



A New Species of *Aporia* (Lepidoptera: Pieridae) from Northwest Yunnan, China with Taxonomic Notes on Its Similar Sympatric Taxa

SHAO-JI HU^{1,2,5}, HUI-HONG ZHANG³, YANG YANG⁴

¹ Yunnan Key Laboratory of International Rivers and Transboundary Eco-security, Yunnan University, Kunming, 650500, China.

² Institute of International Rivers and Eco-security, Yunnan University, Kunming, 650500, China.

³ School of Agriculture, Yunnan University, Kunming, 650500, China.

✉ leozhanghh@foxmail.com; <https://orcid.org/0000-0003-1992-8901>

⁴ Room 515, Building No. 10, Anwai Xiaoguanbeili, Chaoyang District, Beijing, 100029, China.

✉ robert64ed@gmail.com; <https://orcid.org/0000-0002-1417-4154>

⁵ Corresponding author: ✉ shaojiu@hotmai.com; <https://orcid.org/0000-0002-1615-9601>

Abstract

The northwest corner of Yunnan Province, China is the world-class diversity centre of the genus *Aporia* Hübner, 1819 (Lepidoptera: Pieridae). During our expeditions to this area in 2019 and 2020, a new species of *Aporia* was discovered from the upper Yangtze River valley in northwest Yunnan, China. The new taxon, *A. chunhaoi* sp. nov., is similar to *A. lthamo* (Oberthür, 1893) but can be easily identified by larger size, much paler (creamy) male hindwing, as well as different genitalic structures. Our field surveys and comparative studies also confirmed a new range of *A. lthamo* in the upper Yangtze River valley, and the morphological variation of this species is discussed herein.

Keywords: *Aporia lthamo*; genitalic structure; upper Yangtze River valley; upper Lancang-Mekong River valley; subalpine habitats

Introduction

The genus *Aporia* Hübner, 1819 (Lepidoptera: Pieridae) consists of an iconic group of Palaearctic butterflies with the Himalaya-Hengduan Mountains being its diversity centre (Della Bruna *et al.* 2013). Although some species are found in a wider range, e.g., *A. crataegi* (Linnaeus, 1758), many species and subspecies are geographically restricted, occupying only a certain section of a mountain range or river valley (Della Bruna *et al.* 2013). Radiative speciation due to complex geographic conditions is believed to be the driving force underpinning this distribution pattern (Kanoh *et al.* 2016). The complex distribution pattern and high diversity resulted in cryptic species, and a good number of taxa were described in the last four decades (Koiwaya 1989, Hsu & Chou 1999; Koiwaya 1993; Della Bruna *et al.* 2003, 2009).

Aporia lthamo (Oberthür, 1893) is an unmistakable species among all known *Aporia* species due to its extensively darkened wings, conspicuously small size, and very limited distribution range in the upper Lancang-Mekong valley in N.W. Yunnan (Della Bruna *et al.* 2013). However, during the curation of *Aporia* specimens collected in 2019 and 2020, the authors noticed a series of rather paler '*A. lthamo*' collected in the upper Yangtze River valley in N.W. Yunnan, showing constant morphological differences from the typical *A. lthamo* collected near its type locality in the upper Lancang-Mekong valley. During the course of literature analysis, the first author discovered that the paler '*A. lthamo*' from Weixi County was illustrated in Wu & Hsu (2017). After a discussion with the photo provider and the owner of that particular specimen, the specific collecting site was determined to be in the upper Yangtze River valley (C. H. Wang, pers. comm.).

Based on the above-mentioned findings, the authors performed analyses on morphological characters and genitalic structures. The results suggested the paler '*A. lthamo*' from upper Yangtze River valley deserves recognition as a good species new to science. Its morphology is described herein with a taxonomic note on the sympatric similar taxa in N.W. Yunnan.

Materials and methods

Taxon sampling

The focal species and *A. lhamo* were sampled for both morphological and molecular analyses. For a better understanding of the intra- and inter-specific differences and variations of the sympatric taxa in N.W. Yunnan (Della Bruna *et al.* 2013), *A. procris* (Leech, 1890) and *A. uedai* Koiwaya, 1989 from N.W. Yunnan were also included. The specimens used in this study are listed in Appendix 1. The holotype and six paratypes were deposited in the collection of Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (accession numbers 0127556–0127562). The remaining ten paratypes are kept in the private collection of the first author, and will be transferred to the collection of KIZ in the future. Specimens of *A. lhamo*, *A. procris*, and *A. uedai* are kept in the private collections of the authors as noted in Appendix 1.

Morphological comparisons

Spread specimens were photographed with medium grey background and the photos were adjusted using Adobe Photoshop CS (Adobe, USA). Diagnostic characters of the new taxa were also denoted using Adobe Photoshop CS. For comparison between taxa, the lengths of forewing were measured to 0.5 mm precision. Average lengths and the standard deviations were also calculated when $n \geq 3$. Terminology of wing venation follows Hsu *et al.* (2018).

