Description of the bioluminescent emission spectrum of *Bicellonycha amoena* Gorham, 1880 (Coleoptera: Lampyridae) in Guatemala

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\textbf{Abstract.} \textit{Bicellonycha amoena} (Gorham, 1880) (Coleoptera: Lampyridae) usually flies one meter above ground level over grass, water, or open areas, where males produce a simple single flash every 2–4 seconds, having their most active flashing period from mid to late dusk and early night. In addition, nothing else is known about the behavior of this species. We conducted field observations at the “Mayan Golf Club” in Guatemala Department, and Santiago Sacatepéquez, Sacatepéquez Department, Guatemala; and analyzed \textit{B. amoena} flashes with a spectroscope. Fireflies displayed a lime-green bioluminescence color. The male flashing activity began ~30 minutes after sunset and lasted approximately 70 minutes. For \textit{B. amoena}, the spectral composition of the flash is intermediate between those known from twilight-active fireflies and nocturnal-active fireflies.

\textbf{Key words.} Fireflies, spectroscopy, flash pattern.

\textbf{Resumen.} \textit{Bicellonycha amoena} (Gorham, 1880) (Coleoptera: Lampyridae) por lo general, vuela a un metro del nivel del suelo sobre pasto, agua o áreas abiertas, donde los machos producen un destello simple cada 2–4 segundos, siendo más activos desde que inicia a oscurecer hasta principios de la noche. Además de esto, no se conoce más sobre el comportamiento de esta especie. Realizamos observaciones de campo en el “Mayan Golf Club” en el Departamento de Guatemala, y en Santiago Sacatepéquez, Departamento de Sacatepéquez, Guatemala; y analizamos los destellos de \textit{B. amoena} con un espectroscopio. Las luciérnagas mostraron un color de bioluminiscencia verde limón. La actividad de destello del macho comenzó ~30 minutos después de la puesta del sol, y duró aproximadamente 70 minutos. Para \textit{B. amoena}, la composición espectral de su destello es intermedia entre la conocida para luciérnagas de actividad crepuscular y de actividad nocturna.

\textbf{Palabras clave.} Luciérnagas, espectroscopía, patrón de destello.

\textbf{ZooBank registration.} urn:lsid:zoobank.org:pub:512FB867-8E8F-47E6-AD02-B3B13C97C25D
Introduction

Fireflies (Coleoptera: Lampyridae) can be found in various habitats including forests, savannas, grasslands, and swamps; the majority of lampyrids are nocturnal and begin their flight and flashing behavior in the evening twilight (Zaragoza-Caballero and Pérez-Hernández 2014). All lampyrids produce light at some stage in their life cycle. All known lampyrid larvae are bioluminescent, with the possible exception of a lanternless, and allegedly dark undetermined species recently reported from the Amazonian tepuis (Kok et al. 2019). Bioluminescence is produced in the paired larval light organ usually located on the abdomen (Riley et al. 2021). In contrast, adult lampyrids vary greatly in the presence, location, shape, and use of the light organs (Branham and Wenzel 2003). Some lampyrids do not produce light as adults and communicate mainly with pheromones for sexual reproduction (Lloyd 1997; Branham and Wenzel 2003). Larval bioluminescence's main function has been suggested to serve as a defense mechanism or to attract prey (Zaragoza-Caballero and Pérez-Hernández 2014). It has also been suggested that bioluminescence in adult lampyrids is a carryover from the larval stage that eventually evolved into a sexual signal (McDermott 1964; Sivinski 1981; Branham and Wenzel 2003). By morphological data, it is suggested that bioluminescence evolved early during the process of the evolution of cantharoid beetles, and lampyrids seem to have retained bioluminescence from one of these early ancestors (Branham and Wenzel 2001, 2003).

Bioluminescence can be defined as light emission through a chemical reaction produced by living organisms, such as fireflies, certain dipteran larvae, bacteria, fungi, dinoflagellates, and other beetles (such as Elateridae and Phengodidae). The biochemistry of this process consists of the luciferin-luciferase reaction, where a luminous substance (luciferin) is oxidized by the catalytic action of an enzyme (luciferase). The indispensable components of this system are ATP and magnesium (Ugarova and Brovko 1981; Ilyina et al. 1998; Branchini et al. 2010).

