# Research Article

# Solitary Fireflies of Kangkawat Research Station, Imbak Canyon, Sabah

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### Abstract

A survey on solitary fireflies was carried out at Kangkawat Research Station in October 2018. A total of 33 fireflies were collected; three adult males, 13 adult females and 17 larvae. Three fireflies species were identified from male specimens, namely *Pygoluciola wittmeri*, *Luciola* sp. and most larvae consists of *Pyrocoelia* sp. This is a new record, mapping the existence of the species in Imbak, hence expanding the geographical knowledge of *Pygoluciola*, *Luciola* and *Pyrocoelia*. There are five morphospecies of unidentified females and three morphospecies of larvae. Three videos of larvae showed an emission of a long glow while the adult female in captivity exhibited single-peaked pulsing light. Male flashes were caught flying between trees while larvae were found among twigs, leaf litter and leaves.

**Keywords:** Solitary firefly, *Pygoluciola wittmeri*, *Luciola* sp., *Pyrocoelia* sp., Lampyridae.

# Introduction

Firefly (Coleoptera: Lampyridae) is a subject of fascination for many because of its ability to emit light. It is also a tourism attraction in Borneo (Mahadimenakbar et al., 2014; Syazlina et al., 2016). Various research projects have been conducted focusing on the mangrove fireflies (Mahadimenakbar et al., 2018; Mahadimenakbar & Fiffy Hanisdah, 2016; Foo & Mahadimenakbar, 2015, 2016, 2017) because of their ability to flash en masse and function as a tourism magnet. However, much about fireflies remains a mystery, even information as basic as species distribution. There are more than 2,000 species of firefly described worldwide (Hu & Fu, 2018; Mu et al., 2016; Silveira & Mermudes, 2014) with several new species discovered in forested areas year after year. For example, in Malaysia, the genus *Emasia* (Lampyridae: Ototrinae) was erected from a firefly found in Gunung Emas, Tambunan, Sabah (Bocakova & Janisova, 2010). A new species of *Pygoluciola dunguna* was described by Nada & Ballantyne (2018) found at a lowland dipterocarp forest of Dungun, Peninsular Malaysia. New records were also documented, such as *Pteroptyx tener* (Swatdipong et al., 2015) and *Australoluciola* sp. that were newly found in Thailand (Sartsanga et al., 2017). These findings are due to explorations in new and unexplored territories.

As much as mangrove fireflies are revered, information regarding the solitary firefly is falling behind. In Borneo, especially Sabah, there are only a few places in which non-mangrove fireflies were discovered such as in Maliau Basin (*Luciola* sp. found; Muslim et al., 2010), Tabin Wildlife Reserve (*Pyrocoelia opaca*; written as *Lychnuris opaca* in Chung & Binti, 2008), Mahua (*Pygoluciola guigliae* and *wittmeri*; Ballantyne & Lambkin, 2006), Kionsom (*Pygoluciola wittmeri*; Chey, 2008), Ulu Kimanis (Chung et al., 2002) and Mesilau (*Pygoluciola kinabalua*; Ballantyne & Lambkin, 2001). This left a huge unchecked area in the interior part of Sabah for firefly species distribution especially the solitary fireflies. Hence, this study is conducted to survey firefly species in Imbak Canyon to expand our geographical knowledge pertaining to the solitary fireflies.

# Methodology

### Sampling

Sampling was carried out for two hours (8-10pm) along Kawang Trail (5<sup>th</sup> October 2018) and Nepenthes Trail (6<sup>th</sup> and 8<sup>th</sup> October 2018) during the Borneo Geographic Expedition Kangkawat, 2018, organised by UMS. Fireflies were collected opportunistically using sweep net (for flying adults) and forceps (for larvae) by three people equivalent to six man-hours sampling effort. Each location of sampled fireflies was recorded using a GPS receiver and temperature and humidity were measured using the Kestrel Weather Station. Sampling was more focused on catching the adult firefly as soon as possible to avoid escape and flash recordings were not prioritized but only taken when they were sedentary using Canon EOS 100D. Live fireflies and larvae were killed and stored in 70% ethanol.

#### Species Identification

In general, female fireflies are unidentified due to lack of identification key hence species identification is based on male samples. Intact samples and male genitalia were photographed using Zeiss Image Analyzer at the Institute for Tropical biology and Conservation, UMS. All samples were identified by Wan Faridah Wan Jusoh up to genus level and later by the authors using key provided by Nada & Ballantyne (2018) for *Pygoluciola*. Flash patterns were extracted using FFMPEG and ImageJ by measuring grayscale frame-by-frame (modified method from Konno et al., 2016).

