Advances in the Systematics and Evolutionary Understanding of Fireflies (Coleoptera: Lampyridae)

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Advances in the Systematics and Evolutionary Understanding of Fireflies

(Coleoptera: Lampyridae)

Gavin Jon Martin

A dissertation submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

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ABSTRACT

Advances in the Systematics and Evolutionary Understanding of Fireflies (Coleoptera: Lampyridae)

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Doctor of Philosophy

Fireflies are a cosmopolitan group of bioluminescent beetles classified in the family Lampyridae. The first catalogue of Lampyridae was published in 1907 and since that time, the classification and systematics of fireflies have been in flux. Several more recent catalogues and classification schemes have been published, but rarely have they taken phylogenetic history into account. Here I infer the first large scale anchored hybrid enrichment phylogeny for the fireflies and use this phylogeny as a backbone to inform classification. Several classification changes are made throughout the group with emphasis on morphological traits that support the AHE hypothesis.

Building off of this classification work, and in an effort to help correct taxonomic issues that have plagued the Lampyridae, I also present an electronic identification tool to the firefly genera of the world. This tool is built in Lucid and incorporates 23 characters (features) and 76 character states. These characters are inspired by current and historic literature. Emphasis was given to characters and states that are easily located and do not require complex dissection. The key currently works for 113 of the 146 known lampyrid genera. As such, it should be noted that it is a provisionary attempt at identification, and all identifications should be checked against primary literature.

Fireflies, like many organisms, rely on sensory cues from their environment and are an ideal system for studying sensory niche adaptation. This is due in large part to the dependence of many species on bioluminescent sexual communication. Using transcriptomics, I examine the phototransduction pathway and provide some of the first evidence for positive selection in beetles, of components of the phototransduction pathway beyond opsins.

Based on preliminary data gathered in several BYU Bio-100 courses for non-majors, I observed that many students come to class with a human-centric view of the world. In addition to this, and perhaps as an explanation, students also come to class without a firm understanding of natural history collections and their roles both to the general public and specifically to science. Therefore, in two sections of BIO-100 at BYU students were given an online module as part of their normal homework. This module was designed to use fireflies from the Monte L Bean Science Museum to introduce students to the concept of natural history museums and to give an example of an organism at risk for extinction. Unfortunately, no gain in pro-environmental thinking was observed post-intervention, however, I did observe gains in student’s appreciation of the importance of natural history collections to both the general public and to scientific research.

Keywords: classification, lucid, key, phototransduction, natural history collections
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Chapter 1: Higher-level phylogeny and reclassification of Lampyridae (Coleoptera: Elateroidea)

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ABSTRACT

Fireflies (Lampyridae: Rafinesque) are a diverse family of beetles which exhibit an array of morphologies including varying antennal and photic organ features. Due in part to their morphological diversity, the classification within the Lampyridae has long been in flux. Here we use an anchored hybrid enrichment approach to reconstruct the most extensive molecular phylogeny of Lampyridae to date (436 loci and 98 taxa) and use this phylogeny to evaluate the higher-level classification of the group. None of the currently recognized subfamilies were recovered as monophyletic with high support. We propose several classification changes supported by both phylogenetic and morphological evidence: (1) *Pollaclasis* Newman, Vestini McDermott (incl. *Vesta* Laporte, *Dodacles* Olivier, *Dryptelytra* Laporte, and *Ledocas* Olivier), *Photoctus* McDermott, and *Araucariocladus* Silveira & Mermudes are transferred to Lampyridae incertae sedis, (2) *Psilocladinae* McDermott, 1964 status novum is reestablished for the genus *Psilocladus* Blanchard, (3) Lamprohizini Kazantsev, 2010 is elevated to Lamprohizinae Kazantsev, 2010 status novum and *Phausis* LeConte is transferred to Lamprohizinae, (4) *Memoan* Silveira and Mermudes is transferred to Amydetinae Olivier, and (5) *Scissicauda* McDermott is transferred to Lampyrinae Rafinesque.
INTRODUCTION

Fireflies (Lampyridae Rafinesque, 1815) are a cosmopolitan group of beetles with approximately 2200 species (Branham 2010) and a varied and confounded history of classification (Olivier 1907 & 1910a, Green 1959, McDermott 1966, Crowson 1972, Nakane 1991, Jeng 2008). Higher-level firefly classification schemes (e.g. Green 1956) have traditionally been framed in the context of morphological structures related to the production and detection of sexual signals (e.g., photic organs, eyes and antennae). The morphological evolution of these features appears to have been driven by species-specific sexual communication, such as pheromones, glows, and flashes (Buck 1937, Lloyd 1971, Ohba 1983, Branham and Wenzel 2003, Stanger-Hall and Lloyd 2015, Stanger-Hall et al. 2018). Recent work suggests that bioluminescent sexual signals have evolved multiple times during firefly evolution and have been repeatedly lost, with a likely reversal to the use of pheromones for a sexual signal (Branham and Wenzel 2003, Stanger-Hall et al. 2007, Martin et al. 2017). Therefore, morphologies related to these signals (production/reception) may represent instances of convergent evolution and the continued dependence on these morphologies to support firefly classification and taxonomy (Branham and Wenzel 2003) needs to be reconsidered.

The first catalogue of Lampyridae was authored by Ernst Olivier in 1907 and updated in 1910. In this initial taxonomic effort Olivier included nine subfamilies (based almost entirely on antennal morphology) and approximately 1000 species. Drawing on the earlier work of Green (1948, 1959), McDermott (1964) rearranged the taxa into seven subfamilies and added a tribal classification for the Lampyrinae. However, he noted that his classification, while logical, did not reflect phylogeny and was “more or less arbitrary,” as it relied heavily on characters such as antennae and photic organ morphology. This work lead to McDermott’s (1966) supplement to
the Olivier catalogue. Since McDermott, there have been two revised classification schemes for Lampyridae (Crowson 1972, Nakane 1991; see Table 1). The classification of the family by Crowson (1972) was incomplete as it did not include all lampyrid genera, and the work of Nakane (1991; published in Japanese) has largely been overlooked by current taxonomic workers. Recent phylogenetic efforts have provided deeper insight into the classification of fireflies by expanding morphological data sets (Branham and Wenzel 2001, 2003, Jeng 2008); molecular data sets (Stanger-Hall et al. 2007); or both (Martin et al. 2017). All of these studies highlighted the need to update the higher-level classification of this group within a phylogenetic framework. While focused on the evolution of specific traits, each of these studies recovered non-monophyly of at least one subfamily. We are presenting here the first classification of Lampyridae that uses extensive taxon sampling and explicitly takes phylogenetic history into account.
MATERIALS AND METHODS

Taxon Sampling

Our ingroup sample included 53 (of approximately 145) lampyrid genera and 88 species, representing seven of the eight subfamilies sensu Nakane 1991 (Table 2). However, the Ototretadrilinae has since been synonymized under the Ototretinae (Janisova and Bocakova 2013). DNA grade material for the Cheguevarinae was not available. The appropriate sister taxon to Lampyridae has been a topic of debate (Branham and Wenzel 2001, 2003, Stanger-Hall et al. 2007, Jeng 2008, Bocakova et al. 2007, Kundrata et al. 2014, Martin et al. 2017), therefore ten outgroup taxa from several closely related elateroid families were sampled: Omethidae, Eucnemidae, Cantharidae, Lycidae, Elateridae, Rhagophthalmidae, and Phengodidae (Table 2).

DNA Extraction

Specimens were collected in the field, preserved in 95% ethanol and stored at –80 °C for long-term storage. Each specimen was independently identified by at least two co-authors. Muscle tissue was removed from a single metacoxae, when possible, for each specimen. In some instances, the small size of the specimen necessitated more of the specimen to be used. DNA was then extracted using a Qiagen DNeasy extraction kit. DNA samples were sent to the Center for Anchored Phylogenomics at Florida State University, Tallahassee, FL, USA for anchored hybrid enrichment sequencing. The specimens and remaining DNA samples were deposited as vouchers in the BYU Insect Genomics Collection in the Monte L. Bean Museum (BYU) of Natural History, Provo, UT, USA, the Lampyridae cryo-collection in the Stanger-Hall lab (UGA), Athens, GA, USA, and the Coleção Entomológica Prof. José Alfredo Pinheiro Dutra,
Departamento de Zoologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil (DZRJ); see table 2.

Probe Design

In collaboration with the Center for Anchored Phylogenomics (www.anchoredphylogeny.com), an AHE kit representing the diversity of Lampyridae was developed, targeting a subset of the low-copy AHE 941 loci (exons) that Haddad et al. (2018) developed for Coleoptera. To this end, genomic resources, assembled genomes, assembled transcriptomes, and unassembled low-coverage genomic reads (unpublished; see Supp. Table 1 for details), for 14 species of Lampyridae were gathered. In order to collect the low coverage genomic reads, Illumina libraries were prepared following Lemmon et al. (2012) and each was sequenced at 5x coverage on an Illumina HiSeq 2500 with a 150bp paired-end protocol with an 8bp indexing read. After demultiplexing with no mismatches tolerated, overlapping reads were merged following Rokyta et al. (2012).

Target regions appropriate for Lampyridae were identified by following the general methodologies described in Hamilton et al. (2016). The assembled genomes and transcriptomes were scanned using all of the 941 Tribolium castaneum reference sequences developed by Haddad et al. (2018). After reads were mapped and extended to the same references, the reads were extended up to 2000bp in each direction to produce longer sequences. The resulting sequences were then aligned in Mafft v. 7.407 (Katoh and Standley 2013) and visually inspected in Geneious. Well-aligned regions were selected for downstream analysis. Of the 941 beetle exons identified by Haddad et al. (2018), only 586 regions of >180bp in which at least 16 of 19
taxa were present were identified. Seventy overlapping-loci were removed. Repetitive regions were masked using analyses of kmer distributions (see Hamilton et al. 2016 for details).

The final target regions were comprised of 516 target loci covering 578,322 bp. Probes were tiled at 2x coverage across each of the 19 lineages represented in the alignments. After removing redundant probes, 56979 probes were submitted to Agilent Technologies for synthesis (SureSelect XP kit).

Library Preparation

From extracted DNA, libraries with standard Illumina adapters were prepared following Lemmon et al. (2012) and Prum et al. (2015) using a Beckmann Coulter FXp liquid-handling robot. After adding 8bp indexes, libraries were pooled in groups of ~16 and enriched using the Lampyridae-specific enrichment kit described above. Enriched library pools were then pooled and sequenced on three Illumina 2500 sequencing lanes with C-bot clustering and a paired-end 150bp protocol (~100Gb of raw data were collected).

