WORLDWIDE FIREFLY PHYLOGENY (COLEOPTERA: LAMPYRIDAE), EVOLUTION OF SEXUAL SIGNALING

by

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(Under the Direction of Kathrin F. Stanger-Hall)

ABSTRACT

Fireflies (Coleoptera: Lampyridae) are a family of beetles that exhibit great diversity. This diversity is present both in their morphology and in their sexual signaling. While fireflies are known for their bioluminescent flashes and glows used to find mates, not all adult fireflies produce light. Non-bioluminescent adults use pheromones to attract and locate mates instead. These differences in sexual signals are accompanied by differences in firefly morphology, specifically in the sensory organs used to detect light signals or pheromones. My dissertation aimed to investigate the morphological diversity of these signal sensors (i.e., eyes, antennae, and antennal sensilla), as well as the light producing structures (i.e., light organs) of fireflies. My second chapter focused on the diversity of antennal sensilla in fireflies and on identifying potential sensilla used to detect pheromone signals. I found that sensilla morphotypes varied greatly across species. I also found that diurnal (pheromone signaling) firefly species have more sensilla overall, including more chemosensilla that can be used to detect chemicals (including pheromones), than nocturnal (light signaling) fireflies. These findings reflect the importance of chemosensilla in pheromone detection for diurnal species. Interestingly, the specific type of sensilla likely used to detect pheromones varies between species. The third chapter established the most comprehensive worldwide firefly phylogeny to date with 339 species and based on 458 anchored hybrid enrichment loci. This phylogeny determinesd the history of pheromones and bioluminescence signaling in adults and the evolution of light organ shapes using ancestral state reconstruction. I found that both bioluminescence and the diverse light organ shapes evolved multiple times independently during firefly evolution. I also tested the influence of phylogeny and signal type on the evolution of eye, antenna, and light organ size in a phylogeny-based analysis. Aspects of eye and antenna size are significantly influenced by phylogeny and by sexual signal type. This study not only sheds light on the evolutionary relationships of fireflies, and their morphological structures associated with sexual signaling, it also provides a valuable foundation for future evolutionary studies of the remarkable morphological and behavioral diversity in this beetle family.

INDEX WORDS: Firefly, Lampyridae, sensilla, sexual signaling, bioluminescence, light organ, morphological diversity, flashing, glows, pheromones

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DEDICATION

This work is dedicated to my ancestors that did not have the same educational opportunities as me. Thank you for raising the generation that are the reason I am able following my passion.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Firefly sexual signaling

Fireflies (Coleoptera: Lampyridae) exhibit extraordinary diversity across species, including diversity of sexual signals and related morphological structures. Fireflies are well known around the world for their mesmerizing bioluminescent displays. They produce light via a chemical reaction in specific tissue, known as the light organ. All larval fireflies bioluminesce, they use bioluminesence as an aposematic warning to predators (Underwood et al., 1997; De Cock and Matthysen, 2003; Sagegami-Oba et al., 2007). Adult bioluminescence is used as a sexual signal by nocturnal species; that emit flashes or glows. However, not all fireflies bioluminesce, these species are diurnal and use pheromones to attract and find mates. Bioluminescent sexual signaling has been extensively studied including the use of species-specific flash patterns (Lloyd, 1966; Stanger-Hall and Lloyd, 2015), population-specific light colors (Lower et al., 2018), and the visual proteins used to detect these signals (Martin et al., 2015; Sander and & Hall, 2015). While pheromone signaling is known to be the ancestral state of firefly sexual signaling (Branham & Wenzel, 2003; Stanger-Hall et al., 2007; Martin et al., 2017), little is known about pheromone signaling species. To date, researchers have only identified sex pheromones for 5 species, two undetermined *Diaphanes*, *Pyrocelia praetexta*, *Lamprigera tenebrosa* and *Photinus corruscus* (Wattanachaiyingcharoen et al., 2020; Lower et al., 2023). It is hypothesized that diurnal fireflies use their antennae to detect pheromone signals, like most other insects. This is supported by differences in antennal size between diurnal and nocturnal fireflies. Diurnal fireflies have larger antennae (relative to body size) than nocturnal fireflies, while nocturnal fireflies have larger eyes (relative to body size) than diurnal fireflies (Stanger-Hall et al., 2018). This suggests that there is selection on sensory organ size used to detect sexual signals. The size of the antennae provides the surface area for pheromone detection and as a next step we need to identify the specific receptors used to detect pheromones.

Antennae are covered in microscopic hair-like structures called sensilla. Sensilla are the first point of contact for environmental stimuli and different sensilla house the sensory cells for different stimuli, including temperature, pressure, humidity, contact chemicals, and volatile chemicals like pheromones (Schneider, 1964). Antennae and their pheromone specific sensilla play a vital role in non-bioluminescent males' search for a mate by detecting volatile pheromones. Recently the first pheromone sensilla were identified in *Photinus corruscus* (Lower et al., 2023), but it is unknown if these same types of sensilla are used in all fireflies. The aim of chapter two is to document the diversity of the antennal sensilla across seven firefly species and to identify potential pheromone sensilla. This work provides insight on how fireflies interpret their environment through their antenna and also suggests pheromone sensilla candidates that can be tested in functional studies as more pheromone compounds are identified for fireflies.

Lampyrid phylogenetics and signal evolution

The dichotomy of firefly sexual signaling (bioluminescence versus pheromones) provides an excellent model system to study not only the evolution of these sexual signal modes , but also the morphological structures used for signal detection (eyes, antennae, and sensilla) and signal production. However, a well-supported phylogeny of the family is needed to study the evolutionary relationships of fireflies and analyses of character evolution. Historically evolutionary relationships among fireflies have been difficult to resolve due to limited taxonomic sampling, exclusive use of morphological data or a limited number of loci (Suzuki, 1997; Branham and Wenzel, 2001; Stanger-Hall et al., 2007; Jeng, 2008). Martin et al. (2017) attempted to address this with a combined molecular and morphological phylogeny. However, this phylogeny had low support for many of the subfamily relationships and was based on only six loci. More recently, next generation sequencing data, including 463 anchored hybrid enrichment loci, was used to reconstruct the most current phylogeny of Lampyridae (Martin et al., 2019). At the time of publication, this phylogeny included eight of the then nine firefly subfamilies and elevated the tribe Lamprohizini to the subfamily Lamprohizinae, resulting in a total of 10 subfamilies. Since then, a new subfamily has been described, Chespiritoinae Ferreira 2020, resulting in a total of 11 Lampyridae subfamilies (Ferreira et al., 2020). While Martin et al. (2019) presents the most comprehensive phylogenetic analysis to date, taxonomic sampling focused heavily on the subfamilies Lampyrinae and Luciolinae and primarily sampled species with adult bioluminescence. The aim of chapter three was to establish the most diverse Lampyridae phylogeny to date, providing an essential tool for evolutionary studies and to investigate the evolution of firefly sexual signaling, including shifts in signal mode and changes in eye and antenna size. My taxon sampling includes the most extensive representation of non-bioluminescent species and includes 10 of 11 firefly subfamilies to establish a more robust firefly phylogeny.

Previous phylogenetic studies have indicated that bioluminescence sexual signals have evolved independently multiple times within the family and the number of the gains and losses of adult bioluminescence vary based on taxon sampling (Branham and Wenzel, 2003; Stanger-Hall et al., 2007; Martin et al., 2015). Determining the shifts in signal mode is the first step in understanding the evolutionary history of firefly sexual signaling and will provide evolutionary context for studying signal receptors, including which lineages had recent gains or losses of bioluminescence.

Stanger-Hall et al. (2018) showed that bioluminescent fireflies tend to have larger eyes with smaller antennae, relative to body size, while pheromone-using fireflies tend to have larger antennae and smaller eyes; however, it is unknown if the same morphological trends persist throughout Lampyridae. Additionally, Stanger-Hall et al. (2018) found that eye and antenna size was strongly influenced by phylogenetic relatedness. This work also investigates the evolution of firefly sexual signaling on the broadest taxonomic scale to date, identifying shifts in signal mode and changes in eye and antenna size and shape of light organs.

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CHAPTER 2

ANTENNAL SENSILLA DIVERSITY IN DIURNAL AND NOCTURNAL FIREFLIES (COLEOPTERA: LAMPYRIDAE)

Introduction

Antennae are major sensory organs of insects and are remarkably diverse in both form and function. Antennae are composed of multiple segments (antennomeres) whose numbers range from 2-62 across beetle groups (Minelli, 2017; Nunes et al., 2020). There is morphological and functional variation between individual antennomeres: the basal two antennomeres (scape and pedicel) contain muscle attachments that are used to sustain and move the antennae, and the antennomeres that make up the remainder of the antenna (the flagellomeres) are involved with sensing the environment. The flagellomeres can be highly modified (Snodgrass, 1928, Loudon, 2009), creating diverse antennal shapes across insect groups (Elgar et al., 2018).

While the diversity of antennal types showcases different ways of increasing antennal surface to collect environmental information, the first point of contact for the different environmental stimuli are the sensilla, microscopic sensory structures on the antennal surface. Sensilla are extensions of the insect cuticle (Schneider, 1964) and each sensillum represents a specialized accessory structure that translates specific environmental stimuli for specific sensory neurons (Kiel & Steinbrecht, 1984). Functional sensilla types include mechanoreceptors (pressure, touch), chemoreceptors (volatile and/or contact chemicals), thermoreceptors and hygroreceptors (humidity and air pressure) (Schneider, 1964; Kiel & Steinbrecht; 1984, Steinbrecht, 1987).

The antennal sensilla of insects are diverse in both form and function, but the basic sensilla anatomy includes a stalk, which can emerge directly from the antennal surface or from an elevated base. The stalk can vary in shape and length, in the presence or absence of grooves along the length of the stalk, and may lack or bear pores (Schneider, 1964). These characteristics, along with the cell morphology of their sensory neurons, are traditionally used to classify sensilla into broad morphological groups. In his seminal review, Schneider (1964) described nine morphological groups of sensilla found in all insects. Some of these morphotypes are typically associated with specific functions across insects. For example, chaetica and campaniform sensilla tend to be primarily mechanoreceptors (Snodgrass, 1926; Schneider, 1964; Steinbrecht, 2007), coeloconica and capitular sensilla tend to be thermo- or hygro- receptors (Schneider 1964; Altner & Loftus 1985; Yao et al., 2005; Tichy & Kallina, 2010), and trichodea, basiconica, and placodea sensilla are typically chemoreceptors (Schneider, 1964). Additional sensilla morphotypes have been described for different insect groups, either as new variants of described insect morphotypes, or as entirely new sensilla types. For example, a wide variety of sensilla morphotypes have been identified within beetles (25 in Cerambycidae: Haddad et al., 2023; 16 in *Agriotes* elaterids: Faucheux et al., 2020; 16 in Scarabaeidae: Bohacz et al., 2020) with sensilla chaetica, basiconica, and trichodea representing the most common sensilla types. However, sensilla morphotypes greatly varied between these families and even between species.

The specific function of individual sensilla morphotypes has been studied in relatively few beetle species. Sensilla chaetica are well established as mechanoreceptors (Schneider, 1964; Altner & Loftus, 1985) and specific examples in Coleoptera include *Oryzaephilus surinamensis* (Silvanidae) and *Limonius aeruginosus* (Elateridae; White, 1991; Merivee et al., 1998). Among chemoreceptors most functional studies have focused on the detection of plant volatiles and/or sex pheromones (e.g., Lopes et al., 2002). For example, sensilla trichodea detected sex pheromones in the pine weevil *Hylobius abietu* (Curculionidae; Mustaparta et al., 1975), and sensilla basiconica were shown to detect sex pheromones in the firefly *Photinus corruscus* (Lower et al., 2023). In addition, sensilla coeloconica have been identified as thermoreceptors in *Siagona europaea* (Carabidae) (Giglio et al., 2010), and as hygroreceptors in the firefly *Luciola cruciata* (Iwasaki et al., 1995).

Increasing antennal sensitivity for enhanced stimulus detection –

Given the importance of antennae and their sensilla for insects to sense their environment, strong natural or sexual selection for the improved detection of relevant stimuli is expected (Endler, 1992). One way to improve stimulus detection is by using more sensors. This could be achieved by increasing the surface area of the antennae (e.g. Spaethe et al., 2007; Johnson et al., 2017), thus increasing the number of sensilla and their associated sensory neurons on the antennal surface (Chapman, 1982), while at the same time increasing the air space that the antennae can sample. The surface area of the antennae can be increased in one of three ways: (1) Increased number of antennal segments, (2) increased length of individual segments, or (3) the addition of side-branches to segments (Schneider, 1964; Nunes et al., 2020). In addition, stimulus detection could be further improved by (4) increasing the density of the sensilla on a given antennal surface area (Spaethe et al., 2007; Gill et al. 2013) and/or by (5) increasing the size of the individual sensilla to maximize interaction with environmental stimuli and thus their sensitivity at threshold levels (Steinbrecht, 2007).

Fireflies

We focus here on the antennal sensilla diversity of adult Lampyridae. Firefly species occur worldwide (except Antarctica) and exhibit a striking range of antennal diversity (Ohba, 1978; Nunes et al., 2020). This diversity includes both antennal shape and antennomere number. Within Coleoptera, the typical number of antennomeres is eleven (Thomas, 2000), and indeed most beetle families have this fixed number across all species (Minelli et al., 2017). Among fireflies the number ranges from 7-62 antennomeres, with eleven being the most common (Fig. 2.1; Nunes et al., 2020). Within-species variation is known to occur in several genera, including *Alecton*, *Microphotus*, *Pleotomus* and genera of Amydetinae (Nunes et al., 2020). To date, antennal sensilla diversity in fireflies has only been studied in the bioluminescent males of one firefly species, *Luciola cruciata* (Iwasaki et al., 1995) from Asia. Iwasaki et al. (1995) described seven sensilla morphotypes, including four mechanoreceptors, two chemoreceptors and one hygroreceptor. It is unknown whether the morphotypes of *L. cruciata* are representative of other firefly species and whether there are differences in sensilla types, numbers, or densities between males and females and/or between bioluminescent (nocturnal) and non-bioluminescent (diurnal) firefly species. Filling this gap is important, because the absence or presence of adult bioluminescence has implications for how firefly antennae are used.

Nocturnal firefly species are active at dusk or at night and are bioluminescent. They use prolonged glows (De Cock et al., 2014) or species-specific flash patterns (e.g., Lloyd, 1969; Ohba, 2004; Stanger-Hall & Lloyd, 2015) as visual signals to attract and recognize conspecific mates. In contrast, most diurnal firefly species have non-bioluminescent adults that rely exclusively on pheromones to identify and locate a conspecific mate. In both signaling systems males actively look for females during mate search, while females are sedentary, and correlated with this behavior, males have significantly larger eyes than their conspecific females. Furthermore, bioluminescent males that navigate through vegetation at low light levels to locate their bioluminescent females, have significantly larger eyes than the males of diurnal species

(Stanger-Hall et al., 2018). In contrast, diurnal males have significantly longer antennae than their conspecific females and usually also significantly longer antennae than males of bioluminescent species (Stanger-Hall et al., 2018), reflecting the importance of male antennae for the detection of pheromones in diurnal species.

It is currently unknown whether this antennal size dimorphism is also reflected in antennal sensilla diversity, and/or in the number and density of different sensilla types, including olfactory sensilla that are important for pheromone detection. We also presently do not know to what extent bioluminescent species retain the use of long-range pheromones used during mate search, (e.g., to lead males close enough to females, so both can detect and respond to the light signals of their potential mates). During firefly evolutionary history, there were several independent reversals from nocturnal activity with use of light signals to diurnal activity with pheromones as the main mating signal (Branham & Wenzel, 2003; Stanger-Hall et al., 2007; Martin et al., 2017), and such reversals also took place several times within the genus *Photinus* (Stanger-Hall et al., 2015). This suggests that bioluminescent species likely retain the ability to use pheromones, at least to some degree, facilitating the reversal to exclusive pheromone use. Combinations of pheromone and bioluminescent signaling has been indicated at least once in the following genera; *Cyphonocerus*, *Pyrocoelia*, *Erthrolychnia*, *Phaenolis*, *Phausis*, and *Pleotomus* (Lloyd, 1971; Branham and Wenzel, 2003; Ohba 2004, Stanger-Hall et al., 2007). Additionally, field observations suggest that the Blue Ghost firefly, *Phausis reticulata,* may use both light signals and pheromones during mate search (De Cock et al., 2014: supplement). The unusual bioluminescent, but diurnal firefly species *Phosphaenus hemipterus* uses pheromones as the primary sexual signal and its faint bioluminescent glow as an aposematic defense signal rather than for mating (De Cock & Matthysen, 2005; Majka & MacIvor, 2009).

While the bioluminescent mating signals of nocturnal species have been studied extensively, so far little is known about the pheromone signaling system in fireflies. The specific chemical compounds that females emit as pheromones have been identified for only two diurnal firefly species *Pyrocelia oshimana* (Shibue et al., 2000) and *Photinus corruscus* (Lower et al., 2023). The specific antennal sensilla that respond to sex pheromones in *P. corruscus*, the diurnal winter firefly, have recently been identified as sensilla basiconica (Lower et al., 2023). Once in close physical contact, both bioluminescent and non-bioluminescent fireflies will "antennate" each other intensively before mating, suggesting the sampling of contact chemicals, possibly cuticular hydrocarbons (CHCs) with gustatory receptors, for a final verification of a conspecific mate. South et al. (2008) found CHCs on the pronotum and elytra of the diurnal species *P. corruscus* and *Lucidota atra* but found only low or undetectable levels of CHCs in the nocturnal species *Photinus greeni*, *P. ignitus*, and *P. obscurellus* (it is unclear to what extent this may have been influenced by the different extraction methods used). *Photinus corruscus* males were able to distinguish between the CHCs of conspecific and heterospecific (*L. atra*) females, suggesting that CHCs are possibly used for reproductive isolation in this species (South et al., 2008).

In this study we documented the sensilla diversity of males and females of seven species of fireflies, including three diurnal and four nocturnal species. An interesting challenge was the identification of pheromone sensilla, especially in the absence of functional verification with electrophysiology. The first pheromone sensilla were just recently identified in *P. corruscus* (Lower et al. 2023) and we used this opportunity to test our set of predictions for identifying potential sex pheromone sensilla in fireflies based on morphology alone. Given the importance of pheromones for diurnal firefly species, we predicted to find (1) more chemoreceptors (including pheromone receptors) in diurnal species. If nocturnal species have completely lost the ability to use pheromones during mate search, we would expect to find (a) at least one sensilla type among the chemoreceptors that is only present in diurnal fireflies; if nocturnal species retain the ability to use pheromones we would expect (b) at least one sensilla type among the chemoreceptors that is present in greater numbers and possibly greater density on the antennae of diurnal species, compared to nocturnal species. We would further expect that (2) diurnal males, that are tracking a pheromone plume to conspecific females would have greater numbers, and possibly a greater density, of a pheromone receptor than their females. Finally, (3) pores in the sensilla surface are necessary to allow volatile chemicals to enter the internal sensilla space and bind to receptor proteins on the sensory neurons (Schmidt & Benton, 2020), therefore any candidate for a pheromone sensilla should have pores. In the case of gustatory chemoreceptors that pick-up contact chemicals during antennation, we would not predict a difference between diurnal and nocturnal fireflies, since both diurnal and nocturnal species engage in antennation behavior before mating. We also would not predict a sex difference in gustatory chemoreceptors since both sexes engage in antennation behavior; gustatory sensilla also should have pores. Our proposed pheromone and gustatory chemoreceptor sensilla candidates will facilitate future functional testing of different sensilla morphotypes, an important next step towards understanding how fireflies use their antennae to perceive their world.

Methods

Taxon sampling

The seven firefly species in this study were selected to represent phylogenetic diversity (i.e., six genera in four subfamilies: Luciolinae, Lampyrohizinae, Photurinae, and Lampyrinae), sexual signal diversity (i.e., bioluminescent and non-bioluminescent), and differences in dial activity (i.e., nocturnal and diurnal). The seven taxa include four bioluminescent species (in four genera), with three flashing species: *Photinus pyralis*, *Photuris lucicrescens,* Luciolinae sp. (an unidentified species from Africa in the Luciolinae subfamily), and one glowing species: *Phausis* sp. (a new species, discovered by Sarah Lower, that remains undescribed; voucher specimens KSH8663 and KSH8667 are stored in the Stanger-Hall lab at UGA: Lower et al. 2017), as well as three non-bioluminescent species (in three genera): *Lucidota punctata*, *Pyropyga nigricans*, and *Photinus corruscus* (formerly *Ellychnia corrusca,* Zaragoza-Caballero et al. 2020). Species were determined to be bioluminescent or non-bioluminescent as adults by the presence or absence of a light organ on ventrites 5, 6, and/or 7. Flashing and glowing bioluminescent species were differentiated based on field observations and records in the literature (Table 2.1). The antennae of three males and three females of each species were examined. Vouchers are kept in the Stanger-Hall lab at the University of Georgia,

Scanning electron microscopy (SEM)

Specimens were prepared by removing both the left and right antennae from the head of each specimen. Antennae of the first several specimens were washed using soapy water and an ultrasonicator to remove debris. This method was effective for several specimens, but damaged others. Damaged specimens were not further examined, and a new specimen was washed with a new method: antennae were placed in a 0.01% solution of KOH at 50^{o} C for 2 hours. The antennae were then rinsed in distilled water and allowed to air dry for ~10 minutes (both methods), before mounting them on SEM stubs, with one antenna facing dorsal side up and the other facing ventral side up, providing both a dorsal and a ventral view of the antennae for each individual firefly. Antennae were further air dried on their stubs for a minimum of 72 hours to ensure even sputter-coating. The antennae were sputter-coated with 30 nm of gold using the Lecia ACE600. Sputter-coated antennae were then imaged using the FE-SEM Thermo Fisher

Teneo with an EDT and T1 detectors at the Georgia Electron Microscopy laboratory. For each individual antenna, SEM images were obtained for each individual antennomere. Each image contained a scale bar (generated by the Teneo software) for subsequent antennal area measurements.

Antennal area

All taxa sampled have filiform or moderately serrate (*Lucidota punctata*) antennae with 11 antennomeres (Fig. 2.1), except *Phausis* sp. females which are paedomorphic (lacking both elytra and metathoracic wings but with paired pretarsal claws and a pair of stemmata; see Cicero, 1988) and have only three antennomeres. The area of each antennomere was measured (in mm^2) using the polygon selection tool in ImageJ v1.52 (Rasband, 2015) and the scale bars produced during imaging. The area of all (11 or 3) antennomeres was then summed for each specimen to determine the total antennal area for each side of the antenna. The area measurements for the dorsal and ventral sides of each individual antenna were then added to determine the total antennal area (per antenna) of each specimen. Since larger fireflies tend to have larger antennae, we accounted for the influence of body size on antenna size by measuring pronotum length as a proxy for body size (Stanger-Hall et al., 2018) and used scaled antennal area (antenna area/pronotum length) to test for differences in antennal area between diurnal and nocturnal fireflies when accounting for body size (note: due to the lack of pronotal expansions in Luciolinae, pronotum length will underestimate body size for Luciolinae compared to other fireflies). Pronotum length was measured from the anterior to the posterior edge along the midline of the pronotum.

Sensilla morphotypes, diversity and evolution

To describe sensilla morphotypes within and across our focal firefly species, we used key morphological characteristics as defined by Schneider (1964) for different insect sensilla morphotypes. These characteristics included: shape of sensilla base (raised or not raised), shape of stalk (equal width throughout length or unequal width), length of stalk relative to base (stalk length equal to base or stalk length > 1x base length), and the presence or absence of grooves and/or pores. These morphological characteristics were used to separate sensilla morphotypes into the 9 major groups defined by Schneider (1964). In addition, we consulted Faucheux et al. (2020) who examined the sensilla morphology of *Agriotes* (Elateridae) and synonymized sensilla nomenclature across Elateridae, a beetle family closely related to Lampyridae (Martin et al., 2019; McKenna et al., 2019). We used the Elateridae naming scheme to classify each of the sensilla morphotypes for Lampyridae. Sensilla morphotypes that were not previously described or named, were named using the next consecutive number(s) of the Faucheux naming scheme. We report all sensilla morphotypes found in males and females of each species. To identify a potential phylogenetic influence on the distribution of sensilla morphotypes in our seven study species and *Luciola cruciata* (Iwasaki et al., 1995), we plotted all sensilla morphotypes on a cladogram of all 8 species (pruning all other species from the phylogeny of Martin et al., 2019).

Sensilla Diversity Measures

Sensilla richness, or the number of sensilla types present, was counted for each specimen. To compare sensilla diversity across specimens and across firefly species we used the proportions of each sensilla type present (p_i) on the antenna of a given specimen to calculate two different diversity indices that provide different measures of diversity. The general Simpson dominance index (D) is calculated as $D = \sum(p_i)^2$ and favors dominant sensilla types over rarer sensilla types. In contrast, the Shannon Index (H) is calculated as $H=\sum[-\log(p_i)](p_i)$ and favors rare sensilla types, allowing for a more even contribution of each sensilla type to the diversity index (Daly 2018). Since the Shannon and Simpson indices for different species cannot be directly compared or combined for analysis, we transformed all index values into their effective numbers (E) using E_D (Shannon)=10^H and E_H (Simpson)=1/D (Daly, 2018). These effective numbers linearize values calculated from non-linear indices and represent how much more or less diverse in sensilla types one antenna is compared to another. We then used parametric t-Tests for E_D (normally distributed) and non-parametric Wilcoxon rank-sum tests for E_H (not normally distributed) to compare the average effective numbers ($E_{D)}$ and E_H) of each species to determine if there was a difference in sensilla diversity between diurnal and nocturnal species.

Sensilla Counts and Sensilla Density

For each specimen (three females and three males for seven species = 42 specimens) we counted the total number of sensilla for each sensilla morphotype on each antennomere (both dorsal and ventral side). To calculate the total sensilla number for each morphotype on one antenna we added the counts across the 11 antennomeres (three for *Phausis* females) of each individual specimen. We also generated counts for all functional categories (all mechanoreceptors and all chemoreceptors). To determine how closely sensilla were packed on the antennal surface, we calculated sensilla density by dividing the total sensilla counts (per morphotype and category) by the total antennal area of the respective specimen.

For summary statistics we calculated the mean ($\bar{x}$±stdev) sensilla number and mean sensilla density for each sex and each species (for all sensilla, all mechanoreceptor sensilla, and all chemoreceptor sensilla: Tables 2.1 & Supp. Table 2.1), and individual sensilla morphotypes (Tables 2.4, 2.5, Supp. Table 2.2, 2.3). To capture the variation of individuals within species we used the individual data (counts and densities for the 6 individuals per species) for statistical comparisons, rather than species means.

Since count and density data were not normally distributed, we used non-parametric tests. With our small sample size (3 males and 3 females) for each species we could not test each species for sex differences in sensilla counts and densities, so we tested for sex differences across species with a paired Wilcoxon signed-rank test in JMP Pro v.16.0.0 (SAS Institute Inc. Cary, NC) that pairs individual females and males within a species. If no sex difference was found, female and male data were used together for the subsequent comparison of diurnal and nocturnal fireflies; if a sex difference was identified, males and females were analyzed separately. To determine if diurnal firefly species differed in their total sensilla counts and densities, as well as in their mechanoreceptor and chemoreceptor sensilla counts and densities from nocturnal fireflies, we compared the species in these two groups using a non-parametric Wilcoxon rank-sum (unpaired two sample) test with a normal approximation for large sample sizes (JMP Pro v16.0). Due to our small sample sizes (4 nocturnal and 3 diurnal species with 3 males and 3 females each) it is important to note that we may lack the statistical power to correctly reject the Null hypothesis, and therefore non-significant results should be considered preliminary until more species are available for testing.

For both analyses (sex differences and diurnal/nocturnal differences) of sensilla counts we used two comparisons: (1) to directly compare the *overall sensory capacity* of the firefly antennae (for all stimuli, all mechanical stimuli, or all chemical stimuli), we tested the total recorded numbers (and densities) for all sensilla, all mechanoreceptors, and all chemoreceptors between males and females and between diurnal and nocturnal taxa; (2) to compare sensilla numbers (and densities) *relative to the actual antenna size* of individual fireflies, we computed the residuals from a linear regression of sensilla type counts or densities with the measured antennal area for all individual fireflies and tested these for differences (deviation from the regression line).

Sensilla distribution

To assess whether different parts of the antennae could be used for different functions (e.g., mechanoreception, chemoreception), we determined how the different sensilla morphotypes were distributed across the length of the antenna of each individual specimen. To visualize this distribution of the sensilla in each functional group and each morphotype, we plotted the mean number of sensilla per antennomere for each sex and each species across the 11 antennomeres.

Results

Antennal area

The measured antennal areas across our seven species of fireflies varied greatly (Table 2.2). *Phausis* sp. females (three antennomeres) and males (11 antennomeres) had the smallest antennal area (x̄±stdev) with $\bar{x}_{Female}$=0.02mm^2 ± 0.0009 and $\bar{x}_{Male}$ = 0.2mm^2 ± 0.04; *Ph. lucicrescens* females had the largest antennal area ($\bar{x}_{Female}$ = 2.6mm^2 ± 0.6; Table 2.2). The antennal areas of males (x̄ =1.49±0.9 mm^2) and females (x̄ =1.26± 0.9 mm^2) did not differ significantly across our study species (Wilcoxon signed-rank test: N=21, S=34.5, df=20, p=0.24). Similarly, the antennal areas of the nocturnal (x̄ =1.28±1.07 mm^2) and diurnal (x̄ =1.50±0.6 mm^2) fireflies in our study did not differ significantly from each other (Wilcoxon rank-sum test: N= 24,18, S=424, Z=0.9277 p=0.35). However, the antennal areas of fireflies were significantly correlated with pronotum length, a proxy for body size (R^2 =0.467, Spearman's rho =0.668,

$p < 0.0001$), which means larger fireflies tended to have larger antennae. Females of nocturnal *Photuris lucicrescens* and *Photinus pyralis* had the largest measured antennal areas (Table 2.2); however, when body size was taken into account, the males of diurnal *Lucidota punctata* and nocturnal *P. pyralis* had relatively larger (scaled) antennal areas. Overall, males ($\bar{x}=0.86\pm 0.55$mm) had significantly larger scaled antennal areas (antennal area/pronotum length) than their conspecific females ($\bar{x}=0.62\pm0.34$mm; Wilcoxon signed-rank test: N=21, S=-69.5, df=20, p=0.012; Figure 2.2A). In contrast, diurnal and nocturnal fireflies in our study did not differ significantly in their scaled antennal areas (Wilcoxon rank-sum test: N=18, 24, S=463, Z=1.91, p=0.055; Fig. 2.2B). Similarly, when we tested whether the different groups deviated (residuals) randomly from the linear regression line between antennal area and body size (pronotum length), the residuals of males and females differed significantly from each other (Wilcoxon signed-rank test: N=21, S=67.5, df=20, p=0.015), while nocturnal and diurnal fireflies were not significantly different (Wilcoxon rank-sum test: N= 18, 24, S=424, Z=0.928, p=0.35; Supp. Fig. 2.1AB).

Sensilla morphology

We identified a total of 14 different sensilla morphotypes across the seven firefly species. Two of these 14 morphotypes were first identified in *L. cruciata* (Iwasaki et al., 1995). One morphotype was extremely rare and was not counted, two other morphotypes were found in only one sex, or in only a few specimens within a species. Eleven morphotypes, if present at all, were present in both sexes (Table 2.2). Based on their morphology (Schneider, 1964; Faucheux et al. 2020), we identified 3 mechanoreceptor and 8 chemoreceptor morphotypes among the 11 most common sensilla morphotypes in our 7 species (the three rare morphotypes are likely chemoreceptors).

Mechanoreceptors

Sensilla chaetica, also known as the bristle or spine sensilla (Schneider 1967), are slender sensilla arising directly from the antennal cuticle, with no modified ring around the base. These sensilla vary in length, but all gradually taper towards the distal end. The walls of these sensilla can be smooth or bear grooves that run parallel to the length of the stalk. We found two sensilla chaetica variants (types) in fireflies, (Fig.2. 3A, B). (1) Sensilla chaetica type 1 (C1, Fig. 2.3A): C1 are long bristle sensilla with grooves running parallel lengthwise along the stalk. These are the longest sensilla found on firefly antennae and were located on all 11 antennomeres. They were distributed evenly across the surface of each individual antennomere. C1 sensilla were found in all seven species examined. (2) Sensilla chaetica type 2 (C2, Fig. 2.3B): C2 sensilla have a short smooth stalk that comes to a distinct point. These sensilla were found in all seven species examined.

Sensilla campaniform. We identified one type of sensilla campaniform (SC; Fig. 2.3C). These sensilla consist of a ring slightly raised above the antennal surface, with a raised disc, equal in height, inside the center of the ring. SC sensilla were found in low numbers relative to the other mechanoreceptors (Table 2.2). They were found on the antennae of all species except *Phausis* sp.; they were absent in male *Lucidota punctata* and female *Photuris lucicrescens* (Table 2.3).

Chemoreceptors

Sensilla basiconica, also known as peg or cone sensilla, are characterized by their raised base with a peg or cone arising from that base. The length and width of the peg varies between morphotypes. Peg and cone walls may or may not have pores and may have grooves that run parallel along the length of the peg (Schneider 1967). Basiconica sensilla were found in all seven firefly species and we identified six different variants (types): (1) Sensilla basiconica type 1 (B1,

Fig. 2.4A) are peg sensilla with a well-defined dome shaped base and a short peg with a single pore at the distal end. The peg length is ~3-4 times the height of the dome base, the width of the peg is uniform throughout the length and the distal end of the peg is rounded. We found no pores along the length of B1 sensilla, however B1 sensilla identified by Lower et al. (2023) possessed pores along the sides the peg. B1 sensilla were found in the diurnal (non-bioluminescent) species *P. corruscus*, *Py. nigricans*, and the nocturnal (bioluminescent) species *P. pyralis*. (2) <u>Sensilla basiconica type 2</u> (B2, Fig. 2.4B) are peg sensilla with a well-defined dome base and a moderate length peg (~4-5 times the height of the dome base). The peg is widest at the base and gradually tapers in width coming to a blunt point at the distal end, no visible pores were found on the peg. B2 sensilla were found in the diurnal species *P. corruscus*, and the nocturnal species *Ph. lucicrescens* and Luciolinae sp. (3) <u>Sensilla basiconica type 3</u> (B3, Fig. 2.4C) are peg sensilla with a modified dome base, with a flattened top, and a peg (~5 times the height of the base). The width of the peg is equal throughout its length and bears pores in high density along the distal 3⁄4. The peg comes to a blunt point at its distal end. B3 sensilla were found in the diurnal species *P. corruscus*, *Py. nigricans,* and the nocturnal species *P. pyralis*. (4) <u>Sensilla basiconica type 7</u> (B7, Fig. 2.4D) are peg sensilla with a dome base and peg (~2-3 times the height of the base). The peg is equal in width for the first 2/3 of its length and then tapers to a distinct point. The last 1/3 of the peg has parallel grooves that run along the length of the peg and meet at the distal point. There were no visible pores on the peg of B7 sensilla. B7 sensilla were found in all studied species except *Phausis* sp. (5) <u>Sensilla basiconica type 10</u> (B10, Fig. 2.4E) are cone sensilla with a raised collared base and a broad cone. The length of the cone is 2 times the height of the collared base, pores are present on the cone. The cone is equal in width to the collared base for the first ½ of the length and then tapers gradually to a point. B10 sensilla were only found in the diurnal species *L. punctata*. (6) Sensilla basiconica type 11 (B11, Fig. 2.4F) are modified cone sensilla with a collared base and a rounded cone. The length of the cone is ~1.5-2 times the length of the base. The proximal end of the cone is equal in width to the base, the sides then gradually diverge, increasing the width of the cone, with a rounded end. The distal end of the cone is broadly rounded to a shallow dome shape, the cone lacks visible pores. B11 sensilla were only found in the diurnal species *L. punctata*. (7) Sensilla basiconica type 12 (B12, Fig. 2.5A) have a membranous base with a short peg that gradually comes to a point at the distal end which bears a single pore. The length of the peg is about equal to the width at the base; these sensilla were only found in the males of Luciolinae sp. (8) Sensilla basiconica type 13 (B13, Fig. 2.5B) closely resemble B3 sensilla, but completely lack pores; these sensilla were only found in Luciolinae sp.

Sensilla coeloconica (X1, Fig. 2.4G) consist of pegs that arise from inside a pit, a rounded depression in the cuticle, therefore the proximal end of the peg is not visible inside the pit. The sensilla coeloconica in Lampyridae are characterized by a circular pit with a B7 type peg that comes to a point at the distal end with multiple longitudinal grooves meeting at the end and no visible pores.

Sensilla trichodea (T1, Fig. 2.4H) are simple hair like sensilla without a modified base, directly arising from the cuticle surface. We found only one type of sensilla trichodea (T1) in fireflies and exclusively in the nocturnal species *Phausis* sp. These sensilla lack pores and are short, with the width of the stalk at the base ~8 times the width of the stalk at the distal end. The width of the sensilla gradually decreases before coming to a blunt point.

In addition, we found *cone sensilla* (Schneider 1964) in very low numbers and not consistently present across all individuals of a species. These sensilla were seen in one or two individual per species, with 1-3 cones present per individual. Cone sensilla have no raised base and are shaped like cones with the width at the base equal to the height. They were found on *P. corruscus*, *Py. nigricans*, and Luciolinae sp. antennae, but not in all specimens of any species. We also found clusters of large cuticular pores on the antennal surface of the Luciolinae sp., *P. pyralis., Phausis* sp*.,* and *Py. nigricans*. These pores were not associated with sensilla but could often be found at the base of C1 sensilla. These pores closely resemble the glandular pores associated with C1 sensilla in *Agriotes* (Faucheux et al., 2020).

Sensilla Diversity across Species

We documented between three and eight sensilla morphotypes within an individual firefly species. The distribution of individual morphotypes varied greatly between species. Two mechanoreceptor types (Sensilla chaetica types C1 and C2) were the only sensilla morphotypes found in all seven firefly species (Table 2.3). Three different mechanoreceptor sensilla types (sensilla chaetica C1, C2 and sensilla campaniform SC), nine different chemoreceptor sensilla types (sensilla basiconica types B1, B2, B3, B7, B10, B11, B12, B13, and sensilla trichodea T1), as well as one potential temperature/humidity receptor sensilla type (sensilla coeloconica X1), were identified across the seven firefly species in this study. Among the chemoreceptors the B7 sensilla type was shared by most species (except *Phausis* sp.) and found in both males and females (Table 2.3). Other chemoreceptor sensilla were shared by three firefly species (B1, B3), by two species (B2) or they were unique for a single species (B10, B11: *L. punctata*; B12, B13: Luciolinae sp.; T1: *Phausis* sp). A potential thermo/hygroreceptor sensilla type (X1) was shared by three firefly species (Table 2.3). There was no single chemoreceptor morphotype found exclusively in diurnal species, or shared by all diurnal and nocturnal species, and the different chemoreceptor types varied greatly between species (Table 2.3). Our comparisons of sensilla diversity based on effective numbers for Shannon and Simpson diversity indices showed no significant difference in antennal sensilla diversity between diurnal and nocturnal fireflies, no matter whether rare (Shannon's index effective numbers, t-Test; $\bar{x}$Diurnal=2.37±0.53, $\bar{x}$Nocturnal=2.37±0.86, df=13, p=0.99) or common (Simpson's index effective numbers, Wilcoxon rank-sum, $\bar{x}_{\text{Diurnal}}$ =1.62±0.41, $\bar{x}_{\text{Nocturnal}}$ =1.95±0.47, df=13, p=0.061) sensilla types were favored.

Sex differences in sensilla types

Almost all sensilla morphotypes were found in both males and females of their respective species, except for SC and B12 sensilla. SC sensilla were found in both sexes for the two *Photinus* species, *P. nigricans*, and Luciolinae sp., but they were only found in *L. punctata* females and *Ph. lucicrescens* males. B12 sensilla were exclusively found in Luciolinae sp. males (Table 2.3).

Evolution of sensilla morphotypes

Using the phylogeny of Martin et al. (2019) to extract the evolutionary relationships of the eight firefly species in six genera studied to date, it is possible to hypothesize the ancestral pattern of sensilla. B7 appears to be the ancestral sensillum for the common ancestor of all 8 firefly species and was subsequently lost in *Phausis* (Fig. 2.6). Both B1 and B3 sensilla most likely evolved in the common ancestor of *Pyropyga* and *Photinus* fireflies. Other sensilla types evolved independently in individual lineages, e.g., B2 evolved independently in *Photuris lucicrescens* and *P. corruscus*, B10 and B11 evolved within *Lucidota,* B12 and B13 evolved within Luciolinae sp., and T1 evolved within *Phausis*. X1 evolved independently in *Photuris*, *Pyropyga* and *P. corruscus;* alternatively, it could have evolved in *Photuris* and the common ancestor of *Pyropyga* and *Photinus* and was subsequently lost in *Photinus pyralis.* For *Luciola* *cruciata*, unique pored sensilla chaetica, capitular sensilla and gemmiform sensilla are reported in the literature (Iwasaki et al., 1995).

Sensilla counts and densities

Total sensilla counts were positively correlated with antennal area across the seven firefly species (N=42, R^2 =0.389, Spearman's rho =0.6677, p<0.0001; Fig. 2.7A), which means larger antennae had more sensilla. In contrast, sensilla densities were negatively correlated with antennal area (R^2 = 0.542, Spearman's rho= -0.7094, df = 1, p<0.0001, Fig. 2.7B), which means that larger antennae had fewer sensilla per area than smaller antennae. As a result, for our comparative analyses we used both (1) the total sensilla counts and densities on an individual firefly antenna, reflecting the sensory potential for the respective firefly, and (2) the residuals from a regression of the total number of sensilla (or sensilla density) versus antennal area, representing the deviation of that firefly (given its total antennal area) in sensilla counts (or densities) from the common regression line.

The total antennal sensilla counts ($\bar{x}$ ± stdev) for a single firefly antenna ranged from 49 ± 7 total sensilla per antenna in *Phausis* sp. females to 6984 ± 301 sensilla in *Lucidota punctata* males (Table 2.2). Sensilla densities in our species sample ranged from an average of 1090 ± 235 per mm^2 in *Ph. lucicrescens* females to an average of 5955 ± 803 per mm^2 in *Phausis* sp. males (Table 2.2).

Diurnal fireflies had significantly more antennal sensilla than nocturnal fireflies (Wilcoxon rank-sum test, N=18,24, S=565, Z=4.51, p<0.0001; Fig. 2.8A), but the sensilla densities of diurnal (N=18, $\bar{x}$ =3392±907 per $mm^{2;}$ Figure 6A) and nocturnal (N=24, $\bar{x}$ =2949±1766 per mm^2) fireflies did not differ significantly from each other (Wilcoxon rank-sum test, S=441, Z=1.36, p=0.1739; Fig. 2.8B). When taking the total antennal area of the fireflies into account (residuals), diurnal fireflies had significantly more sensilla than nocturnal fireflies (Wilcoxon rank-sum test: N=18,24, S=572, Z=4.689, p<0.0001), and a higher sensilla density for a given total antennal area (Wilcoxon rank-sum test: S=481, Z=2.376, p=0.0175).

Males (N=21, $\bar{x}$=3776±1919) and females (N=21, $\bar{x}$=2995±1638) significantly differed in their total sensilla counts (Wilcoxon signed-rank test: N=21, S= -56.0, p=0.049; Fig. 2.9A), but not in their sensilla densities ($\bar{x}_{\text{Male}}$ =3414±1720 per mm^2, $\bar{x}_{\text{female}}$ =2864±1128 per mm^2; Wilcoxon signed-rank test: N=21, S=42.5, p=0.14; Fig. 2.9B). Given their antennal area, males had relatively higher sensilla counts (Wilcoxon signed-rank test: N=21, S=-62.5, p=0.026), and also higher densities than their females (Wilcoxon signed-rank test: N=21, S=64.5, p=0.021).

The total sensilla counts included counts of three types of mechanoreceptors, nine types of chemoreceptors and one possible thermo-/hygro- receptor (Tables 2.4, 2.5). Overall, there were many more mechanoreceptors (in 1000s) than chemoreceptors (in 100s) on individual firefly antennae. *Phausis* had lower numbers (in 10s) for both sensilla types, but still ~4 times more mechanoreceptors than chemoreceptors (Table 2.2). Potential thermo-/hygro- receptors (X1) occurred in only a few individuals and, if present, in low numbers (N=1-22; Table 2.6). To test our predictions that males should have more chemoreceptors (including pheromone receptors) than females, and that diurnal species should have more chemoreceptors than nocturnal species, we conducted a comparative analysis of mechanoreceptors (no differences expected) and chemoreceptors (differences expected).