To observe the male and female genitalia, the abdomen was treated with 1 mL 10% sodium hydroxide solution to digest soft tissue at 70 °C for 1 h, and then dissected in a water-filled Petri dish under a stereoscope. The genitalia were then transferred to 80% glycerol for 12 h to render them transparent. For female genitalia, 2% chlorazol black solution was used to dye the membranous parts for 10 min in order to obtain better results in photographing. Photographs were taken with a Nikon SMZ1500 stereoscope (Nikon, Japan) and automatically stacked using Helicon Focus 7.5.8 (Helicon Software, USA). After observation and photography, all parts of the genitalia were fixed on a glue card and pinned with the specimen to avoid confusion.

Terminology of the general structures of genitalia follows Hu *et al.* (2018), while those specialised parts of genus *Aporia*, *e.g.*, fovea (the hole inside the sclerotized lining of valve) and trochanter (the sclerotized projection at the base of aedeagus) follows Della Bruna *et al.* (2013). The valve, uncus (especially the dorsal view), fovea, aedeagus, and juxta in male genitalia and signum in female genitalia are characteristics for species delimitation and are described and compared herein.

Results

Aporia chunhaoi Hu, Zhang & Yang sp. nov.

Aporia lhamo (Oberthür, 1893); in Wu & Hsu (eds.). 2017. Butterflies of China, 1: 375 (text), 376 (plate), figs. 2–4.

Description:

Male (Figures 1A–C). Forewing length 23.0–28.0 mm (mean = 25.6 ± 1.3 mm, $n = 14$). Body blackish covered with grey hair, ventral side of abdomen covered by whitish scales. Head small; labial palpi slender, with third segment as long as second; antenna half of forewing length, club abrupt, black with a small white spot at tip. Forewing cell elongate, over half of forewing length; four radial veins: R_2 and R_1 rise freely from cell, R_{4+5} and R_3 on a long stalk; M_1 rises from stalk of R_{4+5} and R_3 . Forewing upperside: ground colour grey with black veins and paler subterminal oval spots, five faintly defined blackish Y-shape streaks (arrow heads) embedded at base of those subterminal spots in cells CuA_1 to R_5 , forming a wavy band. Forewing underside: similar to upperside, but marked with even paler stripes in all cells, apical area and neighbouring termen tinged with yellow. Hindwing vein $Sc+R_1$ ends beyond origin of vein M_1 . Hindwing upperside: ground colour creamy yellow, veins black with whitish scales running through and black scales peppered along sides, six blackish Y-shape streaks in postdiscal area in cells CuA_2 to R_5 , three well defined Y-shape streaks in cells CuA_1 to M_2 , others faint. Hindwing underside: ground colour deep

yellow with thin and clear black veins, five clearly defined black Y-shape streaks in cells CuA₁ to R₅, that in cell CuA₂ usually rather faint.

Female (Figure 1D). Only one female available. Forewing length 26.0 mm. Similar to male with rounded forewing apex and broader wing shape. Upperside of both wings paler, with less blackish scales. Underside of hindwing creamy yellow with faintly marked Y-shape streaks.