Read Assembly and Orthology Assessment

Raw reads passing the CASAVA high-chastity filter were merged following Rokyta et al. (2012) and assembled using the quasi-de novo approach described by Hamilton et al, (2016), with probe region sequences from Phausis reticulata, Pyractomena borealis, Photinus pyralis, Photinus scintillans, Phausis reticulata and a soldier beetle (Cantharidae), serving as references. Assembly clusters derived from less than 42 reads were removed from downstream analyses in order to mitigate the effects of any low-level contamination (clusters were typically comprised of 100-1000 reads). Homologous consensus sequences were derived from the remaining clusters,
with ambiguous base calls being made for each site with a base pattern that could not be
explained by sequencing error, which was assumed to be no more than 1%. For each of the 516
target loci, orthologous sets of homologs were determined using a distance matrix (constructed
by comparing kmer distributions) and a neighbor-joining approach for clustering (with at most
one sequence per individual allowed in each ortholog cluster). After removing ortholog sets with
less than 50% species representation (<51 of 98), 436 loci remained (Supp. Table 2).

Alignment

The sequences obtained for individual loci were aligned via Mafft v. 7.407 (Katoh and
Standley 2013) using the default automatic alignment option. For Maximum Likelihood (ML)
analyses, the alignments of the individual loci included flanking regions (head, tail, or both) of
each locus and were concatenated before analysis and resulted in an alignment length of
1,833,533 base pairs. Breinholt et al. (2017) showed that trimming data from the probe or
flanking region has little effect on the final topology, consequently, we elected not to trim these
regions (Lemmon et. al. 2012). However, it is possible that missing data may bias phylogenetic
reconstruction (Lemmon et al. 2009; however, see also Wiens and Morrill 2011), therefore, we
generated a second dataset utilizing a portion of the pipeline of Breinholt et al. (2017)
(alignment_DE_trim.py) to remove sites from the alignment with <75% density, (i.e.
completeness), and with entropy >1.5 (to account for sites estimated to be nearly random). This
resulted in a trimmed alignment of 401,423 base pairs.
Phylogenetic reconstruction

Maximum Likelihood reconstruction was performed in IQ-Tree v 1.6.8 (Nguyen et al. 2015, Chernomor et al. 2016) with 1,000 ultrafast bootstrap iterations (UFBoot; Hoang et al. 2018). Model estimation for concatenated locus datasets was carried out using ModelFinder (Kalyaanamoorthy et al. 2017) implemented within IQ-Tree, using BIC criteria. GTR+F+R9 was selected as the best-fit model for the untrimmed dataset and GTR+R9 was selected for the trimmed dataset. To account for the potential effect of incomplete lineage sorting on our phylogenetic reconstruction we also used a coalescent approach to estimate a species tree via ASTRAL-III (Zhang et al. 2018) for each dataset (trimmed and untrimmed). ML gene tree topologies for each locus were inferred via IQ-Tree (sensu Breinholt et al. 2017) before estimating the ASTRAL topology for each dataset. We used localized posterior probabilities (Sayyari and Mirarab 2016) to assess nodal support for the coalescent species trees as assessed in ASTRAL-III.
RESULTS

Phylogenetic reconstruction

Maximum Likelihood Reconstruction

Within the maximum likelihood (ML) reconstruction of the **untrimmed dataset** (Topology 1 (T1); Fig. 1), we find strong support for a sister group relationship between the Elateridae, Rhagophthalmidae, and Phengodidae with the Lampyridae (Fig. 1). The Luciolinae + *Lamprigera* Motschulsky were recovered as the sister lineage to all other firefly subfamilies with strong support (UFBoot 100%). Within the lineage of the remaining subfamilies, a clade comprised of (*Pterotus* LeConte + *Pollaclasis* Newman) + the monophyletic Ototretinae forms the sister group to the remaining fireflies. However, neither this clade, nor the Ototretinae clade are well supported (UFBoot: 72% for both nodes). The clade (*Lamprohiza* Motschulsky + *Phausis* LeConte) is recovered as sister to the clade (*Psilocladus* Blanchard + (*Amydetinae + Photurinae)) + Lampyrinae) with UFBoot support of 100% at each node.

The ML reconstruction of the **trimmed dataset** (Topology 2 (T2); Supp. Fig. 1) was largely identical to T1, with the exception of the placement of the paraphyletic Ototretinae and *Pollaclasis* in T2. In T2, Ototretinae is rendered paraphyletic, as *Stenocladius* Fairmaire in Deyrolle and Fairmaire is recovered as the sister taxon to all other Lampyridae, while *Drilaster* Kiesenwetter is recovered as the sister taxon to a clade comprised of *Pterotus* + *Pollaclasis*, albeit with low support (UFBoot 77%). Compared to T1, there is also lower support along the backbone of T2 (Supp. Fig. 1).
Coalescent Reconstruction

The coalescent reconstruction of the **untrimmed dataset** (Topology 3 (T3); Supp. Fig. 2) was largely congruent with the ML topology of the same dataset, except for the placement of the Ototretinae (incl. *Pterotus*). In the coalescent analysis (T3), the Ototretinae are rendered polyphyletic by inclusion of *Pterotus*, and this clade is recovered as the sister group to all other fireflies, while in the ML analysis (T1) this position was held by Luciolinae + *Lamprigera* (Fig. 1; Localized Posterior Probability (LPP): 0.95).

The coalescent reconstruction of the **trimmed dataset** (Topology 4 (T4); Supp. Fig. 3) was identical in respect to subfamilial relationships in the untrimmed data set (T3), with only minor variation in species relationships within the major clades (i.e. placement of the lucioline clade comprised of *Abscondita cerata* and *Curtos* sp.; Supp. Figs. 3 & 4).

Consensus among analyses

In all analyses, Lampyridae was reconstructed as monophyletic with support values of UFboot: 100% or LPP: 1. With the exception of the Ototretinae (discussed separately below), our four different analyses yielded no topological differences that impact the monophyly of the subfamilies among Lampyridae. Therfore we will use Topology 1 (Fig. 1), to discuss the phylogenetic relationships among the seven subfamilies sampled. Topology 1 (T1) is also the best supported (via ultrafast bootstrap analysis), and is based on the full, untrimmed dataset.

In T1, the Luciolinae are reconstructed as paraphyletic with the inclusion of *Lamprigera*. The Ototretinae were represented by two genera (*Drilaster* and *Stenocladius*) in our analyses, and these formed a monophyletic clade, albeit with low support (72%). T1 is the only topology to recover the Ototretinae as monophyletic and sister to a clade comprised of *Pterotus* and
**Pollaclasis.** In both T2 and T4 (Supp Figs. 1 & 3) *Stenocladius* was recovered as the sister lineage to all other fireflies with low support (UFboot: 77% & LPP: 0.75), while in T3 (Supp. Fig. 2) a clade consisting of *Stenocladius + (Drilaster + Pterotus)* is recovered as sister to the remaining fireflies (LPP: 0.95). Given this incongruence, the exact position of the Ototretinae among firefly subfamilies remains uncertain. However, in all phylogenetic reconstructions, *Pterotus* is either sister to, or a lineage within, the Ototretinae. The subfamily Amydetinae is recovered as polyphyletic and its taxa can be found closely associated with diverse subfamilies: *Psilocladus* is recovered as sister to the Amydetinae + Photurinae, *Pollaclasis* forms a clade with *Pterotus* (Pterotinae), *Vesta* Laporte is recovered within the photurine clade, and *Cladodes* Solier, *Ethra* Laporte, and *Scissicauda* McDermott are recovered within the Lampyrinae. The Photurinae are rendered paraphyletic by inclusion of the old-world *Vesta* (see Martin et al. 2017). Lampyrinae is recovered as polyphyletic: *Lamprigera* is recovered within the Luciolinae, and there is high support (UFboot: 100%) for a monophyletic *Lamprohiza + Phausis* clade outside the Lampyrinae (see above). In addition, several amydetine taxa (*Cladodes, Ethra, Scissicauda*) cluster within the remaining Lampyrinae.

As such, in T1, the only subfamily recovered as monophyletic, as currently classified, is the Ototretinae. However, across all other topologies, even the Ototretinae are also rendered non-monophyletic.
DISCUSSION

The higher-level classification of Lampyridae is in need of revision. Our molecular analyses confirm that a few morphological characters related to sexual communication, many likely convergent, cannot be central to diagnosing a natural classification of Lampyridae. The use of these characters in subfamilial classification needs to be reassessed in light of a more complete phylogenetic history of the group. Our phylogenetic analyses based on 436 loci and 98 taxa provide a robust framework for a revision of the classification of Lampyridae.

Luciolinae

The Luciolinae are a subfamily of fireflies that have received much attention over the years (for a taxonomic treatment see Ballantyne et al. 2015) and seem to share stable morphological characters such as a reduced number of abdominal ventrites in the males (see discussion of Lamprigera below).

Our analyses largely agree with recent morphological analyses in terms of the major groupings of the Luciolinae (Ballantyne and Lambkin, 2013). Thanks in large part to the effort of Ballantyne and Lambkin (Ballantyne 1987a, Ballantyne and Lambkin 2009, 2013), there also exists a discussion of the aedeagal sheath, which has provided evidence in support of the current taxonomic framework for these taxa. Many of the morphological characters used in these analyses (Ballantyne and Lambkin 2009, 2013, Ballantyne et al. 2015, 2016) and the phylogenetic patterns they support, correlate well with the phylogenetic hypotheses generated from our molecular data. However, some minor differences between previous phylogenetic work and our overall topology do exist. For example, the Atyphella complex is not recovered as the the most highly derived lineage, as it is in Ballantyne and Lambkin (2013). Our molecular dataset
recovers this lineage as more basal compared to the other Luciolinae. In addition, the lampyrine genus *Lamprigera* is recovered as a lineage within the lucioline clade.

Jeng et al. (2000) remarked that the systematic position of *Lamprigera* was uncertain and would hopefully be more accurately defined through future phylogenetic investigation. Previous analyses have recovered *Lamprigera* in various positions within the Lampyridae. In 2006, on the basis of the 16S mitochondrial marker, Li et al. performed a phylogenetic reconstruction of the Lampyridae. In this analysis, *Stenocladius* was recovered as the basal lineage, while *Lamprigera* was recovered in a surprising clade together with the amydetine genus *Vesta* sister to the remaining fireflies (Li et al. 2006), albeit with low support. In his thesis, Jeng (2008) recovered *Lamprigera* sister to the *Phausis + Lamprohiza* using >400 morphological characters. Wang et al. (2017) recovered *Lamprigera* as a member of the Lampyrinae on the basis of 13 mitochondrial genes. It should be noted, however, that the Wang et al. (2017) analysis suffered from a limited taxon sampling (six lucioline taxa and two additional lampyrine taxa from a single genus). Martin et al. (2017) recovered *Lamprigera* as sister to the monophyletic Luciolinae with high support and placed it as Lampyridae *incertae sedis*.

