Molecular phylogenetic and morphological studies on the systematic position of *Heracula discivitta* reveal a new subfamily of Pseudobistonidae (Lepidoptera: Geometroidea)

HOU SHUAI WANG¹, JEREMY D. HOLLOWAY², NIKLAS WAHLBERG³®, MIN WANG¹ and SÖREN NYLIN⁴

¹Department of Entomology, College of Agriculture, South China Agricultural University, Guangzhou, China, ²Department of Life Sciences, Natural History Museum, London, U.K., ³Department of Biology, Lund University, Lund, Sweden and ⁴Department of Zoology, University of Stockholm, Stockholm, Sweden

Abstract. *Heracula discivitta* Moore is an uncommon moth species currently recorded from India, Nepal and China. Although this species has traditionally been placed in Lymantriinae, its systematic position in Macroheterocera has been enigmatic due to its unique morphological features. Here we used molecular and morphological data to explore the systematic position of *H. discivitta*. Our molecular phylogenetic analyses indicate that this species is sister to *Pseudobiston pinratanai* Inoue, a member of a recently established monotypic family Pseudobistonidae. The examinations of morphological features further show that *H. discivitta* shares synapomorphies with Pseudobistonidae. Based on the analysis results, we propose a new subfamily of Pseudobistonidae (*Heraculinae subfam.n.*) to accommodate *H. discivitta*. The resemblance of the habitus to that of the brahmaeid genus *Calliprogonos* Mell & Hering is discussed.

This published work has been registered on ZooBank, http://zoobank.org/urn:lsid:urn:lsid:zoobank.org:pub:63D17850-6D51-4E03-A5D6-P9EF6E7AF402.

Introduction

Lepidoptera, with over 157 000 recognized extant species, are one of the most speciose insect orders (Mitter *et al.*, 2017; Triant *et al.*, 2018). They play an important role in the natural ecosystem as major herbivores, pollinators and prey, and serve as model organisms for studying ecology, genetics, physiology and evolutionary biology (Wagner, 2001; Roe *et al.*, 2010). Although the deep-level phylogeny of Lepidoptera remains largely unclear, recent advances in molecular phylogenetic studies are gradually contributing to our understanding of the phylogeny and evolution of such a huge insect order (Regier *et al.*, 2009, 2013; Mutanen *et al.*, 2010; Bazinet *et al.*, 2013; Kawahara & Breinholt 2014, Timmermans *et al.*, 2014; Mitter *et al.*, 2017), and have allowed us to address many previously unresolved problems, particularly for the systematic positions of mysterious taxa with unique morphological traits in some major Lepidopteran lineages. Some further examples, both molecular and morphological, in the Lymantriinae, Geometroidea and more widely in the Lepidoptera, are reviewed in the Discussion. Macroheterocera represent a mega-diverse Lepidopteran lineage of more than 72 000 known species (Nieuwerken *et al.*, 2011; Mitter *et al.*, 2017). Within Macroheterocera, a rare and mysterious taxon, *Heracula discivitta* Moore, is currently known from India, Nepal and China, and has persisted as a monotypic genus as the type species in Lymantriinae of Noc-tuoidea since its original description in 1866 (Moore, 1866; Kishida, 1993; Chao, 2003). However, the systematic placement of this species has been problematic due to its distinctive morphological traits. For instance, *H. discivitta* has a pair of filiform antennae and a well-developed proboscis, whereas the antennae of Lymantriinae are strongly bipectinate and their proboscis is usually absent. Moreover, *H. discivitta* lacks distinctive metathoracic tympanal organs that are viewed as an apomorphic character of Noctuoidea. A molecular study of higher classification in Lymantriinae (Wang *et al.*, 2015) that initially included *Heracula* showed that it was an outlier from the rest of the group and probably misplaced.

Correspondence: Min Wang, Department of Entomology, College of Agriculture, South China Agricultural University, 483 Wushan Road, Tianhe District, Guangzhou 510642, China. E-mail: min wang@scau.edu.cn

© 2019 The Authors. *Systematic Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
Fig. 1. Phylogenetic tree from ML analysis of 114 taxa based on eight genes (6365 sequenced sites). Bootstrap support values of ML analysis and posterior probabilities of BI analysis are indicated at the nodes as BP/PP, respectively. [Colour figure can be viewed at wileyonlinelibrary.com].

© 2019 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 44, 211–225
Fig. 2. Head characters of male *H. discivitta*: (a) labial palpus, (b) anterior view (black arrow: cranial pit); (c): posterior view; (d) galea (black arrow: maxillary palpus); (e) anterolateral view with the mouthpart area (black arrow: labrum; white arrow: maxillary palpus); (f) dorsal view (black arrow: cranial pit); (g) dorsal view of antenna; (h) ventral view of antenna; (i) basal area of antenna (white arrow: intercalary sclerite); (j): an area near the postdorsal edge of the right compound eye (white arrow: chaetosemata). Scale bar = 500 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

© 2019 The Authors. *Systematic Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. 44, 211–225
Recently, the molecular phylogenetic study of an eight-gene dataset by Mutanen et al. (2010) addressed many long-standing questions of lepidopteran systematics. This base has been used to investigate the relationships of higher taxa such as families and superfamilies, especially in the so-called Macroheterocera clade (Nieuwerken et al., 2011; Sihvonen et al., 2011; Zahiri et al., 2011, 2012, 2013a,b; Rajaei et al., 2015). In addition to confidently delineating families of Macroheterocera, the molecular data have led to the discovery of a new, currently monotypic family, Pseudobistonidae, consisting of the SE Asian species *Pseudobiston pinratanai* (Rajaei et al., 2015). In order to explore the systematic position of *H. discivitta*, we added the molecular data of this species to the dataset of Mutanen et al. (2010), augmented with sequences from Rajaei et al. (2015) and re-ran the molecular phylogenetic analyses, and also re-examined morphological characters of *H. discivitta*. Based on the results of our analyses, we propose here a new subfamily of Pseudobistonidae to accommodate *H. discivitta*.