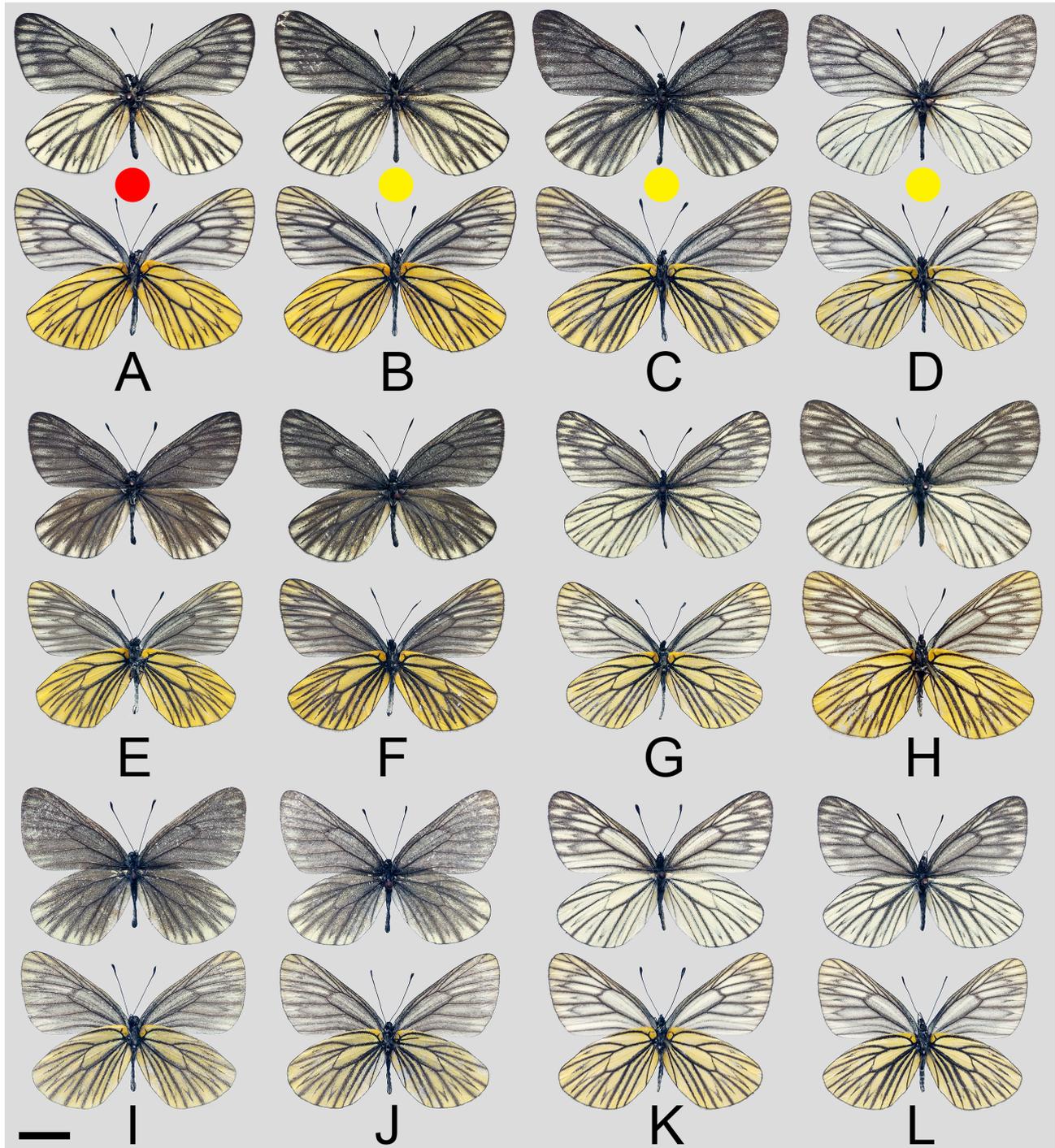


FIGURE 1. A–D: Types of *Aporia chunhaoi* Hu, Zhang & Yang **sp. nov.** A: ♂, **HOLOTYPE**, Tacheng, Yulong County; B: ♂, **PARATYPE**, the same locality as the holotype; C: ♂, **PARATYPE**, Shiba, Deqen County; D: ♀, **PARATYPE**, the same locality as the holotype. E–L: *Aporia lhamo* (Oberthür, 1893). E and F: ♂, Zha'an, Deqen County; G: ♀, near Yubeng, Deqen County; H: ♀, Badi, Weixi County; I and J: ♂, Xiao Xueshan, Zhongdian County; K and L, ♀, the same locality. Scale bar = 10 mm.

Male genitalia (Figure 2A). Heavily sclerotized. Ring slender, straight, perpendicular with saccus and tegumen; uncus broad at base, gradually narrowed into a pointed tip; saccus short and broad. Valve nearly triangulate, with dorsal base concave, ventral margin convex and tip falcate, fovea larger and dorsoventrally elongate. Aedeagus robust, evenly curved with a trochanter at its ventral base. Juxta V-shaped with two arms widely apart.

Female genitalia (Figure 2B). Papillae anales round and short, covered with dense setae. Apophyses posteriores in short rod shape, apophyses anteriores in short conical shape. Lamella antevaginalis connects a pair of smooth pouch-like sterigma, lamella postvaginalis slightly sclerotized. Ductus bursae tubular and membranous, rather slender. Corpus bursae oval with appendix bursae attached anteriorly, signum in narrow dumbbell shape, with median part asymmetrically concaved; signum entirely covered with spines.