Here we recover *Lamprigera* as a member of the Luciolinae for the first time with both strong support and congruence between all of our analyses. However, this placement is based on a single *Lamprigera* species and major morphological differences between Lamprigera and the luciolines, e.g. the number of abdominal ventrites, need to be addressed. A major, long-standing morphological synapomorphy for Luciolinae has been males with six abdominal ventrites, whereas all other Lampyridae have seven or eight abdominal ventrites. Males of *Lamprigera* exhibit the “typical” abdominal morphology of Lampyridae with seven ventrites.
In certain elateroid lineages, ventrite number is known to vary greatly, even within recently derived tribes/subfamilies (Kundrata & Bocak 2019). In contrast, ventrite number has not been shown to vary in Luciolinae. To rigorously test the classification of Lamprigera relative to the Luciolinae, an expanded taxon sampling including deeper species coverage within the Lamprigera, combined with an in-depth morphological investigation, including the plasticity of ventrite number across these taxa will be needed. Until these analyses can be done, we elect to keep Lamprigera as Lampyridae incertae sedis (Martin et al., 2017).

Pterotinae, Cyphonocerinae, and Psilocladinae (McDermott, 1964) stat. nov.

Jeng et al. 1998 & 2006, summarizing the work of McDermott (1966), Crowson (1972), and Nakane (1991), was the first to formally delineate the Psilocladinae (previously known as Cyphonocerinae), by identifying the constituents of the group (Cyphonocerus Kiesenwetter, Psilocladus, and Pollaclasis) and laying out nine morphological features uniting the group. Jeng et al. (1998) also recognized Cyphonocerinae as a subjective synonym of Psilocladinae based on priority. In 2016, Silveira et al. treated Scissicauda as a member of the amydetine subtribe Psilocladina sensu McDermott 1964, distinguishing Scissicauda from the other members of the group (Ethra, Photoctus McDermott, Psilocladus, and Pollaclasis). In 2017, another genus (Araucariocladus Silveira & Mermudes) was added to the Psilocladina sensu McDermott. As a consequence of recognizing Psilocladina sensu McDermott instead of Psilocladinae sensu Jeng/Nakane, Cyphonocerus was left as the sole member of the Cyphonocerinae.

Our phylogenetic analyses support in part the classification presented by Crowson (1972) and Nakane (1991) and supports the classification of Psilocladus as a separate lineage from the Amydetinae. Without Cyphonocerus in our taxon sample, the placement of Psilocladus within
the Cyphonocerinae cannot truly be tested, however, given that *Psilocladus* did not form a monophyletic lineage with Pollaclasis, we formally recognize the subfamily Psilocladinae, distinguished by the antennae with 11 articles, articles 2–11 with two weak, ciliate branches, with the only constituent genus being *Psilocladus*.

Following McDermott (1966) and Silveira et al. (2016), *Pollaclasis* is classified as a member of the Amydetinae. However, both our ML and coalescent analyses challenge this classification. The ML analysis places *Pollaclasis* as a sister taxon to the North American *Pterotus*, the sole member of the Pterotinae (Fig. 1). In contrast, the coalescent analysis places *Pollaclasis* as sister to a *Phausis* + *Lamprohiza* clade (Supp. Fig. 2). Hypothesized to be a close relative of *Cyphonocerus*, *Pollaclasis* has previously been classified in the Psilocladinae based on the morphology of antennae, mandibles, and abdominal segmentation (see Jeng et al. 1998). The present analyses do not support the monophyly of *Pollaclasis* and *Psilocladus*. Based on this evidence, as well as the absence of *Cyphonocerus* in our taxon sample, we transfer *Pollaclasis* to Lampyridae *incertae sedis*. Future efforts need to be made to ascertain whether *Pollaclasis* is indeed a member of the Cyphonocerinae, or as the ML analyses suggest, more closely related to *Pterotus*.

Ototretinae

Our analyses indicate that there may be a close relation between the Ototretinae and the Pterotinae. Recently, the Ototretinae were revised, and their taxonomic placement was addressed (Janisova and Bocakova 2013). In that study, the monotypic Ototretadrilinae were synonomized under the Ototretinae, and the ototretine lineage was transferred from Elateriformia *incertae sedis* to Lampyridae. In addition, the genus *Stenocladius* was transferred from Elateriformia
incertae sedis to Ototretinae. Our analyses corroborate these transfers, however, as our taxon sample included only two of eighteen genera from the Ototretinae, *Stenocladius* and *Drilaster*, a wider generic sampling is needed to address the phylogenetic history of this lineage.

Two other genera, *Anadrilus* Kirsch, 1875 and *Pachytarsus* Motschulsky, 1861, were both excluded from the Ototretinae by Janisova and Bocakova (2013) on the basis of lack of type material to examine. As we were also unable to sample these genera, they remain Lampyridae incertae sedis. Of note, when he described *Pachytarsus*, Motschulsky (1861) was apparently unaware of the true bug genus by the same name, having been described the year before: *Pachytarsus* Fieber, 1860 (for current usage see Ballal et al. 2018). Due to the name *Pachytarsus* being preoccupied, we propose the replacement name *Crassitarsus* Martin.

*Crassitarsus* Martin

**NEW REPLACEMENT NAME**

*Pachytarsus* Motschulsky, 1861

Included species: *Crassitarsus basalis* (Motschulsky, 1861) **NEW COMBINATION,**

*Crassitarsus* bicolor (Pic, 1929) **NEW COMBINATION,** *Crassitarsus* bryanti (Wittmer, 1940) **NEW COMBINATION,**

*Crassitarsus* lateralis (Motschulsky, 1861) **NEW COMBINATION,**

*Crassitarsus* longicornis (Pic, 1921) **NEW COMBINATION,** *Crassitarsus* minutus (Pic, 1933) **NEW COMBINATION,**

*Crassitarsus* obscurus (Pic, 1927) **NEW COMBINATION,**

*Crassitarsus* testaceus (Motschulsky, 1861) **NEW COMBINATION.**

Etymology: *Crassus*, a synonym for *pachy*, both meaning thick, seems an apt replacement to conserve the original thoughts of Motschulsky.
Lamprohizinae Kazantsev, 2010 stat. nov.

There is strong support for a Lamprohiza + Phausis clade in all our topologies. This is supported by a high degree of morphological similarity between these two genera (Fig. 2). *Phausis* was erected for *Lampyris reticulata* Say by LeConte (1852) and *Lamprohiza* was erected in 1853 by Motschulsky for *Lampyris splendidula* Linnaeus. *Lamprohiza* was later synonymized with *Phausis* by Lacordaire in 1857. In contrast, Mulsant, 1862 treated *Lamprohiza* as an independent genus, but in 1881 LeConte wrote “[*Phausis*] is not sufficiently distinct from the European *Lamprohiza*, and in fact the European species seems to have been naturalized in Maryland and Illinois” and included *L. splendidula* within *Phausis*. This classification was accepted until 1964 when McDermott separated *Lamprohiza* from *Phausis* by the “minute appendage on the 11th antennal article” of the latter. However, Fender (1966) treated the two genera as one in his treatment on the “*Phausis*” of North America, while Miksic (1969) followed McDermott in treating them as separate genera. From a phylogenetic perspective, Stanger-Hall et al. (2007) found *Phausis* as sister to Photurinae + Lampyrinae, similar to our results. In 2008, Jeng found support for these genera as members of Lampyrinae, however, he noted that they differed from the traditional Lampyrinae in the “unmodified mandibles *sensu* Green (1949), dorsal abdominal spiracles, and a symmetrical aedeagal sheath.” Our analyses, based on the type species of each genus, strongly support the rank of subfamily and we herein elevate Lamprohizini Kazantsev, 2010 to Lamprohizinae Kazantsev, 2010 stat. nov.

Diagnosis

The Lamprohizinae are distinguished from all other subfamilies with the following combination of characters, based on adult males: mandibles unmodified (i.e. not reduced in size);
antennae filiform, 11-segmented, with or without terminal sensorium, if without then posterior margin of ventrite 7 with weak to strong medial projection, projection emarginate at midline; tarsal claws simple, not bifid; abdomen with seven–eight ventrites; abdominal spiracles dorsal; aedeagal sheath symmetrical.

Key to the genera of Lamprohizinae

Terminal antennomere with small, beadlike sensorium; abdominal ventrite 7 unmodified; ventrite 8 apparent .................................................................Phausis

Terminal antennomere without small, beadlike sensorium; abdominal ventrite 7 with weak to strong medial projection; ventrite 8 not apparent .....................................................Lamprohiza

Amydetinae

McDermott (1964) recognized the Amydetini as a tribe within the Lampyrinae based on the mandibles being “normal, arcuate, regularly narrowing to tips,” antennae bearing rami or branches, and lack of secondary elytral pubescence of the males. To further classify the rather heterogenous group of genera included in the Amydetini, McDermott (1964) recognized three subtribes based on variation in antennal morphology: Amydetina (“Antennae with more than 14 articles, uniramose”), Vestina (“Antennae with 11 articles, Antennae uniramose, rami remiform, relatively broad, folding like a fan”), and Psilocladina (“Antennae with 11 articles, Antennae bi- or uniramose, rami either long and diffuse, or if short and straight, not fan-folding”). In his 1966 update to the classification, McDermott elevated the Amydetini to the subfamily level. Nakane (1991) reduced the Amydetinae to Amydetes Hoffmannsegg in Illiger and Magnoculus McDermott, however Silveira and Mermudes (2013) described the new genus Memoan Silveira
and Mermudes and, based on it having features of both the Amydetinae and the Lampyrinae, placed it in Lampyridae incertae sedis.

Due to the phylogenetic position of Memoan as sister to Amydetes, and the morphological features shared by Memoan with other taxa within the Amydetinae (as identified by Silveira and Mermudes, 2013), including a “continuous glow, pleuralventral suture, ventral approximate eyes, deep punctures on pronotum and scutellum, and absence of tibial spurs,” we formally transfer Memoan to the Amydetinae, thus the Amydetinae now include Amydetes, Magnoculus, and Memoan.