Among fireflies, mate attraction signals are composed of chemical signals (pheromones), glows (continuous light signals), and flashes (short intermittent light signals); the last of which being the most observed (Lloyd 1997; Stanger-Hall et al. 2007). Flashing fireflies tend to be more active at dusk, night, or both, where males and females use species-specific light signals precisely timed to communicate with each other in an interactive visual Morse-code manner that encodes information concerning species identity and sex (Stanger-Hall et al. 2007; Lewis and Cratsley 2008). Males usually initiate the signaling, while in flight. Once a female responds by flashing, a reciprocal courtship dialogue occurs, in which both sexes exchange flash signals. Females flash usually in response to males and remain stationary. Courtship continues until males contact females and copulation occurs. Colors of firefly bioluminescence range widely from green (~546 nm) through yellow and orange (~590 nm), even though the luciferin is identical in all known species (Lewis and Cratsley 2008). The color of the emitted light is only caused by the species-specific enzymes involved in light production (Seliger and McElroy 1964).

To be able to communicate through bioluminescence, fireflies use screening and visual pigments that vary among species allowing variation in the sensitivity of the eye, depending on the time when each species is active (Lall and Lloyd 1989; Cronin et al. 2000). Visual pigments are composed of an opsin (responsible for light detection and overall vision), bound to a chromophore (the structure responsible for light absorption), being R-type opsin primarily responsible for arthropod vision (Wald 1968; Porter et al. 2012; Martin et al. 2015). The screening pigments tune firefly sensitivity in both nocturnal and twilight species (Cronin et al. 2000). It seems likely that when bioluminescence first assumed a role in firefly sexual communication, the luciferase enzyme was selected to produce green bioluminescence emissions to coincide with the spectral sensitivity of the species visual system (Lall et al. 1980, 1982; Seliger et al. 1982a,b).

Molecular evidence suggests that the short wavelength (SW) opsin class has been lost from several beetle lineages, including fireflies (Lampyridae) (Martin et al. 2015; Sander and Hall 2015; Sharkey et al. 2017). All beetles may lack this opsin class (which generally confers visual sensitivity to blue wavelengths in insects); however, physiological studies have revealed that some beetles do have blue sensitive photoreceptors (Hassellmann 1962; Lin and Wu 1992; Lin 1993; Döring and Skorupski 2007; Lord et al. 2016). A recent study has shown that SW opsin class was lost prior to the early evolutionary history of beetles, ~300 million years ago, and blue light sensitivity was regained in numerous lineages of beetles independently of the ancestral SW opsin (Sharkey et al. 2017). Presumptive loss of tri- or di-chromatic color vision under low-light or spectrally-attenuated conditions has little
impact on fitness (Jacobs 2013). It is now known, by analysis through genome and transcriptome sequencing, that fireflies possess only one copy of two opsin classes: long-wavelength-sensitive (LWS) and ultraviolet-sensitive (UVS) (Martin et al. 2015; Sander and Hall 2015; Sharkey et al. 2017).

It has been reported that *Bicellonycha amoena* (Gorham 1880) (Fig. 1) usually flies one meter or less above ground level over the grass and open areas, and males produce a simple single flash every 2–4 seconds at 22–25°C (L. Faust, pers. comm.). This species is distributed from México to Panama (Bohórquez 1996). In Guatemala it occurs at sea level on the Pacific and Atlantic coasts; and in Guatemala City, at 1500m alt. Also, you can see it flashing during at least 10 months of the year depending on the site (Schuster 1997). There has been no published research in Guatemala on *Bicellonycha* since Gorham 1880, in the Biologia Centrali-Americana, except for

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**Figure 1.** Dorsal and ventral view of *B. amoena*. A–B) Male and female from "Mayan Golf Club", Villa Nueva, Guatemala. C–D) Two males from Hacienda Sac Chich near Merida, Mexico (credit: Lynn Faust).
Schuster (1997). In general, research on fireflies in Guatemala has been almost non-existent. Firefly behavior and flash patterns can be very important to species identification without collecting them.

Materials and Methods

**Field observations of male flash pattern.** In October 2015, we observed the courtship behavior of two populations of *B. amoena* (identification done by Dr. Jack Schuster, using an unpublished thesis on a taxonomic review of the genus by Bohórquez (1996)). One population at the “Mayan Golf Club” (MGC) (3 nights) (Fig 1A–B), located at Villa Nueva, Guatemala Department, at 1330 m alt.; and the other one at Santiago Sacatepéquez (SS) (3 nights), Sacatepéquez Department, at 2100 m alt. (Fig. 2). To characterize the flash pattern, we measured the time lapse in seconds between 10 flashes of 5 males, with a chronometer in each site and determined the average time between each flash. A total of 50 flashes were measured in each site, with a slight observed variation. The same person did all this to avoid bias. We also measured the environmental temperature with an iPhone using The Weather Channel app (www.weather.com/). To describe the flashing activity, we express the time as “Minutes after sunset”. Voucher specimens were collected and deposited in La Colección de Artrópodos de la Universidad del Valle de Guatemala (UVGC), Guatemala City.

