





https://doi.org/10.11646/zootaxa.5105.1.2

http://zoobank.org/urn:lsid:zoobank.org:pub:2C1A9465-05E2-4E4F-9658-CF5A539718D4

# A new species of *Rhodambulyx* Mell, 1939 (Lepidoptera: Sphingidae) from Southwest Chongqing, China

ZHEN-BANG XU<sup>1</sup>, TOMÁŠ MELICHAR<sup>2</sup>, JI-BAI HE<sup>3</sup>, CHAO ZHANG<sup>4,5</sup>, XIN-YU ZHANG<sup>4,6</sup>, DU FENG<sup>4,7</sup> & SHAO-JI HU<sup>8,9</sup>\*

<sup>1</sup>Institute of Resource Plants, Yunnan University, Kunming, 650500, China. *Institute of Resource Plants, Yunnan University, Kunming, 650500, China. Institute of Resource Plants, Yunnan University, Kunming, 650500, China.* 

<sup>2</sup>Sphingidae Museum, Příbram, 7926101, Czech Republic. 🖃 sph.melichar@seznam.cz; 💿 https://orcid.org/0000-0001-8589-8531

<sup>3</sup>swild, Chengdu, 610000, China. 🖻 hejibai@swild.cn; 💩 https://orcid.org/0000-0002-8507-8258

<sup>4</sup>Simianshan Forest Resource Service Center, Jiangjin District, Chongqing, 402296, China

<sup>5</sup> mythimna@foxmail.com; <sup>6</sup> https://orcid.org/0000-0001-6069-1059

<sup>6</sup> = 979638735@qq.com; <sup>6</sup> https://orcid.org/0000-0001-7648-7677

<sup>7</sup> = 353639921@qq.com; <sup>10</sup> https://orcid.org/0000-0003-1377-2950

<sup>8</sup>Yunnan Key Laboratory of International Rivers and Transboundary Eco-security, Yunnan University, Kunming, 650500, China

<sup>9</sup>Institute of International Rivers and Eco-security, Yunnan University, Kunming, 650500, China.

\*Corresponding author. 🖃 shaojihu@hotmail.com; 💿 https://orcid.org/0000-0002-1615-9601

# Abstract

A new species of the genus *Rhodambulyx* Mell, 1939, *Rhodambulyx xinyuae* **sp. nov.**, is described from Simianshan Nature Reserve in Southwest Chongqing, China. This species is similar to *R. davidi* Mell, 1939 and *R. kitchingi* Brechlin, 2015 in habitus, but can be distinguished by a different wing pattern, male genitalia structure and DNA barcode sequence. In addition, *Rhodambulyx namvui* Eitschberger & Nguyen, 2017 is removed from synonymy with *R. kitchingi* and synonymized instead with *R. davidi*, although whether it would be better treated as a subspecies of *R. davidi* requires further investigation.

Key words: Rhodambulyx, DNA barcode, Vietnam, hawk moth, genital structure

# 摘要

本文记述了红鹰天蛾属*Rhodambulyx* Mell, 1939一新种——芯语红鹰天蛾*Rhodambulyx xinyuae* **sp. nov.**, 其标本 采集于重庆西南部的四面山自然保护区。该种与已知的近似物种大卫红鹰天蛾*R. davidi* Mell, 1939 和凯氏红鹰 天蛾*R. kitchingi* Brechlin, 2015 相似, 但本种的翅面斑纹、雄性生殖器以及DNA条形码序列与上述2个近似种存 在明显差异。另外,本文将*Rhodambulyx namvui*由*R. kitchingi*的异名变为了*R. davidi*的异名,但将*R. namvui*作为 *R. davidi*的亚种对待是否是最佳处理方式需要进一步研究。

关键词: 红鹰天蛾; DNA条形码; 越南; 天蛾; 生殖器结构

# Introduction

The genus *Rhodambulyx* Mell, 1939 (Lepidoptera: Sphingidae: Smerinthinae) comprises a group of brown-bodied Oriental hawk moths that are commonly found in South China, and North Indochina in spring (Figure 1). The genus was originally established for a single species, *Rhodambulyx davidi* Mell, 1939, described from North Fukien [Fujian, China], and which has since also been found in Hunan, Guangdong, and Guizhou. Subsequently, four more species have been described: *R. schnitzleri* Cadiou, 1990 from Chiang Mai, Thailand (also found in S. W. Yunnar; Chen & Ou 2008 and N. Thailand); *R. hainanensis* Brechlin, 2001 from Wuzhi Shan, Hainan (currently endemic to the mountains of Hainan Island); *R. haxairei* Melichar, Řezáč & Horecký, 2014 from Ba Na Hills, Da Nang, Viet-

48 Accepted by R. Zahiri: 11 Jan. 2022; published: 2 Mar. 2022

Licensed under Creative Commons Attribution-N.C. 4.0 International https://creativecommons.org/licenses/by-nc/4.0/

nam; and *R. kitchingi* Brechlin, 2015 from the Ba To Mountains, Quang Ngai, Vietnam (also known from Central Vietnam). In addition, Eitschberger and Nguyen (2017) described *R. namvui* from northern Vietnam but it was then later synonymized with *R. kitchingi* by Eitschberger (2018) but without stating any reasons for so doing.



**FIGURE 1.** Distributions of the species of *Rhodambulyx*. The type localities are indicated by the black spots. In central Vietnam, the red area indicates the distribution of *R. kitchingi*, the blue area indicates the distribution of *R. haxairei*.

In 2019 and 2020, a series of brownish *Rhodambulyx* specimens was collected by the fourth, fifth and sixth authors from Simianshan Nature Reserve in southwest Chongqing, China. The specimens were initially identified as *R. davidi*, given its similar wing shape and occurrence in southeast China. However, after curation of these specimens, the first and third authors noticed that their wing pattern was different from that of *R. davidi* collected from Guangdong and Guangxi. Later, a series of *R. davidi* collected from its type locality were examined by the second author and he found *R. davidi* and the specimens from Chongqing could be distinguished by the presence of a paler and more clearly delimited subquadrate postmedial patch on the costa of the forewing upperside of the latter. We also considered that *R. kitchingi* from central Vietnam was a close relative and so included specimens in the morphological and genital comparisons and molecular analyses. The results suggested that the '*R. davidi*' from Simianshan were a separate, undescribed species and we describe it below.