Cheguevariinae

Kazantsev (2006) described two enigmatic species in the genus Cheguevaria Kazantsev, 2006, one from Puerto Rico, and one from Dominican Republic. In the description, he remarks on the similarity of Cheguevaria to the Phengodidae, however, he does note the differences, and allies Cheguevaria with the Lampyridae based on the structure of the genital capsule. While he suspected that the genus represented a unique lampyrid subfamily, given the peculiar morphology of the genus, as well as the lack of phylogenetic stability at the time, Kazantsev placed the Cheguevariini as Lampyridae incertae sedis (Kazantsev, 2006). Recent phylogenetic analyses have corroborated the placement of Cheguevaria within the Lampyridae and, confirming the suspicion of Kazantsev, elevated the tribe to subfamilial status (Ferreira, et al. 2019). We were unable to sample the Cheguevariinae for the present analyses.
Photurinae and Lampyrinae

All photurine genera in our analyses (we were unable to sample Presbyolampis Buck, 1947) were recovered in a monophyletic lineage with the old-world Vesta. Known only from the New World, the Photurinae are typically characterized by bifurcate claws in males, and eyes expanding beyond the hypomeron. Nakane (1991) transferred the members of the Vestina (Vesta, Cladodes, Ledocas Olivier, Dodacles Olivier, and Dryptelytra Laporte) to the Lampyrinae, and moved Psilocladus to the Cyphonocerinae (see above) and Ethra to the Lampyrinae; he did not mention Scissicauda or Photoctus. Our analyses agree with Nakane (1991) in the placement of Cladodes and Ethra, however, the old-world Vesta sampled for our analyses formed a monophyletic lineage within the Photurinae, a new-world clade. Vesta is a speciose genus with representatives in both the Old and New World. McDermott (1964) recognized that Vesta might not be a natural group. In his 1966 catalogue he continued to treat the new-world taxa as potentially independent from the old-world taxa. As we were unable to sample the new-world Vesta, or the other members of the Vestini, our analyses are inconclusive with regard to the subfamilial placement of the Vesta. Therefore, we transfer Vesta and the other members of the Vestini (Dodacles, Dryptelytra, and Ledocas) to Lampyridae incertae sedis pending further morphological and phylogenetic analyses.

The Lampyrinae are a diverse assemblage of genera lacking any stable morphological characters (McDermott 1964; Jeng 2008). It is in part due to this that we believe so many “lampyrine” genera are/have been mis-classified. Based on our present phylogenetic analyses, we transfer the genus Scissicauda from Amydetinae into Lampyrinae. Since neither Photoctus nor Araucariocladus were included in the present analyses we transfer these to Lampyridae incertae sedis, pending further phylogenetic analyses.
CONCLUSIONS

With 88 lampyrid taxa in 53 genera for seven of the eight previously recognized subfamilies, these analyses represent the largest molecular phylogeny of the group, thereby providing a unique opportunity to robustly test the monophyly of each subfamily. The results of our four phylogenetic reconstructions were largely congruent, with the only major difference concerning the placement of the Ototretinae, which were under-sampled in this study. The inclusion of additional ototretine taxa will likely aid the resolution of their placement. Aside from the monotypic Pterotinae, among the subfamilies in the current analyses, only the Ototretinae (as currently defined) were reconstructed as monophyletic (T1). As a result, we propose several changes to the higher-level classification of Lampyridae, with an updated higher-level classification scheme for the family. Pollaclasis, Vesta (as well as the other constituents of the Vestini: Dodacles, Dryptelytra, and, Ledocas), Photoctus, and Araucariocladius are transferred to Lampyridae incertae sedis. Psilocladinae is re-established for Psilocladus. Amydetinae is limited to Amydetes, Magnoculus, and Memoan with Scissicauda being transferred to the Lampyrinae. Lamprohizini is elevated to the rank of subfamily, Phausis is transferred to Lamprohizinae. This comprehensive classification of firefly lineages will provide the phylogenetic scaffold in support of future studies into the evolution of the fascinating morphology and behavior of fireflies.

List of Genera

Subfamily Luciolinae Lacordaire, 1857

*Abscondita* Ballantyne, Lambkin, & Fu in Ballantyne et al., 2013

*Luciola terminalis* Olivier, 1883: 330
Aquatica Fu, Ballantyne, and Lambkin, 2010

Aquatica wuhana Fu, Ballantyne, and Lambkin, 2010: 8

Aquilonia Ballantyne in Ballantyne and Lambkin, 2009

Luciola costata Lea, 1921: 66

Asymmetricata Ballantyne in Ballantyne and Lambkin, 2009

Luciola circumdata Motschulsky, 1854b: 50

Atyphella Olliff, 1890

Atyphella lychnus Olliff, 1890: 647

Australoluciola Ballantyne in Ballantyne and Lambkin, 2013

Lampyris australis Fabricius, 1775: 201

Colophotia Motschulsky, 1853

Lampyris praeusta Eschscholtz, 1822: 57

Convexa Ballantyne in Ballantyne and Lambkin, 2009

Luciola wolfi Olivier, 1910b: 343

Curtos Motschulsky, 1845

Curtos mongolicus Motschulsky, 1845: 36

Emarginata Ballantyne in Ballantyne et al., 2019

Luciola trilucida Jeng and Lai in Jeng et al., 2003: 248

Emeia Fu, Ballantyne and Lambkin, 2012

Curtos pseudoauteri Geisthardt, 2004: 1

Inflata Boontop in Ballantyne et al., 2015

Luciola indica Motschulsky, 1854b: 53

Kuantana Ballantyne in Ballantyne et al., 2019
Kuantana menayah Ballantyne in Ballantyne et al., 2019: 82

Lampyroidea Costa 1875

Lampyroidea syriaca Costa 1875: CLXIX

Lloydiiella Ballantyne in Ballantyne and Lambkin, 2009

Luciola majuscula Lea, 1915: 495

Luciola Laporte, 1833

Luciola pedemontana Motschulsky, 1853: 53

Magnalata Ballantyne in Ballantyne and Lambkin, 2009

Luciola limbata Blanchard, 1853: 73

Medeopteryx Ballantyne in Ballantyne and Lambkin, 2013

Pteroptyx effulgens Ballantyne, 1987b: 141

Missimia Ballantyne in Ballantyne and Lambkin, 2009

Missimia flavida Ballantyne in Ballantyne and Lambkin, 2009

Pacifica Ballantyne in Ballantyne and Lambkin, 2013

Atyphella salomonis Olivier, 1911c: 172

Photuroluciola Pic, 1931

Photuroluciola deplanata Pic, 1931: 12

Pteroptyx Olivier, 1902

Luciola testacea Motschulsky, 1854b: 48

Pygatyphella Ballantyne, 1968

Atyphella obsoleta Olivier, 1911c: 174

Pygoluciola Wittmer, 1939

Pygoluciola stylifer Wittmer, 1939: 22
**Pyrophanes** Olivier, 1885

*Pyrophanes similis* Olivier, 1885: 370

**Sclerotia** Ballantyne *in* Ballantyne et al., 2016

*Luciola aquatilis* Thancharoen *in* Thancharoen et al., 2007: 56

**Serratia** Ballantyne *in* Ballantyne et al., 2019

*Serratia sibuyania* Ballantyne *in* Ballantyne et al., 2019: 147

**Triangulara** Pimpa Salee *in* Ballantyne et al., 2016

*Triangulara frontoflava* Pimpa Salee *in* Ballantyne et al., 2016: 242

**Trisinuata** Ballantyne *in* Ballantyne and Lambkin, 2013

*Trisinuata caudabifurca* Ballantyne *in* Ballantyne and Lambkin, 2013: 117

**Tribe Pristolycini Kazantsev, 2010**

**Pristolycus** Gorham, 1883

*Pristolycus sagulatus* Gorham, 1883: 407

**Subfamily Pterotinae LeConte, 1861**

**Pterotus** LeConte, 1859

*Pterotus obscuripennis* LeConte, 1859: 86

**Subfamily Ototretinae McDermott, 1964**

**Baolacus** Pic, 1915

*Baolacus lajoyei* Pic, 1915: 21

**Brachylampis** Van Dyke, 1939

*Brachylampis sanguinicollis* Van Dyke, 1939: 16

**Brachypterodrilus** Pic, 1918
*Brachypterodrilus pallidipes* Pic, 1918: 1

*Ceylanidrilus* Pic, 1911

*Ceylanidrilus bipartitus* Pic, 1911a: 187

*Drilaster* Kiesenwetter, 1879

*Drilaster axillaris* Kiesenwetter, 1879: 311

*Emasia* Bocáková and Janišová, 2010

*Emasia dentata* Bocáková and Janišová, 2010: 61

*Eugeusis* Westwood, 1853

*Eugeusis palpator* Westwood, 1853: 239

*Falsophaeopterus* Pic, 1911

*Falsophaeopterus fruhstorferi* Pic, 1911a: 187

*Flabellopalpodes* Bocakova and Bocak, 2016

*Flabellopalpodes flavus* Bocakova and Bocak, 2016: 372

*Flabellototreta* Pic, 1911

*Flabellototreta fruhstorferi* Pic, 1911b: 156

*Gorhamia* Pic, 1911

*Gorhamia compressicornis* Pic, 1911a: 187

*Harmatelia* Walker, 1858

*Harmatelia bilinea* Walker, 1858: 281

*Hydaspoides* Janišová and Bocáková, 2013

*Hydaspoides kanarensis* Janisova and Bocakova, 2013: 7

*Hyperstoma* Wittmer, 1979

*Hyperstoma marginata* Wittmer, 1979: 86
Lamellipalpodes Maulik, 1921

Lamellipalpodes annandalei Maulik, 1921: 584

Lamellipalpus Maulik, 1921

Eugeusis nigripennis Pascoe, 1887: 10

Ototretadrilus Pic, 1921

Ototretadrilus atritarsis Pic, 1921: 13

Picodrilus Wittmer, 1938

Ototreta drescheri Pic, 1937: 138

Stenocladius Fairmaire in Deyrolle and Fairmaire, 1878

Stenocladius davidis Fairmaire in Deyrolle and Fairmaire, 1878: 113

Subfamily Lamprohizinae Kazantsev, 2010

Phausis LeConte, 1852

Lampyris reticulata Say, 1825: 163

Lamprohiza Motschulsky, 1853

Lampyris splendidula Linnaeus, 1767: 644

Subfamily Cyphonocerinae Crowson, 1972

Cyphonocerus Kiesenwetter, 1879

Cyphonocerus ruficollis Kiesenwetter, 1879: 312

Subfamily Psilocladinae McDermott, 1964

Psilocladus Blanchard, 1846

Psilocladus miltoderus Blanchard, 1846: 122

Subfamily Amydetinae Olivier in Wytsman, 1907

Tribe Amydetini Olivier in Wytsman, 1907
*Amydetes* Hoffmannsegg *in* Illiger, 1807

