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# Systematic review of the firefly genus *Emeia* Fu, Ballantyne & Lambkin, 2012 (Coleoptera: Lampyridae) from China

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# Systematic review of the firefly genus *Emeia* Fu, Ballantyne & Lambkin, 2012 (Coleoptera: Lampyridae) from China

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## Running head

## SYSTEMATIC REVIEW OF EMEIA FIREFLIES

### Abstract

The Luciolinae genus *Emeia* Fu, Ballantyne & Lambkin, 2012 is reviewed. Firefly species tree is reconstructed based upon phylogenetic analysis of CO1 barcoding sequences from 42 fireflies and 2 outgroup species, including three main Lampyridae sub-families: Luciolinae, Photurinae and Lampyrinae. The genus *Emeia* belongs to Luciolinae based on morphological and DNA sequence level evidences, the diagnostic features for male adults include pink-red pronotum with a black median stripe, subparallel-sided pronotum, un-developed elytral humeral carina. In this study, a new *Emeia* species, *Emeia pulchra* sp. nov. is described and illustrated from the wetland of Lishui, Zhejiang, China. The new species is sister to *E. pseudosauteri* in the *Emeia* genus which is supported by characteristic morphological features and DNA barcoding data. The two species are separated geographically as shown on the distribution map. A key to the males of the species of *Emeia* is also provided.

**Key words:** Cytochrome oxidase subunit I, DNA barcoding, *Emeia*

### Introduction

The genus *Emeia* (Coleoptera, Lampyridae, Luciolinae) was established by Fu et al. (2012) with *Emeia pseudosauteri* Fu, Ballantyne & Lambkin, 2012 as type species. *E. pseudosauteri* was first described from Mount Emei, Sichuan, China by Michael Geisthardt in the genus *Curtos* Motschulsky, 1845 (Geisthardt, 2004), and then transferred to *Emeia* based on morphological and molecular evidence (Fu et al. 2012). The genus *Emeia* Fu, Ballantyne & Lambkin had only one species (*E. pseudosauteri*) recorded in China before this study. Fu (Fu, Ballantyne, & Lambkin, 2012) first described the diagnostic phenotypic features of this new genus *Emeia* based on the *Emeia pseudosauteri* samples he collected, one feature of this genus is the trilobite-like larva. Specifically, the thoracic and abdominal terga of *Emeia* larvae are quite different, the lateral thoracic tergal margins are broad similar to that of a trilobite “cephalon”, while the abdomen is narrow and curve posteriorly. However, since the establishment of this genus in 2012, there has been only one species *Emeia pseudosauteri*, which makes the characteristics description of this genus insufficient.

In this study, based on specimens collected from Lishui, Zhejiang, China, we characterized

a new *Emeia* species, *Emeia pulchra* sp. nov, both morphologically and molecularly. We compared it with previously described *E. pseudosauteri*. The new species is morphologically similar to *E. pseudosauteri*, but can be distinguished by body color, male genitalia, wing features and DNA barcoding sequences. We provide complementary information on adult wing feature to the type species *E. pseudosautari* for the first time. With detailed examination of both species, we present a systematic review of the genus *Emeia* and provide a key to species.

## Materials and methods

### Abbreviations

**BOLD** Barcode of Life Data system

**EL** elytral length

**EW** elytra width

**PL** pronotal length

**BL** body length

**BW** body width

**T7, 8** visible abdominal tergites number

**V6, 7** abdominal ventrites number, including hidden, nonvisible ventrites

Samples of both male and female *Emeia pseudosautari* were collected from Leshan, Sichuan Province in April, 2021. The adult male *Emeia pulchra* were collected from Jiulong National Wetland Park, Lishui, Zhejiang Province in April, 2020. Holotypes and prototypes 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 the genitalia were taken using a Nikon D7500 camera mounted on an SZ650 microscope (Chongqing Optec Instrument Co., Ltd) under reflection or transmission light. Images were edited using Adobe Photoshop CS6. The dissected genital structures were preserved in pure glycerol in small vials with corresponding specimens.