Sex Differences in Sensilla Counts and Densities

Across diurnal and nocturnal fireflies, females and males did not differ significantly in their total numbers of mechanoreceptors on their antennae ($\bar{x}_{\text{Female}}$ = 2419±1418, $\bar{x}_{\text{Male}}$ = 2586±1248, Wilcoxon signed-rank (paired), S = -48.0, df = 20, p = 0.10: Fig. 2.10A). However, males did have significantly more chemoreceptors than their females ($\bar{x}_{\text{Male}}$ = 1188±1041, $\bar{x}_{\text{Female}}$ = 573±307; Wilcoxon signed-rank: S = 77.0, df = 20, p = 0.0043; Fig. 2.10C). The two sexes also did not differ significantly in overall mechanoreceptor density ($\bar{x}_{\text{Female}}$ = 2306±915 per mm^2, $\bar{x}_{\text{Male}}$ = 2265±1102 per mm^2, Wilcoxon signed-rank, S= 4.5, df = 20, p = 0.88; Fig. 2,10B), but males had a significantly higher density of chemoreceptors on their antennae than females ($\bar{x}_{\text{Male}}$ = 1147±952 per mm^2, $\bar{x}_{\text{Female}}$ = 556±273 per mm^2; Wilcoxon signed-rank, S = -104.5, df = 20, p < 0.0001; Fig. 2.10D).

Both mechanoreceptor (R^2=0.313) and chemoreceptor (R^2=0.223) sensilla counts were positively correlated with antennal area, which means larger antennae had more sensilla of each type. Considering the total antennal area of individual fireflies (residuals), females and males did not significantly differ in the number of their mechanoreceptors (Wilcoxon signed-rank: N= 21,21; df=20, S=-19.5, p=0.51; Fig. 11.2A), but males had significantly more chemoreceptors on their antennae, given their antennal area (Wilcoxon signed-rank: df=20, S=94.5, p=0.0002; Fig.11.2B).

Both mechanoreceptor (R^2= 0.549) and chemoreceptor (R^2= 0.199) sensilla densities were negatively correlated with antennal area; this means that smaller antennae tended to have higher densities of both sensilla types. Considering the total antennal area of individual fireflies (residuals), females and males did not significantly differ in their mechanoreceptor densities (Wilcoxon signed-rank: df=20, S=31.5, p=0.28; Fig. 2.11C), but given their antennal area, males had significantly higher chemoreceptor densities on their antennae than females, (Wilcoxon signed-rank: df=20, S=-82.5, p=0.0018; Fig. 2.11D).

Sensilla of Nocturnal and Diurnal Fireflies

Diurnal fireflies ($\bar{x}_{\text{Diurnal}}$= 3503 ± 1753) had significantly more mechanoreceptors on their antennae than nocturnal fireflies ($\bar{x}_{\text{Nocturnal}}$ = 1753 ± 1009, Wilcoxon rank-sum, S = 565, Z=4.51, df = 41, p< 0.0001; Fig. 2.12A). In contrast, there was no significant difference in mechanoreceptor densities between diurnal and nocturnal fireflies ($\bar{x}_{\text{Diurnal}}$= 2576/mm^2 ± 827. $\bar{x}_{\text{Nocturnal}}$ = 2068/mm^2 ± 1079), Wilcoxon rank-rum, S = 451, Z=1.61, df=41, p = 0.11; Fig. 12.2B). Relative to their total antennal areas diurnal fireflies tended to have more mechanoreceptors on their antennae (Wilcoxon rank-sum test of residuals: S=571, Z=4.66, p<0.0001) and also a higher mechanoreceptor density than nocturnal fireflies (Wilcoxon rank-sum test: S=481, Z=2.37, p=0.0175).

Since males and females differed significantly in their chemoreceptor counts, the two sexes were tested separately for differences in chemoreceptor counts and densities between diurnal and nocturnal fireflies. Diurnal females ($\bar{x}_{\text{Diurnal}}$ = 660 ± 259) did not differ significantly from nocturnal females ($\bar{x}_{\text{Nocturnal}}$ = 507 ± 334) in total number of chemoreceptors (Wilcoxon rank-sum, S = 110, Z=0.75, df = 41, p = 0.46; Fig. 2.13A), but diurnal males ($\bar{x}_{\text{Diurnal}}$ = 1862 ± 1326) had significantly more chemoreceptors than nocturnal males ($\bar{x}_{\text{Nocturnal}}$ = 801 ± 213, Wilcoxon rank-sum, S = 139, Z=2.81, df = 41, p = 0.005; Fig. 2.13C). In contrast, diurnal and nocturnal females ($\bar{x}_{\text{Diurnal}}$ = 559/mm^2±317, $\bar{x}_{\text{Nocturnal}}$ = 523/mm^2±243, Wilcoxon rank-sum, S = 109, Z=0.68, df = 41, p = 0.50), or diurnal and nocturnal males ($\bar{x}_{\text{Diurnal}}$ = 1027/mm^2±548, $\bar{x}_{\text{Nocturnal}}$ = 1236/mm^2±1187, Wilcoxon rank-sum, S = 110, df = 41, p = 0.46), did not significantly differ in their chemosensilla densities (Fig. 2.13B,D), Relative to their total antennal areas (residuals) diurnal and nocturnal females did not differ in their chemoreceptor sensilla counts (Wilcoxon rank-sum, females: N=9,12: S = 119, Z=1.38, p = 0.16) or chemoreceptor densities (Wilcoxon rank-sum test, females: N=9,12: S = 95, Z=-0.25, p = 0.8). There was also no significant difference between diurnal and nocturnal male chemoreceptor counts (Wilcoxon rank-sum: N=12,9, S = 125, Z=1.81, p = 0.07) or densities when their total antennal areas were considered (Wilcoxon rank-sum: N=12,9, S=104, Z=0.319, p=0.75).

Sensilla Distribution on Firefly Antennae

To determine which portions of firefly antennae may be used for mechanoreception and chemoreception, we examined the distribution of sensilla numbers across antennomeres, for mechanoreceptors, chemoreceptors and for each sensilla morphotype. Mechanoreceptors were most abundant at the base of the antenna. C1 mechanosensilla occurred in high numbers on the scape, dropping to the lowest numbers on the pedicel (the second and smallest antennomere) in all species, except *Phausis* sp.(Fig. 2.14G). C1 sensilla sharply increased in number from the pedicel to the third antennomere and occurred at similar numbers between antennomeres 4 and 10 for all species. Numbers further increased in *P. corruscus*, *L. punctata*, and *P. pyralis* on antennomere 11 (Fig. 2.14A-F). C2 sensilla were exclusively located on the scape and pedicel in all species, with about twice as many C2 sensilla found on the scape (Fig. 2.14H-N). SC sensilla were absent in *Phausis* and in some sexes of other species (Fig. 2.14O-T). SC sensilla were found on all 11 antennomeres but occurred in low numbers and were not evenly distributed. The antennomeres with the highest SC numbers differed between individuals of the same species and between species.

In contrast, chemosensilla were entirely absent from the scape and pedicel. They tended to be most abundant in the middle of the firefly antennae. B1 sensilla increased in number between antennomeres 3 to 6 and then decreased from antennomere 6 to 10 in *P. corruscus* and *P. pyralis*. In contrast, the distribution of B1 sensilla in *Py. nigricans* were overall consistent across antennomeres 3-8 (Fig. 2.15A-C). The number of B2 sensilla also increased between antennomeres 3 and 6 and then decreased from antennomere 6 to 11 (Fig. 2.15D-E). B3 sensilla numbers varied between antennomeres 3 to 8 and gradually decreased in number in each subsequent antennomere (Fig. 2.15F-H). B7 sensilla were found on antennomere 3 in all species except *P. pyralis*. B7 numbers varied between antennomeres 3 to 10 with a sharp increase on antennomere 11 in all species, except *P. pyralis* which had a sharp decrease in numbers on antennomere 11 (Fig. 2.15I-N). B10 sensilla (*Lucidota punctata*) were most abundant between antennomeres 4 and 6 (Fig. 2.16A), slowly decreasing in number towards the distal antennomere; males have about twice as many B10 on each antennomere (peak average of 99 sensilla on antennomere 4) compared to their females (peak average of 56 on antennomere 6). B11 sensilla (*Lucidota punctata*) were rare on female antennae with most (average 9 sensilla) located on the last antennomere; in contrast males consistently averaged between 300 and 350 B11 sensilla on antennomeres 3-11 (Fig. 2.16B.). B12 sensilla (Luciolinae sp.) were present in low numbers on antennomeres 3-10 of male antennae, with the highest number (average 2.7 sensilla) on antennomeres 3 and 4 and the lowest number (average 0.33 sensilla) on antennomere 5 (Fig. 2.16C). B12 sensilla were absent in females. B13 sensilla (Luciolinae sp.) were present on antennomeres 3 to 11 on male antennae, with the highest number on antennomere 5 (average 81 sensilla), then slowly declining in numbers to antennomere 11 (average 17 sensilla; Fig. 2.16D). In parallel to males, B13 sensilla of females were present on antennomeres 3 to 11, with the highest number on antennomere 5 (average 71 sensilla), slowly declining to antennomere 11 (average 14 sensilla; Fig. 2.16D). T1 sensilla (*Phausis* spec.) were present on the 2nd antennomere (of 3) on female antennae (3-10 sensilla each; Fig. 2.16E). *Phausis* males had T1 sensilla on antennomeres 3-11, and they were evenly distributed (ranging on average 55-70 sensilla on each antennomere) between antennomeres 3 to 11 (Fig. 2.16E). X1 sensilla were only found in three species: *P. corruscus, Pyropyga nigricans*, and *Photuris lucicrescens*, and only on individual fireflies. In *Pyropyga nigricans* and *P. corruscus* they were present exclusively on the ventral side of the antenna, and on both dorsal and ventral sides in a single female *Photuris lucicrescens*.

Discussion

We report here a total of 14 sensilla morphotypes across seven species of Lampyridae. Twelve of these morphotypes are new for Lampyridae. The two other morphotypes were previously reported for *Luciola cruciata* (Iwasaki et al., 1995), along with 5 morphotypes not found in our study species. These 5 morphotypes include different variants of sensilla campaniform, sensilla basiconica, sensilla trichodea, and two additional sensilla categories: capitular sensilla and gemmiform sensilla, the latter being a new type for beetles. This results in a total of 19 recorded sensilla morphotypes for 8 species of Lampyridae and suggests that with the further addition of firefly taxa new morphotypes await to be discovered. The majority of these 19 morphotypes are also found in other beetle groups, except for gemmiform sensilla in *L. cruciata* and B10 and B11 sensilla of *Lucidota punctata*, which are reported and described for the first time here. These B10 and B11 sensilla are unique in their wide-collared base and the shape of their peg (Fig. 2.4F).

In contrast to the more diverse chemoreceptors, there are only three types of mechanoreceptors on firefly antennae (two variants of sensilla chaetica and one sensilla campaniform). These tend to occur in much greater numbers (in 1000s) than chemoreceptors (in 100s; Table 2.2), and the three mechanoreceptor types are widely distributed across fireflies. For example, both males and females of all eight firefly species studied so far have sensilla chaetica types C1 and C2 on their antennae. C1 mechanosensilla are present on all 11 antennal segments

(Figure 2.14A-F). They tend to occur in high numbers on the scape, drop in numbers on the pedicel (the smallest antennomere in all species, with the exception of *Phausis* sp. males and females), then sharply increase in numbers on antennomere 3, and remain at relatively high numbers on the other antennomeres. This places C1 sensilla in a good position to process mechanical stimuli from the environment along the entire length of the antennae. C1 sensilla are also consistently found in in closely related Elateridae species, in more distantly related beetles (e.g., Chrysomelidae and Carabidae), and across Insecta (e.g., Blattodea, Hemiptera) in general (Callahan, 1975; Ritcey & McIver, 1990; Merivee et al., 2002; Fukuda et al., 2016; Nowińska1& Brożek, 2017; Faucheux et al., 2020). Like in other elaterid groups (Faucheux & Kundrata, 2017; Faucheux et al., 2020), the C2 sensilla of fireflies were exclusively found on the first two antennomeres (scape and pedicel), where the major muscles for antennal movement are located (Nunes et al., 2020), supporting their function in antennal proprioception (Schneider, 1967; Merivee et al., 1999; Gonzaga-Segura et al., 2013).

The third type of mechanoreceptor sensilla, sensilla campaniform (SC sensilla), is relatively rare in fireflies. Six of the eight firefly species studied to date (except *Phausis* sp. and *L. cruciata*) have SC sensilla (Table 2.3), but they were absent in *Lucidota punctata* males and *Photuris lucicrescens* females. This could be possibly due to the overall low numbers of SC sensilla on firefly antennae (a single SC sensillum was found on the antenna of *P. corruscus* and Luciolinae sp.). SC sensilla were most prevalent in *P. pyralis* and *Py. nigricans* (Table 2.4). SC sensilla are commonly found on insect legs and insect wings and typically function as strain sensors within the exoskeleton (Dickerson et al., 2021). When stimulated, SC sensilla have been found to trigger muscle contraction for stability or propulsion (Zill et al., 2004), however, whether SC sensilla on insect antennae could stimulate the antennal support muscles in the scape and pedicel remains to be tested.

With nine sensilla morphotypes, chemoreceptors are much more diverse than mechanoreceptors on firefly antennae, and their distribution across species is unexpectedly variable (Table 2.3). Chemoreceptor sensilla were absent from the scape and pedicel of firefly antennae, but present on all other antennomeres (3-11), with the exception of *Phausis* sp. females. They tend to be most numerous in the middle and/or the distal end of the antenna (Figure 2.15). The chemoreceptor sensilla type shared by most species (except *Phausis* sp.) were B7 sensilla, which are found in both male and female fireflies, but in very low numbers (Table 2.5). Their function in fireflies is unknown, but B7-like sensilla in Cerambycidae and Curculionidae are hypothesized as thermo-/hygro- receptor sensilla, based on their internal cell structure (Hallberg, 1982; Zhang et al., 2011).

The other chemoreceptor sensilla types were shared by three firefly species (B1, B3), two firefly species (B2 sensilla), or they were unique for a single species (B10, B11, B12, B13, T1). Sensilla basiconica B1, B2, B3, and B7 are also present in Elateridae (Faucheux et al. 2020), in Chrysomelidae (Bartlet et al., 1999), and Cerambycidae (Di Palma et al., 2019). Most sensilla basiconica are hypothesized to be olfactory sensilla based on the presence of pores (Zacharuk & Shields, 1991). B1 and B2 like sensilla were hypothesized as olfactory sensilla in Chrysomelidae (Ma et al., 2022). B3 sensilla have been hypothesized as olfactory sensilla in both Cerambycidae: *Xylotrechus* and Elateridae: *Tetrigus* (Chen et al., 2014; Ren et al., 2014). B7 sensilla is hypothesized as either olfactory or thermoreceptive sensilla in Cerambycidae: *Aromia* (Di Palma et al., 2019).

Sensilla trichodea (T1) detect sex pheromones in pine weevils (*Hylobius abietu*, Curculionidae; Mustaparta et al., 1975) and are the only chemosensilla in *Phausis* fireflies. Whether they are used by *Phausis* to sense pheromones (De Cock et al., 2014) remains to be tested. Sensilla coeloconica (X1) have been identified as temperature sensors in *Siagona europaea* (Carabidae; Giglio et al., 2010). They are present at low numbers on the antenna of *Photinus corruscus*, *Pyropyga nigricans*, and *Photuris lucicrescens* and it remains to be tested whether the X1 neurons in these species respond to temperature or humidity stimuli. Interestingly, X1 sensilla are restricted to the ventral antennal surface of *Photinus corruscus* and *Pyropyga nigricans* and the functional significance of this sensilla distribution remains unknown. Sensilla coeloconica are also hypothesized as thermoreceptors in Diptera (Hu et al., 2010).

Evolution of sensilla types

Given our taxon sampling of 8 taxa in 6 genera and their phylogenetic relationships (Martin et al., 2019), it appears that mechanoreceptor sensilla types are relatively conserved and shared by all (C1) or almost all (C2, SC) firefly species studied to date. In contrast, their chemoreceptor sensilla types are much more diverse and only a single sensilla type (B7) seems to be inherited from the common ancestor of all our study species (Figure 4). The spotty distribution of chemosensilla types across firefly genera suggests a high evolutionary lability of chemoreceptor sensilla types among the six firefly genera in our study. However, these genera are relatively distantly related and belong to three different lampyrid subfamilies. On the other hand, the two *Photinus* species shared many of the same sensilla types, despite their different (diurnal/nocturnal) activity times. To further investigate this seeming evolutionary lability of chemoreceptor sensilla types across genera and the conservation of sensilla types within a genus, more species in each genus need to be studied, as well as additional genera that are closely related to our study taxa (Martin et al., 2019).

Optimizing sensitivity

Antennal sensitivity to relevant environmental stimuli can be increased through additional sensilla, thus increasing the probability of capturing physical or chemical stimuli. This can be achieved by increasing antenna size (surface area) or by packing sensilla more tightly (density). In fireflies antennal sensitivity (sensilla numbers) seems to be mainly increased by increasing the antennal surface area. Larger antennae also have the advantage of sampling a larger airspace, which increases the probability of detecting the pheromones of a conspecific female (e.g., Johnson et al., 2017). Not surprisingly, firefly males have larger antennae (relative to their body size) and significantly higher total sensilla counts compared to their females. The evolution of larger firefly antennae for improved sampling area seems to be supported by the negative correlation between antennal area and sensilla density: as firefly antennae increase in size, sensilla numbers do not increase in a 1:1 relationship with antennal area, resulting in a lower density. However, there may be other limiting factors for sensilla density on firefly antennae, such as limited space in the antennomeres that supports a limited supply system for the receptor neurons in each sensillum (Schneider, 1964).

The antenna areas of the diurnal and nocturnal species in our study greatly overlapped and did not significantly differ, however, we cannot rule out that this may be due to our relatively small species sample and lack of statistical power. A previous large-scale analysis of eye and antenna size in 101 firefly taxa showed that the males of diurnal firefly taxa (N= 26) tend to have significantly longer antennae (and smaller eyes) than the males of nocturnal taxa (N=75; Stanger-Hall et al., 2018) with activity time (and type of mating signals) accounting for

13% of the observed variation in male antenna size, while phylogenetic relatedness (genus) accounted for 63% (Stanger-Hall et al., 2018). Similarly, in a phylogeny-based analysis of 43 North American firefly species antenna length (phylogenetic signal lambda=0.859) was significantly correlated with body size (pronotum length, $p<0.0001$) and activity time (mating signal: $p=0.037$). Combined with the results from the present study, it appears that the evolution of a larger antenna size is an important factor for the increased sensilla counts in diurnal fireflies. However, when fireflies change their activity time from nocturnal to diurnal over evolutionary time, and along with it from bioluminescent signals to the exclusive use of pheromones as mating signal, the selection on antenna size seems to be relatively weak, resulting in a relatively slow change in antenna size when compared to changes in eye size (Stanger-Hall et al., 2018).

Stanger-Hall et al. (2018) found that the first response to selection for improved pheromone detection may occur through sensilla numbers instead, resulting in an increased sensilla density on relatively short antennae. An analysis of our data for *P. pyralis* and *P. corruscus* shows that this is indeed the case. The diurnal firefly *P. corruscus*, which belongs to a clade of fireflies (formerly *Ellychnia*: Zaragoza-Caballero et al., 2020) that split from their common ancestor with nocturnal *Photinus* fireflies ~13 and 25 mya (Höhna et al., 2021) or ~6-12 mya (Powell et al., 2022) has significantly shorter antennae (scaled with body size: Wilcoxon rank-sum test: $S=56$, $Z=2.64$, $p=0.008$) than the nocturnal *P. pyralis*, but it has significantly more sensilla (Wilcoxon rank-sum test: $S=21$, $Z=-2.80$, $p=0.005$) and a significantly higher sensilla density (Wilcoxon rank-sum: $S=21$, $Z=-2.8$, $p=0.005$). This is due to significantly higher mechanosensilla numbers ($\bar{x} = 4506 \pm 456$ versus $\bar{x} = 3015 \pm 222.5$, Wilcoxon rank sum $N=6,6$, $S=21$, $Z=-2.8$, $P=0.005$) and densities ($\bar{x} = 2851 \pm 352$ per mm^2 versus $\bar{x} = 1381 \pm 263$ per mm^2; Wilcoxon rank sum $N=6,6$, $S=21$, $Z=-2.8$, $P=0.005$). Interestingly, the total chemosensilla counts of *P. corruscus* and *P. pyralis* did not differ (N=6,6, x̄= 794±154 versus x̄ = 806±155; Wilcoxon rank sum N=6,6, S=41, Z=0.24, P=0.81), but *P. corruscus* (with its shorter antennae) has a significantly higher chemosensilla density (x̄= 504±109 per mm^2 versus x̄= 365±65 per mm^2; Wilcoxon rank sum N=6,6, S=25, Z=-2.16, P=0.031). Given their similar chemoreceptor numbers, this raises the question whether (or to what degree) *P. pyralis* may still utilize pheromones during mate search.

In the present study diurnal fireflies (males and females) had significantly more mechanoreceptors on their antennae than nocturnal fireflies, and thus a much higher sensitivity for mechanical stimuli. In diurnal fireflies this was further optimized by a higher than expected mechanoreceptor sensilla density (given their antenna size). This finding was unexpected, since both diurnal and nocturnal fireflies need to navigate through their environment during mate search, to deposit fertilized eggs, and from and to their daily resting places. The males and females of diurnal (or nocturnal) fireflies in our study did not differ significantly in their mechanoreceptor counts, suggesting that the selection for high mechanoreceptor counts in diurnal fireflies is not driven by one sex. In contrast, firefly males had significantly more chemoreceptor sensilla on their antennae and a higher chemoreceptor density than their females. As predicted based on their exclusive reliance on pheromones during mate search, diurnal males had significantly more chemoreceptor sensilla on their antennae than nocturnal males, and thus a significantly higher sensitivity for chemical stimuli (including pheromones), while diurnal and nocturnal females did not differ from each other.

Identification of pheromone sensilla candidates in fireflies

Diurnal firefly species had three to five different chemoreceptor sensilla types on their antennae, while nocturnal species had one or three (two types were reported for the nocturnal species *L. cruciata*: Iwasaki et al. 1995). This trend directly reflects the importance of chemoreceptors for diurnal species and is further supported by a significantly higher number of chemoreceptors in diurnal males compared to nocturnal males (Figure 8C-D). However, the absence of a specific chemoreceptor sensilla morphotype found exclusively in diurnal firefly species suggests that pheromone sensilla may not be unique for diurnal species. If pheromone sensilla are not unique to diurnal species, the most likely pheromone sensilla candidate is B7, a grooved peg sensilla type that is shared by 6 of the 7 species in our study (except *Phausis* sp.). However, B7 sensilla were found in relatively low numbers compared to other chemoreceptors and with inconsistent differences between males and females (Table 2.4). Furthermore, B7 sensilla may be used as thermoreceptors and/or hygroreceptors, as is known for other grooved peg sensilla (Altner and Prillinger, 1980; Altner et al., 1977; Zacharuk, 1985; Di Palma et al., 2019). If this were indeed the case for fireflies, B7 would serve an essential function for the survival of these soft-bodied and thus easily dehydrated beetles. However, *L. cruciata* fireflies are using their unique capitular sensilla for that purpose (Iwasaki et al., 1995), which raises the question what their B7 sensilla are used for. If *Phausis* is able to sense temperature or humidity with antennal sensilla, it would be using T1 sensilla, its only non-mechanoreceptor sensilla type. All these possibilities await future functional testing.

The absence of universal "firefly pheromone sensilla" for diurnal species, or for both diurnal and nocturnal species and the limited overlap of the different chemoreceptor sensilla types between genera, suggests that pheromone sensilla, if present, may be genus- or even species-specific. Therefore, we applied our predictions for pheromone sensilla candidates to the most abundant chemical sensilla morphotypes found in each species (Table 2.4) to propose specific sensilla types for future testing. B1 sensilla are the most abundant chemoreceptors in diurnal *P. corruscus* fireflies and males have almost twice as many B1 than their females. Males have on average ~50 B1 sensilla on most of their antennomeres (antennomeres 4-10), but B1 numbers peak to an average of ~75 sensilla on the distal (11) antennomere (Figure 15), enabling them to sample a large airspace with their two antennae and increasing their chances to catch molecules from the female pheromone plume. Most importantly, basiconica sensilla that look most like the B1 sensilla in our study were identified through electro-antennograms as pheromone sensilla in diurnal *P. corruscus* (Lower et al., 2023). However, pores, if present, are not visible in our SEM images; this could possibly be due to the gold-coating obstructing small pores (e.g., Joel et al., 2018). Based on their numbers, B2 and B3 sensilla could be two other pheromone sensilla candidates, but in both cases females have more B2 and B3 sensilla than males. Furthermore, sensilla similar in morphology to B3 sensilla were unresponsive to *P. corruscus* sex pheromones (Lower et al., 2023), confirming that B3 sensilla are not used for the detection of sex pheromones in this species.

The nocturnal *P. pyralis,* a closely related congener of *P. corruscus* in this study, also has high numbers of B1 sensilla, and males have ~ 30% more B1 sensilla than their conspecific females (Table 2.4), suggesting that both *Photinus* species may use B1 sensilla as pheromone sensilla. However, in contrast to *P. corruscus*, in *P. pyralis* males most B1 sensilla (>50/antennomere) are located in the center of their antennae (4-9; Figure 2.15C). The next closest relative to these two *Photinus* taxa in our study is *Pyropyga nigricans,* a diurnal firefly species. It also has B1 sensilla, possibly inherited from a common ancestor with *Photinus* fireflies (Figure 2.6, Martin et al., 2019), however at very low numbers (~5/antennomere) and, contradictory to our pheromone sensilla predictions, females have twice as many B1 sensilla than males. This makes B1 sensilla an unlikely pheromone sensilla candidate for *Pyropyga*. In contrast, pored B3 sensilla are present in great numbers (>100/antennomere on 3-11; Figure 2.15G), and *Py. nigricans* males have ~1.5 times more B3 sensilla on their antennae than their females (Table 2.4), suggesting B3 as pheromone sensilla candidate for *Py. nigricans*.

The species-specific B10 and B11 sensilla make good candidates as pheromone sensors for the diurnal species *L. punctata*. Males have ~1.5 times more B10 sensilla and ~100 times more B11 sensilla than their conspecific females. In addition, B10 sensilla have obvious pores. B10 sensilla are found in the highest numbers between antennomere three and eight in both males and females, while B11 sensilla are found in equal numbers across antennomeres three to eleven in both males and females, but females have a slight increase in B11 sensilla on their distal antennomere (11). In the nocturnal Luciolinae species, the species-specific B12 and B13 sensilla are both candidates as pheromone receptors. B12 were found in low numbers (13±3) exclusively on the antennae of males between the pedicel and antennomere 9. B13 sensilla occur in much higher numbers in males (488±29 per antenna) but are also present in just slightly lower numbers (442±29) on the antenna of females, with the highest numbers on antennomere 5 in both sexes (Fig 2.16: distribution B12 and B13). Based on our prediction of sexual dimorphism this makes B12 a slightly more likely pheromone sensilla candidate, however the very low numbers would suggest that pheromones do not play an important role for the Luciolinae species. Alternatively, based on total sensilla numbers (antennal sensitivity), B13 would be a good pheromone candidate, if pheromones are indeed used by *Luciolinae* sp., which remains to be studied.

The only chemoreceptor sensilla found in nocturnal *Phausis sp*. were species-specific T1 sensilla. These were found in much greater numbers (~50x) in *Phausis* sp. males compared to their females, making T1 sensilla the likely (and only) candidate for pheromone detection in this species (De Cock et al., 2014). In nocturnal *Ph. lucicrescens*, B2 sensilla are the most prevalent chemoreceptor type, but females have ~100 times more B2 sensilla than their males. This either suggests that nocturnal *Ph. lucicrescens* rely exclusively on bioluminescence and therefore do not have any pheromone sensilla, alternatively B2 sensilla may serve more than one function. The predatory females of *Ph. lucicrescens* may use their B2 sensilla to recognize prey species, specifically *Photinus* fireflies, which sequester lucibufagins (Eisner et al., 1997; Gonzalez et al., 1999), potent defense chemicals. The greater abundance of B2 sensilla in *Ph. lucicrescens* females compared to their conspecific males may aid these predatory females to identify captured *Photinus* prey (via cuticular hydrocarbons or other low-volatile chemicals). Whether this is indeed the case, and whether the same sensilla are also used by males to detect female pheromones (if any), remains to be tested.

The intensive antennation behavior that precedes mating in fireflies, led us to hypothesize the sampling of chemical cues by gustatory (contact) chemoreceptors to verify a conspecific mate immediately before mating. Based on this reciprocal behavior we predicted no sex differences in sensilla that pick up on these cues. We presently do not know (1) to what extent individual firefly species use contact chemicals during mating, and (2) whether the same sensilla could function both as pheromone sensilla (olfactory receptors stimulated by high-volatile chemicals) and gustatory sensilla (taste receptor cells stimulated by low-volatile contact chemicals on the cuticle or released during mating), or whether different sensilla types process these different stimuli. For example, the same sensilla type could be used to process both stimuli with specialized olfactory and gustatory receptors on their sensory neurons at the base of the sensillum (Gomez-Diaz et al., 2019). In this case, we would predict a less pronounced sexual dimorphism in candidate sensilla numbers compared to sensilla that function exclusively as pheromone sensilla. Alternatively, if different sensilla types are used, we would predict one chemosensilla type with a pronounced sexual dimorphism (olfactory pheromone sensilla) and a second chemosensilla type (gustatory contact chemical sensilla) without sexual dimorphism, or even skewed towards females, because they incur a larger cost for a mating mistake. Based on these criteria, we propose the following candidates for future functional testing as gustatory sensilla: For separate (specialized) gustatory sensilla we propose B2 or B3 for *Photinus corruscus*, B3 for *P. pyralis*, B1 for *Py. nigricans*, B10 for *L. punctata*, and B13 for Luciolinae sp. If no pheromones are used by *Photuris lucicrescens*, B2 would test as a gustatory sensilla, with a sexual dimorphism based on predatory behavior of females. However, if pheromones are used by *Photuris lucicrescens*, B2 sensilla represent a potentially mixed sensilla type that processes both female pheromones and gustatory prey signatures in this species. For *Phausis* sp. with a single chemosensilla type, T1 sensilla would both function as pheromone sensilla, as well as gustatory sensilla used by males during their antennation behavior (De Cock et al., 2014).

As an important next step in understanding how fireflies perceive the world through their antennae and how this influences their behavior, all these pheromone and gustatory sensilla candidates need to be tested in functional studies. One key question in this context is the extent to which the pheromone sensilla candidates remain functional in nocturnal firefly species. Our morphological data suggest that B1 sensilla are pheromone sensors in both *Photinus* species: *P. pyralis*, a nocturnal bioluminescent species, and *P. corruscus*, which split from nocturnal *Photinus* ~6-25 mya (Höhna et al., 2021; Powell et al., 2022) and returned to diurnal activity with the exclusive use of pheromones for mate search (Stanger-Hall et al., 2007; Stanger-Hall & Lloyd, 2015). Other repeated losses of bioluminescence and reversals to mate search exclusively with pheromones during firefly evolutionary history (Branham & Wenzel, 2003; Stanger-Hall et al. 2007), suggest that pheromone sensilla may remain functional at least in some nocturnal species, especially in those clades with recent reversals to diurnal activity, facilitating the switch from nocturnal to diurnal activity. If functional in nocturnal species, pheromones would increase mating opportunities, because they would attract and direct males towards the bioluminescent display sites of females and facilitate the location of a conspecific mate by visual cues over shorter distances, especially if the environment is not conducive to visually finding females.

For example, considering their antennal areas, the males of diurnal *Lucidota* and of nocturnal *Phausis* both have relatively high chemosensilla (but not mechanosensilla) densities on their antennae. *Lucidota punctata* (5-6mm body length) and *Phausis reticulata* (6-9mm) are both relatively small fireflies and the males of both species search for conspecific females in the low vegetation on the forest floor, navigating around leaves to find their females (Faust, 2017). The diurnal *L. punctata* males have relatively large antennae, combined with a relatively high chemosensilla density to locate their tiny females in shady forests. The nocturnal *Phausis reticulata* (blue ghosts) males fly at night and glow while trying to locate their tiny flightless females. *Phausis* females emit a weak glow (visible within 10 feet, but almost invisible when moonlight is reflected from wet leaves: Faust, 2017) and *Phausis* males seem to use female pheromones to get close to females, and if they cannot locate the females by their weak glows, males may use their own glows as “spotlights” to locate their females (De Cock et al., 2014).

Why are chemosensilla so diverse across fireflies?

The individual firefly species in our study had between one to four different chemoreceptor sensilla types on their antennae. The number of types seem to be correlated with diurnal or nocturnal activity, however, a puzzling insight from our study is the diversity of antennal sensilla types in fireflies, and how little overlap there is between genera. The adult fireflies in our study tend to survive for only a few weeks (except for the winter firefly *P. corruscus* which may live several months, Faust, 2012) for the sole purpose of mating, and diurnal and nocturnal species face very similar challenges: prevent dehydration, locate a conspecific mate, mate, and in the case of females, find a good egg deposition site. So why are their antennal chemosensilla so different? We identified three different pheromone sensilla candidates in our three diurnal firefly species (B1, B3, B10/B11), which raises the question of how it is possible that these different sensilla morphotypes converged on an apparently similar function?

In *Drosophila* trichoid sensilla are required for pheromone detection, while their basiconic sensilla mostly detect food-derived odors (Schmidt and Benton 2020). Similarly, trichoid sensilla respond to female pheromones in click beetles (Elateridae; Merivee et al., 1999) and in Asian longhorned beetles (Cerambycidae; Wei et al., 2018), while placoid sensilla detect pheromones in Japanese beetles (Scarabidae; Kim & Leal, 2000). In fireflies (Lampyridae) we identified trichoid sensilla as pheromone sensilla candidates for nocturnal *Phausis*, however, trichoid sensilla are absent in all other firefly species studied to date. Instead, sensilla basiconica are used for pheromone detection in the firefly *Photinus corruscus* (Lower et al., 2023). *P. corruscus* females emit (1*S*)-*exo*-3-hydroxycamphor (hydroxycamphor), which in single sensillum recordings elicited a neuronal response from a pheromone-sensitive olfactory sensory neuron in a basiconica sensillum on the male antenna (Lower et al., 2023). We identified this sensillum as sensilla basiconica type 1. A possible model for how pheromone detection can switch between major sensilla types is the Asian longhorned beetle *Anoplophora glabripennis* (Cerambycidae), whose pheromone consists of two components (Wei et al., 2018). Trichoid sensilla are the pheromone sensors that respond to both components, but basiconica sensilla respond to one of the two components (along with plant compounds that enhance male attraction). Even though there is no pheromone specific-info relayed by these basiconica sensilla, this observation suggests a possible mechanism for sensilla type switching as pheromone compounds diverge in closely related species. Similarly, in the moth *Ostrinia nubilalis* (Crambidae) pheromone receptors that respond to different pheromone compounds are split between two olfactory sensory neurons in two sensillum subtypes, which is thought to reflect an ongoing evolution of this sensillum type as two *O. nubilalis* strains diverge (Koutrompa et al., 2014). A similar mechanism could account for the diversification of B-sensilla in fireflies (Lampyridae) and the diversity of pheromone sensilla candidates across diurnal firefly species. Sensilla type switching may not be limited to fireflies, but reflect the evolutionary dynamics of pheromone signals and their receptors in insects in general; evolutionary studies with a broad taxon sampling across closely related species in different genera will be required to test this.

Conclusions

This study presents the most comprehensive description of the antennal sensilla of Lampyridae to date. We identified 12 new firefly sensilla morphotypes, for a total of 19 morphotypes documented now for 8 species in 6 genera. We documented these sensilla types for both males and females of our 7 study species. Mechanoreceptor sensilla were the most abundant sensilla on firefly antennae, but chemoreceptor sensilla were unexpectedly diverse. Fireflies increase the sensitivity of their antennae to environmental stimuli by increasing their antennal area, resulting in relatively larger antennae (Stanger-Hall et al., 2018) and this translates into more sensilla, as documented here. Diurnal fireflies had significantly more mechanoreceptors and a significantly higher mechanoreceptor density on their antennae than nocturnal fireflies. The males and females of fireflies in our study did not differ in mechanoreceptor counts or densities, suggesting that the selection for high mechanoreceptor counts in diurnal fireflies is not driven by one sex. But as predicted, males had significantly higher chemosensilla counts and densities than their females, underlining the importance of males and their chemoreceptors (including pheromone sensilla) for locating a conspecific female based on her pheromone plume. Similarly, reflecting their exclusive reliance on pheromones during mate search diurnal males had significantly more chemosensilla (but not higher densities) than nocturnal males, while diurnal and nocturnal females did not differ. An increase in chemoreceptor sensilla density may be utilized in male fireflies with relatively small antennae (e.g., *Phausis*), or where females are difficult to locate in their environment (e.g., *Phausis, Lucidota*). We did not identify a "universal pheromone sensilla" candidate for diurnal (and/or nocturnal) fireflies, but we used our predictions for pheromone sensilla to propose candidate sensilla types for the different species based on their respective sensilla numbers and morphology. These pheromone sensilla candidates will facilitate functional testing in future studies. Similarly, we identified potential candidates for gustatory recognition (if any) of conspecifics during antennation, which could be utilized by different species. It is currently not known whether gustatory chemicals are sampled during antennation or whether this behavior serves as purely physical stimulation in preparation for mating. While our study was limited to firefly species with filiform antennae, our study revealed an unexpected diversity of sensilla types in fireflies. Most of the antennal forms known for beetles (except clavate and plumose) occur within the firefly family Lampyridae (Ohba 1978, Nunes et al., 2020). We predict that future studies will uncover an even greater sensilla diversity across Lampyridae and also inform us whether antennal sensilla cover the entire surface of more complex firefly antennae or whether these enhanced 3-dimensional structures serve other functions, e.g., the direction of airflow across the antennae (Loudon & Davis, 2005). We propose both morphological and functional studies of antennal sensilla with a broad taxon sampling of closely related species across genera to illuminate the dynamics of sensilla evolution as pheromone blends diverge.

FIGURES, TABLES, AND LEGENDS

Table 2.1: Taxon sampling for SEM, sexual (mating) signal, and adult morphology, and SEM preparation method. BL: bioluminescence, LO: light organ

Species	Subfamily	Sexual Signal	Adult LO	SEM washing method
Photinus pyralis	Lampyrinae	BL: Flash	present	Ultrasonicator
Photuris lucicrescens	Photurinae	BL: Flash	present	KOH wash
Luciolinae sp.	Luciolinae	BL: Flash	present	KOH wash
Phausis sp.	Lamprohizinae	BL: Glow	present	KOH wash
Photinus corruscus	Lampyrinae	Pheromone only	absent	Ultrasonicator
Lucidota punctata	Lampyrinae	Pheromone only	absent	KOH wash
Pyropyga nigricans	Lampyrinae	Pheromone only	absent	KOH wash

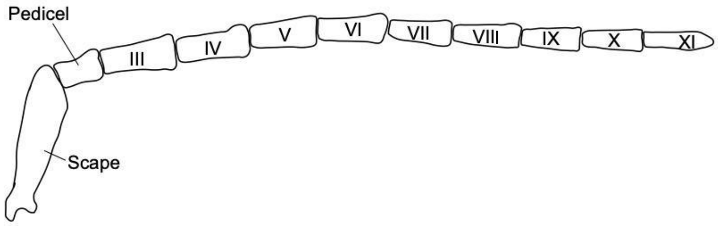


Figure 2.1: Adult firefly antennae with 11 antennomeres. Scape and pedicel represent the first two antennomeres.

Table 2.2: Sensilla counts (mean ± stdev) per antenna for all sensilla types, all mechanoreceptors, and all chemoreceptors of each species (F: 3 females, M: 3 males, D: diurnal, N: nocturnal).

Species	Sex	Active	All sensilla (N)	All mechano sensilla (N)	All chemo sensilla (N)	Pronotum length (mm)	Antennal area (mm^2)
P. corruscus	F	D	5239 ± 381	4481 ± 559	757 ± 193	2.8 ± 0.1	1.6 ± 0.2
	M	D	5361 ± 572	4531 ± 454	830 ± 133	2.5 ± 0.2	1.6 ± 0.5
Py. nigricans	F	D	4487 ± 566	3665 ± 388	822 ± 279	1.4 ± 0.3	1.1 ± 0.4
	M	D	3978 ± 1141	2800 ± 671	1178 ± 470	1.5 ± 0.3	1.5 ± 0.9
L. punctata	F	D	2535 ± 177	2133 ± 158	402 ± 21	1.3 ± 0.04	0.9 ± 0.1
	M	D	6984 ± 301	3405 ± 243	3579 ± 277	1.4 ± 0.2	2.3 ± 0.3
Ph. lucicrescens	F	N	2787 ± 164	1947 ± 116	840 ± 54	3.2 ±0.2	2.6 ± 0.6
	M	N	2710 ± 444	1988 ± 118	722 ± 240	2.8± 0.06	1.9 ±0.2
P. pyralis	F	N	3708 ± 285	2997 ± 190	711 ± 99	1.9 ± 0.1	2.0 ± 0.6
	M	N	3937 ± 354	3034 ± 292	903 ± 151	1.8 ± 0.3	2.5 ± 0.2
Luciolinae sp.	F	N	2143 ± 152	1675 ± 135	468 ± 16	1.4 ± 0.1	0.5 ± 0.05
	M	N	2307 ± 131	1789 ± 68	517 ± 29	1.1 ± 0.1	0.6 ± 0.02
Phausis sp	F	N	49 ± 7	39 ± 4	10 ± 4	0.8 ± 0.06	0.02 ± 0.00
	M	N	1131 ± 114	559 ± 115	572 ± 24	0.9 ± 0.1	0.2 ± 0.04

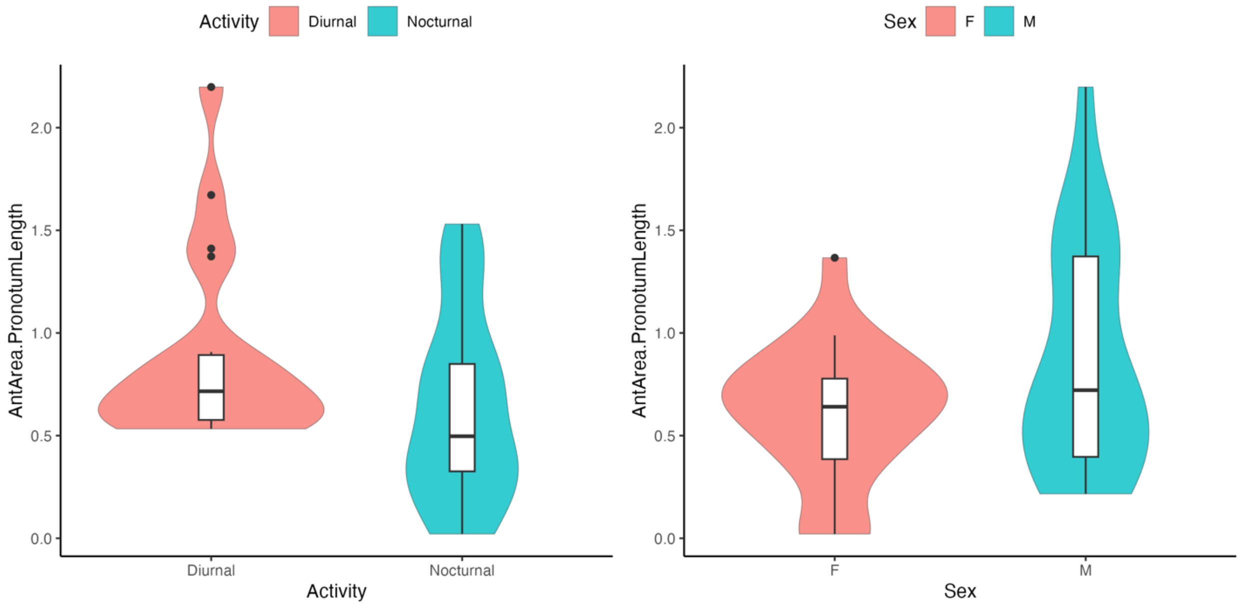


Figure 2.2: Antennal areas scaled by body size of A: female and male fireflies, and B: diurnal and nocturnal fireflies. AntArea/PL: antenna area/pronotum length

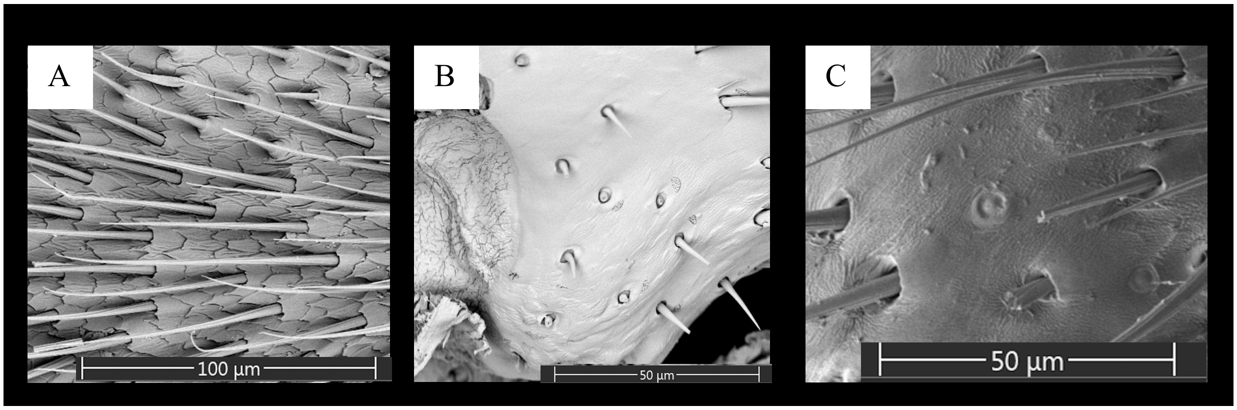


Figure 2.3. Mechanoreceptor sensilla of fireflies (species represented in picture is noted). A: Sensilla chaetica type 1 (C1; *P. corruscus*), B: Sensilla chaetica type 2 (C2; *Py. nigricans*), C: Sensilla campaniform (SC; *Py. nigricans*).