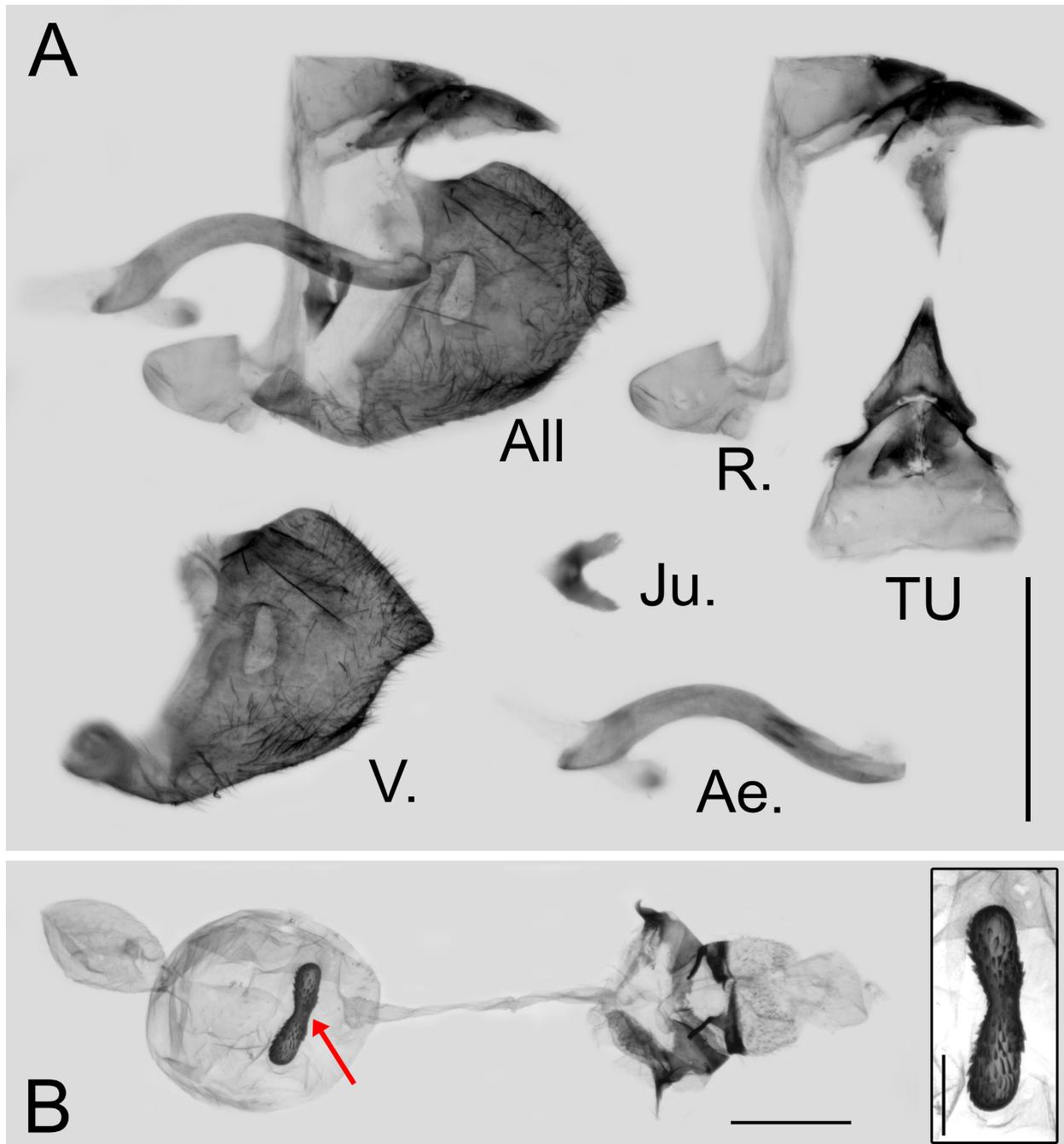


FIGURE 2. A: Male genitalia of *Aporia chunhaoi* Hu, Zhang & Yang **sp. nov.** from Yulong County (specimen A in Figure 1, KIZ 0127556). All: lateral view of the whole genitalia with left valve removed; R.: lateral view of ring; TU: dorsal view of tegumen and uncus; V.: inner lateral view of right valve; Ae.: lateral view of aedeagus; Ju.: posterior view of juxta. Scale bar = 1 mm. B: Female genitalia of *Aporia chunhaoi* Hu, Zhang & Yang **sp. nov.** from the same locality (specimen D in Figure 1, KIZ 0127562), scale bar = 1 mm; signum enlarged in the black box, scale bar = 0.5 mm.

Type materials: HOLOTYPE: ♂, Tacheng (2,800–3,000 m), Yulong County, Lijiang Prefecture, N.W. Yunnan, China, 2020–VII–15–20, Y. Yang *leg.* [KIZ 0127556]. **PARATYPES:** 4♂♂, the same data as the holotype, Y. Yang *leg.* [KIZ 0127557–0127560]; 7♂♂, the same data as the holotype, Y. Yang *leg.* [SJH]; 2♂♂, the same locality as the holotype, 2020–VII–25–30, Y. Yang *leg.* [SJH]; 1♀, the same collecting data, [KIZ 0127562]; 2♂♂, Ludian to Weixi Road (3,000 m), Weixi County, Diqing Prefecture, N.W. Yunnan, China, 2014–VI–13, C. H. Wang *leg.* [CHW]; 1♂, ditto, 2014–VI–13, X. M. Jing *leg.* [CHW]; 1♂, Shiba (2,600–2,700 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2020–VII–5–6, Y. Yang *leg.* [KIZ 0127561]; 1♂, the same collecting data, [SJH].

Voltnism: Univoltine, the adults fly from mid June to late July.

Distribution: Currently confined to N.W. Yunnan in the upper Yangtze River Valley.

Deravtio nominis: The name of this new species is dedicated to Mr. Chun-Hao Wang (Beijing, China), a Chinese butterfly expert who has great enthusiasm for genus *Aporia*. The specific name is a noun in apposition.



FIGURE 3. Male antennal tips of the five sibling *Aporia* taxa from N.W. Yunnan, China. A: *A. procris procris* (Leech, 1890); B: *A. procris lancangica* Della Bruna, Gallo & Sbordoni, 2003; C: *A. uedai* Koiwaya, 1989; D and E: *A. lhamo* (Oberthür, 1893); F: *A. chunhaoi* Hu, Zhang & Yang **sp. nov.** Scale bar = 1 mm.