Total DNA of the described species was isolated using DNeasy Blood and Tissue Kit (Zhejiang Easy-Do Biotech CO., LTD) according to manufacturer's protocol. Primers LCO 1490 and HCO 2198 (Folmer et al. 1994) were used to amplify barcoding fragment of the mitochondrial gene for cytochrome c oxidase subunit I (CO1). We amplified in a 25 uL reaction mix containing 1× PCR buffer, 1 uL of each primer in a final concentration of 1uM, 1 uL of template, 0.2 mM of each dNTPs and 0.5 units of Taq polymerase (Takara Biomedical Technology CO., LTD). The PCR thermal regime consisted of an initial denaturation at 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 checked by electrophoresis in 1% agarose gel, at 170 V for 20 min, and visualized under a UV transilluminator with nucleic acid dye (Cofitt life science, HK). The PCR products were cleaned using Easy Gel Extraction & Clean-up kit (Zhejiang Easy-Do Biotech CO., LTD). The cleaned product was sequenced at ABI 3730XL sequencer (Applied Biosystems, California, USA) in Zhejiang Sunya Biotechnology CO., LTD.

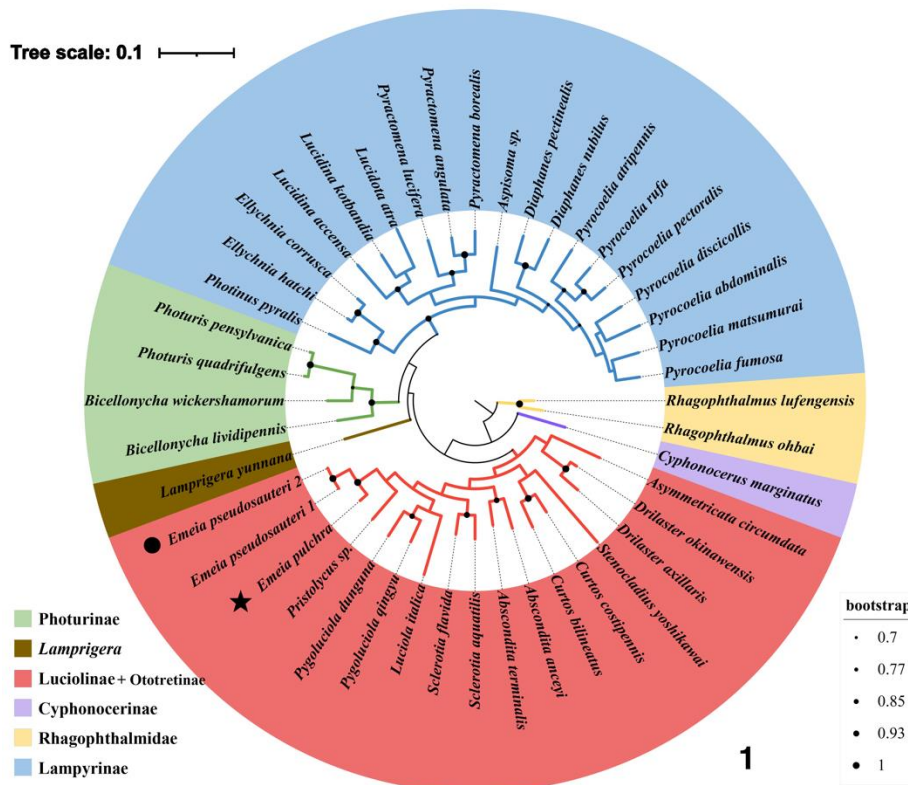
MEGA6 (Tamura et al. 2013) was used for phylogenetic reconstruction. CO1 barcoding sequences from three main sub-families, *i.e.* Luciolinae, Photurinae and Lampyrinae, were included, and sequences from family Rhagophthalmidae were used as outgroups (Table 1). Maximum likelihood method was used with 100 bootstrap replicates, and bootstrap values greater than 70 were shown on the tree. The phylogenetic relationships were displayed using

iTOL (v6; <https://itol.embl.de/>). The new CO1 sequences from *Emeia* have been deposited to Genbank (accession numbers OK144132 and OK103803).