Table 2.3: Presence (+) or absence (-) of 13 sensilla morphotypes in eight species of Lampyridae (3 males and 3 females, except for *L. cruciata*: males only); [1]*L. cruciata* data from Iwasaki et al., 1995; [2] B12 sensilla are only present in male *Luciola sp*.

	P. corruscus	*Py. nigricans*	*L. punctata*	*P. pyralis*	*Ph. lucicrescens*	*Phausis* sp	Luciolinae sp.	*L. cruciata*[1]
Sensilla type	Diurnal	Diurnal	Diurnal	Nocturnal	Nocturnal	Nocturnal	Nocturnal	Nocturnal
C1	+	+	+	+	+	+	+	+
C2	+	+	+	+	+	+	+	-
SC	+	+	+	+	+	-	+	-
B1	+	+	-	+	-	-	-	-
B2	+	-	-	-	+	-	-	-
B3	+	+	-	+	-	-	-	-
B7	+	+	-	+	+	-	+	+
B10	-	-	+	-	-	-	-	-
B11	-	-	+	-	-	-	-	-
B12	-	-	-	-	-	-	+[2]	-
B13	-	-	-	-	-	-	+	-
X1	+	+	-	-	+	-	-	-
T1	-	-	-	-	-	+	-	-

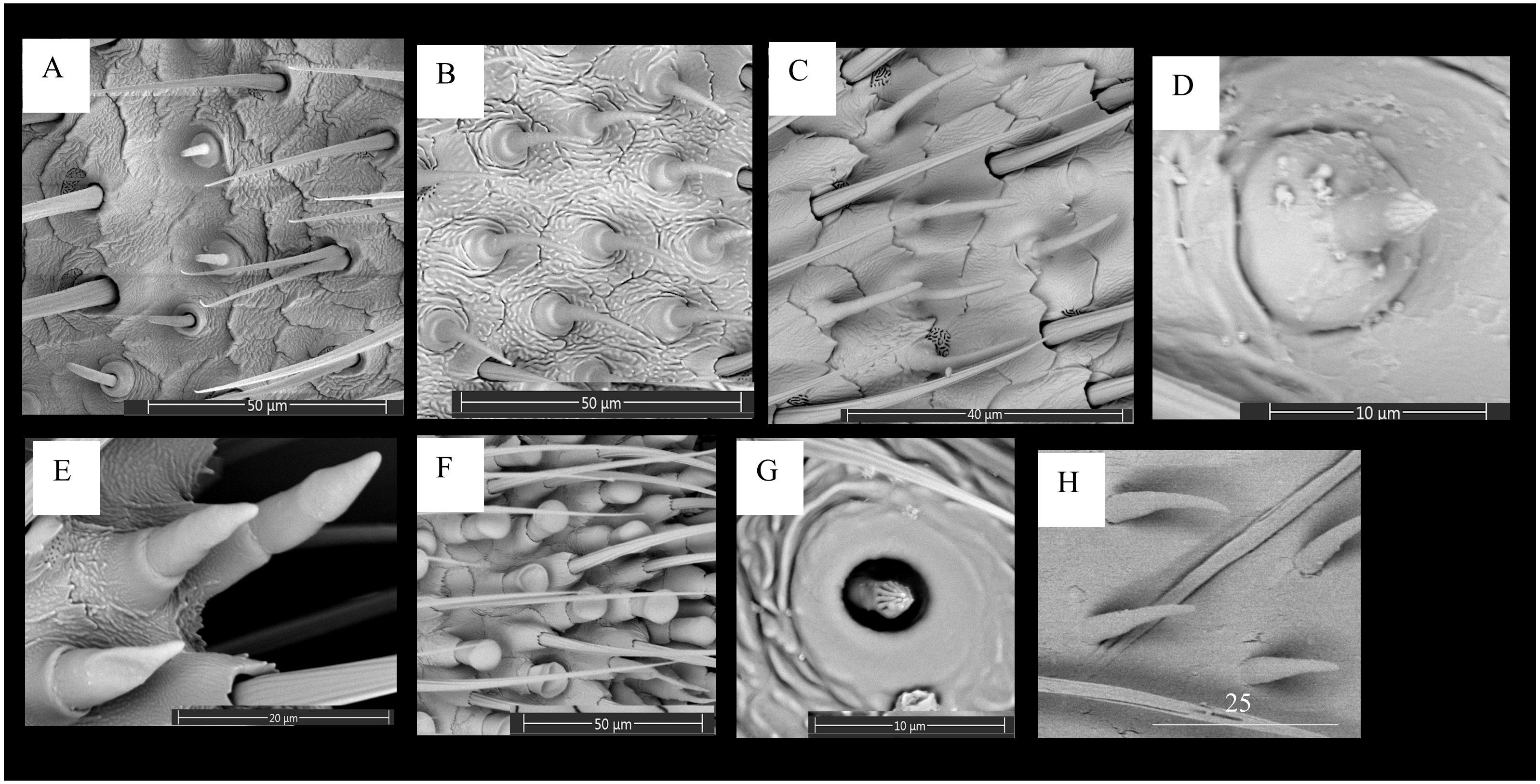


Figure 2.4 Chemoreceptor sensilla of fireflies (species represented in picture is noted). A: Sensilla basiconica type 1 (B1; *P. corruscus*), B: sensilla basiconica type 2 (B2; *P. corruscus*), C: Sensilla basiconica type 3 (B3; *P. nigricans*), D: Sensilla basiconica type 7 (B7; *P. corruscus*), E: Sensilla basiconica type 10 (B10; *L. punctata*), F: Sensilla basiconica type 11 (B11; *L. punctata*), G: Sensilla coeloconica (X1; *P. corruscus*), H: Sensilla trichodea (T1; *Phausis* sp.).

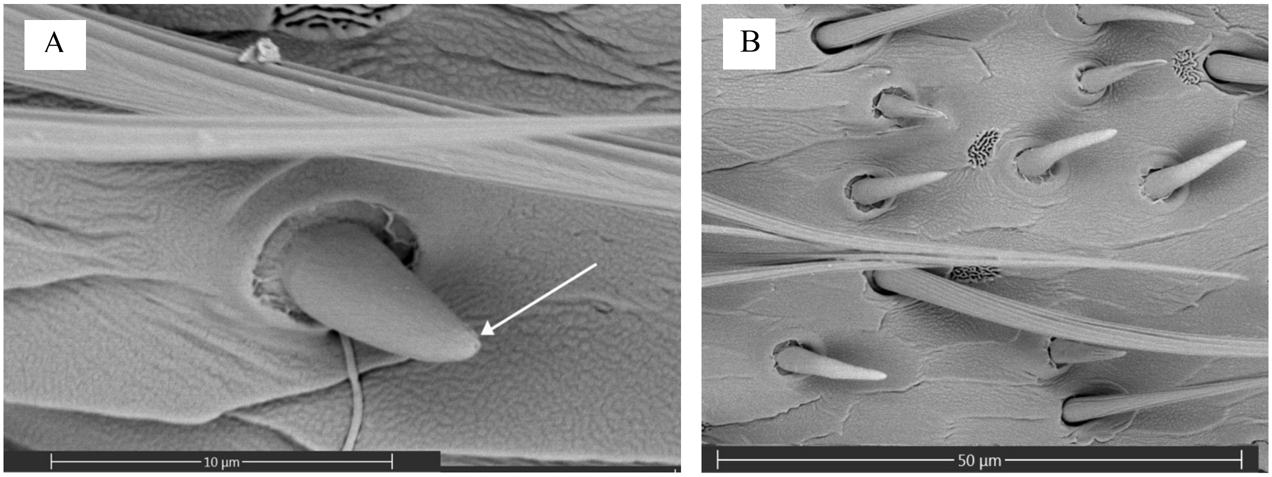
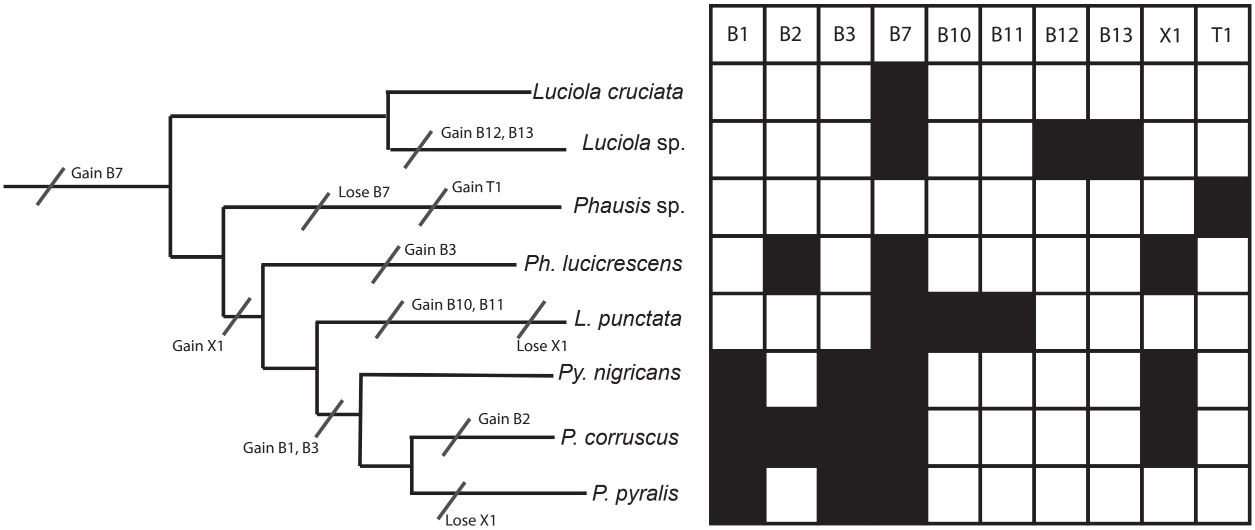


Figure 2.5. Rare sensilla types of fireflies (species represented in picture is noted). A: Sensilla basiconica type 12 (B12; Luciolinae sp.), arrow points to pore; B: sensilla basiconica type 13 (B13; Luciolinae sp.).



	B1	B2	B3	B7	B10	B11	B12	B13	X1	T1
Luciola cruciata	□	□	□	■	□	□	□	□	□	□
Luciola sp.	□	□	□	■	□	□	■	■	□	□
Phausis sp.	□	□	□	□	□	□	□	□	□	■
Ph. lucicrescens	□	■	□	■	□	□	□	□	■	□
L. punctata	□	□	□	■	■	■	□	□	□	□
Py. nigricans	■	□	■	■	□	□	□	□	■	□
P. corruscus	■	■	■	■	□	□	□	□	■	□
P. pyralis	■	□	■	■	□	□	□	□	□	□

Figure 2.6. Cladogram of study taxa with presence (black square)/absence (white square) of each chemosensilla type.

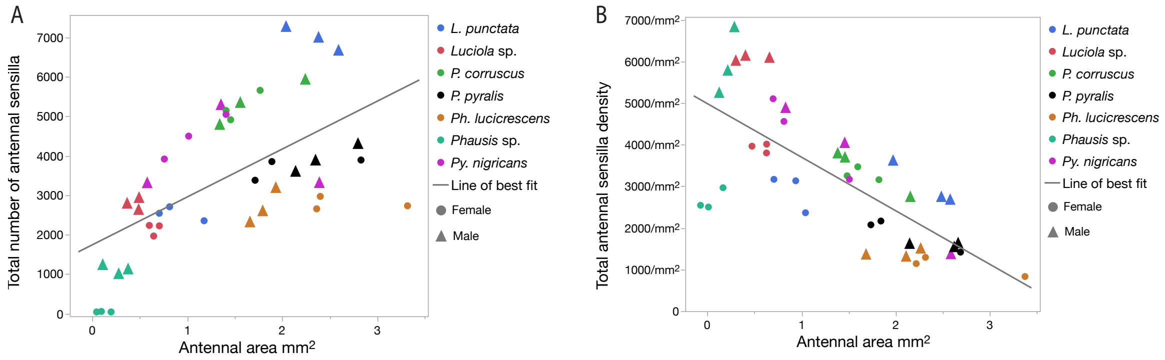


Figure 2.7. A) Total number of sensilla by antennal area. Antennal area and sensilla number are positively correlated (linear regression, $R^2 = 0.37$, df = 41, $p<0.0001$). B) Total sensilla density by antennal area. Antennal area and sensilla density are negatively correlated (linear regression, $R^2 = 0.53$, df = 41, $p<0.0001$).

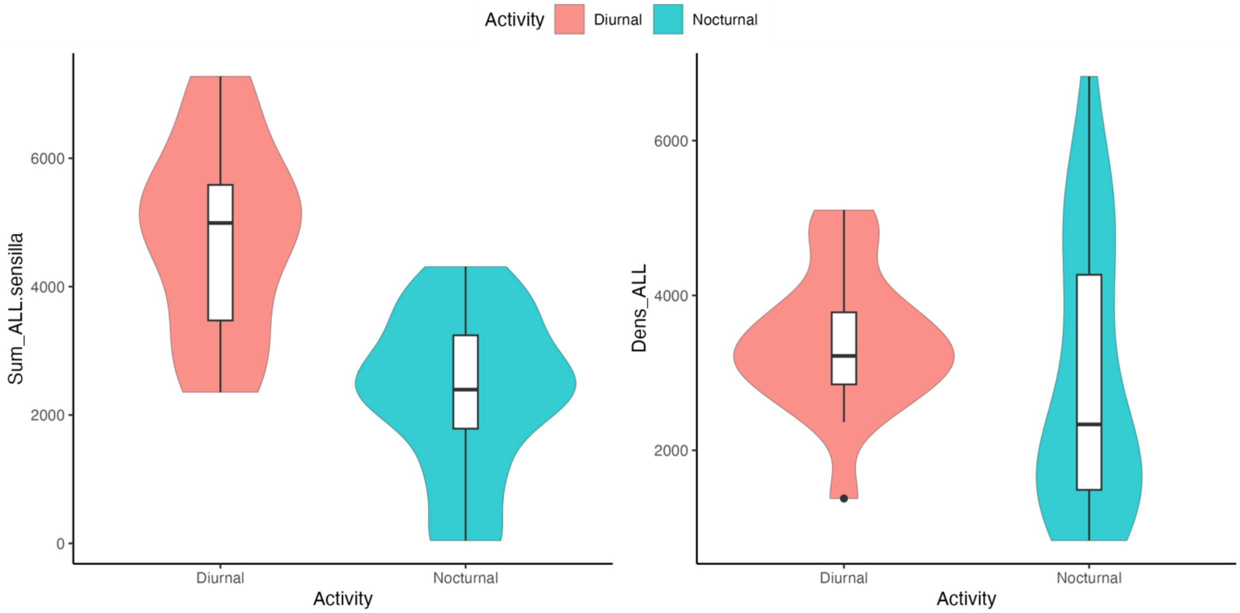


Figure 2.8: Comparisons of total sensilla counts (A) and densities (B) in diurnal and nocturnal fireflies.

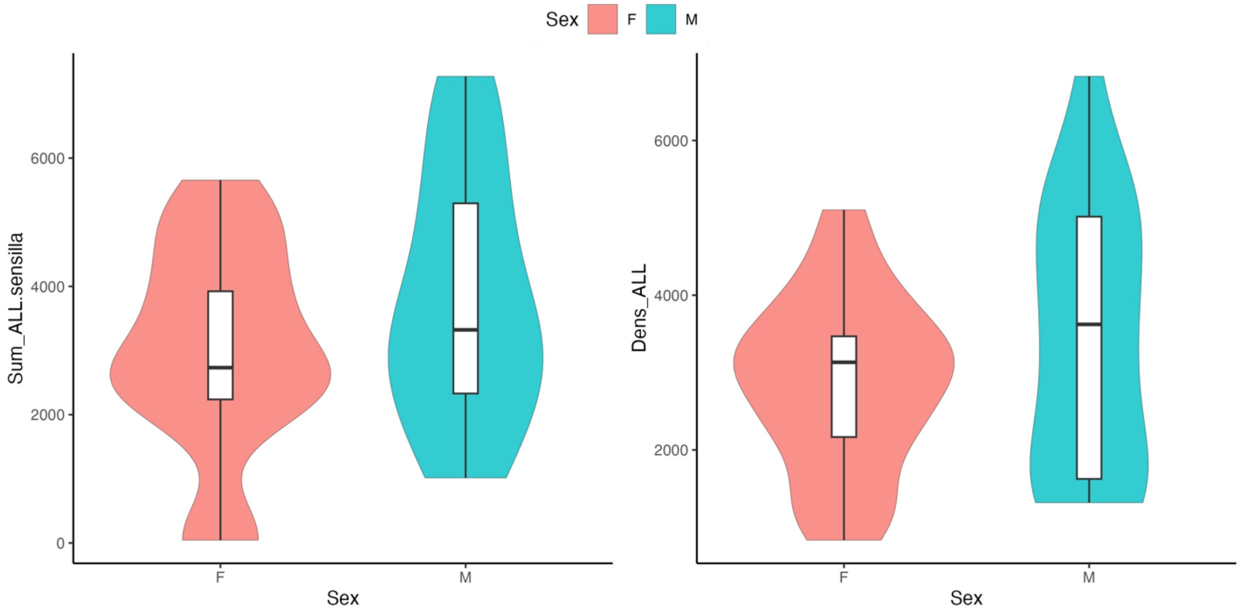


Figure 2.9: Comparisons of total sensilla counts (A) and densities (B) in female and male fireflies.

Table 2.4: Individual mechanoreceptor (C1, C2, SC) counts (mean ± stdev) for each species (F: 3 females, M: 3 males, D: diurnal, N: nocturnal).

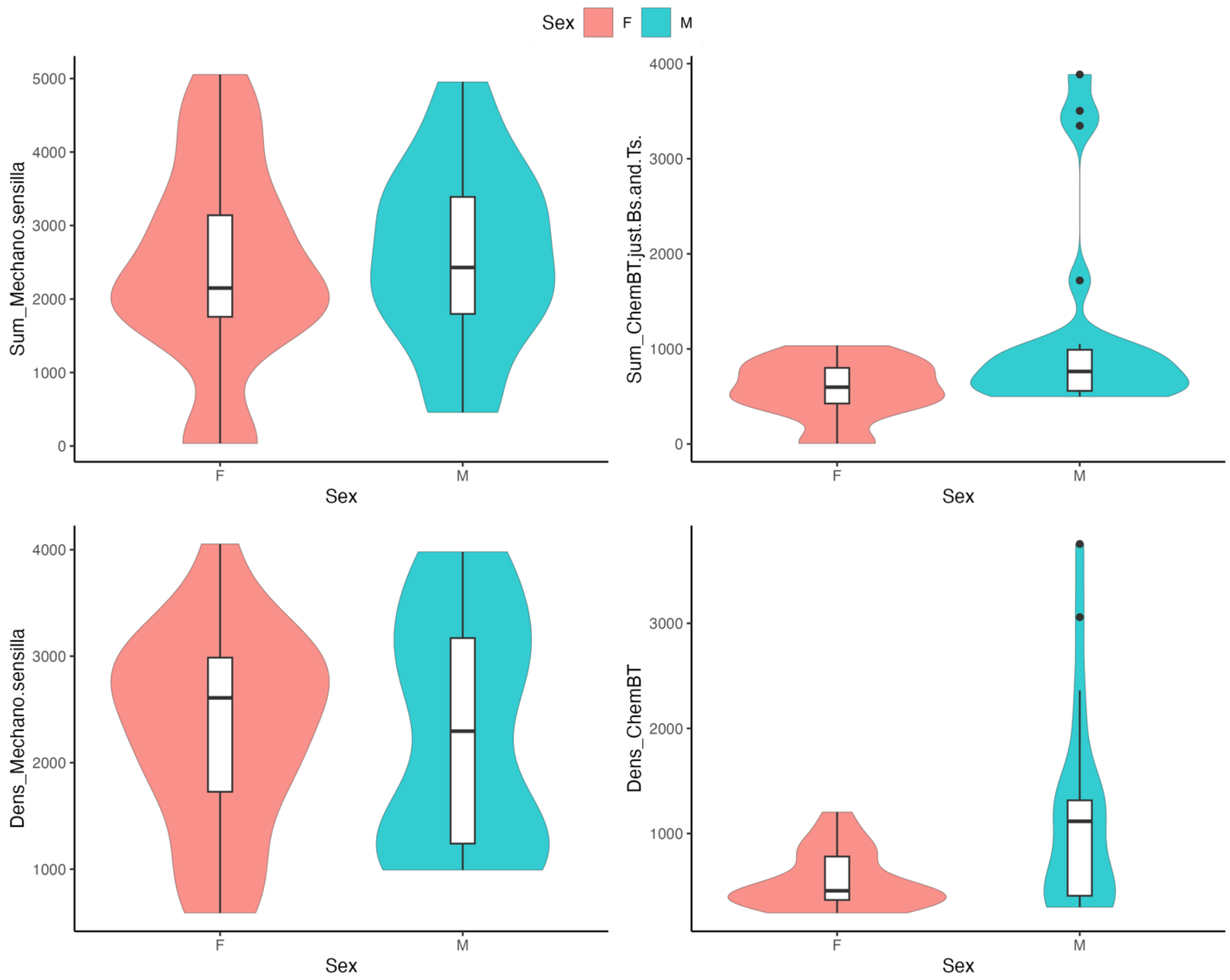
Species	Sex	Activity	C1 (N)	C2 (N)	SC (N)
P. corruscus	F	D	442 7± 555	54 ± 15	1 ± 1
	M	D	4464 ± 435	66 ± 21	1 ± 1
Py. nigricans	F	D	3599 ± 843	49 ± 22	17 ± 10
	M	D	2732 ± 664	67 ± 7	1 ± 1
L. punctata	F	D	2082 ± 154	43 ± 12	8 ± 7
	M	D	3351 ± 236	54 ± 12	0
Ph. lucicrescens	F	N	1860 ± 112	87 ± 7	0
	M	N	1895 ± 1492	92 ± 22	1 ± 2
P. pyralis	F	N	2892 ± 175	82 ± 14	23 ± 6
	M	N	2935 ± 296	74 ± 19	24 ± 4
Luciolinae sp.	F	N	1622 ± 137	51 ± 10	3
	M	N	1721 ± 124	67 ± 6	1 ± 1
Phausis sp	F	N	36 ± 3	2 ± 1	0
	M	N	525 ± 103	33 ± 12	0

Table 2.5: Individual chemoreceptor (B1-B3, B7, B10-B13, T1) counts (mean ± stdev) for each species (F: 3 females, M: 3 males, D: diurnal, N: nocturnal).

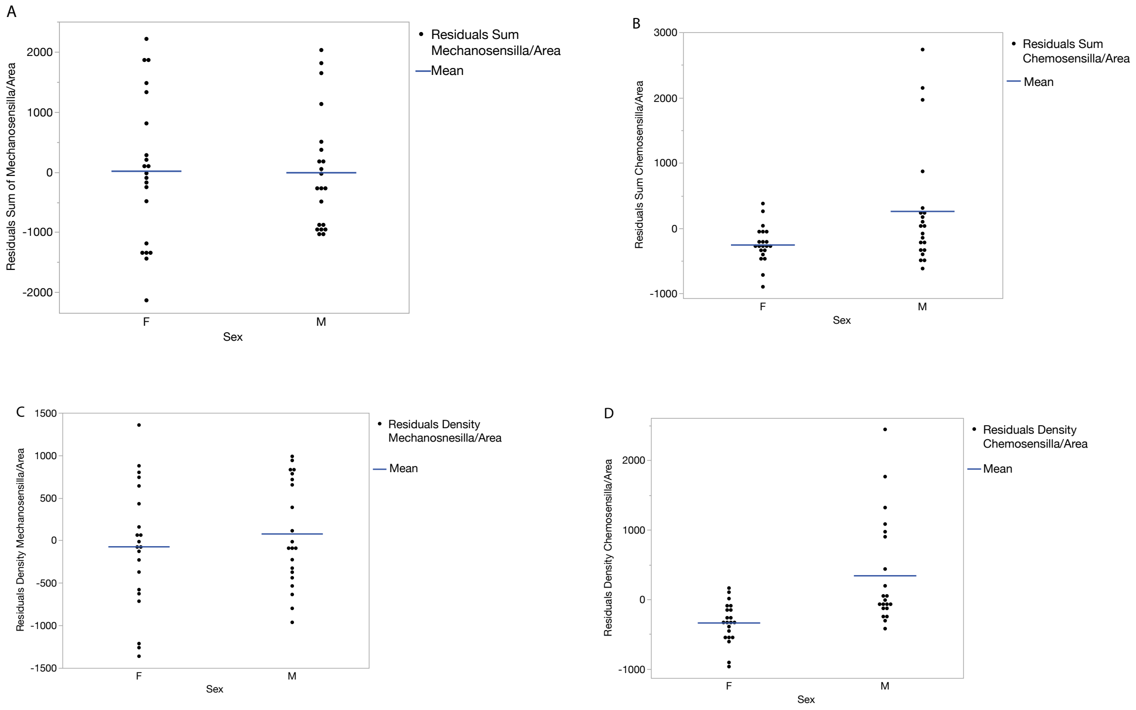
Species	Sex	Active	B1 (N)	B2 (N)	B3 (N)	B7 (N)	B10 (N)	B11 (N)	B12	B13	T1 (N)
P. corruscus	F	D	191± 101	295 ± 77	267 ± 53	4 ± 4	0	0	0	0	0
	M	D	410 ± 141	185 ± 26	206 ± 51	29 ± 9	0	0	0	0	0
Py. nigricans	F	D	32 ± 19	0	755 ± 303	35 ± 14	0	0	0	0	0
	M	D	15 ± 12	0	1151 ± 478	12 ± 10	0	0	0	0	0
L. punctata	F	D	0	0	0	23 ± 18	350 ± 31	29 ± 13	0	0	0
	M	D	0	0	0	35 ± 23	506 ± 64	2881 ± 139	0	0	0
Ph. lucicrescens	F	N	0	825 ± 52	0	15 ± 5	0	0	0	0	0
	M	N	0	704 ± 252	0	17 ± 19	0	0	0	0	0
P. pyralis	F	N	482 ± 90	0	207 ± 10	21 ± 6	0	0	0	0	0
	M	N	622 ± 90	0	269 ± 103	12 ± 14	0	0	0	0	0
Luciolinae sp.	F	N	0	0	0	27 ± 30	0	0	0	442±29	0
	M	N	0	0	0	29 ± 2	0	0	13±3	488±29	0
Phausis sp	F	N	0	0	0	0	0	0	0	0	10 ± 4
	M	N	0	0	0	0	0	0	0	0	572 ± 24

Table 2.6: Mean counts and densities (mean ± standard deviation) for X1 sensilla by species and sex. *X1 sensilla were not present in all specimens within a species, specimens lacking these sensilla were excluded from mean and standard deviation calculations.

Species	Sex	Active	X1 counts*	X1 density*	Sample size
P. corruscus	F	D	22	15	1
	M	D	13 ± 4	8 ± 3	3
Py. nigricans	F	D	4 ± 4	4 ± 5	2
	M	D	5	7	1
L. punctata	F	D		-	-
	M	D	0	-	-
Ph. lucicrescens	F	N	16	7	1
	M	N	4	2	1
P. pyralis	F	N	0	-	-
	M	N	0	-	-
Luciolinae sp.	F	N	0	-	-
	M	N	0	-	-
Phausis sp.	F	N	0	-	-
	M	N	0	-	-



Figures 2.10 Comparisons of male and female (A) mechanoreceptor and (B) chemoreceptor sensilla counts and (C) mechanoreceptor and (D) chemoreceptor densities



Figures 2.11 Given antennal areas (and residuals from regression line): Comparisons of male and female (A) mechanoreceptor and (B) chemoreceptor sensilla counts and (C) mechanoreceptor and (D) chemoreceptor densities.

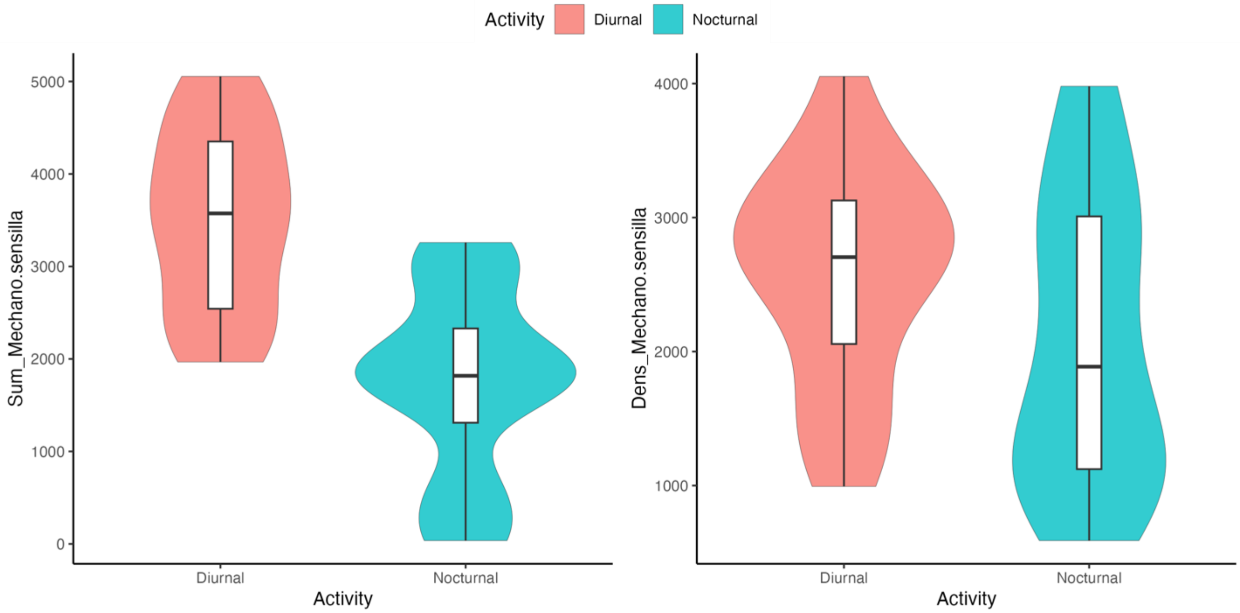


Figure 2.12 Comparisons of (A) mechanoreceptor counts and (B) densities between diurnal and nocturnal fireflies.

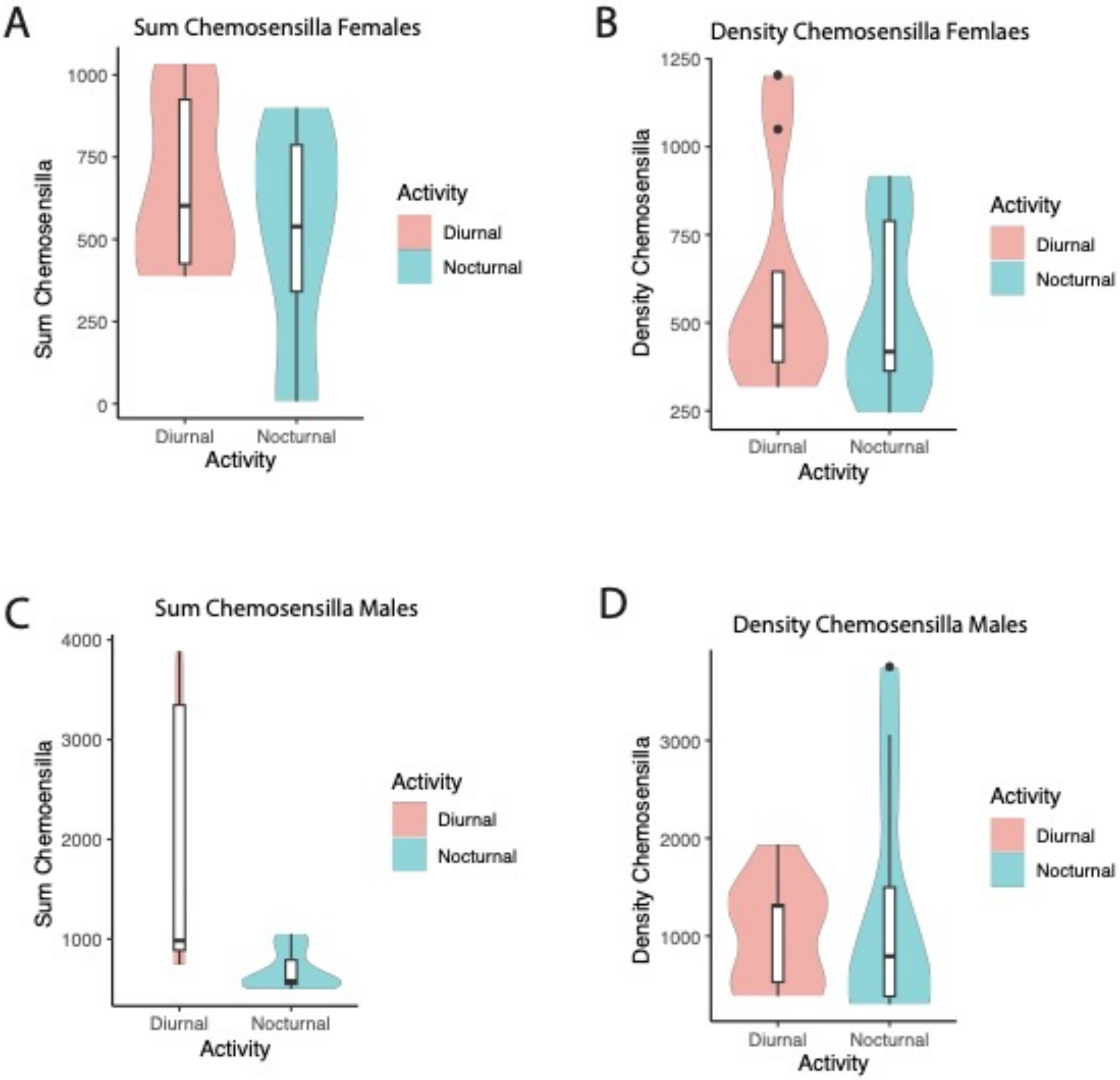


Figure 2.13 Comparisons of chemoreceptor counts and densities (by sex): (A) chemoreceptor counts and (B) densities of diurnal and nocturnal females; (C) chemoreceptor counts and (D) densities of diurnal and nocturnal males.

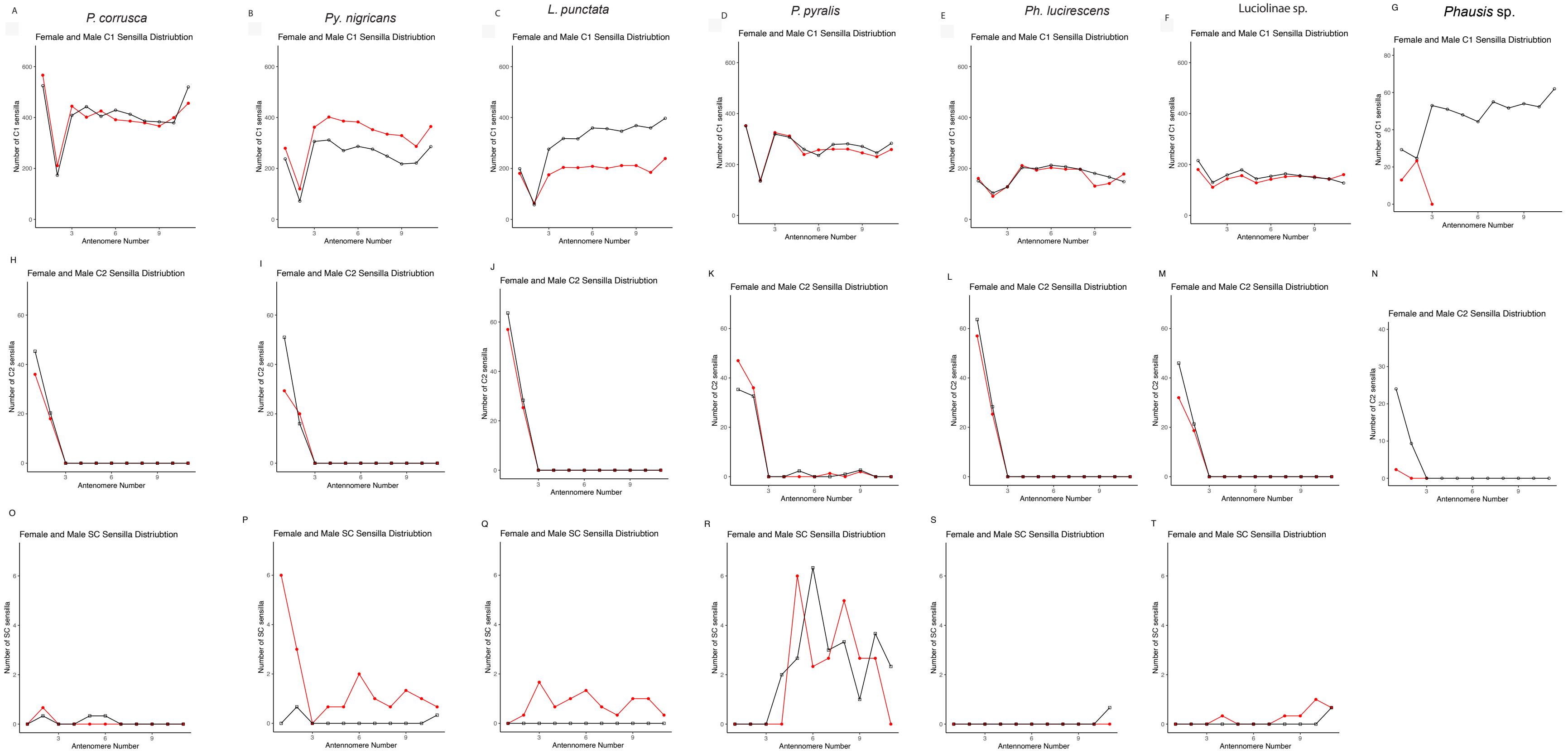


Figure 2.14: Distribution of mechnosensilla for females (red) and males (black) for each species.

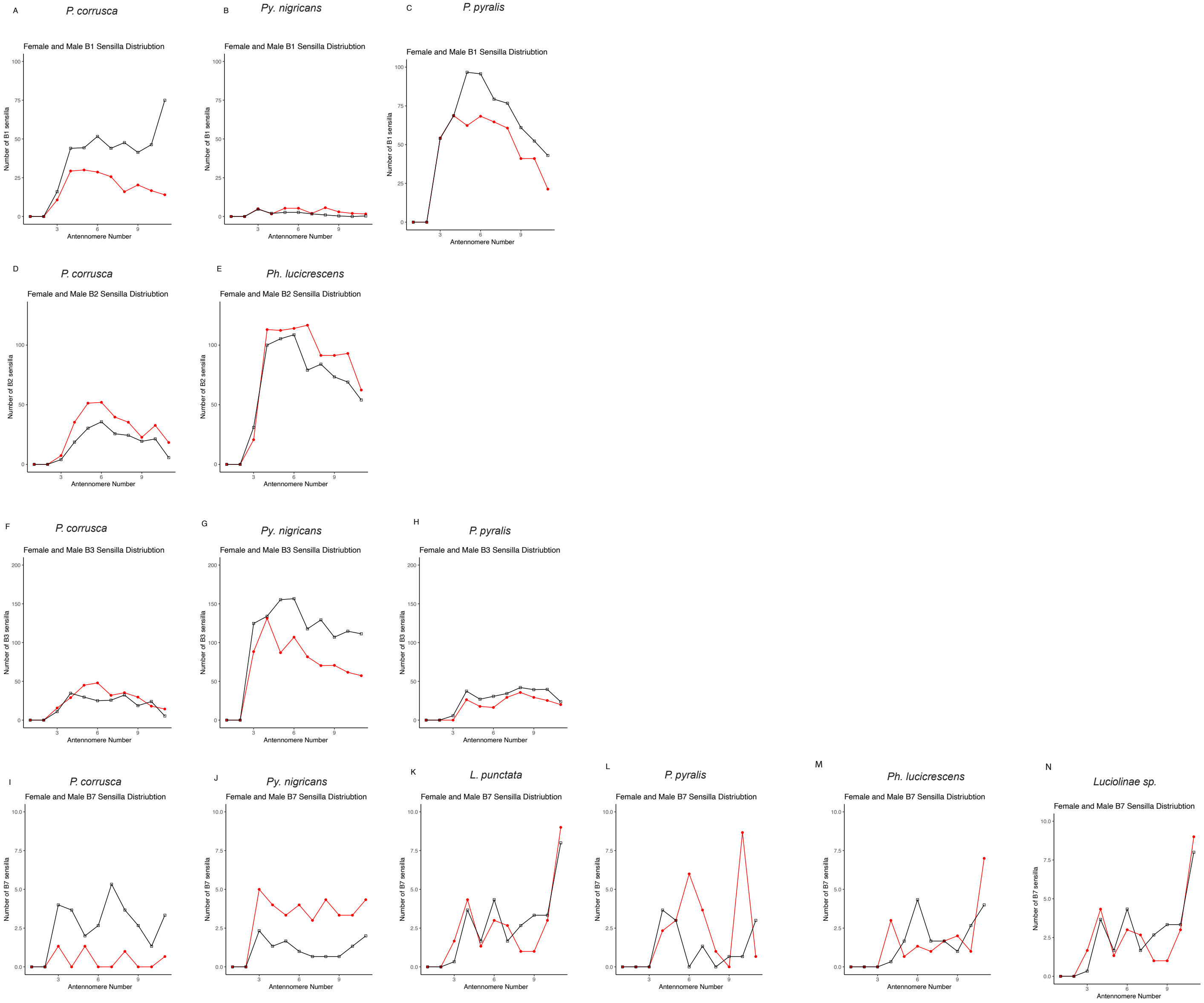


Figure 2.15: Basiconica sensilla distribution for females (red) and males (black) of each respective species.

