

Morphology and DNA barcodes show that *Calybites hauderi* does not occur in the British Isles (Gracillariidae)

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Abstract. Evidence is presented that all British specimens of *Calybites hauderi* (Rebel, 1906) are not that species but the first brood of bivoltine *Caloptilia semifascia* (Haworth, 1828). *C. hauderi* is removed from the British list and its occurrence in Belgium is questioned. *C. semifascia* is normally univoltine in the British Isles but bivoltine populations are now spreading in southern counties.

Zusammenfassung. Der Nachweis wird erbracht, daß es sich bei allen britischen *Calybites hauderi* (Rebel, 1906) um die erste Generation der bivoltinen *Caloptilia semifascia* (Haworth, 1828) handelt. *C. hauderi* wird von der britischen Liste gestrichen und das Vorkommen in Belgien wird angezweifelt. *C. semifascia* ist auf den Britischen Inseln normalerweise univoltin doch breiten sich in den südlichen Grafschaften zunehmend bivoltine Populationen aus.

Introduction

Calybites hauderi (Rebel, 1906) was first recorded from Britain in 1933 by L. T. Ford under the name *Gracilaria* [sic] *pyrenaeella* (Chrétien, 1908) as identified by E. Meyrick (Ford 1933: 230). Ford had reared nine adults in early July 1933 from a quantity of the characteristic cones he had collected on 9 June on field maple (*Acer campestre* L.). The single locality was a small area of woodland with much *Acer campestre* at St Helen's, near the coast in the east of the Isle of Wight. The first record from the English mainland was in 1991, when the species was discovered in West Sussex (Agassiz et al. 1993: 162). It appeared in Hampshire in 2000 (Langmaid & Young 2001: 244), since when it has been recorded there regularly, in Surrey in 2007 (JRL, pers. obs.), Oxfordshire in 2008 (Sims 2009: 169) and Kent in 2009 (P. A. Sokoloff, pers. comm.).

In the British literature this species was variously recorded as *Gracilaria* [*sic*] *pyrenaeella* (Ford 1933: 230), *Caloptilia pyrenaeella* (Fletcher 1940: 8; Wakely, 1960: 247), *Euspilapteryx* (*Gracilaria*) *pyrenaeella* (Wakely 1962: 120), *Calybites pyrenaeella* (Bradley et al. 1972: 9; Emmet 1979: 53), *Calybites hauderi* (Emmet et al. 1985: 273) and *Caloptilia hauderi* (Bradley 1998: 9). It is worth noting that Emmet et al. (loc. cit.) expressed doubt about the validity of *Calybites* Hübner, 1822, and *Caloptilia* Hübner, 1825, as distinct genera in view of their extremely similar biology.

Gracilaria [sic] *hauderi* was originally described from a pair of specimens collected by Hauder in Austria, Oberösterreich, Kirchdorf [not Kirschdorf!] on 18.viii.1904 (male) and 13.iv.1905 (female). *Gracilaria* [sic] *pyrenaeella* was originally described from the French Basses-Pyrénées from an unspecified number of adults reared by Chrétien from larvae that lived on *Acer campestre*, much in the manner of other '*Gracilaria*' species,

Fig. 1. *Caloptilia falconipennella* first brood f. oneratella.

i.e. initially mining and later producing the characteristic cones. The adults emerged in late June and July and Chrétien did not observe a second brood although he suspected there might be one. For an English translation of Chrétien's description see Fletcher (1940: 8). Subsequently, Leraut (1983: 36) synonymized *G. pyrenaeella* with *G. hauderi*, after designating lectotypes for both, and transferred the species to the genus *Calybites*. It is currently listed in the Global Taxonomic Database of Gracillariidae (http://gc.bebif.be) as *Calybites hauderi* (= *pyrenaeella*) and its distribution is given as Austria, Belgium, Czech Republic, France, Hungary, Italy, Romania, Switzerland and United Kingdom.