## Results

### Phylogenetic analysis

We sequenced the 658 bp DNA barcoding sequences of CO1 gene from *Emeia pseudosauteri* and *E. pulchra*. The CO1 barcoding sequences of *E. pseudosauteri* and *E. pulchra* share 94% similarity. Phylogeny constructed from fireflies CO1 sequences formed three main clades, i.e. Lampyrinae, Photurinae and Luciolinae (Fig. 1). *Emeia pseudosauteri* and *E. pulchra* belong to the subfamily Luciolinae. Based on the CO1 phylogeny, *E. pulchra* is a closest sister species to *E. pseudosauteri*, with strong support (100%).



**Figure 1.** Maximum likelihood tree of *Emeia pulchra* sp. n. (black star), *E. pseudosauteri* (black dot) and related genera, based on CO1 barcoding sequences. Bootstrap values are shown on the tree (100 bootstrap replicates).

### Taxonomic treatment

#### *Emeia pulchra* Zhu & Zhen, sp. nov.

(Figs. 2, 3, 4, 5)

**Description.** *Male:* BL 10.0–10.4 mm; BW 3.5–3.7 mm. Body elongate. Pronotum pink-red, with a black median stripe, subparallel-sided; Elytral humeral surface smooth without longitudinal carina.

Antennae pectinate and in black, 11 segments, almost as long as 2/3 body length; first antennomere cone-shaped; second short and cylindrical; third to tenth compressed, without

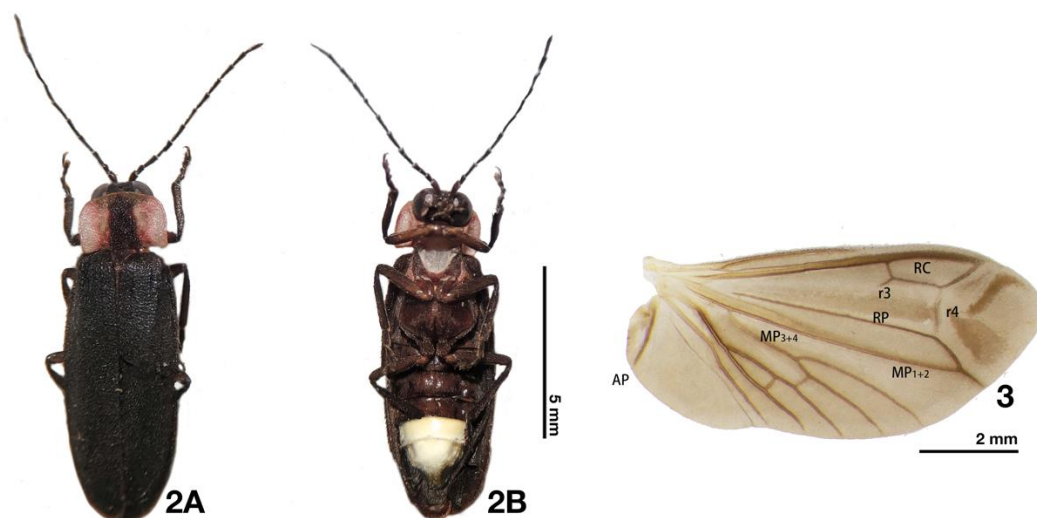
inconspicuous branches originated; eleventh almost 1.5 times as long as the proceeding segment, slightly dilated from base to apex.

Head moderately depressed between eyes, eyes large and almost occupy the whole head, eyes above labrum moderately separated, cannot fully contract to the pronotum. Mouthparts functional, strongly flattened. Clypeolabral flexible, outer edges of labrum reach inner edges of closed mandibles.