**Spectroscopic measure of bioluminescence.** Five males and five females from each site were collected with a butterfly net. In the optic laboratory of the Universidad del Valle de Guatemala (UVG), we determined with a spectroscope (© WINSCO) and with the help of rapid sequential photos the spectrum composition of bioluminescence of 20 *B. amoena* individuals in total. To do this, we held each firefly and placed its light organ on the lens of the spectroscope and induced their glowing behavior by touching their bodies gently with a feather (this was done in a dark room). We also determined whether there was a significant difference between populations in the bioluminescent spectrum using a Wilcoxon-Mann-Whitney U test.

![Figure 2. Sampling locations for both populations of *B. amoena* in Guatemala and Sacatepéquez.](image)
Results

**Male flash pattern.** The time of most flashings of *B. amoena* was from mid to late dusk and early night. The male flashing activity began ~30 minutes after sunset and lasted approximately 70 minutes. We observed that males of the MGC population flew about a meter from the ground surrounding females while females stayed on the grass. In contrast, males of the SS population, similarly to females, remained at the tip of the leaves of the grass. The flash of the males lasts a few milliseconds, while that of the females lasts approximately one second. At 17°C, males in the SS population flashed every 6.21 sec, and at 20°C, at the MGC population, every 6.02 sec.

**Bioluminescent emission spectrum.** The light from the lantern of *B. amoena* appears yellowish-green. Spectro-module measurements confirm a lime-green color. On average, the shortest wavelength present in the spectrum of bioluminescence of both males and females at the SS population is 530 nm; and the longest is 626 nm. At MGC population for both sexes, the shortest average is 526 nm, and the largest is 615 nm. There is no significant difference between populations in terms of which wavelength was the shortest and largest within the wavelength range of the bioluminescence spectrum. On average, the shortest wavelength present in the bioluminescence spectrum of *B. amoena* is 528 nm, and the largest is 621 nm. The wavelength of maximum intensity (peak wavelength) is 565 nm (Fig. 3). It is comparable to that of the North American species *Photinus consanguineous* (LeConte, 1852), *Photinus consimilis* (Green, 1956), *Photinus sabulosus* (Green, 1956), and *Photinus umbratus* (LeConte, 1878) (Fig. 4).

Discussion

Although it is known that *B. amoena* flies approximately one meter over the grass (L. Faust, pers. comm.), in the SS population, both males and females remain at the tip of the leaves of the grass until a female responds to a male. After this, the successful male goes to where the female is. This strange behavior probably occurs in this population because of their thermal limit for flight. Fireflies rarely fly below 10°C, and some tropical/semi-tropical species have trouble even flying at 18°C (Faust 2017). However, fireflies have been observed flying and flashing at 11°C in Guatemala (J. Schuster, pers. obs.). Fireflies in this population were active at 17°C or less the nights they were observed. Santiago Sacatepéquez is located at a high elevation (2100m alt.), and in October to January, it has temperatures of 11.8–14.3°C min. and 22.8–23.3°C max. (INSIVUMEH 2022). The finding of

![Graph 3](image-url)  
*Figure 3.* Bioluminescence emission spectrum of *B. amoena*. A) SS population. B) MGC population.
these fireflies in Santiago Sacatepéquez at 2100m alt. represents the highest altitude reported for *B. amoena* in Guatemala (J. Schuster, pers. obs.).

It is known that the bioluminescence spectrum, in general, varies according to activity time. For example, nocturnal fireflies use shorter wavelengths than crepuscular ones (Lall et al. 1980; Booth 2004). Twilight-active species tend to produce lemon-yellow to yellow-orange flashes, in contrast to nocturnal species which more commonly possess lime-green light (Lall et al. 1980; Seliger et al. 1982a,b). *Bicellonycha. amoena* is a twilight species (species that initiates flashing late at twilight and continue into the night) (Lall et al. 1988, 2023); it presents a lime-green bioluminescence color. Assuming that the spectral emission reflects the spectral sensitivity, we infer *B. amoena* has a visual spectral sensitivity intermediate between twilight and nocturnal-active fireflies, with only some attenuation of sensitivity in the green portion of light.