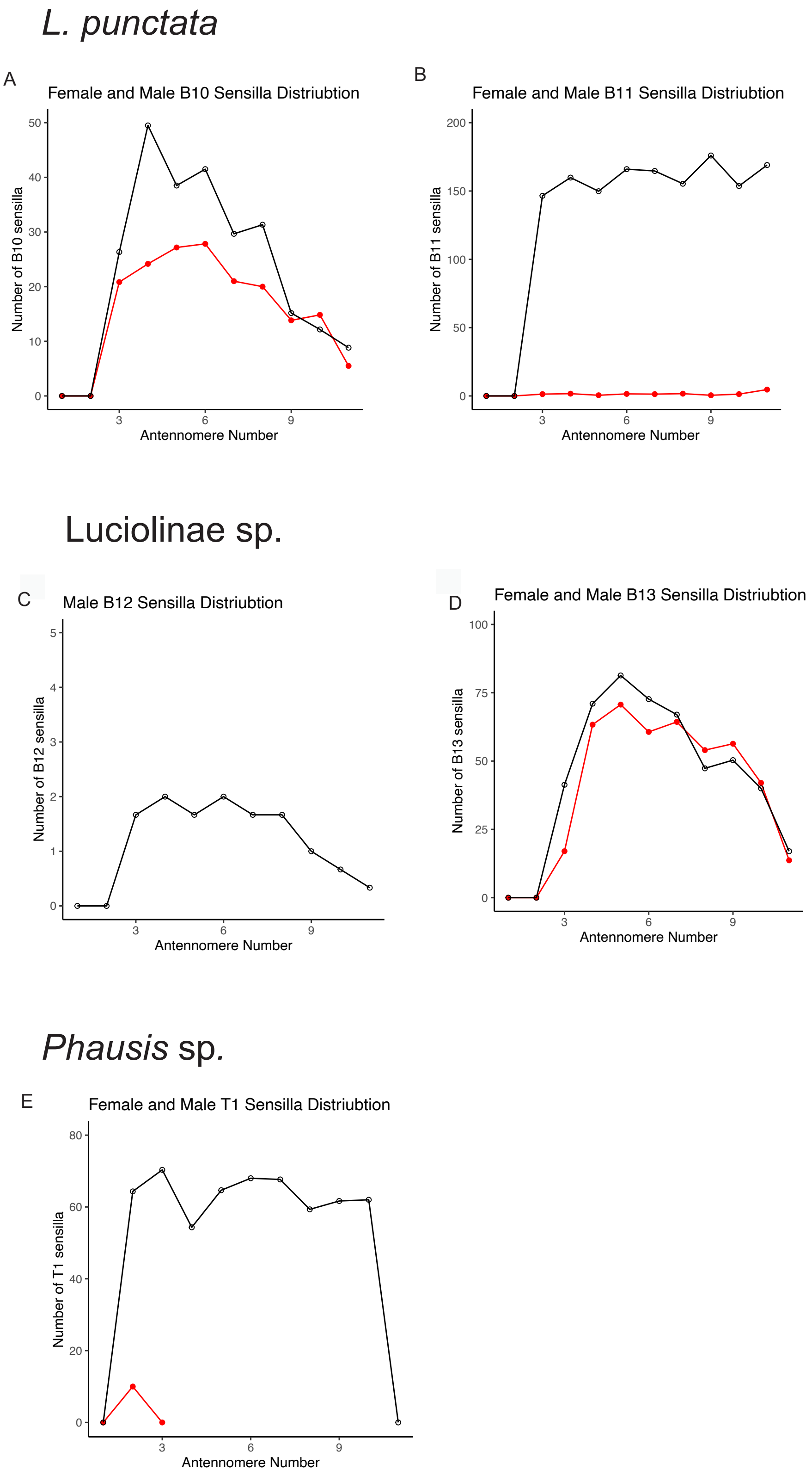


Figure 2.16 Average number of (A) B10, (B) B11, (C) B12, (D) B13, and (E) T1 sensilla per antennal segment.

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Supplemental Table 2.1: Sensilla density (mean ± stdev) per antenna for all sensilla types, all mechanoreceptors, and all chemoreceptors of each species. (F: 3 females, M: 3 males, D: diurnal, N: nocturnal).

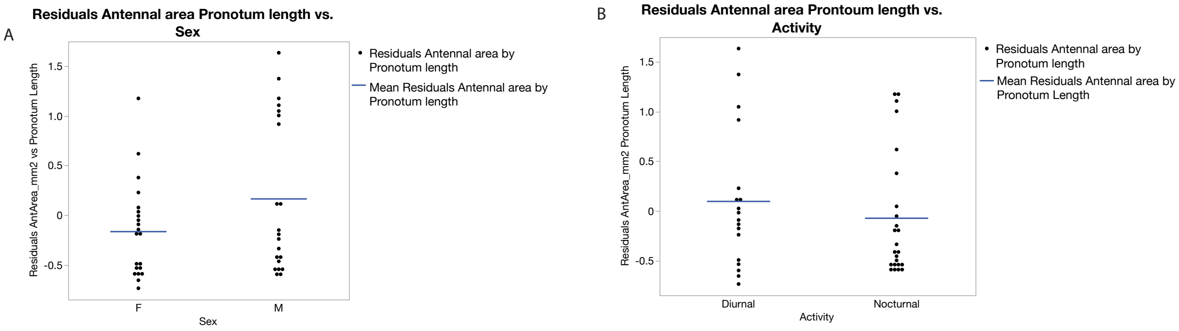
Species	Sex	Activity	All sensilla (N/mm^2)	All mechano-sensilla (N/mm^2)	All chemo-sensilla (N/mm^2)
P. corruscus	F	D	3294 ± 159	2810 ± 196	483 ± 155
	M	D	3416 ± 577	2892 ± 516	524 ±67
Py. nigricans	F	D	4277 ± 998	3420 ± 604	857 ± 473
	M	D	3438 ± 1834	2435 ± 1319	1002 ± 536
L. punctata	F	D	2889 ± 454	2431 ± 394	458 ± 59
	M	D	3020 ± 524	1467 ± 208	1553 ± 334
Ph. lucicrescens	F	N	1090 ± 235	761 ± 158	330 ± 76
	M	N	1399 ± 101	1031 ± 34	369 ± 87
P. pyralis	F	N	1887 ± 408	1526 ± 331	360 ± 83
	M	N	1604 ± 50	1235 ± 16	369 ± 61
Luciolinae sp.	F	N	3927 ± 111	2431 ± 394	458 ± 59
	M	N	5023 ± 41	3896 ± 76	1127 ± 55
Phausis sp.	F	N	2671 ± 256	2127 ± 71	544 ± 207
	M	N	5955 ± 803	2897 ± 174	3058 ± 697

Supplemental Table 2.2 Individual mechanoreceptor (C1, C2, SC) density (mean ± stdev N/mm^2) of each species (F: 3 females, M: 3 males, D: diurnal, N: Nocturnal). (-) type absent.

Species	Sex	Activity	C1 (N/mm^2)	C2 (N/mm^2)	SC (N/mm^2)
P. corruscus	F	D	2776 ± 203	34 ± 8	0.4 ± 0.8
	M	D	2850 ± 512	401 ± 12	0.5± 0.5
Py. nigricans	F	D	3354 ± 592	51 ± 33	15 ± 10
	M	D	2376 ± 1287	58 ± 31	1 ± 0.9
L. punctata	F	D	2373 ± 386	49 ± 14	10 ± 9
	M	D	1443 ± 200	23 ± 8	-
Ph. lucicrescens	F	N	727 ± 153	33 ± 5	-
	M	N	983 ± 41	47 ± 8	0.4 ± 0.7
P. pyralis	F	N	1474 ± 324	41 ± 8	11 ± 2
	M	N	1195 ± 19	30 ± 8	10 ± 2
Luciolinae sp.	F	N	2970 ± 101	93 ± 30	5 ± 6
	M	N	3747 ± 95	147 ± 21	2 ± 3
Phausis sp.	F	N	200 ± 57	128 ±25	-
	M	N	2728 ± 170	169 ± 26	-

Supplemental Table 2.3: Individual chemoreceptor (B1-B3, B7, B10-B13, T1) density (mean ± stdev) of each species (F: 3 females, M: 3 males, D: diurnal, N: nocturnal); (-) type absent.

Species	Sex	Active	B1 (N/mm^2)	B2 (N/mm^2)	B3 (N/mm^2)	B7 (N/mm^2)	B10 (N/mm^2)	B11 (N/mm^2)	T1 (N/mm^2)
P. corruscus	F	D	122 ± 70	188 ± 61	171 ± 48	3 ± 3	-	-	-
	M	D	253 ± 43	123 ± 47	131 ± 35	18 ± 5	-	-	-
Py. nigricans	F	D	28 ± 15	-	797 ± 473	31 ± 4	-	-	-
	M	D	9 ± 3	-	980 ± 530	13 ± 17	-	-	-
L. punctata	F	D	-	-	-	28 ± 23	396 ± 27	34 ± 18	-
	M	D	-	-	-	15 ± 9	216 ± 8	1245 ± 203	-
Ph. lucicrescens	F	N	-	323 ± 74	-	6 ± 3	-	-	-
	M	N	-	360 ± 95	-	9 ± 9	-	-	-
P. pyralis	F	N	244 ± 61	-	106 ± 27	10 ± 2	-	-	-
	M	N	256 ± 51	-	108 ± 34	5 ± 5	-	-	-
Luciolinae sp.	F	N	-	-	-	49 ± 53	-	-	-
	M	N	-	-	-	63 ± 7	-	-	-
Phausis sp.	F	N	-	-	-	-	-	-	128 ± 25
	M	N	-	-	-	-	-	-	170 ± 26



Supplemental Figure 2.1: Comparison of residuals for the regression line of antennal area by pronotum length between (A) females and males and (B) diurnal and nocturnal species.

CHAPTER 3

WORLDWIDE FIREFLY PHYLOGENY (COLEOPTERA LAMPYRIDAE): SEXUAL SIGNALING AND SIGNAL RECEPTOR EVOLUTION

Introduction

Phylogeny

A phylogenetic hypothesis is an essential tool in not only understanding the evolutionary relationships of species but it is also used to answer a wide range of evolutionary questions (e.g., systematics, biodiversity, biogeography, character evolution, and lineage dating). Phylogenetic reconstruction methods have changed significantly over the past few decades to account for the increased availability of genetic data. Next generation sequencing (NGS) methods allow for large amounts of molecular data quickly and without the need to assemble genomes. Target DNA enrichment, one type of NGS data, allows researchers to target specific types of genetic data based on their needs; this method uses loci probes designed specifically for the group of interest to obtain sequences (Mamanova et al., 2010; Kozarewa et al., 2015). Target loci can range from ultraconserved to regions to highly variable regions of the genome. Anchored hybrid enrichment sequencing targets highly conserved loci with less conserved flanking regions to provide data needed for both deep and shallow relationships within a phylogeny (Lemmon et al., 2012).

The increase of data availability has also influenced tree reconstruction methods. Analysis of large NGS data sets are can be extremely computationally intensive for character based reconstruction methods such as maximum likelihood, in which best practices suggest individual partitions and models of evolution for each locus (Kapli et al., 2020). Alternatively, multispecies coalescent (MSC) methods use gene trees from each locus to infer a species tree, not only does this require less computational demand but it attempts to account for incomplete lineage sorting (Degnan, et al., 2009).

The beetle family Lampyridae (fireflies) is composed of 2,200+ species and taxonomic work continues to identify firefly diversity, with new species discoveries and descriptions. As researchers quantify the great diversity of this group an evolutionary framework is needed to determine how fireflies have changed over time. I use both coalescent and a maximum likelihood super matrix (of concatenated locus alignments) reconstruction methods along with firefly-specific anchored hybrid enrichment data to hypothesize the relationships between species around the world and establish a valuable tool for evolutionary studies within the group.

The first molecular phylogeny of Lampyridae was presented by Suzuki (1997) for 29 Japanese firefly species in 3 subfamilies (Ototretinae, McDermot 196, Lampyrinae, Rafinesque, 1815, and Cyphonocerinae, Crowson 1972) utilizing the 16S locus for mitochondrial ribosomal RNA. This analysis recovered the subfamilies Lampyrinae and Ototretinae as polyphyletic. Subsequent analyses with increased taxonomic diversity also recovered Lampyrinae and Ototretinae as polyphyletic; including a morphological analysis using 5 (of then 6) subfamilies (Branham & Wenzel, 2003) and a molecular phylogeny using 3 loci (18s, 16s, and COI; Stanger-Hall et al., 2007). Additional diversity was sampled using 8 subfamilies and morphological data that again recovered Lampyrinae as polyphyletic and a lack of resolution between 5 subfamilies (Lampyrinae, Psilocladinae, McDermot, 1964, Amydetinae, Olivier 1907, Chegeuvariinae, Kazanstev, 2007, and Photurinae, Lacordaire 1857 ; Jeng, 2008); while a molecular phylogeny using nine subfamilies and 6 loci (12, 16s, 18s, 28s, COI, and wingless) again recovered a polyphyletic Lampyrinae. Until this point relationships between the subfamilies varied greatly based on analyses (taxon sampling and loci used) and the subfamily Lampyrinae was consistently recovered as non-monophyletic. The most extensive phylogeny of fireflies to date (Martin et al., 2019) was based on 436 anchored hybrid enrichment (AHE) loci and included 88 firefly species from 53 genera in eight subfamilies worldwide. Based on this analysis Martin et al. (2019) established systematic changes, describing a new firefly subfamily Lampyrohizinae, Kazantsev, 2010 and reestablishing the subfamily Psilocladinae, McDermott, 1964 resulting in a total of 10 recognized firefly subfamilies. Since Martin et al. (2019) two additional lampyrid phylogenies have been published to determine the phylogenetic placement of Cheguevariinae and the recently described subfamily Chespiritoinae Ferreira et al., 2020. Using three loci (nuclear 18S rRNA, and mitochondrial rrnL and cox1) and 71 firefly species in 5 of now 11 subfamilies, Cheguevariinae was recovered as sister to the (Lampyrinae + Photurinae) subfamilies (Ferreira et al., 2019). Chespiritoinae was described as a new eleventh Lampyridae subfamily and as a sister subfamily to Cyphonocerinae and Luciolinae (Ferreira et al. 2020). This placement was based on a phylogenetic analysis of 68 firefly species in 8 subfamilies, utilizing four loci (18S rRNA, 28S rRNA, rrnL and cox1).

The relationship of Cheguevariinae and Chespiritoinae to the rest of the Lampyridae in a broad taxonomic and molecular framework is still undetermined. Additionally, the monophyly of some subfamilies remains questionable due to limited representation in previous studies (i.e., Psilocladinae, Amydetinae, Ototretinae, and Lamprohizinae. Previous phylogenetic estimates included a majority representation of Nearctic, Indomalayan, and Australasian taxa due to increased taxonomic work and availability of specimens for these areas, however, Neotropical and Afrotropical taxa have been poorly represented in worldwide estimates of lampyrid phylogenies.

Here we present the most comprehensive Lampyridae phylogeny to date including 342 firefly taxa in 90 genera from ten of the eleven subfamilies described to date. Our analysis did not include Cyphonocerinae, but it included for the first time the subfamilies Cheguevariinae and Chespiritoinae in a large-scale genomic analysis. In addition, we also greatly increased the taxon sampling for several subfamilies (e.g., Photurinae, and Ototretinae, and Amydetinae) and genera (e.g., *Vesta*) to address questions regarding monophyly and taxonomic placement within Lampyridae (Martin et al., 2019; Vaz et al., 2020; Bocakova et al., 2022). This taxon sampling represents the broadest geographic sampling of Lampyridae to date, including the Neotropics, a biogeographic region which was previously underrepresented in molecular phylogenies. This taxon sampling allowed us to test specific hypotheses on firefly evolution, including the evolution of sexual signals and their associated morphological traits.

Evolution of Bioluminescence

Fireflies are one of the most well-known bioluminescent organisms. All firefly larvae produce bioluminescent glows as aposematic signals to warn predators of their chemical defenses, but as adults only some firefly species use bioluminescence flashes or glows to attract and identify conspecific mates. The other firefly species lack bioluminescence as adults and exclusively rely on pheromones for mate search, and some species likely use a combination of pheromones and glows (Branham & Wenzel, 2003; Stanger-Hall et al., 2007; De Cock et al., 2014). It is established that the ancestral sexual signal of Lampyridae was pheromone signaling (Branham & Wenzel, 2003; Stanger-Hall et al. 2007; Martin et al., 2017), but studies disagree on the number of independent origins of adult bioluminescent signals and the number of subsequent losses of bioluminescence and reversals back to pheromone use. Dependent on taxon sampling, between one and five independent origins of adult firefly bioluminescence are hypothesized and between two and ten subsequent losses. However, these estimates included limited representation of generic diversity and limited non-bioluminescent taxa (12-28 taxa) among bioluminescent (48-83) taxa. Our extended phylogeny, with its increased taxon sampling of 64 pheromone signaling and 229 bioluminescent species (50 species with an unknown signal type), provided a great opportunity to investigate the evolution of firefly sexual signals and to update recent estimates on the number of times bioluminescence originated and was lost in Lampyridae, along with the number of times bioluminescent signals shifted between glows and flashes. This knowledge will give us the opportunity to understand how easily shifts in sexual signal methods can occur in a group.

Eye and antenna size

The diversity of Lampyridae sexual signals is accompanied by diversity in the receptor organs for their sexual signals. For example, fireflies use their eyes to detect bioluminescent signals and their antennae to detect pheromones. The size of firefly eyes and antennae are correlated with their sexual signals (Stanger-Hall et al., 2018). Specifically, the nocturnal fireflies that use bioluminescent signals have relatively larger eyes than diurnal firefly species. This is likely driven by selection for better vision at night, both for navigating through a darker environment and for the detection of light signals. In contrast, species that exclusively use pheromones tend to have relatively larger antennae (Stanger-Hall et al., 2018) and thus more chemoreceptors and a larger sampling area for pheromones (Pacheco et al. *in prep*.). While the size of these sensory organs is associated with the type of signals used, it is also influenced by body size and phylogenetic relatedness. Stanger-Hall et al. (2018) documented a strong phylogenetic signal in a morphological analysis of 46 North American firefly eyes and antennae. In addition, genus was used as a proxy for phylogenetic relatedness of 101 species and 32 genera, because at this time there was no published worldwide phylogeny. In this analysis genus (representing phylogeny) also had the greatest influence on male firefly eye and antennal size for worldwide taxonomic sampling of 101 species and 32 genera (Stanger-Hall et al., 2018). With our worldwide phylogeny we are now able to use a phylogeny-based analysis to study the evolution of firefly eyes and antennae in 112 taxa across 7 subfamilies and 56 genera, including 37 diurnal and 51 nocturnal (bioluminescent) firefly taxa, on a global scale. We determine if phylogenetic signal of eye and antenna size is present in our more diverse data set, while accounting for variation due to evolutionary relationships, and if signal type (pheromone/flash/glow) can be predicted across Lampyridae based on eye and antenna measurements. Stanger-Hall et al. (2018) showed that the signal mode (flashes versus glows versus pheromones) could be predicted based on eye and antennal size among 46 North American firefly taxa (in three subfamilies). However, their sampling at the time included only a single glowing species and with our extended data set of seven glowing, 44 flashing, and 37 dark (i.e. relying exclusively on pheromones) taxa, we aim to test the predictability of signals across a taxonomically more diverse data set.

Light organ size and shape

The light organ shapes and sizes of bioluminescent fireflies vary greatly between species. In most firefly species, the male light organ is located on the fifth and six ventrite (visible ventral abdominal segments), and males of some species have a light organ restricted to the seventh ventrite (Branham & Wenzel, 2003). Light organs can cover the entire ventrite(s) or only part of a ventrite(s) and come in different shapes. Branham & Wenzel (2003) described four basic shapes of light organs: 1. occupying the entire ventrite (full light organs), 2. a single spot per ventrite, 3. two spots per ventrite, and 4. a central strip per ventrite. They noted that all four shapes appear to have multiple evolutionary origins across Lampyridae and suggested that light organ shape may be associated with bioluminescent signal type (glows versus flashes) and specifically that partial light organs may be present in glowing species, while light organs that cover the entire ventrite(s) may be used by flashing species (Branham & Wenzel, 2003). We used our large molecular phylogeny to reconstruct the evolution of firefly light organ shapes and to identify different origins and/or transitions of light organ shapes and sizes.

This study aimed to establish a robust phylogeny of the beetle family Lampyridae that includes all 10 of 11 subfamilies (except the monogeneric Cyphonocerinae) and the most diverse generic and biogeographical taxon sampling to date. We used this phylogeny to reconstruct the evolution of the sexual signals used by fireflies as well as the evolution of their light organ shapes. We used phylogeny-based analyses to test the influence of phylogeny and signal type (pheromones or bioluminescence) on eye and antenna size.

Materials and Methods

Taxon sampling

We sampled firefly species from 10 of 11 lampyrid subfamilies, representing 90 (of ~150) genera (Table 3.1). My analysis included 342 ingroup taxa and 3 outgroup taxa (Rhagopthalmidae sp. and *Zarhipis* sp. (Phengodidae) and *Ampedus pomonae* (Elateridae). To reduce the risk of random rooting (DeSalle et al. 2023) we ran our analyses first with two closely related outgroup taxa *Rhagophthalmus motschulski* (Ragophthalmidae) *Zarhipis* sp. (Phengodidae) and then added *Ampedus pomonae*, a more distantly related Elateridae species.

Library preparation

Muscle tissue was extracted from the left side of the thorax, just under the coxa of the metathoracic leg. DNA was extracted from the muscle tissue using the Qiagen DNeasy Blood and Tissue Kit. DNA concentration was determined via Invitrogen Qubit assay. DNA was submitted to Rapid Genomics Gainesville, FL USA for library preparation and Illumina sequencing. In the Rapid Genomics pipeline DNA was sheared to a mean length of 500bp, fragments were end-repaired and A-tailed Illumina adaptors unique to Lampyridae (Martin et al. 2019) were added to the fragments. The fragments were then enriched by PCR and samples were sequenced on a NovaSeq S4 flow cell.

Sequence assembly and Phylogeny

Raw sequences were trimmed of adapters using fastp (Chen et al., 2018) and assembled with Hybpiper (Johnson et al., 2016). Hybpiper was used to map the raw reads to reference sequences, of 516 loci assembled from transcriptomes and genomes of 13 species of fireflies, using the Burrows-Wheeler Alignment tool (BWA), reads were assembled to a single contig for each locus for each taxon. We used the hybpiper_stats option to retrieve information about the number of loci recovered for each taxon. We tested for paralogs within the assembled sequences using the Hybpiper paralog_retrieve option. To limit missing data, we examined the sequence coverage across taxa and across loci. As a first step we removed all taxa with assembled sequences for less than 50% of the 516 loci, resulting in a total of 342 ingroup taxa and 3 outgroup taxa (Fig. 3.1). As a second step we determined the taxon coverage for each locus and removed all loci with less than 50% taxon coverage (present in < 172 taxa) from the data set, resulting in 481 loci remaining in our analysis. The individual locus sequences were aligned in MAFFT v. 7.470 (Khatoh & Standley, 2013) on the University of Georgia Sapelo 2 computing cluster. The lengths of the assembled sequences varied across taxa for each locus, therefore as a third step to further limit missing data (within loci) we removed any site that had less than 50% taxon coverage, for a total of 455,954 sites in the data set. In addition, we assembled a second data set retaining only loci with at least 75% taxon coverage (step 2), and sites with at least 50% taxon coverage (step 3), for a total of 324 ingroup taxa, 381 loci, and 364,661 sites.

The individual locus alignments were used for both coalescent and maximum likelihood super matrix (ML) tree reconstruction. For coalescent tree reconstruction individual gene trees were reconstructed for each locus in IQ-Tree v2.2.0 (Schmidt et al., 2014) using the GTR+I+G model, gene trees were then used to assemble the coalescent species tree in ASTRAL-Pro (Zhang et al., 2020). Nodal supports in the Astral tree were assessed using local posterior probabilities. For maximum likelihood super matrix reconstruction the 481 loci were concatenated into a super matrix in Geneious 10.2.4 and analyzed as a single partition using the GTR+I+G model in IQ-Tree (Schmidt et al., 2014) with 1000 ultrafast bootstrap replicates to assess nodal support (Hoan et al., 2018).

To determine the number of gene trees that contributed to each branch of our coalescent topology we calculated gene concordance factor (gCF) using IQ-Tree v2.2.0 (Minh et al., 2020). We used gCF values to determine which gene tree topologies had high discordance with the species tree. To assess their influence on our phylogenetic reconstruction we removed the 5% of gene trees with the largest discordance (i.e., outlier gene trees) and reconstructed a second (more concordant tree with 458) coalescent species tree. We compared the original coalescent tree to the new coalescent tree (outlier gene trees removed) to determine if, and to what extent, the outlier gene trees affected the species tree topology. To determine the agreement among sites in our 481 loci super matrix for each branch in our maximum likelihood super matrix topology we calculated the site concordance factor (sCF) in IQ-Tree v2.2.0 (Minh et al., 2020) averages over 100 quartet scores.

Evolution of Sexual Signals

Specimens were identified as male or female by a combination of morphological characters 1. reproductive organs, 2. light organ morphology, in some groups females only have a light organ on a single ventrite while males have light organs on two ventrites (e.g., *Heterophotinus* and *Pleotomus;* Branham and Wenzel, 2003), and 3. paedomorphism, which is present only in female fireflies of select species. We used our coalescent phylogeny with 50% taxon coverage and outlier loci removed to reconstruct the gains and losses of adult bioluminescence and the use of glows, flashes and/or pheromones as sexual signals during the evolution of our taxon sample. To determine where these shifts occurred on the phylogeny we used parsimony ancestral state reconstruction in Mesquite v 3.80 (Madison and Madison, 2023) and different coding schemes for signal mode (pheromones or bioluminescence) and bioluminescent signal type (flashes or glows). For each analysis, any taxa with unknown states were treated as missing data.

1.Presence/absence of adult bioluminescence. We coded the presence or absence of adult bioluminescence (BL) in our study taxa based on the absence (0) or presence (1) of an adult light organ in our specimens, supplemented by records in the literature, character states were coded as unordered. The majority of our DNA voucher specimens were male fireflies (N=330) and only a few species were represented by females (N=12). We coded males and females separately, but if either sex had a light organ we coded adult bioluminescence as present for the respective taxon. If light organs (and thus bioluminescence) were absent in a species this indicated that the respective firefly species relied exclusively on pheromones during mate search. This data set included 294 taxa (Table 3.1).

2.Mating signals: Flashes versus glows versus pheromones. Bioluminescent fireflies can produce continuous glows or short flashes arranged in species-specific flash patterns. To capture this diversity, we used the literature to code taxa that produce pheromones only (0), glows (1), or flashes (2) during mate search; character states were considered unordered. This data set included 230 taxa (Table 3.1).

Morphological measurements of eyes, antennae, and light organ

To investigate the evolution of eyes and antennae as the sensory organs that detect sexual signals, we took eye and antenna measurements from 112 DNA voucher specimens (36 diurnal and 76 nocturnal and 55 genera) that were included in our phylogeny. These included images for dorsal and ventral habitus, the frontal view of the head and eyes, the dorsal pronotum (as a proxy for body size), and antennae. Images were taken with the Leica DFC290 imaging system. We obtained morphological measurements using the straight line, segmented line, and polygon tools in ImageJ (Rasband, 2015). Measurements included (Fig. 3.2): pronotum length (distance between the most anterior and most posterior point of the pronotum at the midline), antennal length (average of the length of both fully intact antennae from the base of the scape to the distal end of the final antennomere; if one antennae was damaged we used only the measurement of the fully intact antenna), the max eye span (the distance between the two most lateral points of the eyes at the widest point of the eyes), the minimum eye span (the distance between the interior margins of the eye at the widest point of the eyes), and eye distance (distance between the interior margins of the eye at the top of the head).

Light organ shape.

To reconstruct the evolution of male light organ (LO) shapes we classified and coded the shape of the 180 adult male light organs in our data set using four categories (Branham and Wenzel 2003): (0) single spot (per ventrite), (1) 2 paired spots (per ventrite), (2) central strip (no light organ tissue on the anterior and posterior margins of the ventrite), and (3) entire (light organ occupying the total area of the ventrite). If species were represented by female vouchers, we used records in the literature to determine the shape of the male light organ. This data set included 242 taxa (Table 3.1).

Light organ size.

The majority of adult male bioluminescent fireflies have light organs (LO) located on the fifth and sixth ventrites (visible ventral abdominal plate); while the males of a few species (e.g., in the genera *Pleotomus, Robopus, Heterophotinus*) have LOs located solely on ventrite seven (Branham and Wenzel, 2003). We took images of the LOs of 27 male DNA voucher specimens with a Leica DFC290. Due to sexual dimorphism in light organ size in many fireflies (Marshad et al., 2008; Fu et al., 2012; Souto et al., 2019), we excluded species from our analysis that were represented by female vouchers. We used these images and the polygon tool in ImageJ (Rasband, 2015) to measure the LO area for each ventrite. The LO tissue can occupy a portion or the entire surface area of the ventrite and can be distinguished by its smooth yellow to ivory appearance from the rest of the ventrite which matches the color of the other abdominal ventrites. For species that had LO tissue on two ventrites (i.e., ventrites five and six) we added the LO area from both ventrites for a total LO area measurement. To quantify how much of the potential LO area on the respective ventrites that was actually used as a light organ by the different morphological LO variants, we calculated the relative LO area for the different morphological LO variants we measured the total ventrite (with LOs) areas and divided it by the actual LO area.

Phylogenetic generalized least squares

We pruned the coalescent Astral tree to include only the taxa that had no missing data for our eye and antenna measurements (112 males). We calculated Pagel's λ for each morphological measurement (antennal length, max eye span, minimum eye span, eye distance, and eye area) using the phylosig() function in the R package phytools (Revell, 2012). To calculate the phylogenetic signal of the categorical variable of signal type (pheromones/bioluminescence) we used the phylo.d() function in the R v 4.3.1 (R Studio Team, 2020) package caper (Orme et al., 2023) to determine the D statistic. To determine the variation due to sexual signal type and body size for each of our eye and antenna measurements, while accounting for phylogenetic relatedness we used phylogenetic generalized least squares (PGLS).

We used the comparative.data() function in caper to construct a comparative data set for our measurements with a variance covariance matrix. We used the pgls() function in caper to account for variation in eye and antenna measurements due to phylogeny and body size (pronotum length). We tested five models (Table 3.2), one for each measurement variable, 1. Log(average antennal length), 2. Log(maximum eye span), 3. Log(eye distance), 4. Log(minimum eye span), 5. Log(average eye area), (example: Log(antennal length) ~ signal type + Log(pronotum length)). In instances where measurements for an individual was 0 (eye distance and minimum eye span) we transformed all measurements for this character by adding 1, then we took the log of the transformed value.

To investigate the evolution of light organ size, we used our data set of 27 bioluminescent males and pruned the coalescent Astral tree to contain only taxa in this analysis. We determined phylogenetic signal of relative and absolute light organ size and determined the variation in light organ size due to bioluminescent type (flash/glow) and body size, while accounting for phylogeny. We calculated Pagel's λ for relative and absolute light organ measurements using phylosig() and used phylo.d() to calculate the D-statistic for bioluminescent type. We used the comparative.data() function to account for phylogeny and determine the correlation between light organ size and bioluminescent type. We tested models for both relative and absolute light organ size with the explanatory variables of bioluminescent type and body size (example: Log(relative light organ area) ~ bioluminescence type + Log(pronotum length)).

Species that flash have periods of darkness between flashes, as opposed to continuous glows, this means that flash signals are not always visible and could go undetected by conspecific mates if not noticed at the right time. One way to increase light intensity and visibility would be to increase the surface area of the LO. A larger area of light emission may increase likelihood for detection from conspecifics. We predict that flashing species will have larger light organs than glowing species.

Discriminant analysis

Stanger-Hall et al. (2018) showed that signal type could be predicted with eye and antennal measurements in a phylogenetic discriminant analysis of 46 North American taxa. We coded the signal type for 93 of our 109 worldwide taxa with eye and antenna measurements, for the other 16 taxa we were unable to find records on signal type in the literature. As a result, character states for signal type were coded as pheromone, flash, glow, or unknown. We used 74 of the 93 taxa that had known signal method as training taxa for a phylogenetic discriminant analysis with the program phylo.FDA.v0.2 in R, this included 36 flashing, 4 glowing, and 34 pheromone taxa. We selected training taxa to represent our distribution of both signal type and taxonomic diversity, with most taxa representing the subfamilies Lampyrinae (N=53) and Luciolinae (N=25). The remaining 35 were used as test taxa, 16 of the test taxa had known signal types and were used to verify the discriminate analysis, with the other 19 taxa had truly unknown signal types, with no records in the literature. Of the 16 known test taxa there were 7 flashing, 5 glowing, and 4 pheromone species. We used measurement of eye and antennal size and body size (pronotum length) to inform the discriminate analysis, along with our coalescent phylogeny to account for relatedness.

Results

Coalescent topology

The final coalescent topology included 458 loci, once outlier gene trees were removed, with 342 taxa Fig. 3.3), the topology did not significantly differ from the coalescent topology that included outlier loci. The final maximum likelihood super matrix topology included 435,727 sites from 458 loci and 342 taxa (Fig. 3.4). The coalescent phylogeny recovered 2 of the 10 subfamilies as monophyletic (Lamprohizinae, and Amydetinae) and 3 subfamilies (Pterotinae, Chespiritoinae, and Cheguevariinae), were represented by only one individual, therefore their monophyly cannot be assessed. The five other subfamilies were not supported as monophyletic: Cheguevariinae was recovered within Photurinae. Luciolinae was recovered as non-monophyletic due to the placement of *Lampyris zenkeri* (Lampyrinae) within Luciolinae and *Asymmetricata circumdata* (Luciolinae) was recovered within the Photurinae. In addition, multiple Lampyrinae genera were recovered within the Photurinae, (e.g., *Lucidota*, *Dilychnia,* and *Erythrolychnia*), rendering the subfamilies non-monophyletic. A single *Psilocladus* sp. was recovered within the Lampyrinae, rendering Psilocladinae as non-monophyletic. The Ototretinae were recovered in two clades as a base of the tree rendering the subfamily non-monophyletic. The same relationships regarding monophyly were recovered in our maximum likelihood super matrix phylogeny (Fig. 3.4).

In the coalescent analysis the two Ototretinae clades were recovered as the most basal and the second most basal clades, sister to the remaining Lampyridae (Fig. 3.5). Pterotinae was the next lineage to diverge, followed by *Polliclasis* + Chespiritoinae, then Luciolinae. From the remaining subfamilies Lampyrinae was recovered sister to Psilocladinae, this grouping was recovered as sister to Amydetinae + Photurinae. Cheguevariinae was recovered within Photurinae. The same subfamily relationships were recovered in our maximum likelihood super matrix topology (Fig. 3.4).

The genus *Crassitarsus*, previously designated as incertae sedis (Martin et al., 2019) was recovered within the second clade of Ototretinae. While *Lamprigera* was recovered within Luciolinae and *Polliclasis* was sister to Chespiritoinae. We also recovered *Cladodes*, *Ethra*, and Vestini which were previously designated as Lampyrinae incertae sedis, within Photurinae, while *Scissicauda* was recovered in Lampyrinae. These genera were placed in the same subfamilies in our maximum likelihood super matrix topology (Fig. 3.4).

Topology support

We assessed nodal support for our coalescent topology using two measures, posterior probability, and gene concordance factor (gCF; Fig. 3.3). Posterior probabilities were high (1) for all subfamily relationships. Most nodes within the subfamilies had posterior probabilities >0.90, those with lower support are noted in Fig 3.3. Gene concordance factors are a measure of the number of decisive gene trees that support a node (Minh et al. 2020), our highest gCF values was 99.04 meaning that 99.04% of gene trees were concordant with the species tree and decisive for that node, our lowest gCF values was 1.43 for the relationships between two clades within the Lampyrinae. gCF values for subfamilies ranged from 31.1 for the sistergrouping of (Psilocladinae + Lampyrinae) + (Amydetinae + Photurinae) to 8.1 for the relationships between

Lampyrinae and Psilocladinae. Nodal support for the maximum likelihood super matrix topology was measured using 1000 ultrafast bootstrap replicates and site concordance factor (sCF). Bootstrap values for subfamily relationships were high (1). Site concordance factors are a measure of the number of decisive sites supporting a node (Minh et al. 2020), our highest sCF values was 97.86 meaning that 97.86% of the 435,727 sites are both concordant and decisive for the species tree.

Signal evolution

Ancestral state reconstruction supported pheromones as the ancestral firefly sexual signal. Adult bioluminescence evolved five - eight times, while it was lost seven - thirteen times (Fig. 3.6). There have been five - nine origins of flashing signals and six origins of glowing signals (Fig. 3.7). Notable shifts in bioluminescence type included the evolution of glowing from non-bioluminescent ancestors of Lamprohizinae and flashing ancestors in the Amydetinae. Our analysis also indicates that the Pterotinae were the first lineage to evolve adult bioluminescence in the form of flashing. The ancestral state of Lampyrinae is flashing bioluminescence with three independent shifts to glowing and at least three shifts back to pheromones. The common ancestor of the Photurinae was also a flashing firefly, with at least one loss of bioluminescence in the Vestini (Fig. 3.7).

Light organ shape

Analysis of light organ shape indicated ten to eleven independent origins of the single spots shape, ten to eleven origins of the paired spots, 15-17 of the bar shape, and 10-12 origins of the light organ occupying the entire ventrite (Fig. 3.8). The ancestral state for Luciolinae light organ shape was identified as entire, with three shifts in *Pygatphella*, *Lampryoidae*, and *Luciola* *lusitanica* to the bar shape and one to paired dots (*Lamprigera*). The ancestral state of the Lamprohizinae was the bar shape. There were multiple changes of light organ shape within the Lampyrinae and Photurinae, with the majority of light organ shapes being entire and paired spots

Phylogenetic signal and PGLS

Pagel's λ was calculated for phylogenetic signal of each continuous variable (Table3.3), where $\lambda = 0$ means the characters is independent of phylogeny while $\lambda=1$ means the character is exclusively dependent on phylogeny. In the full eye and antennae data set (N=112 males) phylogenetic signal was detected in two of our eye and antenna measurements, antennal length ($\lambda= 0.776$, $p=3.465e-16$) and eye area ($\lambda= 0.778$, $p=1.662e-5$). Signal type (pheromones/bioluminescence) also showed phylogenetic signal (D=0.313). In our data set of male bioluminescent species (N=27) relative light organ size had strong phylogenetic signal ($\lambda=0.999$, $p=0.00466$) while absolute light organ size did not indicate phylogenetic signal ($\lambda=7.332e-05$, $p=1$). Bioluminescent type (flashes/glows) showed a relatively weak phylogenetic signal in our data set of males with light organs (N=27, D=0.007).

We used the simplest model of evolution to test for the influence of signal type while accounting for phylogeny and body size for each measurement character (character ~ signal type + pronotum length; model tests Table 3.2; measurements supplemental Table 1). Signal type was significant for all eye and antennal characters except minimum eye span, (antennal length: $\lambda=0.863$, $R^2=0.150$, $p=0.000163$; maximum eye span: $\lambda=0$, $R^2=0.102$, $p=2.2e-6$; eye distance; $\lambda=0$, $R^2=0.102$, $p=0.00250$; eye area: $\lambda=0.259$, $R^2=0.533$, $p=2.2e-16$; Table 2). Bioluminescent signal type was not significantly correlated with relative light organ size (LO relative size ~ bioluminescent signal type + pronotum length, N=27; $\lambda=0.938$, R2=0.145, $p=0.458$) but it was significantly correlated with absolute light organ size (LO absolute size ~ bioluminescent type +

pronotum length, N=27, λ=0, R2=0.239, p=0.0145; model tests Table 3.4; measurements Supp. Table 3.2).

Discriminant analysis

The discriminant analysis incorrectly predicted the signal method six of out 16 known training taxa (Supp. Table 3.3). Four glowing taxa were incorrectly predicted as flashing species and two flashing species were predicted to use pheromones. Additionally, the discriminate analysis predicts the signal method for the training taxa to verify accuracy, 18 of the 74 training taxa were predicted incorrectly (Supp. Table 3.4). Pheromone taxa were incorrectly predicted as flashing taxa and true flashing taxa were predicted as glowing, while true glowing species were predicted as either pheromone or flashing.

Discussion

This study presents the most comprehensive phylogeny of Lampyridae to date, with 459 loci and 342 taxa, to date. This study uses the most robust representation of subfamilies (10 out of 11) and extensive sampling of Ototretinae, Photurinae, and Amydetinae, with a large molecular data set. Notably we determine the placement of Chespiritoinae and Cheguevariinae using our extensive molecular data set.

The lack of monophyly in the lampyrid subfamilies suggests that taxonomic work is necessary across the group. While the monophyly of Luciolinae genera has been supported by morphological work (Ballantyne & Lambkin, 2013; Ballantyne et al. 2016; Ballantyne et al., 2019), our morphological data suggest that further taxonomic work is necessary to further revise the systematic placement of species within these genera. With the extensive morphological data available for many of these species and our molecular data, this presents an ideal opportunity to join both the data types for a more comprehensive taxonomic study of the Luciolinae subfamily.

Additional groups that need furth taxonomic work include *Nyctophila* and *Lampyris* which form a clade, the Photurinae *Vesta, Dodacles,* and *Dilychnia,* and the genus *Lucidota*. The lack of monophyly in these groups makes apparent the need for additional morphological expertise in Lampyridae.

The low gCF values for some nodes in the topology may be indicators of high discordance among gene trees or weak phylogenetic signal (Minh et al. 2020). Additionally, gCF values tend to be lower with increased number of loci and short branch lengths (Minh et al. 2020), as seen in the coalescent topology with 496 genes.

Our ancestral state reconstruction of sexual signal method and bioluminescence type is consistent with previous hypotheses of sexual signal evolution in Lampyridae (Branham and Wenzel, 2003; Stanger-Hall et al. 2007, Martin et al. 2017). While pheromone use stands as the ancestral sexual signal in fireflies, the first bioluminescent adults used flashes as a sexual signal and glows evolved in later lineages. Additionally, we observed that light organ shape is convergent across Lampyridae. It is important to note that light organs presenting as paired spots could be vestigial larval light organs and not functional for sexual signaling, this highlights the importance of behavior records. Our analysis of sexual signal and light organ evolution provides the largest database of sexual signal characters for fireflies.

I identified phylogenetic signal in all antennal length, eye area, and relative light organ size, however, not in maximum eye span and eye distance which was shown in Stanger-Hall et al., (2018). This suggests that eye and antennal size do not change in the same way across Lampyridae, with shifts in signal method. We also determined that bioluminescent type (flashes and glows) has very weak phylogenetic signal, suggesting that the evolution of these traits is due to selective pressures rather than relatedness.

The incorrect predictions in our discriminate analysis suggests that patterns in eye and antennal size in relation to signal type or not consistent across Lampyridae. Stanger-Hall et al. (2018) was able to correctly predict signal type for taxa from three subfamilies. Our taxon sampling included seven subfamilies. This increased diversity likely increases morphological variation altering the parameters of the discriminate analysis. Subfamily-specific discriminate analyses may have increased accuracy because it reduces variation of characters that are strongly influenced by phylogeny (e.g., antennal size and eye area). Five of the seven families in our data set have limited representation, preventing subfamily specific analyses in this study, more extensive and diverse taxonomic sampling could provide further insight on the ability to predict signal type with morphological measurements. This would be valuable tool for studies of signal evolution when behavior information is lacking.

This study provides a valuable tool for future lampyrid systematic and evolutionary research. Our phylogeny will allow for the placement work focused on specific taxonomic groups (e.g., tribes and genera) in a broad systematic context and provides insight into groups that need additional taxonomic work. It also allows for additional evolutionary studies on subjects such as flash pattern, olfactory sensilla used for pheromone detection, and biogeography.

FIGURES, TABLES, AND LEGENDS

Table 3.1 Taxon sampling for phylogenetic reconstruction and signal type (pheromones/bioluminescence), bioluminescence type (Flash/Glow), and light organ character coding.