**Materials and methods**

**Morphological examination**

The specimens examined in this study are deposited in the Insect Collection, Department of Entomology, South China Agricultural University (SCAU), Guangzhou, China, except for the holotype and the only female specimen of *H. discivitta* which are kept at the Natural History Museum, London, UK (NHMUK). Photos of adults were taken using a Nikon Coolpix S8000 digital Camera. Adults were entirely dissected in pure ethanol and were stained with Chlorazol Black E after the whole body (except wings) was macerated in hot 10% sodium hydroxide in an aqueous solution for 2–8 min. Wing venation was observed after removing the scales in absolute ethanol and staining the wings in an acetocarmine solution. The dissected structures of adults (including head, thoracic exoskeleton, abdomen, legs, genitalia and wing venation) were mounted on microscope slides in glycerin, and photographed using a KEYENCE VHX-5000 digital microscope or a Zeiss Discovery V12 stereo-scope. All images of the female *H. discivitta* were obtained from NHMUK. All of the images were edited with Adobe PHOTOSHOP CS6. Label data for the illustrated specimens in this study are given in Table S1. Terminology largely follows Scoble (1992) and Kristensen (2003). Some terms for the cephalic and thoracic morphology follow Matsuda (1970), Brock (1971), Fänger (1999), Kawahara et al. (2012) and Minet (2003). The naming of the vein venation follows Wootton’s (1979) system.

**Molecular data analyses**

The DNA sequences of Macroheterocera in the study of Mutanen et al. (2010) were downloaded from NCBI Genbank, including also four taxa of Pyraloidea to root the tree. In addition, the sequences of *Pseudobiston* from the study of Rajaei et al. (2015) were downloaded from NCBI Genbank. For *H. discivitta*, total genomic DNA was extracted from two
legs of a dried adult specimen, using Qiagen DNeasy tissue extraction kit (Hilden, Germany) following the manufacturer’s protocol. The eight gene regions (COI, EF-1α, RpS5, GAPDH, MDH, CAD, IDH and Wingless) used by Mutanen et al. (2010) were amplified using PCR. DNA amplification and sequencing protocols followed Wahlberg & Wheat (2008). All sequences and accession numbers used in this study are listed in Table S2. The sequences obtained were edited and aligned using BioEdit v7.2.0 (Hall, 1999). Aligned DNA matrix used in the phylogenetic analyses of this study is provided in File S1. Phylogenetic analyses were conducted using two model-based methods: maximum-likelihood (ML) and Bayesian inference (BI). Eight partitions were made by gene regions. The GTR + G + I model was identified as the best-fit model of sequence evolution for each gene partition using the Akaike information criterion (AIC) implemented in MrModelTest2 (Nylander, 2004). BI analysis was performed using the GTR + G + I model in MrBayes v3.2.2 (Ronquist et al., 2012) on the CIPRES Science Gateway (Miller et al., 2010), with the default parameters and four independent runs for 50 million generations, each with one cold chain, sampled every 1000th generation, and three heated chains. For ML analysis, the GTR + G model was used, as the strong correlation between parameters G and I confounds their use together (Ren et al., 2005). ML analysis was executed with the web-server RAxML (Stamatakis et al., 2008) on CIPRES, using 1000 bootstrap replicates. Tree files and DNA matrices are deposited in TreeBASE with a study accession URL: http://purl.org/phylo/treebase/phylobase/study/TB2:S22416.

Results

The data matrix used in the analyses comprised 114 taxa and 6365 nucleotide sites (1476 bp of COI, 1240 bp of EF-1α, 691 bp of GAPDH, 722 bp of IDH, 407 bp of MDH, 603 bp of RpS5, 826 bp of CAD and 400 bp of Wingless). The IDH sequence of H. discivitta was not successfully amplified. Some genes are missing for some taxa (Table S2). The two phylogenetic analyses (ML and BI) of the eight-gene dataset strongly support
that Heracula Moore is sister to Pseudobiston Inoue which is the type genus of a recent established new family Pseudobistonidae (Fig. 1: BP = 99; PP = 1.00). Based on the molecular analyses and morphological examination, we here assign the genus to a new subfamily of Pseudobistonidae with the following description.

Heraculinae Wang and Holloway subfam.n.

http://zoobank.org/urn:lsid:zoobank.org:act:73400E25-C543-40AD-9C20-773B3DD76DFE

Type genus. Heracula Moore 1866.

Material examined. Holotype, ♂, Darjeeling, INDIA, leg. A. E. Russell, NHMUK. 1♀, Darjeeling, INDIA, leg. A. E. Russell, NHMUK. 2♂, Nacheng near Nilgiri, NEPAL, 12–14.vi.1969, leg. T. Miyashita, SCAU; 2♂, Lete near Nilgiri, NEPAL, 2400 m a.s.l., 23.vi.1969, leg. T. Miyashita, SCAU; 1♂, Gaoligong mountain, Yunnan province, CHINA, 21–24.vii.2006, leg. Min Wang & Xiaoling Fan, SCAU; 1♂, Chongxing village, Longdong Town, Baoxing county, Yaan city, Sichuan province, CHINA, 30°28.254′N, 102°38.233′E, 1850 m a.s.l., 20.vi.2011, leg. Houshuai Wang & Haiming Xu, SCAU; 1♂, Erlang mountain, Sichuan province, CHINA, 29°38.540′N, 102°07.011′E, 2124 m a.s.l., 23.vi.2012, leg. Houshuai Wang & Xiaohua Deng, SCAU; 1♂, Dongla mountain, Baoxing county, Yaan city, Sichuan province, CHINA, 30°28.106′N, 102°38.340′E, 1844 m a.s.l., 16.vi.2012, leg. Houshuai Wang & Xiaohua Deng, SCAU; 1♂, Dongla mountain, Baoxing county, Yaan city, Sichuan province, CHINA, 30°27.247′N, 102°39.058′E, 1550 m a.s.l., 17.vi.2012, leg. Houshuai Wang & Xiaohua Deng, SCAU; 1♂, Anzihe Natural Reserve, Chongzhou, Sichuan province, CHINA, 1500 m a.s.l., 19.vi.2016, leg. Houshuai Wang & Shuqin Ji, SCAU; 1♂, Motuo County, Xizang Zang Autonomous Region, CHINA, 2820 m a.s.l., 23.vii.2017, leg. Shuqin Ji, SCAU.

