Luminescent characteristics and mitochondrial COI barcodes of nine cohabitated Taiwanese fireflies

King-Siang Goh1, Liang-Jong Wang2, Jing-Han Ni3 and Tzi-Yuan Wang4

1 Genomics Research Center, Academia Sinica, Taipei, Taiwan
2 Forest Protection Division, Taiwan Forestry Research Institute, Taipei, Taiwan
3 Department of Ecological Humanities, Providence University, Taichung, Taiwan
4 Biodiversity Research Center, Academia Sinica, Taipei, Taiwan

ABSTRACT

Background. Over 50 Taiwanese firefly species have been discovered, but scientists lack information regarding most of their genetics, bioluminescent features, and cohabitating phenomena. In this study, we focus on morphological species identification and phylogeny reconstructed by COI barcoding, as well as luminescent characteristics of cohabited Taiwanese firefly species to determine the key factors that influenced how distinct bioluminescent species evolved to coexist and proliferate within the same habitat.

Methods. In this study, 366 specimens from nine species were collected in northern Taiwan from April to August, 2016–2019. First, the species and sex of the specimens were morphologically and genetically identified. Then, their luminescent spectra and intensities were recorded using a spectrometer and a power meter, respectively. The habitat temperature, relative humidity, and environmental light intensity were also measured. The cytochrome oxidase I (COI) gene sequence was used as a DNA barcode to reveal the phylogenetic relationships of cohabitated species.

Results. Nine species—eight adult species (Abscondita chinensis, Abscondita cerata, Aquatica ficta, Luciola curtithorax, Luciola kagiana, Luciola filiformis, Curtos sauteri, and Curtos costipennis) and one larval Pyrocoelia praetexta—were morphologically identified. The nine species could be found in April–August. Six of the eight adult species shared an overlap occurrence period in May. Luminescent spectra analysis revealed that the $\lambda_{\text{max}}$ of studied species ranged from 552–572 nm (yellow–green to orange–yellow). The average luminescent intensity range of these species was about 1.2–14 lux (182.1–2,048 nW/cm$^2$) for males and 0.8–5.8 lux (122.8–850 nW/cm$^2$) for females, and the maximum luminescent intensity of males was 1.01–7.26-fold higher than that of females. Compared with previous studies, this study demonstrates that different $\lambda_{\text{max}}$, species-specific flash patterns, microhabitat choices, nocturnal activity time, and/or an isolated mating season are key factors that may lead to the species-specific courtship of cohabitated fireflies. Moreover, we estimated that the fireflies start flashing or flying when the environmental light intensity decreased to 6.49–28.1 lux. Thus, based on a rough theoretical calculation, the sensing distance between male and female fireflies might be 1.8–2.7 m apart in the dark. In addition, the mitochondrial COI barcode identified species with high resolution and suggested that most of the studied species have been placed correctly with congeners in previous phylogenies. Several cryptic species were revealed by the COI barcode with 3.27%–12.3% variation.
This study renews the idea that fireflies’ luminescence color originated from the green color of a Lampyridae ancestor, then red-shifted to yellow-green in Luciolinae, and further changed to orange–yellow color in some derived species.

**Subjects** Conservation Biology, Ecology, Entomology, Taxonomy, Zoology

**Keywords** Molecular phylogeny, COI, Luciola, Aquatica, Abscondita, Wavelength, Pyrocoelia, Curtos, Firefly

**INTRODUCTION**

Among terrestrial bioluminescent insects, fireflies (Lampyridae) have the most charismatic shine, which they use for mating or aposematic signals at night (Oba, Branham & Fukatsu, 2011). Fireflies in Coleoptera are the most diverse terrestrial group of bioluminescent organisms. Over 2,100 firefly species have been reported in temperate and tropical regions, including Eurasia, America, New Zealand, and Australia. Firefly life history and bioluminescence have been studied for over a century and have offered bioinspiration for many inventions and methods, such as a method for detecting gene expression (biomedical), improvements in LED technology (industrial), and algorithms (mathematical) (Kaskova, Tsarkova & Yampolsky, 2016; Kim et al., 2016; Yang, 2009). Fireflies are also considered to be an environmental indicator species for assessing light, water, and soil pollution. Moreover, some of their larvae—such as *Pyrocoelia pectoralis*, which eat invasive snails (Fu & Meyer-Rochow, 2013)—are used as biological controls in some species. Firefly population sizes are dramatically affected by changes in land-use, as habitat deterioration and artificial night lighting decrease their populations (Firebaugh & Haynes, 2016; Owens, Meyer-Rochow & Yang, 2018).

The phylogeny of Lampyridae (fireflies) has been reassessed several times (Ballantyne et al., 2013; Ballantyne & Lambkin, 2013; Ballantyne et al., 2019; Chen et al., 2019; Martin et al., 2017; Martin et al., 2015; Martin et al., 2019; Stanger-Hall, Lloyd & Hillis, 2007; Wang, Wu & Wang, 2021). These studies identified the following subfamilies: Ototretinae, Cyphonocerinae, Luciolinae (incl. Pristolycus), Pterotinae, Lamprohizinae, Psicoladinae, Amydetinae, Photurinae, and Lampyrinae. The most comprehensive study used 436 genomic loci to reconstruct a consensus phylogeny of fireflies with paraphyletic subfamilies, except Ototretinae with *Drilaster* and *Stenocladius* (Martin et al., 2019). For example, this study reassessed Luciolinae as paraphyletic with *Lamprigera*, and the higher-level classification of Lampyridae was revised accordingly. However, only few Asian species were included. In addition, the reassessed phylogeny might influence the hypothesis of previous bioluminescent evolution (Martin et al., 2017; Oba et al., 2020).

Previous phylogeny of fireflies reveals the evolution of their bioluminescence (Martin et al., 2017; Oba et al., 2020). Studies show that luminescence appeared in the common ancestor of Lampyridae about 100–200 million years ago (Oba et al., 2020; Zhang et al., 2020). In the forests of the mid-Cretaceous, the first luciferase gene evolved from acyl-CoA synthetase (acyl-CoA synthetase) to produce yellow luminescence that may be due to nocturnal predation. The ancestral Lampyrinae fireflies later evolved to have green
luminescence, while the ancestral Luciolinae fireflies evolved a red-shifted yellow-green luminescence; more species need to be studied to confirm this evolutionary distinction.

Most fireflies glow during the larval stage (1–2 years), but bioluminescent courtship behavior only occurs during the short adult stage (2–4 weeks) (Buck, 1948; Riley, Rosa & Lima da Silveira, 2021). All known luminous signals of adult fireflies can be roughly divided into flashing and continuous glowing (Lloyd, 1966; Seliger et al., 1964). Research suggests that each species has its own specific flash pattern, determined by differences in flash duration, flash frequency, and flash color (Lewis & Cratsley, 2008; Seliger et al., 1964). The wavelength ($\lambda_{\text{max}}$) of most fireflies’ flash color range from yellow-green (538 nm) to orange-red (622 nm) (He et al., 2021).

The mitochondrial Cox1 (COI) barcode is a powerful biomarker for estimating large-scale species richness, determining the potential for beta-diversity studies, and setting conservation priorities. However, error rates can be high for some individual genera, especially when very recent species form nonmonophyletic clusters (Bergsten et al., 2012; Hendrich et al., 2015; Pentinsaari et al., 2016). The comprehensive COI barcode databases and the Barcode Index Number (BIN) system are well-established and regularly updated (Adamowicz, 2015; Adamowicz et al., 2017; Hendrich et al., 2015; Ratnasingham & Hebert, 2013; Roslin et al., 2022; Rulik et al., 2017). In insects such as beetles, the mitochondrial COI barcode has proven an effective molecular marker for species identification (Hendrich et al., 2015; Pentinsaari, Hebert & Mutanen, 2014; Raupach et al., 2020; Roslin et al., 2022). The COI barcode can also be used to establish firefly phylogeny, biogeography, and population genetics, as well as to identify cryptic species (Choi et al., 2003; Dong et al., 2021; Han et al., 2020; Jusoh et al., 2014; Kim et al., 2001; Lee et al., 2003; Muraji, Arakaki & Tanizaki, 2012; Stanger-Hall, Lloyd & Hillis, 2007; Usener & Cognato, 2005). Thus, the COI barcode is a cheaper and more convenient biomarker for firefly identification. However, until the past two years, only a few Asian species had been sequenced (Choi et al., 2003; Dong et al., 2021; Han et al., 2020; Kim et al., 2001; Lee et al., 2003; Liu & Fu, 2020; Sriboonlert & Wonnapinij, 2019).