Scutellum black and ligulate. Elytra elongate, brownish in black, apices not deflexed, lateral margins slightly convex-sided, elytra humeral carina absent. Hind wing well developed, r3 short than r4 (Fig. 3).

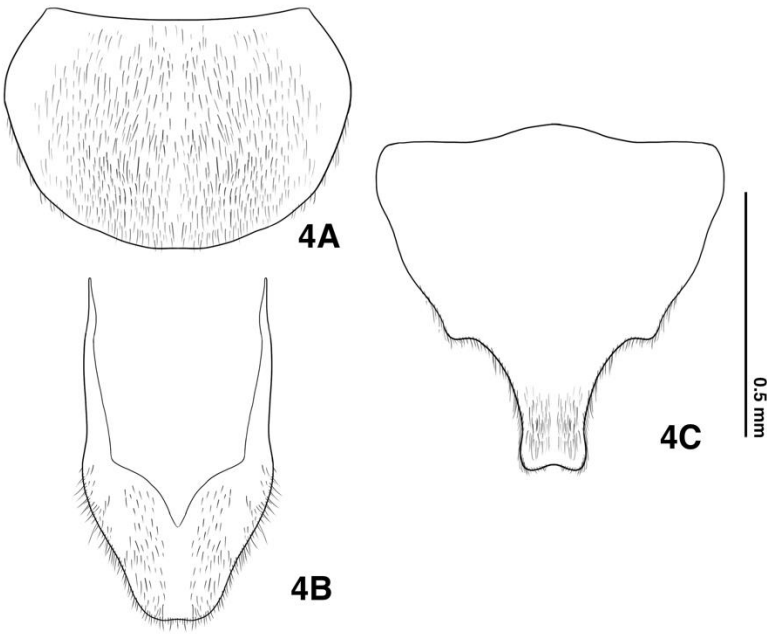
Legs long and thick, brown blackish, dense white hairs on the surface.

Abdominal terga shorter than elytra; abdomen in brownish, gradually smaller from basal to apical segments. Photoc organs in yellowish white, occupying almost all V6 and half of V7, not reaching to posterior margins at V7. V6 and V7 rounded (Fig. 4), posterior half of V7 not arched, shrink into a leaf-like protrusion with a little bifurcation (Fig. 4C). T7 rounded, without anterolateral corners (Fig. 4A); T8 symmetrical with concealed anterolateral arms, widest across middle with lateral margins subparallel-sided in anterior half, tapering evenly in posterior half to a rounded and partly truncate posterior margin (Fig. 4B). Abdominal spiracles on lateral edges of each abdominal segments. EL/EW 2.26–2.27; EL/PL=3.92–4.12.



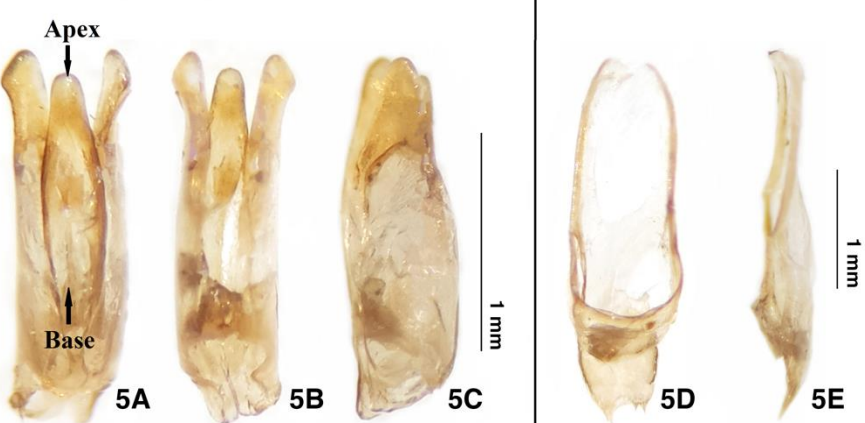
**Figure 2-3. *Emeia pulchra* Zhu & Zhen, sp. nov, male. 2 Habitus of holotype. (A) dorsal view. (B) ventral view. Scale bar: 5 mm. 3 Right wing. Dorsal view. Scale bar: 2 mm.**