# Results

### Firefly Species, Composition and Distribution

Thirty-three fireflies were caught made up of three adult males, 13 adult females and 17 larvae (Figure 1). Most individuals were caught within Nepenthes Trail (25 individuals), five from Kawang Trail and three by the river near the base camp (Figure 2). Male caught within the Nepenthes Trail was identified by WFWJ as *Pygoluciola* sp. (Figure 3-5) and the two remaining males were *Luciola* sp. (Figures 6 and 7). The *Pygoluciola* specimen is consistent with *Pygoluciola wittmeri* described in Nada and Ballantyne (2018) by having no tibiae curved, bifurcate median posterior projection of ventrite 7 (V7 henceforth) at apex and deeply emarginate apex visible from the ventral view. Its light organ (LO) on V7 is also consistent with *Pygoluciola wittmeri* described in Ballantyne & Lambkin (2006) by having shallow anteromedian emargination and not posteromedially emarginated (Figure 4; arrow). The V7 light organ was also described by Chey (2008) as a heart-shaped light organ.

Females are unidentified. However, based on pronotum, elytra and abdominal shape, female specimens were clustered into five morphospecies (Figures 8-17) and larvae were distinctively grouped into four morphospecies (Figures 18-29). Eleven out of 17 larvae are from the genus *Pyrocoelia* sp. which is marked as morphospecies 2 in Figures 21-23.



#### No. of Individual by Firefly Form

Figure 1. Numbers of fireflies sampled according to their form



Firefly Distribution in Kangkawat, Imbak Canyon Conservation Area (ICCA)

Figure 2. Locations of fireflies sampled from Kangkawat, ICCA. Five individuals were caught along Kawang Trail, 25 from Nepenthes Trail and three by the river near the



**Figure 3 - 11.** 3 - 5 male *Pygoluciola wittmeri* (red arrow on 4 showing LO in V7, tip of abdomen shown in 5); 6, 7 male *Luciola* sp.; 8, 9 female morphospecies 1; 10, 11 female morphospecies 2. 3, 6, 8, 10 dorsal, 5 right lateral, 4, 7, 9, 11 ventral. Scale line is 1



**Figure 12 - 20.** 12 - 13 female morphospecies 3; 14, 15 female morphospecies 4; 16, 17 female morphospecies 5; 18 - 20 larva morphospecies 1. 12, 14, 16, 18 dorsal, 13, 15, 17, 19, 20 ventral, 20 posterior end. Scale line is 1 mm.



**Figure 21 - 29.** Larvae. 21 - 23 morphospecies 2 identified as *Pyrocoelia* sp., 22 larval abdomen curved ventrally (red arrow on 23 showing caudal grasping organ); 24 - 26 morphospecies 3; 27 - 29 morphospecies 4. 21, 24, 27 dorsal, 22, 23, 25, 28 ventral, 26,

### General Ecology and Behaviour

Adult fireflies and larvae occupied different spaces and substrates. For instance, a majority of the adult fireflies were caught during flight and rarely found perching on leaves, but larvae were found on leaf litter (Figures 30 and 31), twigs (Figure 32) and leaves (Figure 33). Larvae in morphospecies 1, 3 and 4 were all found on leaf litter. *Pyrocoelia* sp. larvae (morphospecies 2) were glowing among leaf litter with only two individuals found on a twig and a leaf. Figures 34 and 35 show that fireflies may be found in places with almost similar temperature and humidity. Male fireflies were found in places with 27.13  $\pm$  0.32 °C and highly humid environment (95.23  $\pm$  2.11 % relative humidity). The results are similar with female (26.97  $\pm$  0.7 °C and 97.19  $\pm$  2.34 %RH) and larvae (26.87  $\pm$  0.32 °C and 98.18  $\pm$  2.21 %RH).

In terms of flash patterns, there are only four recordings available for analysis; a single video of captured female and three of moving *Pyrocoelia* sp. larvae. The female was sedentary in a specimen bottle and emitted single-peaked pulses (Figure 36) and the *Pyrocoelia* sp. larvae emitted a long glow before it paused into darkness (Figure 37 - 39) and moved slowly by pushing its body forward using its caudal grasping organ at the posterior end (arrow on Figure 23 and Figure 32).



Figure 30. Larva morphospecies 1 found on leaf litter. Photo credit: Azrie Aliamat.



Figure 31. Pyrocoelia sp. larva caught moving on leaf litter. Photo credit: Azrie Aliamat.



**Figure 32.** *Pyrocoelia* sp. larva inching along a twig. It uses the caudal grasping organ on its posterior end to push itself forward (arrowed). Photo credit: Azrie Aliamat.



Figure 33. Pyrocoelia sp. larva found on a leaf (arrowed).



### Habitat Temperature

Figure 34. Boxplots of ambient temperature for each firefly form.



Habitat Humidity

Figure 35. Boxplots of relative humidity for each firefly form.



**Figure 36.** Female flashes in captivity. Scale line on upper right is two seconds. Ordinate: relative brightness (grayscale value), Abcissa: time in 0.04 seconds interval.



**Figure 37 - 39.** Larval glow. Scale line on upper left is 1.36 seconds for fig 37, 1.28 s for fig 38 and 39. Ordinate: relative brightness (grayscale value), Abcissa: time in 0.04 seconds interval.

