



## Two new species of *Pyrocoelia* Gorham (Coleoptera: Lampyridae) from Southwest China

CHENGQI ZHU<sup>1,2,3,4\*</sup>, XIAODONG XU<sup>2,3,6</sup> & YING ZHEN<sup>2,3,5\*</sup><sup>1</sup>College of Life Sciences, Zhejiang University<sup>2</sup>Westlake Laboratory of Life Sciences and Biomedicine, Key Laboratory of Structural Biology of Zhejiang Province, School of Life Sciences, Westlake University, Hangzhou, Zhejiang, China.<sup>3</sup>Institute of Biology, Westlake Institute for Advanced Study, Hangzhou, Zhejiang Province, China.<sup>4</sup>✉ [zhuchengqi@westlake.edu.cn](mailto:zhuchengqi@westlake.edu.cn); <https://orcid.org/0000-0001-5920-8824><sup>5</sup>✉ [zhenying@westlake.edu.cn](mailto:zhenying@westlake.edu.cn); <https://orcid.org/0000-0001-8382-2533><sup>6</sup> <https://orcid.org/0000-0002-3682-9967>

\*Corresponding authors

### Abstract

Two new species of the genus *Pyrocoelia* Gorham, 1880, *Pyrocoelia cenwanglaoensis* **sp. nov.** and *P. rubrothorax* **sp. nov.** were described from Mt. Cenwanglaoshan of Guangxi, Southwest China. *Pyrocoelia cenwanglaoensis* is morphologically similar to *P. pectoralis* Olivier, 1883 and *P. amplissima* Olivier, 1886, while *Pyrocoelia rubrothorax* resembles *P. praetexta* Olivier, 1911 and *P. sanguiventer* Olivier, 1911 respectively, but these species can be distinguished based on external morphological characters and male genitalia. The diagnostic features of the two new species are described and illustrated based on morphological features. Phylogenetic reconstruction using *cox1* barcoding sequences confirms the two new species belong to genus *Pyrocoelia*. We further summarized the distribution of accepted *Pyrocoelia* species currently known in China.

**Key words:** *Pyrocoelia*, Cytochrome c oxidase subunit I, DNA barcoding, firefly

### Introduction

*Pyrocoelia* Gorham, 1880 is a genus of Asian Lampyridae and includes more than 60 known species (Jeng *et al.* 1999; McDermott, 1966). Most of the fireflies in this genus live in humid environments, and their larvae feed on snails (Osozawa *et al.* 2015). Species in this genus are sexually dimorphic in that males are alate, while females have vestigial elytra and lack hind wings (Jeng *et al.* 2011).

The genus *Pyrocoelia* and *Diaphanes* Motschulsky, 1852 are often found in the Oriental and Eastern Palearctic regions and have similar morphological characteristics (vitreous spots on pronotum, lantern tissues on 5<sup>th</sup> and 6<sup>th</sup> sternites). According to Gorham's definition (Gorham, 1880), *Pyrocoelia* could be distinguished from *Diaphanes* by three major morphological characteristics, *i.e.* serrate antennae, length of antennae longer than pronotum and at least half of body length, and length of 2<sup>nd</sup> antennomere shorter than 3<sup>rd</sup> and later segments. In addition, Jeng *et al.* (2001) pointed out that the ratio of head size to pronotum, and ratio of eyes to head in *Pyrocoelia* are smaller than that of *Diaphanes*. Molecular evidence, including DNA barcoding sequences and genomic data, are now often used as aids to new species identification, complementing traditional morphological classification. Recently, a collection of papers described new species based on the integrative approach of combining morphological and molecular data (Dong *et al.* 2021; Martínez-Villar *et al.* 2020; Wang *et al.* 2021).

In this study, we describe and illustrate two new species, *Pyrocoelia cenwanglaoensis* **sp. nov.** and *P. rubrothorax* **sp. nov.** based on specimens collected from Mt. Cenwanglaoshan, Baise, Guangxi, China. These two new species were identified as *Pyrocoelia* using an integrative approach of combining both morphological characters and DNA barcoding sequences. We compare them with the morphologically similar species, *P. pectoralis*, *P. amplissima*, *P. praetexta* and *P. sanguiventer*, and identify diagnostic features of the two new species. At last, we compile a checklist of accepted *Pyrocoelia* species from China and provide a distribution map of these species.

## Materials and methods

### Abbreviations

<b>BOLD</b>	Barcode of Life Data system
<b>EL</b>	elytral length
<b>EW</b>	elytral width
<b>PL</b>	pronotal length
<b>BL</b>	body length
<b>BW</b>	body width

Adult males of the two new species were collected in October 2019, near the Mt. Cenwanglaoshan of Baise, Guangxi, Southwest China. Holotypes and paratypes used in this study are stored at School of Life Sciences, Westlake University, Hangzhou, Zhejiang.