*Homalisus apicalis* Germar, 1824: 67

*Magnoculus* McDermott, 1964

*Megalophthalmus bennetti* Gray, 1832: 371

*Memoan* Silveira and Mermudes, 2013

*Memoan ciceroi* Silveira and Mermudes, 2013: 84

**Subfamily Cheguevariinae Kazantsev, 2006**

**Tribe Cheguevariini Kazantsev, 2006**

*Cheguevaria* Kazantsev, 2006

*Cheguevaria taino* Kazantsev, 2006: 370

**Subfamily Photurinae Lacordaire, 1857**

*Bicellonycha* Motschulsky, 1853

*Bicellonycha deleta* Motschulsky, 1854b: 58

*Photuris* Dejean, 1833

*Lampyris versicolor* Fabricius, 1798: 123

*Presbyolampis* Buck, 1947

*Presbyolampis immigrans* Buck, 1947: 75

*Pyrogaster* Motschulsky, 1853

*Pyrogaster grylloides* Motschulsky, 1853: 53

**Subfamily Lampyrinae Rafinesque, 1815**

**Tribe Cratomorphini Green, 1948**

*Aspisoma* Laporte, 1833

*Lampyris ignita* Linnaeus, 1767: 645
Aspisomoides Zaragoza-Caballero, 1995

Aspidosma bilineatum Gorham, 1880b: 86

Cassidomorphus Motschulsky, 1853

Cassidomorphus silphoides Motschulsky, 1853: 36

Cratomorphus Motschulsky, 1853

Photinus fabricii Laporte, 1840: 268

Micronaspis Green, 1948

Micronaspis floridana Green, 1948: 63

Paracratomorphus Zaragoza-Caballero, 2013

Paracratomorphus reyesi Zaragoza-Caballero, 2013: 145

Pyractomena Melsheimer, 1846

Pyractomena lucifera Melsheimer, 1846: 304

Tribe Lamprocerini Olivier, 1907

Alecton Laporte, 1833

Alecton discoidalis Laporte, 1833: 135

Lamprocera Laporte, 1833

Homalisus grandis Sturm, 1826: 58

Lucernuta Laporte,1833

Lampyris fenestrata Germar, 1824: 66

Lucio Laporte, 1833

Lucio abdominale Laporte, 1833: 136

Lychnacris Motschulsky, 1853

Lychnacris triguttula Motschulsky, 1853: 33
Tenaspis LeConte, 1881

Hyas angularis Gorham, 1880: 7

Tribe Lampyrini Rafinesque, 1815

Afrodiaphanes Geisthardt, 2007

Lampyris marginipennis Boheman, 1851: 439

Diaphanes Motschulsky, 1853

Lampyris luniger Motschulsky, 1853: 45

Lampyris Geoffroy, 1762

Lampyris noctiluca Linnaeus, 1767: 643

Lychnobius Geisthardt, 1983

Lampyris conspicua Gyllenhal in Schönherr, 1817: 20

Microlampyris Pic, 1956

Microlampyris basilewski Pic, 1956: 193

Microphotus LeConte, 1866

Microphotus dilatatus LeConte, 1866: 90

Nelsonphotus Cicero, 2006

Nelsonphotus aridus Cicero, 2006: 201

Nyctophila Olivier, 1884

Lamprotomus caucasica Motschulsky, 1854a: 19

Ovalampis Fairmaire, 1898

Ovalampis crissipacollis Fairmaire, 1898: 404

Paraphausis Green, 1949

Paraphausis eximia Green, 1949: 4
Pelania Mulsant, 1860

Lampyris mauritanica Linnaeus, 1767: 645

Petalacmis Olivier, 1908

Petalacmis praeclarus Olivier, 1908: 187

Prolutacea Cicero, 2006

Lampyris pulsator Cicero, 1984: 322

Pyrocoelia Gorham, 1880

Lampyris bicolor Fabricius, 1801: 100

Tribe Photinini LeConte, 1881

Ankonophallus Zaragoza-Caballero and Navarrete-Heredia, 2014

Ankonophallus zuninoi Zaragoza-Caballero and Navarrete-Heredia, 2014: 126

Aorphallus Zaragoza-Caballero and Gutierrez-Carranza, 2018

Aorphallus cibriani Zaragoza-Caballero and Gutierrez-Carranza, 2018: 160

Callopisma Motschulsky, 1853

Lampyris rufa G.A. Olivier, 1790: 28

Calotrechelum Pic, 1930

Calotrochelum olivieri Pic, 1930: 88

Dadophora Olivier in Wytsman, 1907

Dadophora hyalina Olivier in Wytsman, 1907: 27

Dilychnia Motschulsky, 1853

Dilychnia basalis Motschulsky, 1853: 30
Ellychnia Blanchard, 1845

Lampyris corrusca Linnaeus, 1767: 644

Erythrolychnia Motschulsky, 1853

Erythrolychnia dimidiatipennis Motschulsky, 1853: 29

Heterophotinus Olivier, 1894

Photinus limbipennis Du Val, 1857: 86

Jamphotus Barber, 1941

Jamphotus tuberculatus Barber, 1941: 11

Lucidina Gorham, 1883

Lucidina accensa Gorham, 1883: 408

Lucidota Laporte, 1833

Lucidota banoni Laporte, 1833: 137

Lucidotopsis McDermott, 1960

Lucidota cruenticollis Fairmaire, 1889: 38

Luciuranus Silveira, Khattar, and Mermudes in Silveira et al., 2016

Luciuranus josephi Silveira, Khattar, and Mermudes in Silveira et al.,

2016 377

Macrolampis Motschulsky, 1853

Macrolampis longipennis Motschulsky, 1853: 37

Microdiphot Barber, 1941

Microdiphot cavernarum Barber, 1941: 13

Mimophotinus Pic, 1935

Mimophotinus angustatus Pic, 1935: 9
Oliviereus Pic, 1930

Oliviereus flavus Pic, 1930: 88

Phosphaenopterus Schauffuss, 1870

Phosphaenopterus metzneri Schauffuss, 1870: 61

Phosphaenus Laporte, 1833

Lampyris hemiptera Geoffroy in Fourcroy, 1785: 58

Photinoides McDermott, 1963

Photinoides penai McDermott, 1963: 87

Photinus Laporte, 1833

Lampyris pallens Fabricius, 1798: 124

Platylampis Motschulsky, 1853

Platylampis latiuscula Motschulsky, 1853: 44

Pseudolychnuris Motschulsky, 1853

Pseudolychnuris vittata Motschulsky, 1853: 32

Pyractonema Solier in Gay, 1849

Pyractonema compressicornis Solier in Gay, 1849: 446

Pyropyga Motschulsky, 1852

Lampyris nigricans Say, 1823: 179

Pyropygodes Zaragoza-Caballero, 2000

Pyropygodes huautlae Zaragoza-Caballero, 2000: 20

Robopus Motschulsky, 1853

Robopus roseicollis Motschulsky, 1853: 42

Rufolychnia Kazantsev, 2006
Callopisma boreconca Leng and Mutchler, 1922: 440

*Uanauna* Campello-Gonçalves, Souto, Mermudes, and Silviera, 2019

*Uanauna angaporan* Campello-Gonçalves, Souto, Mermudes, and Silviera, 2019: 67

*Ybytyramoan* Silveira and Mermudes, 2014

*Ybytyramoan praeclarum* Silveira and Mermudes, 2014: 328

**Tribe Pleotomini Summers, 1875**

*Calyptocephalus* Gray in Griffith and Pidgeon, 1832

*Calyptocephalus fasciatus* Gray in Griffith and Pidgeon, 1832: 70

*Ophoelis* Olivier, 1911

*Ophoelis impura* Olivier, 1911: 48

*Phaenolis* Gorham, 1880

*Phaenolis laciniatus* Gorham, 1880: 10

*Pleotomodes* Green, 1948

*Pleotomodes needhami* Green, 1948: 65

*Pleotomus* LeConte, 1861

*Pleotomus pallens* LeConte, 1866: 88

*Roleta* McDermott, 1962

*Roleta coracina* McDermott, 1962: 69

**Lampyrinae incertae sedis**

*Cladodes* Solier in Gay, 1849

*Cladodes flabellatus* Solier in Gay, 1849: 445

*Ethra* Laporte, 1833
Cladophorus marginatus Gray in Griffith and Pidgeon, 1832: 371

Scissicauda McDermott, 1964

Lucidota disjuncta Olivier, 1896: 1

Lampyridae incertae sedis

Anadrilus Kirsch, 1875

Anadrilus indus Kirsch, 1875: 37

Araucariocladus Silveira and Mermudes, 2017

Araucariocladus hiems Silveira and Mermudes, 2017: 209

Crassitarsus Martin, nom. nov.

Pachytarsus basalis Motschulsky, 1861: 135

Lamprigera Motschulsky, 1853

Lamprigera boyei Motschulsky, 1853: 48

Oculogryphus Jeng, Engel, and Yang, 2007

Oculogryphus fulvus Jeng in Jeng, Engel, and Yang, 2007: 7

Photoctus McDermott, 1961

Photoctus boliviae McDermott, 1961: 174

Pollaclasis Newman, 1838

Pollaclasis ovatus Newman, 1838: 385

Subtribe Vestini McDermott, 1964

Dodacles Olivier, 1885

Dodacles elegans Olivier, 1885: 141

Dryptelytra Laporte, 1833

Dryptelytra cayennenis Laporte, 1833: 139
Ledocas Olivier, 1885

Ledocas parallelus Olivier, 1885: 140

Vesta Laporte, 1833

Vesta chevrolati Laporte, 1833:133
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CHAPTER 1 TABLES

Table 1. Comparison of major classification schemes in Lampyridae.

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Table 2. Taxa included in current study.