In addition, two samples of *R. namvui* from the type locality, including the holotype, were included in the morphological and phylogenetic analysis. The results showed that *R. namuvi* should be considered a synonym of *R. davidi*, not *R. kitchingi* (see Results section below).

# Materials and methods

# Abbreviations of specimen depositories

KIZ collection of the Kunming Institute of Zoology, Chinese Academy of Science, Kunming, Yunnan, China.

SFU collection of the Southwest Forestry University, Kunming, Yunnan, China.

EMM collection of Entomologischen Museum, Marktleuten, Germany.

SMCR Sphingidae Museum, Příbram, Czech Republic.

ZBX Private collection of Zhen-Bang Xu, Kunming, Yunnan, China.

ZHJ Private collection of Zhuo-Heng Jiang, Hangzhou, Zhejiang, China.

**Taxon sampling.** Specimens of the two focal species, *R. davidi* and *R. kitchingi* were sampled for both morphological and molecular analyses, including one from northern Vietnam potentially identified as *R. namvui*. Three *R. haxairei* were also included to better understand the interspecific differences of the sympatric taxa in central Vietnam. Specimen data of all specimens used in this study are listed in Appendix 1.

For each individual used in the molecular analysis, two legs from the same side were taken for DNA extraction before the specimens were rehydrated for spreading. An individual of *Rhodoprasina callantha callantha* Jordan, 1929 and one of *Cypoides chinensis* (Rothschild & Jordan, 1903) collected by the first author were chosen as outgroups for phylogenetic analyses, following the '*Cypa* genus-group' which is a putatively monophyletic group identified by Kitching & Cadiou (2000). In addition, seven sequences, including three individuals of *R. davidi*, two individuals of *R. schnitzleri* and two individuals of *R. hainanensis*, were downloaded from the Barcode of Life Database v.4 (BOLD) (http://www.boldsystems.org). Four unpublished sequences, including two individuals of *R. kitchingi* and two individuals of *R. haxairei* deposited in the BOLD database were also included in this study, courtesy of Mr. Jean Haxaire (Muséum national d'Histoire naturelle, Paris, France). These sequences will be published by Mr. Haxaire in due course. The collection data, GenBank accession numbers and BOLD sample IDs are listed in Table 1.

**Morphological comparisons.** Specimens were spread for examination, and species identification was undertaken prior to molecular analysis based on habitus. Specimens were photographed using a Canon EOS 70D digital camera (Canon, Japan), and the exposure adjusted using Adobe Photoshop CS (Adobe, USA).

The male genitalia were prepared following Hu *et al.* (2018). Forewing lengths were measured to 0.5 mm precision using a ruler. The whole abdomen was removed and placed into a 1.5 mL microcentrifuge tube, treated with 1 mL 10% sodium hydroxide solution to digest soft tissue for 1h at 70 °C. The treated abdomen then was neutralized with 2% acetic acid and dissected in a water-filled Petri dish under a stereoscope to remove residual tissues, scales, and hair. The genitalia were transferred to 80% glycerol for 12 h to render them transparent. Photographs of the genital structures were taken with a KUY NICE E3ISPM (KUY NICE, China) and automatically stacked using Helicon Focus 7 (Helicon Software, USA). Finally, all parts of the genitalia were fixed onto a glue card with watersoluble white glue and pinned with the specimen.

**DNA Extraction and Amplification.** The phenol-chloroform protocol was used to extract genomic DNA from two legs removed from the same side of each specimen sampled. The legs were homogenized in protease buffer containing 450  $\mu$ L STE (10mmol/L Tris-HCl, 1 mmol/L EDTA, 100 mmol/L NaCl, pH= 8.0), 25  $\mu$ L Proteinase K (20 mg/mL) and 75  $\mu$ L SDS (10%) and incubated at 55 °C for 12 h to rehydrate and lyse the tissue. The subsequent extraction protocol followed Hu *et al.* (2018) and the resultant genomic DNA preserved at -40 °C.

DNA amplification followed Xu *et al.* (2020). The polymerase chain reaction (PCR) was carried out in a 25  $\mu$ L system using the TaKaRa Ex *Taq* Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The system contained 2.5  $\mu$ L 10 × PCR buffer, 2.0  $\mu$ L MgCl<sub>2</sub>(2.5 mmol/L), 2.0  $\mu$ L dNTP mixture (2.5 mmol/L each). The mitochondrial *cox1* gene fragment (the DNA barcode) was amplified and sequenced with the primers LCO1490 (5'- GGT CAA ATC ATA AAG ATA TTG-3') and HCO2198 (5'- TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.*, 1994). The PCR thermal profile consisted of an initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 72 °C for 1 min; then a final elongation at 72 °C for 5 min. Sequencing was undertaken using an ABI Prism 3730 sequencer (Applied Biosystems, Foster City, CA, USA).