Species (with taxon ID)	**Family**	**Subfamily**	**Signal type**	**Bioluminescent type**	**Light organ shape**
CO2248_Memoan_sp._nov.	Lampyridae	Amydetinae	Bioluminescence	Glow	Entire
KSHX1020_Memoan_new_sp	Lampyridae	Amydetinae	Bioluminescence	Glow	
KSHX1419_Amydetes_fucata	Lampyridae	Amydetinae	Bioluminescence		
KSHX1420_Cladodes_cincticollis	Lampyridae	Amydetinae			
CO1212_Amydetes_fastigiata	Lampyridae	Amydetinae	Bioluminescence	Glow	
CO1214_Memoan_ciceroi	Lampyridae	Amydetinae	Bioluminescence	Glow	
CO1990_Magnoculus_or_near_	Lampyridae	Amydetinae	Pheromones	Pheromones	-
CO2230_Magnoculus__sp.3	Lampyridae	Amydetinae	Pheromones	Pheromones	-
CO2247_Magnoculus__sp.2	Lampyridae	Amydetinae	Bioluminescence	Glow	Bar
KSHX1021_Magnoculus_obscurus	Lampyridae	Amydetinae	Bioluminescence	Glow	
KSHX1025_Amydetes_apicalis	Lampyridae	Amydetinae	Pheromones	Pheromones	-
CHEZ002_Cheguevaria_sp	Lampyridae	Cheguevariinae	Pheromones	Pheromones	-
WIBF041527_Chespirito_zaragozai	Lampyridae	Chespiritinae	Pheromones	Pheromones	-
CO1299_Vesta_saturnalis	Lampyridae	incertae sedis	Pheromones	Pheromones	-
CO1471_Vesta__c.f._concolor	Lampyridae	incertae sedis	Bioluminescence	Pheromones	Single spot
CO1935_Vesta_sp	Lampyridae	incertae sedis	Bioluminescence	Pheromones	paired spots
CO1953_Crassitarsus?_	Lampyridae	incertae sedis			
CO1955_Crassitarsus?_	Lampyridae	incertae sedis			
CO1989_Dodacles__plumosa	Lampyridae	incertae sedis	Bioluminescence		paired spots
KSH385_Pollaclasis_bifaria	Lampyridae	incertae sedis	Pheromones	Pheromones	-
KSHX1007_Vesta_cf._thoracica	Lampyridae	incertae sedis	Bioluminescence	Pheromones	-
KSHX1412_Lamprigera_taimoshana	Lampyridae	incertae sedis	Bioluminescence		paired spots
KSHX1422_Dodacles_emissus	Lampyridae	incertae sedis			

KSHX1423_Dodacles_remixtus	Lampyridae	incertae sedis			
KSHX1424_Dryptelytra_cayannensis	Lampyridae	incertae sedis	Pheromones	Pheromones	-
KSHX259_Lamprigera_yunnana	Lampyridae	incertae sedis	Bioluminescence		paired spots
KSHX439_Dodacles_sp.	Lampyridae	incertae sedis			
LS124_Vesta_despecta	Lampyridae	incertae sedis	Pheromones	Pheromones	-
LS148_Vesta__sp._2	Lampyridae	incertae sedis	Pheromones	Pheromones	-
LS162_Vesta__"rubricollis"	Lampyridae	incertae sedis	Pheromones	Pheromones	-
LS472_Vesta__arcta	Lampyridae	incertae sedis	Pheromones	Pheromones	-
CO1549_Phausis_reticulata	Lampyridae	Lamprohizinae	Bioluminescence	Glow	
CO1926_Lamprohiza_sp	Lampyridae	Lamprohizinae	Bioluminescence	Glow	Bar
CO1931_Lamprohiza_sp	Lampyridae	Lamprohizinae	Bioluminescence	Glow	Bar
CO1937_Lamprohiza_sp	Lampyridae	Lamprohizinae	Bioluminescence	Glow	Bar
CO1963_near_Photinus/Photinoides_	Lampyridae	Lamprohizinae	Bioluminescence		Entire
CO1964_Pyropyga?_	Lampyridae	Lamprohizinae	Bioluminescence		paired spots
KSHX26_Lamprohiza_splendidula	Lampyridae	Lamprohizinae	Bioluminescence	Glow	Bar
CO_ARG_Pyractonema_sp._2	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1057_Photinus_stellaris	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1060_Lamprocera_sp._1	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1063_Pyractonema_sp._1	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1067_Heterophotinus_sp.	Lampyridae	Lampyrinae	Bioluminescence	Flash	
CO1069_Aspisoma_sp.	Lampyridae	Lampyrinae	Bioluminescence	Flash	
CO1071_Lampyris_noctiluca	Lampyridae	Lampyrinae	Bioluminescence	Glow	
CO1074_Photinus_macdermotti_1	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1080_Pyropyga_nigricans	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1092_Microphotus_sp.	Lampyridae	Lampyrinae	Bioluminescence	Glow	paired spots
CO1197_Ellychnia_sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1211_Cladodes_illigeri	Lampyridae	Lampyrinae	Pheromones	Pheromones	-

CO1213_Ethra_axillaris	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1215_Scissicauda_disjuncta	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1223_Diaphanes_sp._1	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1227_Petalacmis_sp.	Lampyridae	Lampyrinae	Bioluminescence		
CO1231_Lamprocera_sp._4	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1232_Lucio_blattinum	Lampyridae	Lampyrinae	Bioluminescence		
CO1234_Cratomorphus_sp.	Lampyridae	Lampyrinae	Bioluminescence		
CO1269_Scisiccauda_sp.	Lampyridae	Lampyrinae	Pheromones		-
CO1270_Photinus_sp._8	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1271_Lucidotina__sp.	Lampyridae	Lampyrinae	Bioluminescence		Entire
CO1306_Tenaspis_angularis	Lampyridae	Lampyrinae	Pheromones		
CO1307_Pleotomodes_needhami	Lampyridae	Lampyrinae	Bioluminescence	Glow	
CO1457_Lamprocera_sp._3	Lampyridae	Lampyrinae	Pheromones		paired spots
CO1459_Lamprocera_sp._2	Lampyridae	Lampyrinae	Pheromones		paired spots
CO1476_Lamprocerini_sp.	Lampyridae	Lampyrinae	Bioluminescence		Single spot
CO1496_Platylampis_explanata	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1516_Diaphanes_sp._2	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1523_Diaphanes_sp._4	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1524_Diaphanes_sp._3	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1528_Photinus_sp._2	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1546_Photuris_congener	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1547_Photinus_pyralis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1551_Lucidota_atra	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1552_Pyropyga_decipiens	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1928_Nyctophila_sp	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1929_Microphotus_sp	Lampyridae	Lampyrinae	Bioluminescence	Glow	paired spots
CO1932_Nyctophila_sp	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1933_Nyctophila_sp	Lampyridae	Lampyrinae			
CO1934_Nyctophila_sp	Lampyridae	Lampyrinae			

CO1936_Photinus_sp	Lampyridae	Lampyrinae		Flash	
CO1941_Tenaspis_OR_Lychnacris_	Lampyridae	Lampyrinae	Bioluminescence		Bar and spot
CO1942_Callopisma_sp	Lampyridae	Lampyrinae	Bioluminescence	Glow	Single spot
CO1943_Aspisomoides_sp	Lampyridae	Lampyrinae	Bioluminescence		Entire
CO1944_Lychnacris__sp	Lampyridae	Lampyrinae	Bioluminescence		Entire
CO1945_Pyrocoelia_sp	Lampyridae	Lampyrinae			
CO1946_Lucidina_sp	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1948_Lampyrini/Cratomorphini_	Lampyridae	Lampyrinae	Bioluminescence		Single spot
CO1949_Diaphanes_sp	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1952_Pyrocelia_sp	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1958_Lucidota_bella	Lampyridae	Lampyrinae	Pheromones	-	-
CO1959_Heterophotinus_sp	Lampyridae	Lampyrinae	Bioluminescence	Flash	paired spots
CO1960_Callopisma_sp	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1966_Pleotomus_sp	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1966_Pleotomus_sp.	Lampyridae	Lampyrinae	Bioluminescence		
CO1967_Lamprocerini/Pleotomini_-new_genus-_	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1968_Cratomorphus/Nyctocera_	Lampyridae	Lampyrinae	Bioluminescence		partial, Bar
CO1969_Nyctophila__reichii	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO1971_Phosphaenus_hemipterous	Lampyridae	Lampyrinae	Bioluminescence	Glow	check
CO1974_Lucidina_biplagiata	Lampyridae	Lampyrinae		Pheromones	-
CO1975_Photinus_signaticollis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1977_Callopisma_emarginata	Lampyridae	Lampyrinae	Bioluminescence	Flash	
CO1979_Erythrolychnia_roseimargo	Lampyridae	Lampyrinae	Bioluminescence		Single spot
CO1980_Heterophotinus_sp	Lampyridae	Lampyrinae	Bioluminescence	Flash	
CO1986_Robopus_nigrifrons	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO1987_Micronaspis_floridana	Lampyridae	Lampyrinae	Bioluminescence	Flash	
CO1988_Cratomorphus_signativentris	Lampyridae	Lampyrinae	Bioluminescence		Bar and spots
CO2124_Microphotus_sp.	Lampyridae	Lampyrinae	Bioluminescence	Glow	paired spots

CO2226_Aspisoma_c.f._pallens	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO2227_Aspisoma_lineatum	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO2229_Rufolychnia_borencona	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO2259_Phaenolis__sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO2260_Dilychnia_c.f._guttala	Lampyridae	Lampyrinae	Bioluminescence		Single spot
CO2261_Lucidina_sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
CO2262_Cratomorphus_sp.	Lampyridae	Lampyrinae	Bioluminescence		Bar and spots
CO2263_Pyractonema_sp.	Lampyridae	Lampyrinae			
CO2287_Aspimodes?__sp.	Lampyridae	Lampyrinae			
CO2288_Heterophotinus_sp.	Lampyridae	Lampyrinae	Bioluminescence	Flash	
CO2290_Callopisma__sp.	Lampyridae	Lampyrinae			
CO2291_Petalacmis_sp.	Lampyridae	Lampyrinae	Bioluminescence		
CO2299_Lampyrini__sp.	Lampyridae	Lampyrinae			
CO2302_Lucidota_c.f._aurantiaca	Lampyridae	Lampyrinae	Bioluminescence		paired spots
CO2304_Ototretinae__sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSH1006_Photinus_floridanus	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH10104_Pyractomena_linearis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1028_Photinus_australis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1053_Pyractomena_lucifera	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1078_Pyractomena_angulata	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1313_Microphotus_angustus	Lampyridae	Lampyrinae	Bioluminescence	Glow	paired spots
KSH1461_Photinus_granulatus	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1765_Photinus_ardens	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1766_Photuris_flavicollis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH1779_Pyractomena_angulata	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH2096_Pyractomena_linearis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH2099_Pyractomena_dispersa_DD	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH2100_Pyractomena_dispersa	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH2125_Pyractomena_ecostata	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire

KSH24_Pleotomus_pallens	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSH382_Lucidota_punctata	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSH4007_Pyractomena_dispersa	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH4008_Pyractomena_punctiventris	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH4012_Pyractomena_borealis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH4029_Pyractomena_dispersa	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH408_Pyractomena_borealis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH412_Pyractomena_limbicollis	Lampyridae	Lampyrinae	Bioluminescence	Flash	paired spots
KSH472_Paraphausis_eKSHXimius	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSH474_Microphotus_dilatatus	Lampyridae	Lampyrinae	Bioluminescence	Glow	paired spots
KSH5850_Pyractomena_sp._3	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH5852_Pyractomena_sp._1	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH609_Ellychnia_corrusca	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSH713_Pyractomena_angulata	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH8115_Pyractomena_borealis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH8126.1_Pyractomena_marginalis	Lampyridae	Lampyrinae	Bioluminescence	Flash	
KSH8715_Photinus_not_macdermotti_2	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH9013_Photinus_brimleyi	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH9224_Pyractomena_angulata	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH9348_Pyractomena_marginalis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSH9670_Photinus_carolinus	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHDNA10A_Pyractomena_palustris	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHX1001_Luciuranus_jameshooki	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1003_Phaenolis_basalis	Lampyridae	Lampyrinae			
KSHX1005_"Lucidota"_sp	Lampyridae	Lampyrinae			
KSHX1008_Costalampis_delicata	Lampyridae	Lampyrinae			
KSHX1009_Micronaspis_gabrielae	Lampyridae	Lampyrinae	Bioluminescence	Glow	
KSHX1011_Uananna_angaporan	Lampyridae	Lampyrinae			
KSHX1013_Platylampys_diversithorax	Lampyridae	Lampyrinae			

KSHX1014_Pyractonema_sp_nov?	Lampyridae	Lampyrinae			
KSHX1015_Dilychnia_propinqua	Lampyridae	Lampyrinae	Bioluminescence		
KSHX1017_Dilychnia_succensa	Lampyridae	Lampyrinae	Bioluminescence		
KSHX1018_Dilychnia_dumasi	Lampyridae	Lampyrinae	Bioluminescence		
KSHX1019_Lucernuta_savignii	Lampyridae	Lampyrinae	Bioluminescence	Glow	
KSHX1023_Lucidota_flabellicornis	Lampyridae	Lampyrinae			
KSHX1024_Luciuranus_josephi	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1026_Cladodes_stellata	Lampyridae	Lampyrinae			
KSHX1027_Cladodes_minor_group_sp_nov1	Lampyridae	Lampyrinae			
KSHX1029_Ybytyramoan_sp_nov_IG	Lampyridae	Lampyrinae	Bioluminescence	Flash	
KSHX1030_Ybytyramoan_diasi	Lampyridae	Lampyrinae		female	
KSHX1031_Ybytyramoan_praeclarum	Lampyridae	Lampyrinae	Bioluminescence	Flash	Bar
KSHX1032_Ybytyramoan_diasi	Lampyridae	Lampyrinae	Bioluminescence	Flash	Single spot
KSHX1033_Ybytyramoan_monteirorum	Lampyridae	Lampyrinae	Bioluminescence	Flash	Bar
KSHX1034_Ybytyramoan_sp_nov_PB	Lampyridae	Lampyrinae	Bioluminescence	Flash	
KSHX1036_Ybytyramoan_sp_nov_Campos	Lampyridae	Lampyrinae	Bioluminescence	Flash	
KSHX1275_Photinus_signaticollis	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHX1276_Nyctophila__heydeni	Lampyridae	Lampyrinae			
KSHX1279_Nyctophyla_reichii	Lampyridae	Lampyrinae			
KSHX1286_Lampyris_iberica/noct?	Lampyridae	Lampyrinae	Bioluminescence	Glow	paired spots
KSHX1299_L/Pheromones_zenkeri	Lampyridae	Lampyrinae			
KSHX1302_Lucidota__amablilis	Lampyridae	Lampyrinae	Bioluminescence		Single spot
KSHX1304_Lucidota__apicalis	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1332_Nyctophila_syriaca	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSHX1333_Lampyris__sp._3_-new	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSHX1334_Lampyris/Nyctophila_pseudozenkeri	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSHX1378_Lampyris__fuscata_apuliae	Lampyridae	Lampyrinae		Glow	
KSHX140_Phosphaenopterus_metzneri	Lampyridae	Lampyrinae	Bioluminescence		

KSHX1415_Pyrocelia_lunata	Lampyridae	Lampyrinae	Bioluminescence		Bar
KSHX1421_Cladodes_ater	Lampyridae	Lampyrinae			
KSHX1426_Macrolampis_acicularis	Lampyridae	Lampyrinae	Bioluminescence		
KSHX1427_Macrolampis_cf._acicularis	Lampyridae	Lampyrinae	Bioluminescence		
KSHX1428_Pseudolychnuris_vittatus	Lampyridae	Lampyrinae			
KSHX1429_Ybytyramoan_sp_nov	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHX1430_Lucidota_luteicollis	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1431_Lucidota_banoni	Lampyridae	Lampyrinae	Bioluminescence		Single spot
KSHX1434_Platylampis_latiuscula	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1436_Ethra_sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1437_Phaenolis_cf._ustulatus	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1438_Photinus_sp._Peru	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHX1439_Photinus_sp._tobago	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHX1443_Afrodiaphanes_sp.	Lampyridae	Lampyrinae			
KSHX1449_Lucio_sp._1	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1450_Lucio__sp._2	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1451_Roleta__sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1452_Lucernuta__savignii	Lampyridae	Lampyrinae	Bioluminescence	Glow	Bar
KSHX1456_Haplocauda_yasuni	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSHX1457_Calyptocephalus_sp.	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX1461_Alychnus_suturalis	Lampyridae	Lampyrinae	Pheromones	Pheromones	-
KSHX188_Photinus__interdius	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
KSHX250_Diaphanes_pectinealis	Lampyridae	Lampyrinae	Bioluminescence	Flash	paired spots
KSHX256_Pyrocoelia_pygidialis	Lampyridae	Lampyrinae	Bioluminescence	Flash	
KSHX299_Lampyris_raymondi	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSHX3_Pyrocoelia_formosana_	Lampyridae	Lampyrinae	Bioluminescence		Entire
KSHX300_Lampyris_sardiniae	Lampyridae	Lampyrinae	Bioluminescence		paired spots
KSHX301_Lampyris_zenkeri/germariensis?	Lampyridae	Lampyrinae	Bioluminescence		paired spots

KSHX321_Lampyris_fuscata_fuscata	Lampyridae	Lampyrinae			
KSHX386_"Lucidota_like_antennae"_	Lampyridae	Lampyrinae	Bioluminescence		Bar
KSHX421_Aspisoma_trilineata_	Lampyridae	Lampyrinae	Bioluminescence	Flash	Bar
LS166_Lucidota_discolor	Lampyridae	Lampyrinae			
LS387_Lucidota_bella	Lampyridae	Lampyrinae			
LS416_Rufolychnia_borencona	Lampyridae	Lampyrinae	Bioluminescence	Flash	Entire
CO1222_Australoluciola_sp.	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1225_Luciola_sp._2	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1229_Trisinuata_sp	Lampyridae	Luciolinae	Bioluminescence		
CO1255_Abscondita_pallescens	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1297_Asymmetricata_circumdata	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1303_Curtos_obsuricolor	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1505_Uncertain_Luciolinae_nr._Luciola_1	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1509_Uncertain_Luciolinae_nr._Luciola_4	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1510_Uncertain_Luciolinae_nr._Luciola_3	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1517_Pteroptyx_sp.	Lampyridae	Luciolinae	Bioluminescence	Flash	
CO1518_Atyphella_olivieri	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1520_Australoluciola_nigra	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1525_Uncertain_Luciolinae_nr._Luciola_2	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1534_Atyphella_leucura	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1939_Luciolinae_sp	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1954_Luciolinae_sp.	Lampyridae	Luciolinae	Bioluminescence		Entire
CO1970_Atyphella_maritimus?	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1972_Medeopteryx_	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO1973_Sclerotia_brahmina	Lampyridae	Luciolinae	Bioluminescence		Entire
CO1976_Magnalata_limbata	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO2295_Aquatica__lateralis	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
CO2301_Erythrolychnia_sp.	Lampyridae	Luciolinae	Bioluminescence		Entire

KSHX11_Abscondita_cerata	Lampyridae	Luciolinae	Bioluminescence	Flash	
KSHX1287_Lucioloa_lusitanica	Lampyridae	Luciolinae	Bioluminescence	Flash	
KSHX1288_Luciola__italica	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1290_Photinus__signaticollis	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1294_Luciola__lusitanica	Lampyridae	Luciolinae	Bioluminescence	Flash	Bar
KSHX1322_Lampyroidea_nigrescens	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1324_Lampyroidea_maculicollis	Lampyridae	Luciolinae	Bioluminescence	Flash	Bar
KSHX1326_Lampyroidea_sp._2_-new	Lampyridae	Luciolinae	Bioluminescence	Flash	Bar
KSHX1328_Lampyroideae__sp._4	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1337_Luciola_novaki	Lampyridae	Luciolinae	Bioluminescence		Entire
KSHX1389_Aquatica_leii	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1392_Abscondita__terminalis	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1394_Medeopteryx_honkongensis	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1409_Pteroptyx_maipo	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1418_Luciolinae_sp.	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1446_Pyrophanes_similissima	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1447_Pygatyphella_obsoleta	Lampyridae	Luciolinae	Bioluminescence	Flash	Bar
KSHX1448_Pygatyphella_uberia	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire/split
KSHX1459_Convexa_wolfi	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX1460_Luciola__sp._Cameroon	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX266_Emeia_pseudosauteri	Lampyridae	Luciolinae	Bioluminescence	Flash	Bar
KSHX268_Pygoluciola_qinqyu	Lampyridae	Luciolinae	Bioluminescence	Flash	
KSHX281_Curtos_bilineatus	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX332_Luciola_lusitanica	Lampyridae	Luciolinae	Bioluminescence		Entire
KSHX454_Abscondita__sp.	Lampyridae	Luciolinae	Bioluminescence	Flash	Entire
KSHX484_Luciola__lusitanica	Lampyridae	Luciolinae	Bioluminescence	Flash	
KSHX1441_Harmatelia_bilinea	Lampyridae	Otoretinae	Pheromones	Pheromones	-
CO1302_Stenocladius_shirakii	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO1308_Drilaster_sp.	Lampyridae	Ototretinae			paired spots

CO1927_Ototretinae__sp	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO1930_Stenocladius_sp	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2123_Brachylampis__sanguinicollis	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2254_Ototretinae_sp._1	Lampyridae	Ototretinae		Pheromones	-
CO2255_Ototretinae_sp._2	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2256_Ototretinae_sp._3	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2257_Ototretinae_sp._4	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2258_Ototretinae_sp._5	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2289_Lamellipalpus_pacholatkoi_pacholatkoi	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO2303_Ototretinae__sp.	Lampyridae	Ototretinae	Pheromones	Pheromones	-
KSHX1404_Stenocladius_bicoloripes	Lampyridae	Ototretinae			
KSHX1406_Stenocladius_sp.	Lampyridae	Ototretinae	Pheromones	Pheromones	-
CO1049_Photuris_divisia	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
CO1062_Pyrogaster_sp.	Lampyridae	Photurinae	Bioluminescence	Flash	
CO1093_Becellonycha_wickershamorum	Lampyridae	Photurinae			
CO1228_Bicellonycha_sp._2	Lampyridae	Photurinae	Bioluminescence	Flash	
CO1940_Bicellonycha_sp	Lampyridae	Photurinae	Bioluminescence	Flash	Single spot
CO1947_Pyrogaster_sp	Lampyridae	Photurinae	Bioluminescence	Flash	Bar
CO1965_Photuris_sp	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
CO1985_Presbyolampis_vagaensis	Lampyridae	Photurinae			
KSH1477_Photuris_lucicrescens	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
KSH406_Photuris_frontalis	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
KSH8870_Photuris_quadrifulgens	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
KSHX1004_Photuris_femoralis	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
KSHX1010_Pyrogaster_telephorinus	Lampyridae	Photurinae	Bioluminescence	Flash	
KSHX1012_Pyrogaster_vestitus	Lampyridae	Photurinae	Bioluminescence	Flash	
KSHX1264_Photuris_vitta	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
KSHX1308_Bicellonycha__sp.	Lampyridae	Photurinae	Bioluminescence	Flash	

KSHX167_Bicellonycha_sp._1	Lampyridae	Photurinae	Bioluminescence	Flash	
LS22_Photuris__sp._1	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
LS474_Bicellonycha__sp._2	Lampyridae	Photurinae	Bioluminescence	Flash	
LS476_Photuris__sp._3	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
LS478_Photuris__sp._2	Lampyridae	Photurinae	Bioluminescence	Flash	Entire
LS48_Bicellonycha_discicollis	Lampyridae	Photurinae	Bioluminescence	Flash	
LS8_Pyrogaster__sp._1	Lampyridae	Photurinae	Bioluminescence	Flash	
LS94_Bicellonycha__sp._3	Lampyridae	Photurinae	Bioluminescence	Flash	
CO1216_Psilocladus_sigillatus	Lampyridae	Psilocladinae	Bioluminescence	Flash	
CO1245_Psilocladus_sp._1	Lampyridae	Psilocladinae	Bioluminescence	Flash	
KSHX1309_Psilocladus_sp._7	Lampyridae	Psilocladinae	Bioluminescence	Flash	Bar
KSHX1310_Psilocladus_sp._8	Lampyridae	Psilocladinae	Pheromones	Pheromones	-
KSHX1311_Psilocladus_sp._9	Lampyridae	Psilocladinae	Bioluminescence	Flash	Single spot
KSHX1314_Psilocladus_sp._11	Lampyridae	Psilocladinae	Bioluminescence	Flash	Single spot
KSHX1315_Psilocladus_sp._4	Lampyridae	Psilocladinae	Bioluminescence	Flash	paired spots
KSHX1432_Psilocladus_miltoderus	Lampyridae	Psilocladinae	Bioluminescence	Flash	
CO1550_Pterotus_obscuripennis	Lampyridae	Pterotinae	Bioluminescence	Flash	Entire
CO1310_Ampedus_pomonae	Elateridae		Pheromones	Pheromones	-
CO1961_???_	Lampyridae		Pheromones	Pheromones	-
CO1962_???_	Lampyridae		Bioluminescence		Single spot
CO1991_New_Genus_EQ_1__	Lampyridae		Pheromones	Pheromones	-
CO2293_Paraguay__sp.	Lampyridae				
CO2294_Paraguay__sp._2	Lampyridae				
CO2296_Brunei_sp._1_sp.	Lampyridae				
CO2297_Paraguay__sp._3	Lampyridae		Bioluminescence		Entire
CO2298_China_sp.__sp.	Lampyridae		Pheromones	Pheromones	-
CO2300_Indonesia__sp.	Lampyridae				
COVL_uncertain_Vietnam	Lampyridae				
COVM_uncertain_Vietnam	Lampyridae		Bioluminescence	Flash	

COVS_uncertain_Vietnam	Lampyridae			
KSH1894_Zarhipis_integripennis	Phengodidae	Bioluminescence	Glow	
KSHX1006_New?_	Lampyridae			
KSHX1022_Gen_nov_12_antnm_sp_nov?	Lampyridae			
KSHX111_KSHX111_	Lampyridae	Bioluminescence		Entire
KSHX1401_Rhagophthalmus_motschulski	Rhagopthalmidae	Bioluminescence	Glow	Bar

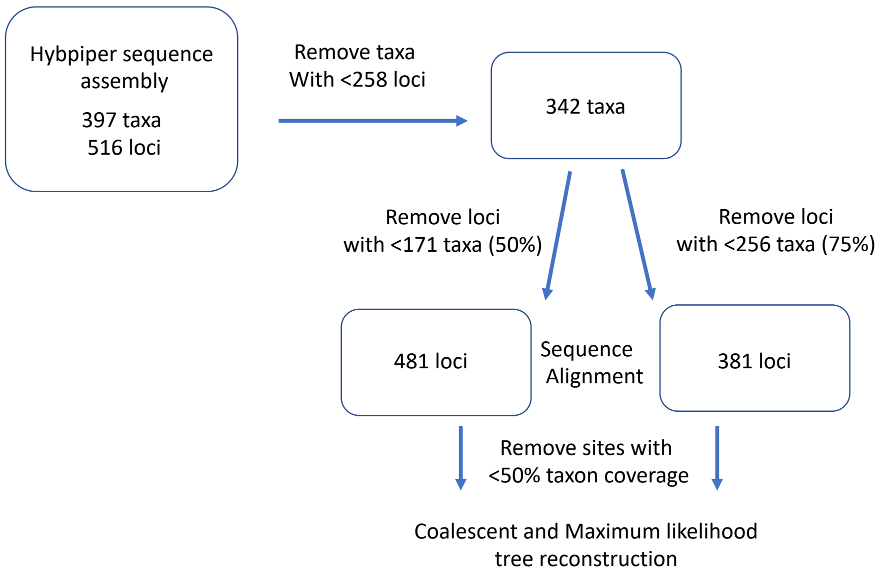


Figure 3.1 Flow chart of data filtering process.

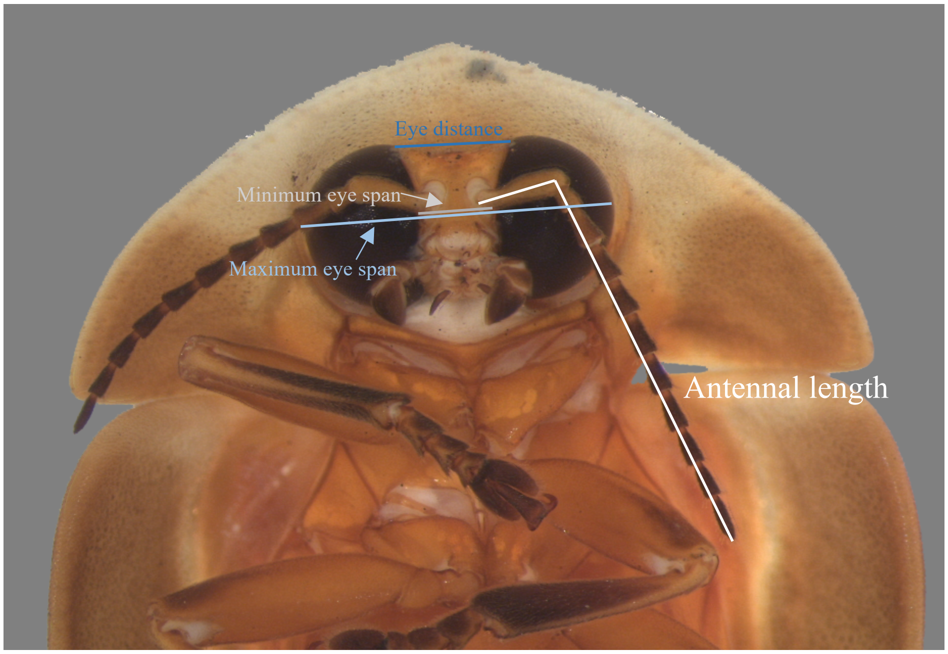
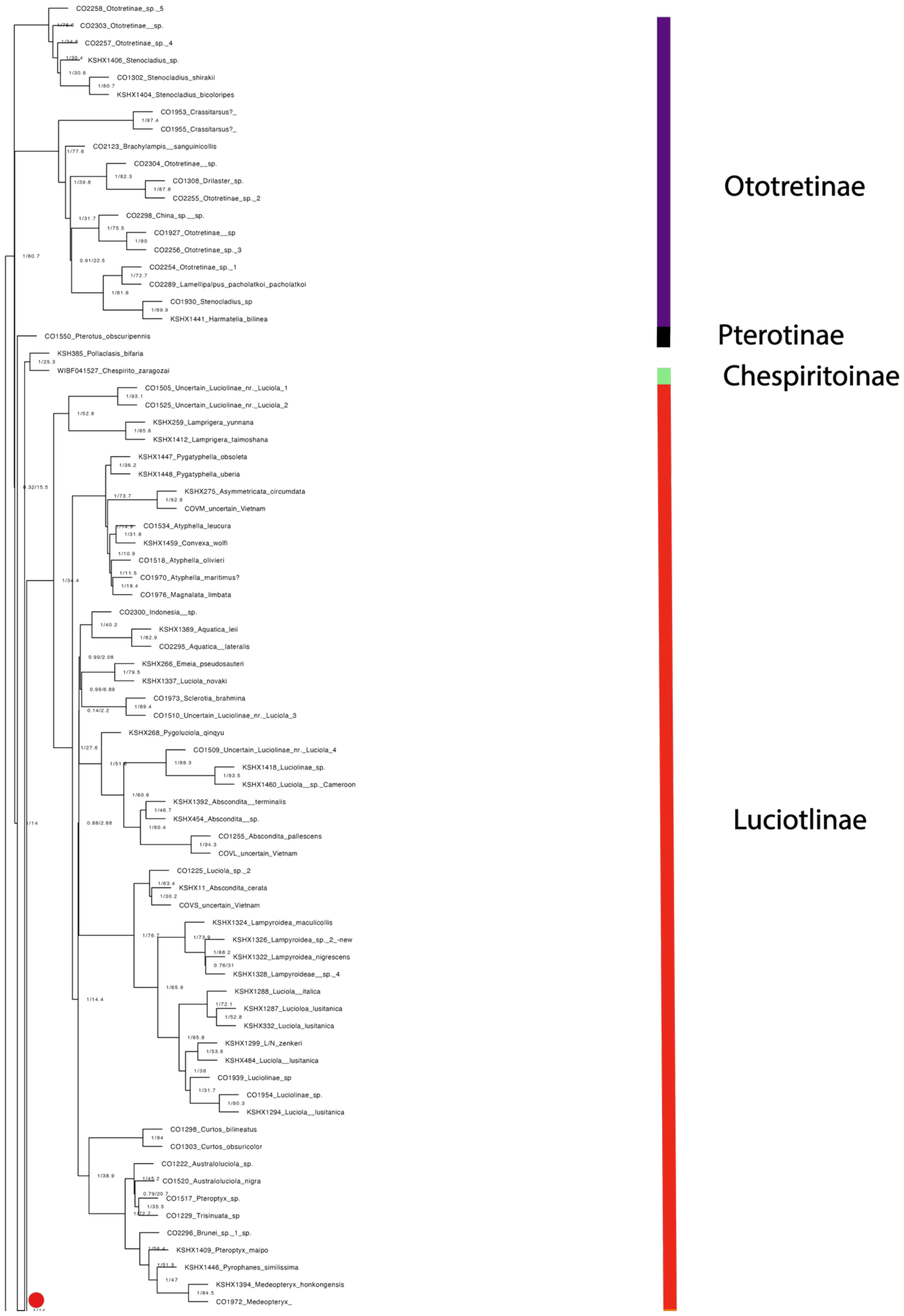


Figure 3.2 Eye and antennal measurements for PGLS.

Table 3.2 PGLS models for each eye and antennal measurement, N=112. Signal type is represented by activity time diurnal = pheromones and nocturnal = bioluminescence.

Model: Antennal Area	Model: Max eye span	Model: Eye distance	Model: Min eye span	Model: Eye Area
Log(AntArea) ~ Activity + Log(pronotum length)	Log(MaxEye) ~ Activity + Log(pronotum length)	Log(EyeDistance) ~ Activity + Log(pronotum length)	Log(MinEye) ~ Activity + Log(pronotum length)	Log(EyeArea) ~ Activity + Log(pronotum length)
Residuals:	Residuals:	Residuals:	Residuals:	Residuals:
Min 1Q Median 3Q Max	Min 1Q Median 3Q Max	Min 1Q Median 3Q Max	Min 1Q Median 3Q Max	Min 1Q Median 3Q Max
-0.154 -0.0338 0.00829 0.0623 0.157	-0.0943 -0.0129 0.00376 0.0179 0.117	-0.0824 -0.0120 -0.00182 0.00782 0.0649	-0.106 -0.016 -0.00142 0.0161 0.446	-0.414 -0.0582 -0.00439 0.0454 0.237
Branch length transformations:	Branch length transformations:	Branch length transformations:	Branch length transformations:	Branch length transformations:
kappa [Fix] : 1.000, delta [Fix] : 1.000	kappa [Fix] : 1.000, delta [Fix] : 1.000	kappa [Fix] : 1.000, delta [Fix] : 1.000	kappa [Fix] : 1.000, delta [Fix] : 1.000	kappa [Fix] : 1.000, delta [Fix] : 1.000
lambda [ML] : 0.863	lambda [ML] : 0.000	lambda [ML] : 0.000	lambda [ML] : 0.000	lambda [ML] : 0.259
lower bound : 0.000, p = 2.085e-13	lower bound : 0.000, p = 1	lower bound : 0.000, p = 1	lower bound : 0.000, p = 1	lower bound : 0.000, p = 0.030183
upper bound : 1.000, p = 0.0023861	upper bound : 1.000, p = 4.1644e-11	upper bound : 1.000, p = 2.4549e-06	upper bound : 1.000, p = 6.3207e-12	upper bound : 1.000, p = 7.7905e-07
95.0% CI : (0.658, 0.968)	95.0% CI : (NA, 0.336)	95.0% CI : (NA, 0.583)	95.0% CI : (NA, 0.304)	95.0% CI : (0.020, 0.645)
Coefficients:	Coefficients:	Coefficients:	Coefficients:	Coefficients:
Estimate Std.Error t value Pr(>|t|)	Estimate Std.Error t value Pr(>|t|)	Estimate Std.Error t value Pr(>|t|)	Estimate Std.Error t value Pr(>|t|)	Estimate Std.Error t value Pr(>|t|)
(Intercept) 0.220 0.0775 2.843 0.00538	(Intercept) 0.0312 0.0239 1.306 0.195	(Intercept) 0.201 0.0156 12.891 < 2.2e-16	(Intercept) 0.253 0.0504 5.0095 2.217e-06	(Intercept) -1.051 0.0934 -11.252 < 2.2e-16
Nocturnal 0.0345 0.0350 0.985 0.327	Nocturnal 0.0925 0.0225 4.115 7.711e-05	Nocturnal 0.00554 0.0147 0.378 0.706	Nocturnal 0.00193 0.0474 0.0408 0.968	Nocturnal 0.397 0.0619 6.406 4.314e-09
Activity_unknown -0.0369 0.0900 -0.410 0.683	Activity_unknown 0.0168 0.0706 0.237 0.813	Activity_unknown 0.0214 0.0461 0.464 0.644	Activity_unknown 0.00518 0.149 0.0348 0.972	Activity_unknown 0.137 0.176 0.790 0.438
LogPrL 0.4403 0.0980 4.490 1.835e-05	LogPrL 0.514 0.0526 9.781 2.220e-16	LogPrL 0.133 0.0343 3.875 0.000186	LogPrL -0.136 0.111 -1.227 0.222	LogPrL 1.374 0.161 8.509 1.326e-13
Residuals(SE) = 0.0704 (df) = 105	Residuals(SE) = 0.02999 (df) =105	Residuals(SE) = 0.01955 (df) =105	Residuals(SE) = 0.06322 (df) =105	Residuals(SE) = 0.08219 (df) =105
Multiple R-squared: 0.173 Adjusted R-squared: 0.150	Multiple R-squared: 0.521 Adjusted R-squared: 0.507	Multiple R-squared: 0.127 Adjusted R-squared: 0.102	Multiple R-squared: 0.0142 Adjusted R-squared: -0.014	Multiple R-squared: 0.546 Adjusted R-squared: 0.533
F-statistic: 7.34 on 3 and 105 DF, p-value: 0.000163	F-statistic: 38.07 on 3 and 105 DF, p-value: < 2.2e-16	F-statistic: 5.088 on 3 and 105 DF, p-value: 0.00250	F-statistic: 0.5031 on 3 and 105 DF, p-value: 0.681	F-statistic: 42.08 on 3 and 105 DF, p-value: < 2.2e-16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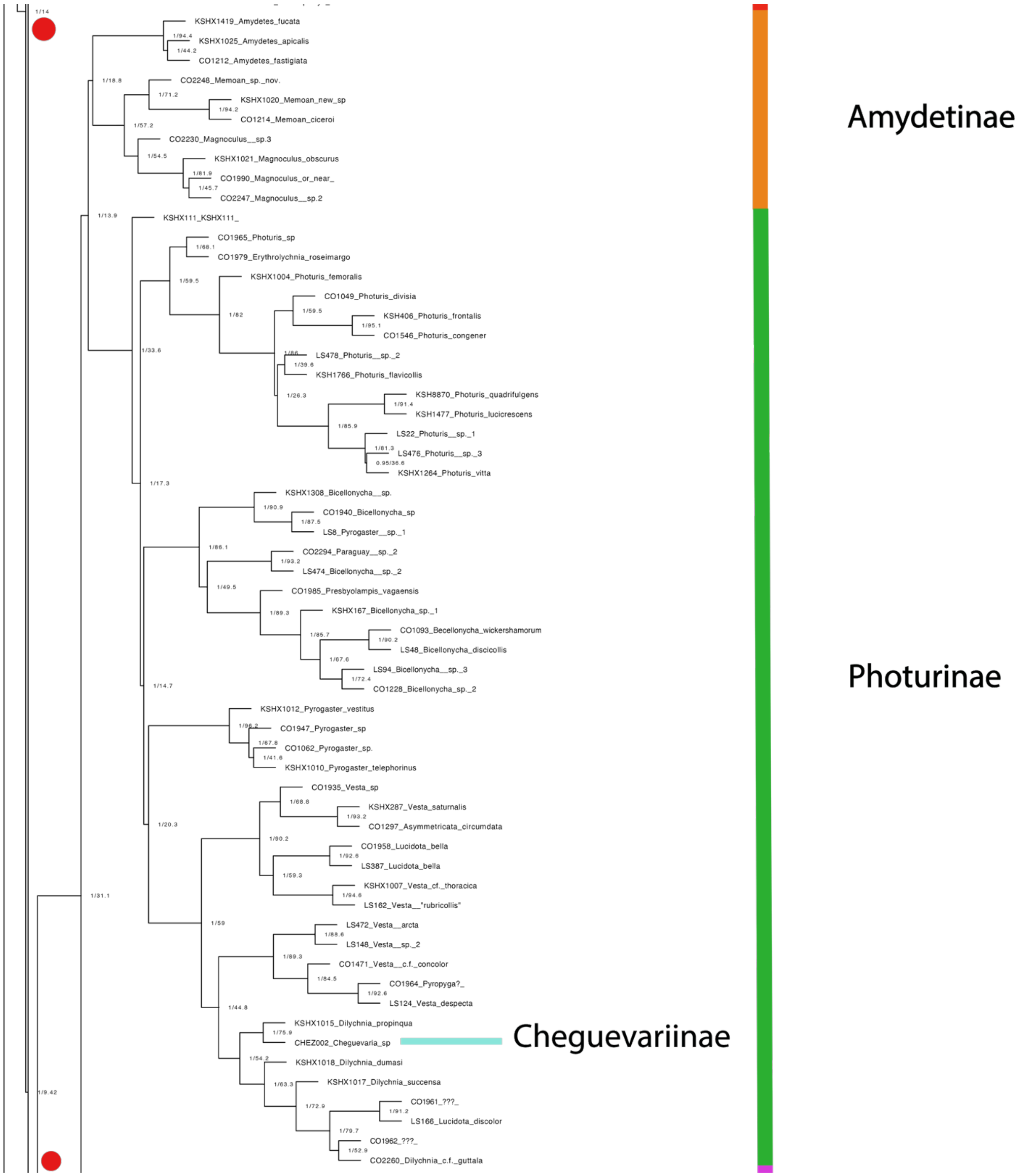
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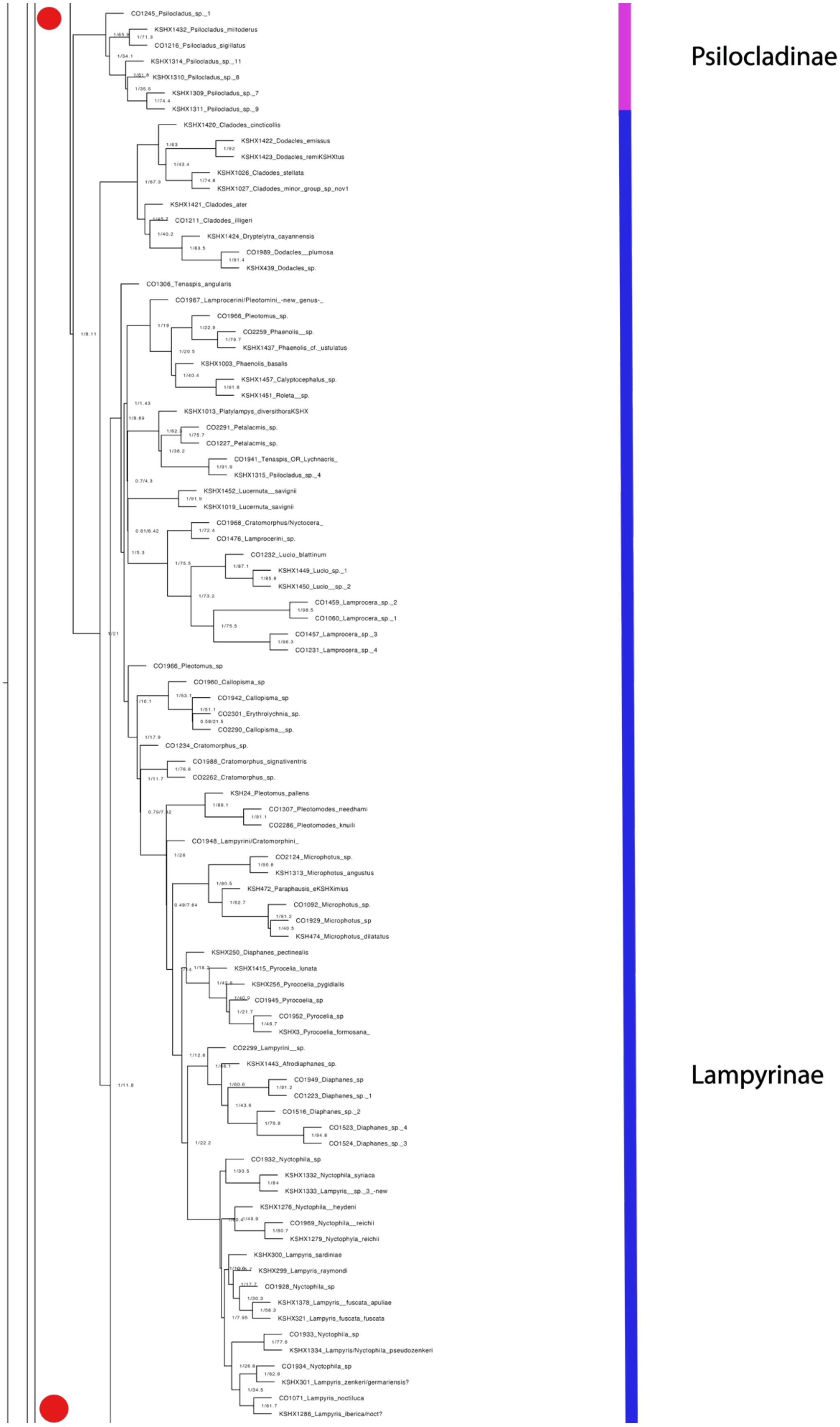
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KSHX1450_Lucio__sp._2
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1/98.5
CO1060_Lamprocera_sp._1
1/75.5
CO1457_Lamprocera_sp._3
1/21
1/96.3
CO1231_Lamprocera_sp._4
CO1966_Pleotomus_sp
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CO2290_Callopisma__sp.
1/17.9
CO1234_Cratomorphus_sp.
CO1988_Cratomorphus_signativentris
1/76.6
1/11.7
CO2262_Cratomorphus_sp.
KSH24_Pleotomus_pallens
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0.79/7.42
1/91.1
CO2286_Pleotomodes_knuili
CO1948_Lampyrini/Cratomorphini_
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CO1952_Pyrocelia_sp
1/46.7
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Psilocladinae
Lampyrinae

