)	5.4–6.8 mm mean 6.0 mm (n = 18) Overall aspect of specimens in almost any case distinctly smaller than *O. arcuella*; only 1 specimen was in the size range of *O. arcuella*	Small specimens: 6.5–8.5 mm* mean: 7.2 mm (n = 45)* Overall aspect of specimens in almost any case distinctly larger than *O. subtilana*
Male terminalia (Fig. 2) Valva	Ventral margin simple, not markedly irregular nor conspicuously edged. Tuft of bristles on sacculus located close to cucullus	Sacculus with a strong, conspicuous outer edge and an irregularly scalloped margin posterior of edge. Tuft of bristles on sacculus located closer to basal opening than to cucullus
Phallus	Vesica simple, cornuti absent	Vesica containing ~10 small cornuti, usually visible at the posterior tip of phallus
Uncus	Distinctly notched at apex	Barely notched at apex
Female terminalia (Fig. 2)	Antrum simple, cylindric to funnel-shaped, with ring-like ostium bursae	Posterior part of antrum protruded, conspicuously swollen around ostium bursae, like a knob

Distribution, habitats and phenology. Distribution and frequency of *O. subtilana* in Central Europe are still poorly understood.

Our efforts to verify *O. subtilana* from C and W European countries other than Germany were thus far in vain (see chapter 'Material' above), but we have checked the available material from our own collections only, and no systematic survey on a larger scale has been undertaken yet.

Our present observations suggest that *O. subtilana* might be a quite local and possibly rare species. All known places of occurrence are within, or at least closely associated to, mountainous regions (Alps, Bavarian Forest, Thuringian Forest, and Palatine Forest) (Fig. 3) and lie generally below 900 m altitude. Adults are active during day, flying in deciduous wood- and scrubland of different types (including alluvial forests, bog forests, trivial woodland and parks). Behaviour and biotopes are obviously similar to those of *O. arcuella*, and in most places both species occur syntopically.

The bulk of specimens known to date is from south eastern Bavaria but this may well be an artefact: the records from other, widely separated parts of Germany (Thuringia, Palatinate) rather indicate that the species may have a much wider distribution than previously thought. We expect to find further populations especially in the low mountain ranges of Germany as well as in adjacent countries, but possibly also in more distant areas.

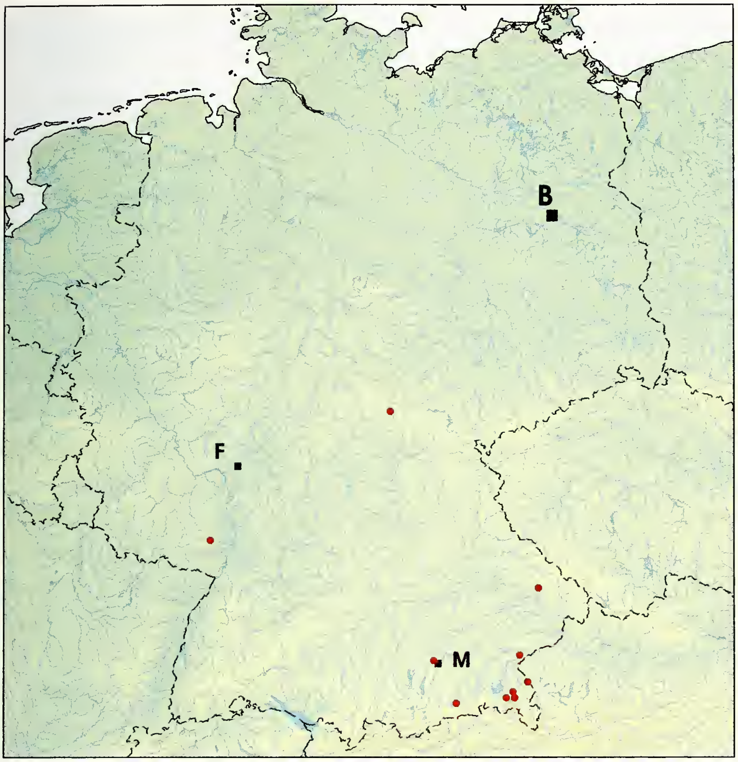

Fig. 3. Records of *O. subtilana* in Germany (red). To facilitate orientation, the cities of Berlin (B), Frankfurt/Main (F) and Munich (M) are displayed in black (Map produced with GPS Visualizer).

Most specimens of *O. subtilana* were caught within the first half of June, about one week later than the peak flight of *O. arcuella*; this observation, however, is statistically not well supported and may be artificial due to small sample size (Fig. 4).

Discussion

Though *O. subtilana* was already known from Eastern Europe (north-western and central Russia: Kuznetsov 1987), the identity of *O. arcuella* as the sole species of the group occurring in Central Europe was obviously never in doubt until Razowski (2003) and Aarvik (2010). This may at least in part be due to the fact that *O. arcuella* is very common and widely distributed and cannot be mistaken for any other palearctic tortricid, except for the two 'eastern' species mentioned above. Thus, visual diagnosis has been considered to be safe and unequivocal for identification of central European '*O. arcuella*' and there seemed to be no need for any closer examination of series by collectors. As a result, *O. subtilana* has been overlooked in Central Europe for decades. Retrospectively, its occurrence in Germany is not completely surprising. It seems quite evident that the zoogeographic pattern of its distribution refers to a so called 'eurosiberian' species, the range of which reaches further to the west than previously thought (Lattin 1967: 378). The question of the exact western and southern boundaries of its range must be left open at present.