TABLE 1. Sampling information, GenBank study.	<b>IABLE 1.</b> Sampling information, GenBank accession no/BOLD SampleID of the DNA barcode sequences (COI) of the <i>Rhodambulyx</i> specimens and outgroups used in this study.	oarcode sequences (COI)	of the <i>Rhodambulyx</i> specimens	s and outgroups used in this
Taxon (Sample Code)	Locality	<b>Collection date</b>	GenBank Accession No.	SampleID
Rhodambulyx kitchingi	Thua Thien Hue, Vietnam	no collecting date		BC-Hax5254
Rhodambulyx kitchingi	Thua Thien Hue, Vietnam	no collecting date		BC-Hax5255
Rhodambulyx haxairei	Lam Dong, Vietnam	no collecting date		BC-Hax5249
Rhodambulyx haxairei	Thua Thien Hue, Vietnam	no collecting date		BC-Hax5250
Rhodambulyx haxairei (RXL02)	Da Nang, Lam Dong, Vietnam	2019–111	MZ606148	
Rhodambulyx haxairei (RXL03)	Lam Dong, Vietnam	2016–III	MZ606149	
Rhodambulyx xinyuae <b>sp. nov.</b> (RYS01) (Paratype)	Simianshan, Chongqing, China	2019–III	MZ606150	
Rhodambulyx xinyuae <b>sp. nov.</b> (RYS02) (Paratype)	Simianshan, Chongqing, China	2019–III	MZ606151	
Rhodambulyx davidi	Dayao Shan, Guangxi, China	2004–III		BC-Hax0492
Rhodambulyx davidi	Dayao Shan, Guangxi, China	2005–III		BC-EMEM1002
Rhodambulyx davidi (RDX01)	Shunhuang Shan, Hunan, China	2021–III	MZ606147	
Rhodambulyx davidi 'namvui'(RDC01) (Holotype)	Cao Bang, Vietnam	2017-II	MZ664282	
Rhodambulyx davidi 'namvui' (RDC02)	Cao Bang, Vietnam	2018–III	OL416032	
Rhodambulyx schnitzleri	Doi Pha Hom Pok, Chiang Mai, Thailand	2006–II		BC-EMEM0999
Rhodambulyx schnitzleri	Doi Pha Hom Pok, Chiang Mai, Thailand	2004–III		BC-Mel0059
Rhodambulyx hainanensis	Wuzhi Shan, Hainan, China	2000–III		BC-RBP-1313
Rhodambulyx hainanensis	Wuzhi Shan, Hainan, China	2000–III		BC-RBP-1314
Rhodoprasina callantha (RCB01)	Xishuangbanna, Yunnan, China	2021–III	MZ606146	
Cypoides chinensis (CYN01)	Nanjing, Jiangsu, China	2021–X	MZ606145	

**Phylogenetic Analyses and Species Delimitation.** We proofread and aligned the raw sequences with ClustalW (Thompson *et al.* 1994) in BioEdit 7.0.9 (Hall 1999) by examining the chromatograms for polymorphic sites. MEGABLAST was used to check the identities of all sequences against genomic references and nucleotide collections in the BOLD and GenBank databases, and amino acid translation was done with the invertebrate mitochondrial criterion in MEGA 7.0 (Kumar *et al.* 2016) to detect possible *Numts* (nuclear copies of mtDNA fragments) (Song *et al.* 2008; Bertheau *et al.* 2011). A search for non-synonymous mutations, in-frame stop codons and indels was also carried out to detect possible cryptic *Numts*. The Kimura two-parameter (Kimura 1980) distances between taxa were calculated in MEGA 7.0.

All sequences were included in the phylogenetic reconstructions without pruning identical haplotypes to test the phylogenetic integrity of the species as identified using morphological characters (Ivshin *et al.* 2018; Cheng *et al.* 2017; Nässig *et al.* 2010). The phylogeny was reconstructed using a Bayesian Inference (BI) criterion as implemented in PhyloSuite 1.2.2 (Zhang *et al.* 2020). We used a reversible-jump Markov Chain Monte Carlo (rj-MCMC) method to allow sampling across the entire substitution rate model space (Huelsenbeck *et al.* 2004). BI analyses consisted of two independent runs, each with eight rj-MCMC running for five million generations (sampled every 1000<sup>th</sup> generation) to calculate the clade posterior probabilities (PP). The marginal likelihood estimate was performed with stepping-stone sampling (Xie *et al.* 2011), as implemented in MrBayes with 100 steps, each with 10 million generations, and a diagnostic frequency of 1000. Finally, Monophylizer (http://monophylizer.naturalis.nl/) (Mutanen *et al.* 2016) was applied to the resultant BI tree to test the monophyly of each identified taxon.

# Results

# **Molecular Phylogenetics**

Bayesian phylogenetic analyses converged well, as indicated by an average standard deviation of split frequencies close to 0 (0.004874), potential scale reduction factors equal to 1 (maximum = 1.005), and an effective sample size >> 200 for all parameters. The phylogeny recovered three major clades, each with a maximal PP value (Figure 2).

In the first of these clades, comprising *R. davidi*, *R. davidi* 'namvui', *R. kitchingi* and *R. xinyuae* **sp. nov.**, *R. kitchingi* and *R. xinyuae* **sp. nov.** are sister to each other, forming a monophyletic subgroup, and *R. davidi* and *R. davidi* 'namvui' together forming another. Sister to these four taxa is a clade comprising *R. schnitzleri* from northern Thailand and *R. hainanensis* from Hainan, with *R. haxairei* from central Vietnam being sister to all other species of this genus (Figure 2).

#### **Molecular species delimitation**

The Kimura two-parameter (K2P) distances among all taxa ranged from 0.77–6.16%, with that between *R. davidi* '*namvui*' and *R. davidi* being the smallest (0.77%), and that between *R. kitchingi* and *R. schnitzleri* being the greatest (6.61%) (Table 2). The distance between the new species and *R. kitchingi* is 2.16%. The distances between the new species and other known *Rhodambulyx* species are all above this value.

	1	2	3	4	5	6	7
1. Rhodambulyx xinyuae sp. nov.							
2. Rhodambulyx kitchingi	2.16						
3. Rhodambulyx davidi 'namvui'	2.56	2.88					
4. Rhodambulyx davidi	3.04	3.04	0.77				
5. Rhodambulyx hainanensis	4.83	5.16	4.75	4.92			
6. Rhodambulyx haxairei	4.99	5.23	5.12	5.29	3.13		
7. Rhodambulyx schnitzleri	5.15	6.16	5.58	5.42	2.97	4.85	

**TABLE 2.** Kimura two-parameter (K2P) distances (in percentages) between all taxa of genus *Rhodambulyx* calculated from the DNA barcode sequences (COI), with species identified as in the Bayesian phylogenetic tree in Figure 2.

The Monophylizer analysis supported all morphologically analysed species of two or more samples as monophyletic on the BI tree (Table 3). The results showed that the samples of the new species also formed a monophyletic group.

Overall, given the aforementioned molecular evidence, we propose that the *Rhodambulyx* from Chongqing is a new species and describe it below.



**FIGURE 2.** Bayesian inference phylogenetic tree of *Rhodambulyx* based on the DNA barcode sequences (COI) and rooted on *Rhodoprasina callantha* and *Cypoides chinensis* as outgroups. Values at the nodes indicate posterior probabilities.