C. hauderi was recorded in Belgium on the strength of two photographs taken in 2004 of a moth that had subsequently been released (De Prins et al. 2005: 53–54, fig. 1). However, with its sub-triangular rather than sub-quadrate costal blotch in the forewing, the specimen looks more like the first brood form *oneratella* Zeller, 1847, of *Caloptilia falconipennella* (Hübner, 1813) (Fig. 1) and the occurrence of *C. hauderi* in Belgium must be considered as unconfirmed. All records of *C. hauderi* elsewhere should be reassessed in the light of our findings.

Material and Methods

Suspicions that all was not as it seemed arose in 2008 when a huge abundance of *Caloptilia* spinnings was found in a small grove of *Acer campestre* trees on some common land in Portsmouth, Hampshire, in early June. These spinnings produced moths in July all of which were, apparently, '*hauderi*' (Fig. 3). This was followed in August of the same year with an equally great abundance of spinnings at the same locality (Fig. 2) all of which produced specimens typical of *C. semifascia* (Haworth) (Fig. 4) in September of that year. Although this was the situation in Portsmouth, which is on the south coast of England, it was different 50 km inland at Farnham in Surrey. Here, between 19 May and 12 June 2007, a large number of *Caloptilia* spinnings were collected in the hope of breeding *C. hauderi*. Of approximately one hundred moths that were reared, only two were '*hauderi*' whereas all others were typical *semifascia*.

Many British specimens of what was thought to be *hauderi* (Fig. 3), including some from Ford's locality in the Isle of Wight, and typical *semifascia* (Fig. 4) were dissected following standard protocol.

DNA was extracted from adult specimens (dry hind legs) using the routine protocol of the CCDB (Ivanova et al. 2006 and the CCDB website: www.dnabarcoding.ca/pa/ge/research/protocols). The 'DNA barcode' region of COI was amplified, sequenced and analysed following the protocol described in De Prins et al. 2009.

In total ten individuals of *Caloptilia* were barcoded (Tab. 1); *Caloptilia stigmatella* (Fabricius, 1781) is used as outgroup for the analysis.

Fig. 2. *Caloptilia semifascia* larval spinnings on *Acer campestre*.

Records for those specimens are gathered within the project 'Gracillariidae – PUBLIC records' (code GRPUB) in the Published Projects section of the Barcode of Life Data systems (BOLD; www.barcodinglife.org) (Ratnasingham & Hebert 2007). Information on specimen vouchers (field data and GPS coordinates) and sequences (nucleotide composition, trace files) are found in this project by following the 'view all records' link and clicking on the 'specimen page' or 'sequence page' links for each individual record. Sequences are also available on GenBank (Tab. 1).

Results

The female genitalia show no differences between the two British forms, '*hauderi*' and *semifascia*. In the males (Figs 5–7) there are only minor differences in the shape of the cucullus between what we were now recognising as the two superficially distinguishable broods of a single species, *C. semifascia*, and even those differences are not consistent. A request was then made to the Naturhistorisches Museum, Vienna, for the loan of the lectotype male and paralectotype female genitalia slides of *Calybites hauderi*. Examination of them showed that our British specimens definitely did not belong to that species and were not even congeneric with it. The true *hauderi* male is distinct in having a strong ventral spine at about the middle of the valva; such a spine is absent in *Caloptilia*. The *hauderi* female has only one signum in the corpus bursae in contrast to the pair of signa present in *semifascia* and other *Caloptilia* species.

DNA barcodes were obtained for ten specimens of *Caloptilia*; all sequences are complete barcodes of 658 bp except for one (GRACI353-08), which is 398 bp long. Two haplo-

Figs 3–4. *Caloptilia semifascia.*
3. First brood form.
4. Second brood form.

types are reported; one occurs in Denmark (GRPAL118-10) and the Czech Republic (GRACI449-09) and is distinct by a single nucleotide substitution. The mean genetic variation within *Caloptilia semifascia* is 0.05, with a maximum distance of 0.15 between the nine individuals analysed. Interspecific distances are high in *Caloptilia* with up to 11.8 between *C. semifascia* from Denmark (GRPAL118-10) and *C. stigmatella*. The interspecific distances observed within the genus *Caloptilia* are on average 10.73 but can go up to 15.64 (Lopez Vaamonde unpublished data). These values are as high as those found in the genus *Phyllonorycter* (De Prins et al. 2009).