**Figure 4.** Male abdominal ventrites (V) and tergites (T) of *Emeia pulchra*. (A) T7 (B) T8 (C) V7. Scale bar: 0.5 mm.

*Male genitalia* (Fig. 5): Aedeagal sheath (Fig. 5D–E) about 3.15 mm long; anterior half of sternite broad, apically rounded; tergite without protrusion along posterior margin of T9. Aedeagus (Fig. 5A–C) about 1.61 mm long, trilobate in form. Median lobe short and thick, broadest at midlength, becoming thinner in apical and base, little shorter than parameres (lateral lobes). Parameres robust and stretch in apical, subparallel-sided, symmetric, little curved outside from about 1/3 length of parameres.



**Figure 5.** Male aedeagal of *Emeia pulchra*. (A) dorsal view (B) ventral view (C) lateral view. Scale bar: 1 mm; Male aedeagal sheath of *E. pulchra*. (D) dorsal view (E) ventral view. Scale bar: 1 mm.

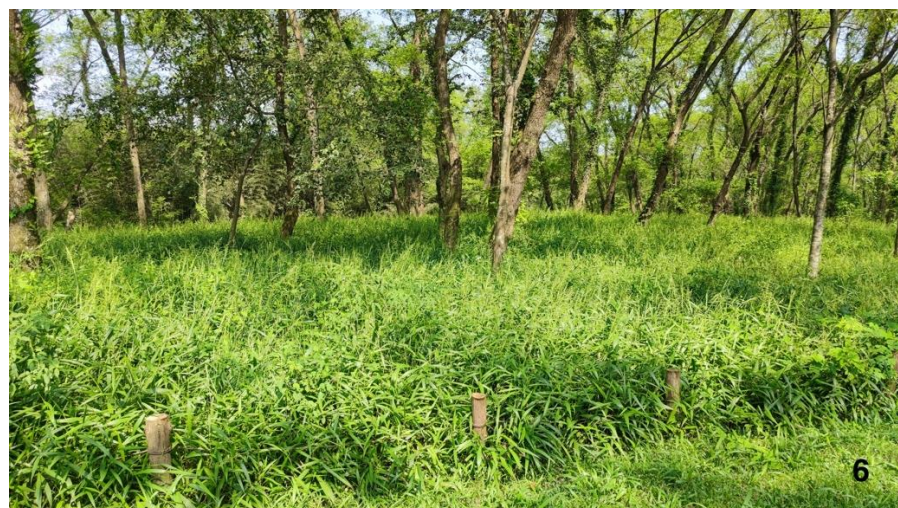
**Etymology.** The specific name *pulchra* refers to bright color of its pronotum.

**Holotype:** CHINA·1♂; Zhejiang, Lishui; 28°37.56'N; 119°49.7'E; H: 60 m, 2. IV. 2020; Chengqi Zhu leg.; 'HOLOTYPE (red), ♂, *Emeia pulchra* sp. n., det. Zhu, Zhen, 2021' (Westlake University).

**Paratype:** CHINA·1♂; Zhejiang, Lishui; 28°37.56'N; 119°49.7'E; H: 60 m, 2. IV. 2020; Chengqi Zhu leg.; 'PARATYPE (yellow), ♂, *Emeia pulchra* sp. n., det. Zhu, Zhen, 2021' (Westlake University).

**Distribution.** China: Zhejiang Province.

**Habitat and occurrence.** The males were found in an open forest dominant with Chinese wingnut [*Pterocarya stenoptera* C. DC.] (Fig. 6). The ground of *Emeia pulchra* habitat is covered with lush weeds with a height of 20-30 cm. We also found that there are many terrestrial snails and slugs in this habitat, which may be potential food for *Emeia pulchra* larvae. Combining descriptions from local people and our field observations, adult fireflies are usually observed in mid-March every year. With the restoration of habitat and strengthening of protection measures, the observed population size of fireflies has increased from 2014 to 2019 (Fan, 2019).