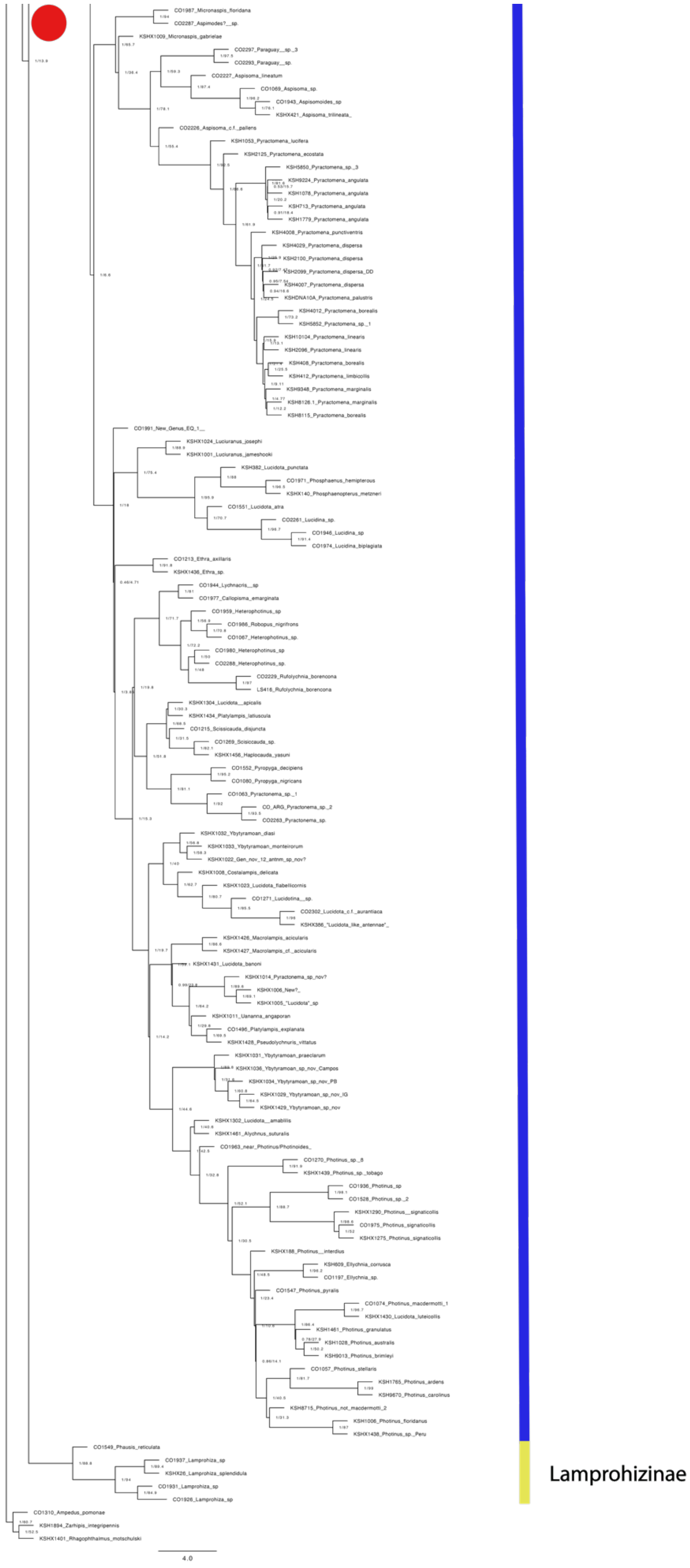
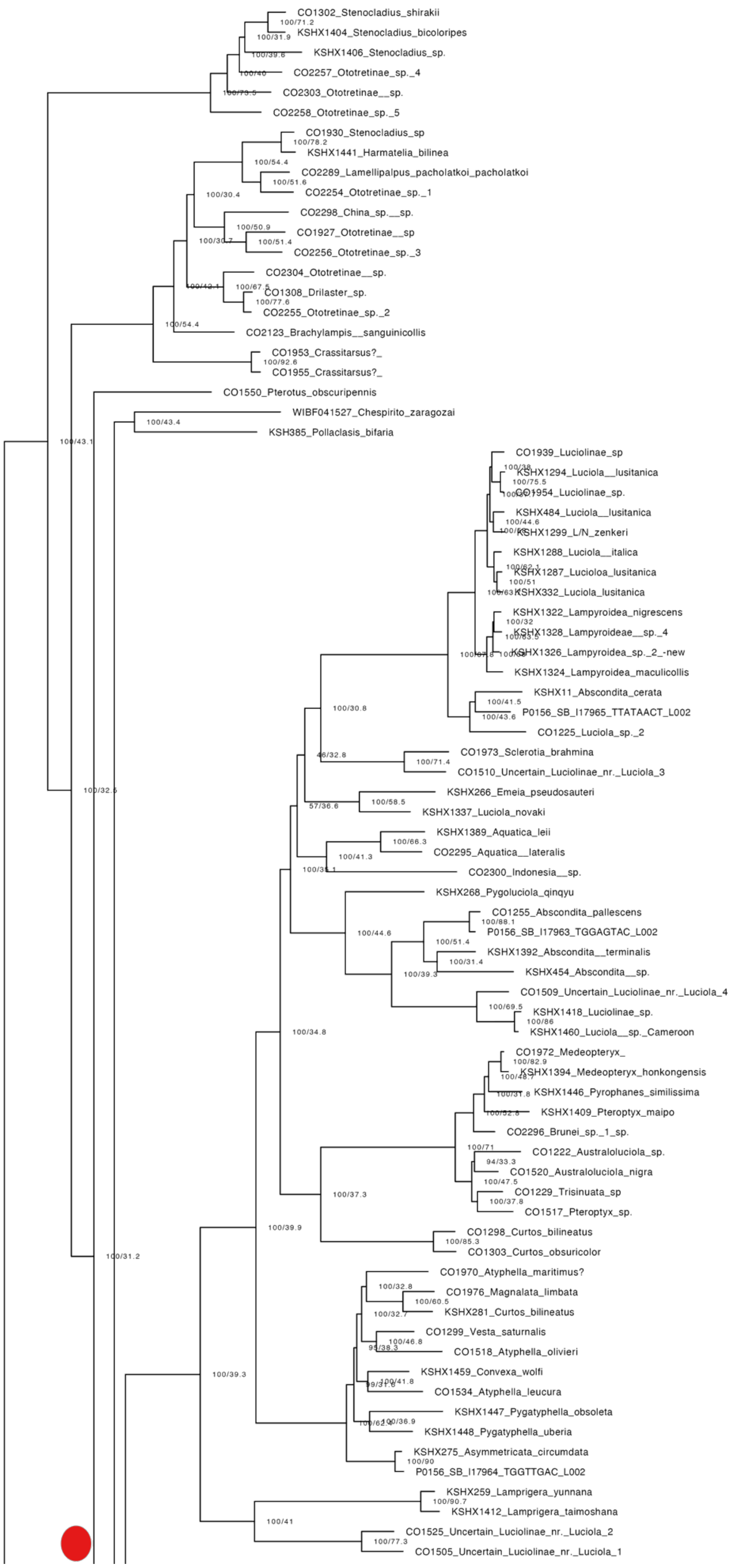


Figure 3.3 Coalescent topology of 342 species with posterior probability/gCF values for nodal support. Circles indicate where tree backbone matches with previous and following pages.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100/40
CO2303_Ototretinae__sp.
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CO2258_Ototretinae_sp._5
CO1930_Stenocladius_sp
100/78.2
KSHX1441_Harmatelia_bilinea
100/54.4
CO2289_Lamellipalpus_pacholatkoi_pacholatkoi
100/51.6
CO2254_Ototretinae_sp._1
100/30.4
CO2298_China_sp.__sp.
100/50.9
CO1927_Ototretinae__sp
100/30.7
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CO2256_Ototretinae_sp._3
CO2304_Ototretinae__sp.
100/42.1
100/67.5
CO1308_Drilaster_sp.
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CO2255_Ototretinae_sp._2
100/54.4
CO2123_Brachylampis__sanguinicollis
CO1953_Crassitarsus?_
100/92.6
CO1955_Crassitarsus?_
CO1550_Pterotus_obscuripennis
WIBF041527_Chespirito_zaragozai
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KSH385_Pollaclasis_bifaria
100/43.1
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100/38
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100/75.5
CO1954_Luciolinae_sp.
100/37.7
KSHX484_Luciola__lusitanica
100/44.6
100/58
KSHX1299_L/N_zenkeri
KSHX1288_Luciola__italica
100/62.1
KSHX1287_Lucioloa_lusitanica
100/51
100/63
KSHX332_Luciola_lusitanica
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100/71
CO1222_Australoluciola_sp.
94/33.3
CO1520_Australoluciola_nigra
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100/37.3
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CO1298_Curtos_bilineatus
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CO1970_Atyphella_maritimus?
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CO1976_Magnalata_limbata
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100/32.7
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CO1534_Atyphella_leucura
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100/90.7
KSHX1412_Lamprigera_taimoshana
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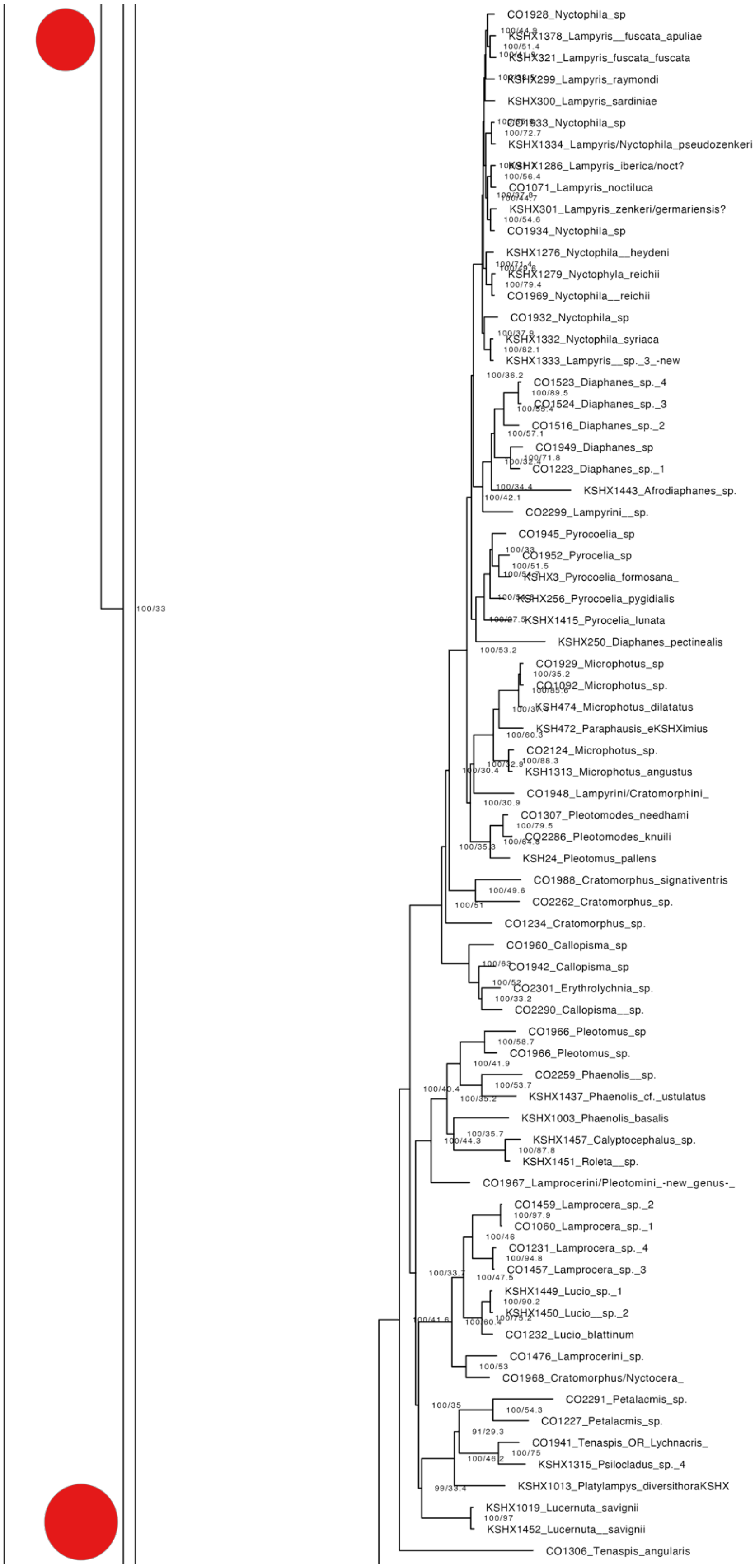
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CO1933_Nyctophila_sp
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KSHX1286_Lampyris_iberica/noct?
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100/37.8
100/44.7
KSHX301_Lampyris_zenkeri/germariensis?
100/54.6
CO1934_Nyctophila_sp
KSHX1276_Nyctophila__heydeni
100/71.4
100/49.6
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100/79.4
CO1969_Nyctophila__reichii
CO1932_Nyctophila_sp
100/37.9
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100/82.1
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100/89.5
CO1524_Diaphanes_sp._3
100/55.4
CO1516_Diaphanes_sp._2
100/57.1
CO1949_Diaphanes_sp
100/71.8
100/32.4
CO1223_Diaphanes_sp._1
100/34.4
KSHX1443_Afrodiaphanes_sp.
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CO1945_Pyrocoelia_sp
100/33
CO1952_Pyrocelia_sp
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KSHX256_Pyrocoelia_pygidialis
100/27.5
KSHX1415_Pyrocelia_lunata
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CO1092_Microphotus_sp.
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CO1234_Cratomorphus_sp.
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100/63
CO1942_Callopisma_sp
100/52
CO2301_Erythrolychnia_sp.
100/33.2
CO2290_Callopisma__sp.
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CO1060_Lamprocera_sp._1
100/46
CO1231_Lamprocera_sp._4
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CO1457_Lamprocera_sp._3
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100/60.4
100/75.2
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CO1476_Lamprocerini_sp.
100/53
CO1968_Cratomorphus/Nyctocera_
100/35
CO2291_Petalacmis_sp.
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CO1227_Petalacmis_sp.
91/29.3
CO1941_Tenaspis_OR_Lychnacris_
100/75
100/46.2
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100/97
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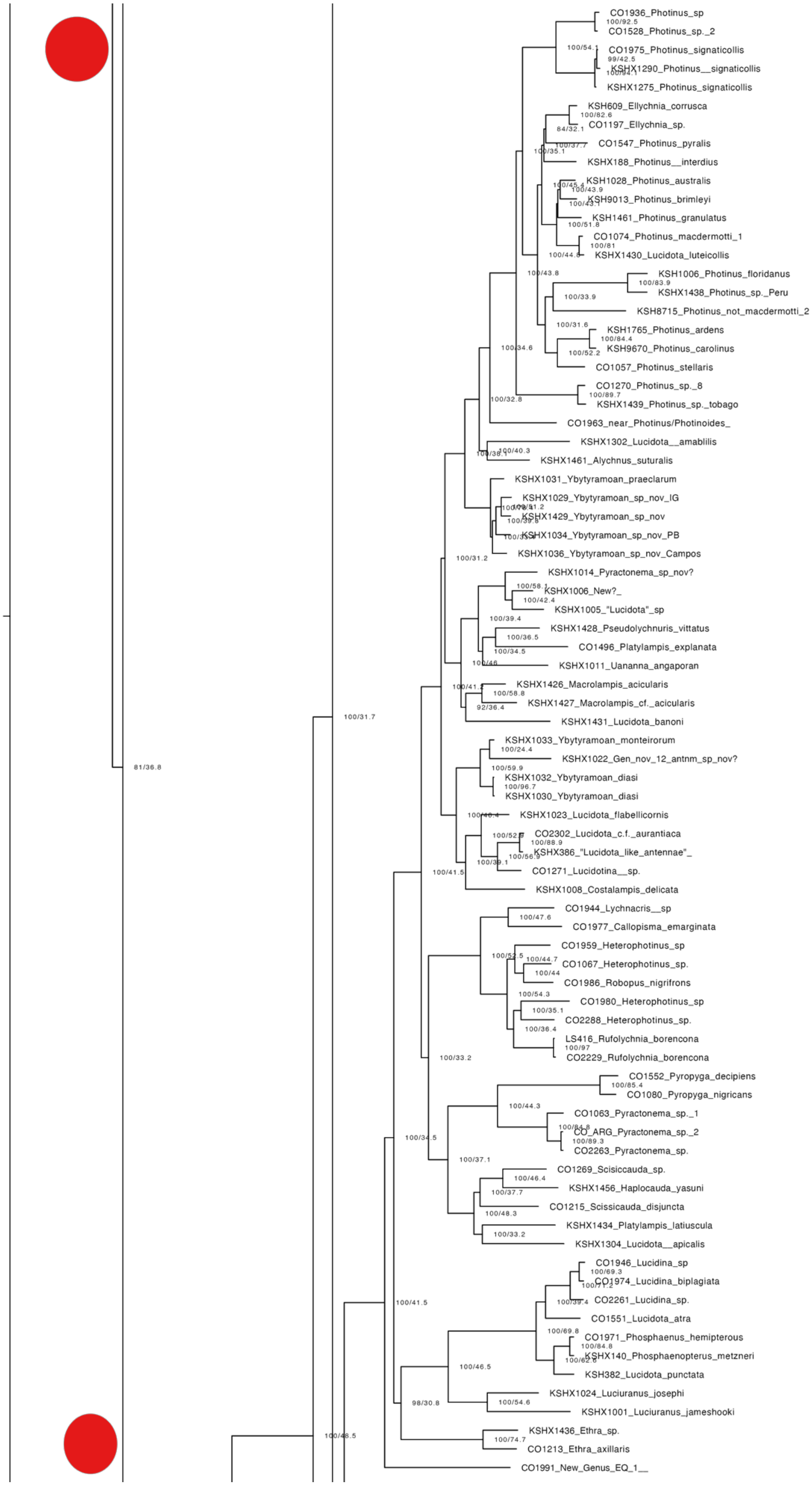
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100/94.1
KSHX1275_Photinus_signaticollis
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CO1547_Photinus_pyralis
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KSH1461_Photinus_granulatus
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KSHX1030_Ybytyramoan_diasi
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100/44
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CO2288_Heterophotinus_sp.
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81/36.8


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48/35.4
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100/32.5
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100/88.2
100/60.9
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KSHX1421_Cladodes_ater
KSHX1424_Dryptelytra_cayannensis
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100/31.7
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CO1245_Psilocladus_sp._1
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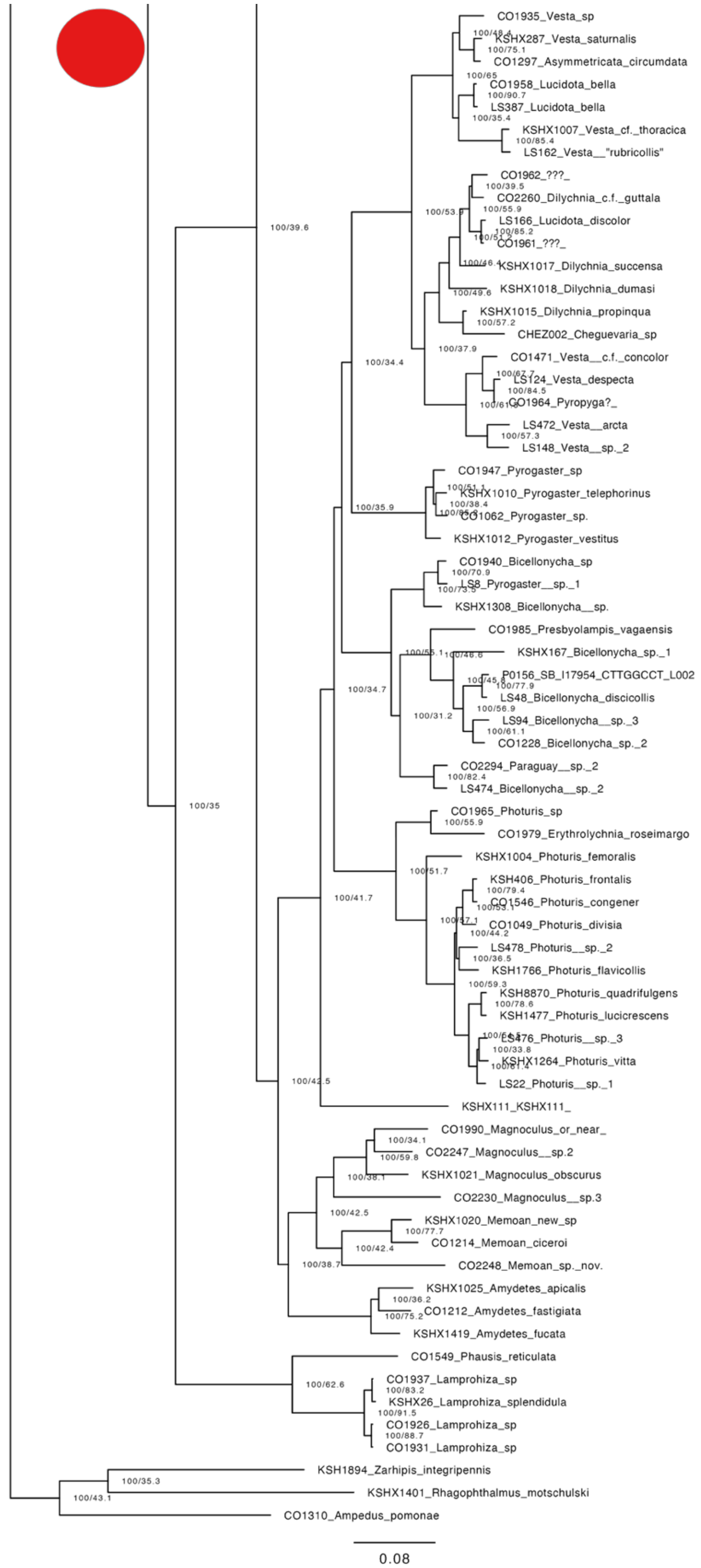


Figure 3.4 Maximum likelihood super matrix topology of 342 species with bootstrap/sCF values for nodal support.

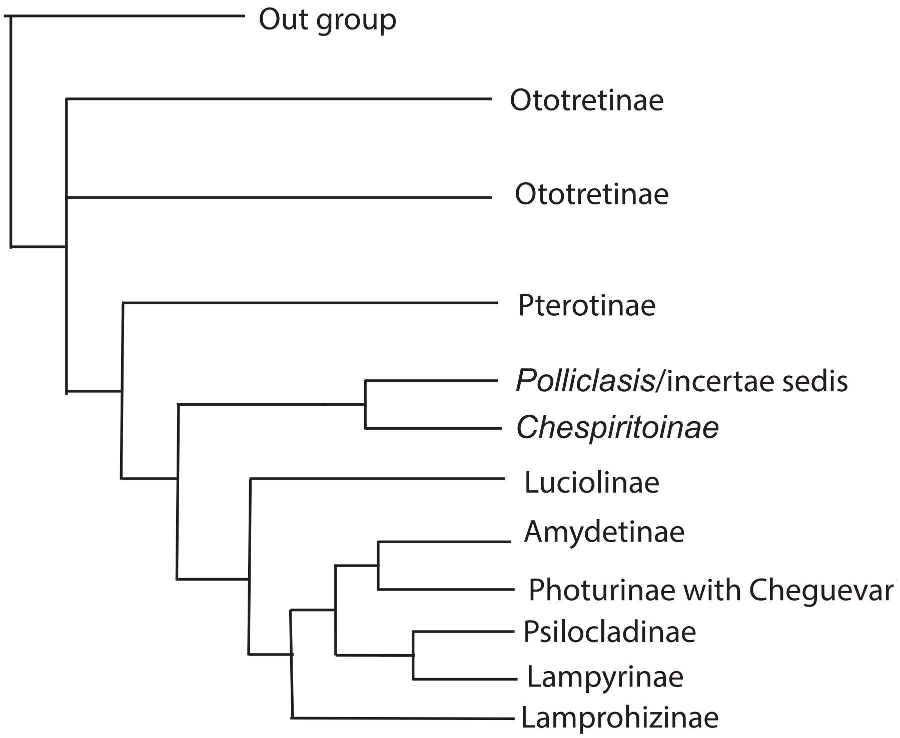
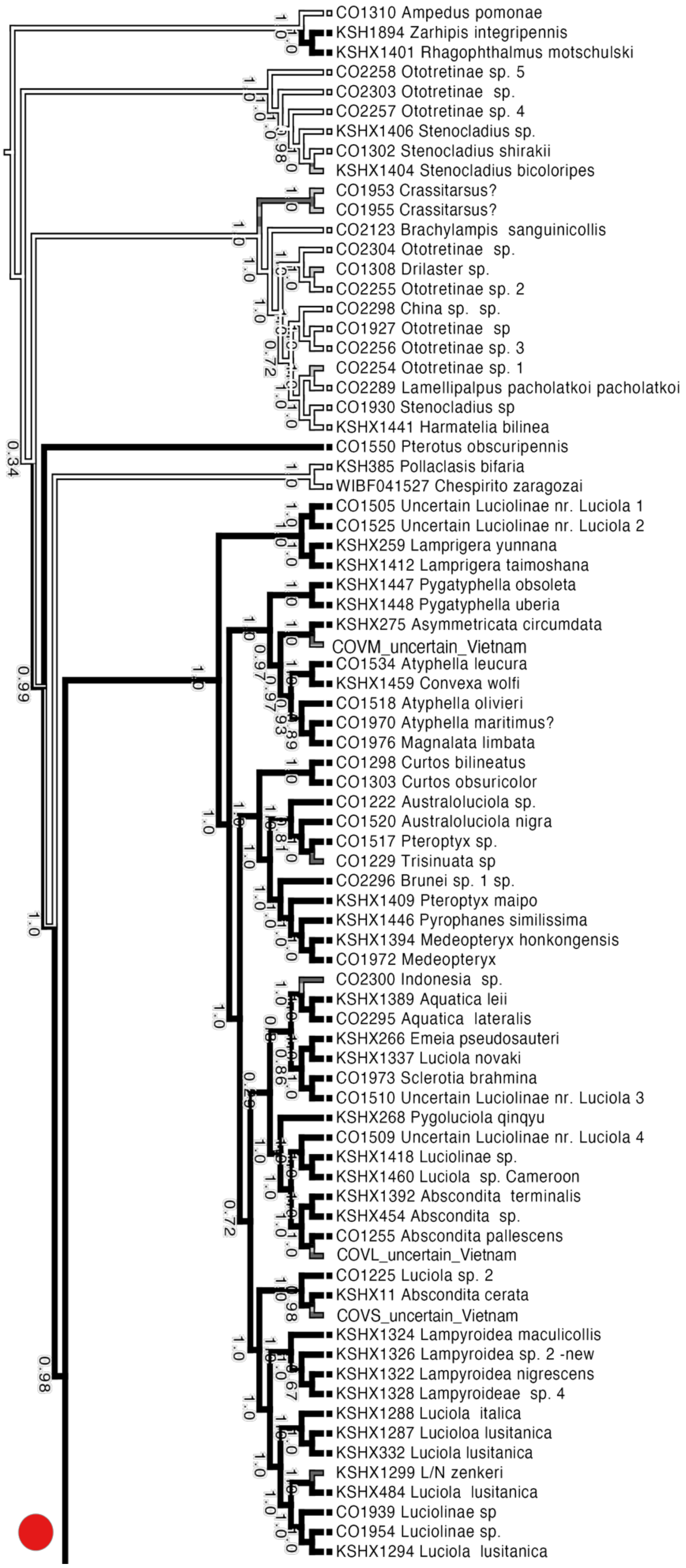


Figure 3.5 Cladogram of subfamily relationships.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CO2257 Ototretinae sp. 4
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CO1302 Stenocladius shirakii
KSHX1404 Stenocladius bicoloripes
CO1953 Crassitarsus?
CO1955 Crassitarsus?
CO2123 Brachylampis sanguinicollis
CO2304 Ototretinae sp.
CO1308 Drilaster sp.
CO2255 Ototretinae sp. 2
CO2298 China sp. sp.
CO1927 Ototretinae sp
CO2256 Ototretinae sp. 3
CO2254 Ototretinae sp. 1
CO2289 Lamellipalpus pacholatkoi pacholatkoi
CO1930 Stenocladius sp
KSHX1441 Harmatelia bilinea
CO1550 Pterotus obscuripennis
KSH385 Pollaclasis bifaria
WIBF041527 Chespirito zaragozai
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CO1525 Uncertain Luciolinae nr. Luciola 2
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KSHX1412 Lamprigera taimoshana
KSHX1447 Pygatyphella obsoleta
KSHX1448 Pygatyphella uberia
KSHX275 Asymmetricata circumdata
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KSHX1459 Convexa wolfi
CO1518 Atyphella olivieri
CO1970 Atyphella maritimus?
CO1976 Magnalata limbata
CO1298 Curtos bilineatus
CO1303 Curtos obsuricolor
CO1222 Australoluciola sp.
CO1520 Australoluciola nigra
CO1517 Pteroptyx sp.
CO1229 Trisinuata sp
CO2296 Brunei sp. 1 sp.
KSHX1409 Pteroptyx maipo
KSHX1446 Pyrophanes similissima
KSHX1394 Medeopteryx honkongensis
CO1972 Medeopteryx
CO2300 Indonesia sp.
KSHX1389 Aquatica leii
CO2295 Aquatica lateralis
KSHX266 Emeia pseudosauteri
KSHX1337 Luciola novaki
CO1973 Sclerotia brahmina
CO1510 Uncertain Luciolinae nr. Luciola 3
KSHX268 Pygoluciola qinqyu
CO1509 Uncertain Luciolinae nr. Luciola 4
KSHX1418 Luciolinae sp.
KSHX1460 Luciola sp. Cameroon
KSHX1392 Abscondita terminalis
KSHX454 Abscondita sp.
CO1255 Abscondita pallescens
COVL_uncertain_Vietnam
CO1225 Luciola sp. 2
KSHX11 Abscondita cerata
COVS_uncertain_Vietnam
KSHX1324 Lampyroidea maculicollis
KSHX1326 Lampyroidea sp. 2 -new
KSHX1322 Lampyroidea nigrescens
KSHX1328 Lampyroideae sp. 4
KSHX1288 Luciola italica
KSHX1287 Lucioloa lusitanica
KSHX332 Luciola lusitanica
KSHX1299 L/N zenkeri
KSHX484 Luciola lusitanica
CO1939 Luciolinae sp
CO1954 Luciolinae sp.
KSHX1294 Luciola lusitanica
0.34
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0.29
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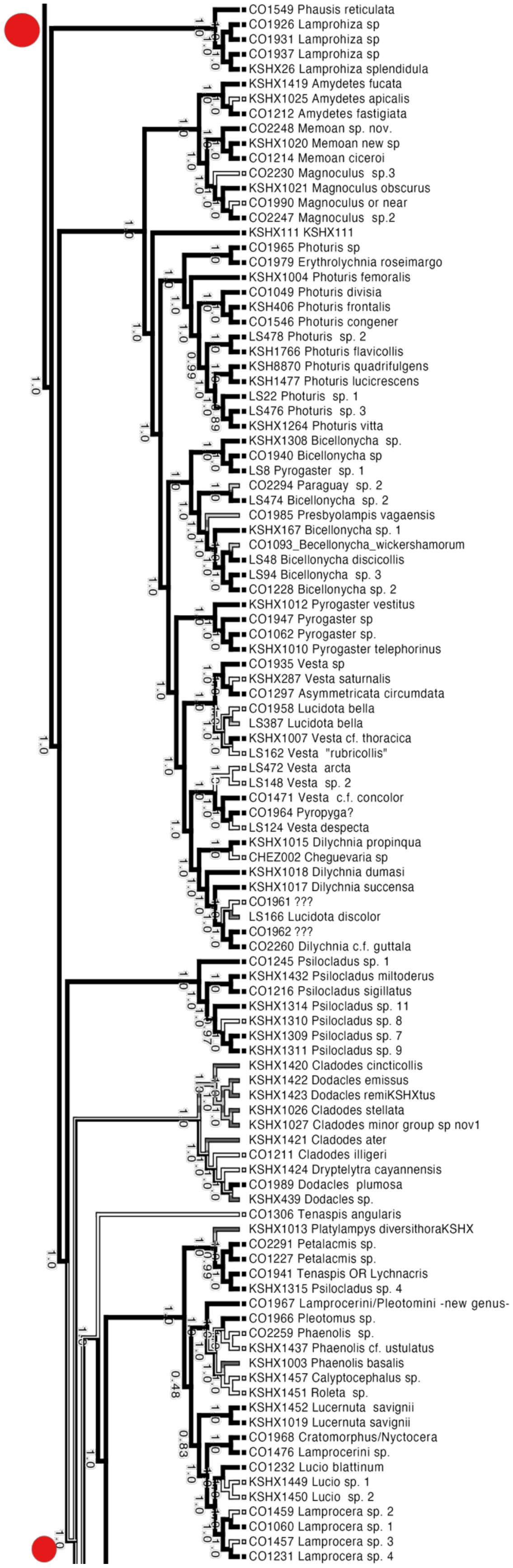
0.93
0.89
0.81
0.86
0.67
0.98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CO1926 Lamprohiza sp
CO1931 Lamprohiza sp
CO1937 Lamprohiza sp
KSHX26 Lamprohiza splendidula
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KSHX1025 Amydetes apicalis
CO1212 Amydetes fastigiata
CO2248 Memoan sp. nov.
KSHX1020 Memoan new sp
CO1214 Memoan ciceroi
CO2230 Magnoculus sp.3
KSHX1021 Magnoculus obscurus
CO1990 Magnoculus or near
CO2247 Magnoculus sp.2
KSHX111 KSHX111
CO1965 Photuris sp
CO1979 Erythrolychnia roseimargo
KSHX1004 Photuris femoralis
CO1049 Photuris divisia
KSH406 Photuris frontalis
CO1546 Photuris congener
LS478 Photuris sp. 2
KSH1766 Photuris flavicollis
KSH8870 Photuris quadrifulgens
KSH1477 Photuris lucicrescens
LS22 Photuris sp. 1
LS476 Photuris sp. 3
KSHX1264 Photuris vitta
KSHX1308 Bicellonycha sp.
CO1940 Bicellonycha sp
LS8 Pyrogaster sp. 1
CO2294 Paraguay sp. 2
LS474 Bicellonycha sp. 2
CO1985 Presbyolampis vagaensis
KSHX167 Bicellonycha sp. 1
CO1093_Becellonycha_wickershamorum
LS48 Bicellonycha discicollis
LS94 Bicellonycha sp. 3
CO1228 Bicellonycha sp. 2
KSHX1012 Pyrogaster vestitus
CO1947 Pyrogaster sp
CO1062 Pyrogaster sp.
KSHX1010 Pyrogaster telephorinus
CO1935 Vesta sp
KSHX287 Vesta saturnalis
CO1297 Asymmetricata circumdata
CO1958 Lucidota bella
LS387 Lucidota bella
KSHX1007 Vesta cf. thoracica
LS162 Vesta "rubricollis"
LS472 Vesta arcta
LS148 Vesta sp. 2
CO1471 Vesta c.f. concolor
CO1964 Pyropyga?
LS124 Vesta despecta
KSHX1015 Dilychnia propinqua
CHEZ002 Cheguevaria sp
KSHX1018 Dilychnia dumasi
KSHX1017 Dilychnia succensa
CO1961 ???
LS166 Lucidota discolor
CO1962 ???
CO2260 Dilychnia c.f. guttala
CO1245 Psilocladus sp. 1
KSHX1432 Psilocladus miltoderus
CO1216 Psilocladus sigillatus
KSHX1314 Psilocladus sp. 11
KSHX1310 Psilocladus sp. 8
KSHX1309 Psilocladus sp. 7
KSHX1311 Psilocladus sp. 9
KSHX1420 Cladodes cincticollis
KSHX1422 Dodacles emissus
KSHX1423 Dodacles remiKSHXtus
KSHX1026 Cladodes stellata
KSHX1027 Cladodes minor group sp nov1
KSHX1421 Cladodes ater
CO1211 Cladodes illigeri
KSHX1424 Dryptelytra cayannensis
CO1989 Dodacles plumosa
KSHX439 Dodacles sp.
CO1306 Tenaspis angularis
KSHX1013 Platylampys diversithoraKSHX
CO2291 Petalacmis sp.
CO1227 Petalacmis sp.
CO1941 Tenaspis OR Lychnacris
KSHX1315 Psilocladus sp. 4
CO1967 Lamprocerini/Pleotomini -new genus-
CO1966 Pleotomus sp.
CO2259 Phaenolis sp.
KSHX1437 Phaenolis cf. ustulatus
KSHX1003 Phaenolis basalis
KSHX1457 Calyptocephalus sp.
KSHX1451 Roleta sp.
KSHX1452 Lucernuta savignii
KSHX1019 Lucernuta savignii
CO1968 Cratomorphus/Nyctocera
CO1476 Lamprocerini sp.
CO1232 Lucio blattinum
KSHX1449 Lucio sp. 1
KSHX1450 Lucio sp. 2
CO1459 Lamprocera sp. 2
CO1060 Lamprocera sp. 1
CO1457 Lamprocera sp. 3
CO1231 Lamprocera sp. 4
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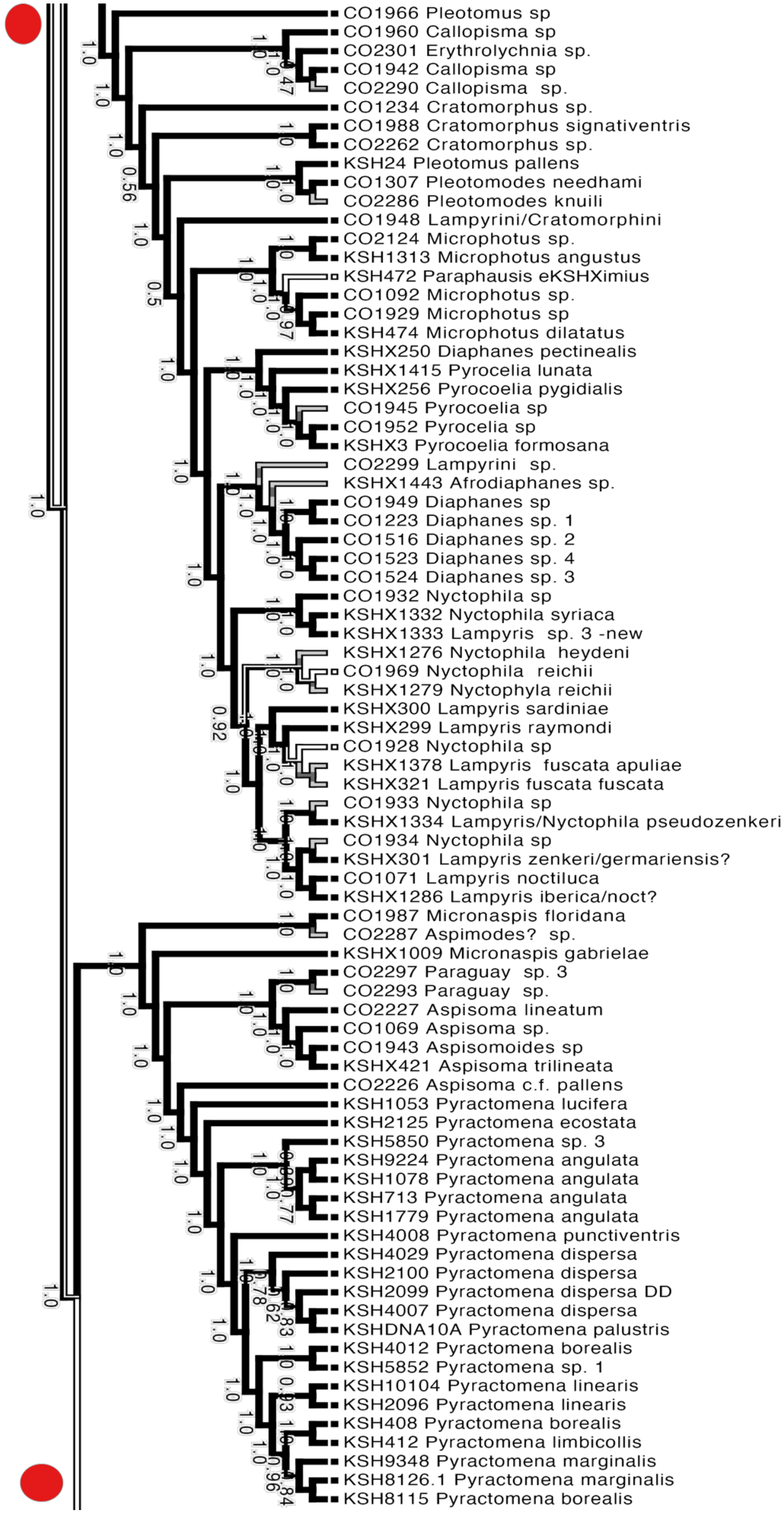
0.89
0.97
0.48
0.83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CO1960 Callopisma sp
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CO1942 Callopisma sp
CO2290 Callopisma sp.
CO1234 Cratomorphus sp.
CO1988 Cratomorphus signativentris
CO2262 Cratomorphus sp.
KSH24 Pleotomus pallens
CO1307 Pleotomodes needhami
CO2286 Pleotomodes knuili
CO1948 Lampyrini/Cratomorphini
CO2124 Microphotus sp.
KSH1313 Microphotus angustus
KSH472 Paraphausis eKSHXimius
CO1092 Microphotus sp.
CO1929 Microphotus sp
KSH474 Microphotus dilatatus
KSHX250 Diaphanes pectinealis
KSHX1415 Pyrocelia lunata
KSHX256 Pyrocoelia pygidialis
CO1945 Pyrocoelia sp
CO1952 Pyrocelia sp
KSHX3 Pyrocoelia formosana
CO2299 Lampyrini sp.
KSHX1443 Afrodiaphanes sp.
CO1949 Diaphanes sp
CO1223 Diaphanes sp. 1
CO1516 Diaphanes sp. 2
CO1523 Diaphanes sp. 4
CO1524 Diaphanes sp. 3
CO1932 Nyctophila sp
KSHX1332 Nyctophila syriaca
KSHX1333 Lampyris sp. 3 -new
KSHX1276 Nyctophila heydeni
CO1969 Nyctophila reichii
KSHX1279 Nyctophyla reichii
KSHX300 Lampyris sardiniae
KSHX299 Lampyris raymondi
CO1928 Nyctophila sp
KSHX1378 Lampyris fuscata apuliae
KSHX321 Lampyris fuscata fuscata
CO1933 Nyctophila sp
KSHX1334 Lampyris/Nyctophila pseudozenkeri
CO1934 Nyctophila sp
KSHX301 Lampyris zenkeri/germariensis?
CO1071 Lampyris noctiluca
KSHX1286 Lampyris iberica/noct?
CO1987 Micronaspis floridana
CO2287 Aspimodes? sp.
KSHX1009 Micronaspis gabrielae
CO2297 Paraguay sp. 3
CO2293 Paraguay sp.
CO2227 Aspisoma lineatum
CO1069 Aspisoma sp.
CO1943 Aspisomoides sp
KSHX421 Aspisoma trilineata
CO2226 Aspisoma c.f. pallens
KSH1053 Pyractomena lucifera
KSH2125 Pyractomena ecostata
KSH5850 Pyractomena sp. 3
KSH9224 Pyractomena angulata
KSH1078 Pyractomena angulata
KSH713 Pyractomena angulata
KSH1779 Pyractomena angulata
KSH4008 Pyractomena punctiventris
KSH4029 Pyractomena dispersa
KSH2100 Pyractomena dispersa
KSH2099 Pyractomena dispersa DD
KSH4007 Pyractomena dispersa
KSHDNA10A Pyractomena palustris
KSH4012 Pyractomena borealis
KSH5852 Pyractomena sp. 1
KSH10104 Pyractomena linearis
KSH2096 Pyractomena linearis
KSH408 Pyractomena borealis
KSH412 Pyractomena limbicollis
KSH9348 Pyractomena marginalis
KSH8126.1 Pyractomena marginalis
KSH8115 Pyractomena borealis
1.0
0.47
0.56
0.5
0.97
0.92
0.77
0.78
0.62
0.83
0.93
0.96
0.84