Taxon	Assessment	Tanglees	
R. kitchingi	monophyletic	_	
R. xinyuae sp. nov.	monophyletic	_	
R. davidi	monophyletic	_	
R. davidi 'namvui'	monophyletic	—	
R. hainanensis	monophyletic	_	
R. schnitzleri	monophyletic	—	
R. haxairei	monophyletic	_	

TABLE 3. Monophylizer assessment of *Rhodambulyx* species used in this study.

# Rhodambulyx xinyuae Xu, Melichar & He sp. nov. 芯语红鹰天蛾

HOLOTYPE: ♂, Dawopu (1,200–1,300 m), Simianshan, Jiangjin District, S. W. Chongqing, China, 2019–III–3, C. Zhang *leg*. [KIZ 0136260]. PARATYPES: 2♂♂, the same data as the holotype, C. Zhang *leg*. [ZBX][GenBank accession no.: MZ606150 and MZ606151]; to be transferred to KIZ at a later date]; 1♂, the same collecting data, [KIZ 0136261]; 1♂, Tudiyan (1,100 m), Simianshan, Jiangjin District, S. W. Chongqing, China, 2021–II–22, C. Zhang *leg*. [KIZ 0136262].

# **Description:**

**Male** (Figure 3 A–D). Forewing length 39–42 mm, ground colour brownish, with some individuals more reddish. Body brownish. Antenna about one-third of forewing length, beige with brownish pectinations. Forewing upperside: Ground colour brownish-beige, with three transverse darker brown bands overlain by a faintly silvery sheen. Discal spot creamy yellow. Subapical patch paler, running from costa to vein  $M_3$ , crossed by the postdiscal band, immediately adjacent to which is a brownish apical patch. Terminal band inwardly wavy with silvery sheen. Forewing underside: Light buff-brown peppered with darker scales, basal half covered with reddish hair forming a patch. Discal band darker, running from costa into the basal reddish patch. Submarginal line rather thin, Y-shaped running from apex and costa to cell CuA<sub>1</sub>, bifurcating in cell M<sub>1</sub>. Hindwing upperside: Ground colour brownish-red. Darker postmedial line rather obscure. Narrow black outer margin fades inwardly. Hindwing underside: Postmedial and submarginal bands brownish-red, distinctly delineated. Cilia creamy yellow.

Female. Unknown.



**FIGURE 3.** *Rhodambulyx xinyuae* Xu, Melichar & He **sp. nov.** (A–D) and three similar taxa, *R. kitchingi* Brechlin, 2015 (E–H) *R. davidi* Mell, 1939 (I–J), and *R. davidi* '*namvui*' (K–L). A–B:  $\mathcal{J}$ , HOLOTYPE, Simianshan, Chongqing, China; C–D:  $\mathcal{J}$ , PARA-TYPE, same locality as holotype; E–F:  $\mathcal{J}$ , Da Nang, Lam Dong, Vietnam; G–H:  $\mathcal{J}$ , Bach Ma, Thua Thien Hue, Vietnam; I–J:  $\mathcal{J}$ , Dayao shan, Guangxi China; K–L:  $\mathcal{J}$ , HOLOTYPE, Phia Den, Cao Bang, Vietnam, © Ulf Eitschberger. Scale bar = 10 mm.

**Male genitalia.** In total, five genitalia were dissected. Overall, heavily sclerotized. Tegumen and vinculum broad, the latter with a very short saccus (Figure 4A and B); uncus broadest at base, elongate, gradually narrowing towards a slightly bifurcate tip; apical third ventrally with a median ridge that bifurcates into two apical ridges (Figure 7A), and which is individually variable in shape (see Figure 7D). Valve broad in basal half, nearly rectangular, elongate and more membranous in the distal half, covered with dense setae, harpe triangular, broad at base and gradually narrowed, then abruptly upturned into hooked tip (Figure 7B and E). Juxta thin, rising from base of valve. Phallus of moderate length, straight; basal half narrower, distal half approximately of equal width; tip pointed with smooth cornuti (Figure 7C and F).

Female genitalia. Unknown.

**Voltinism:** Univoltine, adults appearing from mid-February to early March. In the same area, no adults have been found in other months, and no other species of the same genus have been found.

Distribution: Currently known only from the Simianshan, S. W. Chongqing.

**Derivatio nominis:** The species is dedicated to Ms Xin-Yu Zhang (Chongqing, China), wife of Chao Zhang. Zhang decided to express his love for his wife and her unselfish companionship and support over the years.

**Biology:** The adults appear in February and March, flying in Subtropical Evergreen Broad-leaved Forest with foggy weather. Larval hostplants unknown.



**FIGURE 4.** Male genitalia of *Rhodambulyx xinyuae* Xu, Melichar & He **sp. nov.**, Simianshan, Chongqing, China (A–B); *R. kitchingi* Brechlin, 2015, Thua Thien Hue, Vietnam (C–D); and *R. davidi* Mell, 1939, Dayao shan, Guangxi, China (E).



**FIGURE 5.** Photo of live *Rhodambulyx xinyuae* Xu, Melichar & He **sp. nov.** Taken in Tudiyan, Simianshan, Jiangjin District, S. W. Chongqing, China, 2021–II–22, by Chao Zhang.

#### Morphological comparison with similar species

Since *R. xinyuae* **sp. nov.** is readily distinguishable from *R. hainanensis*, R. *haxairei*, and *R. schnitzleri* (Figure 6), the present study hereafter only compares it with the two most similar species, namely *R. davidi* and *R. kitchingi*.



FIGURE 6. Photos of *Rhodambulyx schnitzleri* Cadiou, 1990 (A–B), *R. hainanensis* Brechlin, 2001 (C–D), and *R. haxairei* Melichar, Řezáč & Horecký, 2014 (E–F). A–B: ♂, Doi Pha Hom Pok, Chiang Mai, Thailand, © Ulf Eitschberger; C–D: ♂, HO-LOTYPE, Wuzhi Shan, Hainan, China, © The Trustees of the Natural History Museum, London. https://sphingidae.myspecies. info/taxonomy/term/2493; E–F: ♂, Da Nang, Lam Dong, Vietnam.