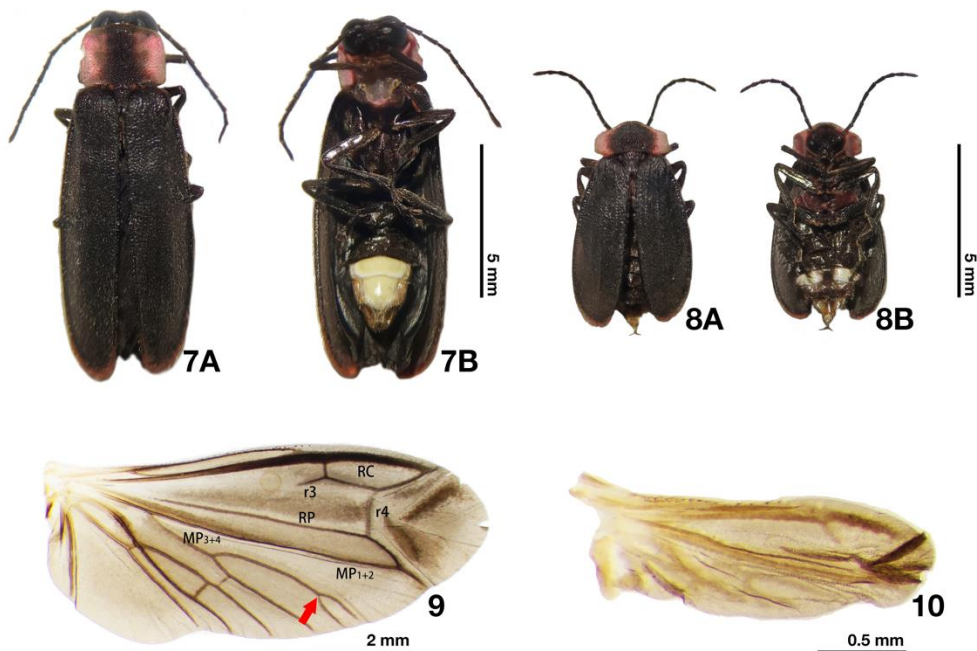


**Figure 6.** Habitat of *Emeia pulchra*.

**Behavioral remarks.** There are two obvious luminous bands at the terminal end of the adult male abdomen. The two adult male luminous bands both emit intermittent bright light during courtship. The male courtship behavior usually starts at 17:00 (approximately 1h after sunset), and peaks at about 20:30. Adult males rest on higher weeds and emit yellow and green flashing light. Males are not good at flying; the distance of each flight ranges from 0.5 to 5 m.

***Emeia pseudosauteri* Fu, Ballantyne & Lambkin, 2012**  
(Figs. 7, 8, 9, 10, 11)

*Emeia pseudosauteri* Fu, Ballantyne & Lambkin, 2012. *Zootaxa* (3403), 1-53. TL: 'Mt. Tiantai Shan'



**Figure 7-10. *Emeia pseudosauteri* Fu, Ballantyne & Lambkin, 2012.** Male and female. **7** Habitus of male. **(A)** dorsal view **(B)** ventral view. Scale bar: 5 mm. **8** Habitus of female. **(A)** dorsal view **(B)** ventral view. Scale bar: 5 mm. **9** Right wing of male. Dorsal view. Scale bar: 2 mm. **10** Right wing of male. Dorsal view. Scale bar: 0.5 mm.



**Figure 11. Male aedeagal of *Emeia pseudosauteri*.** **(A)** dorsal view **(B)** ventral view **(C)** lateral view. Scale bar: 1 mm; Male aedeagal sheath of *E. pseudosauteri*. **(D)** dorsal view **(E)** ventral view. Scale bar: 1 mm.