Fifty-six species have been described from Taiwan to date (Jeng, Lai & Yang, 2003; Jeng, Yang & Engel, 2007; Jeng et al., 1998), but few reports have been made on their biodiversity, ecological habitats, comparative morphology, life cycle, or behavior (Ballantyne et al., 2013; Ballantyne et al., 2015; Ballantyne & Lambkin, 2013; Ballantyne et al., 2019; Goh, Lee & Wang, 2022; Goh & Li, 2011; Ho et al., 2010; South et al., 2008). Taiwan has Luciola; Curtos; the reassessed Aquatica (Fu, Ballantyne & Lambkin, 2010), Abscondita (Ballantyne et al., 2013; Ballantyne et al., 2019), and Sclerotia (Ballantyne et al., 2016) species of Luciolinae; and some Lampyrinae species (Ballantyne et al., 2013; Goh, Lee & Wang, 2022; Jeng, Lai & Yang, 1999; Jeng, Yang & Engel, 2007; Jeng et al., 1998; Ohba & Yang, 2003; Wang, Wu & Wang, 2021). The endemic Abscondita cerata (formerly known as Luciola cerata) is the most abundant species, widely distributed from low altitude to medium-high altitude (1,500 m) in Taiwan. During its breeding season, several sympatric fireflies could be found (Goh, Lee & Wang, 2022; Jeng, Lai & Yang, 1999; Ohba & Yang, 2003). A recent study revealed that LED light intensity can influence the flash pattern of Aquatica ficta (Owens, Meyer-Rochow & Yang, 2018). These are the only two species of Taiwanese firefly for which systematic
Table 1. Luminescent spectrum ($\lambda_{\text{max}}$) and intensity (nW/cm$^2$) of nine cohabitated species from two habitats in northern Taiwan.

| Species                  | Sex     | Individuals (n) | $\lambda_{\text{max}}$ (nm) | Luminescent intensity$^{b,c}$ (nW/cm$^2$) |
|--------------------------|---------|-----------------|-------------------------------|-------------------------------------------|
|                          |         |                 |                               | Mean | Maximum                          |
| Abscondita cerata        | Female  | 17              | 562.2 ± 0.4                  | 122.8 ± 19.3 | 282.2                           |
|                          | Male    | 28              | 563.6 ± 0.3                  | 406.6 ± 96.5 | 2,048                           |
| Abscondita chinensis     | Female  | 3               | 571.3 ± 0.3                  | 245.7 ± 83.9 | 329.7                           |
|                          | Male    | 2               | 572.0 ± 0.0                  | 332.1 | 332.1                           |
| Aquatica ficta          | Female  | 5               | 564.0 ± 0.5                  | 569.4 ± 101.1 | 850                             |
|                          | Male    | 17              | 564.4 ± 0.3                  | 525.7 ± 71.1 | 1,102                           |
| Curtos costipennis      | Female  | 1               | 554                           | 462 | ND                              |
|                          | Male    | –               | –                            | – | –                               |
| Curtos sauteri          | Female  | 5               | 554.0 ± 0.3                  | 187.7 ± 55.7 | 349.3                           |
|                          | Male    | 3               | 552.7 ± 0.9                  | 347.3 ± 95.9 | 536.7                           |
| Luciola curtithorax     | Female  | 12              | 566.3 ± 0.4                  | 157.9 ± 30.4 | 301.3                           |
|                          | Male    | 26              | 572.5 ± 0.2                  | 356.1 ± 48.0 | 814.1                           |
| Luciola filiformis      | Female  | –               | –                            | – | –                               |
|                          | Male    | 12              | 567.3 ± 0.2                  | 182.1 ± 31.2 | 323.8                           |
| Luciola kagiana         | Female  | 3               | 574.3 ± 0.3                  | ND | ND                              |
|                          | Male    | 3               | 574.0 ± 1.0                  | 5.4 ± 4.8 | 10.2                           |
| Pyrocoelia praetexta    | Larva$^a$ | 3              | 552.7 ± 0.9                  | ND | ND                              |

Notes.
$^a$Luminescent spectra were only successfully recorded from larvae.
$^b$Mean and maximum values were obtained as described in Methods and materials.
$^c$‘‘–’’; no sample; ‘‘ND’’; not detectable.

studies have been conducted based on luminescence spectrum and DNA barcoding. Therefore, this study investigated nine cohabitated species in northern Taiwan for species identification by COI barcode, flash color and luminescent intensity to determine the key factors through which distinct bioluminescent species evolved to coexist and proliferate within the same habitat.

**MATERIALS & METHODS**

**Specimen collection and habitat**

366 specimens of eight adult and one larval species were randomly collected in flight or from vegetation using hand dip nets from two habitats in the suburbs of Taipei, Taiwan—Nankang (25°01’40.4”N 121°38’02.6”E) and Miaoli County, Nanzhuang (24°37’53.5”N 121°01’37.0”E)—at 18:30–19:30 from April to August 2016–2019 (Table 1). After the bioluminescent spectrum/intensity measurement, the specimens were deposited in a laboratory freezer. For DNA extraction, several specimens were then stored at $-80$ °C. The remaining specimens were stored at $-20$ °C for species identification.

The environmental conditions before and after the fireflies began flashing and/or flying were investigated to understand what environmental factors may trigger their nocturnal activity. The temperature, relative humidity, and light intensity (lux) of the firefly habitat...
were recorded during the period before flashing/flying (18:20∼18:40) and the period after the fireflies began flashing/flying (18:30∼18:50) using HOBO U12-012 data loggers (Onset Computer Corp., Bourne, MA, USA) at 10-sec intervals.

Species identification and morphological measurements
The specimens were collected as previously described in Goh, Lee & Wang (2022). The material collected in this study was identified by LJ Wang on the species level through the use of available references (Ballantyne et al., 2013; Chen, 2003; Jeng, Lai & Yang, 2003; Jeng, Yang & Lai, 2003; Jeng et al., 1998). Five morphological characteristics of the specimens (body length, pronotum length, pronotum width, front wing length, and front wing width) were measured (see Table S3) using a dissecting microscope and photographed with a digital video camera as previously described in Goh, Lee & Wang (2022). During the survey, the specimens were chilled on ice. All surveys were completed within two days of the collection. One to five identified specimens were sacrificed and stored at −20 °C in the Biodiversity Research Center, Academia Sinica, Taipei, Taiwan (contact person: TY Wang, tziyuan@gmail.com).

Bioluminescence spectrum/intensity measurement
The wavelength ($\lambda_{\text{max}}$) and luminescent intensity (nW/cm$^2$) of the light flashes produced by the living samples were measured by a USB2000+ spectrometer (Ocean Optics) and a PD300 power meter (Ophir), respectively. All surveys were completed within two days of the collection. The wavelength and luminescent intensity measurements were performed in a dark room by directly attaching the detector of the USB2000+ spectrometer or PD300 to the light organ of a trapped firefly (Fig. 1 modified from Goh, Lee & Wang (2022)). The average wavelength peak and $\lambda_{\text{max}}$ were obtained from an average of 3–5 measurements in complete darkness at 25 °C with 75% humidity. The luminescent intensity of the flash was obtained by averaging each flash from 3–10 min of recording data with a PD300 power meter. To compare the luminescent intensity data from PD300 and HOBO U12-012 using the same units, all data in the energy unit nW/cm$^2$ were converted into lux via the conversion 1 lux = 1.464E−07 W/cm$^2$ = 146.41 nW/cm$^2$ (at 555 nm).

Statistics
The differences in bioluminescence spectrum among the specimens were determined by the Chi-square test between two species.

DNA barcode sequencing
Crude DNA was extracted from thoracic muscles via the ZR Tissue & Insect DNA MicroPrep™ kit (D6015). Two beetle-specific primers (ClepFolF 5′-ATTCAACCAATCATAAAGATATTGG-3′ and ClepFolR 5′-TAAACTTCTGGA TGTCCTAAAAATCA-3′) were designed based on the comprehensive DNA barcode database of beetles (Hendrich et al., 2015) to amplify a 620-bp segment including the cytochrome oxidase I (COI) gene. Polymerase chain reactions (PCRs) in 50-µL volumes were performed with a dNTP concentration of 200 µM and a primer concentration of 0.3 µM, with 50 ng of genomic DNA, one unit of TaKaRa Taq™ DNA Polymerase, and the
buffer supplied by the manufacturer. The PCR was run for 35–40 cycles under the following conditions: denaturation at 95 °C for 30 s, annealing at 50–55 °C for 40 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The product mixture was used as a template for DNA sequencing (Genomics Ltd., Taipei, Taiwan). Haplotype sequences were deposited into GenBank under accession numbers MT534191–MT534201, ON209457 (Table 2).

**Molecular phylogeny**

The COI sequences of closely-related species and/or species with known λ\(_{\text{max}}\) of luminescence were downloaded from GenBank based on previous studies (Adamowicz, 2015; Arnoldí, Neto & Viviani, 2010; Cassata, 2020; Dong et al., 2021; Han et al., 2020; He et al., 2021; Hendrich et al., 2015; Jusoh et al., 2021, 2018; Jusoh et al., 2014; Kim et al., 2001; Li, Yang & Fu, 2022; Liu et al., 2017; Liu & Fu, 2020; Martin et al., 2017; Martin et al., 2015; Muraji, Arakaki & Tanizaki, 2012; Oba, Branham & Fukatsu, 2011; Oba et al., 2020; Osozawa et al., 2015; Roslin et al., 2022; Rulik et al., 2017; Stanger-Hall, Lloyd & Hillis, 2007; Usener & Cognato, 2005; Wilcox, 2021; Zhang et al., 2018). Sequences were
Table 2  DNA barcodes (COI) of studied fireflies. Only haplotype sequences were submitted to GenBank for the same species.

| Species                  | Accession number of haplotype (individual number) | Reference       |
|--------------------------|--------------------------------------------------|-----------------|
| Luciolinae               |                                                  |                 |
| *Abscondita cerata*      | MT534192 (6), MT534199 (3)                       | present study   |
| *Abscondita chinensis*   | MT534196 (3), ON209457 (1)                       | Present study   |
| *Aquatica ficta*         | MT534197 (2)                                     | Present study   |
| *Curtos sauteri*         | MT534198 (1)                                     | Present study   |
| *Luciola curtithorax*    | MT534191 (1), MT534193 (1), MT534195 (1)         | Present study   |
| *Luciola filiformis*     | MT534201 (1)                                     | Present study   |
| *Luciola kagiana*        | MT534200 (1)                                     | Present study   |
| Lampyrinae               |                                                  |                 |
| *Pyrocoelia praetexta*   | MT534194 (1)                                     | Present study   |

then aligned using the ClustalX program (Thompson, Gibson & Higgins, 2002) with the default setting in MEGA X (Kumar, Stecher & Tamura, 2016), followed by length trimming due to different amplicons. After trimming, the short-length sequences were removed. At most, three representative sequences were kept for each species to simplify the tree topology. There were a total of 520 positions and 161 nucleotide sequences in the final dataset (Table S1). Neighbor-joining (NJ) (Saitou & Nei, 1987) and maximum-likelihood (ML) trees were constructed using GTR+G+I distances in MEGA X with 500 bootstrap replications (Felsenstein, 1985). The substitution model (parameter) used to calculate GTR+G+I distances (Nei & Kumar, 2000) was selected using Modeltest v3.7 (Posada & Crandall, 1998). The differences in the composition bias among sequences were considered in the evolutionary comparisons (Tamura & Kumar, 2002).