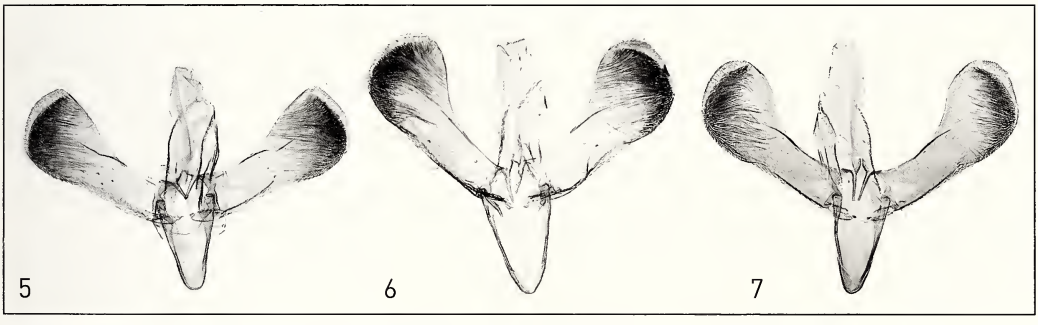
These divergences were calculated using Kimura's 2 parameter model (K2P), since it takes into account the possibility that the rates of substitutions (transitions and transversions) per site may vary. This is clearly the case for mitochondrial DNA where transitions are generally more frequent than transversions and therefore this model is normally used in Barcode studies to calculate distance values. Please see Hall (2008) and Page & Holmes (1998) for further details about the K2P model and other distance measures for nucleotide sequences.

DNA barcoding, that is the use of a single genetic marker (i.e. COI) to assign the name of a known species to a specimen of unknown identity has been criticised among other things because sometimes two different species may have the same DNA barcode, for instance some *Grammia* Rambur, 1866 (Arctiidae) (Schmidt & Sperling, 2008) and *Agrodiaetus* Hübner, 1822 (Lycaenidae) species (Wiemers & Fiedler, 2007). However, species pairs with 0% interspecific divergence are rare as shown by Hebert et al. (2009) in a large survey of more than 1300 Lepidoptera species from the eastern half of North America. They found only nine pairs of species that shared the same barcode. These cases always involved closely related species. In our case the similarity of both DNA barcodes and genitalia morphology among all nine individuals examined clearly indicates that the British material of '*C. hauderi*' belongs to *C. semifascia*.

It is therefore apparent that *C. semifascia*, which was previously thought to be univoltine in Britain, was actually bivoltine in the Isle of Wight locality at the time of Ford's discovery in 1933. It has become so also in some southern counties of the English mainland over the past twenty years. Furthermore, the massive infestation of the *Acer campestre* trees in Portsmouth, together with the observation that only a single parasitic hymenopteran was bred from a hundred or so spinnings, might indicate a recent invasion of the bivoltine form of *semifascia* from either continental Europe or possibly the "*hauderi*" locality in the Isle of Wight. The fact that the first brood larvae from Portsmouth all

Tab. 1. Samples used for the DNA barcoding analysis. The SampleID code is a unique identifier linking the record in the BOLD database and the voucher specimen from which the sequence is derived. Additional collecting and specimen data are accessible in BOLD's public project GRPUB, as well as all sequence data.