Head (Fig. 2). Antenna (in the male) filiform; scapus and pedicellus nearly cylindrical, entirely scaled; scapus thin basally, c.2x the length of the pedicellus; intercalary sclerite fairly small; flagellum dorsally scaled and ventrally covered with tiny sensory setae. Frontoclypeus, vertex and occiput densely covered with piliform scales. Compound eyes well-developed, naked. Ocelli absent. Chaetosemata small, remote from each other, sparsely covered with yellow hairs. Two discrete, minute cranial pits present on the vertex and each side of the postgenal-occipital area, respectively. Maxillary palpus rather short, three-segmented. Labrum scaled, without distinct projection medially; pilifers absent. Mandibles absent. Labial palpus porrect, covered with dense scales, three-segmented; basal segment almost as long as median one; distal segment short, <1/3 of median one in length. Proboscis present, unscaled; galea with a row of brush-like setae along the anterior-lateral margin.

Prothorax (Fig. 3). Patagia approximately kidney-shaped, anterior surface sclerotized, posterior surface membranous. No distinct parapatagia (they may be membranous or reduced). Pronotal anteromedian plate small, articulated with a short finger-like posteromedian process. Anterolateral pronotal plates c.3x as long as wide. Laterocervicale consists of well-developed anterodorsal and ventral arms, the former relatively straight and the latter concave laterally. Katepisternum small, subtriangular; trochantin absent. Propleuron with a weakly sclerotized epimeron caudad of the ventral half of the pleural sulcus. Dorsal processes of furca tapering toward apex, shorter than a digitate prospinasternum that is articulated with median longitudinal sulcus on the posterior margin of pro sternum.

Mesothorax (Figs 4, 5). Tegula falcate, broader at anterodorsal surface, covered with dark brown fine hairs. A pair of prescutal clefts convergent dorsally, their dorsal extremities almost as wide as the length of the antennal sulcus. Anterolateral margins of prescutum prolonged along the prescutal clefts, forming
long finger-like prealar arms. Phragma 1 membranous, inconspicuous, slightly bilobed. A complete median longitudinal sulcus present on the mesoscutum. Subtegula elongated, fused to a tergopleural apodeme. Suralare short, forming an anterior notal wing process, being separated from the mesoscutum by an anterolateral scutal sulcus that produces an internal ridge and ends posteriorly in a short transverse bar. Median notal wing process well-developed, anterobasally bordered by a small notal incision, and associated with a short posterior notal wing process through a plate-like sclerite. Mesoscutellum approximately rhombic in dorsal view, slightly round at the middle of posterior margin. Mesopostnotum concave anteriorly. Phragma 2 large, with a small notch on the ventral-medial edge. Laterophragmata 2 well-developed. Anepisternum relatively large, its ventral margin being clearly demarcated by an anapleural cleft that extends backwards to the pleural sulcus. Kaepepisternum small, triangular with posterior margin almost equal to that of the preepisternum in length. The marginopleural sulcus short, anastomosing anteriorly with an oblique precoxal sulcus. The parepisternal flexion zone broad, extending upward to the anterior edge of the anapleural cleft. Parepisternum moderately sclerotized, connected to the basisternum, ventral half of posterior margin bordered by a parepisternal sulcus. Mesoclidium shaped like an inverted-Y, with the divergent ventral arms reaching to the anterolateral area of the basisternum. Subalare spacious, irregularly sclerotized at the dorsal side. Epimeron deeply concave on the upper margin; its anterior arm narrow, tapering dorsally. A large subtriangular sclerite located between the posterior area of the epimeron and meron. The furcal arm large, strongly sclerotized, fused with the posterior arm of the epimeron. Meron dorsally broad, separated from eucoxa by a long mesocoxal sulcus.

Fig. 6. Metathorax of male *H. discivitta*: (a) posterior view (the upper part); (b) posterior view (the lower part); (c) lateral view (the lower part of the left side); (d) posteroventral view (the lower part). ec, eucoxa; eu, euphragma; fa, furcal apophyses; fe, foramen of euphragma; ffb, furco-furcal band; fl, fenestrae lateralis; lp, laterophragma; me, meron; pvl, posterior ventral lamina; s, strut; scm, scutum; sf, stem of furca; sfa, secondary furcal arm; suc, supraphragmal sclerite. [Colour figure can be viewed at wileyonlinelibrary.com].
Fig. 7. Legs of *H. discivitta* (male): (a) fore leg; (b): middle leg; (c) hind leg. Scale bar = 500 μm. [Colour figure can be viewed at wileyonlinelibrary.com].

Fig. 8. Pretarsus of *H. discivitta* (male): (a) ventrolateral view; (b) dorsal view. a, arolium; b, pseudopodial bristle; c, claw; p, pulvillus. Scale bar = 100 μm. [Colour figure can be viewed at wileyonlinelibrary.com].
Metathorax (Figs 5, 6). Metascutum concealed mesally under the metascutellum; its lateral part large and broad, with a pigmented sulcus arising near the anterolateral margin of the metascutellum. No distinct fenestra media. Fenestrae laterals minute. Suprathoraxal sclerite elongate, delimited by an internal ridge ventrally. Laterosternal region with a well-defined subtriangular sclerite (termed ‘laterophragma’ by Brock, 1971) that is ventrally adjacent to the true lobe-like laterophragma. Euphragma (def.: Fänger, 1999) developed only in the lateral region along the tergal rim (sensu Brock, 1971), with a large, central oval foramen. Anepisternum small. Preepisternum gradually narrowed toward the ventral surface. Parepisternal sulcus long, reaching to the anepisternal cleft. Marginopleural sulcus moderately long, linking the pleural sulcus posteriorly. Subalare well-developed.

In lateral view, an unsclerotized flexion zone across the posteroventral arm of the metepimeron. Furco-furca band (sensu Minet, 2003) broad. The internal ridges of stem of furca relatively slender, extending dorsally to the secondary furcal arms (termed ‘laminae of secondary arms’ by Brock, 1971). A pair of struts (sensu Brock, 1971) connected ventrally with the stem of furca, their dorsal extremities reaching to the posterior ventral laminae that unite anteriorly with the furcal apophyses and merge posteriorly into an enlarged medial region between the secondary furcal arms. Eucoxa and meron bordered by a metacoxal sulcus.