RESULTS

Cohabitated species composition at Nanzhuang and Nankang
From April to August 2016–2019, we collected 366 flying specimens from two firefly habitats (Nanzhuang and Nankang) in northern Taiwan (Table 1 & S2 Fig. 2), and morphologically identified them to the species level (Table S3). These specimens comprised nine different species, including adult males and/or females of *Aquatica ficta*, *Luciola filiformis*, *Abscondita cerata*, *Luciola kagiana*, *Luciola curtithorax*, *Abscondita chinensis*, *Curtos sauteri*, and *Curtos costipennis*. Only *Pyrocoelia praetexta* was observed as larvae from April to August. Five of the species—*Aq. ficta*, *L. filiformis*, *Abs. cerata*, *L. kagiana* and *P. praetexta*—were found in both habitats. *Luciola curtithorax* was collected only in Nankang, and *Abs. chinensis*, *C. sauteri*, and *C. costipennis* were collected only in Nanzhuang (Table S2). To simplify the results, Table 1 combined all specimens from the two habitats for further analysis.

Occurrence periods of the cohabitated species
Figure 3 shows the estimated occurrence periods of the eight adult species in Nankang and Nanzhuang based on the collection dates of the specimens. *Abscondita cerata* and *L. kagiana* occurred in April–May, while *C. sauteri* and *Aq. ficta* occurred in May–August.
L. curtithorax occurred in May-July while L. filiformis occurred in May–June. Abscondita chinensis was found only in June, while C. costipennis was found only in August. Six of the eight studied species shared an overlap occurrence period in May: C. sauteri, Aq. ficta, Abs. cerata, L. kagiana, L. filiformis, and L. curtithorax. The occurrence periods of this study overlapped with the occurrence periods of previous studies based in other habitats in Taiwan (Chen, 2003; Chen & Jeng, 2012).

**Differences in the luminescence spectrum between the cohabitated species**

The average $\lambda_{\text{max}}$ from the luminescent spectra of nine studied species (eight adult species and larval P. praetexta) ranged from about 552 nm (green-yellow) to 575 nm (yellow-orange) (Table 1, Fig. S1). Excluding insufficient data on three species, the average $\lambda_{\text{max}}$ of five species showed no significant difference between intraspecific males and females. The average $\lambda_{\text{max}}$ of L. curtithorax was significant different between female and male. Ignoring the sexual differences, the pairwise comparison of interspecific $\lambda_{\text{max}}$ (Table 3) showed that the studied species commonly displayed significant difference ($p$-values <0.05) in $\lambda_{\text{max}}$ to
most other studied species. No significant difference was found between *Abs. chinensis* and *L. curtithorax* \((p\text{-value} = 0.0604)\) or between *C. sauteri* and *P. praetexta* \((p\text{-value} = 0.161)\).

To determine the courtship behaviors of cohabited fireflies based on sex, we further compared the \(\lambda_{\text{max}}\) between six interspecific adult females (Table 4) and males (Table 5). Most studied species revealed significant differences in \(\lambda_{\text{max}}\) between interspecific females. However, no significant difference has found between females of *Aq. ficta* and *Abs. cerata* \((p\text{-value} = 0.0742)\). In contrast, the \(\lambda_{\text{max}}\) comparison between interspecific adult males (Table 5) showed no significant difference between *Aq. ficta* and *Abs. cerata* \((p\text{-value} = 0.672)\), between *L. curtithorax* and *Abs. chinensis* \((p\text{-value} = 0.626)\), between *L. kagiana* and *P. praetexta* \((p\text{-value} = 0.161)\).
Table 4  Differences in pairwise $\lambda_{\text{max}}$ (p-value) of adult female between species. The statistics were calculated using $\lambda_{\text{max}}$ of adult females. Numbers in boldface are not significantly different.

|               | Abs. cerata | Abs. chinensis | Aq. ficta | C. sauteri | L. filiformis | L. curtithorax | L. kagiana |
|---------------|-------------|----------------|-----------|------------|---------------|---------------|-----------|
| Abs. cerata   |             | 0.0000         |           |            |               |               |           |
| Abs. chinensis| 0.0742      |                |           |            |               |               |           |
| Aq. ficta     |             | 0.0000         |           | 0.0000     |               |               |           |
| C. sauteri    | 0.0000      | 0.0000         |           | 0.0000     |               |               |           |
| L. filiformis*| NA$^b$      | NA             | NA        | NA         | NA            | NA            | NA        |
| L. curtithorax| 0.0000      | 0.0000         | 0.0095    | 0.0000     | NA            |               |           |
| L. kagiana    | 0.0000      | 0.0000         | 0.0000    | 0.0000     | NA            | 0.0000        | 0.0000    |

Notes.

$^a$Without adult female.

$^b$''NA'': not analysis due to lack of female.

Table 5  Differences in pairwise $\lambda_{\text{max}}$ (p-value) of adult males between species. The statistics were calculated using $\lambda_{\text{max}}$ of males. Numbers in boldface are not significantly different.

|               | Abs. cerata | Abs. chinensis | Aq. ficta | C. sauteri | L. filiformis | L. curtithorax | L. kagiana |
|---------------|-------------|----------------|-----------|------------|---------------|---------------|-----------|
| Abs. cerata   |             | 0.0000         |           |            |               |               |           |
| Abs. chinensis| 0.0672      |                |           |            |               |               |           |
| Aq. ficta     |             | 0.0028         | 0.0021    | 0.0031     |               |               |           |
| C. sauteri    | 0.0000      | 0.0000         | 0.0000    | 0.0022     |               |               |           |
| L. filiformis*| 0.0000      | 0.0626         | 0.0000    | 0.0010     | 0.0000        |               |           |
| L. curtithorax| 0.0052      | 0.1835         | 0.0075    | 0.0001     | 0.0177        | 0.2606        |           |
| L. kagiana    | 0.0000      | 0.0000         | 0.0000    | 0.0000     | NA            | 0.0000        |           |

and Abs. chinensis (p-value = 0.1835), and between L. kagiana and L. curtithorax (p-value = 0.2606).

**Correlation between firefly luminescent intensity and environmental photic intensity**

This study was performed during an Abs. cerata massive occurrence (April to May) in Nankang and Nanzhuang. During the studied periods, it was estimated that the average environmental light intensity during twilight, the ten-minute period before the fireflies started flashing or flying in the habitats, was in a range of 35.7–136.5 lux (Table 6). The suitable environmental light intensity for fireflies flashing and/or flying was in a range of 6.49–28.1 lux.

With the exception of L. kagiana and P. praetexta due to abnormal behavior (no glowing or glowing in extremely low light intensity), the luminescent intensity of seven adult species was about 1.2–14 lux (182.1–2,048 nW/cm$^2$) in male fireflies and nearly 0.8–5.8 lux (122.8–850 nW/cm$^2$) in female fireflies (Table 1). The results showed that the male fireflies have higher luminescent intensity than the females, which might be related to their courtship behaviors.