Sample ID	Species	Country	Barcode Number (BOLD)	Accession number (NCBI GENBANK)
CLV0409	*Caloptilia semifascia* (identified as *C. hauderi*)	UK	IBERO004-09	GU695245.1
CLV0309	*Caloptilia semifascia* (identified as *C. hauderi*)	UK	IBERO003-09	GU695244.1
CLV24508	*Caloptilia semifascia* (identified as *C. hauderi*)	UK	GRACI353-08	HQ171490
CLV0109	*Caloptilia semifascia*	UK	IBERO001-09	GU695242.1
CLV21808	*Caloptilia semifascia*	UK	GRACI326-08	HQ171489
CLV0209	*Caloptilia semifascia*	UK	IBERO002-09	GU695243.1
G09semi	*Caloptilia semifascia*	Czech Rep.	GRACI449-09	HQ171488
DP09127	*Caloptilia semifascia*	Denmark	GRPAL117-10	HM392581.1
DP09128	*Caloptilia semifascia*	Denmark	GRPAL118-10	HM392582.1
G08stigm	*Caloptilia stigmatella*	Portugal	GRACI448-09	HQ171491



Figs 5–7. Male genitalia of *Caloptilia semifascia*. **5–6.** First brood. **7.** Second brood.

produced the form resembling *hauderi* and the ones from Farnham mostly produced specimens typical of the second brood of *semifascia* lends some weight to that possibility. *C. semifascia* is recorded from almost all European countries and Morocco, Tajikistan, Turkey and Turkmenistan (De Prins & De Prins 2010). Unfortunately we are unable at this stage to comment on its bivoltinism in continental Europe because of confusion with the first brood of *C. falconipennella* and the true *Calybites hauderi*.

It should be noted that the name *onustella* Hübner, 1813, was sometimes applied to a form of *semifascia* Haworth, 1828, and would, of course, antedate the latter (Karsholt 1996: 303). In fact, World Catalogue of Insects (De Prins & De Prins 2005: 110) and the Global Taxonomic Database of Gracillariidae (Lepidoptera) (http://gc.bebif.be) record *Caloptilia onustella* Hübner as a valid species, with *semifascia* in synonymy. Serious doubt about the identity of *onustella* with *semifascia* was raised by JRL, and a recent reassessment does indeed indicate that Hübner's name does not apply to a *Caloptilia*

species (Bengtsson 2010: 106). We therefore continue to use *semifascia*, the name universally applied to this species in the British entomological literature.

Acknowledgements

Thanks are due to Sabine Gaal-Haszler (Naturhistorisches Museum, Vienna), for the loan of the genitalia slides of *Calybites hauderi*. We would also like to thank Bengt Å. Bengtsson (Färjestaden), Jurate De Prins (Tervueren), Ole Karsholt (Copenhagen), Martin Honey (Natural History Museum, London), Zdeněk Laštůvka (Brno), David Lees (Orléans), Rodolphe Rougerie (Rouen), Ian Sims (Reading), Paul Sokoloff (Orpington), Poul Szyska (Gedser), Ian Thirlwell (Southsea) and Gerry Tremewan (Truro), for information, specimens, logistic support, discussions and comments on the manuscript. Rodolphe Rougerie (Rouen), is also thanked for processing the DNA samples at Guelph Barcoding Center. Sequencing of DNA barcodes was supported by a grant from Genome Canada to the international Barcode of Life project (iBOL).

References

Agassiz, D., R. Heckford & J. Langmaid 1993. Microlepidoptera Review for 1991. – Entomologist's Record and Journal of Variation **105** (7–8): 159–170.

Bengtsson, B. Å. 2010. *Caloptilia jurateae* sp. n., a sibling species of *C. semifascia* (Haworth, 1828) (Lepidoptera Gracillariidae). – Entomologisk Tidskrift **131** (2): 105–112.

Bradley, J. D. 1998. Checklist of Lepidoptera Recorded from the British Isles. – Private Publication, Fordingbridge, Hants. vi+106 pp.

Bradley, J. D., D. S. Fletcher & P. E. S. Whalley 1972. Lepidoptera. – *In*: G. S. Kloet & W. D. Hincks, A Check List of British Insects, Second Edition, Part 2. viii+153 pp. Royal Entomological Society, London.