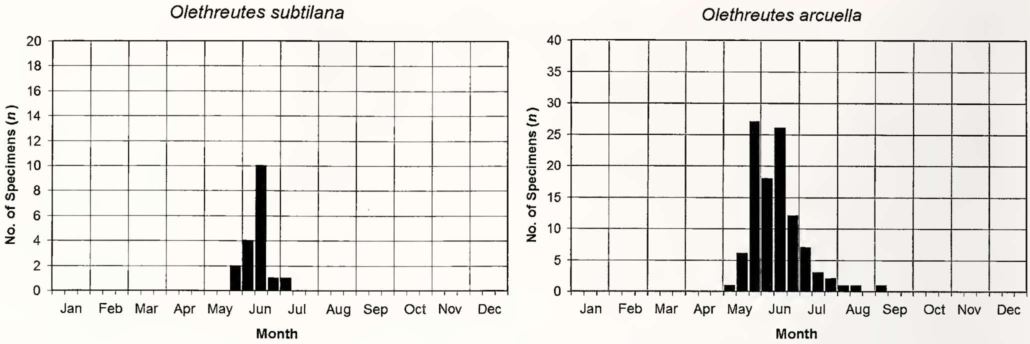

Fig. 4. Phenograms of adult flight of *O. subtilana* (n = 18) and *O. arcuella* (n = 105), as calculated from the data of the specimens listed in section 'Material'.

O. subtilana does not seem to be a neozoon currently expanding its area. Instead, there are records dating back to the 1940s and probably the 1920s (see chapter 'Material'). The seeming accumulation of records in recent years in south-eastern Bavaria is most probably a bias caused by enhanced microlepidoterological collecting activities in this area since the 1990s.

In order to elucidate the pattern of distribution, we encourage European collectors to check their series of '*O. arcuella*', and to collect very small '*O. arcuella*' systematically in the field.

Beyond being a finding of mere zoogeographical and faunistic interest, the detection of *O. subtilana* in Germany is also an issue of more general impact:

First, the finding adds substance to a growing body of literature corroborating the potential of DNA barcoding for species identification, biodiversity assessment, biogeography and other disciplines. The late detection of *O. subtilana* in Central Europe despite the extensive work of so many lepidopterists is clearly a case where DNA barcoding has effectively and efficiently enhanced the knowledge of the German fauna, demonstrating that the method is well capable of challenging pre-existing hypotheses about the spectrum of species occurring within a certain area. Although the demands and efficacy of DNA barcoding have been a matter of controversy (e.g., Janzen 2004; Meyer & Paulay 2005; Will et al. 2005; DeSalle 2006; Rubinoff 2006; Rubinoff et al. 2006, Elias et al. 2007), and there are some papers reporting poor performance and pitfalls in some groups (e.g., Hurst & Jiggins 2005; Whithwort et al. 2007; Wiemers & Fiedler 2007), the general benefits for identification of species are clearly evident. As a preliminary result of the BFB project, the method is suitable for unambiguously identifying approximately 98% of the Bavaria moth species (Hausmann, Segerer, unpublished). The remainder corresponds to interesting cases of barcode sharing. In contrast, about 5% of the species show deep infraspecific COI-splits (Hausmann, unpublished), but this is not affecting re-identification because these haplotypes do not match with other species. Such genetic polymorphisms, including possible cryptospecies need to be studied in detail in the future. Secondly, the detection of *O. subtilana* in Central Europe is a textbook example of the importance of scientific collecting. Though there is general agreement that collecting is an indispensable scientific standard in entomology, it has been and is consistently claimed even by some professionals that in-field identification of 'unmistakable' species (either by sight or photography) is equivalent to collecting, in terms of both efficiency, protection of natural resources, and nature conservation. Following this argument, *O. subtilana* would not have been detected in Bavaria, due to confusion with another species hitherto thought to be "absolutely unmistakable". Rather, it was the presence of large series of '*O. arcuella*' collected from many different places that eventually led to the discovery of *O. subtilana*. Countless numbers of '*O. arcuella*' have been recorded by in-field sighting of which the accuracy has to be questioned now.

A third lesson that can be learned is about the importance of sufficiently large sample sizes in barcoding (or any other faunistic) studies. The particular project design of BFB generally limits the number of specimens to be barcoded to four per species, preferably from geographically distinct and widely separated areas of Bavaria. This strategy is evidently sufficient for most, but not all cases: While *O. arcuella* is very common in Germany, *O. subtilana* apparently is not. The occurrence of the latter would have been definitely overlooked in the course of BFB simply for statistical reasons, unless we had not decided to extend the database of *O. arcuella* to a further specimen of somewhat 'strange' appearance. We conclude that some entomological intuition can be important and helpful when selecting specimens for studies restricted to small sample sizes.

Acknowledgements

We thank Paul Hebert and his colleagues at the Biodiversity Institute of Ontario (Canada) for their enthusiastic support of our project and the agencies that support their work: Genome Canada, the Ontario Ministry of Research and Innovation, and the Natural Sciences and Engineering Research Council of Canada (NSERC). We are highly grateful to Axel Hausmann (ZSM) for invaluable discussions and critical comments on the paper. Further thanks to Miki Sakamoto (Neuötting) for translating Japanese literature, Olga Schmidt (ZSM) for her help with Russian literature, Paul Sokoloff (Kent, UK) for improving the English, and Ulf Buchsbaum and Mei-Yu Chen (both: ZSM) for excellent technical support. The current study is part of the „Barcoding Fauna Bavarica" project (BFB) which is financially supported by the Bavarian Ministry of Science, Research and Art (Bayerisches Staatsministerium für Wissenschaft, Forschung und Kunst, Munich, Germany).

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