Herein, we argue that firefly luminescent intensity is correlated with environmental photic intensity. For examples, among the studied male species, male A. cerata produced the brightest flashes, measuring up to 14 lux (or 2,048 nW/cm$^2$). In contrast, female A.
Table 6  The environmental temperature, relative humidity, and environmental light intensity of the habitats around twilight and when Abs. cerata starts flashing/flying.

| Date          | Nocturnal activity time        | Temp (°C) ± SD | RH (%) ± SD | Environmental light intensity (lux) ± SD |
|---------------|-------------------------------|----------------|-------------|----------------------------------------|
| A. Nankang, Taipei: | Twilight (18:20–18:30)        | 24.9 ± 0.68   | 84.3 ± 3.32 | 56.1 ± 26.8                            |
|               | Start flashing/flying (18:30–18:40) | 23.8 ± 0.12   | 90.7 ± 0.76 | 10.5 ± 4.55                            |
| 4/29/2017     | Twilight (18:16–18:26)        | 18.7 ± 0.31   | 81.0 ± 1.28 | 136.5 ± 66.2                           |
|               | Start flashing/flying (18:26–18:36) | 17.7 ± 0.21   | 85.2 ± 1.03 | 6.49 ± 3.74                            |
| 5/1/2017      | Twilight (18:22–18:30)        | 23.2 ± 0.36   | 81.8 ± 1.83 | 68.1 ± 21.0                            |
|               | Start flashing/flying (18:30–18:40) | 22.1 ± 0.28   | 87.5 ± 1.46 | 19.4 ± 8.65                            |
| 5/18/2017     | Twilight (18:31–18:41)        | 22.7 ± 0.07   | 90.3 ± 0.40 | 41.7 ± 15.97                           |
|               | Start flashing/flying (18:41–18:51) | 22.5 ± 0.04   | 91.5 ± 0.35 | 12.6 ± 5.34                            |
| B. Nanzhuang, Miaoli: | Twilight (18:26–18:30)        | 20.3 ± 0.46   | 71.2 ± 1.85 | 122.5 ± 26.3                           |
|               | Start flashing/flying (18:30–18:40) | 19.0 ± 0.46   | 77.2 ± 2.52 | 28.1 ± 24.2                            |
| 5/7/2017      | Twilight (18:30–18:40)        | 23.2 ± 0.07   | 92.8 ± 0.44 | 40.52 ± 18.2                           |
|               | Start flashing/flying (18:40–18:50) | 23.0 ± 0.07   | 93.6 ± 0.20 | 8.69 ± 4.15                            |
| 5/8/2017      | Twilight (18:27–18:37)        | 22.7 ± 0.06   | 93.35 ± 0.18| 35.7 ± 14.0                            |
|               | Start flashing/flying (18:37–18:47) | 22.5 ± 0.05   | 93.8 ± 0.12 | 9.21 ± 4.01                            |

Notes.

*a* For comparison, the environmental light intensity of twilight was estimated with ten minutes before fireflies flashing or flying in habitats. The recording interval is 10 s per time (n = 60).

*b* Recording time postponed due to unexpected schedule in field trip.

**ficta** emitted the brightest flashes among the studied female species, measuring up to 5.8 lux (or 850 nW/cm²). In addition, the maximum luminescent intensity emitted from the five kinds of adult males was 2.3–14 lux (332.1–2,048 nW/cm²), which is 1.01–7.26-fold higher than that of conspecific females (1.9–5.8 lux or 282.2–850 nW/cm²). Thus, this result clearly shows that the range of the environmental light intensity (6.49–28.1 lux) when fireflies begin to flash partially overlaps with the luminescent intensity of fireflies. In addition, during 18:00–19:30, the change in average environmental temperature and relative humidity were in a range of 17.1–25.0 °C and 71.2–95.8%, respectively.

**Molecular phylogeny of Lampyridae inferred by COI barcodes**

To reveal how bioluminescence evolved, it is important to compare the luminescence spectrum and molecular phylogeny. The COI barcodes of eight studied species (except C. costipennis) were successfully sequenced for phylogenetic analysis (Table 2). All are new COI barcodes of Taiwanese fireflies sequenced in this study. Their haplotype sequences were deposited in GenBank under accession numbers MT534191–MT534201, ON209457.

The NJ tree (Fig. S2) and ML tree (Fig. S3) indicate that the studied genera Abscondita, Curtos, Aquatica, and Luciola belong to Luciolinae, while the genus Pyrocoelia belongs to Lampyrinae, a monophyly supported by previous mitogenomic phylogeny (Wang, Wu & Wang, 2021). However, the short COI sequences showed incongruence grouping among subfamilies in the high-level phylogeny. For example, Rhagophthalmus (Rhagophthalmidae) was placed close to the Luciolinae with a low bootstrap value; Stenocladius did not form a
Figure 4 Neighbor-Joining tree using the COI gene (520 bp) with bootstrap test results (500 replicates) at the nodes. The optimal tree with the sum of branch length = 5.58552373 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei & Kumar, 2004) with number of base substitutions per site. The rate variation among sites was modeled with gamma distribution (shape parameter = 1.079137891). All positions with less than 95% site coverage were eliminated. See Fig. S2 for a detailed NJ tree.

clade with Drilaster as Ototretinae. Nevertheless, most studied species are placed correctly with congeners (Figs. 4 and 5).

There are several monophyletic clades supported by medium or high bootstrap values. Lampyrinae was a monophyletic clade with Pyrocoelia, Diaphanes, Lampyris, Microphotus,
Figure 5  Maximum Likelihood tree using the COI gene (520 bp) with bootstrap test results (500 replicates) at the nodes. The evolutionary history was inferred using the Maximum Likelihood method based on the General Time Reversible model. The tree with the highest log likelihood (−11653.0821) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with the superior log likelihood value. A discrete gamma distribution was used to model differences in evolutionary rates across sites (four categories (+G, parameter = 0.5737)). The rate variation model allowed some sites to be evolutionarily invariable (1+I, 37.4868% sites). The tree is drawn to scale, with branch length measurements based on the number of substitutions per site. All positions with less than 95% site coverage were eliminated. See Fig. S3 for a detailed ML tree.
Aspisoma, Photinus, Pleotomodes, Ellychnia, Lucidina, Phosphaenus, Pyractomena, and Pyropyga, although these genera did not form stable sister groups with each other. Photuris and Bicellonycha (as Photurinae) formed a clade with Pyropyga nigricans.

A previous study indicated that Luciolinae is not monophyletic, even when 436 gene loci were used (Martin et al., 2019). Thus, it is reasonable to see polyphyletic Luciolinae in the COI gene tree. The Luciolinae complex included the monophyletic genera Luciola, Aquatica, Pterophyx, Sclerotia, Abscondita, Pygoluciola, Curtos, and Lamprigera, which comprises Pristolycus, Vesta, and Emeia pseudosauteri. There are still several monophyletic clades with medium bootstrapping values. Excluding L. cruciata, the COI barcode grouped 12 Luciola species as a monophyly supported by a medium bootstrapping value (61/54), including the type species (L. italic). Luciola cruciata formed a stable clade with five Aquatica species. In addition, Pygoluciola clustered with Abscondita while Curtos clustered with Lamprigera supported only with a low bootstrapping value.

Large COI sequence variations can be found between and/or within geographically distinct species. For example, two COI sequences of Chinese Emeia pseudosauteri were separated into distinct clades. Emeia pseudosauteri is restricted to central China and isolated among mountains. Such habitat isolation caused great mitochondrial DNA variation (Liu & Fu, 2020). Accordingly, there might be cryptic species and a need to reclassify some other species. A detailed analysis of Taiwanese fireflies will be discussed later.

DISCUSSION

We identified five cohabitated species from Nankang and eight from Nanzhuang (Table S2). The evenings after sunny days with high humidity and cool temperature are the most suitable for firefly nocturnal activity (Table 6). Along with their morphological and genetic identification, we also measured the luminescence spectrum and luminescent intensity of firefly flashes, which might be related to the recognition of cohabitated fireflies. The biology of communication with flash patterns in fireflies is well outlined (Lewis, Cratsley & Deiner, 2004; Stanger-Hall & Lloyd, 2015). More than 10 cohabitated species can search for a conspecific mate at the same time via specific flash patterns (Lloyd, 1969). The males use this conspecific flash delay signaling for a particular female while females respond to male flashes with a species-specific response delay (Lewis, Cratsley & Deiner, 2004; Lloyd, 1966; Lloyd, 1968). A recent study (Goh, Lee & Wang, 2022) also recorded the species-specific flash patterns of three sympatric male fireflies (Abs. cerata, L. kagiana, and L. curtithorax).

At least one previous study (Ohba & Yang, 2003) showed that the communication system of abundant Abs. cerata is classified as an HP system in which the female responses to the flying male flashes lasted about 0.24 s. Previous studies already revealed that flash patterns play an important role in conspecific fireflies’ mating behavior. However, recording flash patterns in the field is not easy, especially when there is a short nocturnal activity period with a high population density of different cohabitated fireflies. This study further focuses on the flash color, luminescent intensity, and habitat environments to reveal other important factors that were previously lacking due to limited records of male–female communication signals. In addition, the COI phylogeny of the studied species revealed large genetic variation within known species in Taiwan and/or between adjacent regions.
Differences in luminescence spectra among cohabitated fireflies

The $\lambda_{\text{max}}$ values of the luminescence spectra were similar within the same species, but different between species (Table 3). The pairwise comparison also showed significantly different interspecific $\lambda_{\text{max}}$, except between adult Abs. chinensis and adult L. curtithorax and between adult C. sauteri and larval P. praetexta. A recent study (Goh, Lee & Wang, 2022) recorded species-specific flash patterns of three sympatric male fireflies (Abs. cerata, L. kagiana, and L. curtithorax); both that study and the present study (Table 5) showed that males of L. kagiana and L. curtithorax have similar $\lambda_{\text{max}}$ values but still retain their own unique flash patterns. Thus, the four studied species with similar $\lambda_{\text{max}}$ might also have species-specific flash patterns, which need further study in the future. The above results might imply that most cohabitating fireflies distinguish between each other based on different luminescence spectra and/or specific flash patterns. This implication is important to consider when previous literature (Chen, 2003; Chen & Jeng, 2012) and our findings indicate that all studied adult fireflies appear simultaneously from April to June (Fig. 3). Thus, various cohabitated species may have evolved species-specific recognition to improve male–female searching within such a densely populated area over such a short nocturnal activity time.