Legs (Figs 7, 8). Femora covered with long dense piliform scales at the inner side. Midfemora slightly longer than the other femora. Tibiae and tarsi smoothly scaled. Foretibiae with a stubby epiphysis. Tarsi composed of five tarsomeres that bear irregularly arranged spines on their inner surface. The tibial spur formula: 0-2-4 (hind leg with a minute pair of subapical spurs that are c. 0.5× length of the apical pair). Pretarsi with a pair of simple, robust claws; pulvilli finger-like, with numerous minute setae; pseudempodial setae fairly long; arolium heavily pigmented, membranous apically.

Wings (Figs 9–11). Forewing apex round, outer margin somewhat convex. Upperside of male forewing with a red-brown spot at the base, and a large brown reniform patch at the distal one-third area; medial transverse band broad, deep brown, interior margin surrounding the basal spot, exterior margin slightly concave, both interior and exterior margin with a narrow pale white border. Upperside of male hindwing dark brown, with a red-brown marginal band. Underside of both wings brown, without wing patterns; frenulum present at the humeral angle of the hindwing, interlocking with a retinaculum at the base of the forewing. Female larger than male in size; forewing with yellow brown spot and patch, and with a much wider pale white border, hindwing with a yellow marginal band. Underside of both wings brown, with out wing patterns; frenulum present at the humeral angle of the hindwing, interlocking with a retinaculum at the base of the forewing. Female larger than male in size; forewing with yellow brown spot and patch, and with a much wider pale white border, hindwing with a yellow marginal band. Forewing venation with a rather long Sc that extends beyond the middle of the costa; R free, arising just before the upper angle of the discal cell; Rs1 to Rs5 stalked, and Rs6 branching before Rs1 + 2 + 3; areole absent; M1 free, arising near the upper angle of the cell; M2 arising closer to M1 than M1 ; M2 and CuA1 separated; CuA2 branching from the inner margin of the cell; 1 + 2A arising from the base of the forewing, Sc + R in the hindwing arising from the base, connecting with the costa of the cell at the basal area and forming a small basal cell; Rs and M1 separated, branching from

Fig. 9. Adults of Heraculinae (Pseudobistonidae) and Calliprogonos (Brahmaeidae): (a) male of H. discivitta; (b) female of H. discivitta (copyright of NHMUK); (c) male of C. miraculosa. Scale bar = 1 cm. [Colour figure can be viewed at wileyonlinelibrary.com].
well pigmented. Sternum A2 sclerotized except the posterior area; its anterolateral processes short, without connection to the marginotergites. Sterna A3–A8 almost unpigmented, with the similar width as their corresponding terga. The abdominal segments from A5 to A8 being gradually narrower.

Male genitalia (Fig. 13a). Uncus broad basally, tapering toward the apex, slightly acute at the apex. Gnathos laterally sclerotized along the posterior margin of tegument, with a large inverted triangular plate mesally. Valva simple, elongated, broad at basal half, being narrow from the distal half of sacculus; costa somewhat straight; cucullatus round; a short weakly sclerotized process located near the middle area of sacculus. Cristae of juxta with a short arm dorsally and a long finger-like one ventrally. Saccus small. Aedeagus short, vesica simple.

Female genitalia (Fig. 13b). Papillae anales round, sclerotized, with numerous setae. Anterior apophyses almost as long as posterior apophyses. Eighth tergite sclerotized, slightly shorter longitudinally than transversely. Ostium pocket-shaped, strongly sclerotized. Ductus bursae short, straight, membranous and more flimsy. Corpus bursae ovate, thinly membranous, without signum.

Subfamily diagnosis. The subfamily Heraculinae can be clearly separated from its sister group Pseudobistoninae by filiform antennae in the males and the hind tibia with four spurs. Pseudobistoninae have a pair of bipectinate antennae in the males and their hind tibia only possess two spurs. Another distinct difference between the two subfamilies lies in the position of vein M2: this vein arises closer to M1 than to M3 in the hindwings of Heraculinae, whereas it arises closer to M1 than to M3 in the hindwings of Pseudobistoninae.

Etymology: The subfamily name is derived from that of the type genus, Heracula Moore.

Discussion

Our molecular phylogenetic analyses show that Heracula and Pseudobiston (the type genus of the monotypic family Pseudobistonidae established by Rajaei et al., 2015) form a strongly supported clade, which itself is the sister clade to the family Epicopeiidae with moderate support (Fig. 1). Although the systematic position of the Pseudobistonidae + Epicopeiidae clade has not been robustly resolved based on our eight-gene molecular analyses or the analyses of Rajaei et al. (2015), more extensive molecular evidence indicates that at least Epicopeiidae belongs to the superfamily Geometroidea (Bazinet et al., 2013; Regier et al., 2013; Heikkilä et al., 2015).

Molecular phylogenetic analyses from this study and Rajaei et al. (2015) indicate that Geometroidea are comprised of two main clades (namely, Pseudobistonidae, Epicopeiidae and Sematuridae form a clade, which is sister to Geometriidae + Uranidae), with weak support for the monophyly of this superfamily. Rajaei et al. (2015) proposed five morphological autapomorphies supporting the monophyly of Geometroidea, and provided a number of characters as derived traits among geometroid families, although several of these characters were
Heraculinae, a new subfamily of Pseudobistonidae

considered to be continuous in nature (Heikkilä et al., 2015). Rajaei et al. (2015) also provided two synapomorphies for Pseudobistonidae + Epicopeiidae + Sematuridae as well as four synapomorphies for Geometridae + Uraniidae, but they did not compare the variation of male abdominal tympanal organs for the two clades. In fact, the male abdominal tympanal organs are present in Geometridae and Uranidae, but absent in the remaining three families of Geometroidea (Minet & Scoble, 1998; Rajaei et al., 2015), this could be regarded as a diagnostic character if the sister relationship between the two clades is accepted.