# Rhodambulyx davidi Mell, 1939

**Male** (Figure 3 I–J). Forewing length 42–54 mm, ochre. Body brownish. Antenna about one-third of forewing length, beige with brownish pectinations. Forewing upperside: Ground colour reddish-beige with three transverse light brown bands overlain by a clear silvery sheen. Discal spot creamy yellow. Postdiscal band wavy, running from costa to inner margin. Terminal band inwardly wavy with strongly silvery sheen. Brownish apical patch distinct. Forewing underside: Light buff-brown peppered with darker scales, basal half covered with faintly brownish-beige hair forming a patch. Discal band obscure, running from costa into the basal brownish-beige patch. Submarginal line thin, Y-shaped running from apex and costa, gradually disappearing to cell  $CuA_1$ , bifurcating in cell  $M_1$ . Faintly black terminal band slightly disappeared to basal. Hindwing upperside: Ochre-beige with narrow black outer margin. Darker postdiscal line rather disappeared. Hindwing underside: Postdiscal and submarginal bands ochre, slightly indistinct. Cilia reddish beige.

**Male genitalia.** In total, seven genitalia were dissected. Overall sclerotized. Tegumen and vinculum broad, the latter with a short saccus (Figure 3C and D); uncus broad at base, elongate, triangular shaped with blunt tip (Figure 7M). Valve broad in basal half, nearly oval, elongate and more membranous in the distal half, covered with dense setae, harpe triangular, broad at base and gradually narrowed, then abruptly upturned into hooked tip (Figure 7N). Juxta thin, rising from base to valve. Phallus straight, basal half and distal half approximately of equal width; distal half gradually narrowed with teardrop-shaped tip (Figure 7O).

# Rhodambulyx kitchingi Brechlin, 2015

**Male** (Figure 3 E–H). Forewing length 43–49 mm, brownish ochre. Body brownish. Antenna about one-third of forewing length, beige with brownish pectinations. Forewing upperside: Ground colour reddish-beige, with three distinctly transverse darker brown bands overlain by a faintly silvery sheen. Discal spot creamy yellow. Postdiscal band straight, running from costa to inner margin. Terminal band inwardly wavy with silvery sheen. Apical patch

brownish with obscure boundary inwardly. Forewing underside: Buff-brown peppered with darker scales, basal half covered with reddish hair forming a patch. Discal band darker and distinct, running from costa into the basal reddish patch. Submarginal line distinct, Y-shaped running from apex and costa to cell  $CuA_1$ , bifurcating in cell  $M_1$ . Hindwing upperside: Ground colour brownish-red. Darker postdiscal line obscure, with narrow black outer margin. Hindwing underside: Postdiscal and submarginal bands brownish-red, distinctly delineated. Cilia creamy yellow.

**Male genitalia.** In total, seven genitalia were dissected. Overall heavily sclerotized. Tegumen and vinculum broad, the latter with a short saccus (Figure 3C and D); uncus broadest at base, elongate towards a bifurcate tip (Figure 7G); apical third ventrally with a median ridge that bifurcate into two apical ridges, and which is individually variable in shape (see Figure 7J). Valve broad in basal half, nearly rhombic, elongate and more membranous in the distal half, covered with dense setae, harpe triangular, broad at base and gradually narrowed, then abruptly downward into hooked tip (Figure 7H and K). Phallus of moderate length, straight; basal half narrower; distal half approximately of equal width; tip elongated and pointed with smooth cornuti (Figure 7I and L).

**Diagnostic characters:** The two known species (*R. davidi* and *R. kitchingi*) can be distinguished by the following characters: (1) *R. kitchingi* is more reddish than *R. davidi* in external appearance. (2) The brownish apical patch of forewing upperside in *R. davidi* more distinct than *R. kitchingi*. (3) The postdiscal band of forewing in *R. kitchingi* is straighter than that of *R. davidi*. (4) The discal band on the forewing underside in *R. kitchingi* is more distinct than that of *R. davidi*. (5) The postdiscal and submarginal bands in hindwing underside are more distinct in *R. kitchingi* (Figure 3). Genitally: (1) The pointed triangular tip in the uncus of *R. davidi* is far less obvious, appearing almost rounded. (2) The harpe is thinner and sharper in *R. davidi* than in *R. kitchingi* (Figure 4 and Figure 7).

The new species resembles *R. kitchingi* from central Vietnam but can be distinguished from the following characteristics: (1) Its smaller size on average, (2) the brownish apical patch of forewing upperside more distinct than *R. kitchingi*, (3) the duller silvery sheen on the forewing upperside in new species. (4) The discal band on the forewing underside in the new species is not as distinct as that in *R. kitchingi*. (5) The narrow black margin fades inwardly in hindwing upperside of new species, but this feature is not obvious in *R. kitchingi* (Figure 3). In male genitalia, the diagnostic differences are: (1) the uncus of the new species is not gradually tapered distally as it is in *R. kitchingi* (2) the dorsal margin of harpe is smoother rather than wavy in *R. kitchingi* (3) the phallus is shorter with a shorter tip (Figure 4 and Figure7).



**FIGURE 7.** Enlarged uncus, valve, and phallus of male genitalia of *Rhodambulyx xinyuae* Xu, Melichar & He **sp. nov.**, Simianshan, Chongqing, China (A–F); *R. kitchingi* Brechlin, 2015, Thua Thien Hue, Vietnam (G–L); and *R. davidi* Mell, 1939, Dayao Shan, Guangxi, China (M–O). A, D, G, J, and M: ventral-lateral view of uncus; B, E, H, K, and N: posterior view of valve and harpe; C, F, I, L, and O: lateral view of phallus.

*Rhodambulyx xinyuae* **sp. nov.** is also similar to *R. davidi*. However: (1) The delimited subquadrate creamy yellow patch on the forewing in the new species is more evident than that in *R. davidi*. (2) The postdiscal band of fore-

wing upperside in the new species, straighter than that in *R. davidi*. (3) The postmedial and submarginal bands on hindwing underside of the new species are more distinct than those of *R. davidi*. (4) The narrow black outer margin of the hindwing fades inwardly in the new species, which is not obvious in *R. davidi* (Figure 3). In male genitalia, the diagnostic differences are: (1) The pointed triangular tip in the uncus of *R. davidi* is far less obvious, appearing almost rounded. (2) The harpe is sharper in *R. davidi* than in *R. xinyuae* **sp. nov.** (Figure 4 and Figure 7).