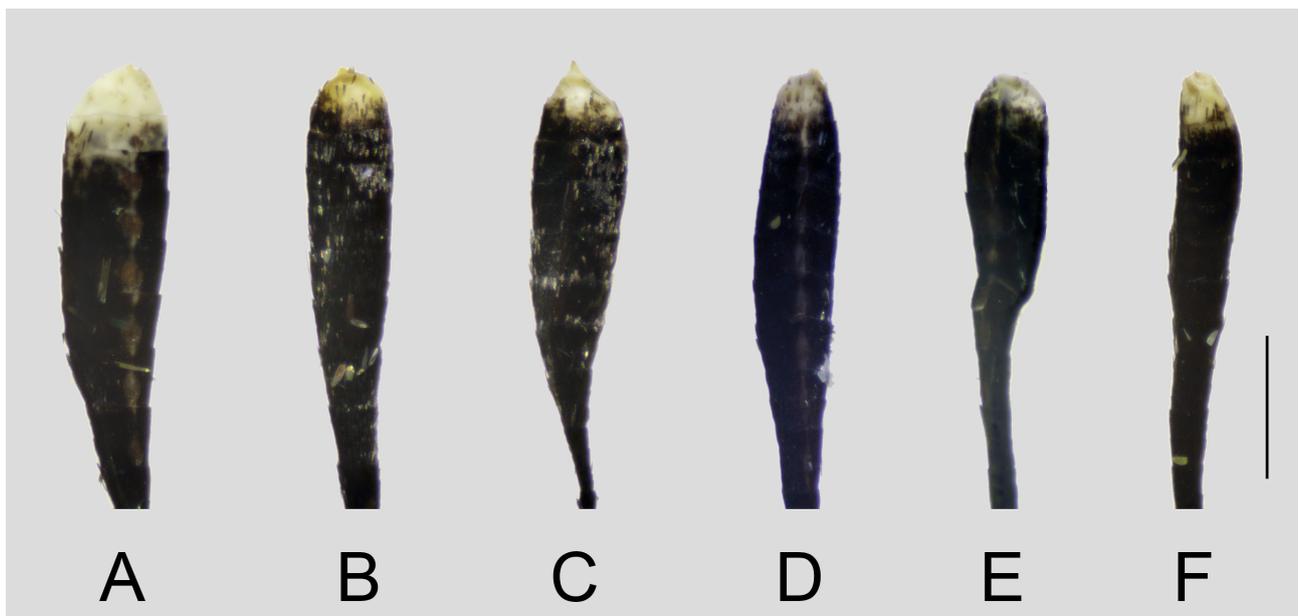


FIGURE 4. Female antennal tips of the five sibling *Aporia* taxa from N.W. Yunnan, China. A: *A. procris procris* (Leech, 1890); B: *A. procris lancangica* Della Bruna, Gallo & Sbordoni, 2003; C: *A. uedai* Koiwaya, 1989; D and E: *A. lhamo* (Oberthür, 1893); F: *A. chunhaoi* Hu, Zhang & Yang **sp. nov.** Scale bar = 1 mm.

Diagnostic characters: This new species can be easily distinguished from the similar species *A. lhamo* by the following characters: (1) slightly larger, overall colour much paler, the hindwing upperside is creamy yellow with only black veins, even in the males (Figure 1); (2) the tip of antennal club with a small white spot, while in *A. lhamo* it is completely black, or only poorly indicated (Figures 3 and 4); (3) the black Y-shape streaks (arrow heads) on the underside of hindwing evidently shorter, only half as long as those in *A. lhamo* (Figures 1E–K); (4) male genitalia distinct: ring almost perpendicular with the saccus (right angled in *A. lhamo*, *A. procris*, and *A. uedai*), saccus shorter and broader (longer and narrower in *A. lhamo*, *A. procris*, and *A. uedai*), the tip of uncus gradually narrowed into a pointed end (abruptly narrowed in *A. lhamo* and *A. uedai*, but less abrupt in *A. procris*), fovea much larger and dorsoventrally elongated (much smaller and rounded in *A. lhamo*, *A. procris*, and *A. uedai*), aedeagus robust like in *A. lhamo* and *A. procris*, but not as slender in *A. uedai*; juxta larger with arms more widely apart (thinner in *A. lhamo* and *A. uedai*) (Figures 2A and 5A–C); (5) in female genitalia: signum more medially concave than that of *A. lhamo* and *A. procris*, while less concave than that of *A. uedai* (Figures 2B and 5D–F).