Habitus images were taken using a Nikon D7500 camera. Images of genitalia were taken under the microscope using OLYMPUS cellSens Dimension software (v 3.1.1). Images were edited using Adobe Photoshop CS6. Morphological characteristics were measured and described using methods and abbreviations in Zhu *et al.* (2021). Specifically, male body length (**BL**) is the sum of pronotal length (**PL**) and elytral length (**EL**) (**BL=PL+EL**). The abbreviations **EW** and **BW** (**BW=2EW**) denote elytral width and body width. The aedeagal sheath is enclosed by the tergite of the sheath (TS), including abdominal tergite 9 (T9), abdominal tergite 10 (T10) and abdominal sternite 9 (S9). The dissected genital structures were preserved in pure glycerol in small vials with the corresponding specimens.

Genomic DNA of the described species was isolated using DNeasy Blood and Tissue Kit (Zhejiang Easy-Do LTD) following the manufacturer's protocol. We partially amplified the DNA barcoding sequences of the mitochondrial gene cytochrome c oxidase subunit I (*cox1*). Primers used were LCO 1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO 2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer *et al.* 1994). The 25  $\mu$ L reaction mix consisted of 1 $\times$  PCR buffer, 1  $\mu$ L of each primer in a concentration of 1  $\mu$ M, 1  $\mu$ L of template, 0.2 mM of each dNTP and 0.5 unit of Taq polymerase (Takara Biomedical Technology CO., LTD). The PCR amplification protocol was as follows: 95 °C for 3 min; 30 cycles of 30 s at 94 °C, 30 s of 48 °C and 30 s at 72 °C, followed by a 5 min final extension at 72 °C. The PCR products were evaluated by electrophoresis in 1% agarose gel, at 170 V for 20 min, and visualized under ultraviolet light. PCR products were cleaned using Easy Gel Extraction & Cleanup kit (Zhejiang Easy-Do Biotech CO., LTD) and sequenced by Zhejiang Sunya Biotechnology CO., LTD. These sequences have been deposited to Genbank with accession numbers MW883609 and MW883615.

DNA barcoding sequences were analyzed in MEGA6 (Tamura *et al.* 2013), and a Maximum Likelihood analysis conducted with 1000 bootstrap replicates for the phylogenetic tree reconstruction. *Cox1* barcoding sequences from species in the subfamilies Lampyrinae, and Luciolinae (genera *Aquatica* and *Curtos*), and the family Rhagophthalmidae were download from Genbank and used as outgroups (Table 1). The phylogenetic relationships were displayed in FigTree (v1.4.4) (<https://github.com/rambaut/figtree/releases>).

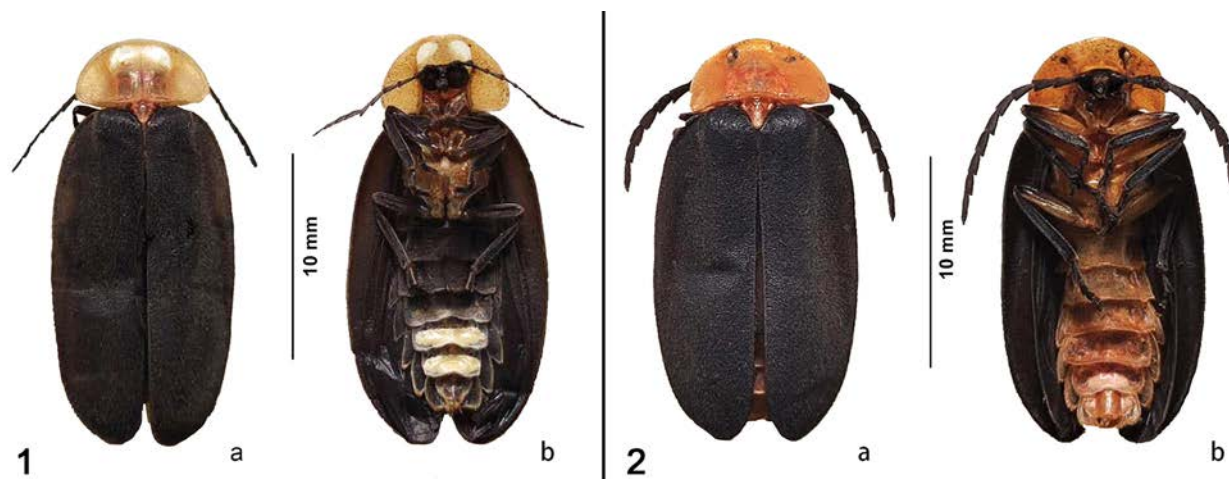
## Results

### *Pyrocoelia cenwanglaensis* Zhu & Zhen, sp. nov.

(Figs. 1, 3, 5, 6)

**Description.** *Male* (Figs. 1, 3): BL 18.6–20.8 mm; BW 9.2–11.0 mm; EL/EW 1.48–1.56; EL/PL=3.16–3.48 (six individuals).

**Head.** Antenna serrate, black, almost 1/3 as long as body length; first antennomere cone-shaped; second short and cylindrical; third to tenth compressed, with inconspicuous branches originating from inner side; eleventh almost 1.5 times as long as the tenth antennomere, slightly dilated from base to apex; antennal sockets broadly separated from each other. Eyes moderately separated above the labrum, weakly oviform laterally. Clypeus and labrum fused together and elongate oval. Mandibles shorter than clypeus and labrum.



