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Hypobapta tachyhalotaria spec. nov. from Tasmania – an example of a new species revealed by DNA barcoding

(Lepidoptera, Geometridae)

Axel Hausmann, Manfred Sommerer, Rodolphe Rougerie & Paul Hebert

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In Tasmanian *Hypobapta percomptaria* Guenée, 1858, slightly bigger and clearer grey specimens without a rosy tinged underside were hitherto deemed to reflect intraspecific variation. However, clear-cut differences in the mtDNA sequences (COI; 5' barcoding fragment; 648 bp) support the assumption of a separate species beside *H. percomptaria*: *H. tachyhalotaria* **spec. nov.** is diagnosed and figured. The original type specimen of *H. percomptaria*, for which a DNA barcode was successfully obtained, is included in the tree-diagram illustrating the sequence similarities/differences of all specimens of *Hypobapta* species that were barcoded in the "Australia" campaign of the All-Leps project. The potential for rapid biodiversity assessment is exemplified by the discovery of this new species hitherto hidden under *H. percomptaria*.

Dr. Axel Hausmann, Zoologische Staatssammlung München, Münchhausenstr. 21, 81247 Munich, Germany; e-mail: Axel.Hausmann@zsm.mwn.de

Résumé

Chez le géomètre *Hypobapta percomptaria* Guenée, 1858, l'existence de spécimens légèrement plus grands et d'un gris plus pâle, dont le dessous des ailes ne montre pas de teinte rosée, avait jusqu'à aujourd'hui été considérée comme le reflet du spectre de variation intraspécifique de ce papillon. Des différences marquées au niveau de l'ADN mitochondrial (COI; code barre ADN; 648 bp) ont démontré l'existence d'une nouvelle espèce différente de *H. percomptaria*: *H. tachyhalotaria* **spec. nov.**, ici décrite et figurée. Le spécimen type original de *H. percomptaria*, pour lequel un code barre ADN a pu être obtenu, est inclus dans l'arbre illustrant les similarités et différences entre les séquences de tous les spécimens du genre *Hypobapta* dont un code barre a été obtenu lors de la campagne de DNA barcoding australienne du projet canadien. Cette découverte d'une nouvelle espèce jusqu'alors confondue avec *H. percomptaria* illustre le potentiel de cette approche pour une évaluation rapide de la biodiversité.

Introduction

Hypobapta percomptaria Guenée, 1858 was originally described from 'Nouvelle Hollande' ([Australia] according to Scoble 1999). All subsequent papers on that 'species' considered it to be a widespread species in Australia including Tasmania (e. g. Common 1990, Fig. 37), and recognized it as being clearly distinct from its congeners *H. barnardi* Goldfinch, 1929, *H. diffundens* Lucas, 1891 (with syn. *H. eugramma* Lower, 1892), and *H. xenomorpha* Lower, 1915. Within the genus, '*H. percomptaria*' was the only species known to occur in Tasmania. However, collecting activities near Hobart during the Forum Herbulot, organized in Tasmania in January 2006, yielded some specimens which differed from the usual *H. percomptaria*, by their larger body size, a clearer grey coloration, and by their lack of a rosy tinge on the underside. Such differences were originally thought to represent nothing more than intraspecific variation. The present study interprets them as marking different species.

Abbreviations

BIO	Collection of the Biodiversity Institute of Ontario, Guelph, Canada.
CCDB	Canadian Centre for DNA Barcoding
IZBE	Institute of Agronomy and Environmental studies, Estonian University of Life Sciences, Tartu, Estonia
MNHN	Muséum national d'Histoire naturelle, Paris, France
ZFMK	Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany
ZSM	Zoologische Staatssammlung, Munich, Germany

Material and Methods

The study was based on 72 specimens of "*H. percomptaria*" (sensu auctorum) from Tasmania and 21 specimens from different regions (New South Wales, Queensland) of mainland Australia, including the type specimen of *H. percomptaria* from the MNHN. Furthermore, 17 Australian specimens of *H. diffundens*, 21 of *H. barnardi*, and 1 of *H. xenomorpha* were included. All 132 specimens were barcoded in the "Australia" campaign of the All-Leps project of CCDB (see http://www.lepbarcoding.org) and the results could be used for this study by courtesy of CCDB. Pictures and details of the specimens examined are made accessible from the following sources: (1) the taxonomy browser (http://www.barcodinglife.com/views/taxbrowser.php) and (2) the published data projects of BOLD (http://www.barcodinglife.org, projects GZPAG and GZPPT), (3) GenBank, and (4) the accompanying website on Tasmanian geometrids (http://www.zsm.mwn.de/lep/tasmania.htm).