Most fireflies have significantly different $\lambda_{\text{max}}$ between interspecific females (Table 4). Only those of Aq. ficta and terrestrial Abs. cerata females were not significantly different. However, the microhabitat of Aq. ficta and Abs. cerata was in the aquatic habitat and moist forest, respectively (Chen, 2003; Jeng, Yang & Lai, 2003). The flying males could still have better chance to find the conspecific females in their specific microhabitat.

In contrast, similar $\lambda_{\text{max}}$ were found in males of L. curtithorax, Abs. chinensis, and L. kagiana (Table 5), but males of L. curtithorax and L. kagiana have their own flash patterns (Goh, Lee & Wang, 2022). Thus, flash pattern is another key for cohabited female fireflies to recognize conspecific males (Lewis & Cratsley, 2008; Lower, Stanger-Hall & Hall, 2018). In addition, Abs. cerata and L. kagiana have different nocturnal activity time, while L. curtithorax is restricted to the dark ground layer of forest (Goh, Lee & Wang, 2022).

Based on the above phenomena, different $\lambda_{\text{max}}$, species-specific flash patterns, microhabitat choices, nocturnal activity time, and/or isolated mating seasons are key factors that may lead to the species-specific courtship of cohabitated fireflies.

Luminescent intensity of flashes implies sensing distance

Fireflies seem to be very sensitive to the photic environment in the evening. Artificial light pollution is a major force influencing firefly proliferation, mating, and growth (Costin & Boulton, 2016; Firebaugh & Haynes, 2016; Haynes & Firebaugh, 2019; Owens, Meyer-Rochow & Yang, 2018). The environmental light intensity and the light sensitivity of the fireflies influence whether the fireflies will flash. Therefore, the luminescent intensity of flashes emitted by fireflies could be an ecological indicator for evaluating light pollution to fireflies. This study further investigated this issue based on the first flash time of abundant Abs. cerata (Table 6). Fireflies start flashing or flying (nocturnal activity) when the environmental light intensity decreases to 6.49–28.1 lux (≈950–4,114 nW/cm²). The luminescent intensity of male Abs. cerata ranges from the average (2.1–3.4 lux or 406.6
± 96.5 nW/cm²) to the maximum (14 lux or 2,048 nW/cm²), which overlaps with the environmental light intensity suitable for their nocturnal activity (Table 1). A previous study also showed that the abundant Abs. cerata begins flashing when the photic environment decreases to 0.04–1.38 lux (Ohba & Yang, 2003). All imply that Abs. cerata could tolerate environmental light intensity around 28.1 lux but wait until 6.49 lux to start nocturnal activity in the evening at twilight. Another study also revealed that most male Abs. cerata start to fly in the evening at twilight while L. kagiana starts its nocturnal activity later (Goh, Lee & Wang, 2022), which Table 1 indeed showed lower luminescent intensity of L. kagiana. In addition, another study (Owens, Meyer-Rochow & Yang, 2018) revealed that half of the Aq. ficta specimens stopped flashing under bright exposure (∼20 and 200 lux). Table 1 further shows that the luminescent intensity of male Aq. ficta ranged from the average (3.1–4.1 lux or 525.7 ± 71.1 nW/cm²) to the maximum (7.5 lux or 1102 nW/cm²), which we also observed the Aq. ficta appeared with Abs. cerata during the same period of nocturnal activity in Nanzhuang. Such differences in luminescent intensity of the three species might imply another adaptation factor for the different nocturnal activity time among species.

Next, we measured the putative sensing distance between males and females. During a typical courtship, the flying males flash to attract perched females. Then, the female responds and flashes to the flying male. The male fireflies close and lands near the female; each displays different flash patterns for communication. As they court each other, the paired fireflies stop flashing on perch. Communication between female and male fireflies relies on the illumination of their light organ in the dark. Usually, the average luminescent intensity emitted by most females (the light organ from single tagma) is around half that of males (the light organ from double tagmata). The differences in luminescent intensity between sex could be due to their courtship behavior for sensing each other. The male needs a higher intensity exposure for females to find him while the female needs to save energy for later proliferation and only responds to male signals with detectable intensity.

The sensing distance between a female and male could be relative to their bioluminescent intensity. So, using the luminescent intensities of male and female, we could estimate the sensing distance. The assumption is the females have higher sensitivity while males have higher luminescent intensity. So, the luminescent intensity difference between male and female could be the sensing ability for a female to detect a male or vice versa. Thus, the maximum luminescent intensity might represent the maximum sensing distance between females and males, assuming that the minimum sensing distance (r, meter) is around the same luminescent intensity between females and males.

We can estimate the sensing distance using the example of the Abs. cerata. The males have a maximum luminescent intensity of 2,048 nW/cm² (14 lux) and the females have a maximum luminescent intensity of 282.2 nW/cm² (∼1.93 lux). Using the formula $14 / (r^2) = 1.93$, we can estimate the maximum sensing distance (r) for this species to be around 2.7 m. Using the same formula calculation with average luminescent intensities, we estimated the average sensing distance to be around 1.8 m. In other words, the putative sensing distance for female Abs. cerata could range from 1.8 to 2.7 m, which may also be the sensing distance for a flying male searching for a female. That said, it is important to
note that most females prefer to perch as males fly to approach them (Goh, Lee & Wang, 2022), since perched females should flash less than what we measured. Thus, the sensing distance between males and females may actually be shorter. Nevertheless, the luminescent intensity could be an indicator of the sensing distance between flying males and perched females. After all, a previous study revealed that male Photinus carolinus use a 15–30 cm landing distance when approaching perched females (Copeland, Moiseff & Faust, 2008), which is a reasonable sensing distance in our estimation. Further behavior experiments should investigate these issues.

**Monophyly of Luciola sensu stricto**
Both the mitogenome (Jusoh et al., 2021) and COI barcode (this study) revealed that each of the studied L. species form a clade, except for L. cruciata. Luciola cruciata and genus Aquatica were grouped together. The other genera of Luciolinae (Curtos, Pteroptyx, Sclerotia, Abscondita, Pygoluola) are distinct.

**Lamprigera is not within Lampyrinae**
Both the mitogenome (Wang, Wu & Wang, 2021) and 436 nuclear loci (Martin et al., 2019) indicated genus Lamprigera groups within Luciolinae instead of Lampyrinae. The COI phylogeny (Figs. 4 and 5) also showed that Lamprigera is a sister group to Curtos and separate from Lampyrinae. In addition, the morphology and COI sequences of eight native species (Dong et al., 2021) further revealed that Lamprigera should be closer to Luciolinae.

**Cryptic species implied by mitochondrial COI barcode variation**
The mitochondrial genetic variation of fireflies within a population or adjacent regions has fewer genetic differences—e.g., the desert-based Microphotus octarthrus (Usener & Cognato, 2005), the widespread Photinus pyralis (Lower, Stanger-Hall & Hall, 2018), and the Korean Aquatica lateralis (Kim et al., 2001; Suzuki et al., 2004). Previous biogeographical study revealed that the two studied sites in Northern Taiwan are within the same geographical regions; thus, we sequenced 1–3 individuals, except the abundant Abs. cerata, in which only one SNP site between two haplotypes could be found from nine Abs. cerata individuals of two habitats. Herein, the COI barcode showed a genus-level resolution for species identification in Figs. 4 and 5, although COI phylogenies in higher-level topologies are not consistent with those of previous morphological studies (Ballantyne et al., 2013; Ballantyne et al., 2015; Ballantyne & Lambkin, 2013; Martin et al., 2017; Stanger-Hall, Lloyd & Hillis, 2007) and molecular phylogeny (Chen et al., 2019; Martin et al., 2017; Martin et al., 2019; Wang, Wu & Wang, 2021). Nevertheless, the COI barcode could successfully identify most species at the genera-to-species level (Figs. 4 and 5). The COI phylogeny showed that the studied genera Abscondita, Curtos, Aquatica, and Luciola belong to Luciolinae, while Pyrocoelia belongs to Lampyrinae as expected.

The COI sequence variations revealed several cryptic species in Taiwan. For example, 62 SNP sites (~11.9% variation) were found in the COI sequences between Taiwanese and northern Chinese Abs. chinensis. Sixty-four SNP sites (~12.3% variation) were found in the COI sequences between Abs. terminalis and Chinese (Taiwanese) Abs. chinensis, respectively. Building on a previous study (Ballantyne et al., 2013), this study further
showed that the $\lambda_{\text{max}}$ of the *Abs. chinensis* lantern spectrum (flash color) is different between Taiwanese (572 nm) and northern Chinese (565 nm) individuals, though there might be unknown environmental effects that cause the flash color variation in widespread species, like with the North American firefly, *Photinus pyralis* (*Lower, Stanger-Hall & Hall, 2018*). Thus, the new evidence reveals that Taiwanese *Abs. chinensis* may be a distinct species to Chinese *Abs. chinensis* (Figs. 4D and 5D).

Large COI variation was also found in six Asian species: *P. praetexta* (Figs. 4A and 5A), *C. costipennis* (Figs. 4E and 5E), *Aq. ficta* and *Aq. lateralis* (Figs. 4C and 5C), and *L. curtithorax* and *L. filiformis* (Figs. 4B and 5B). The COI barcode also indicated 17 SNP sites (~3.27% variation) between Taiwanese and northern Chinese *Aq. ficta* (Figs. 4C and 5C). One study indicated that the characterization of the Chinese *Aq. ficta* differed slightly from the Taiwanese *Aq. ficta* (*Ballantyne & Lambkin, 2009*). In addition, *Aq. leii* was considered as a different species to the Chinese *Aq. ficta* (*Fu, Ballantyne & Lambkin, 2010*). However, there are only two SNP sites (~0.39% variation) between Chinese *Aq. ficta* and *Aq. leii*. The Taiwanese *Aq. ficta* may be a cryptic species with a large variation (17 SNP sites).