It is possible that this intermediate visual spectral sensitivity allows *B. amoena* a strategy of early emergence to avoid predation pressure from dark-active aggressive mimics of the genus *Photuris* (Lloyd 1975), which have been reported in Guatemala (Gorham 1881, Schuster 1997); and thus balance the reproductive success of the species with the loss of general visual efficiency. While there is no published reference of *Photuris* predation on *Bicellonycha*, there is an observation by Dr. Schuster of *Photuris* sp. 1 eating *Bicellonycha* sp. 1 at Puerta Parada, Santa Catarina Pinula, Guatemala at 1840m alt. during field observations. He also observed that *Bicellonycha* and *Photinus* species from Puerta Parada flash 30 minutes before *Photuris* species, overlapping signaling periods at night. At Puerta Parada, *Bicellonycha* sp. 1, *Photuris* sp. 1 and sp. 2 coincide in April to May; *Bicellonycha* sp. 2 and *Photuris* sp. 1 coincide in June to July; and *Bicellonycha* sp. 2 and *Photuris* sp. 3 coincide in August to September (Schuster 1997), showing overlapping phenologies between genera.

Loss of general visual efficiency occurs when the visual spectral sensitivity is further narrowed and shifted to operate during twilight (Seliger et al. 1982b). This is because, while night-active fireflies utilize a single broad peak sensitivity across the green portion of the visible spectrum, twilight-active fireflies utilize a narrow peak sensitivity in the yellow portion, in addition to a marked attenuation in the green portion by screening pigments (Lall et al. 1980, 1982, 1988; Lall and Worthy 2000). This characteristic gives the twilight-active fireflies an improved detection ability of bioluminescent signals by an increased spectral specificity to filter a significant amount of environment non-informative light, but this also represents a tradeoff with general visual efficiency (Seliger et al. 1982a, 1982b; Lall and Lloyd 1989).

Another possible explanation for intermediate spectral emission and bioluminescence may be that having a higher sensitivity to the green portion of the visible spectrum than the pure twilight-active fireflies allows for a little less specificity in signals detection but a greater ability to detect low-intensity signals and also detect flashes in a green foliage environment in low illumination conditions when contrast with the background is less important (Seliger et al. 1982a; Lall and Lloyd 1989; Booth 2004; Lall et al. 2009). It would be interesting to observe the behavior of this firefly during the totality phase of the next solar eclipse on April 8, 2024; to understand better and elucidate the findings of this research (Branham and Faust 2019).

We registered a simple single flash every 6.21 sec. at 17°C from \( B. \text{amoena} \) males in the SS population, and every 6.02 sec. at 20°C from \( B. \text{amoena} \) males in the MGC population, instead of the pattern of a simple single flash every 2–4 seconds registered at a 22–25°C temperature range by Lynn Faust (pers. comm.). This phenomenon is well known and has been observed for other firefly species. The flash response delay in females and the flash-dark interval from one flash to the next in males are very temperature dependent. Both change inversely with temperature; the hotter the environment, the faster it will be, and the colder, the slower (Lloyd 1966; Faust 2017).

We did not find a significant difference in wavelength between populations. The wavelength range of their bioluminescence spectrum led us to hypothesize that for \( B. \text{amoena} \), the spectral composition of bioluminescence enhances the ability of the firefly to detect the flash pattern, thus enabling the identification of a potential mate of the suitable species for copulation. The fact before mentioned also supports this; the flash response delay in females and flash-dark interval in males is very temperature dependent (Lloyd 1966; Faust 2017). It is possible that \( B. \text{amoena} \) can distinguish light signals based on the spectrum composition (chromaticity) and discriminate what light signal and pattern belong to a member of his own species, as was demonstrated by Booth (2004) and colleagues with \( \text{Lampyris noctiluca} \) (L.). It is important to mention that this is an exploratory study, and as such, further work is required to understand and determine the visual spectral sensitivity, spectral tuning, and color vision of \( B. \text{amoena} \) by electroretinogram experimentation.

**Conclusions**

In the SS population, both males and females remained at the tip of the leaves of the grass until a female responded to a male. This strange behavior may occur in this population because of their thermal limit for flight. Fireflies rarely fly below 10°C, and some tropical/semi-tropical species have trouble even flying at 18°C, so it is not surprising that this population does this because fireflies in this population were at 17°C or less during the nights they were observed.

The presence of the SS population at 2100m represents an extension in the altitudinal range since the highest altitude reported in Guatemala for \( B. \text{amoena} \) was 1500m alt.

In this study, we infer \( B. \text{amoena} \) has a visual spectral sensitivity intermediate between twilight and nocturnal-active fireflies, with only some attenuation of sensitivity in the green portion of light.

Further work is required to understand and determine the visual spectral sensitivity, spectral tuning, and color vision of \( B. \text{amoena} \). Another question that needs to be resolved for Guatemalan fireflies is: Do species that fly simultaneously have similar spectral characteristics?

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