**Key to species (adult male)**

1. Body elongate; elytral humeral carina not developed; pronotum pink-red, with a black median stripe, subparallel-sided; male aedeagal median lobe little shorter than parameres.....*Emeia* Fu, Ballantyne & Lambkin
2. Lateral margins of elytra orange (Fig. 7A); the upper vein of MP<sub>3+4</sub> forks and reaches the margin of hind wings (Fig. 9), the median lobe and parameres tubbiness, approx. 2 times



as long as wide (Fig. 11A) .....*E. pseudosauteri* Fu, Ballantyne & Lambkin  
 - The lateral margins of elytra is almost black (Fig. 2A); the upper vein of MP<sub>3+4</sub> reaches the  
 margin of hind wings, but without forks (Fig. 3), the median lobe and parameres slender,  
 approx. 3 times as long as its width (Fig. 5A) ..... *E. pulchra* Zhu & Zhen

## Discussion

We examined the type species *Emeia pseudosauteri* and tried to look for other undescribed species in *Emeia* in major collections of National Animal Collection Resource Center (<http://museum.ioz.ac.cn/index.html>), and we didn't find any sample that may belong to *Emeia*. Based on adult males of two identified *Emeia* species, we summarize the diagnostic features of the *Emeia* genus. A distinct external feature is the pronotum that is subparallel-sided and pink-red with a black median stripe. In contrast with other firefly species, *Luciola* and *Pteroptyx* species have bright orange pronotum and dark elytra (Ballantyne, 1987; Ballantyne & Lambkin, 2009), and *Aquatica wuhana* and *A. lateralis* have black marks on pronotum (Fu, Ballantyne, & Lambkin, 2010), however, the *Emeia* species have a relatively red pronotum with broad black stripe that is easily distinguished from the other species (Fig 2; Fig 7; Fig 8).

*Emeia pulchra* sp. nov. is morphologically similar to *E. pseudosauteri* Fu, Ballantyne & Lambkin, 2012 from Sichuan of Southwestern China. The main morphological differences between these two species are in antennae, elytra, pronotum and male genitalia. The antennae of male *E. pulchra* (Fig. 2) is narrower than that of *E. pseudosauteri* (Fig. 7); The female *E. pseudosauteri* is about 2/3 in body length compared to male and have normal elytra (Fig. 8), but its hind wings are small and shrunken, about ~1/4 length of male hind wings (Fig. 9-10). For male, we find hind wing of *E. pseudosauteri* is relatively narrower and longer than *E. pulchra*, and the upper vein of MP<sub>3+4</sub> forks and reaches the margin of wing (marked in red arrow in Fig. 9). The lateral margins of elytra is orange in *E. pseudosauteri* while black in *E. pulchra*. Typically, the body size of *E. pseudosauteri* (BL 6.6–7.2 mm; BW 2.7–2.9 mm) is much smaller than *E. pulchra* (BL 10.0–10.4 mm; BW 3.5–3.7 mm). The pronotum of the new species is more pink and narrower than *E. pseudosauteri*. For male genitalia, the median lobe and parameres of new species (Fig. 5A) are slender than *E. pseudosauteri* (Fig. 11A), and parameres are not as curved as those in *E. pseudosauteri* (Fig. 5B; Fig. 11B). In addition, the new species is only known from S. Zhejiang while *E. pseudosauteri* is only found in SW. Sichuan. There is broad geographical isolation between two localities (Fig. 12).



**Figure 12.** Distribution map of the genus *Emeia* from China. Black star indicates the *E. pulchra* sp. n., black dot indicates the *E. pseudosauteri* (map of China from: <http://bzdt.ch.mnr.gov.cn/>).

In this paper, we report one new species, *Emeia pulchra* sp. nov and present a systematic review of the genus *Emeia*. The discovery of this new species increases the number of species in genus *Emeia*. These two sister species share high morphological similarity, but could be readily distinguished using morphological characters and CO1 barcoding sequences. To date, there are only very few CO1 submissions in Lampyridae and further efforts are needed to fill this gap.