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Fig. 1. Maximum Likelihood reconstruction of full, untrimmed dataset aligned in MAFFT; log-likelihood = -26321164.240. Numbers at nodes indicate UFBoot support. Unlabelled nodes have UFBoot values of 100%, colors represent the newly proposed classification. Star at node depicts Lampyridae; A.-I. Dorsal habitus of genus representatives: A. Lamprigera yunnana; B. Luciola cyathigera; C. Stenocladius davidii; D. Amydetes fastigiata; E. Vesta chevrolati; F. Diaphanes sp.; G. Aspisoma gentile; H. Ellychnia aurora; I. Photinus laticollis;
Fig. 2. Lamprohizinae habitus images; A: *Phausis reticulata* dorsal; B: *Phausis reticulata* ventral; C: *Lamprohiza delarouzei* dorsal; D: *Lamprohiza delarouzei* ventral.
Chapter 2: Lampyrid-ID: An interactive LUCID based electronic identification key to the genera of the Lampyridae (Coleoptera) of the world

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Keywords: bioluminescence, fireflies

ABSTRACT

Fireflies (Coleoptera: Lampyridae) are a cosmopolitan group of beetles with a troubled taxonomic history and diverse morphology. Because of this identification resources are lacking. Here, an electronic Lucid-based identification tool is presented for the genera of Lampyridae (Coleoptera: Elateroidea). General features used in the key are discussed.
INTRODUCTION

Fireflies (Coleoptera: Lampyridae) are a cosmopolitan group of beetles (Branham 2010) that are perhaps most well known for their bioluminescence as adults for most species and larvae for all species (Lloyd 1971, Lewis and Cratsley 2008). This bioluminescence is produced by specialized photic organs normally located on the ventral surface of the abdomen in adults.

While many species of firefly are bioluminescent, there are numerous genera that have no adult light organ. Those that are bioluminescent as adults have a variety of photic organ morphologies (Branham and Wenzel 2001, 2003). These include light organs that take up entire ventrites and others that appear as isolated stripes across a single ventrite or as various arrangements of spots (Fig. 1). Fireflies also possess highly diverse antennal morphology. This diversity in morphology occurs not only between species but also between genders. For this reason, several species of firefly are known only from males or females (Barber 1941). While a few identification keys exist for geographical regions (Ballantyne et al. 2019) or for specific tribes (e.g. Silveira et al. 2019), there is no comprehensive resource available for identifying fireflies to genus. This may be a contributing factor to the rampant misclassification of the group (Martin et al. 2019). With current genomic tools, large sets of data are becoming available to elucidate the evolutionary relationships of life on Earth. However, taxonomic expertise in general are becoming more and more rare. This electronic resource will help stem this trend in the fireflies.
MATERIALS AND METHODS

Project Description

This identification key is based on the males of the type species (where possible) for each genus and is focused on including as many genera as possible. Several types are either destroyed or reside in unknown locations. For these reasons, we were unable to include several genera at this time.

General Features

The data matrix for this identification key is comprised of 23 morphological and distributional characters with a combined 76 character states. Most characters coded represent external morphological features that do not require dissection or extensive preparation. These characters were derived and/or modified from existing literature (i.e. Olivier 1907, McDermott 1966, Jeng 2008). When possible, scorings were confirmed by examination of type and other material, comprised of several specimens for each genus with an emphasis on the type species. Specimens were examined from several museums: California Academy of Sciences (CAS), Sacramento, CA, USA; Florida State Collection of Arthropods (FSCA), Gainesville, FL, USA; National Museum of Natural History (USNM), Washington D.C., USA; Australian National Insect Collection (ANIC), Canberra, Australia; Muséum National d'Histoire Naturelle (MNHN), Paris, France; The Natural History Museum (NHM), London, United Kingdom; Naturhistorisches Museum (NHMB), Basel, Switzerland; and Coleção Entomológica Prof. José Alfredo Pinheiro Dutra (DZRJ), Rio de Janeiro, Brazil.

List of characters used in key

- Geographic distribution;
- Number of ventrites (six/seven/eight);
**Antennal features:**

Antenna (filiform/serrate/uniflabellate/biflabellate/highly modified, terminal antennomere elongate, longer than all preceding antennomeres);
Sensorum on apical antennomere (absent/present);
Relative length of scape/antennomere 1 (longer than pedicel (antennomere 2)/shorter than pedicel (antennomere 2));
Number of antennomeres (fewer than 11/11/12/17 to 18/more than 22);

**Head features:**

Eye separation in ventral aspect (widely separated (interocular distance wider than width of eye)/moderately separated but not touching (interocular distance narrower than width of eye)/narrowly separated to contiguous);
Mandibles (large and arcuate/moderately sized and arcuate/small and needle-shaped/highly reduced/not visible);
Number of labial palpomeres (4/3/2/1);

**Thoracic features:**

Anterior margin of pronotum (flat, not reflex/weakly reflexed/strongly reflexed);
Prothoracic trochantin (glabrous/setose);

**Leg features:**

Hindleg tibial spurs (absent/2 small spurs (barely longer than surrounding setae)/2 large spurs);
Tarsal pad (absent/present on tarsomere 4);
Hindleg claws (apically entire/both claws bifid apically/one claw bifid apically);
Metafemoral comb (absent/present);
**Elytral features:**

Elytral costae (absent/present);

Apex of elytra (more or less independently rounded/somewhat acute/with ventral back-folds);

**Abdominal features:**

Ventrete 6, if last ventrite, median posterior projection (absent/prolonged into paired, asymmetric hooks/present);

Ventrete 7, posterior pointed projection (absent/present);

Abdominal spiracles (ventral/dorsal).

**Light organ features:**

Light organ on abdominal ventrite 5 (absent/fully occupying ventrite 5/a transverse stripe/a large, transversely elliptical central spot/a small round spot);

Light organ on abdominal ventrite 6 (absent/occupying full or nearly full ventrite/bipartate/restricted to anterior portion of ventrite/confined to center of ventrite/two small round spots/);

Light organ on abdominal ventrite 7 (absent/present, two small lateral spots);

**Software technical specifications**

Application: Lucid™ Builder 3.3 ([www.lucidcentral.org](http://www.lucidcentral.org))

Key version: 1.0

Requirements for use: Java-enabled browser and internet connectivity

License for use of the key: Creative Commons Attribution License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided original author and source are credited
Web location: https://keys.lucidcentral.org/keys/v3/lampyridae/
CONCLUSIONS

Fireflies have a long and convoluted taxonomic history. Many genera were described inadequately in the late 1800’s and early 1900’s (Olivier 1907). This has made understanding generic limits and identification of Lampyridae difficult. This identification key, based primarily on the type species for each genus, should facilitate the identification of specimens to genus and advance future studies of the family Lampyridae. Future taxonomic efforts will surely describe currently unknown biodiversity as well as further refine our concepts of taxonomic groups within the family Lampyridae. It is our hope that this identification key to genera will be expanded and refined to integrate such contributions. Several known genera have yet to be included in this identification key or are coded from species other than the type species. This is either due to the type species/specimens being lost (e.g. *Anadrilus*), or extremely rare (e.g. *Cyphonocerus*). Future editions of this identification key will include as many of these genera as possible, while also working to improve the morphological character coding used to support identification.
ACKNOWLEDGEMENTS

We greatly appreciate the curators and collection managers who provided specimens critical to this study: Cate Lemann (ANIC), Chris Grinter (CAS), Paul Skelley and Kyle Schnepf (FSCA), Azadeh Taghavian (MNHN), Max Barclay and Michael Geiser (NHM), Isabelle Zuercher (NHMB), and Floyd Shockley (USNM). We also thank Gareth Powell, Robert Erickson, and Kristin Dunn for assistance on collection visits. We thank Oliver Keller, Natalie Saxton and the Bybee Lab group for preliminary testing of identification resources. This work was funded by NSF: DEB-1655981 (SMB), DEB-1655936 (MAB), and DEB-1655908 (awarded to Kathrin Stanger-Hall in support of L.F.L.D.S.).
LITERATURE CITED


reclassification of Lampyridae (Coleoptera: Elateroidea). Insect Systematics and Diversity 3(6): ixz024.


Table 1: Genera and species used for coding of key-matrix. Type species in **bold**.

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Figure 1: Ventral habitus of various species showing diversity of photic organ and antennal morphology. A. *Magnalata rennelia*; B. *Pyractomena vexillaria*; C. *Luciola amplipennis*; D. *Photuris excavaticeps*; E. *Dilychnia disparilis*; F. *Cladodes illigeri*; G. *Lucidota aurantiaca*; H. *Vesta rustica*; I. *Psilocladus pulcher*; J. *Amydetes fastigiata*
Chapter 3: The genes of the phototransduction pathway among fireflies (Coleoptera: Lampyridae)

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Keywords: Arrestin-2, inaC, positive selection, vision, sensory niche

ABSTRACT

Background:

Most organisms are dependent on sensory cues from their environment for survival and reproduction. Fireflies (Coleoptera: Lampyridae) represent an ideal system for studying sensory niche adaptation due to many species relying on bioluminescent communication, as well as a diversity of ecology. Here, using transcriptomics, we examine the phototransduction pathway in this non-model organism, and provide some of the first evidence for positive selection in the PT pathway beyond opsins in beetles.

Results:
Gene duplications are found in calmodulin, inactivation no afterpotential C, inactivation no afterpotential D, and transient receptor potential. We also find strong support for positive selection in arrestin-2, inactivation no afterpotential D, and transient receptor potential, with weak support for positive selection in guanine nucleotide-binding protein G(q) subunit alpha and neither inactivation nor afterpotential C.

Conclusions:

Our results show positive selection in certain genes in diurnal fireflies. This is one of the first times positive selection is investigated in the beetle phototransduction pathway. Taken with other recent work in flies, butterflies, and moths, this represents an exciting new avenue of study as we seek to further understand diversification and constraint on the PT pathway in light of organism ecology.
INTRODUCTION

Background

Organisms rely on sensory input from the environment to inform their basic ecology. Inputs occur across different channels, such as auditory (e.g. song), tactile (e.g. mechanosensory stimulation), gustatory (e.g. tastes), olfactory (e.g. pheromones, kairomones), and visual (e.g. bright colors, behavioral displays) channels, and are detected by different sensory structures and underlying molecular pathways. While many animals integrate input from several sensory channels depending on the circumstances, often a single channel dominates (e.g. vision in primates: [1], dragonflies and damselflies [2]), perhaps due to tradeoffs in investment in sensory structures [3, 4] and the interplay of selection and constraint on the underlying molecular players (e.g. [5]). The extent to which selection and constraint play a role in adaptation to an organism’s specific sensory niche, particularly with respect to the underlying molecular mechanisms, remains a fascinating question that has been investigated mostly by work in model systems. Modern sequencing technologies enable interrogation of these questions in non-model organisms, which present unique opportunities to study evolutionary forces in systems where evolutionary changes in primary sensory niche (i.e. nocturnal vs. diurnal) are known.