# Discussion

Our findings showed that *Rhodambulyx xinyuae* **sp. nov.** from Simianshan is a species different from others in the genus *Rhodambulyx*. The molecular data showed the genetic distinctness and integrity of the new species (Figure 2; Table 3). Although the K2P genetic distance (2.16%) is smaller than that between many Smerinthini at the species level (which often exceeds 4%; Řezáč, 2018), we consider that the evidence from the morphological analyses of the genital structures and wing patterns and the monophyly shown in the phylogenetic analysis is sufficient that *Rhodambulyx xinyuae* **sp. nov.** can be treated as a good species (Meyer *et al.* 2005; Řezáč 2018). The paler subapical costal patch on the forewing upperside serves as a good diagnostic feature of the new species.

The K2P genetic distance between *R. xinyuae* **sp. nov.** and *R. kitchingi* is small for two species in the genus *Rhodambulyx*, which may imply a possibly recent divergence of the two taxa. Currently, *R. xinyuae* **sp. nov.** is only known from its type locality, and its full geographic range is still unclear. Although the distributions of all *Rhodambulyx* species are geographically well separated, with *R. davidi* occurring between *R. xinyuae* **sp. nov.** and *R. kitchingi* (Figure 1), we cannot rule out the possibility of at least partial overlapping distributions of the three species. Future surveys will be necessary to confirm or refute this, as well as to determine the full range of *R. xinyuae* **sp. nov.** However, similar biogeographical patterns have been reported in *Graphium* subgenus *Pazala* Moore, 1888, *e.g., Graphium (Pazala) confucius* Cotton & Hu, 2018, *G. (P.) daiyuanae* Hu & Cotton, 2018, and *G. (P.) wenlingae* Hu, Cotton & Monastyrskii, 2019 in Southwest China and Vietnam (Hu *et al.* 2018; Hu *et al.* 2019), as well as in Saturniidae moths, where *Actias chapae* Mell, 1950 has two subspecies in this area, with the nominotypical subspecies occupying central, southwest and south China, as well as North Vietnam, with another subspecies found in south Vietnam (Wu & Naumann 2006; Sochivko & Ivshin 2008; Zhang 2013). A similar pattern is seen in *Rhodoprasina callantha* Jordan, 1929, which has two subspecies in southwest China and a third in Central Vietnam (Brechlin 2015).

We would note that although R. davidi 'namvui' forms a separate branch on the Bayesian tree from R. davidi (Figure 2; Table 3), the K2P genetic distance is relatively low (Table 2). In the male genitalia, the tip of phallus in R. davidi 'namvui' is shorter than that of R. kitchingi (Figure 8J, N, and R), the apex of uncus in R. kitchingi has a distinct fold that is absent in R. davidi 'namvui' (Figure 8K, O, and S), the valve of R. davidi 'namvui' more elliptical, unlike the downward beak-shaped in R. kitchingi (Figure 8L, P, and T). Although the apex of uncus in R. davidi *'namvui*' is slightly different from that of *R. davidi* in its gradually tapered shaft and a blunt tip, it is still too soon to conclude whether such differences are merely individual variation between populations due to the limited number of specimens examined. Overall, all these aforementioned genital characteristics of R. davidi 'namvui' are closer to R. davidi rather than to R. kitchingi. Moreover, the external morphological appearance of R. davidi 'namvui' is similar to R. davidi, but different from R. kitchingi (Figure 3). Originally, R. davidi 'namvui' was described as a distinct species (Eitschberger & Nguyen 2017) but was then later synonymized with R. kitchingi (Eitschberger 2018). Our analysis does not support R. davidi 'namvui' being a junior synonym of R. kitchingi but rather shows it has a closer relationship with R. davidi. Considering the spatial distribution pattern of the three taxa, we believe that R. namvui is a southern population of R. davidi. However, whether it is a subspecies of R. davidi or just a junior synonym of that species requires further investigation with additional material and DNA data. For now, we provisionally treat R. namvui syn. nov. as a junior synonym of R. davidi.

*Rhodambulyx xinyuae* **sp. nov.** is the northernmost species of the genus, relatively isolated from the other species, which occur further south in southern China and northern Indochina. The presence of a species of *Rhodambulyx* in this region of southern Chongqing may be attributable to the Simianshan being a transition zone between the Yunnan-Guizhou Plateau and the Sichuan Basin. By reason of a favorable climate and sufficient rainfall, the entire zone is luxuriant subtropical evergreen broad-leaved forests (Li & Hou, 2004) suitable for these tropical hawkmoths. The distribution of *Rhodambulyx* in Chongqing, Guizhou, and Sichuan certainly needs further investigation.



**FIGURE 8.** Male genitalia of *Rhodambulyx davidi* Mell, 1939, from Shunhuang Shan, Hunan, China (A–D, © Zhuo-Heng Jiang) and Wuyi Shan, Fujian, China (E–H, © Ulf Eitschberger); *R. davidi 'namvui*', Phia Den, Cao Bang, Vietnam (I–L, © Ulf Eitschberger), *R. kitchingi* Brechlin, 2015, Thua Thien Hue, Vietnam (M–T). A, E, I, M, and Q: lateral view of the whole genitalia with left valve removed; ,B, F, J, N, and R: lateral view of phallus; C, G, O, and S: dorsal view of uncus; K: dorsal view of uncus; D, H, L, P, and T: lateral view of left valve.

### Acknowledgements

The authors would like to thank Dr. Ian. J. Kitching (Natural History Museum, London, UK) for his valuable comments, Mr. Jean Haxaire (Muséum national d'Histoire naturelle, Paris, France), Dr. Ulf Eitschberger (Entomologischen Museum, Marktleuten, Germany) and Mr. Zhuo-Heng Jiang (Westlake University, Hangzhou, China) for sample collection and information; Prof. Chuan-Hui Yi and Prof. Qiu-Ju He (Southwest Forestry University, Kunming, China) for allowing specimen examination; and Prof. Qin Qiao (Yunnan University, Kunming, China) and Tian-Tian Yu (South China Agricultural University, Guangzhou, China) for their valuable opinions and assistance. We also thank Si-Yao Huang (Zhuhai, China) and Hui-Hong Zhang (Kunming, China) for giving constructive suggestions to this study.