### Discussion

Due to its infrequent discovery, Pygoluciola fireflies were once dubbed as South East Asian rare fireflies (Ballantyne, 2008). Later, as the record expanded, it was revealed that *Pygoluciola* firefly was found in Malaysia (Ballantyne & Lambkin, 2006; Nada & Ballantyne, 2018), Philippines (Ballantyne, 2008), Australia, China (Fu & Ballantyne, 2008) and Thailand (Wattanachaiyingcharoen et al., 2016). Five out of nine species of Pygoluciola can be found in Borneo (Sabah and Sarawak). Pyrocoelia fireflies are widespread in Asia (Jeng et al., 2011; Li et al., 2005; Osozawa et al., 2015; Wang et al., 2007) and in Malaysia it was recorded in Tasik Chini (Roslan & Sulaiman, 2015), Fraser's Hill (Nada et al., 2013) and Tabin wildlife Reserve (Chung & Binti, 2008). This study presents new records of Pygoluciola wittmeri, Luciola sp. and Pyrocoelia sp. in Imbak Canyon and their distribution in Borneo. This has broadened the geographical knowledge of the species and the genus in general. Moreover, the species P. wittmeri was previously known to occupy Mahua River (Ballantyne & Lambkin, 2006) and Kionsom (Chey, 2008) but not other places in Sabah. Unfortunately, the ecology and habitat of Pygoluciola wittmeri are still currently unknown due to its rarity.

However, its rarity may be explained by its solitary behaviour. This is because in fireflies, there are two general classifications on how males search for their counterparts; congregating on a tree or travelling alone which is known as roving (Mahadimenakbar et al., 2018; Lewis & Cratsley, 2008). Roving can be seen in species such as *Photinus* sp. that wanders without specific direction (Lewis et al., 2004: Lewis et al., 2004). Another possibility is habitat requirement. Longton & Hedderson (2000) discuss rarity by lending 'rarity factors' set by Rabinowitz (1981) as a complex mixture of (1) strict geographical range, (2) highly specific habitat and (3) low population size. Any species that falls into the spectrum of the three criteria might become rare species and require intervention. The case with *Pygoluciola* is not obvious in this study other than the shallow number of individuals. From the distribution data, their population is small with imbalanced portion of male to female ratio. Ballantyne & Lambkin (2006) also argued that rarity might be contributed to the unexplored areas of the species and when it favours to habituate dangerous terrain. This makes them more difficult to be surveyed, hence making them rare.

Our findings also indicate that the forest floor is an important space for the growing larva. Larval stage is when they are hunting for food such as snails or slugs on the ground and mould into several instar stages (Fu & Meyer-Rochow, 2012, 2013; Liew & Schilthuizen, 2014; Madruga Rios & Hernández Quinta, 2010). Fireflies spend a good amount of time in larval form before becoming adults. For example, Aspisoma lineatum spends 16-19 days in larval form before going into prepupal stage (Viviani, 2001), up to 13 days for Pyrocoelia pectoralis (Wang et al., 2007) and 328 days for Luciola ficta (Ho et al., 2010). The significance of a long glow in firefly larvae had been hypothesised as a form of aposematic signal to tell predators that they are unpalatable (Vencl et al., 2016), but it is also a possible tactic to lure prey (Bechara & Stevani, 2018). The glowing strategy as aposematism tool is used by the firefly until they reach adulthood. In an experiment by Leavell et al., (2018), they demonstrated that bioluminescent is used by fireflies to ward off predators. Hence, it is very important to note that firefly conservation must start from the ground to allow feeding and growth.

# Conclusion

There are three species of fireflies in Imbak Canyon; the rare *Pygoluciola wittmeri*, *Luciola* sp. and the larval form *Pyrocoelia* sp. This makes Imbak Canyon a suitable place to discover more about both species which are widely unknown, in terms of ecology, mating behaviour and feeding habit. Though the rarity of *Pygoluciola* is due to their low population, there is a possibility it was caused by the limitations faced in this study. For example, the length of study might yield different results if carried out longer than three nights or when sampling locations are not restricted based on the trail alone. Perhaps, when these limitations are corrected in future research, we will be able to discover more about their population which is true for any other 'rare' species.

# Acknowledgements

The authors would like to thank the Institute for Tropical Biology and Conservation (ITBC) for conducting the Borneo Geographic Expedition Kangkawat 2018 and Universiti Malaysia Sabah (UMS) for providing financial support (Grant No. SDK0043-2018). Thank you also to Sabah Biodiversity Council for Access License Ref. No. JKM/MBS.1000-2/JLD.3 (246), Imbak Canyon Management Committee for research permission, Yayasan Sabah staff especially to Mr. Jack Lee (ICCA staff), and ITBC staff especially to Mr. Nashrul Molius and Mr. Leonardo Jainih for their assistance in the field. We would also like to thank Dr. Wan Faridah Wan Jusoh for the species confirmation and Mrs. Arnie Abd. Hamid (ITBC staff) for her assistance in mapping.

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