Heracula and Pseudobiston share four imaginal synapomorphies: (i) prothoracic katepisternum appears subtriangular in anterior view (Fig. 3c, k; Rajaei et al., 2015: fig. 7, white arrow); (ii) prothorax has a large and weakly sclerotized epimeron caudad of the pleural sulcus (Fig. 3c, em; Rajaei et al., 2015: fig. 7, black arrow); (iii) the dorsal extremity of parepisternal sulcus in the mesothorax is located along the anterodorsal half of the parepisternum (Fig. 5b, pas; Rajaei et al., 2015: fig. 11, white arrow); (iv) Anterolateral brush organs are absent in the male abdomen (Fig. 12a, e; Rajaei et al., 2015: fig. 22).

The loss of anterolateral brush organs also has been considered as a synapomorphy for the clade Uranidae + Geometridae, which may be an instance of parallel evolution, as such brush organs are observed in the groundplan of Epicopeiidae and in the male of Apropropgon hesperistis of Sematuridae (Minet, 2003; Rajaei et al., 2015). Heracula also shares two derived traits with Pseudobiston, Epicopeiidae and Sematuridae: (i) the areole is absent from the forewing venation (Fig. 11; Rajaei et al., 2015: fig. 31); (ii) the neotergite is fused laterally with the marginotergites on the abdominal dorsum A1 (Fig. 12a; Rajaei et al., 2015: fig. 22).

In addition, the chaetosemata are present and remote from each other in both Heracula (Fig. 2i) and Pseudobiston, but they are not regarded as a synapomorphy of the two taxa, as similar structures also occur in many other Macroheterocera including some epicopeiid taxa (Minet, 2003; Rajaei et al., 2015). The chaetosemata are usually a small bundle of long setae in Epicopeiidae (Minet & Scoble, 1998), but absent in Mimaporia (Wei & Yen, 2017), and well-developed in Develeia with a transverse shape (Minet, 2003). The long setae of chaetosemata often overhang the eyes in Sematuridae (Minet & Scoble, 1998). In Geometridae and Uranidae, the well-developed, transverse chaetosemata have been treated as a synapomorphy of the two families, even though their sizes and shapes are variable in Geometridae (Scoble & Krüger, 2002; Rajaei et al., 2015).

At least nine morphological characters can be used to diagnose Heracula in the present paper. (i) The vertex, on each side
of the postgenal-occipital area, has two discrete, minute cranial pits (Fig. 2b, f). The cranial pits are proposed in Libytheinae, a subfamily of nymphalid butterflies, and are suspected to be an attachment site for a sensillum (Kawahara et al., 2012: figs 3, 4). These structures also are found on the vertex between the antennae in many geometrid moths (see the ‘extensions’ of Sihvonen, 2005: fig. 100), but are not recorded in Pseudobiston. (ii) Maxillary palpus is rather small, consisting of three segments (Fig. 2d, black arrow). There is only one segment in Pseudobiston, and one or two segments in Uraniaidae and Geometridae (Minet & Scoble, 1998; Rajaei et al., 2015). (iii) Antennae are filiform in the males. The filiform antennae are seen widely in the males of Geometroidea (Minet & Scoble, 1998), whereas the male antennae are bipectinate in Pseudobiston (Rajaei et al., 2015: Fig. 5). (iv) Ocelli are absent. The absence of ocelli is common in the Scoble, 1998; Rajaei Sematuridae, as well as most taxa of Geometroidea, but they are often present in Sematuridae, reduced in family-group taxa of Geometroidea, and is absent in the sematurid genus Sematura (Fänger, 1999; Rajaei et al., 2015). (v) Euphragma and meron of the metathorax are bordered only by a single metacoxal sulcus (Fig. 6c, d). In Pseudobiston, merocosta is divided by a short gap into two sections that delimit laterodorsally a subtriangular region of the meron (Rajaei et al., 2015: Fig. 9). (vi) hind tibia possess four spurs (Fig. 7c). The same situation also occurs in Epicopeiidae, Sematuridae, as well as most taxa of Geometridae and Uraniaidae (Minet & Scoble, 1998), however, only two hind tibial spurs appear in Pseudobiston (Rajaei et al., 2015: fig. 26). (vii) Vein M2 arises closer to M3 than to M1 in both fore- and hindwings (Fig. 11). In Pseudobiston, M2 arises closer to the upper angle of the cell than to the base of M3 in the forewings, and closer to M1 than to M3 in the hindwings (Rajaei et al., 2015: fig. 31). (ix) Sockets are absent in the male genitalia (Fig. 13a). These structures are very distinctive in Pseudobiston (Rajaei et al., 2015), and are considered as a part of the ground plan in several superfamilies including the Geometroidea (Schmidt, 2017).

As mentioned above, although Heracula and Pseudobiston form a well-supported monophyletic clade from our molecular analysis, morphological evidence shows that there exist great differences between two taxa. Heracula can be easily distinguished from Pseudobiston by the nine morphological characters enumerated in the above paragraph. Many of these characters are conservative in Lepidoptera, and their variations are often regarded as derived characters of higher-level taxa (Scoble, 1992; Kristensen, 2003). In addition, the shared synapomorphies of Heracula and Pseudobiston, except for the absence of anterolateral brush organs, are three thoracic features, which are usually not clear-cut and difficult to observe without a dissection of thoracic skeleton. For these reasons, here we formally propose two independent monotypic subfamilies (Heraculinae and Pseudobistoninae) of Pseudobistonidae to accommodate Heracula and Pseudobiston, respectively.

With regard to the clade Pseudobistonidae + Epicopeiidae, Rajaei et al. (2015) interpreted its sister relationship using seven imaginal synapomorphies, among which a dorsal foramen of the euphragma cannot now be used as synapomorphy, because Heraculinae, with an oval central foramen (Fig. 6a), are placed as a subfamily of Pseudobistonidae. Epicopeiidae currently include 26 species in 10 genera, and exhibit high morphological diversity and various complex mimicry features (Wei & Yen, 2017). The monophyly of Epicopeiidae is well studied based on morphological traits (Minet, 2003; Rajaei et al., 2015), but it has not been verified by molecular data. Recently, Wei & Yen (2017) did a preliminary molecular phylogenetic analysis of Epicopeiidae with incomplete sampling, but their results show significant differences from those of the morphological analysis in Minet (2003). Further molecular phylogenetic studies are needed for clarifying the monophyly of Epicopeiidae and the relationships among the genera of this family.