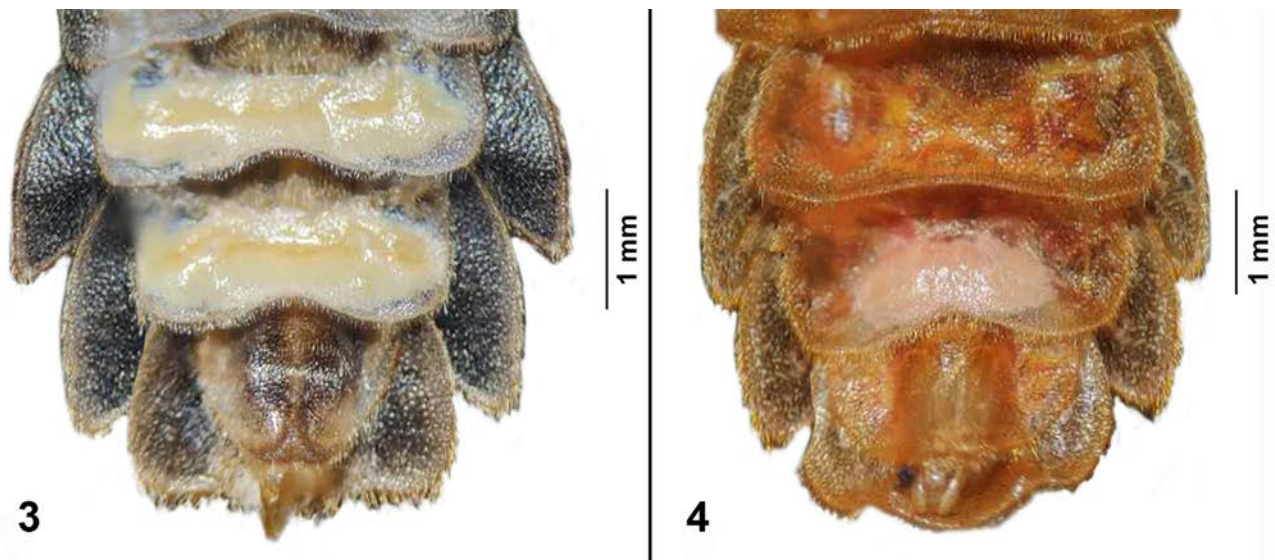
**FIGURE 1–2.** 1) Habitus of male holotype of *Pyrocoelia cenwanglaensis* Zhu & Zhen, sp. nov (a. dorsal view; b. ventral view). Scale bar = 10 mm. 2) Habitus of male holotype of *Pyrocoelia rubrothorax* Zhu & Zhen, sp. nov (a. dorsal view; b. ventral view). Scale bar = 10 mm.

**TABLE 1.** *Cox1* sequences used for phylogenetic reconstruction.

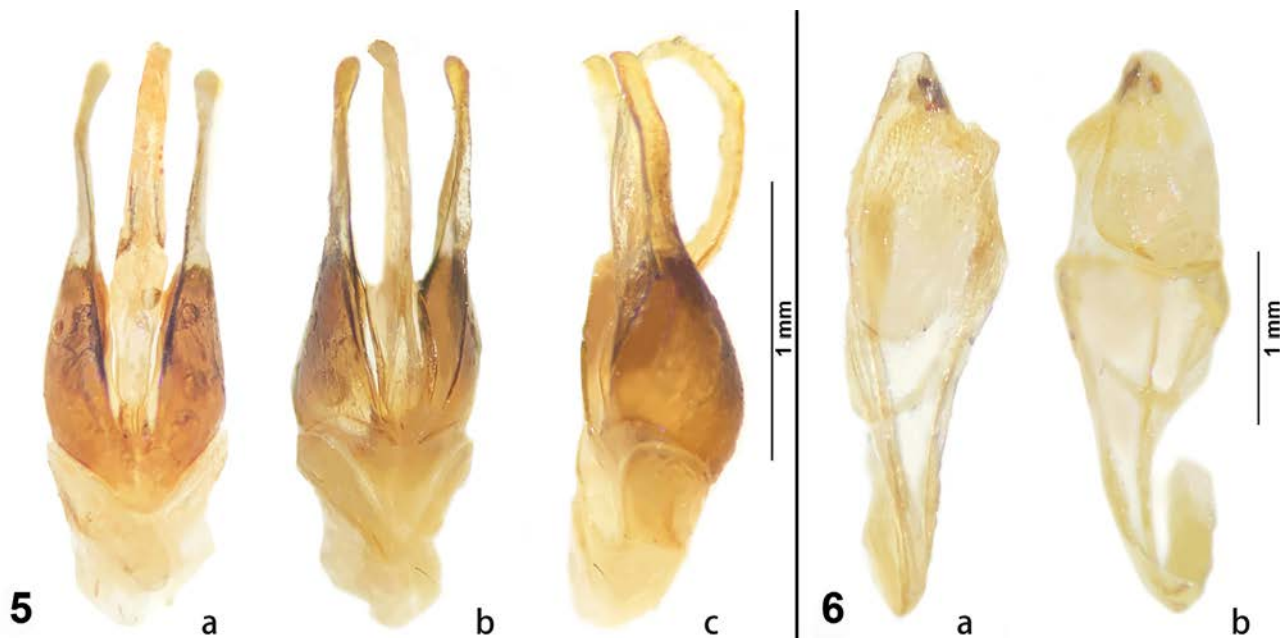
Species	Family	Sub-family	Genbank ID
<i>Pyrocoelia abdominalis</i>	Lampyridae	Lampyrinae	AB608766.1
<i>Pyrocoelia atripennis</i>	Lampyridae	Lampyrinae	AB608767.1
<i>Pyrocoelia discicollis</i>	Lampyridae	Lampyrinae	AB608768.1
<i>Pyrocoelia fumosa</i>	Lampyridae	Lampyrinae	AB608769.1
<i>Pyrocoelia matsumurail</i>	Lampyridae	Lampyrinae	AB608770.1
<i>Pyrocoelia pectoralis</i>	Lampyridae	Lampyrinae	KP763467.1
<i>Pyrocoelia praetexta</i>	Lampyridae	Lampyrinae	NC_0044790.1
<i>Pyrocoelia thibetana</i>	Lampyridae	Lampyrinae	NC_0044792.1
<i>Pyrocoelia pygidialis</i>	Lampyridae	Lampyrinae	MG200081.1
<i>Pyrocoelia rufa</i>	Lampyridae	Lampyrinae	AF452048.1
<i>Pyrocoelia cenwanglaensis</i>	Lampyridae	Lampyrinae	MW883609.1
<i>Pyrocoelia rubrothorax</i>	Lampyridae	Lampyrinae	MW883615.1
<i>Diaphanes nubilus</i>	Lampyridae	Lampyrinae	MG200080.1
<i>Diaphanes pectinealis</i>	Lampyridae	Lampyrinae	NC_044793.1
<i>Aquatica ficta</i>	Lampyridae	Luciolinae	KP763456.1
<i>Aquatica leii</i>	Lampyridae	Luciolinae	KP763457.1
<i>Curtos costipennis</i>	Lampyridae	Luciolinae	AB608764.1
<i>Rhagophthalmus lufengensis</i>	Rhagophthalmidae	-	DQ888607.1
<i>Rhagophthalmus ohbai</i>	Rhagophthalmidae	-	AB608775.1