In contrast, the COI barcode indicated a large variation of 10% (52 SNP sites) between Korean and Japanese *Aq. lateralis* (Figs. 4C and 5C). There are 58 SNP sites (~11.2% variation) between Taiwanese and northern Chinese *L. curtithorax* (Figs. 4B and 5B). The COI barcode indicated 58 SNP sites (~11.2% variation) between Taiwanese *L. filiformis* and Japanese *L. filiformis yayeyamana* (Figs. 4B and 5B). The COI barcode indicated 57 SNP sites (~11% variation) between Taiwanese and southwestern Chinese *P. praetexta* (Figs. 4A and 5A). The COI barcode indicated 33 SNP sites (~6.35% variation) between southern Japan and eastern Chinese *C. costipennis* (*AB608764 and MK609965 in Figs. 4E and 5E*). All these examples indicate large COI variations between two geographical isolates. Further investigations are needed to reclassify these geographically isolated species.

Bioluminescent evolution inferred from mitochondrial COI barcodes and known phylogeny of Lampyridae

The contracted high-level phylogeny (*Chen et al., 2019; Martin et al., 2019*) and Luciolinae grouping (*Jusoh et al., 2021*) correspond well with our bioluminescent evolution phylogeny (Fig. 6). Another study revealed the bioluminescent evolution via recombinant luciferases and suggested the origin of beetle bioluminescence (*Oba et al., 2020*). Accordingly, this study gives a detailed summary on the evolution of bioluminescence in Lampyridae based on the $\lambda_{\text{max}}$ of its luminescence spectrum (*Arnoldi, Neto & Viviani, 2010; Goh, Lee & Wang, 2022; He et al., 2021; Oba et al., 2020; Wilcox, 2021*). Our studied species further revealed that the fireflies’ luminescence color was originally a green color in a Lampyridae ancestor, then red-shifted to a yellow-green in Luciolinae and is now an orange-yellow color in some derived species (Fig. 6).

CONCLUSION

This study establishes the bioluminescent spectrum and intensity of nine cohabitated fireflies and can be referenced to ensure that light pollution in habitats does not become high enough to disrupt firefly mating. The mitochondrial COI barcode revealed a genus-level
Figure 6  Bioluminescent evolution of fireflies. The phylogenetic topology and lantern wavelength ($\lambda_{\text{max}}$) were adopted from our new data and previous studies (Chen et al., 2019; He et al., 2021; Jusoh et al., 2021; Martin et al., 2019; Oba et al., 2020).
resolution for species identification and six cryptic species that need to be further studied. Combined with previous literature, this study supports the argument that bioluminescent evolution has red-shifted to yellow-green in Luciolinae and specified to orange-yellow color in some derived species.

**ACKNOWLEDGEMENTS**

We are grateful to Miss Xian-Ju Chang for her sampling assistance. Thanks also to Noah Last of Third Draft Editing for his English language editing. We extend our deepest thanks to reviewer Christine Lambkin, Queensland Museum, for her valuable comments.

**ADDITIONAL INFORMATION AND DECLARATIONS**

**Funding**

This research was funded by Academia Sinica, Taiwan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Grant Disclosures**

The following grant information was disclosed by the authors:

Acadia Sinica, Taiwan.

**Competing Interests**

The authors declare there are no competing interests.

**Author Contributions**

- King-Siang Goh conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Liang-Jong Wang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Jing-Han Ni performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Tzi-Yuan Wang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

**Data Availability**

The following information was supplied regarding data availability:

The COI sequences are accessible at GenBank: MT534191–MT534201, ON209457.

**Supplemental Information**

Supplemental information for this article can be found online at [http://dx.doi.org/10.7717/peerj.14195#supplemental-information](http://dx.doi.org/10.7717/peerj.14195#supplemental-information).
REFERENCES

Adamowicz SJ. 2015. International barcode of life: evolution of a global research community. Genome 58:151–162 DOI 10.1139/gen-2015-0094.

Adamowicz SJ, Hollingsworth PM, Ratnasingham S, Van der Bank M. 2017. International barcode of life: focus on big biodiversity in South Africa. Genome 60:875–879 DOI 10.1139/gen-2017-0210.

Arnoldi FGC, Neto AJD, Viviani VR. 2010. Molecular insights on the evolution of the lateral and head lantern luciferases and bioluminescence colors in Mastinocerini railroad-worms (Coleoptera: Phengodidae). Photochemical & Photobiological Sciences 9:87–92 DOI 10.1039/b9pp00078j.

Ballantyne L, Fu XH, Lambkin C, Jeng ML, Faust L, Wijekoon WMCD, Li DQ, Zhu TF. 2013. Studies on South-east Asian fireflies: Abscondita, a new genus with details of life history, flashing patterns and behaviour of Abs. chinensis (L.) and Abs. terminalis (Olivier) (Coleoptera: Lampyridae: Luciolinae). Zootaxa 3721:1–48 DOI 10.11646/zootaxa.3721.1.1.

Ballantyne LA, Lambkin C. 2009. Systematics of Indo-Pacific fireflies with a redefinition of Australasian Atypella Olliff, Madagascan Photuroluciola Pic, and description of seven new genera from the Luciolinae (Coleoptera: Lampyridae). Zootaxa 1997:1–188 DOI 10.11646/zootaxa.1997.1.1.

Ballantyne LA, Lambkin CL. 2013. Systematics and phylogenetics of Indo-Pacific Luciolinae fireflies (Coleoptera: Lampyridae) and the description of new genera. Zootaxa 3653:1–162 DOI 10.11646/zootaxa.3653.1.1.

Ballantyne L, Lambkin CL, Boontop Y, Jusoh WF. 2015. Revisonal studies on the Luciolinae fireflies of Asia (Coleoptera: Lampyridae): 1. The genus Pyrophanes Olivier with two new species. 2. Four new species of Pteroptyx Olivier and 3. A new genus Inflata Boontop, with redescription of Luciola indica (Motsch.) as Inflata indica comb. nov. Zootaxa 3959:1–84 DOI 10.11646/zootaxa.3959.1.1.

Ballantyne LA, Lambkin CL, Ho JZ, Jusoh WFA, Nada B, Nak-Eiam S, Thancharoen A, Wattanachaingcharoen W, Yiu V. 2019. The Luciolinae of S. E. Asia and the Australopacific region: a revisionary checklist (Coleoptera: Lampyridae) including description of three new genera and 13 new species. Zootaxa 4687(1):1–174 DOI 10.11646/zootaxa.4687.1.1.

Ballantyne LA, Lambkin CL, Luan X, Boontop Y, Nak-Eiam S, Pimpasalee S, Silalom S, Thancharoen A. 2016. Further studies on south eastern Asian Luciolinae: 1. Sclerotia Ballantyne, a new genus of fireflies with back swimming larvae 2. Triangulara Pimpasalee, a new genus from Thailand (Coleoptera: Lampyridae). Zootaxa 4170:201–249 DOI 10.11646/zootaxa.4170.2.1.

Bergsten J, Bilton DT, Fujisawa T, Elliott M, Monaghan MT, Balke M, Hendrich L, Geijer J, Herrmann J, Foster GN, Ribera I, Nilsson AN, Barraclough TG, Vogler AP. 2012. The effect of geographical scale of sampling on DNA barcoding. Systematic Biology 61:851–869 DOI 10.1093/sysbio/sys037.
Buck JB. 1948. The anatomy and physiology of the light organ in fireflies. *Annals of the New York Academy of Sciences* 49:397–485 DOI 10.1111/j.1749-6632.1948.tb30944.x.

Cassata SA. 2020. Identification of fireflies (Coleoptera: Lampyridae) at Thayer Farm, Otsego county. NY Master Thesis of Science in Biology, State University of New York, College at Oneonta.

Chen TR. 2003. *The fireflies of Taiwan*. Taipei: Field Image Publisher.

Chen X, Dong ZW, Liu GC, He JW, Zhao RP, Wang W, Peng YQ, Li XY. 2019. Phylogenetic analysis provides insights into the evolution of Asian fireflies and adult bioluminescence. *Molecular Phylogenetics and Evolution* 140:106600 DOI 10.1016/j.ympev.2019.106600.

Chen TR, Jeng ML. 2012. *The fireflies in Siraya*. Tainan: Siraya National Scenic Area Administration.

Choi YS, Bae JS, Lee KS, Kim SR, Kim I, Kim JG, Kim KY, Kim SE, Suzuki H, Lee SM, Sohn HD, Jin BR. 2003. Genomic structure of the luciferase gene and phylogenetic analysis in the *Hotaria*-group fireflies. *Comparative Biochemistry and Physiology B* 134:199–214 DOI 10.1016/s1096-4959(02)00249-x.

Copeland J, Moiseff A, Faust L. 2008. Landing distance in a synchronic North American firefly. *Physiological Entomology* 33:110–115 DOI 10.1111/j.1365-3032.2007.00611.x.

Costin KJ, Boulton AM. 2016. A field experiment on the effect of introduced light pollution on fireflies (Coleoptera: Lampyridae) in the piedmont region of Maryland. *Coleopterists Bulletin* 70:84–86 DOI 10.1649/072.070.0110.