# References

- Bertheau, C., Schuler, H., Krumböck, S., Arthofer, W. & Stauffer, C. (2011) Hit or miss in phylogenetic analyses: The case of the cryptic NUMTs. *Molecular Ecology Resources*, 11, 1056–1059. https://doi.org/10.1111/j.1755-0998.2011.03050.x
- Brechlin, R. (2001) *Rhodambulyx hainanensis*, eine neue Schwärmerart aus China (Lepidoptera: Sphingidae). *Nachrichten des entomologischen Vereins Apollo*, Neue Folge, 22, 145–147.
- Brechlin, R. (2015) *Rhodambulyx kitchingi* n. sp., eine neue Sphingide aus Vietnam (Lepidoptera: Sphingidae). *Entomo-Sat-sphingia*, 8, 5–7.
- Brechlin, R. (2015) Zwei neue Unterarten von *Rhodoprasina callantha* Jordan, 1926 (Lepidoptera, Sphingidae). *Entomo-Sat-sphingia*, 8, 26–30.
- Cadiou, J.-M. (1990) A new sphingid from Thailand: *Rhodambulyx schnitzleri* (Lepidoptera Sphingidae). *Lambillionea*, 90 (2), 42–48.
- Chen, J. & Ou, X.H. (2008) Two new record species of Sphingidae (Lepidoptera) from China. *Entomotaxonomia*, 30 (1), 39-40.
- Cheng, R., Xue, D.Y., Galsworthy, A., & Han, H.X. (2017) Complete mitochondrial genomes throw light on budding speciation in three *Biston* species (Lepidoptera, Geometridae). *Zoologica Scripta*, 46, 73–84. https://doi.org/10.1111/zsc.12184
- Eitschberger, U. & Nguyen, H.B. (2017) *Rhodambulyx namvui* spec. nov., eine neue Schwärmerart aus Vietnam (Lepidoptera, Sphingidae). *Neue Entomologische Nachrichten*, 74, 172–192.
- Eitschberger, U. (2018) Die Taxa des Genus *Rhodambulyx* Mell, 1939 mit neuer Synonymie (Lepidoptera, Sphingidae). *Neue Entomologische Nachrichten*, 75, 94–95.
- 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.
- Huelsenbeck, J.P., Larget, B. & Alfaro, M.E. (2004) Bayesian phylogenetic model selection using reversible jump Markov Chain Monte Carlo. *Molecular Biology and Evolution*, 21, 1123–1133. https://doi.org/10.1093/molbev/msh123
- Hu, S.J., Cotton, A.M., Condamine, F.L., Duan, K., Wang, R.J., Hsu, Y.F., Zhang, X. & Cao, J. (2018) Revision of *Pazala* Moore, 1888: the *Graphium (Pazala) mandarinus* (Oberthür, 1879) group, with treatments of known taxa and descriptions of new species and new subspecies (Lepidoptera: Papilionidae). *Zootaxa*, 4441 (3), 401–446. https://doi.org/10.11646/zootaxa.4441.3.1
- Hu, S.J., Condamine, F.L., Monastyrskii, A.L. & Cotton, A.M. (2019) A new species of the *Graphium (Pazala) mandarinus* Group from Central Vietnam (Lepidoptera: Papilionidae). *Zootaxa*, 4554 (10, 286–300. https://doi.org/10.11646/zootaxa.4554.1.10
- Ivshin, N., Krutov, V. & Romanov, D. (2018) Three new taxa of the genus *Cechetra* Zolotuhin & Ryabov, 2012 (Lepidoptera, Sphingidae) from South-East Asia with notes on other species of the genus. *Zootaxa*, 4450 (1), 1–25. https://doi.org/10.11646/zootaxa.4450.1.1
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111–120. https://doi.org/10.1007/BF01731581
- Kitching, I.J. & Cadiou, J.-M. (2000) *Hawkmoths of the World: an annotated and illustrated revisionary checklist (Lepidoptera: Sphingidae)*. Natural History Museum, London and Cornell University Press, Ithaca, New York, 226 pp., 8 pls.
- Kumar, S., Stecher, G. & Tamura, K. (2016) Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology & Evolution*, 33, 1870–1874.

https://doi.org/10.1093/molbev/msw054

Melichar, T., Řezáč, M. & Horecký, Č. (2014) A new species of the genus *Rhodambulyx* Mell, 1939 (Lepidoptera, Sphingidae) from Vietnam. *The European Entomologist*, 6, 161–165.

- Mell, R. (1939) Beiträge zur Fauna sinica. XVIII. Noch unbeschriebene chinesische Lepidopteren (V). Deutsche Entomologische Zeitschrift, Berlin, 52, 135–152.
- Mell, R. (1950) Aus der Biologie der chinesischen Actias Leach (Argema chapae sp. n., A. sinensis f. virescens f. n.). Entomologische Zeitschrift, 60, 6 & 7, 41–45 & 53–56.
- Meyer, C.P. & Paulay, G. (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biology, 3, e422. https://doi.org/10.1371/journal.pbio.0030422
- Moore, F. (1888) Additional Species Family Papilionidae. In: Hewitson & Moore (1879–1888), Description of New Indian Lepidopterous Insects from the Collection of the Late Mr. W.S. Atkinson, M.A., F.L.S., & c., Director of the Public Instruction, Bengal. Rhopalocera. Part III. Asiatic Society of Bengal, Calcutta, pp. 283–285.
- Wu Y. & Naumann, S. (2006) The preimaginal instars of *Actias chapae* (Mell, 1950) (Lepidoptera: Saturniidae). *Nachrichten des entomologischen Vereins Apollo*, Neue Folge, 27, 1/2, 17–21.
- Mutanen, M., Kivelä, S.M., Vos, R.A., Doorenweerd, C., Ratnasingham, S., Hausmann, A., Huemer, P., Dincă, V., van Nieukerken, E.J., Lopez-Vaamonde, C., Vila, R., Aarvik, L., Decaëns, T., Efetov, K.A., Hebert, P.D.N., Johnsen, A., Karsholt O., Pentinsaari, M., Rougerie, R., Segerer, A., Tarmann, G., Zahiri, R. & Godfray, H.C.J. (2016) Species-level para- and polyphyly in DNA barcode gene trees: strong operational bias in European Lepidoptera. *Systematic Biology*, 65, 1024–1040. https://doi.org/10.1093/sysbio/syw044
- Nässig, W.A., Naumann, S. & Rougerie. (2010) Evidence for the existence of three species in the genus *Archaeoattacus* (Lepidoptera: Saturniidae). *The Journal of Research on the Lepidoptera*, 43, 37–47.
- Řezáč, M. (2018) Notes on the taxonomy of the genus *Rhodoprasina* Rothschild & Jordan, 1903 (Lepidoptera, Sphingidae) with the description of a new species. *The European Entomologist*, 10 (1), 185–206.
- Song, H., Buhay, J.E., Whiting, M.F. & Crandall, K.A. (2008) Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *PNAS*, 105, 13486–13491. https://doi.org/10.1073/pnas.0803076105
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–4680.