**Thorax.** Pronotum orange in yellowish, with two crescent transparent cavities; pronotum semi-elliptical; margins of apical and lateral slightly elevated; longitudinal carina distinct. Scutellum ligulate and covered with orange hairs. Legs long and thick, black. Elytra elongated, in blackish, subparallel and broadest in apical 2/3.

**Abdomen.** Black or dark brown, abdominal terga shorter than elytra; abdomen black, gradually smaller from basal to apical segments; terga wide, with lobate expansion on both sides, apical blunt. Light organ in bright yellow (Fig. 3), occupying almost all fifth and sixth ventrites; seventh ventrite emarginate. Abdominal spiracles on lateral edges of each abdominal segment.



**FIGURE 3–4.** 3) Male abdomen and light organs on each of ventrites 5–6 of *Pyrocoelia cenwanglaoensis*. Scale bar = 1 mm; 4) Male abdomen and light organ only on ventrite 6 of *Pyrocoelia rubrothorax*. Scale bar = 1 mm.



**FIGURE 5–6.** 5) Aedeagus of *Pyrocoelia cenwanglaoensis* (a. dorsal view; b. ventral view; c. lateral view). Scale bar = 1 mm; 6) Aedeagal sheath of *Pyrocoelia cenwanglaoensis* (a. dorsal view; b. ventral view). Scale bar = 1 mm.

**Male genitalia.** (Figs. 5–6) Aedeagal sheath (Fig. 6a–b) about 2.5 mm long, tergite of the sheath (TS) subtrapezoidal, with base broadly rounded. Aedeagus (Fig. 5a–c) about 2.1 mm long, trilobate. Phallus slender, broadest at base, becoming narrower in apical 1/3, then elliptically expanding, dilated weakly at apex, little longer than parameres. Parameres robust in basal 1/3, subparallel-sided, symmetric, apical arm thumb-like, about 1/3 length of parameres.

**Diagnosis.** Body elongated, depressed dorsally, antenna serrate, second antennomere short and cylindrical, elytra subparallel. *Pyrocoelia cenwanglaoensis* sp. nov. is morphologically similar to *P. pectoralis* Olivier, 1883 from Hubei in central China but can be distinguished by the following characters: body size of *P. cenwanglaoensis* (BL=18–20 mm) is larger than *P. pectoralis* (BL=14 mm); pronotum of *P. cenwanglaoensis* is orange in yellowish while pronotum of *P. pectoralis* is orange. As for male genitalia, phallus of *P. cenwanglaoensis* is slender than *P. pectoralis*, and the parameres is much straighter.

*P. cenwanglaoensis* is also similar to *P. amplissima* Olivier, 1886, but the color of the metasternum and ventral

abdomen in *P. amplissima* is orange while in *P. cenwanglaoensis* is orange in yellowish and black; phallus of *P. amplissima* is thicker than *P. cenwanglaoensis*.