© 2019 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, 44, 211–225
Notes on the resemblance of the habitus in Heracula and Calliprogonos

In the course of this work we noted the resemblance of the unusual habitus of Heracula to that of the similarly monobasic bombycoid brahmaid genus Calliprogonos Mell & Hering, reviewed by Naumann (2009), particularly in the large pale ring at the base of the forewing (Fig. 9). However, morphological and molecular evidence precludes any close phylogenetic relationship between the two genera. For instance, the lower edge of hindwing discal cell is longer than vein M3 in Heracula (Fig. 11), but is markedly shorter than vein M3 in Calliprogonos (Lemaire & Minet, 1998: fig. 18.5H). Moreover, male of Heracula has a retinaculo-frenate wing coupling (Fig. 10), whereas male wing coupling is amplexiform in Calliprogonos ((Lemaire & Minet, 1998). Furthermore, DNA barcoding indicates that Calliprogonos is very close to the brahmaid genus Dactylocerus Mell (Naumann, 2009). Therefore, the resemblance between the two genera can only be through convergence. The resemblance may be purely fortuitous were not the two taxa sympatric over the more localized range of the brahmaeid, occurring at similar altitudes of 1500 m a.s.l. upwards, leading to the possibility of some interaction between them, with the ranges suggesting that Heracula would be the model in the case of mimicry. Field observations of the species at rest would be valuable to assess whether the rings might appear as eye spots to a potential predator.

The photograph of Pseudobiston at rest in Rajaei et al., 2015, Fig. 4) shows a resting posture that, if transposed to the other two taxa, would place the pale rings near the front of the moth and, if in the head-up position, could resemble the eyes of a bird, with the pale wing-margins forming a band below the eyes. The upper part of the Pseudobiston at rest does suggest, to a fertile imagination, the head of a bird, with the black-centred pale lunules at the bases of the forewings being the eyes.

Biogeographical distribution of Pseudobistonidae and their relatives

The Pseudobistonidae, Epicopeiidae and Sematuridae are placed in a clade sister to the rest of the Geometroidea: the Uraniiidae and the Geometridae. They are all tropical or subtropical in distribution. Three of the family-group taxa are monobasic: the southern African subfamily of the Sematuridae, Apoprogoninae, and the two subfamilies of the Oriental but allopatric Pseudobistonidae discussed here. Both the Sematurinae and the Epicopeiidae contain several genera, the former mainly Neotropical and the latter mainland Oriental apart from a species of Epicopeia in Sumatra.

The Uraniiidae (Lees & Smith, 1991; Holloway, 1998) are pantropical, a range spanned by the Uraniiinae and the most species-rich subfamily, the Epipleminae. The two other subfamilies, the Auzeinae and the Microniinae, are found only in the Palaeotropics.

The family Geometridae has a cosmopolitan distribution, and is currently divided into eight subfamilies including four major ones: Larentiinae, Sterrhinae, Geometrinae and Ennominae (Sihvonen et al., 2011; Mitter et al., 2017). The Ennominae are the most species-rich, but the Larentiinae are more diverse at high latitudes and, in the tropics, high altitudes.

In the present study we have resolved the systematic position of the mysterious taxon Heracula, transferring it from Noctuoidea to Geometroidea as a new subfamily of Pseudobistonidae, using multilocus molecular phylogenetic data together with the re-examination of its morphological characters. In fact, Heracula is not the only mysterious taxon to have been misclassified in the Lymantriinae. Another example within Macroheterocera is the Oriental genus Allotoma Roepke which was originally placed in the Notodontidae but transferred to Lymantriidae by Kiriakoff (1968, Genera Insectorum 217c: 2) without comment, a placement that still stood in Watson et al.
(1980). The genus was associated with Bombycoidea by Holloway (1999: 153–154), where it cannot be clearly assigned to any particular family based on the existing morphological traits. A more extreme transfer in phylogenetic placement was that of Shisa excellens Strand from the Lymantriae to the cossid family Rataridiae (Owada, 1993).

Mysterious taxa, with dubious placements from morphological information, also have been found in some other Lepidopteran lineages, and molecular phylogenetic data have been increasingly applied in addition to morphology to ascertain their systematic status with some particularly successful cases. For example, the systematic position of the rare Asian butterfly genus Calimaga had long been a topic for debate until it was recently verified as a monobasic subfamily Calinaginae in Nymphalidae based mainly on molecular phylogenetic data (Wahlberg, et al., 2003; Todisco, et al., 2017). The moth tribe Diptychini Janse, with a controversial placement, was demonstrated to be a group of Ennominiae in Geometridae using an eight-gene molecular dataset (Sihvonen, et al., 2015). The mysterious African moth Prodidactis mystica (Meyrick) was finally confirmed as a monotypic family Prodidactidae belonging to Hyblaeoidea using molecular and morphological data (Kaila, et al., 2013). The enigmatic Palaeartic moth genus Lypusa Zeller was historically assigned to Tineoidea, but later clarified as a family-level taxon in Gelechioidea based on the phylogenetic analyses of a seven-gene dataset (Heikkilä & Kaila, 2010; Kaila, et al., 2011). The small Aenigmatinea glatzella Kristensen & Edwards was elucidated as a case of a new extant family Aenigmatineidae sister to Neopseustidae with evidence from 25 genetic loci (Kristensen, et al., 2015). Thus, molecular phylogenetic data, as an additional powerful tool, may shed light on the systematic placements of such mysterious taxa in Lepidoptera where morphological studies have failed.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Aligned DNA matrix used in the phylogenetic analyses.nex.

Table S1. Label data for the illustrated specimens in this study.

Table S2. List of taxa with voucher codes and Genbank accession numbers.