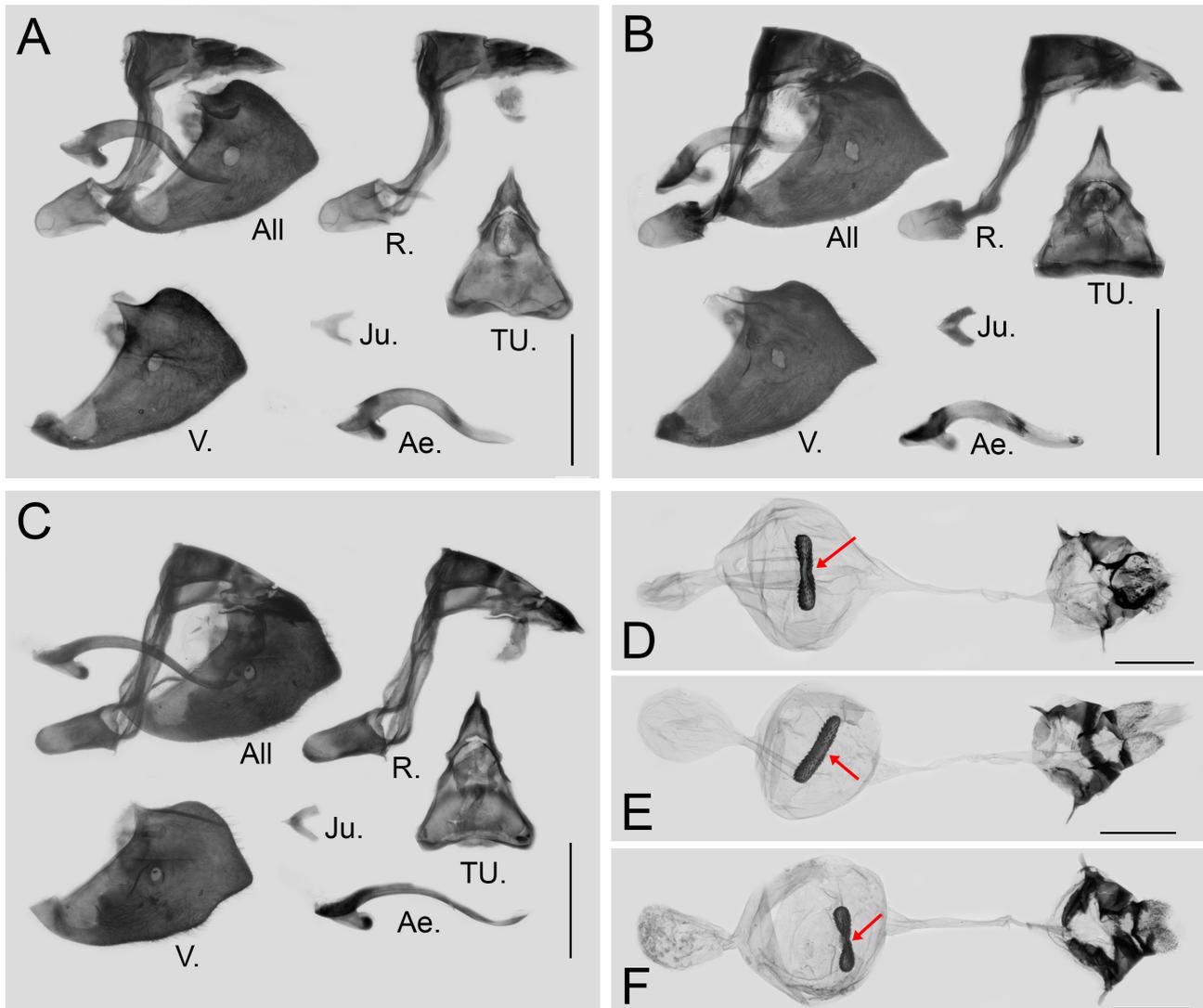


FIGURE 5. Male and female genitalia of the sympatric taxa. A: male genitalia of *Aporia lhamo* (Oberthür, 1893), Zha'an, Deqen County (specimen E in Figure 1); B: male genitalia of *Aporia procris procris* (Leech, 1890), Yulong Xueshan; C: male genitalia of *Aporia uedai* Koiwaya, 1989, Zha'an, Deqen County; D: female genitalia of *Aporia lhamo* (Oberthür, 1893), Badi, Weixi County (specimen H in Figure 1); E: female genitalia of *Aporia procris procris* (Leech, 1890), Yulong Xueshan; F: female genitalia of *Aporia uedai* Koiwaya, 1989, Zha'an, Deqen County. All: lateral view of the whole genitalia with left valve removed; R.: lateral view of ring; TU.: dorsal view of tegumen and uncus; V.: inner lateral view of right valve; Ae.: lateral view of aedeagus; Ju.: posterior view of juxta. Red arrows indicate difference in signum. Scale bars = 1 mm.