**Etymology.** The specific name *cenwanglaoensis* (Chinese name: 岑王老山窗萤) refers to the type locality of this new species.

**Holotype:** CHINA: 1♂, labeled: ‘China: Guangxi, Baise County, Mt. Cenwanglaoshan, 24°29'42"N, 106°24'28"E, H: 1300 m, 10. XI. 2019, Local People leg.’; ‘HOLOTYPE (red), ♂, *Pyrocoelia cenwanglaoensis* sp. n., det. Zhu, Zhen, 2020’ (Westlake University).

**Paratype:** CHINA: 2♂♂, labeled: ‘China: Guangxi, Baise County, Mt. Cenwanglaoshan, 24°29'42"N, 106°24'28"E, H: 1300 m, 10. XI. 2019, Local People leg.’; ‘PARATYPE (yellow), 2♂♂, *Pyrocoelia cenwanglaoensis* sp. n., det. Zhu, Zhen, 2020’ (Westlake University).

**Distribution.** China: Guangxi Province.

### *Pyrocoelia rubrothorax* Zhu & Zhen, sp. nov.

(Figs. 2, 4, 7, 8)

**Description.** *Male* (Figs. 2, 4): BL 18.2–19.5 mm; BW 8.5–9.2 mm; EL/EW 1.67–1.88; EL/PL=3.92–4.28 (five individuals).

**Head.** Antenna black, thick and serrate, almost 1/2 as long as body length; first antennomere cone-shaped; second short and cylindrical; third to tenth compressed, with obvious branches originating from inner side; eleventh almost 1.5 times as long as the tenth antennomere, slightly dilated from base to apex; antennal sockets broadly separated from each other. Eyes small, moderately separated above the labrum, weakly oviform laterally. Clypeus and labrum fused together and elongate oval. Mandibles shorter than clypeus and labrum.

**Thorax.** Pronotum orange in reddish, prosternum and metasternum, hypomera bright orange, with two little transparent cavities; pronotum semi-elliptical; margins of apical and lateral slightly elevated; longitudinal carina distinct. Scutellum ligulate and covered with orange hairs. Elytra in blackish, elongated, subparallel and broadest in apical 2/3. Legs long and thick in blackish, femur orange but rest of the parts in blackish.

**Abdomen.** Orange in reddish, abdominal terga shorter than elytra; gradually smaller from basal to apical segments, terga wide, with lobate expansion on both sides, apical blunt. Light organ in bright orange (Fig. 4), on the sixth ventrite; seventh ventrite emarginate. Abdominal spiracles on lateral edges of each abdominal segment.

**Male genitalia.** (Figs. 7–8) Aedeagal sheath (Fig. 8a–b) about 2.7 mm long, tergite of the sheath (TS) subtrapezoidal, with base broadly rounded. Aedeagus (Fig. 7a–c) about 2.2 mm long, trilobate. Phallus thumb-like, thick and straight, then elliptically expanding, a little shorter than parameres. Parameres robust in basal 1/2, subparallel-sided, becoming narrower towards apical; apical arm thumb-like, about 1/3 length of parameres.

**Diagnosis.** Body elongated, depressed dorsally, antenna serrate, second antennomere short and cylindrical, elytra subparallel. *Pyrocoelia rubrothorax* sp. nov. resembles *P. praetexta* Olivier, 1911 from Taiwan, but the elytral edge and elytral flange of *P. rubrothorax* is black, whereas that of *P. praetexta* is orange. In comparison with *P. praetexta*, *P. rubrothorax* has a more developed male phallus and much straighter parameres.

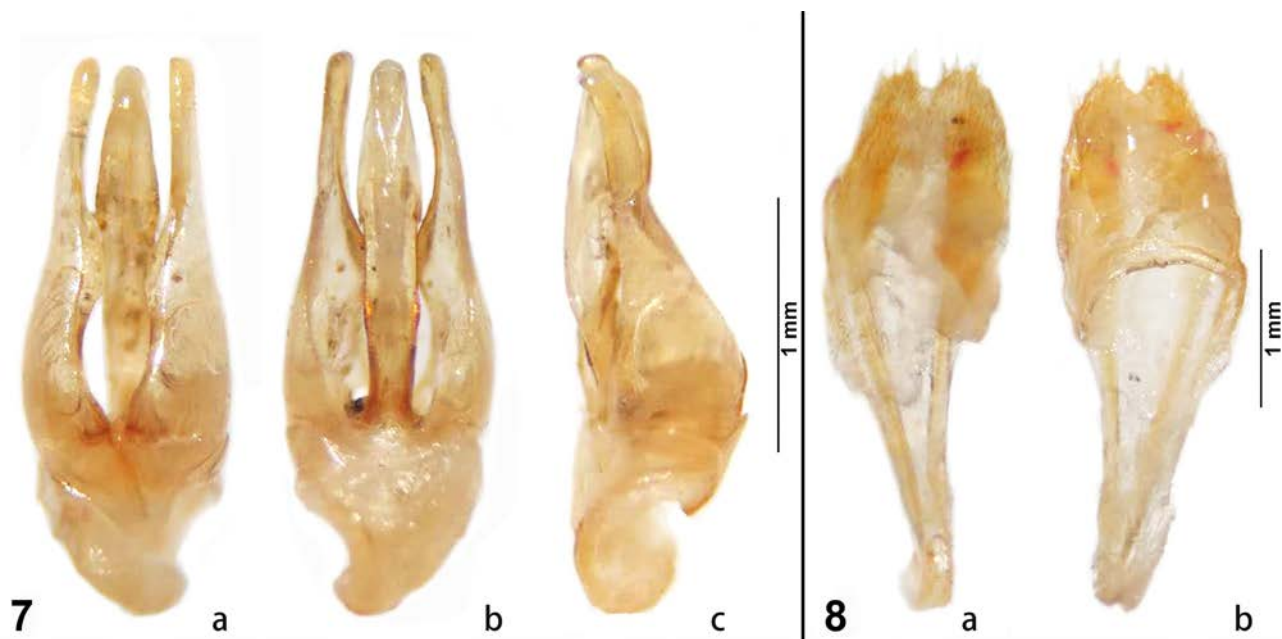
*Pyrocoelia rubrothorax* also similar to *P. sanguiventer* Olivier, 1911, known from Taiwan and Hong Kong, but can be distinguished by the following characters: the pronotum margin of *P. sanguiventer* is black but reddish in *P. rubrothorax*; the color of the metasternum in *P. sanguiventer* is black but reddish in *P. cenwanglaoensis*.