https://doi.org/10.1093/nar/22.22.4673

- Xie, W.G., Lewis, P.O., Fan, Y., Kuo, L. & Chen, M.H. (2011) Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology*, 60, 150–160. https://doi.org/10.1093/sysbio/syq085
- Xu, Z.B., Wang, Y.Y., Condamine, F.L., Cotton, A.M. & Hu, S.J. (2020) Are the Yellow and Red Marked Club-Tail Losaria coon the same species? *Insects*, 11, 392, 1–23. https://doi.org/10.3390/insects11060392
- Zhang, D., Gao, F.L, Jakovlic, Zou, H., Li, W.X. & Wang, G.T. (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources*, 20, 348–355.

https://doi.org/10.1111/1755-0998.13096

Zhang, W.W. (2013) No Title [Introduction to cover photo *Actias chapae*]. *Bulletin of Biology*, 48, 4. https://doi.org/10.1016/j.materresbull.2012.10.030

# APPENDIX 1. Specimens examined in this study.

#### Rhodambulyx xinyuae Xu, Melichar & He sp. nov. 芯语红鹰天蛾

HOLOTYPE: ♂, Dawopu (1,200–1,300 m), Simianshan, Jiangjin District, S. W. Chongqing, China, 2019-III-3, C. Zhang *leg.* [KIZ: 0130260]. PARATYPES: 2♂♂, same data as holotype, C. Zhang *leg.* [ZBX]; 1♂, same collecting data, [KIZ: 0130261]; 1♂, Tudiyan (1,100 m), Simianshan, Jiangjin District, S. W. Chongqing, China, 2021-II-22, C. Zhang *leg.* [KIZ: 0130262].

# Rhodambulyx davidi Mell, 1939 大卫红鹰天蛾

1Å, North Fujian, China, 2003, Sinjaev *et al. leg.* [SMCR]; 1Å, Dayaoshan, Guangxi, China, 2001-III, Sinjaev *et al. leg.* [SMCR]; 1Å, Dayaoshan, Guangxi, China, 2005-III, Sinjaev *et al. leg.* [SMCR]; 1Å, Dayaoshan, Guangxi, China, 2004-III, Sinjaev *et al. leg.* [SMCR]; 1Å, Shunhuangshan, Hunan, China, 2021-III, local catcher *leg.* [ZHJ]; 1Å, Cenwanglaoshan, Guangxi, China, 2018-IX, local catcher *leg.* [ZHJ]; 1Å, Wuyi Shan, Fujian, China, 2006-IV.–VII, local catcher leg. [EMM].

### Rhodambulyx davidi 'namvui' (Eitschberger & Nguyen, 2017)

13, HOLOTYPE, Phia Den, Cao Bang, Vietnam, 2017-II-22, Hoa Binh Nguyen *leg*. [EMM]; 13, same locality as holotype, 2018-III, Hoa Binh Nguyen leg. [EMM].

# Rhodambulyx kitchingi Brechlin, 2015 凯氏红鹰天蛾

1Å, Bach Ma, Thua Thien Hue, Vietnam, 2016-V (collecting date needs confirmation), local catcher *leg.* [ZBX]; 1Å, Bach Ma, Thua Thien Hue, Vietnam, 2015-XIII (collecting date needs confirmation), local catcher *leg.* [ZBX]; 1Å, Bach Ma, Thua Thien Hue, Vietnam, 2016-III, local catcher *leg.* [ZBX]; 3Å, Da Nang, Lam Dong, Vietnam, 2020-I, local catcher *leg.* [ZBX]; 2Å, Kon Tum, Vietnam, 2017-II, local catcher *leg.* [ZBX], 1Å, Bach Ma, Thua Thien Hue, Vietnam, 2015, local catcher *leg.* [SMCR].

# Rhodambulyx schnitzleri Cadiou, 1990 施氏红鹰天蛾

13, Baoshan, Yunnan, China, 2002-IV, C. H. Yi *leg*. [SFU]; 13, Mae Ai, Chiang Mai, Thailand, 2004-III-24, T. Ihle & S. Loeffler *leg*. [SMCR]; 13, Doi Pha Hom Pok, Chiang Mai, Thailand, 2006-II, T. Ihle *leg*. [EMM]; 13, Doi Pha Hom Pok, Chiang Mai, Thailand, 2004-III, T. Ihle *leg*. [EMM].

# Rhodambulyx haxairei Melichar, Řezáč & Horecký, 2014 哈氏红鹰天蛾

1Å, Da Nang, Lam Dong, Vietnam, 2020-I, local catcher *leg.* [ZBX]; 1#, Da Nang, Lam Dong, Vietnam, 2019-III, local catcher *leg.* [ZBX]; 1Å, Lam Dong, Vietnam, 2016-III, local catcher *leg.* [ZBX].

# Rhodambulyx hainanensis Brechlin, 2001 海南红鹰天蛾

13, Wuzhi Shan, Hainan, China, 2000-III-1, Sinjaev leg. [BOLD].