Dong ZW, Yiu V, Liu GC, He JW, Zhao RP, Peng YQ, Li XY. 2021. Three new species of *Lamprigera* Motschulsky (Coleoptera, Lampyridae) from China, with notes on known species. *Zootaxa* 4950:441–468 DOI 10.11646/zootaxa.4950.3.2.

Felsenstein J. 1985. Confidence-limits on phylogenies—an approach using the bootstrap. *Evolution* 39:783–791 DOI 10.2307/2408678.

Firebaugh A, Haynes KJ. 2016. Experimental tests of light-pollution impacts on nocturnal insect courtship and dispersal. *Oecologia* 182:1203–1211 DOI 10.1007/s00442-016-3723-1.

Fu XH, Ballantyne I, Lambkin CL. 2010. *Aquatica* gen. nov from mainland China with a description of *Aquatica Wuhana* sp nov (Coleoptera: Lampyridae: Luciolinae). *Zootaxa* 2530(2530):1–18 DOI 10.11646/zootaxa.2530.1.1.

Fu XH, Meyer-Rochow VB. 2013. Larvae of the firefly *Pyrocoelia pectoralis* (Coleoptera: Lampyridae) as possible biological agents to control the land snail Bradybaena ravida. *Biological Control* 65:176–183 DOI 10.1016/j.biocontrol.2013.02.005.

Goh KS, Lee CM, Wang TY. 2022. Species-specific flash patterns track the nocturnal behavior of sympatric Taiwanese fireflies. *Biography* 11:58 DOI 10.3390/biology11010058.

Goh KS, Li CW. 2011. A photocytes-associated fatty acid-binding protein from the light organ of adult Taiwanese firefly, *Luciola cerata*. *PLOS ONE* 6:e29576 DOI 10.1371/journal.pone.0029576.
Han T, Kim SH, Yoon HJ, Park IG, Park H. 2020. Evolutionary history of species of the firefly subgenus Hotaria (Coleoptera, Lampyridae, Luciolinae, Luciola) inferred from DNA barcoding data. Contributions to Zoology 89:127–145 DOI 10.1163/18759866-20191420.

Haynes KJ, Firebaugh A. 2019. Light pollution may inhibit firefly courtship flashing and mating success: response to Lewis and Owens (2019). Basic and Applied Ecology 35:67–69 DOI 10.1016/j.baae.2019.01.003.

He JW, Liu GC, Dong PX, Dong ZW, Zhao RP, Wang W, Li XY. 2021. Molecular cloning, characterization, and evolution analysis of the luciferase genes from three sympatric sibling fireflies (Lampyridae: Lampyrinae, Diaphanes). Photochemical and Photobiological Sciences 20:1053–1067 DOI 10.1007/s43630-021-00080-4.

Hendrich L, Moriniere J, Haszprunar G, Hebert PDN, Hausmann A, Kohler F, Balke M. 2015. A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species to BOLD. Molecular Ecology Resources 15:795–818 DOI 10.1111/1755-0998.12354.

Ho JZ, Chiang PH, Wu CH, Yang PS. 2010. Life cycle of the aquatic firefly Luciola ficta (Coleoptera: Lampyridae). Journal of Asia-Pacific Entomology 13:189–196 DOI 10.1016/j.aspen.2010.03.007.

Jeng ML, Lai J, Yang PS. 1999. A synopsis of the firefly fauna at six national parks in Taiwan (Coleoptera: Lampyridae). Formosan Entomologist 19:65–91.

Jeng ML, Lai J, Yang PS. 2003. Lampyridae: a synopsis of aquatic fireflies with description of a new species (Coleoptera). Water Beetles of China 3:539–562.

Jeng ML, Yang PS, Engel MS. 2007. The firefly genus Vesta in Taiwan (Coleoptera: Lampyridae). Journal of the Kansas Entomological Society 80:265–280 DOI 10.2317/0022-8567(2007)80[265:Tfgvit]2.0.Co;2.

Jeng ML, Yang PS, Lai J. 2003. Notes on the genus Luciola (Coleoptera, Lampyridae, Luciolinae) of Taiwan. Special Bulletin of the Japanese Society of Coleopterologists, Tokyo 6:247–262.

Jeng ML, Yang PS, Sato M, Lai J, Chang JC. 1998. The genus Curtos (Coleoptera, Lampyridae, Luciolinae) of Taiwan and Japan. The Japanese Journal of Systematic Entomology 4:331–347.

Jusoh WFA, Ballantyne I, Chan SH, Wong TW, Yeo D, Nada B, Chan KO. 2021. Molecular systematics of the firefly genus Luciola (Coleoptera: Lampyridae: Luciolinae) with the Description of a New Species from Singapore. Animals 11(3):687 DOI 10.3390/ani11030687.

Jusoh WFA, Ballantyne I, Lambkin CL, Hashim NR, Wahlberg N. 2018. The firefly genus Pteroptyx Olivier revisited (Coleoptera: Lampyridae: Luciolinae). Zootaxa 4456:1–71 DOI 10.11646/zootaxa.4456.1.1.

Jusoh WFA, Hashim NR, Saaksjarvi IE, Adam NA, Wahlberg N. 2014. Species delineation of Malaysian Mangrove Fireflies (Coleoptera: Lampyridae) using DNA barcodes. Coleopterists Bulletin 68:703–711 DOI 10.1649/0010-065x-68.4.703.
Kaskova ZM, Tsarkova AS, Yampolsky IV. 2016. 1001 lights: luciferins, luciferases, their mechanisms of action and applications in chemical analysis, biology and medicine. *Chemical Society Reviews* 45:6048–6077 DOI 10.1039/c6cs00296j.

Kim JG, Kim I, Bae JS, Jin BR, Kim KY, Kim SE, Choi JY, Choi YC, Lee KY, Sohn HD, Noh SK. 2001. Genetic subdivision of the firefly, *Luciola lateralis* (Coleoptera: Lampyridae), in Korea determined by mitochondrial COI gene sequences. *Korean Journal of Genetics* 23:203–219.

Kim JJ, Lee J, Yang SP, Kim HG, Kweon HS, Yoo S, Jeong KH. 2016. Biologically inspired organic light-emitting diodes. *Nano Letters* 16:2994–3000 DOI 10.1021/acs.nanolett.5b05183.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870–1874 DOI 10.1093/molbev/msw054.

Lee SC, Bae JS, Kim I, Suzuki H, Kim SR, Kim JG, Kim KY, Yang WJ, Lee SM, Sohn HD, Jin BR. 2003. Mitochondrial DNA sequence-based population genetic structure of the firefly, *Pyrocoelia rufa* (Coleoptera: Lampyridae). *Biochemical Genetics* 41:427–452 DOI 10.1023/b:bigi.0000007777.87407.1b.

Lewis SM, Cratsley CK. 2008. Flash signal evolution, mate choice, and predation in fireflies. *Annual Review of Entomology* 53:293–321 DOI 10.1146/annurev.ento.53.103106.093346.

Lewis SM, Cratsley CK, Deiner K. 2004. Mate recognition and choice in *Photinus* fireflies. *Annales Zoologici Fennici* 41:809–821.

Li W, Yang Z, Fu XH. 2022. The complete mitochondrial genome of the firefly *Curtos fulvocapitalis* (Coleoptera: Lampyridae). *Mitochondrial DNA Part B* 7:1–3 DOI 10.1080/23802359.2021.1958080.

Liu GC, Dong ZW, He JW, Zhao RP, Wang W, Li XY. 2017. Genome size of 14 species of fireflies (Insecta, Coleoptera, Lampyridae). *Zoological Research* 38:449–458 DOI 10.24272/j.issn.2095-8137.2017.078.

Liu Q, Fu X. 2020. The genetic variations in the mitochondrial genomes of three Luciolinae fireflies. *Mitochondrial DNA B* 5:3210–3214 DOI 10.1080/23802359.2020.1806126.

Lloyd JE. 1966. *Studies on the flash communication system in Photinus fireflies*. Ann Arbor: Museum of Zoology, University of Michigan.

Lloyd JE. 1968. A new *Photinus* firefly, with notes on mating behavior and a possible case of character displacement (Coleoptera: Lampyridae). *The Coleopterists Bulletin* 22:1–10.

Lloyd JE. 1969. Flashes, behavior and additional species of nearctic *Photinus* fireflies (Coleoptera: Lampyridae). *The Coleopterists Bulletin* 23:29–40.

Lower SE, Stanger-Hall KF, Hall DW. 2018. Molecular variation across populations of a widespread North American firefly, *Photinus pyralis*, reveals that coding changes do not underlie flash color variation or associated visual sensitivity. *BMC Evolutionary Biology* 18:129 DOI 10.1186/s12862-018-1251-9.
Martin GJ, Branham MA, Whiting MF, Bybee SM. 2017. Total evidence phylogeny and the evolution of adult bioluminescence in fireflies (Coleoptera: Lampyridae). *Molecular Phylogenetics and Evolution* **107**:564–575 DOI 10.1016/j.ympev.2016.12.017.

Martin GJ, Lord NP, Branham MA, Bybee SM. 2015. Review of the firefly visual system (Coleoptera: Lampyridae) and evolution of the opsins genes underlying color vision. *Organisms Diversity & Evolution* **15**: 513–526 DOI 10.1007/s13127-015-0212-z.