**Etymology.** The specific name *rubrothorax* (Chinese name: 赤胸窗萤) refers to the reddish pronotum of the species.

**Holotype:** CHINA: 1♂, labeled: ‘China: Guangxi, Baise County, Mt. Cenwanglaoshan, 24°29'42"N, 106°24'28"E, H: 1300 m, 10. XI. 2019, Local People leg.’; ‘HOLOTYPE (red), ♂, *Pyrocoelia rubrothorax* sp. n., det. Zhu, Zhen, 2020’ (Westlake University).

**Paratype:** CHINA: 5♂♂, labeled: ‘China: Guangxi, Baise County, Mt. Cenwanglaoshan, 24°29'42"N, 106°24'28"E, H: 1300 m, 10. XI. 2019, Local People leg.’; ‘PARATYPE (yellow), 5♂♂, *Pyrocoelia rubrothorax* sp. n., det. Zhu, Zhen, 2020’ (Westlake University).

**Distribution.** China: Guangxi Province.



**FIGURE 7–8.** 7) Aedeagus of *Pyrocoelia rubrothorax* (a. dorsal view; b. ventral view; c. lateral view). Scale bar = 1 mm; 8) Aedeagal sheath of *Pyrocoelia rubrothorax* (a. dorsal view; b. ventral view). Scale bar = 1 mm.

### Key to the new species and their related species (adult male)

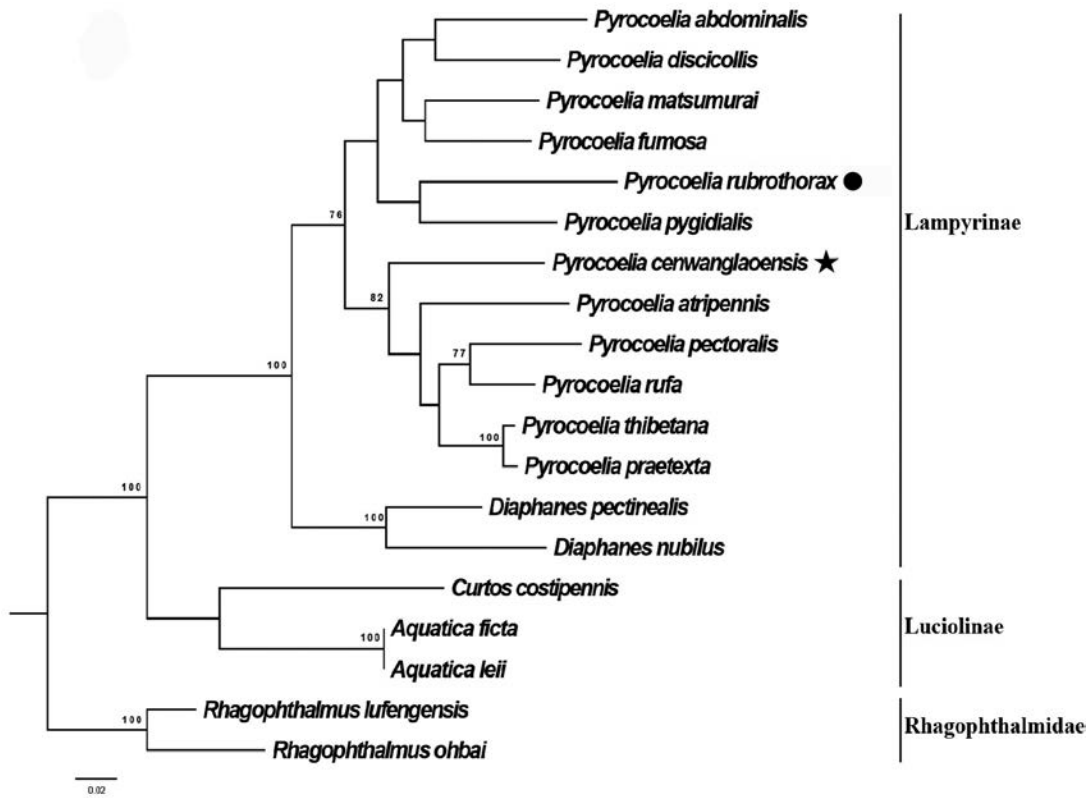
1. Elytra blackish, elytral edge and flange orange in yellowish, pronotum orange in yellowish, metasternum and abdomen yellow in brownish. . . . . *P. praetexta*
- Elytra entirely blackish. . . . . 2.
2. Metasternum blackish; pronotum reddish, the edge of the pronotum is black; abdomen reddish. . . . . *P. sanguiventer*
- Metasternum orange in reddish; pronotum and abdomen orange in reddish, coxa and femur orange but rest of the parts in blackish. . . . . *P. rubrothorax* **sp. nov.**
- Metasternum orange in yellowish. . . . . 3.
3. Abdomen orange in reddish, pronotum orange in yellowish, coxa and femur orange but rest of the parts in blackish. . . . .
- . . . . . *P. amplissima*
- Abdomen blackish. . . . . 4.
4. Phallus slender, large body size (BL= 18-20 mm), pronotum orange in yellowish. . . . . *P. cenwanglaoensis* **sp. nov.**
- Phallus thick, medium body size (BL = 14 mm), pronotum orange. . . . . *P. pectoralis*