The DNA barcodes were obtained at the CCDB 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. Sequence data of all 93 records for *H. percomptaria* and the newly described species are accessible in GenBank (acc. no. FJ429846 to FJ429938); 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), an internet platform that provides a very practical workbench where all key information is attached to each individual specimen record, and where basic analytical tools allow data exploration and analysis. An unlimited number of color images per voucher specimen as well as cartography tools also represent valuable features.

The over 150 year old type specimen of *H. percomptaria* was treated with a new analytical approach using 6 different nested primer pairs (Rougerie pers. obs.; detailed protocol not yet publicly accessible at CCDB). A barcode sequence of 536 bp (vs. the usual full length of 648 bp) was recovered from the specimen and made publicly available to the authors (project GZPPT in BOLD, open access).

Sequence analyses were conducted using MEGA4 (Tamura et al. 2007). A Neighbor Joining (NJ) optimal tree was built based on the three codon positions; we used K2P distances (Kimura 1980), considering transitions and transversions, a homogeneous pattern among lineages, and uniform rates of substitution among sites. A bootstrap analysis with 1000 pseudoreplicates was conducted to evaluate the robustness of clusters inferred from the NJ analysis.

Results

Among the Tasmanian specimens previously lumped in *H. percomptaria*, we observed a clear-cut barcode split congruent with morphological diversity which we interpret as evidence for the existence of an undescribed cryptic species – described below as *H. tachyhalotaria*. The 536 bp sequence obtained from the type specimen of *H. percomptaria* permits the unequivocal assignment of this species name to specimens grouping together in the same genetic cluster of small sized Tasmanian specimens with red suffusion of the underside, a habitus feature of

Tab. 1. Interspecific genetic distances in the genus *Hypobapta*, minimum pairwise distances (in %) and mean of pairwise distances (in parentheses), based on sequences of the mtDNA COI gene (barcode fragment 5', 648 bp).

	1	2	3	4
1 *Hypobapta barnardi*				
2 *Hypobapta diffundens*	9.1 (9.9)			
3 *Hypobapta percomptaria*	11.6 (12.8)	12.5 (14.1)		
4 *Hypobapta tachyhalotaria*	8.2 (9.5)	9.8 (11.1)	8.3 (9.5)	
5 *Hypobapta xenomorpha*	9.4 (9.8)	9.6 (10.1)	9.6 (10.2)	9.5 (9.9)