Acknowledgements

We thank Mr Yasunori Kishida (Tokyo, Japan) for providing specimens. We also thank Maia Vaswani, Mr Alessandro Giusti and Dr Alberto Zilli (Natural History Museum, London, NHMUK) for providing the images of the female adult of H. discivitta and its genitalia. This work was supported by a grant from National Natural Science Foundation of China to HSW (No. 31601879), by overseas study program of Guangzhou Elite Project (Sui jiaoke[2012] 59).

References

Bazinet, A.L., Cummings, M.P., Mitter, K.T. & Mitter, C.W. (2013) Can RNA-seq resolve the rapid radiation of advanced moths and butterflies (Hexapoda: Lepidoptera: Apoditrysa)? An exploratory study. PLoS ONE, 8, e82615.

Brock, J.P. (1971) A contribution towards an understanding of the morphology and phylogeny of the Ditrysian Lepidoptera. Journal of Natural History, 5, 29–102.

Chao, Z.L. (2003) Lepidoptera Lymantriidae. Fauna Sinica, Insecta 30. Science Press, Beijing.

Fanger, H. (1999) Comparative morphology of tergal phragmata occurring in the dorsal thoraco-abdominal junction of ditrysian Lepidoptera (Insecta). Zoomorphology, 119, 163–183.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium Series, 41, 95–98.

Heikkilä, M. & Kaila, L. (2010) Reassessment of the enigmatic Lepidoptera family Lypusidae (Lepidoptera: Tineoidea; Gelechioidea). Systematic Entomology, 35, 71–89.

Heikkilä, M., Mutanen, M., Wahlberg, N., Sihvonen, P. & Kaila, L. (2015) Elusive ditrysian phylogeny: an account of combining systematized morphology with molecular data (Lepidoptera). BMC Evolutionary Biology, 15, 260. https://doi.org/10.1186/s12862-015-0520-0.

Holloway, J.D. (1998) The moths of Borneo (part 8): Castniidae, Callidulidae, Drepanidae, Uranidae. Malayau Nature Journal, 52, 1–155.

Holloway, J.D. (1999) The moths of Borneo (part 5): family Lymantriidae. Malayau Nature Journal, 53, 1–188.

Kaila, L., Mutanen, M. & Nyman, T. (2011) Phylogeny of the mega-diverse Gelechioidea (Lepidoptera): adaptations and determinants of success. Molecular Phylogenetics and Evolution, 61, 801–809.

Kaila, L., Epstein, M., Heikkilä, M. & Mutanen, M. (2013) The assignment of Prodidactidae to Hyblaeoidea, with remarks on Thyridoidea (Lepidoptera). Zootaxa, 3682, 485–494.

Kawahara, A.Y. & Breinhold, J.W. (2014) Phylogenomics provides strong evidence for relationships of butterflies and moths. Proceedings of the Royal Society (B), 281, 20140970.

Kawahara, A.Y., Ortiz-Acevedo, E. & Marshall, C.J. (2012) External morphology of adult Libythea celtis (Laicharting [1782]) (Lepidoptera: Nymphalidae). Zoological Science, 29, 463–476.

Kirkioff, S.G. (1968) Notodontidae III (Indo-Australica). Genera Lepidopterorum 217 c (ed. by P. Wytmsan), pp. 1–269. Genera Lepidopterorum, Kraaimem.

Kishida, Y. (1993) Lymantriidae. Moths of Nepal, Part 2 (ed. by T. Haruta). Tinea, 13 (Suppl.), pp. 80–95. The Japan Heterocerists’ Society, Tokyo.

Kristensen, N.P. (2003) Skeleton and muscles: adults. Handbuch der Zoologie/Handbook of Zoology IV/36, Lepidoptera, Moths and Butterflies, Vol. 2 (ed. by N.P. Kristensen), pp. 39–131. Walter de Gruyter, Berlin.

Kristensen, N.P., Hilton, D.J., Kallies, A. et al. (2015) A new extant family of primitive moths from Kangaroo Island, Australia and its significance for understanding early Lepidoptera evolution. Systematic Entomology, 40, 5–16.

Lees, D.C. & Smith, N.G. (1991) Foodplant associations of the Urania and their systematic evolutionary and ecological significance. Journal of the Lepidopterists Society, 45, 296–347.

© 2019 The Authors. Systematic Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, 44, 211–225
Lemaire, C. & Minet, J. (1998) The Bombycoidea and their relatives. *Lepidoptera, Moths and Butterflies, Vol. 1: Evolution, Systematics and Biogeography* (ed. by N.P. Kristensen), pp. 321–353. Walter de Gruyter, Berlin.

Matuda, R. (1970) Morphology and evolution of the insect thorax. *Memoirs of the Entomological Society of Canada, 76*, 1–431.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *Gateway Computing Environments Workshop (GCE)*, New Orleans, Louisiana, pp. 1–8.

Minet, J. (2003) The Epicoepidei: phylogeny and a redetermination, with the description of new taxa (Lepidoptera: Drepanoidea). *Annales de la Société Entomologique de France (Nouvelle Série), 38*, 463–487.

Minet, J. & Scoble, M.J. (1998) The Drepanoide/Geometroide assemblage. *Lepidoptera, Moths and Butterflies, Vol. 1: Evolution, Systematics and Biogeography* (ed. by N.P. Kristensen), pp. 301–320. Walter de Gruyter, Berlin.

Mitter, C., Davis, D.R. & Cummings, M.P. (2017) Phylogeny and evolution of Lepidoptera. *Annual Review of Entomology, 62*, 265–283.

Moore, F. (1866) On the Lepidopterous insects of Bengal. *Proceedings Zoological Society of London, 1865*, 755–823.

Mutanen, M., Wahlberg, N. & Kaila, L. (2010) Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. *Proceedings of the Royal Society B, 277*, 2839–2848.

Naumann, S. (2009) Notes on the Chinese Calliprogonos miraculosa Mell, 1937 (Lepidoptera: Brahmaeidae). *Nachrichten des Entomologischen Vereins Apollo N.F., 30*, 5–8.

Nieukerken, E.J.V., Kaila, L., Kitching, I.J. et al. (2011) Order Lepidoptera. *Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness* (ed. by Z.-Q. Zhang). Zootaxa, *3148*, 212–221.