### Molecular analyses

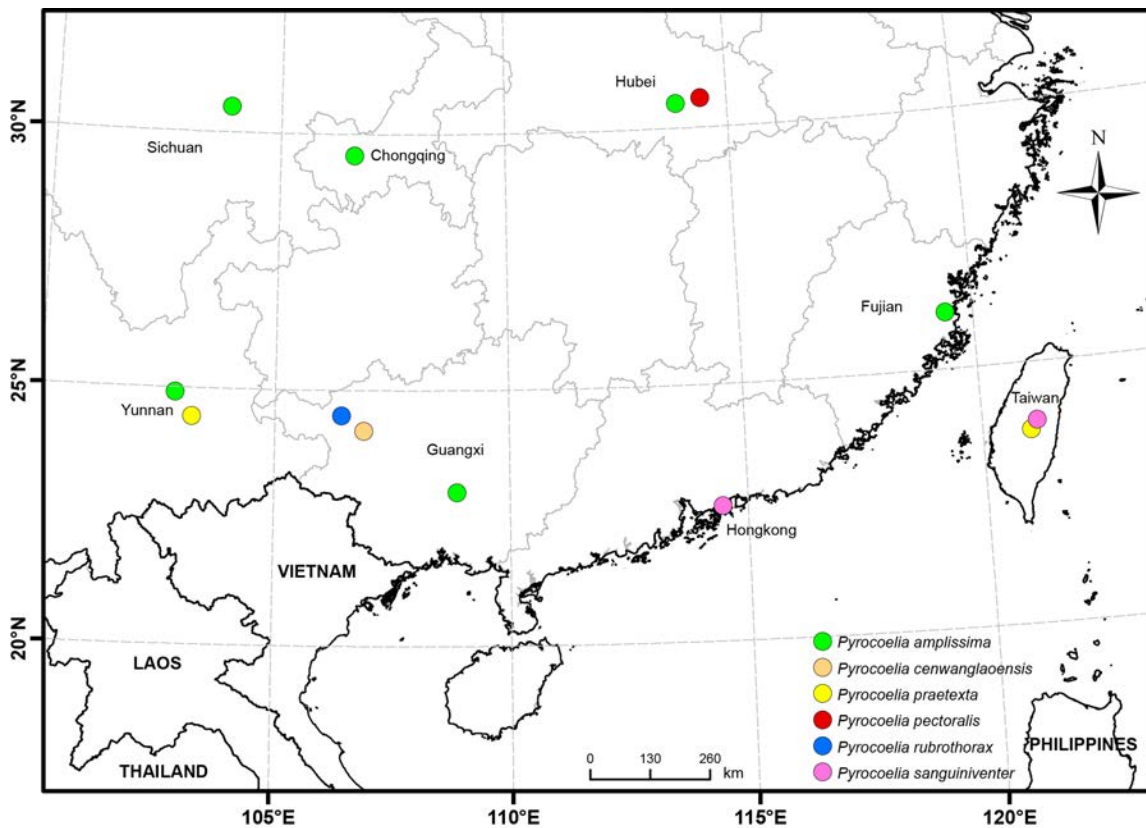
Our phylogenetic analysis of firefly *cox1* barcoding sequences confirms that *Pyrocoelia cenwanglaoensis* and *P. rubrothorax* are placed within the subfamily Lampyrinae, specifically in genus *Pyrocoelia* (Fig. 9).