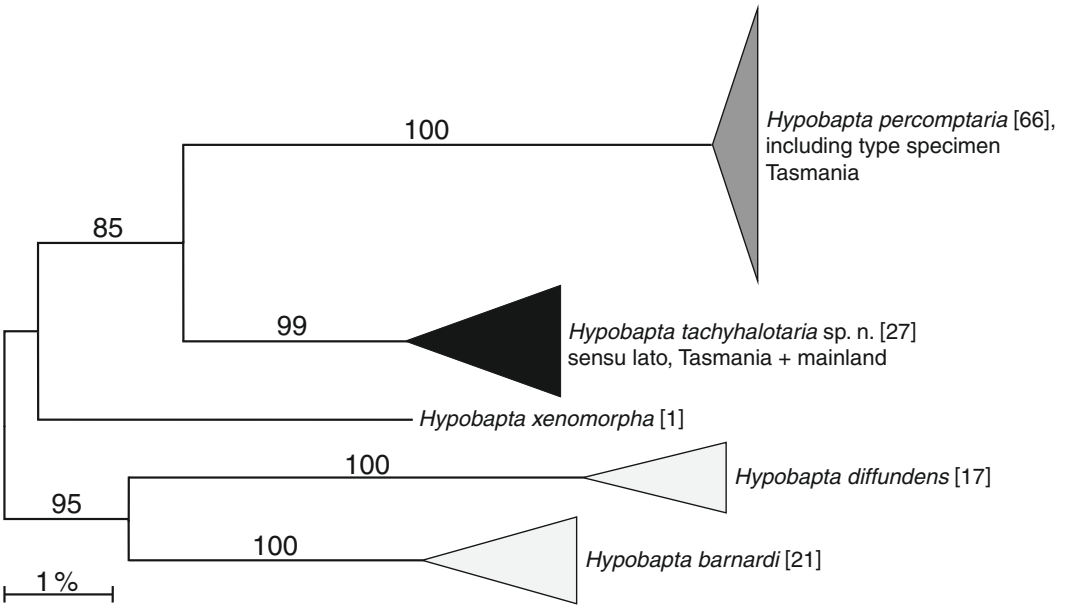


Fig. 1. Neighbor joining tree of the *Hypobapta percomptaria* Guenée, 1858 species-group based on sequences of the mtDNA COI gene (barcode fragment 5', 648 bp); numbers within brackets refer to the number of specimens per cluster, and number above branches are bootstrap support values for each node. The depth of triangles represents the mean intraspecific divergence within each cluster.

the holotype, too. All examined specimens from the Australian mainland hitherto named *H. percomptaria* are, in habitus, reminiscent of Tasmanian *H. tachyhalotaria* and close to that species even in their barcodes, and therefore are considered here as part of the *H. tachyhalotaria* complex sensu lato.

The mtDNA barcodes of 132 specimens of the four already described taxa in *Hypobapta* plus this newly discovered species that were barcoded in the "Australia" campaign of the All-Leps project showed marked differences between the species. Interspecific distances (K2P pairwise distances – calculated in MEGA4) span from approx. 8 % (between *H. percomptaria*, *H. tachyhalotaria* and *H. barnardi*) to more than 14 % (between *H. percomptaria* and *H. diffundens*). The sequence similarities/differences are summarized in Table 1.

There is low intraspecific divergence from 0 to 1.4 % within the 66 Tasmanian specimens analysed of *H. percomptaria*, and only 0 to 0.31 % within the six Tasmanian specimens of *H. tachyhalotaria*. Within the 27 specimens of the *H. tachyhalotaria* complex including 21 specimens from mainland Australian populations, sequence variation is much higher, reaching a maximum of 4.45 %.

The genetic pattern derived from the barcoding results as shown in Table 1 and Fig. 1 indicates that there are two well separated mitochondrial lineages in the *Hypobapta* specimens from Tasmania. Together with the correlated differences in male genitalia and in habitus as pointed out below (Description, Differential diagnosis) the minimum pairwise K2P distance of 8.3 % provides strong evidence that there are in fact two different sympatric species in Tasmania: *H. percomptaria* and the new species *H. tachyhalotaria*. Since the type specimen of *H. percomptaria* with its sufficient long mtDNA sequence is perfectly integrated in one of the lineages and also has the habitus features of that lineage, it is safe to tell which specimens belong to the new species.

None of the barcoded specimens of *H. percomptaria* sensu auctorum from mainland Australia shares the mitochondrial DNA and habitus features of the type specimen of *H. percomptaria*. Although the type of *H. percomptaria* is labelled as from "Nouvelle Hollande", i. e. Australia, no specimens of this species from the Australian mainland could be found, so far. In the context with the other *Hypobapta* specimens and species, the 21 barcoded mainland Australian specimens form a group with the Tasmanian specimens of *H. tachyhalotaria* but show a gradual interpopulational variation in the mitochondrial genetics that reaches 4.45 % in its extreme difference from Tasmanian *H. tachyhalotaria* specimens, thus exceeding the maximum values of about 2 % which are usually observed within morphologically con-

Figs 2-3. *Hypobapta tachyhalotaria* sp. n., Holotype, Tasmania. **2,** upperside; **3,** underside. Scale bar = 1 cm.