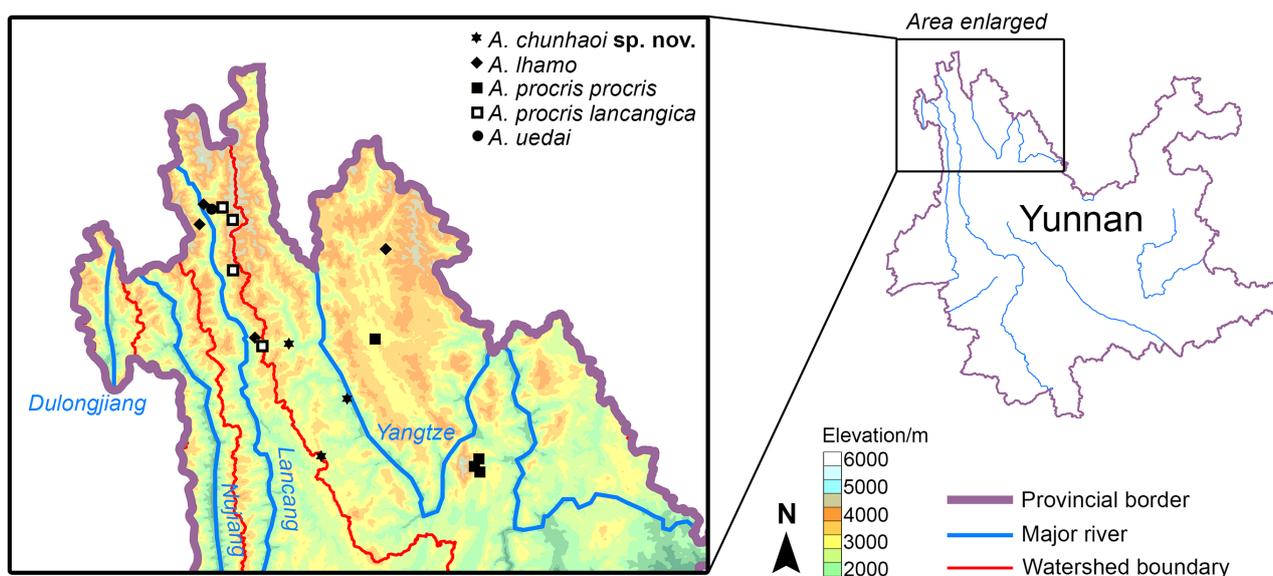


FIGURE 6. Distribution map of *Aporia chunhaoi* Hu, Zhang & Yang **sp. nov.** and sympatric taxa in N.W. Yunnan, China.

Discussion

Our expeditions in 2019 to N.W. Yunnan discovered a broader distribution range of *A. lhamo* in the upper Yangtze watershed, which makes the range of *A. lhamo* overlapping with *A. chunhaoi* **sp. nov.** Sympatric distribution, combined with the stable morphological and genitalic differences between the two *Aporia* species, support *A. chunhaoi* **sp. nov.** as a good species.

The broader distribution range of *A. lhamo* can be logically explained by the following two aspects: 1) the historical survey of *Aporia* in N.W. Yunnan is highly constrained by road accessibility, that in previous decades many areas were inaccessible compared to nowadays; 2) *Aporia lhamo* is not completely isolated in the upper Lancang-Mekong River valley by the mountains to its east, several suitable areas (mountain tracks ca. 3,000 m) are identified to the south of this mountain range (Figure 6), which may provide corridors to the upper Yangtze River valley. This discovery also implies the possibility of finding more *Aporia* species in a wider range in the future, as repeatedly mentioned by Della Bruna *et al.* (2003).

Aporia lhamo and *A. chunhaoi* **sp. nov.** are more variable than *A. procris* and *A. uedai*, especially the degree of melanism which could be different even within the same population. Our analysis discovered two much darkened individuals of *A. chunhaoi* **sp. nov.** collected near the mountain ridge separating the upper Lancang-Mekong and upper Yangtze watersheds, while a series of less darkened individuals with thicker hindwing black veins were also collected within the Yangtze River valley (Figure 1A–C). Such variations are also shown in Wu & Hsu (2017: plate 376, figs. 2–4). Similarly, some female individuals of *A. lhamo* collected in the new locality to the north of Zhongdian County (upper Yangtze watershed) are paler compared to the typical *A. lhamo* from the upper Lancang-Mekong River valley, while the males are more similar (Figure 1E–L). Future biogeographical and ecological research should be applied to answer the question whether such variation is geographically associated. Furthermore, our morphological comparison also confirmed variation in antennal tip in *A. lhamo*, which has been proposed by Della Bruna (2013) as a distinguishing character. In both upper Lancang-Mekong River valley and upper Yangtze River valley, male antennal tips varied from entirely blackish to with an evident whitish spot; the largest size of this whitish spot is approximately the same as that in *A. chunhaoi* **sp. nov.** (Figure 3). Meanwhile, this character is far less useful in females, since the whitish spot is more widely present in *A. uedai* and *A. lhamo*, as well as *A. chunhaoi* **sp. nov.** (Figure 4). Hence, the authors hereby propose that the antennal tip could only be effective when combined with other morphological characters.

During the analyses in this study, we also examined the differences in the DNA barcode sequences (mitochondrial *cox1* gene fragments) of all the sampled taxa. However, the DNA barcode sequences failed to separate these taxa effectively (we also included sequences from the Barcode of Life Database, <http://boldsystems.org/>). There are two

major problems in using DNA barcodes in this group of *Aporia*. First, the genetic distances between species are small (only 0.1–2.3%). Second, each species cannot form monophyly on any phylogenetic tree.

The reason why genetic divergence between these taxa is so limited still remains unclear. One likely speculation is that they underwent fast radiative divergence in the geographically complex Hengduan Mountains in very recent times. Species with lower genetic divergence in DNA barcode sequences have been met and reported in other butterflies, e.g., *Graphium daiyuanae* Hu, Zhang & Cotton, 2018 vs. *G. confucius* Hu, Duan & Cotton, 2018 (Hu *et al.* 2018), and *Papilio (Pterourus) eurymedon* Lucas, 1852 vs. *P. (P.) rutulus* Lucas, 1852 (Sperling 1993; Kunte *et al.* 2011). It appears to the authors that the situation of these *Aporia* may represent another extreme case of such shallow divergence or fits the characters of ‘budding speciation’ as mentioned in some other organisms (Vanderpoorten & Long 2006; Kruckenhauser *et al.* 2014; Kaya & Çiplak 2016; Cheng *et al.* 2017).

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Appendix 1. Specimens examined in this study.