### Discussion

An updated checklist of Chinese *Pyrocoelia* species is provided. We carefully compared the two new species with previously named *Pyrocoelia* species and found that each new species has two related species with relatively more similarity in morphological characters, *i.e.* *Pyrocoelia cenwanglaoensis* is morphologically similar to *P. pectoralis* Olivier, 1883 and *P. amplissima* Olivier, 1886, while *Pyrocoelia rubrothorax* is morphologically similar to *P. praetexta* Olivier, 1911 and *P. sanguiventer* Olivier, 1911. However, they can be distinguished by the body color (pronotum, mesosternum, abdomen, legs), body size or aedeagus as described in species diagnosis. A distribution map of *Pyrocoelia cenwanglaoensis* and *Pyrocoelia rubrothorax* and their morphologically similar species from China are also shown in Fig. 10.



**FIGURE 9.** Maximum likelihood tree of *Pyrocoelia* species, including *Pyrocoelia cenwanglaoensis* sp. n. (star marked), *P. rubrothorax* sp. n. (dot marked) and related genus (*Diaphanes*), based on *coxI* barcoding sequences. Bootstrap values greater than 0.7 are shown in the tree (1000 bootstrap replicates).



**FIGURE 10.** Distribution map of *Pyrocoelia cenwanglaoensis* and *Pyrocoelia rubrothorax* and their morphologically similar species from China.

The *cox1* phylogeny supports that *Pyrocoelia cenwanglaoensis* and *Pyrocoelia rubrothorax* belong to the genus *Pyrocoelia*, which is consistent with the morphological evidence. To date, there are very few *cox1* sequences from the genus *Pyrocoelia* in the BOLD database (<http://v4.boldsystems.org/>). Our *cox1* DNA barcoding sequences from the two new species will contribute to the future identification of *Pyrocoelia* firefly species.

### A checklist of accepted species of *Pyrocoelia* in China

Data source: (Olivier, 1886; Li, 2005; Cao *et al.* 2021)

Taiwan Encyclopedia of Life: <https://taieol.tw/>

BOLD: <http://www.boldsystems.org/>

Catalogue of Life: <https://www.catalogueoflife.org/>

1. *Pyrocoelia abdominalis* Nakane, 1977  
Distribution: Taiwan
2. *Pyrocoelia amplissima* Olivier, 1886  
Distribution: Hubei, Sichuan, Yunnan, Fujian, Chongqing, Guangxi
3. *Pyrocoelia analis* Fabricius, 1811  
Distribution: Hainan, Guangdong, Guizhou, Guangxi, Jiangxi, Fujian, Zhejiang, Jiangxi, Yunnan, Heilongjiang, Hongkong, Taiwan
4. *Pyrocoelia atripes* Pic, 1937  
Distribution: Tibet
5. *Pyrocoelia bicolor* Fabricius, 1801  
Distribution: Yunnan
6. *Pyrocoelia rubrothorax* Zhu & Zhen, **sp. nov.**  
Distribution: Guangxi
7. *Pyrocoelia enervis* Olivier, 1909  
Distribution: Guizhou
8. *Pyrocoelia flaviventrtris* Olivier, 1909  
Distribution: Guangxi, Jiangxi, Yunnan
9. *Pyrocoelia fochowensis* Gorham, 1880  
Distribution: Xiamen
10. *Pyrocoelia formosana* Olivier, 1911  
Distribution: Guangxi, Hubei, Taiwan, Sichuan
11. *Pyrocoelia fumata* Fairmaire, 1886  
Distribution: Beijing
12. *Pyrocoelia grandicollis* Fairmaire, 1891  
Distribution: Hongkong, Hubei
13. *Pyrocoelia kanamarui* Kishida, 1936  
Distribution: Northeast China
14. *Pyrocoelia lunata* Yiu, 2017  
Distribution: Hongkong
15. *Pyrocoelia motschulskyi* Motschulsky, 1853  
Distribution: Beijing, Guizhou, Yunnan
16. *Pyrocoelia moupinensis* Fairmaire, 1889  
Distribution: Sichuan
17. *Pyrocoelia opaca* Olivier, 1885  
Distribution: Sichuan
18. *Pyrocoelia pectoralis* Olivier, 1883  
Distribution: Hubei
19. *Pyrocoelia pekinensis* Gorham, 1881  
Distribution: Beijing



20. *Pyrocoelia praetexta* Olivier, 1911  
Distribution: Taiwan, Yunnan
21. *Pyrocoelia prolongata* Jeng et Lai, 1999  
Distribution: Taiwan
22. *Pyrocoelia pygidialis* Pic, 1926  
Distribution: Yunnan
23. *Pyrocoelia cenwanglaoensis* Zhu & Zhen, **sp. nov.**  
Distribution: Guangxi
24. *Pyrocoelia rufa* Olivier, 1886  
Distribution: Zhejiang, Shandong, Shanxi
25. *Pyrocoelia sanguiniventer* Olivier, 1911  
Distribution: Taiwan, Hongkong
26. *Pyrocoelia scutellaris* Pic, 1926  
Distribution: China
27. *Pyrocoelia signaticollis* Oliver, 1886  
Distribution: Sichuan, Chongqing, Hubei, Anhui, Jiangsu, Fujian, Zhejiang
28. *Pyrocoelia thibetana* Olivier, 1886  
Distribution: Tibet, Yunnan

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