Nylander, J.A.A. (2004) MrModelTest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Owada, M. (1993) The systematic position of Shisa excellens (Lepidoptera: Rataridae). *Japanese Journal of Entomology, 61*, 251–260.

Rajaee, H.S., Greve, C., Letsch, H., Stünig, D., Wahlberg, N., Minet, J. & Misof, B. (2015) Advances in Geometroidea phylogeny, with characterization of a new family based on Pseudobiston pinrutatani (Lepidoptera, Glossata). *Zoologica Scripta*, *44*, 418–436.

Regier, J.C., Zwick, A., Cummings, M.P. et al. (2009) Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. *BMC Evolutionary Biology, 9*, 280.

Regier, J.C., Mitter, C., Zwick, A. et al. (2013) A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). *PLoS ONE, 8*, e58568.

Ren, F., Tanaka, H. & Yang, Z. (2005) An empirical examination of the utility of codon-substitution models in phylogeny reconstruction. *Systematic Biology, 54*, 808–818.

Roe, A.D., Weller, S.J., Baixeras, J. et al. (2010) Evolutionary framework for Lepidoptera model systems. *Genetics and Molecular Biology of Lepidoptera* (ed. by M. Goldsmith and F. Marec). CRC Press, Boca Raton, FL.

Ronquist, F., Teslenko, M., van der Mark, P. et al. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*, 539–542.

Schmidt, O. (2017) Myths and facts about the absence of socii in the male genitalia of Larentiinae (Lepidoptera: Geometridae). *Zootaxa, 4286*, 254–266.

Scoble, M.J. (1992) The Lepidoptera: Form, Function, and Diversity. Oxford University Press, Oxford, U.K.

Scoble, M.J. & Kruger, M. (2002) A review of the genera of Macarini with a revised classification of the tribe (Geometridae: Ennominae). *Zoological Journal of the Linnean Society, 134*, 257–315.

Sihvonen, P. (2005) Phylogeny and classification of the Scopulininae moths (Lepidoptera: Geometridae. Sterrhinae). *Zoological Journal of the Linnean Society, 143*, 473–530.

Sihvonen, P., Mutanen, M., Kaila, L., Brehm, G., Hausmann, A. & Staude, H.S. (2011) Comprehensive molecular sampling yields a robust phylogeny for geometrid moths (Lepidoptera: Geometridae). *PLoS ONE, 6*, e20356.

Sihvonen, P., Staude, H.S. & Mutanen, M. (2015) Systematic position of the enigmatic African cycad moths: an integrative approach to a nearly century old problem (Lepidoptera: Geometridae: Diptychini). *Systematic Entomology, 40*, 606–627.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology, 57*, 758–771.

Timmermans, M.J.T.N., Lees, D.C. & Simonsen, T.J. (2014) Towards a mitogenomic phylogeny of Lepidoptera. *Molecular Phylogenetics and Evolution, 79*, 169–178.

Todisco, V., Nazari, V., Cesaroni, D. & Sbordoni, V. (2017) Preliminary molecular phylogeny and biogeography of the monobasic subfamily Caligninae (Lepidoptera, Nymphalidae). *Zoosystematics & Evolution, 93*, 243–254.

Trient, D.A., Cinel, S.D. & Kawahara, A.Y. (2018) Lepidoptera genomes: current knowledge, gaps and future directions. *Current Opinion in Insect Science, 25*, 99–105.

Wagner, D.L. (2001) Moths. *Encyclopedia of Biodiversity* (ed. by S.A. Levin). Academic Press, San Diego, CA.

Wahlberg, N. & Wheat, C.W. (2008) Genomic outposts serve the phylogenomic pioneers: designing novel nuclear markers for genomic DNA extractions of lepidoptera. *Systematic Biology, 57*, 231–242.

Wahlberg, N., Weingartner, E. & Nylin, S. (2003) Towards a better understanding of the higher systematics of Nymphalidae (Lepidoptera: Papilionoidea). *Molecular Phylogenetics and Evolution, 28*, 473–484.

Wang, H.S., Wahlberg, N., Holloway, J.D. et al. (2015) Molecular phylogeny of Lymanniinae (Lepidoptera, Noctuoidea, Erebidae) inferred from eight gene regions. *Cladistics, 31*, 579–592.

Watson, A., Fletcher, D.S. & Nye, I.W.B. (1980) *The Generic Names of Moths of the World*. Vol. 2. British Museum (Natural History), London.

Wei, C.H. & Yen, S.H. (2017) *Mimaporia*, a new genus of Epicopeidae (Lepidoptera), with description of a new species from Vietnam. *Zootaxa, 4254*, 537–550.

Wootton, R.J. (1979) Function, homology and terminology in insect wings. *Systematic Entomology, 4*, 81–93.

Zahiri, R., Kitching, I.J., Lafontaine, J.D., Mutanen, M., Kaila, L., Holloway, J.D. & Wahlberg, N. (2011) A new molecular phylogeny offers hope for a stable family level classification of the Noctuoidea (Lepidoptera). *Zoologica Scripta, 40*, 158–173.

Zahiri, R., Holloway, J.D., Kitching, I.J., Lafontaine, J.D., Mutanen, M. & Wahlberg, N. (2012) Molecular phylogenetics of Erebidae (Lepidoptera, Noctuoidea). *Systematic Entomology, 37*, 102–124.

Zahiri, R., Lafontaine, J.D., Holloway, J.D., Kitching, I.J., Schmidt, B.C., Kaila, L. & Wahlberg, N. (2013a) Major lineages of Nolidae (Lepidoptera, Noctuoidea) elucidated by molecular phylogenetics. *Cladistics, 29*, 337–359.

Zahiri, R., Lafontaine, J.D., Schmidt, B.C., Holloway, J.D., Kitching, I.J., Mutanen, M. & Wahlberg, N. (2013b) Relationships among the basal lineages of Noctuidae (Lepidoptera, Noctuoidea) based on eight gene regions. *Zoologica Scripta, 42*, 488–507.

Accepted 30 June 2018

© 2019 The Authors. *Systematic Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society. *44*, 211–225.