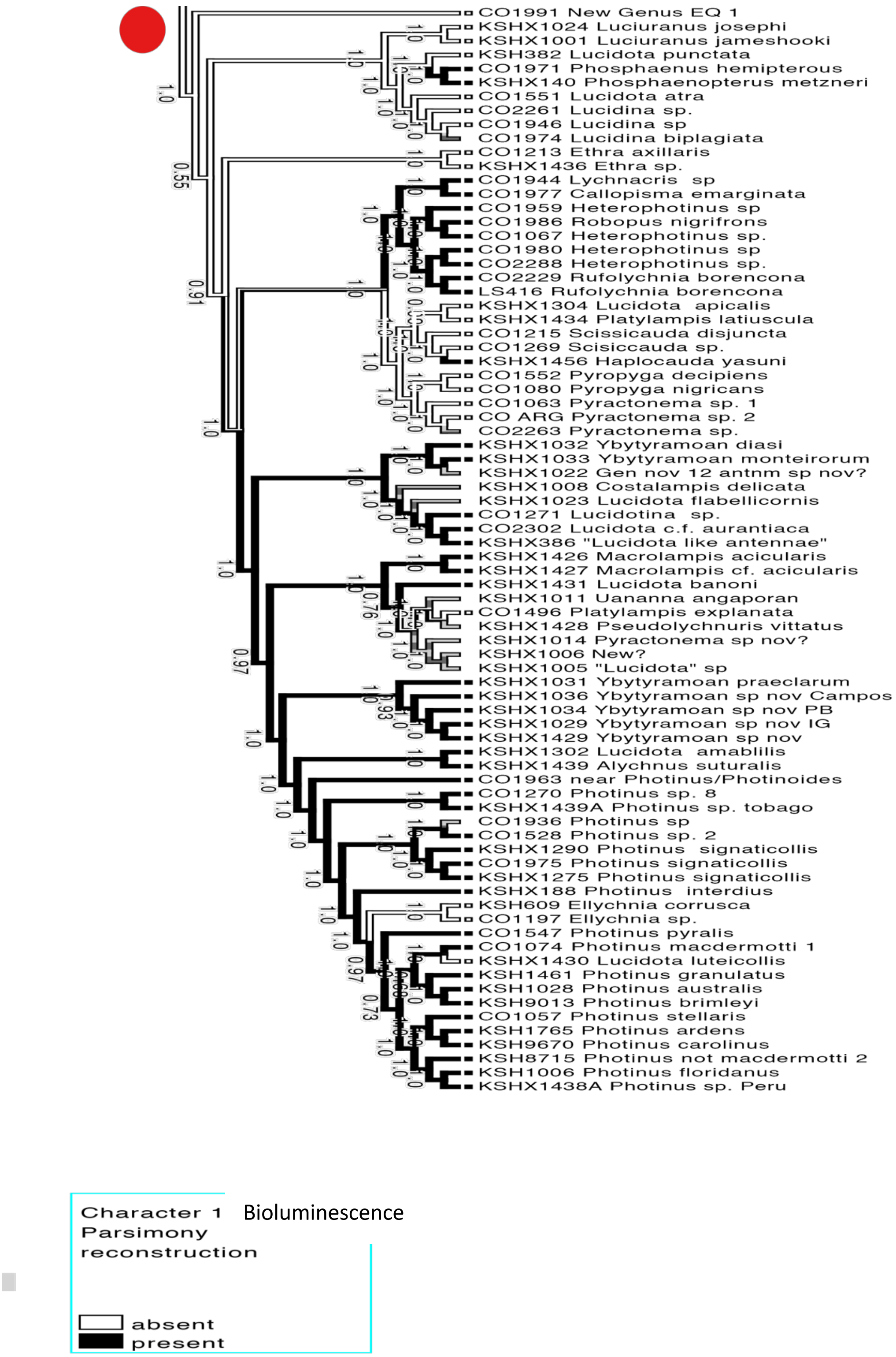
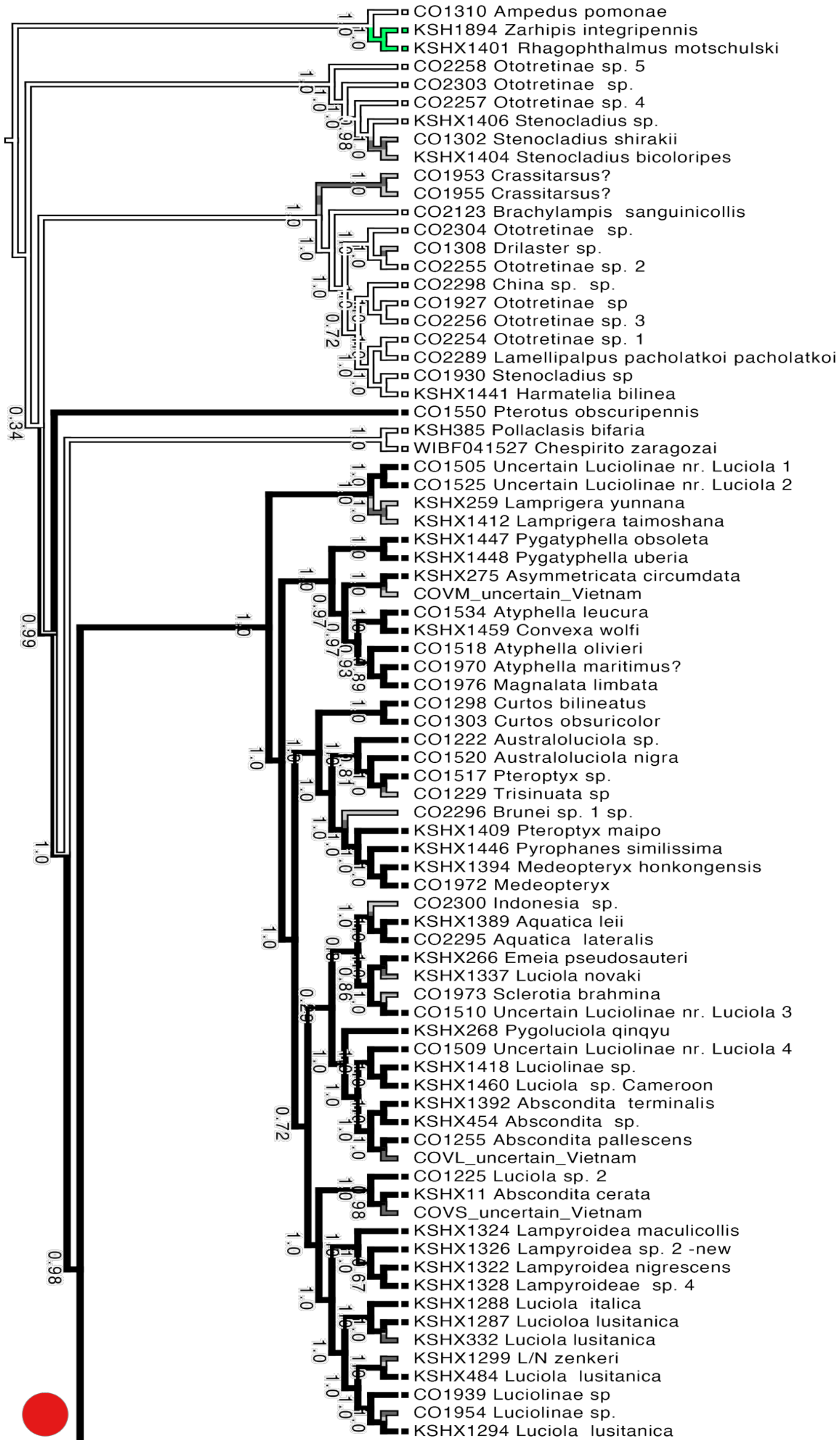


Figure 3.6 Ancestral state reconstruction of pheromones (white) and bioluminescence (black).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KSH1894 Zarhipis integripennis
KSHX1401 Rhagophthalmus motschulski
CO2258 Ototretinae sp. 5
CO2303 Ototretinae sp.
CO2257 Ototretinae sp. 4
KSHX1406 Stenocladius sp.
CO1302 Stenocladius shirakii
KSHX1404 Stenocladius bicoloripes
CO1953 Crassitarsus?
CO1955 Crassitarsus?
CO2123 Brachylampis sanguinicollis
CO2304 Ototretinae sp.
CO1308 Drilaster sp.
CO2255 Ototretinae sp. 2
CO2298 China sp. sp.
CO1927 Ototretinae sp
CO2256 Ototretinae sp. 3
CO2254 Ototretinae sp. 1
CO2289 Lamellipalpus pacholatkoi pacholatkoi
CO1930 Stenocladius sp
KSHX1441 Harmatelia bilinea
CO1550 Pterotus obscuripennis
KSH385 Pollaclasis bifaria
WIBF041527 Chespirito zaragozai
CO1505 Uncertain Luciolinae nr. Luciola 1
CO1525 Uncertain Luciolinae nr. Luciola 2
KSHX259 Lamprigera yunnana
KSHX1412 Lamprigera taimoshana
KSHX1447 Pygatyphella obsoleta
KSHX1448 Pygatyphella uberia
KSHX275 Asymmetricata circumdata
COVM_uncertain_Vietnam
CO1534 Atyphella leucura
KSHX1459 Convexa wolfi
CO1518 Atyphella olivieri
CO1970 Atyphella maritimus?
CO1976 Magnalata limbata
CO1298 Curtos bilineatus
CO1303 Curtos obsuricolor
CO1222 Australoluciola sp.
CO1520 Australoluciola nigra
CO1517 Pteroptyx sp.
CO1229 Trisinuata sp
CO2296 Brunei sp. 1 sp.
KSHX1409 Pteroptyx maipo
KSHX1446 Pyrophanes similissima
KSHX1394 Medeopteryx honkongensis
CO1972 Medeopteryx
CO2300 Indonesia sp.
KSHX1389 Aquatica leii
CO2295 Aquatica lateralis
KSHX266 Emeia pseudosauteri
KSHX1337 Luciola novaki
CO1973 Sclerotia brahmina
CO1510 Uncertain Luciolinae nr. Luciola 3
KSHX268 Pygoluciola qinqyu
CO1509 Uncertain Luciolinae nr. Luciola 4
KSHX1418 Luciolinae sp.
KSHX1460 Luciola sp. Cameroon
KSHX1392 Abscondita terminalis
KSHX454 Abscondita sp.
CO1255 Abscondita pallescens
COVL_uncertain_Vietnam
CO1225 Luciola sp. 2
KSHX11 Abscondita cerata
COVS_uncertain_Vietnam
KSHX1324 Lampyroidea maculicollis
KSHX1326 Lampyroidea sp. 2 -new
KSHX1322 Lampyroidea nigrescens
KSHX1328 Lampyroideae sp. 4
KSHX1288 Luciola italica
KSHX1287 Lucioloa lusitanica
KSHX332 Luciola lusitanica
KSHX1299 L/N zenkeri
KSHX484 Luciola lusitanica
CO1939 Luciolinae sp
CO1954 Luciolinae sp.
KSHX1294 Luciola lusitanica
0.34
0.99
1.0
0.98
0.72
0.29


CO1549 Phausis reticulata
CO1926 Lamprohiza sp
CO1931 Lamprohiza sp
CO1937 Lamprohiza sp
KSHX26 Lamprohiza splendidula
KSHX1419 Amydetes fucata
KSHX1025 Amydetes apicalis
CO1212 Amydetes fastigiata
CO2248 Memoan sp. nov.
KSHX1020 Memoan new sp
CO1214 Memoan ciceroi
CO2230 Magnoculus sp.3
KSHX1021 Magnoculus obscurus
CO1990 Magnoculus or near
CO2247 Magnoculus sp.2
KSHX111 KSHX111
CO1965 Photuris sp
CO1979 Erythrolychnia roseimargo
KSHX1004 Photuris femoralis
CO1049 Photuris divisia
KSH406 Photuris frontalis
CO1546 Photuris congener
LS478 Photuris sp. 2
KSH1766 Photuris flavicollis
KSH8870 Photuris quadrifulgens
KSH1477 Photuris lucicrescens
LS22 Photuris sp. 1
LS476 Photuris sp. 3
KSHX1264 Photuris vitta
KSHX1308 Bicellonycha sp.
CO1940 Bicellonycha sp
LS8 Pyrogaster sp. 1
CO2294 Paraguay sp. 2
LS474 Bicellonycha sp. 2
CO1985 Presbyolampis vagaensis
KSHX167 Bicellonycha sp. 1
CO1093_Becellonycha_wickershamorum
LS48 Bicellonycha discicollis
LS94 Bicellonycha sp. 3
CO1228 Bicellonycha sp. 2
KSHX1012 Pyrogaster vestitus
CO1947 Pyrogaster sp
CO1062 Pyrogaster sp.
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CO1935 Vesta sp
KSHX287 Vesta saturnalis
CO1297 Asymmetricata circumdata
CO1958 Lucidota bella
LS387 Lucidota bella
KSHX1007 Vesta cf. thoracica
LS162 Vesta "rubricollis"
LS472 Vesta arcta
LS148 Vesta sp. 2
CO1471 Vesta c.f. concolor
CO1964 Pyropyga?
LS124 Vesta despecta
KSHX1015 Dilychnia propinqua
CHEZ002 Cheguevaria sp
KSHX1018 Dilychnia dumasi
KSHX1017 Dilychnia succensa
CO1961 ???
LS166 Lucidota discolor
CO1962 ???
CO2260 Dilychnia c.f. guttala
CO1245 Psilocladus sp. 1
KSHX1432 Psilocladus miltoderus
CO1216 Psilocladus sigillatus
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KSHX1310 Psilocladus sp. 8
KSHX1309 Psilocladus sp. 7
KSHX1311 Psilocladus sp. 9
KSHX1420 Cladodes cincticollis
KSHX1422 Dodacles emissus
KSHX1423 Dodacles remiKSHXtus
KSHX1026 Cladodes stellata
KSHX1027 Cladodes minor group sp nov1
KSHX1421 Cladodes ater
CO1211 Cladodes illigeri
KSHX1424 Dryptelytra cayannensis
CO1989 Dodacles plumosa
KSHX439 Dodacles sp.
CO1306 Tenaspis angularis
KSHX1013 Platylampys diversithoraKSHX
CO2291 Petalacmis sp.
CO1227 Petalacmis sp.
CO1941 Tenaspis OR Lychnacris
KSHX1315 Psilocladus sp. 4
CO1967 Lamprocerini/Pleotomini -new genus-
CO1966 Pleotomus sp.
CO2259 Phaenolis sp.
KSHX1437 Phaenolis cf. ustulatus
KSHX1003 Phaenolis basalis
KSHX1457 Calyptocephalus sp.
KSHX1451 Roleta sp.
KSHX1452 Lucernuta savignii
KSHX1019 Lucernuta savignii
CO1968 Cratomorphus/Nyctocera
CO1476 Lamprocerini sp.
CO1232 Lucio blattinum
KSHX1449 Lucio sp. 1
KSHX1450 Lucio sp. 2
CO1459 Lamprocera sp. 2
CO1060 Lamprocera sp. 1
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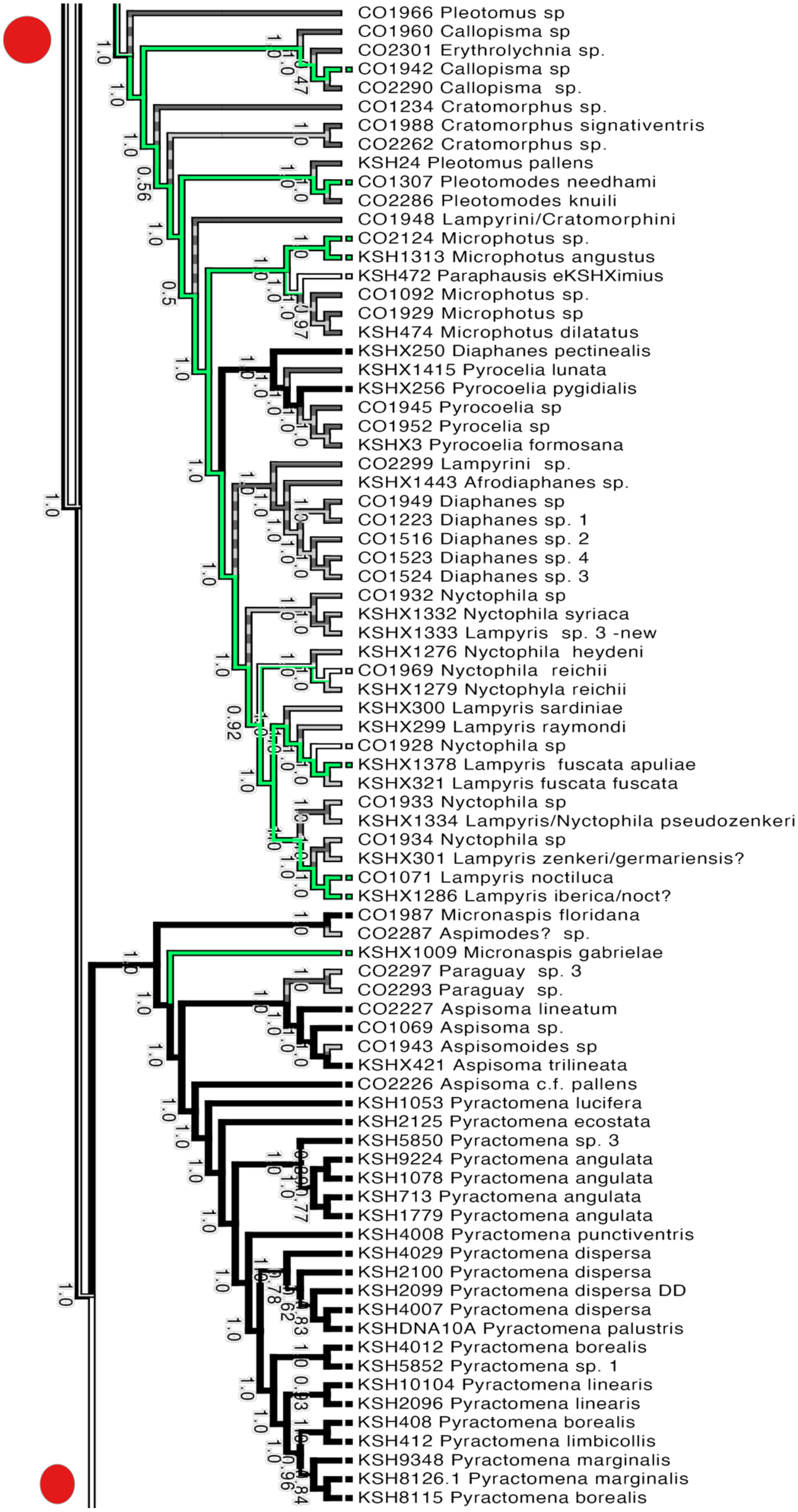
0.99
0.89
0.97
0.48
0.83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CO2262 Cratomorphus sp.
KSH24 Pleotomus pallens
CO1307 Pleotomodes needhami
CO2286 Pleotomodes knuili
CO1948 Lampyrini/Cratomorphini
CO2124 Microphotus sp.
KSH1313 Microphotus angustus
KSH472 Paraphausis eKSHXimius
CO1092 Microphotus sp.
CO1929 Microphotus sp
KSH474 Microphotus dilatatus
KSHX250 Diaphanes pectinealis
KSHX1415 Pyrocelia lunata
KSHX256 Pyrocoelia pygidialis
CO1945 Pyrocoelia sp
CO1952 Pyrocelia sp
KSHX3 Pyrocoelia formosana
CO2299 Lampyrini sp.
KSHX1443 Afrodiaphanes sp.
CO1949 Diaphanes sp
CO1223 Diaphanes sp. 1
CO1516 Diaphanes sp. 2
CO1523 Diaphanes sp. 4
CO1524 Diaphanes sp. 3
CO1932 Nyctophila sp
KSHX1332 Nyctophila syriaca
KSHX1333 Lampyris sp. 3 -new
KSHX1276 Nyctophila heydeni
CO1969 Nyctophila reichii
KSHX1279 Nyctophyla reichii
KSHX300 Lampyris sardiniae
KSHX299 Lampyris raymondi
CO1928 Nyctophila sp
KSHX1378 Lampyris fuscata apuliae
KSHX321 Lampyris fuscata fuscata
CO1933 Nyctophila sp
KSHX1334 Lampyris/Nyctophila pseudozenkeri
CO1934 Nyctophila sp
KSHX301 Lampyris zenkeri/germariensis?
CO1071 Lampyris noctiluca
KSHX1286 Lampyris iberica/noct?
CO1987 Micronaspis floridana
CO2287 Aspimodes? sp.
KSHX1009 Micronaspis gabrielae
CO2297 Paraguay sp. 3
CO2293 Paraguay sp.
CO2227 Aspisoma lineatum
CO1069 Aspisoma sp.
CO1943 Aspisomoides sp
KSHX421 Aspisoma trilineata
CO2226 Aspisoma c.f. pallens
KSH1053 Pyractomena lucifera
KSH2125 Pyractomena ecostata
KSH5850 Pyractomena sp. 3
KSH9224 Pyractomena angulata
KSH1078 Pyractomena angulata
KSH713 Pyractomena angulata
KSH1779 Pyractomena angulata
KSH4008 Pyractomena punctiventris
KSH4029 Pyractomena dispersa
KSH2100 Pyractomena dispersa
KSH2099 Pyractomena dispersa DD
KSH4007 Pyractomena dispersa
KSHDNA10A Pyractomena palustris
KSH4012 Pyractomena borealis
KSH5852 Pyractomena sp. 1
KSH10104 Pyractomena linearis
KSH2096 Pyractomena linearis
KSH408 Pyractomena borealis
KSH412 Pyractomena limbicollis
KSH9348 Pyractomena marginalis
KSH8126.1 Pyractomena marginalis
KSH8115 Pyractomena borealis
1.0
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0.92
0.47
0.97
0.77
0.78
0.62
0.83
0.93
0.96
0.84

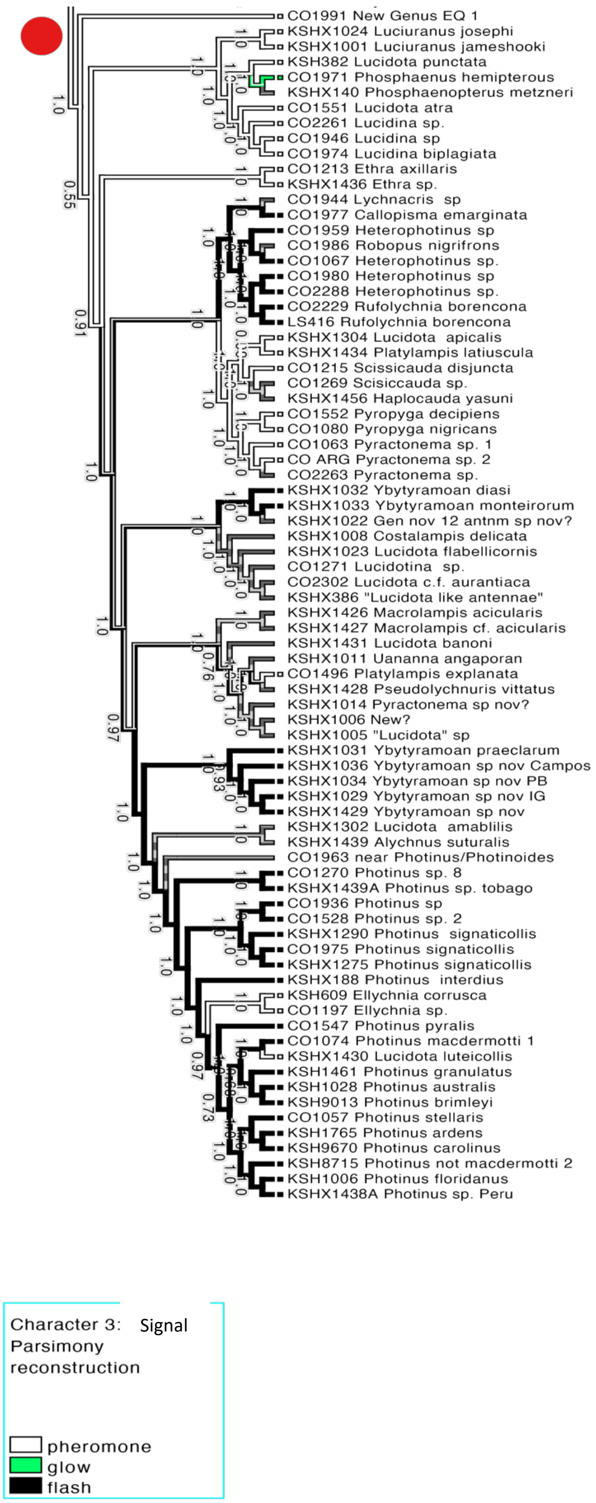
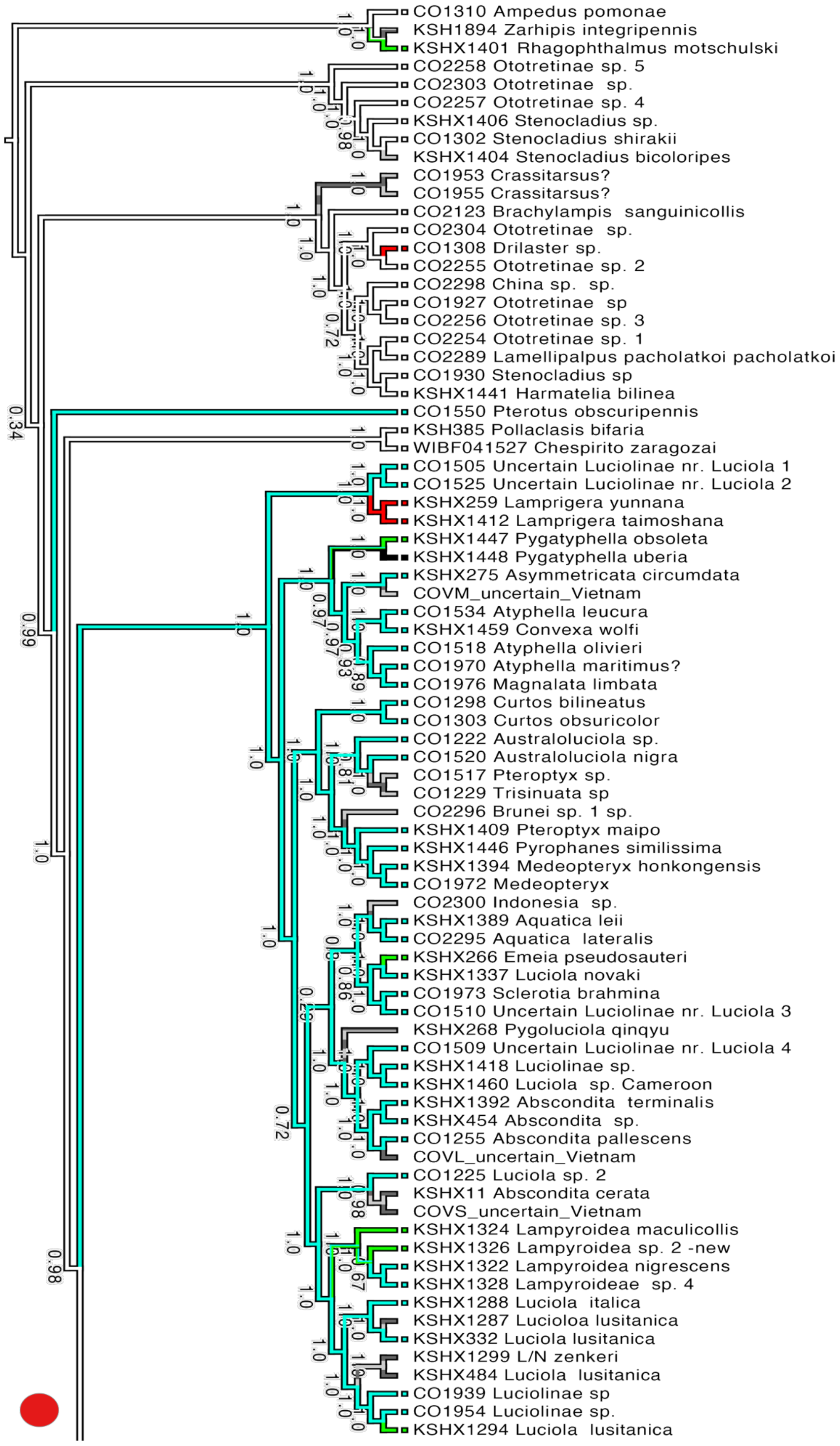


Figure 3.7 Ancestral state reconstruction of bioluminescent type (flashes or glows). Pheromones (white), flashes (black), glows (green).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CO2257 Ototretinae sp. 4
KSHX1406 Stenocladius sp.
CO1302 Stenocladius shirakii
KSHX1404 Stenocladius bicoloripes
CO1953 Crassitarsus?
CO1955 Crassitarsus?
CO2123 Brachylampis sanguinicollis
CO2304 Ototretinae sp.
CO1308 Drilaster sp.
CO2255 Ototretinae sp. 2
CO2298 China sp. sp.
CO1927 Ototretinae sp
CO2256 Ototretinae sp. 3
CO2254 Ototretinae sp. 1
CO2289 Lamellipalpus pacholatkoi pacholatkoi
CO1930 Stenocladius sp
KSHX1441 Harmatelia bilinea
CO1550 Pterotus obscuripennis
KSH385 Pollaclasis bifaria
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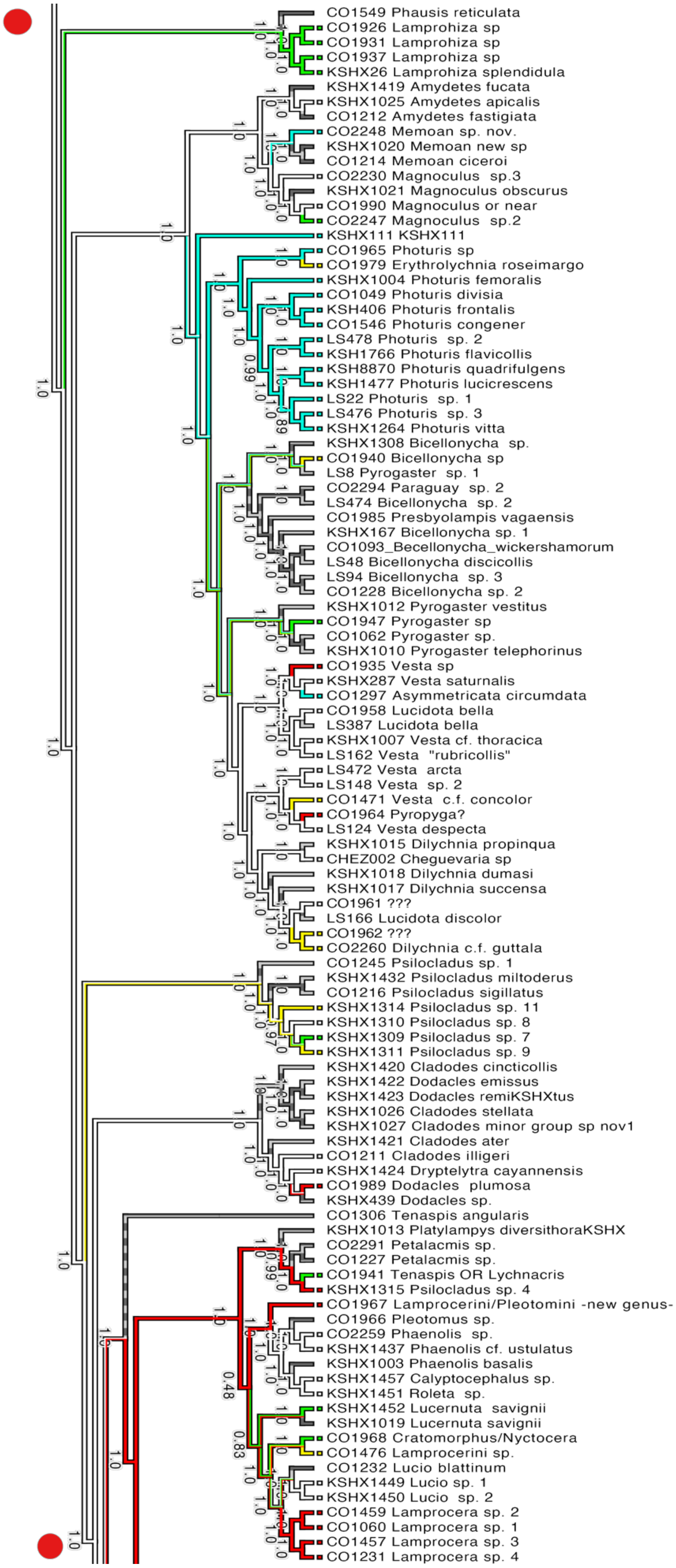
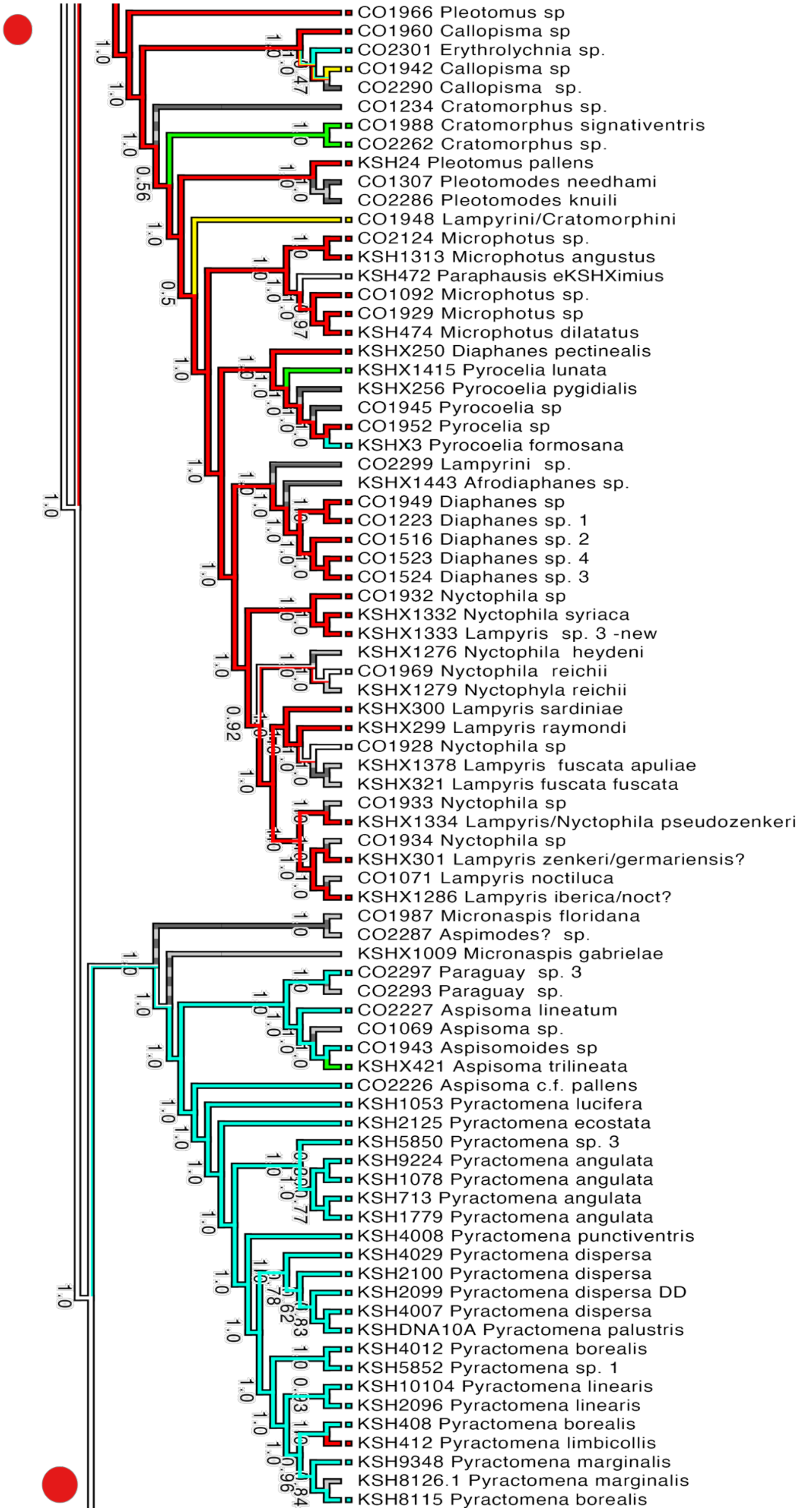
CO1525 Uncertain Luciolinae nr. Luciola 2
KSHX259 Lamprigera yunnana
KSHX1412 Lamprigera taimoshana
KSHX1447 Pygatyphella obsoleta
KSHX1448 Pygatyphella uberia
KSHX275 Asymmetricata circumdata
COVM_uncertain_Vietnam
CO1534 Atyphella leucura
KSHX1459 Convexa wolfi
CO1518 Atyphella olivieri
CO1970 Atyphella maritimus?
CO1976 Magnalata limbata
CO1298 Curtos bilineatus
CO1303 Curtos obsuricolor
CO1222 Australoluciola sp.
CO1520 Australoluciola nigra
CO1517 Pteroptyx sp.
CO1229 Trisinuata sp
CO2296 Brunei sp. 1 sp.
KSHX1409 Pteroptyx maipo
KSHX1446 Pyrophanes similissima
KSHX1394 Medeopteryx honkongensis
CO1972 Medeopteryx
CO2300 Indonesia sp.
KSHX1389 Aquatica leii
CO2295 Aquatica lateralis
KSHX266 Emeia pseudosauteri
KSHX1337 Luciola novaki
CO1973 Sclerotia brahmina
CO1510 Uncertain Luciolinae nr. Luciola 3
KSHX268 Pygoluciola qinqyu
CO1509 Uncertain Luciolinae nr. Luciola 4
KSHX1418 Luciolinae sp.
KSHX1460 Luciola sp. Cameroon
KSHX1392 Abscondita terminalis
KSHX454 Abscondita sp.
CO1255 Abscondita pallescens
COVL_uncertain_Vietnam
CO1225 Luciola sp. 2
KSHX11 Abscondita cerata
COVS_uncertain_Vietnam
KSHX1324 Lampyroidea maculicollis
KSHX1326 Lampyroidea sp. 2 -new
KSHX1322 Lampyroidea nigrescens
KSHX1328 Lampyroideae sp. 4
KSHX1288 Luciola italica
KSHX1287 Lucioloa lusitanica
KSHX332 Luciola lusitanica
KSHX1299 L/N zenkeri
KSHX484 Luciola lusitanica
CO1939 Luciolinae sp
CO1954 Luciolinae sp.
KSHX1294 Luciola lusitanica
0.34
0.99
1.0
0.98
0.72
0.29
0.97
0.93
0.89
0.81
0.86
0.67
0.98


CO1549 Phausis reticulata
CO1926 Lamprohiza sp
CO1931 Lamprohiza sp
CO1937 Lamprohiza sp
KSHX26 Lamprohiza splendidula
KSHX1419 Amydetes fucata
KSHX1025 Amydetes apicalis
CO1212 Amydetes fastigiata
CO2248 Memoan sp. nov.
KSHX1020 Memoan new sp
CO1214 Memoan ciceroi
CO2230 Magnoculus sp.3
KSHX1021 Magnoculus obscurus
CO1990 Magnoculus or near
CO2247 Magnoculus sp.2
KSHX111 KSHX111
CO1965 Photuris sp
CO1979 Erythrolychnia roseimargo
KSHX1004 Photuris femoralis
CO1049 Photuris divisia
KSH406 Photuris frontalis
CO1546 Photuris congener
LS478 Photuris sp. 2
KSH1766 Photuris flavicollis
KSH8870 Photuris quadrifulgens
KSH1477 Photuris lucicrescens
LS22 Photuris sp. 1
LS476 Photuris sp. 3
KSHX1264 Photuris vitta
KSHX1308 Bicellonycha sp.
CO1940 Bicellonycha sp
LS8 Pyrogaster sp. 1
CO2294 Paraguay sp. 2
LS474 Bicellonycha sp. 2
CO1985 Presbyolampis vagaensis
KSHX167 Bicellonycha sp. 1
CO1093_Becellonycha_wickershamorum
LS48 Bicellonycha discicollis
LS94 Bicellonycha sp. 3
CO1228 Bicellonycha sp. 2
KSHX1012 Pyrogaster vestitus
CO1947 Pyrogaster sp
CO1062 Pyrogaster sp.
KSHX1010 Pyrogaster telephorinus
CO1935 Vesta sp
KSHX287 Vesta saturnalis
CO1297 Asymmetricata circumdata
CO1958 Lucidota bella
LS387 Lucidota bella
KSHX1007 Vesta cf. thoracica
LS162 Vesta "rubricollis"
LS472 Vesta arcta
LS148 Vesta sp. 2
CO1471 Vesta c.f. concolor
CO1964 Pyropyga?
LS124 Vesta despecta
KSHX1015 Dilychnia propinqua
CHEZ002 Cheguevaria sp
KSHX1018 Dilychnia dumasi
KSHX1017 Dilychnia succensa
CO1961 ???
LS166 Lucidota discolor
CO1962 ???
CO2260 Dilychnia c.f. guttala
CO1245 Psilocladus sp. 1
KSHX1432 Psilocladus miltoderus
CO1216 Psilocladus sigillatus
KSHX1314 Psilocladus sp. 11
KSHX1310 Psilocladus sp. 8
KSHX1309 Psilocladus sp. 7
KSHX1311 Psilocladus sp. 9
KSHX1420 Cladodes cincticollis
KSHX1422 Dodacles emissus
KSHX1423 Dodacles remiKSHXtus
KSHX1026 Cladodes stellata
KSHX1027 Cladodes minor group sp nov1
KSHX1421 Cladodes ater
CO1211 Cladodes illigeri
KSHX1424 Dryptelytra cayannensis
CO1989 Dodacles plumosa
KSHX439 Dodacles sp.
CO1306 Tenaspis angularis
KSHX1013 Platylampys diversithoraKSHX
CO2291 Petalacmis sp.
CO1227 Petalacmis sp.
CO1941 Tenaspis OR Lychnacris
KSHX1315 Psilocladus sp. 4
CO1967 Lamprocerini/Pleotomini -new genus-
CO1966 Pleotomus sp.
CO2259 Phaenolis sp.
KSHX1437 Phaenolis cf. ustulatus
KSHX1003 Phaenolis basalis
KSHX1457 Calyptocephalus sp.
KSHX1451 Roleta sp.
KSHX1452 Lucernuta savignii
KSHX1019 Lucernuta savignii
CO1968 Cratomorphus/Nyctocera
CO1476 Lamprocerini sp.
CO1232 Lucio blattinum
KSHX1449 Lucio sp. 1
KSHX1450 Lucio sp. 2
CO1459 Lamprocera sp. 2
CO1060 Lamprocera sp. 1
CO1457 Lamprocera sp. 3
CO1231 Lamprocera sp. 4
1.0
0.99
0.89
0.97
0.48
0.83


CO1966 Pleotomus sp
CO1960 Callopisma sp
CO2301 Erythrolychnia sp.
CO1942 Callopisma sp
CO2290 Callopisma sp.
CO1234 Cratomorphus sp.
CO1988 Cratomorphus signativentris
CO2262 Cratomorphus sp.
KSH24 Pleotomus pallens
CO1307 Pleotomodes needhami
CO2286 Pleotomodes knuili
CO1948 Lampyrini/Cratomorphini
CO2124 Microphotus sp.
KSH1313 Microphotus angustus
KSH472 Paraphausis eKSHXimius
CO1092 Microphotus sp.
CO1929 Microphotus sp
KSH474 Microphotus dilatatus
KSHX250 Diaphanes pectinealis
KSHX1415 Pyrocelia lunata
KSHX256 Pyrocoelia pygidialis
CO1945 Pyrocoelia sp
CO1952 Pyrocelia sp
KSHX3 Pyrocoelia formosana
CO2299 Lampyrini sp.
KSHX1443 Afrodiaphanes sp.
CO1949 Diaphanes sp
CO1223 Diaphanes sp. 1
CO1516 Diaphanes sp. 2
CO1523 Diaphanes sp. 4
CO1524 Diaphanes sp. 3
CO1932 Nyctophila sp
KSHX1332 Nyctophila syriaca
KSHX1333 Lampyris sp. 3 -new
KSHX1276 Nyctophila heydeni
CO1969 Nyctophila reichii
KSHX1279 Nyctophyla reichii
KSHX300 Lampyris sardiniae
KSHX299 Lampyris raymondi
CO1928 Nyctophila sp
KSHX1378 Lampyris fuscata apuliae
KSHX321 Lampyris fuscata fuscata
CO1933 Nyctophila sp
KSHX1334 Lampyris/Nyctophila pseudozenkeri
CO1934 Nyctophila sp
KSHX301 Lampyris zenkeri/germariensis?
CO1071 Lampyris noctiluca
KSHX1286 Lampyris iberica/noct?
CO1987 Micronaspis floridana
CO2287 Aspimodes? sp.
KSHX1009 Micronaspis gabrielae
CO2297 Paraguay sp. 3
CO2293 Paraguay sp.
CO2227 Aspisoma lineatum
CO1069 Aspisoma sp.
CO1943 Aspisomoides sp
KSHX421 Aspisoma trilineata
CO2226 Aspisoma c.f. pallens
KSH1053 Pyractomena lucifera
KSH2125 Pyractomena ecostata
KSH5850 Pyractomena sp. 3
KSH9224 Pyractomena angulata
KSH1078 Pyractomena angulata
KSH713 Pyractomena angulata
KSH1779 Pyractomena angulata
KSH4008 Pyractomena punctiventris
KSH4029 Pyractomena dispersa
KSH2100 Pyractomena dispersa
KSH2099 Pyractomena dispersa DD
KSH4007 Pyractomena dispersa
KSHDNA10A Pyractomena palustris
KSH4012 Pyractomena borealis
KSH5852 Pyractomena sp. 1
KSH10104 Pyractomena linearis
KSH2096 Pyractomena linearis
KSH408 Pyractomena borealis
KSH412 Pyractomena limbicollis
KSH9348 Pyractomena marginalis
KSH8126.1 Pyractomena marginalis
KSH8115 Pyractomena borealis

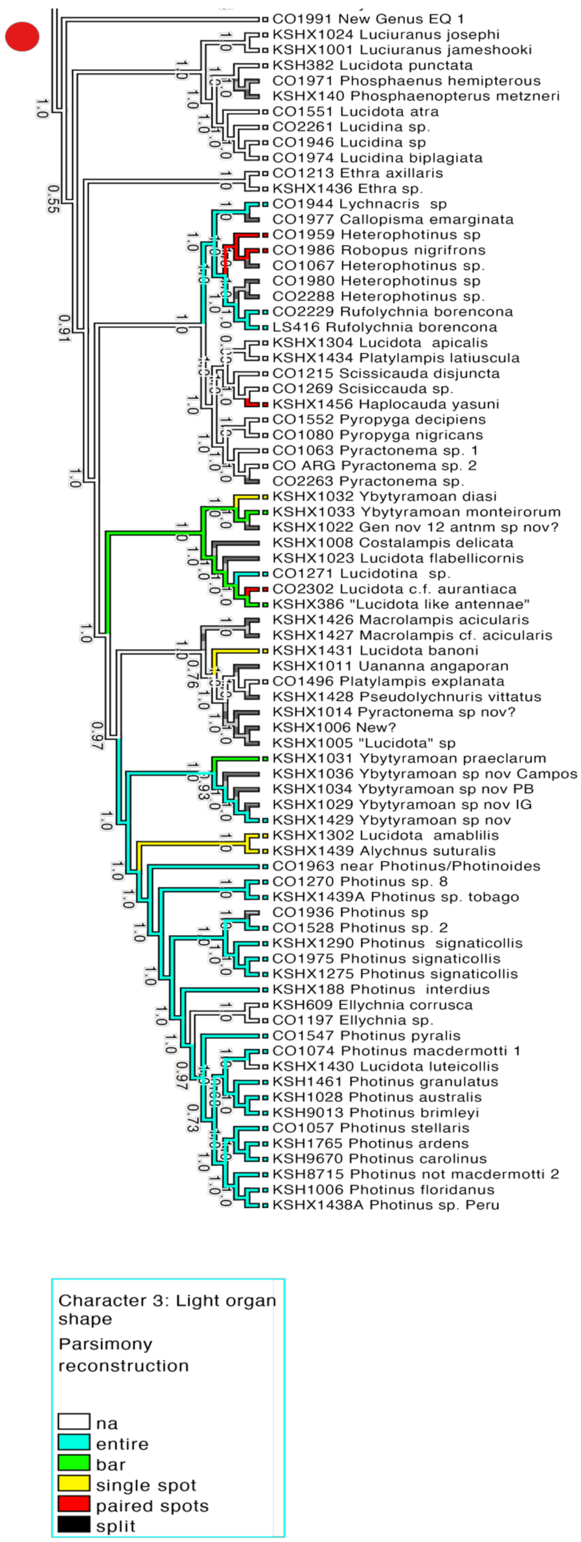


Figure 3.8 Ancestral state reconstruction of light organ shape. Entire segment (blue), bar (green), single spot (yellow), paired spots (red), no light organ (white).