Abbreviations of specimen depositories are listed as follow: [CHW], private collection of Chun-Hao Wang (Beijing, China); [HHZ], private collection of Hui-Hong Zhang (Kunming, China); [KIZ], Entomology Collection of Kunming Institute of Zoology, Chinese Academy of Sciences (Kunming, China); [SJH], private collection of Shao-Ji Hu (Kunming, China).

Aporia chunhaoi Hu, Zhang & Yang sp. nov.

HOLOTYPE: ♂, Tacheng (2,800–3,000 m), Yulong County, Lijiang Prefecture, N.W. Yunnan, China, 2020–VII–15–20, Y. Yang leg. [KIZ 0127556]. **PARATYPES:** 4♂♂, the same data as the holotype, Y. Yang leg. [KIZ 0127557–0127560]; 7♂♂, the same data as the holotype, Y. Yang leg. [SJH]; 2♂♂, the same locality as the holotype, 2020–VII–25–30, Y. Yang leg. [SJH]; 1♀, the same collecting data, [KIZ 0127562]; 2♂♂, Ludian to Weixi Road (3,000 m), Weixi County, Diqing Prefecture, N.W. Yunnan, China, 2014–VI–13, C. H. Wang leg. [CHW]; 1♂, ditto, 2014–VI–13, X. M. Jing leg. [CHW]; 1♂, Shiba (2,600–2,700 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2020–VII–5–6, Y. Yang leg. [KIZ 0127561]; 1♂, the same collecting data, [SJH].

Aporia lhamo (Oberthür, 1893)

1♂, N. of scenic view point of Meili Xueshan (3,100 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2014–VI–17, C. H. Wang leg. [CHW]; 4♀♀, near Yubeng (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2019–VI, Y. Yang leg. [SJH]; 1♀, Zha'an (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2018–VII–30, H. H. Zhang leg. [HHZ]; 1♂, Zha'an (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2019–VI–17, H. H. Zhang leg. [HHZ]; 6♂♂, ditto, 2019–VI–17, H. H. Zhang leg. [SJH]; 1♂, 1♀, ditto, 2019–VI–17, H. H. Zhang leg. [HHZ]; 1♀, Badi, Weixi County, Diqing Prefecture, N.W. Yunnan, China, 2019–VI–19, H. H. Zhang leg. [SJH]; 2♀♀, Zha'an (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2020–VIII–3, H. H. Zhang leg. [SJH]; 2♂♂, 2♀♀, Xiao Xueshan (N.E. of Geza) (3,200 m), Zhongdian County, Diqing Prefecture, N.W. Yunnan, China, 2020–VII–25–30, Y. Yang leg. [SJH].

Aporia procris procris (Leech, 1890)

1♂, Zhongdian County, Diqing Prefecture, N.W. Yunnan, China, 1987–VII–7, Y. X. Gan leg. [KIZ]; 8♂♂, ditto (3,200–3,500 m), Diqing Prefecture, N.W. Yunnan, China, 1987–VI–18, C. L. Li leg. [KIZ]; 1♂, Ganhaizi (3,100 m), Yulong County, Lijiang Prefecture, N.W. Yunnan, China, 2016–VI–15, H. H. Zhang leg. [HHZ]; 1♂, ditto, 2018–VI–21, S. J. Hu leg. [SJH]; 1♂, Sancha He (2,900 m), Yulong County, Lijiang Prefecture, N.W. Yunnan, China, 2018–VI–21, S. J. Hu leg. [SJH]; 2♀♀, Maoni Ping (3,500 m), Yulong County, Lijiang Prefecture, N.W.

Yunnan, China, 2019–VI–14, H. H. Zhang *leg.* [HHZ]; 1♂, Lanyue Gu (2,800), Yulong County, Lijiang Prefecture, N.W. Yunnan, China, 2020–V–31, S. J. Hu *leg.* [SJH].

***Aporia procris lancangica* Della Bruna, Gallo & Sbordoni, 2003**

16♂♂, 1♀ Deqen County (3,400–3,500 m), Diqing Prefecture, N.W. Yunnan, China, 1987–VII–20–22, C. L. Li *leg.* [KIZ]; 13♂♂, 2♀♀, Baima Xueshan (4,100 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2004–VI–28–29, D. Z. Dong *leg.* [KIZ]; 1♂, Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2010–VII–22, Z. G. Wu *leg.* [SJH]; 1♂, Zha'an (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2018–VII–29, H. H. Zhang *leg.* [HHZ]; 1♂, near Yubeng (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2019–VI, Y. Yang *leg.* [SJH]; 2♂♂, Zha'an (3,000 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2019–VI–17, H. H. Zhang *leg.* [HHZ]; 1♀, Tuobake (3,500 m), Weixi County, Diqing Prefecture, N.W. Yunnan, China, 2019–VI–19, H. H. Zhang *leg.* [HHZ].

***Aporia uedai* Koiwaya, 1989**

1♂, Zha'an (2,600 m), Deqen County, Diqing Prefecture, N.W. Yunnan, China, 2018–VII–30, H. H. Zhang *leg.* [HHZ]; 1♂, ditto, 2019–VI–16, H. H. Zhang *leg.* [SJH]; 1♀, ditto, 2019–VI–17, H. H. Zhang *leg.* [HHZ].