Martin GJ, Stanger-Hall KF, Branham MA, Da Silveira LFL, Lower SE, Hall DW, Li XY, Lemmon AR, Lemmon EM, Bybee SM. 2019. Higher-level phylogeny and reclassification of Lampyridae (Coleoptera: Elateroidea). *Insect Systematics and Diversity* **3**(6):11 DOI 10.1093/isid/ixz024.

Muraji M, Arakaki N, Tanizaki S. 2012. Evolutionary relationship between two firefly species, *Curtos costipennis* and *C. okinawanus* (Coleoptera: Lampyridae), in the Ryukyu Islands of Japan revealed by the mitochondrial and nuclear DNA sequences. *Scientific World Journal* **2012**:653013 DOI 10.1100/2012/653013.

Nei M, Kumar S. 2000. *Molecular evolution and phylogenetics*. New York: Oxford University Press.

Oba Y, Branham MA, Fukatsu T. 2011. The terrestrial bioluminescent animals of Japan. *Zoological Science* **28**:771–789 DOI 10.2108/zsj.28.771.

Oba Y, Konishi K, Yano D, Shibata H, Kato D, Shirai T. 2020. Resurrecting the ancient glow of the fireflies. *Science Advances* **6**(49):eabc5705 DOI 10.1126/sciadv.abc5705.

Ohba N, Yang PS. 2003. Flash patterns and communication system of the Taiwan firefly, *Luciola cerata* Olivier. *Science Report of the Yokosuka City Museum* **50**:1–12.

Osozawa S, Oba Y, Kwon HY, Wakabayashi J. 2015. Vicariance of *Pyrocoelia* fireflies (Coleoptera:Lampyridae) in the Ryukyu islands, Japan. *Biological Journal of the Linnean Society* **116**:412–422 DOI 10.1111/bij.12595.

Owens ACS, Meyer-Rochow VB, Yang EC. 2018. Short- and mid-wavelength artificial light influences the flash signals of *Aquatica ficta* fireflies (Coleoptera: Lampyridae). *PLOS ONE* **13**:e0191576 DOI 10.1371/journal.pone.0191576.

Pentinsaari M, Hebert PD, Mutanen M. 2014. Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLOS ONE* **9**:e108651 DOI 10.1371/journal.pone.0108651.

Pentinsaari M, Salmela H, Mutanen M, Roslin T. 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Scientific Reports* **6**:35275 DOI 10.1038/srep35275.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**:817–818 DOI 10.1093/bioinformatics/14.9.817.

Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. *PLOS ONE* **8**:e66213 DOI 10.1371/journal.pone.0066213.

Raupach MJ, Hannig K, Morinieres J, Hendrich L. 2020. A DNA barcode library for ground beetles of Germany: the genus *Pterostichus* Bonelli, 1810 and allied taxa (Insecta, Coleoptera, Carabidae). *Zookeys* **980**:93–117 DOI 10.3897/zookeys.980.55979.
Riley WB, Rosa SP, Lima da Silveira LF. 2021. A comprehensive review and call for studies on firefly larvae. *PeerJ* 9:e12121 DOI 10.7717/peerj.12121.

Roslin T, Somervuo P, Pentinsaari M, Hebert PDN, Agda J, Ahlroth P, Anttonen P, Aspi J, Blagoev G, Blanco S, Chan D, Clayhills T, De Waard J, De Waard S, Elliot T, Elo R, Haapala S, Helve E, Ilmonen J, Hirvonen P, Ho C, Itamies J, Ivanov V, Jakovlev J, Juslen A, Jussila R, Kahanpaa J, Kaila L, Jari P, Kakko A, Kakko I, Karhu A, Karjalainen S, Kjaerandsen J, Koskinen J, Laasonen EM, Laasonen L, Laine E, Lampila P, Levesque-Beaudin V, Lu L, Lahteenmoro M, Majuri P, Malmberg S, Manjunath R, Martikainen P, Mattila J, McKeown J, Metsala P, Miklasevskaja M, Miller M, Miskie R, Muinonen A, Veli M, Naik S, Nikolova N, Nupponen K, Ovaskainen O, Osterblad I, Paasivirta L, Parkko P, Paukkunen J, Penttinen R, Perez K, Pohjoismaki J, Prosser S, Raekunnas M, Rahulan M, Rannisto M, Ratnasingham S, Rinne A, Rintala T, Miranda Romo S, Salmela J, Salokannel J, Savolainen R, Schulman L, Sihvonen P, Soliman D, Sones J, Steinke C, Stahl G, Tabell J, Tiusanen M, Vaheri G, Vaheri M, Väijäinen E, Vikberg V, Viitasaari M, Vilén J, Warne C, Wei C, Winqvist K, Zakharov E, Mutanen M. 2022. A molecular-based identification resource for the arthropods of Finland. *Molecular Ecology Resources* 22:803–822 DOI 10.1111/1755-0998.13510.

Rulik B, Eberle J, Von der Mark L, Thomann J, Jung M, Kohler F, Apfel W, Weigel A, Kopetz A, Kohler J, Fritzlar F, Hartmann M, Hadulla K, Schmidt J, Horren T, Krebs D, Theves F, Eulitz U, Skale A, Rohwedder D, Kleeberg A, Astrin JJ, Geiger MF, Wagele JW, Grobe P, Ahrens D. 2017. Using taxonomic consistency with semi-automated data pre-processing for high quality DNA barcodes. *Methods in Ecology and Evolution* 8:1878–1887 DOI 10.1111/2041-210x.12824.

Saitou N, Nei M. 1987. The neighbor-joining method—a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406–425.

Seliger HH, Buck JB, Fastie WG, Mcelroy WD. 1964. Flash patterns in Jamaican fireflies. *Biological Bulletin* 127:159–172 DOI 10.2307/1539352.

South A, Sota T, Abe N, Yuma M, Lewis SM. 2008. The production and transfer of spermatophores in three Asian species of *Luciola* fireflies. *Journal of Insect Physiology* 54:861–866 DOI 10.1016/j.jinsphys.2008.03.008.

Sriboonlert A, Wonnapinij P. 2019. Comparative mitochondrial genome analysis of the firefly, *Inflata indica* (Coleoptera: Lampyridae) and the first evidence of heteroplasmacy in fireflies. *International Journal of Biological Macromolecules* 121:671–676 DOI 10.1016/j.ijbiomac.2018.10.124.

Stanger-Hall KF, Lloyd JE. 2015. Flash signal evolution in *Photinus* fireflies: character displacement and signal exploitation in a visual communication system. *Evolution* 69:666–682 DOI 10.1111/evo.12606.

Stanger-Hall KF, Lloyd JE, Hillis DM. 2007. Phylogeny of North American fireflies (Coleoptera: Lampyridae): Implications for the evolution of light signals. *Molecular Phylogenetics and Evolution* 45:33–49 DOI 10.1016/j.ympev.2007.05.013.

Suzuki H, Sato Y, Ohba N, Bae JS, Jin BR, Sohn HD, Kim SE. 2004. Phylogeographic analysis of the firefly, *Luciola lateralis*, in Japan and Korea based on mitochondrial
cytochrome oxidase II gene sequences (Coleoptera: Lampyridae). *Biochemical Genetics* 42:287–300 DOI 10.1023/b:bigi.0000039805.75118.8f.

Tamura K, Kumar S. 2002. Evolutionary distance estimation under heterogeneous substitution pattern among lineages. *Molecular Biology and Evolution* 19:1727–1736 DOI 10.1093/molbev/msf017.

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America* 101:11030–11035 DOI 10.1073/pnas.0404206101.

Thompson JD, Gibson TJ, Higgins DG. 2002. Multiple sequence alignment using ClustalW and ClustalX. *Current Protocols in Bioinformatics Chapter 2*:Unit 2.3; 2.3.1-2.3.22 DOI 10.1002/0471250953.bi0203s00.

Usener JL, Cognato AI. 2005. Patterns of mitochondrial diversity among desert firefly populations (Lampyridae: *Microphoton octarthus* Fall). *Coleopterists Bulletin* 59:361–367 DOI 10.1649/796.1.

Wang LJ, Wu YW, Wang TY. 2021. Characterization of the complete mitochondrial genome of *Abscondita cerata* (Olivier, 1911) (Coleoptera: Lampyr- dae) and its phylogenetic implications. *Mitochondrial DNA B* 6:2528–2530 DOI 10.1080/23802359.2021.1959456.

Wilcox A. 2021. A description and examination of fluorescence in nine North American firefly species (Coleoptera: Lampyridae). *Psyche* 2021:8856155 DOI 10.1155/2021/8856155.

Yang XS. 2009. *Firefly algorithms for multimodal optimization. International symposium on stochastic algorithms*. Berlin, Heidelberg: Springer, 169–178.

Zhang R, He J, Dong Z, Liu G, Yin Y, Zhang X, Li Q, Ren Y, Yang Y, Liu W, Chen X, Xia W, Duan K, Hao F, Lin Z, Yang J, Chang Z, Zhao R, Wan W, Lu S, Peng Y, Ge S, Wang W, Li X. 2020. Genomic and experimental data provide new insights into luciferin biosynthesis and bioluminescence evolution in fireflies. *Scientific Reports* 10:15882 DOI 10.1038/s41598-020-72900-z.

Zhang SQ, Che LH, Li Y, Dan L, Pang H, Slipinski A, Zhang P. 2018. Evolutionary history of Coleoptera revealed by extensive sampling of genes and species. *Nature Communications* 9:205 DOI 10.1038/s41467-017-02644-4.