Table 3.3 Phylogenetic signal (Pagel's $\boldsymbol{\lambda}$ and D-statistic) for eye, antenna, and light organ measurements, activity time/signal type (diurnal = pheromone, nocturnal = bioluminescence), and bioluminescence type (flash or glow).

Trait	Pagel's $\boldsymbol{\lambda}$	LRT p value	D-statistic
Log(Pronotum length)			
Log(Antennal length)	0.776146	3.46473e-16	
Log(Max. eye span)	0.4077	0.064816	
Log(Eye distance)	0.109373	0.29804	
Log(Min. eye span)	7.33137e-05	1	
Log(Eye area)	0.778427	1.66197e-05	
Log(Relative LO area)	0.999	0.00466	
Log(Absolute LO area)	7.331e-5	1	
Signal type (pheromone/bioluminescence)			0.313
Probability of E(D) resulting from no (random) phylogenetic structure : 0			
Probability of E(D) resulting from Brownian phylogenetic structure : 0.108			
Bioluminescence type (flash/glow)			0.00772
Probability of E(D) resulting from no (random) phylogenetic structure : 0.034			
Probability of E(D) resulting from Brownian phylogenetic structure : 0.529			

Table 3.4 PGLS for absolute light organ and relative light organ size, Bioluminesce type = flash or glow, N=27

Model: Relative LO Area	Model: Absolute LO Area
Log(relative LO) ~ Bioluminescence type + Log(pronotum Length)	Log(absolute LO) ~ Bioluminescence type + Log(pronotum Length)
Residuals	
Min 1Q Median 3Q Max	Min 1Q Median 3Q Max
-0.128 -0.0259 0.00247 0.0249 0.129	-0.29453 -0.05951 -0.01183 0.06609 0.22304
Branch length transformations:	Branch length transformations:
kappa [Fix] : 1.000, delta [Fix] : 1.000	kappa [Fix] : 1.000, delta [Fix] : 1.000
lambda [ML]: 0.938	lambda [ML]: 0.000
lower bound : 0.000, p 0.0789	lower bound : 0.000, p = 1
upper bound : 1.000, p 0.665	upper bound : 1.000, p = 0.0023717
95.0% CI (NA, NA)	95.0% CI (NA, 0.756)
Coefficients:	Coefficients:
Estimate Std.Error t value Pr(>|t|)	Estimate Std.Error t value Pr(>|t|)
(Intercept) 0.203 0.0940 2.161 0.0409	(Intercept) -0.264 0.140 -1.883 0.0719
Signal_glow -0.0907 0.0745 -1.217 0.235	Signal_glow -0.192 0.145 -1.327 0.197
LogPrL 0.101 0.152 0.663 0.513	LogPrL 1.496 0.482 3.104 0.00484
Residuals (SE) =0.06729 (df)= 24	Residuals (SE) =0.125 (df)=24
Multiple R-squared: 0.06355, Adjusted R-squared: -0.01448	Multiple R-squared: 0.300, Adjusted R-squared: 0.239
F-statistic: 0.8144 on 2 and 24 DF, p-value: 0.455	F-statistic: 5.078 on 2 and 24 DF, p-value: 0.0145

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Supplemental Table 3.1 Taxa and morphological measurements used for phylogenetic signal, PGLS, and discriminate analysis of eye and antennal size.

Species (with taxon ID)	**LogPrL**	**LogAntL**	**LogMaxEye**	**LogEyeDist**	**LogInEye**	**LogEyeArea**	**Activity**
KSHX1429_Ybytyramoan_sp_nov	0.44106641	0.70948197	0.46996921	0.35410844	0.31281183	0.08332342	Nocturnal
KSHX1430_Lucidota_luteicollis	0.14860266	0.63798978	0.09795107	0.2121876	0.17231097	-0.7851562	Diurnal
KSHX1431_Lucidota_banoni	0.3941013	1.02259331	0.27531136	0.24378192	0.26505379	-0.3835245	Nocturnal
KSHX1432_Psilocladus_miltoderus	0.27392678	0.71374248	0.13703746	0.20493352	0.26173855	-0.7110804	Diurnal
KSHX1434_Platylampis_latiuscula	0.09829754	0.68196446	0.03502928	0.18469143	0.18155777	-0.9665762	Diurnal
KSHX1436_Ethra_sp.	0.31722735	0.79768294	0.23552845	0.30168095	0.27392678	-0.7447275	Diurnal
KSHX1437_Phaenolis_cf._ustulatus	0.53007157	0.76064862	0.24748226	0.27137687	0.23426412	-0.4430947	Diurnal
KSHX1438A_Photinus_sp._Peru	0.39058188	0.89340109	0.53453376	0.36059341	0	0.18667387	Nocturnal
KSHX1441_Harmatelia_bilinea	0.20057693	0.68735057	0.18241465	0.2929203	0.29114676	-0.9645703	Diurnal
KSHX1447_Pygatyphella_obsoleta	0.26787542	0.39515159	0.21774707	0.24229291	0.18127177	-0.4424928	Nocturnal
KSHX1448_Pygatyphella_uberia	0.10754913	0.39445168	0.25527251	0.17609126	0.06145248	-0.1463018	Nocturnal
KSHX1449_Lucio_sp._1	0.69757803	0.81378108	0.38273727	0.35276119	0.35024802	-0.2765443	Diurnal
KSHX1450_Lucio__sp._2	0.72271617	0.53237213	0.3079237	0.31449923	0.24328615	-0.2688143	Diurnal
KSHX1452_Lucernuta__savignii	0.60552052	1.01663645	0.45727619	0.37730625	0.33061667	0.06762872	Nocturnal
CO1976_Magnalata_limbata	0.23019338	0.41970798	0.36078269	0.25959388	0.15745677	-0.0336236	Nocturnal
CO1937_Lamprohiza_sp	0.29025727	0.32283927	0.23019338	0.20357678	0.10924097	-0.2498775	Nocturnal
CO1939_Luciolinae_sp	0.23451728	0.60292771	0.32076923	0.28465628	0.23044892	-0.1580152	Nocturnal
CO1958_Lucidota_bella	0.28510703	0.71024433	0.13385813	0.2688119	0.25719843	-0.9100949	Diurnal
CO1940_Bicellonycha_sp	0.2884728	0.75815462	0.32097668	0	0.2757719	-0.4134127	Nocturnal
CO1988_Cratomorphus_signativentris	0.8136477	0.85003326	0.6580114	0.25527251	0.17724784	0.66647118	Diurnal
CO1959_Heterophotinus_sp	0.21484385	0.96710316	0.24895362	0.21590181	0.19284612	-0.3767507	Nocturnal
CO1960_Callopisma_sp	0.34399907	0.75166395	0.25599573	0.28824923	0.26387268	-0.5016894	Diurnal
CO1943_Aspisomoides_sp	0.60820501	0.56837775	0.37730625	0.32592596	0.22219605	-0.0893756	Nocturnal
CO1944_Lychnacris__sp	0.07554696	0.56211435	-0.0357404	0.18012588	0.16643011	-1.1337127	Diurnal
CO1989_Dodacles__plumosa	0.51067903	0.68592079	0.30362798	0.25887663	0.24179543	-0.3372422	Diurnal

CO1961_???_	0.44777801	0.80232894	0.30189772	0.28330123	0.27829621	-0.3840499	Nocturnal
CO1962_???_	0.43424945	0.75446301	0.28126069	0.29358351	0.29622629	-0.410609	Diurnal
CO1945_Pyrocoelia_sp	0.58081097	0.92957218	0.30963017	0.43488812	0.22427401	-0.0105502	Diurnal
CO1946_Lucidina_sp	0.32407658	0.9263939	0.65619406	0.27253778	0.23223352	-0.853872	Diurnal
CO1990_Magnoculus_or_near_	-0.1463018	0.43208699	0.06892761	0.25671775	0.24029958	-1.2924298	Diurnal
CO1963_near_Photinus/Photinoides_	0.41763774	0.79837438	0.34143453	0.21748394	0.21272015	-0.1417637	Nocturnal
CO1964_Pyropyga?__	0.23401082	1.12437379	0.18949031	0.24748226	0.23904909	-0.6382722	Nocturnal
CO1991_New_Genus_EQ_1__	0.34888872	0.43288916	-0.1146388	0.17666993	0.16465022	-1.49485	Diurnal
CO1947_Pyrogaster_sp	0.38756778	0.97744923	0.39532639	0.26528963	0.2367891	-0.0777937	Nocturnal
CO1965_Photuris_sp	0.31217736	0.84877394	0.31534048	0.30984301	0.19200959	-0.2175274	Nocturnal
CO1948_Lampyrini/Cratomorphini_	0.28307498	0.81749926	0.16731734	0.15896526	0.14921911	-0.4400934	Nocturnal
CO1966_Pleotomus_sp	0.34202769	0.78064129	0.06333336	0.20276069	0.17026172	-0.8416375	Diurnal
CO1967_Lamprocerini/Pleotomini_-new_genus-_	0.49164179	0.842422	0.22297645	0.30124709	0.26434551	-0.653647	Diurnal
CO1949_Diaphanes_sp	0.52061452	0.40611419	0.40773073	0.17695898	0.14363924	0.195069	Diurnal
CO1968_Cratomorphus/Nyctocera_	0.74067843	0.79396491	0.67108023	0	0.21537315	0.68489022	Diurnal
CO1471_Vesta__c.f._concolor	0.23350376	0.82249499	0.18497519	0.28488172	0.22814361	-0.6968039	Nocturnal
CO1476_Lamprocerini_sp.	0.40088322	0.972388	0.31764554	0.25333801	0.22089225	-0.1678105	Nocturnal
CO1496_Platylampis_explanata	0.2955671	0.61193563	0.00518051	0.18780264	0.18155777	-1.1135093	Diurnal
CO2247_Magnoculus__sp.2	0.20682588	0.52074547	0.20330492	0.25042	0.20844136	-0.4173686	Nocturnal
CO2248_Memoan_sp.nov.	-0.2915791	0.17666993	-0.3160529	0.08314414	0.04571406	-1.3665315	Nocturnal
CO2254_Ototretinae_sp._1	-0.0545314	0.44870632	0.20002927	0.29863478	0.27966694	-0.7759852	Diurnal
CO2255_Ototretinae_sp._2	0.17260293	0.6751365	0.19228861	0.29863478	0.29358351	-0.8979095	Diurnal
CO2256_Ototretinae_sp._3	-0.0123337	0.70496513	0.0523091	0.22891341	0.1826999	-0.90309	Diurnal
CO2257_Ototretinae_sp._4	-0.0204516	0.46508529	0.08314414	0.16849748	0.15533604	-0.6746896	Diurnal
CO2258_Ototretinae_sp._5	-0.0594835	0.43280901	0.00346053	0.15986785	0.14207646	-0.8601209	Diurnal
CO2260_Dilychnia_c.f._guttala	0.30103	0.86646457	0.31026837	0.23019338	0.20709554	-0.1821042	Nocturnal
KSHX1322_Lampyroidea_nigrescens	0.02694163	0.4542349	0.07664044	0.09725731	0.10002573	-0.5398542	Nocturnal
KSHX1324_Lampyroidea_maculicollis	0.19645254	0.58314207	0.27852497	0.29578694	0.24278981	-0.2992963	Nocturnal

KSHX1328_Lampyroideae__sp._4	0.09516935	0.45278279	0.12645611	0.23653726	0.18695634	-0.6243364	Nocturnal
KSHX1418_Luciolinae_sp.	0.12678058	0.53750428	0.25719843	0.22401481	0.1826999	-0.231732	Nocturnal
KSHX1392_Abscondita__terminalis	0.26552534	0.63367041	0.38649897	0.28216878	0.21695721	0.02897771	Nocturnal
KSHX1394_Medeopteryx_honkongensis	-0.0273344	0.25779853	0.04883009	0.17405981	0.12221588	-0.7022395	Nocturnal
KSHX1406_Stenocladius_sp.	0.09933528	0.53345429	0.14332713	0.23879856	0.19089172	-0.6420652	Diurnal
KSHX1409_Pteroptyx_maipo	0.15926633	0.4817292	0.22401481	0.20817253	0.13449586	-0.2856702	Nocturnal
KSHX1412_Lamprigera_taimoshana	0.57760668	0.3630476	0.52374647	0.27300127	0.19534606	0.38111508	Nocturnal
CO2302_Lucidota_c.f._aurantiaca	0.28578227	0.99561326	0.27392678	0.31952245	0.26173855	-0.4756039	Nocturnal
CO1271_Lucidotina__sp.	0.5289167	0.89558828	0.47041049	0.33685982	0.30963017	0.07059193	Nocturnal
CO2226_Aspisoma_c.f._pallens	0.47099817	0.5378191	0.3047059	0.20221578	0.18780264	-0.1643094	Nocturnal
CO2227_Aspisoma_lineatum	0.44870632	0.60216855	0.41145134	0.28936595	0.20924685	0.02428038	Nocturnal
CO2229_Rufolychnia_borencona	0.32201244	0.76094995	0.2137833	0.24477176	0.2166936	-0.526513	Nocturnal
CO2230_Magnoculus__sp.3	-0.0118872	0.4740705	-0.0056828	0.21298619	0.17811325	-1.1674911	Nocturnal
CO1057_Photinus_stellaris	0.15806079	0.50528567	0.04571406	0.15775889	0.12385164	-0.7328283	Nocturnal
CO1049_Photuris_divisia	0.17260293	0.74640064	0.34537373	0.29578694	0.2166936	-0.1316495	Nocturnal
CO1071_Lampyris_noctiluca	0.32633586	0.38685553	0.30341207	0.27346427	0.21642983	-0.1345999	Nocturnal
CO1069_Aspisoma_sp.	0.49066065	0.55575929	0.24254143	0.29666519	0.25863728	-0.4743075	Nocturnal
CO1063_Pyractonema_sp._1	0.34772022	0.82151353	0.19479176	0.30037807	0.27067884	-0.7772835	Diurnal
CO1060_Lamprocera_sp._1	0.63808972	0.85476136	0.4619485	0.34596154	0.31470969	0.07353507	Nocturnal
CO1067_Heterophotinus_sp.	0.42291798	0.83809314	0.36567514	0.30621051	0.26292547	-0.1668529	Nocturnal
CO1062_Pyrogaster_sp.	0.36828689	0.78515181	0.40208935	0.40636984	0.33223642	-0.4365189	Nocturnal
CO1197_Ellychnia_sp.	0.37088302	0.61909333	0.13576852	0.26221371	0.2828486	-1.0385789	Diurnal
CO1212_Amydetes_fastigiata	0.20736504	0.7906018	0.31555053	0.48685536	0.29534715	-0.3297541	Nocturnal
CO1213_Ethra_axillaris	0.30663944	0.88320703	0.14176323	0.32592596	0.28600712	-0.9892761	Nocturnal
CO1215_Scissicauda_disjuncta	0.33304403	0.76012079	0.09968064	0.2716093	0.2347703	-0.9956786	Diurnal
CO1216_Psilocladus_sigillatus	0.06929801	0.51560895	0.00817418	0.20221578	0.18041263	-0.950782	Nocturnal
CO1229_Trisinuata_sp	-0.0315171	0.43870053	0.12417806	0.18582536	0.10140335	-0.4621809	Nocturnal
CO1228_Bicellonycha_sp._2	0.45697301	0.71883372	0.28802554	0.25935493	0.17868924	-0.4341522	Nocturnal
CO1227_Petalacmis_sp.	0.16106839	0.46337033	0.00173371	0.1360861	0.08206693	-0.6777807	Nocturnal

CO1225_Luciola_sp._2	-0.1745739	0.13385813	0.11193428	0.07736791	2.49831055	-0.4795165	Nocturnal
CO1223_Diaphanes_sp._1	0.42699896	0.35964579	0.35276119	0.15866398	0.0366289	0.16465022	Nocturnal
CO1222_Australoluciola_sp.	0.10105936	0.48486903	0.2121876	0.24278981	0.20628604	-0.4017568	Nocturnal
CO1231_Lamprocera_sp._4	0.6560982	0.96557796	0.44731311	0.33162972	0.26810973	0.01745073	Nocturnal
CO1233_Aspisoma_sticticum	0.70191321	0.68408198	0.50487846	0.36267093	0.24870874	0.19145101	Nocturnal
CO1234_Cratomorphus_sp.	0.7097786	0.87863667	0.47158505	0.3332457	0.24303805	0.16151773	Nocturnal
CO1245_Psilocladus_sp._1	0.25188146	0.65504234	0.26292547	0.24204424	0.19672872	-0.3023348	Nocturnal
CO1457_Lamprocera_sp._3	0.54666603	0.98376156	0.43488812	0.29003461	0.29092456	0.03842145	Nocturnal
CO1459_Lamprocera_sp._2	0.62705846	0.93981866	0.47523522	0.3533391	0.33965016	0.07426774	Nocturnal
CO1505_Uncertain_Luciolinae_nr._Luciola_1	0.20384846	0.56193576	0.25382244	0.25261034	0.18892848	-0.3001623	Nocturnal
CO1509_Uncertain_Luciolinae_nr._Luciola_4	0.37566361	0.76970957	0.41094586	0.34380233	0.29534715	-0.0123337	Nocturnal
CO1510_Uncertain_Luciolinae_nr._Luciola_3	0.29512709	0.61904113	0.39880773	0.25599573	0.13703746	0.10140335	Nocturnal
CO1518_Atyphella_olivieri	0.18383904	0.23185172	0.20709554	0.22427401	0.17172645	-0.3506651	Nocturnal
CO1520_Australoluciola_nigra	-0.0404816	0.3638939	0.10856502	0.19200959	0.14891099	-0.5505216	Nocturnal
CO1528_Photinus_sp._2	0.29578694	0.6613867	0.26552534	0.18412335	0.19451434	-0.2584545	Nocturnal
CO1534_Atyphella_leucura	0.22762965	0.30963017	0.30319606	0.25382244	0.18497519	-0.1369747	Nocturnal
CO1308_Drilaster_sp.	0.08600371	0.7251763	0.15472821	0.28623185	0.28148789	-1.0177288	Diurnal
KSH385_Pollaclasis_bifaria	0.21404868	0.92464105	-0.1784865	0.09621459	0.10243371	-1.6478175	Diurnal
CO1552_Pyropyga_decipiens	0.13798673	0.51646927	0.01199312	0.21005085	0.21272015	-1.19382	Diurnal
CO1547_Photinus_pyralis	0.39304847	0.78756673	0.33825723	0.2664669	0.26102483	-0.2016947	Nocturnal
CO1306_Tenaspis_angularis	0.46805179	0.84772686	0.16731734	0.24427712	0.23299611	-0.6978856	Nocturnal
KSHX11_Abscondita_cerata	-0.0056828	0.44739063	0.15836249	0.22814361	0.15014216	-0.4546929	Nocturnal
KSHX167_Bicellonycha_sp._1	0.28148789	0.63144377	0.28057837	0.27207379	0.17055506	-0.2720523	Nocturnal
KSHX250_Diaphanes_pectinealis	0.48072538	0.95179877	0.33304403	0.23929948	0.19783169	-0.1154878	Nocturnal
KSHX256_Pyrocoelia_pygidialis	0.499137	0.71737921	0.25115134	0.28216878	0.17609126	-0.5590909	Nocturnal
KSHX259_Lamprigera_yunnana	0.60530505	0.27184161	0.36958689	0.20978302	0.1528996	0.11859537	Nocturnal
KSHX266_Emeia_pseudosauteri	0.23375736	0.75518859	0.33385015	0.23754374	0.1708482	-0.1121015	Nocturnal
KSHX268_Pygoluciola_qinqyu	0.26387268	0.68721673	0.28981184	0.29490691	0.24079877	-0.2628074	Nocturnal
KSHX281_Curtos_bilineatus	-0.0310503	0.38614211	0.16286299	0.195069	0.18892848	-0.5294425	Nocturnal

KSHX287_Vesta_saturnalis	0.44575984	0.87693914	0.29819787	0.34458874	0	-0.4094925	Diurnal

Supplemental Table 3.2 Taxa and measurements used for phylogenetic signal and PGLS for light organ size.

Species (with taxon ID)	**LogPrL**	**LogLO (Absolute)**	**LogLO (Relative)**	**signal**
KSHX1429_Ybytyramoan_sp_nov	0.44106641	0.51228406	0.23464882	flash
KSHX1438A_Photinus_sp._Peru	0.39058188	1.01674093	0.30103	flash
KSHX1447_Pygatyphella_obsoleta	0.26787542	-0.5283764	0.06398833	flash
KSHX1448_Pygatyphella_uberia	0.10754913	-0.4386891	0.29877098	flash
KSHX1452_Lucernuta__savignii	0.60552052	0.06792308	0.09142292	glow
CO1976_Magnalata_limbata	0.23019338	-0.2282832	0.30103	flash
CO1937_Lamprohiza_sp	0.29025727	0.00130093	0.20399017	glow
CO1939_Luciolinae_sp	0.23451728	0.07881918	0.30103	flash
CO1940_Bicellonycha_sp	0.2884728	-0.7931741	0.03945277	flash
CO1959_Heterophotinus_sp	0.21484385	-0.2269453	0.02863149	flash
CO1963_near_Photinus/Photinoides_	0.41763774	0.7251763	0.30103	flash
CO1965_Photuris_sp	0.31217736	0.91323087	0.30103	flash
CO1948_Lampyrini/Cratomorphini_	0.28307498	-0.3936186	0.11230529	flash
CO1471_Vesta__c.f._concolor	0.23350376	-0.4614643	0.16309461	glow
CO2247_Magnoculus__sp.2	0.20682588	-0.1850868	0.30103	flash
CO2248_Memoan_sp._nov.	-0.2915791	-0.8096683	0.24273823	flash
CO2260_Dilychnia_c.f._guttala	0.30103	-0.2708352	0.10950578	flash
KSHX1322_Lampyroidea_nigrescens	0.02694163	-0.1567672	0.30103	flash
KSHX1324_Lampyroidea_maculicollis	0.19645254	0.19672872	0.21621896	flash
KSHX1328_Lampyroideae__sp._4	0.09516935	-0.0510982	0.23229444	flash
KSHX1418_Luciolinae_sp.	0.12678058	0.27276959	0.24496552	flash
KSHX1392_Abscondita__terminalis	0.26552534	0.6891312	0.28845072	flash
KSHX1394_Medeopteryx_honkongensis	-0.0273344	-0.0227338	0.30103	flash
KSHX1409_Pteroptyx_maipo	0.15926633	0.46089784	0.30035534	flash
CO2226_Aspisoma_c.f._pallens	0.47099817	0.62920566	0.30087708	flash
CO2227_Aspisoma_lineatum	0.44870632	0.97141502	0.30103	flash
CO1549_Phausis_reticulata	0.10856502	-0.1706962	-0.1706962	glow

Supplemental Table 3.3 Training taxa used for discriminate analysis. *Indicates know taxa that were incorrectly predicted.

Species (with taxon ID)	True class	Predicted class	P(flash)	P(glow)	P(phero)	DA1	DA2
CO1937_Lamprohiza_sp	glow	glow	0.41740655	0.45564476	0.12694869	-0.9651714	-2.6340934
CO2230_Magnoculus__sp.3	glow	pheromone	0.227955	0.16342517	0.60861984	0.66558555	-1.8333188
CO2247_Magnoculus__sp.2	glow	flash	0.60020649	0.20572099	0.19407252	-0.8592207	-1.3728675
CO1049_Photuris_divisia	flash	flash	0.8327534	0.10618106	0.06106554	-1.9063124	-0.5413038
CO1940_Bicellonycha_sp	flash	pheromone	0.38442207	0.01130726	0.60427067	0.44032278	1.53219358
KSHX167_Bicellonycha_sp._1	flash	flash	0.65021289	0.16832396	0.18146315	-0.9535545	-1.0931508
CO1228_Bicellonycha_sp._2	flash	pheromone	0.23675441	0.04782883	0.71541676	0.823527	-0.4452255
CO1947_Pyrogaster_sp	flash	flash	0.74800227	0.03109263	0.2209051	-0.8159728	0.89182056
CO1062_Pyrogaster_sp.	flash	pheromone	0.35474321	0.07533885	0.56991794	0.35305984	-0.6000214
KSHX287_Vesta_saturnalis	pheromone	pheromone	0.34451243	0.0617221	0.59376547	0.41398761	-0.4050607
CO1958_Lucidota_bella	pheromone	pheromone	0.11895933	0.03863168	0.84240899	1.42939637	-0.8221769
CO1471_Vesta__c.f._concolor	pheromone	pheromone	0.36514191	0.06480768	0.57005041	0.34122394	-0.4107142
CO1962_???_	pheromone	pheromone	0.31978688	0.06017934	0.62003378	0.49862911	-0.4393849
CO1245_Psilocladus_sp._1	flash	flash	0.69306628	0.13757115	0.16936257	-1.0380362	-0.8274347
KSHX1432_Psilocladus_miltoderus	pheromone	pheromone	0.28557679	0.05827936	0.65614384	0.62010038	-0.4998787
CO1216_Psilocladus_sigillatus	flash	pheromone	0.34226424	0.2242224	0.43351336	0.1180528	-1.8548898
CO1989_Dodacles__plumosa	pheromone	pheromone	0.30002376	0.07061101	0.62936523	0.54523492	-0.66844
CO1306_Tenaspis_angularis	pheromone	pheromone	0.1174451	0.020227	0.86232789	1.48937072	-0.1312146
CO1967_Lamprocerini/Pleotomini_-new_genus-_	pheromone	pheromone	0.11951075	0.02440033	0.85608892	1.46218017	-0.3189645
KSHX1452_Lucernuta__savignii	glow	flash	0.63641243	0.04186765	0.32171992	-0.4419276	0.48159013
CO1968_Cratomorphus/Nyctocera_	pheromone	flash	0.812903	0.00892054	0.17817646	-0.9671119	2.28305444
KSHX1449_Lucio_sp._1	pheromone	pheromone	0.13377088	0.02607614	0.84015298	1.3672078	-0.2899277

KSHX1450_Lucio__sp._2	pheromone	pheromone	0.1235759	0.08525801	0.79116608	1.31530259	-1.6523275
CO1966_Pleotomus_sp	pheromone	pheromone	0.14940352	0.03503089	0.81556559	1.25369971	-0.5118207
CO1960_Callopisma_sp	pheromone	pheromone	0.39601028	0.07927013	0.52471959	0.21346658	-0.5668249
CO1988_Cratomorphus_signativentris	pheromone	flash	0.77954098	0.02794511	0.19251391	-0.9406906	1.02320451
CO1945_Pyrocoelia_sp	pheromone	flash	0.6249728	0.11415385	0.26087335	-0.6371747	-0.6521614
CO1949_Diaphanes_sp	pheromone	flash	0.6658421	0.17047796	0.16367993	-1.0469501	-1.1014705
CO2227_Aspisoma_lineatum	flash	flash	0.71684478	0.13031521	0.15284001	-1.1344388	-0.7542113
CO1069_Aspisoma_sp.	flash	pheromone	0.21944088	0.09819254	0.68236658	0.8031361	-1.2997775
CO2226_Aspisoma_c.f._pallens	flash	flash	0.5279519	0.11749382	0.35455427	-0.2953453	-0.7897086
CO1991_New_Genus_EQ_1__	pheromone	pheromone	0.00941167	0.01434013	0.9762482	3.33643234	-2.0639392
CO1946_Lucidina_sp	pheromone	pheromone	0.04156386	0.00154188	0.95689426	2.41601125	1.70969763
KSHX1436_Ethra_sp.	pheromone	pheromone	0.26381822	0.04882018	0.68736159	0.71833245	-0.3741066
CO1944_Lychnacris__sp	pheromone	pheromone	0.25638448	0.12939983	0.61421569	0.60368527	-1.4714375
CO1959_Heterophotinus_sp	flash	flash	0.8094206	0.034943	0.1556364	-1.1357488	0.78248771
CO1067_Heterophotinus_sp.	flash	flash	0.69607791	0.06306404	0.24085805	-0.7394116	0.07538438
KSHX1434_Platylampis_latiuscula	pheromone	pheromone	0.41186959	0.09644491	0.49168551	0.1280262	-0.7529055
CO1215_Scissicauda_disjuncta	pheromone	pheromone	0.09856557	0.02675121	0.87468322	1.60589766	-0.592331
CO1552_Pyropyga_decipiens	pheromone	pheromone	0.13810138	0.08728204	0.77461658	1.22193186	-1.578717
CO1063_Pyractonema_sp._1	pheromone	pheromone	0.21506913	0.0363832	0.74854767	0.93747819	-0.2309659
CO1496_Platylampis_explanata	pheromone	pheromone	0.06494654	0.03254878	0.90250467	1.9059363	-1.1833692
KSHX1429_Ybytyramoan_sp_nov	flash	flash	0.7757486	0.10928229	0.11496911	-1.3904432	-0.5367591
CO1528_Photinus_sp._2	flash	flash	0.71489961	0.09242743	0.19267296	-0.9430787	-0.3486379
CO1197_Ellychnia_sp.	pheromone	pheromone	0.03923338	0.01781314	0.94295348	2.31684763	-0.9892643
CO1547_Photinus_pyralis	flash	flash	0.65599195	0.06422588	0.27978217	-0.5886036	0.02494589

CO1057_Photinus_stellaris	flash	flash	0.44464519	0.19591108	0.35944373	-0.1937558	-1.4979946
KSHX1438A_Photinus_sp._Peru	flash	flash	0.88763354	0.05652594	0.05584052	-1.9834992	0.18428065
KSH385_Pollaclasis_bifaria	pheromone	pheromone	0.00439456	0.00049514	0.9951103	4.0508588	0.87420621
CO2258_Ototretinae_sp._5	pheromone	flash	0.5264097	0.20086696	0.27272334	-0.5157145	-1.413597
CO2257_Ototretinae_sp._4	pheromone	flash	0.6383379	0.16865626	0.19300584	-0.8953022	-1.1024156
KSHX1406_Stenocladius_sp.	pheromone	flash	0.52226891	0.13802501	0.33970608	-0.3280039	-0.9804846
CO2255_Ototretinae_sp._2	pheromone	pheromone	0.16915541	0.03376166	0.79708293	1.15319521	-0.3612764
CO2256_Ototretinae_sp._3	pheromone	flash	0.49563542	0.08196473	0.42239985	-0.1033661	-0.4309154
CO2254_Ototretinae_sp._1	pheromone	flash	0.5509697	0.21598299	0.23304732	-0.6673248	-1.475075
KSHX1441_Harmatelia_bilinea	pheromone	pheromone	0.10040269	0.01973301	0.8798643	1.61347988	-0.2456549
CO1505_Uncertain_Luciolinae_nr._Luciola_1	flash	flash	0.62913067	0.11438623	0.2564831	-0.654414	-0.6509603
KSHX1447_Pygatyphella_obsoleta	flash	pheromone	0.31588292	0.12146558	0.5626515	0.39842308	-1.2249035
KSHX1448_Pygatyphella_uberia	flash	flash	0.7580653	0.15425159	0.08768312	-1.593249	-0.9735497
CO1534_Atyphella_leucura	flash	flash	0.59832892	0.23062539	0.17104569	-0.9565776	-1.5193547
CO1976_Magnalata_limbata	flash	flash	0.715167	0.14916847	0.13566452	-1.2281758	-0.9213602
KSHX266_Emeia_pseudosauteri	flash	flash	0.72973124	0.04444974	0.22581902	-0.8013559	0.48646313
CO1510_Uncertain_Luciolinae_nr._Luciola_3	flash	flash	0.78818629	0.0770909	0.1347228	-1.2657135	-0.1198391
KSHX268_Pygoluciola_qinqyu	flash	flash	0.54573269	0.06977355	0.38449376	-0.2308453	-0.1832839
CO1509_Uncertain_Luciolinae_nr._Luciola_4	flash	flash	0.6224715	0.05043776	0.32709074	-0.4241646	0.26265125
KSHX1418_Luciolinae_sp.	flash	flash	0.72055743	0.11720903	0.16223354	-1.0882775	-0.6254276
KSHX1392_Abscondita__terminalis	flash	flash	0.77421503	0.07962189	0.14616308	-1.1947348	-0.1582036
CO1225_Luciola_sp._2	flash	flash	0.83383278	0.05988524	0.10628198	-1.4668562	0.16701345
KSHX11_Abscondita_cerata	flash	flash	0.65644453	0.20259735	0.14095812	-1.1568757	-1.3248812
KSHX1324_Lampyroidea_maculicollis	flash	flash	0.59450856	0.10967649	0.29581495	-0.507598	-0.6348971
KSHX1322_Lampyroidea_nigrescens	flash	flash	0.60679797	0.117887	0.27531503	-0.5786434	-0.7055046
CO1222_Australoluciola_sp.	flash	flash	0.6134931	0.14059188	0.24591502	-0.6790429	-0.9036997

CO1520_Australoluciola_nigra	flash	flash	0.61134341	0.22894657	0.15971001	-1.0217872	-1.5025872
KSHX1409_Pteroptyx_maipo	flash	flash	0.64694244	0.12538732	0.22767024	-0.7666828	-0.7434669

Supplemental Table3. 4 Test taxa used for discriminate analysis and their predictions.

Species (with taxon ID)	Predicted class	P(flash)	P(glow)	P(phero)	DA1	DA2
CO1212_Amydetes_fastigiata*	flash	0.69159261	0.20084888	0.10755851	-1.3925922	-1.3106315
CO1990_Magnoculus_or_near_	pheromone	0.24220947	0.19089992	0.56689061	0.56310919	-1.9566759
CO1965_Photuris_sp	flash	0.70122149	0.08180893	0.21696958	-0.8354459	-0.2156392
CO1961_???_	pheromone	0.31999083	0.0444933	0.63551587	0.53225354	-0.1086148
CO2260_Dilychnia_c.f._guttala	flash	0.77644178	0.05179596	0.17176226	-1.0546979	0.33470326
CO1227_Petalacmis_sp.	flash	0.46607137	0.28406347	0.24986516	-0.5148821	-1.9140531
KSHX1437_Phaenolis_cf._ustulatus	pheromone	0.22211162	0.04857944	0.72930894	0.88089584	-0.5177666
CO1476_Lamprocerini_sp.	flash	0.70193355	0.04484962	0.25321684	-0.6902895	0.45929479
CO1459_Lamprocera_sp._2	flash	0.57844184	0.04474062	0.37681753	-0.2625955	0.34716541
CO1060_Lamprocera_sp._1	flash	0.55128416	0.06222059	0.38649526	-0.2279725	-0.0493641
CO1457_Lamprocera_sp._3	flash	0.67430389	0.03389385	0.29180226	-0.5429629	0.74752367
CO1231_Lamprocera_sp._4	pheromone	0.46377861	0.03452792	0.50169347	0.11498964	0.4693237
CO1234_Cratomorphus_sp.	flash	0.50889438	0.04515471	0.44595092	-0.0501657	0.24613413
CO1948_Lampyrini/Cratomorphini_	flash	0.57279121	0.05427453	0.37293426	-0.2736164	0.12778338
KSHX250_Diaphanes_pectinealis*	flash	0.59003443	0.03046109	0.37950447	-0.2508793	0.78188199
KSHX256_Pyrocoelia_pygidialis*	pheromone	0.11835631	0.0317479	0.8498958	1.44971326	-0.6134348
CO1223_Diaphanes_sp._1*	flash	0.6869714	0.22958391	0.08344469	-1.582968	-1.5017591
CO1071_Lampyris_noctiluca*	flash	0.55572822	0.32897535	0.11529644	-1.2164229	-2.0340084
CO1943_Aspisomoides_sp	pheromone	0.40107891	0.13257271	0.46634838	0.0904732	-1.1295076
CO1213_Ethra_axillaris	pheromone	0.12373198	0.0261029	0.85016512	1.42961888	-0.3609778
CO2229_Rufolychnia_borencona	flash	0.48659599	0.07225128	0.44115273	-0.0519294	-0.3046627
CO1271_Lucidotina__sp.	flash	0.72485626	0.05100212	0.22414162	-0.8094577	0.33054792
CO2302_Lucidota_c.f._aurantiaca	flash	0.59164475	0.0464683	0.36188695	-0.3100512	0.32056462
KSHX1431_Lucidota_banoni	flash	0.53780964	0.02345611	0.43873425	-0.0660202	1.0019928
CO1963_near_Photinus/Photinoides_	flash	0.67673963	0.05195437	0.27130601	-0.6217248	0.27778283
KSHX1430_Lucidota_luteicollis	pheromone	0.43102599	0.12511178	0.44386222	0.00734374	-1.0085084

CO1308_Drilaster_sp.	pheromone	0.18023315	0.02997309	0.78979376	1.1089599	-0.175746
KSHX259_Lamprigera_yunnana	pheromone	0.36137836	0.14742335	0.4911983	0.19510433	-1.3319084
KSHX1412_Lamprigera_taimoshana	flash	0.64508206	0.14116382	0.21375412	-0.81761	-0.8842139
CO1518_Atyphella_olivieri	flash	0.45679919	0.28368234	0.25951847	-0.472909	-1.9250586
KSHX281_Curtos_bilineatus	flash	0.63997664	0.18029319	0.17973017	-0.9533333	-1.1835048
KSHX1328_Lampyroideae__sp._4*	pheromone	0.40660296	0.1547625	0.43863454	0.02759021	-1.2938779
CO1939_Luciolinae_sp	flash	0.67303994	0.094888	0.23207206	-0.7651393	-0.4028987
CO1229_Trisinuata_sp	flash	0.69917028	0.17907542	0.1217543	-1.3022807	-1.1568572
KSHX1394_Medeopteryx_honkongensis	flash	0.43823474	0.29935678	0.26240848	-0.4389658	-2.0195959

CHAPTER 4

CONCLUSIONS

This work investigates key aspects of firefly diversity and and the evolution of sexual signaling, including establishing a worldwide firefly phylogeny, documenting structures used for pheromone detection, estimating the evolutionary history of sexual signaling, and identifying the influence of phylogeny and activity time on signal receptor characteristics. My research provides valuable tools and foundational knowledge for future studies in firefly systematics and evolution and their mating signals.

Chapter two focuses on the diversity of antennal sensilla used by fireflies to collect information from their environment, including detecting and identifying pheromones during mate search. My research documents for the first time the diversity of firefly antennal sensilla and shows that sensilla morphotypes vary greatly between species. As sensilla morphotypes are identified in other firefly species we can determine how often and how quickly morphotypes change between species. Future functional testing of antennal sensilla will also provide evidence on whether or not bioluminescent species also use pheromones during mate search.

Using a set of predictions for pheromone sensilla, I identified at least nine potential pheromone sensilla across seven firefly species. These candidate pheromone sensilla provide a starting point for future functional studies once pheromones are identified for more firefly species. In addition to potential pheromone sensilla, I also identified on basiconica sensilla morphotype as a potential receptors for cuticular hydrocarbons or other contact chemicals, which may be used for mate recognition during reciprocal antennation behavior that precedes mating in fireflies.

Phylogenies are an essential tool for systematic research and also provide a necessary framework for evolutionary studies. The phylogenetic relationships identified in chapter three, represent the most comprehensive picture of the evolution of the family Lampyridae and the relationships of 10 subfamilies to date. It supports the status of seven subfamilies, and also raises awareness for Lampyridae groups that need further investigation and taxonomic work. For example, the placement of *Pterotus* as sister to Chespiritoinae and Cheguevariinae in Photurinae indicates the need for additional sampling from these groups and taxonomic evidence to support these relationships. In addition, the lack of monophyletic genera in Luciolinae and Vestini should be addressed with a combination of molecular and morphological data to identify synapomorphic characters that support these relationships and increase taxon sampling to examine the taxonomic status of these genera.

My evolutionary analyses of light signals and light organ shape indicate that both the evolution of light signals and light organ shape evolved multiple times during firefly evolution. An extended time-calibrated phylogeny (Powell et al. 2022) coupled with ancestral state reconstructions of sexual signals as well as light organ shape would provide estimates on how quickly these changes may occur between species. In addition to the evolution of sexual signaling this phylogeny can be used to answer a wide range of questions about firefly evolution. For example, combined with geographic information for species it can be used to determine dispersal patterns as lineages diverged, and/or calculate species diversification rates.

While my work was unable to correctly predict bioluminescent types (i.e. flashes or glows) based on my morphological measurements across the wide taxonomic range of fireflies in this study, I suggest that further analyses should be done with additional morphological traits to further test the predictability of signal type across the whole family or lineage specific (e.g., subfamily) analyses. Specifically, the ability to predict bioluminescence type (i.e., the use of flashes versus glows) would be a valuable tool in studying the evolution of bioluminescence in fireflies, especially for historic museum specimens with no behavioral data.

REFERENCES

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