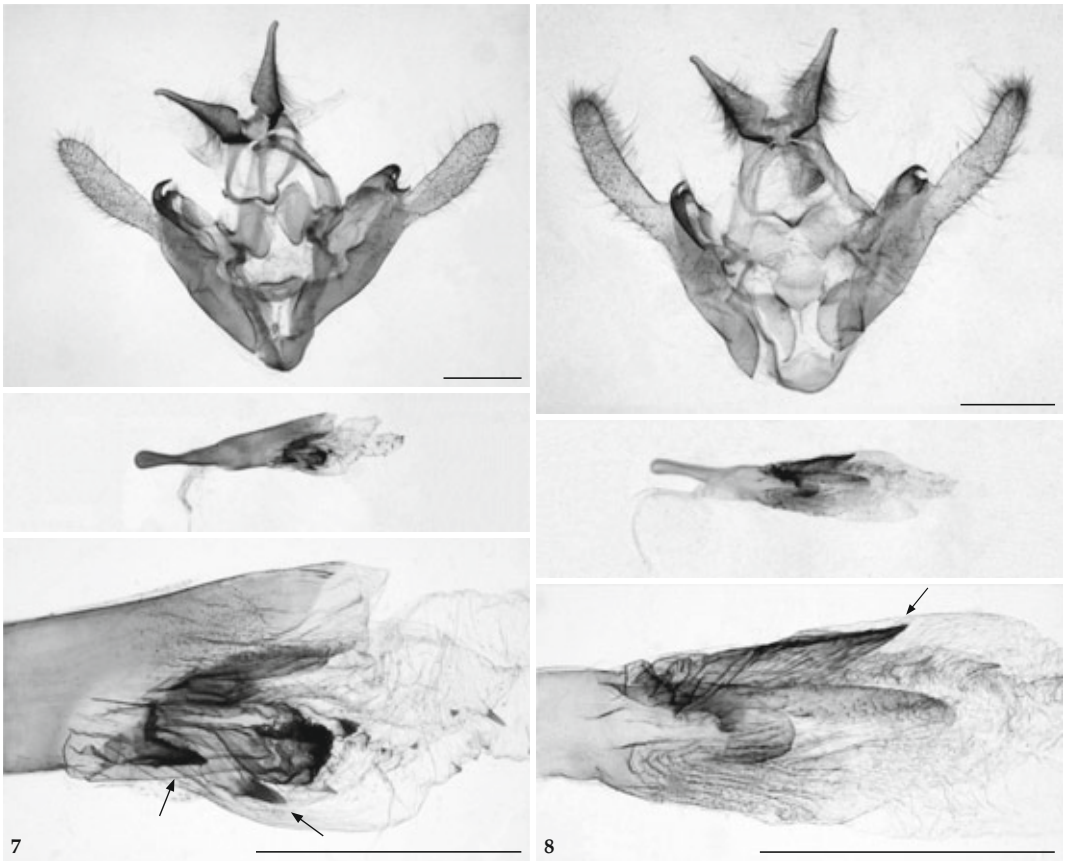
firmed species. While it is clear that those mainland Australian specimens cannot be *H. percomptaria*, they are here only provisionally grouped with *H. tachyhalotaria*, until a revision of *Hypobapta* on the basis of a much larger sample clarifies the taxonomic situation in this respect.

Hypobapta tachyhalotaria
Hausmann & Sommerer, spec. nov.

Holotype. ♂, Tasmania mer., vic. Hobart, Mt. Nelson 300 m, 24.I.2006 leg. M. Sommerer, coll. Zoologische Staatssammlung München (ZSM) (BC ZSM Lep 07996; gen. slide 13974)

Paratypes. 1♂, Tasmania mer., vic. Hobart, Mt. Wellington 320 m, 42°55'S / 147°16'E, 20.I.2006, leg. et coll. M. Sommerer (BC ZSM Lep 07997); 1♀, Tasmania mer. vic. Hobart, Mt. Nelson 300 m, 24.I.2006 leg. et coll. M. Sommerer (BC ZSM Lep 07998). 1♀, Tasmania mer.,

Figs 4-6. *Hypobapta percomptaria* (Guenée, 1858), Tasmania. **4,** upperside; **5,** underside; **6,** holotype, underside (photo J. Minet, MNHN Paris). Scale bar = 1 cm.



Figs 7-8. Male genitalia, aedeagus, and magnified detail of aedeagus. Scale bars = 1 mm. **7,** *Hypobapta tachyhalotaria* sp.n., holotype, prp. ZSM G 13974. **8,** *Hypobapta percomptaria* (Guenée, 1858), Tasmania, prp. ZSM G 13972.

vic. Hobart, Mt. Wellington 320m, 42°55'S / 147°16'E, 20.I.2006, leg. D. Stüning, coll. ZFMK; 1♂, Tasmania, Hobart, College Rd., 18.I.2006, leg. E. Ounap & J. Viidalepp, coll. IZBE (gen. prep. 7632); 1♂, Tasmania, Hobart, Mt. Nelson, 25.I.2006, leg. E. Ounap & J. Viidalepp, coll IZBE; 1♀, Tasmania, Ridgeway/Hobart, 20.I.2006, leg. E. Ounap & J. Viidalepp, coll IZBE; 3♂, Tasmania, Hobart, Kingston Beach, –42.986 / 147.317, alt. 110 m., 26.XII.2005, leg. R. D. Ward, coll. BIO; 1♂, id., 04.I.2006; 1♂, id., 27.XII.2006; 1♂, Mt. Wellington Tas., V.J. Robinson K.D. Fairey, coll. ANIC.