### Acknowledgements

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**Table 1.** CO1 sequences used for phylogenetic reconstruction.

Species	Family	Sub-family	Accession number
<i>Pyrocoelia pectoralis</i>	Lampyridae	Lampyrinae	KP763467.1
<i>Pyrocoelia rufa</i>	Lampyridae	Lampyrinae	AF452048.1
<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>Diaphanes nubilus</i>	Lampyridae	Lampyrinae	MG200080.1
<i>Diaphanes pectinealis</i>	Lampyridae	Lampyrinae	NC_044793.1
<i>Photinus pyralis</i>	Lampyridae	Lampyrinae	KY778696.1
<i>Ellychnia corrusca</i>	Lampyridae	Lampyrinae	KR483038.1
<i>Ellychnia hatchi</i>	Lampyridae	Lampyrinae	JF887410.1
<i>Pyractomena lucifera</i>	Lampyridae	Lampyrinae	MF640134.1
<i>Pyractomena borealis</i>	Lampyridae	Lampyrinae	HQ928227.1
<i>Pyractomena angulata</i>	Lampyridae	Lampyrinae	JN290381.1
<i>Aspisoma</i> sp.	Lampyridae	Lampyrinae	EU009322.1
<i>Lucidina accensa</i>	Lampyridae	Lampyrinae	AB608771.1
<i>Lucidina kotbandia</i>	Lampyridae	Lampyrinae	FJ462784.1
<i>Lucidota atra</i>	Lampyridae	Lampyrinae	HQ984304.1
<i>Photuris pensylvanica</i>	Lampyridae	Photurinae	MF634963.1
<i>Photuris quadrifulgens</i>	Lampyridae	Photurinae	HM433520.1
<i>Bicellonycha lividipennis</i>	Lampyridae	Photurinae	KJ922151.1
<i>Bicellonycha wickershamorum</i>	Lampyridae	Photurinae	EU009302.1
<i>Pristolytus</i> sp.	Lampyridae	Luciolinae	MK292099.1

<i>Sclerotia flavida</i>	Lampyridae	Luciolinae	KP763460.1
<i>Sclerotia aquatilis</i>	Lampyridae	Luciolinae	KP763466.1
<i>Pygoluciola dunguna</i>	Lampyridae	Luciolinae	MT106243.1
<i>Pygoluciola qingyu</i>	Lampyridae	Luciolinae	MK292093.1
<i>Curtos bilineatus</i>	Lampyridae	Luciolinae	NC_044789.1
<i>Curtos costipennis</i>	Lampyridae	Luciolinae	AB608764.1
<i>Abscondita terminalis</i>	Lampyridae	Luciolinae	NC_044776.1
<i>Abscondita anceyi</i>	Lampyridae	Luciolinae	NC_039706.1
<i>Emeia pseudosauteri</i> 1	Lampyridae	Luciolinae	MN722654.1
<i>Emeia pseudosauteri</i> 2	Lampyridae	Luciolinae	OK103803
<i>Emeia pulchra</i>	Lampyridae	Luciolinae	OK144132
<i>Luciola italica</i>	Lampyridae	Luciolinae	KM448530.1
<i>Asymmetricata circumdata</i>	Lampyridae	Luciolinae	NC_032062.1
<i>Drilaster axillaris</i>	Lampyridae	Ototretinae	AB608756.1
<i>Drilaster okinawensis</i>	Lampyridae	Ototretinae	AB608758.1
<i>Stenocladus yoshikawai</i>	Lampyridae	Ototretinae	AB608759.1
<i>Lamprigera yunnana</i>	Lampyridae	Incertae_sedis	MG200082.1
<i>Cyphonocerus marginatus</i>	Lampyridae	Cyphonocerinae	AB608754.1
<i>Rhagophthalmus lufengensis</i>	Rhagophthalmidae	-	DQ888607.1
<i>Rhagophthalmus ohbai</i>	Rhagophthalmidae	-	AB608775.1

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