Fireflies (Coleoptera: Lampyridae) are an excellent system for studying sensory niche adaptation due to variation in their conspicuous bioluminescent mating displays. With over 2000 species distributed around the globe, variation in flash patterns across nocturnal taxa is diverse and serves in both species recognition and mate choice [6, 7]. While the proximate and ultimate reasons for signal diversity in light-using species have been studied for centuries [8–11], many species have lost the ability to produce light as adults. Instead of light, these day-active fireflies
rely on pheromone signals to locate, recognize, and choose mates [12–14]. In contrast, pheromones may be relatively unimportant for nocturnal light-using species [12]. The change from nocturnal, primarily-visual, light signal use to diurnal, primarily-pheromone, signal use, makes fireflies an ideal system for testing hypotheses about the role of selection in these evolutionary transitions in sensory niche.

Previous studies in fireflies highlight the interplay of diversification and constraint in both morphological and molecular adaptations to sensory niche. Nocturnal, light-using fireflies that rely on bioluminescence for communication have larger eyes and smaller antennae than their diurnal, pheromone-using relatives [4]. This inverse relationship between eye and antenna size across sensory niches may indicate developmental constraints that limit investment in antenna at the expense of eyes, and vice versa [15]. At the molecular level, previous research has demonstrated that contrary to the insect eye bauplan, fireflies along with most other beetles and their close relatives have only two opsin copies: one ultraviolet and one long-wavelength [16–18]. [17] found evidence for positive selection in the long-wavelength opsins among diurnal species, but not nocturnal. Additionally, certain fireflies have been shown to vary the expression levels of the long-wavelength opsin in correlation with peak bioluminescent signaling time [19]. However, opsins represent only one of many molecular players in the phototransduction pathway, begging the question - are changes in other members of the pathway associated with evolutionary transitions in sensory niche?

Vision in fireflies, like all insects, is regulated by the components of the phototransduction (PT) pathway (Figure 1). The phototransduction pathway consists of several
key genes and is essentially a seven to eight step process that starts with light activating the insect rhodopsin and is terminated by arrestin-1 and/or -2 binding to the rhodopsin (for a more complete review see [20]. While most of what is known about the insect PT pathway comes from work in the Dipteran fruit fly model organism, *Drosophila melanogaster*, and more recently the Lepidopeteran *Heliconius* [20] little is known about the pathway in other insect groups, with the exception of opsins [21–23]. Opsins have also been well studied in the fireflies [16, 17]. Further, exploring the full phototransduction pathway in fireflies provides one of the first studies of this pathway with respect to signal niche adaptation and potential for selection in insects.

With growing genomic tools for non-model organisms, including fireflies [17, 24], it is possible to examine the entire PT pathway and form hypotheses regarding the visual evolution of these organisms. Here, we search publicly available and newly-sequenced firefly transcriptomes (20 taxa in total) to identify the visual pathway genes and address the following objectives: 1) Identify genes involved in phototransduction in firefly transcriptomes; 2) Examine patterns of duplication and loss as compared to other model insects (i.e. *Drosophila* and *Tribolium*); and 3) Test hypotheses of selection related to sensory niche adaptation (nocturnal vs diurnal). The reliance on bioluminescent communication to find mates for many, but not all, adult fireflies provides an ideal and simple system to study the evolution of both the visual signal (adult bioluminescence) and receiver system (adult eyes).
MATERIALS AND METHODS

**Sampling and Sequencing**

Transcriptomes utilized in this study were taken from [16] and [17]. RNA was extracted from several body parts (i.e. head only, abdomen/light organ only, and whole body). As such, while there are 32 RNA-seq libraries in the study, these represent 20 taxa (Table 1, datadryad.org/stash/share/QRYn_7voIXFgtWFQH6E398eIkEfRW-ZicXx5dHjbWJg).

Both head and body tissue for transcriptome assembly was prepped separately for the following taxa: *Micronaspis floridana* Green, *Pyractomena dispersa* Green, *P. pyralis* Linnaeus (see [16]). Total RNA was extracted from each taxon using NucleoSpin columns (Clontech) and reverse-transcribed into cDNA libraries using the Illumina TruSeq RNA v2 sample preparation that both generates and amplifies full-length cDNAs. Prepped mRNA libraries were sequenced on an Illumina HiSeq 2000 utilizing 101-cycle paired-end reads by the Microarray and Genomic Analysis Core Facility at the Huntsman Cancer Institute at the University of Utah, Salt Lake City, UT, USA. The transcriptomic data allows us to combine current data, as well as gain a knowledge of what parts of the phototransduction pathway are actually expressed in the firefly eye.

**Transcriptome assembly and annotation**

Quality control, assembly, and transcriptome searches were performed using a pipeline constructed from existing computational tools after [60] to facilitate downstream evolutionary analyses. In short, RNA-seq reads were trimmed using the Mott algorithm implemented in PoPoolation version 1.2.2 [61], with a minimum read length=40 and quality threshold=20. The
de novo assembly of the transcriptome contigs was carried out using Trinity version 2.0.6 [62] under the default parameters. Assembled transcriptomes were then annotated using the trinotate release 2013-08-26 pipeline. Detection and filtering for putative phototransduction gene sequences from the Lampyridae transcriptomes were performed against a database using insect visual phototransduction homologous groups from ORTHODB database [63]. Recovered insect sequences from the PT pathway were then aligned using MAFFT v. 7.407 [64] and converted into a profile hidden Markov model (pHMM) database using hmmbuild program of HMMER 3.1b1 [65]. Using this database we screened TransDecoder-predicted proteomes for genes known from the PT pathway against pHMM database using hmmscan with an e-value cutoff of $10^{-5}$. Additionally, we used PIA [66] with default parameters to identify phototransduction genes that may be missed by the HMMER search using the raw Trinity transcriptome assemblies. All redundant (identical) phototransduction gene sequences identified by both approaches were removed using CDHIT [67, 68]. After all putative hits were compiled, sequences were screened for any false positives, including sequences coding for other genes, or duplicate reads not the result of biology, but rather read coverage. Sequences were aligned via MAFFT to remove sequences with >98% similarity. Each unique sequence was then Blast searched using megablast against the NCBI database to confirm gene identity.

**Analyses of positive selection**

Sequences for each gene were aligned independently in MAFFT using the default automatic alignment option. Each subsequent alignment was used for Maximum Likelihood tree inference implemented within IQ-Tree v. 1.6.8 [69]. Tests for positive selection in each gene were performed in PAML v. 4.9 [26]. Using the branch-specific test, the free-ratios model (Ha;
model=2, nsites=0) was tested against the branch model (Ho; model=0, nsites=0). The log likelihood of each model was compared with the likelihood ratio test (LRT) using $X^2$ distributions (3.84 at p-value=0.05) with appropriate degrees of freedom.

Inference of positive selection has been shown to be greatly influenced by the accuracy of MSA [28]. In particular the inference of selection obtained on MSAs produced by MAFFT shows overconfidence in identification of positive selection (i.e. increased rates of false positive results). In order to mitigate that problem we implemented a Bayesian approach utilized in BAli-Phy version 3.4.1 [27] that essentially jointly estimates MSAs and positive selection (in our case branch-specific) and exhibits superior accuracy [28]. To run analyses of branch positive selection in BAli-Phy, for each gene we used the corresponding tree estimated by ML initializing 3 independent MCMC chains with 3000 iterations each as in [28]. MSA was sampled every 5th iteration. The seemingly low number of iterations is explained by the fact that at each MCMC draw BAli-Phy updates multiple parameters compared to other MCMC software where only one parameter is updated per iteration. In order to improve our estimator of positive selection we used Rao-Blackwellization technique, i.e. taking a conditional expectation of the current estimator.

The results of 3 runs were pulled together discarding 15% burn-in for each gene. Then Bayes factors (BF) were calculated to assess support for positive selection. We followed a scoring scheme proposed by [70], where BF > 20 exhibits strong support for positive selection, 20 < BF < 3 exhibits “positive” support and BF < 3 is “not worth more than a bare mention”.

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RESULTS

Transcriptome assemblies recover most conserved genes:

To examine patterns of gene duplication and test for selection in the PT pathway across firefly species, we compiled transcriptome assemblies from published and new datasets. Transcriptomes varied in quality, generally according to input tissue type (Table 1). Transcriptome assemblies captured the majority of genes as full-length transcripts - mean BUSCO completeness across assemblies was 76% (range: 40–95%), the mean N50 was 2,096 bp, and the mean maximum contig length was 17,138 bp. Total number of contigs varied from 19,676 to 77,811. In general, transcriptomes derived from the whole body or the head region were longer and more complete than those derived from abdominal tissue only (Table 1).

Orthologous search strategy identifies duplications in phototransduction genes:

Several gene families in the PT pathway have been shown to contain duplication events within insects [25]. Genes identified in this dataset by orthologous search, followed by manual curation of results, included arrestin-1 (arr1), arrestin-2 (arr2), calmodulin (Cam), guanine nucleotide-binding protein G(q) subunit alpha (Gq), G protein-coupled receptor kinase 1 (Gprk1), inactivation no afterpotential C (inaC), inactivation no afterpotential D (inaD), inactivation no afterpotential E (inaE), neither inactivation nor afterpotential C (ninaC), no receptor potential A (norpA), transient receptor potential (trp), and transient receptor potential-like (trpl). Unfortunately, arr1 and trp were only recovered from three taxa, none of which were diurnal. Therefore we were unable to test the genes further. While not every gene was found in every species (likely due to the incompleteness of the data), putative duplications of various genes were found in several species: Photinus pyralis (Cam & inaC), Photinus australis (Cam),
Photinus macdermotti (inaC), Lucidota atra (inaC), Phausis reticulata (inaC), Bicellonycha wickershamorum (inaC), and Photinus carolinus (inaD); see Table 2. These putative duplicates were confirmed in the Photinus pyralis genome [24], however the putative duplicate of inaD within Photinus carolinus does not seem to be present in the P. pyralis genome, and therefore requires further confirmation.