Additional material examined, but not included in the type series: 6♂♀, Australia, Queensland, various localities, coll. E. Friedrich (all barcoded); 5♂♀, Australia, Queensland, leg. U. Buchsbaum, coll. ZSM; 10♂♀ various localities in Queensland and New South Wales, coll. BIO (all barcoded).

Description (Figs 2-8)

Wingspan ♂ 39-42 mm, ♀ 44-48 mm. Hindwing elongate. Ground color light grey. Basal, antemedial and postmedial lines blackish dark grey. Terminal area darker on all wings, with pale grey wavy line. Cell spot fine, elongate on all wings. Underside whitish, in Tasmanian populations without red, on Australian mainland often with narrow and sharply bordered reddish line basal of the black terminal fascia. Male genitalia comparatively large, socii long, juxta reniform, basodorsal projections of valva ('hemitranstillae') strongly sclerotized and reniform, harpe hooks comparatively large, aedeagus with large basal cornutus and some lateral cornuti beside the sclerites of the vesica which are diagnostic.

Differential diagnosis. Nearest species is *Hypobapta percomptaria* with smaller body size, e.g. wingspan ♂ 32-38 mm, ♀ 38-44 mm, hindwing less elongate. Ground color much darker and with slight brown tinge. Underside of hindwing with extended diffuse red suffusion. Dark ground color may be faded in worn specimens. Rare forms of *H. percomptaria* show a paler medial area of forewing upperside,

but the correlated three features red suffusion of underside, genitalic features (aedeagus sclerites) and DNA barcodes clearly assign these forms to *H. percomptaria*. Male genitalia smaller, socii shorter, juxta broad and rounded, basodorsal projections of valva ('hemitranstillae') less sclerotized, subquadratic, harpe hooks slightly smaller. Aedeagus with a large, tapering sclerite (truncate in *H. tachyhalotaria*), without lateral cornuti beside the long and elongate vesica sclerites (two stout cornuti in *H. tachyhalotaria*). Vesica wrinkled over almost the whole length of the aedeagus.

Distribution. Tasmania. The occurrence of the species in New South Wales and Queensland is only tentative.

Etymology. The name is derived from the Greek word ταχυαλωτος, i.e. rapidly conquered, and is intended to reflect the authors' opinion that appropriately used data from DNA barcoding campaigns can have an accelerating effect on taxonomy.

Discussion

The specimens available from populations from mainland Australia (New South Wales, Queensland) show relatively high interpopulational genetic variation correlated with slight differences in habitus (e. g. a continuous red line bordering the black fascia of hindwing underside) and deserve further taxonomic studies based on a much larger sample size and a better coverage of mainland Australian localities. It is possible that the more or less gradual differences in the mtDNA sequences seen in the small sample available will point, in a broader context, to distinct lineages and, in combination with habitus features to be studied in detail, to the existence of other new taxa on the Australian mainland. Consequently, the type series of *H. tachyhalotaria* has been strictly limited to Tasmanian specimens.

We want to emphasize here how the combination of traditional taxonomic approaches and quickly progressing DNA barcoding campaigns can facilitate the detection and discrimination of species. Given the mass of new species to be found and formally described, we consider that the use of new tools such as DNA barcoding and BOLD offer a very practical way to tackle this task both efficiently and reliably.

Acknowledgements

Joel Minet (MNHN, Paris) kindly took photographs of the type specimen of *H. percomptaria* and sampled a leg of it for DNA analysis. Similarly, Charlie and Kim Mitter (University Maryland) provided photographs and tissues of the only specimen of *Hypobapta xenomorpha*. Thanks are also due to Dieter Stüning (ZFMK, Bonn), Ted Edwards (ANIC, Canberra), Erki Ounap and Jaan Viidalepp (Tartu, Estonia) for including their own specimens into the series of paratypes and for advice. Further samples of *Hypobapta* specimens that were included in BOLD came from ANIC (M. Horak; T. Edwards), the Biodiversity Institute of Ontario (leg. P. Hebert, Bob Ward (CSIRO), Andrew Mitchell), the New South Wales Department of Primary Industries, and research collections of Egbert Friedrich (Jena, Germany) and Graeme V. Cocks (Australia).

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