Chrétien, P. 1908. Microlépidoptères nouveaux pour la faune française. – Le Naturaliste **30**: 245–246.

De Prins, J. & W. De Prins 2010. Global Taxonomic Database of Gracillariidae (Lepidoptera). Royal Museum for Central Africa, Belgian Biodiversity Platform, Tervuren, Brussels, Belgium. – http://gc.bebif.be (accessed 21 April 2010).

De Prins, J., W. De Prins & M. Jacobs 2005. *Caloptilia hauderi*, a new species for the Belgian fauna (Lepidoptera: Gracillariidae). – Phegea **33** (2): 53–54.

De Prins, J., R. Mozūraitis, C. Lopez-Vaamonde & R. Rougerie 2009. Sex attractant, distribution and DNA barcodes for the Afrotropical leaf-mining moth *Phyllonorycter melanosparta* (Lepidoptera: Gracillariidae). – Zootaxa **2281**: 53–67.

Emmet, A. M. 1979. A Field Guide to the Smaller British Lepidoptera. – The British Entomological and Natural History Society, London. 271 pp.

Emmet, A. M., I. A. Watkinson & M. R. Wilson 1985. Gracillariidae. – *In*: J. Heath & A. M. Emmet (eds), The Moths and Butterflies of Great Britain and Ireland. – Harley Books, Colchester. 460 pp.

Fletcher, T. B. 1940. *Caloptilia pyrenaeella*, Chrétien 1908. – Entomologist's Record and Journal of Variation **52** (1): 8–9.

Ford, L. T. 1933. *Gracilaria pyrenaeella*, Chrét., an addition to the British list. – Entomologist **66**: 230.

Hall, B. G. 2008. Phylogenetic trees made easy: a how-to manual. – Sinauer Associates. 233 pp.

Hebert, P. D. N., J. R. deWaard & J.-F. Landry 2009. DNA barcodes for 1/1000 of the animal kingdom. – Biological letters. doi: 10.1098/rsbl.2009.0848)

Ivanova, N. V., J. R. deWaard & P. D. N. Hebert 2006. An inexpensive, automation-friendly protocol for recovering high-quality DNA. – Molecular Ecology Notes **6**: 998–1002.

Karsholt, O. & J. Razowski 1996. The Lepidoptera of Europe. – Apollo Books, Stenstrup. 380 pp.

Langmaid, J. R. & M. R. Young 2001. Microlepidoptera Review of 2000. – Entomologist's Record and Journal of Variation **113** (6): 241–254.

Leraut, P. 1983. Quelques changements dans la nomenclature des Lépidoptères de France [Lep. Tineidae, Gracillariidae]. – Entomologica Gallica **1** (1): 35–36.

Page, R. D. M. & Holmes, E. 1998. Molecular evolution, a phylogenetic approach. – Blackwell Science Ltd. 346 pp.

Ratnasingham S. & P. D. N. Hebert 2007. BOLD: The Barcode of Life Data System (http://www.barcodinglife.org). – Molecular Ecology Notes **7**: 355–364.

Schmidt, B. C. & F. Sperling 2008. Widespread decoupling of mtDNA variation and species integrity in *Grammia* tiger moths (Lepidoptera: Noctuidae). – Systematic Entomology **33**: 613–634

Sims, I. 2009. Exhibit, 2008 Annual Exhibition, British Microlepidoptera. – British Journal of Entomology and natural History **22** (3): 168–169.

Wakely, S. 1960. *Caloptilia pyrenaeella* Chret. and *Gracillaria semifascia* Hw. in the Isle of Wight. – Entomologist's Record and Journal of Variation **72**: 247–248.

Wakely, S. 1962. Further notes on *Euspilapteryx (Gracilaria) pyrenaeella* Chretien. – Entomologist's Record and Journal of Variation **74**: 120–121.

Wiemers, M. & K. Fiedler 2007. Does the DNA barcoding gap exist? – a case study in blue butterflies (Lepidoptera: Lycaenidae). – Frontiers in Zoology **4**: 8.