**Analysis for positive selection identifies genes under positive selection:**

*PAML:* Tests for positive selection were performed in PAML [26] on two sets of trees resulting from two different approaches to alignment. First, PAML was run on the original MAFFT alignment. The branch model test (model=0, nsites=0) for the null hypothesis showed elevated omega values (average ω across all genes 0.076) for Gq (ω=0.114), inaD (ω=0.114), ninaC (ω=0.110), and trpl (ω=0.230). This analysis also resulted evidence for positive selection (lrt > 3.84 at p value = 0.05) in arr2 (lrt=43.59), inaC (lrt=64.35), inaD (lrt=28.04), ninaC (lrt=43.76), and trpl (lrt=16.07) when the alternative hypothesis (model=2, nsites=0) was selection in diurnal lineages. Second, PAML was run on the alignment resulting from BAli-Phy [25, 27] to account for potential overestimation of positive selection in the MAFFT alignment [28]. This analysis resulted in elevated omega values (average ω across all genes 0.046), inaD (ω=0.092), ninaC (ω=0.109), and trpl (ω=0.082). The PAML analysis of the BAli-Phy alignment also resulted in support for evidence of positive selection in arr2 (lrt=56.28), Gq (lrt=5.02), inaD (lrt=22.30), ninaC (lrt=45.10), and trpl (lrt=114.44); (Table 3).

Consistent between the two PAML results arr2, inaD, ninaC, and trpl were identified as possibly evolving under positive selection.
To further explore genes identified by PAML to be under positive selection, we also tested for genes under positive selection using BAli-Phy by examining the Bayes Factor (BF). The genes *arr2* (BF: 9.5), *Gq* (82.28), *inaD* (5.13), and *trpl* (8.59) were again identified as evolving under positive selection (Figure 2; Table 3).

Given these results, there is strong evidence in all three analyses for *arr2*, *inaD*, and *trpl* being under positive selection in diurnal lineages, with additional support in two analyses for positive selection in *ninaC* and *Gq* (Table 3).
DISCUSSION

The insect phototransduction cascade is most well-known and studied in the model insects *Drosophila* and *Heliconius* (reviewed in [29][20]). Here we increase the knowledge of this cascade system by exploring the PT pathway in fireflies. We further explore these data by looking for patterns of selection that are potentially related to the signaling ecology of nocturnal vs diurnal fireflies.

Duplication rates of phototransduction genes have been shown to be higher in arthropods and related groups as opposed to other metazoan organisms [25]. These duplications have been shown in several gene families such as TRP, r-opsin, and arrestin among others [20, 25]. Here we add to the known duplication events in insects by showing evidence for duplications in *Cam*, *inaC*, and *inaD*. What is especially interesting about these findings is the lack of *inaC* within the *Tribolium* genome [30]). While functionally similar paralogues where found in *Tribolium* (one of which is likely homologous to the *inaC* copy recovered in this study), there is quite a divergent biology between the cereal pest *Tribolium* and the fireflies. *InaC* was recovered in the phototransduction survey of the blind, cave-dwelling *Ptomaphagus hirtus* (Coleoptera:Leiodidae [31]). This suggests that the *inaC* gene was likely lost after the divergence of the Staphilinoidea with remaining polyphagan beetles [32]. Future research is needed to determine exactly when this loss occurred, and with which sensory niche the loss is associated. The duplication in the *Cam* gene was recovered in several firefly lineages and seems to be closely allied with a yet uncharacterized neo-calmodulin like gene.
In insect phototransduction, *inaD* acts a scaffold protein that is central to a group of proteins commonly known as the signalplex [33–37]. This signalplex is composed primarily of *inaD, trp, norpA*, and *inaC*, also found in one of our analyses to be under positive selection [29]. If *inaD* is lost or disrupted, it causes a downstream breakup of the signalplex [38]. Further, *inaD* stability is dependent upon *trp*, a Ca\(^{2+}\)-permeable cation within the cell [29, 39, 40]. Further upstream in the cascade *Gq* plays a role in the portion of the cascade that leads to the production of DAG and MAG [41] which are hypothesized to play a critical role in activation of *trp* and *trpl* [20, 42].

The interaction between *trp* and *trpl* in organisms which inhabit low-light vs. high-light environments has received much recent attention. It appears that one or the other can be up- or down-regulated depending on the dominant light environment. In *Drosophila melanogaster* (active primarily during the day) *trp* is not only the more abundant channel, but also flies act blindly with mutated *trp* [43]. In cockroaches (primarily nocturnal) the opposite was shown, *trpl* was the more abundant channel by far [44]. Recently, Macias-Munoz and colleagues. [20] have shown an interesting relationship within the Lepidoptera. The day active *Heliconius melpomene* showed no differentiation in *trp vs trpl* channels, however the night-active *Manduca sexta* showed down-regulation of *trp*, showing a similar pattern to that of the cockroaches [20]. Here, we show strong support for the hypothesis that *trpl* is under more positive selection in the diurnal fireflies *Ellychnia* and *Lucidota*.

*InaD* also forms a protein complex with *ninaC* (also recovered in two of the three tests for positive selection), each of them binding *Cam*. This complex has been shown to mediate Ca\(^{2+}\)
movement, thus greatly increasing the efficiency of arrestin [45, 46]. In order for arrestin movement to take place arrestins must bind to phosphoinositides, which is thought to be mediated via \textit{ninaC} ([47], however see [48]).

There is also strong support for the detection of positive selection in \textit{arr2} in diurnal species of fireflies. This is not surprising given the critical role of \textit{arr2} in light-mediated signaling. The arrestin gene family is well known for mediation in many G protein-coupled receptor signaling cascades [49]. In these systems, its purpose is to arrest or stop signaling [50, 51]. While there are four known arrestin genes in mammalian systems [52], only two, arrestin-1 and arrestin-2 have been found in \textit{Drosophila} [53–56]. Both arrestin genes were expressed in our Lampyridae taxa, however arrestin-1 was only found in a handful of taxa, and not recovered in either of the diurnal species included. In Lepidoptera, it has been shown that \textit{arr2} is more highly expressed than \textit{arr1}. \textit{Arr2} does not reside in the rhabdomere (where phototransduction takes place) but is trafficked into the rhabdomere after phototransduction (see above).
CONCLUSIONS

This study represents one of the first attempts to assess positive selection in the phototransduction genes in a non-model insect beyond the r-opsin gene family. Arr2, inaD, and trpl represent critical components of the insect phototransduction pathway. All three are associated with light-dependent interactions within the eye. While sexual communication in many fireflies is critically dependent on vision, bioluminescent communication tends to take place beginning in the dusk hours and continuing into the night. In this dark environment, regardless of sexual activity, there are simply fewer photons reaching the firefly eye, meaning the PT pathway is not as active. For those lineages (Ellychnia and Lucidota) active during the day, and therefore more dependent on light for survival and reproduction, it is intriguing that these light-activated genes have support for evolving under positive selection, and are therefore diverging from those in the conserved, nocturnal sensory niche.

As we investigate the variation in this system, both with a larger taxon sampling, and with more complete genomic resources, our understanding of insect vision will come into sharper focus. While this study adds an important component in insect visual research of non-model organisms, much more remains to be addressed. For example, an in depth quantification and qualification of PT genes across multiple firefly genomes, as well as expression data from throughout the diel changes to compare potential differences of PT genes expression. Such experiments would also provide insight into dimorphic gene expression patterns as has been found in one species of firefly [19]. Additionally, there is more variation in firefly communication systems that could be investigated. These modes of communication have undergone various classification systems [57, 58] however most recently firefly communication
modes were simplified into four groups: pheromone use only, continuous glow + pheromone, short or long flashes, and pheromone use + weak, daylight glow [59]. Sampling from these communication modes would allow for finer scale look at the evolution of phototransduction genes.

As it stands, we have successfully identified several genes that are strong candidates for further positive selection studies. In the case of inaC, we have also identified a tantalizing area of study into the loss of certain phototransduction genes across the beetle tree of life. Further study will surely help our understanding of positive selection in terms of adaptation to differing sensory niches.
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LITERATURE CITED


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Figure 1: Diagram of the basic insect phototransduction pathway (modified from Macias-Munoz et al. 2019). Components in gray represent parts of the pathway not sampled. Components in orange were sampled but were recovered in too few taxa for selection analysis. All other components are colored to reflect relative omega values as reported via the PAML analysis. Dark blue indicates high relative omega values whereas lighter blue yellow indicates low relative omega values.
Figure 2: Distributions of the PP of positive selection across phototransduction genes. Violin plots represent estimated kernel density MCMC posterior probability of positive selection (blue dots).
Chapter 4: Building appreciation for Natural History Collections from biological collection data.

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ABSTRACT

Natural history collections are currently underfunded and understaffed. There is a growing need to educate the general public on the importance of natural history collections for diverse scientific topics from species discovery to effects of climate change. Here we present an online module aimed at assessing differences in attitudes towards natural history collections as well as the effect on pro-environmental thinking in a group of religious, undergraduate, non-biology major students. We show statistical differences post-intervention suggesting that this module is effective at raising students ranking of importance of natural history collections to both scientific research and the general public.
INTRODUCTION

There is a growing and documented need for inquiry-driven, active science education (NRC, 2003; Brewer and Smith, 2011; Cook et al., 2014). Rutherford (1990) stated: “Science, mathematics, and technology do not create curiosity. They accept it, foster it, incorporate it, reward it, and discipline it—and so does good science teaching…” (Rutherford, 1990). With these two ideas in mind us as educators can ask ourselves the following questions: How was our curiosity fostered in the various “-ology” courses which incorporate specimens? How many of us make use of specimens in our research projects? How can we use specimens in our introductory level classrooms in hands on activities? Natural history collections are the historical repository against which we can measure our rapidly changing world. Changes in policy, land use, technology, population and especially climate mean that the world is more heavily impacted now than ever before. Natural history collections provide the information we need to understand the responses of organisms to these changes (Suarez and Tsutsui, 2004; Winker, 2004; Wandeler et al., 2007). Further, they allow us to harness these collections for genetic studies (for example Neanderthal genomics: Prüfer et al 2014) to not only understand the history of organisms around us but even our own as a species. There are approximately 2–4 billion specimens in natural history collections worldwide (Ariño, 2010). While the public side of natural history museums (i.e., exhibits, activities, etc.) are broadly appreciated by the public, the non-public side of natural history museums, the collections and laboratories, are often as easily accessible and thus are not as fully understood and or appreciated by the general public (Suarez and Tsutsui, 2004). Further, natural history collections also represent a vast resource and network for the improvement of biological education. Increasing efficiency in the biology classroom can best be achieved when mirroring what is done in the laboratory, especially focusing on inquiry-driven science (Feldman
et al., 2012). Scientific teaching seeks to mirror the discovery process in the classroom (Handelsman et al., 2004). Using collections and mirroring the research that happens in collections is an excellent way to increase inquiry-driven science for students while also building a more complete understanding for collections among students that represent the general public.

**Specimens in Science:**

Specimens in natural history collections are extremely valuable to scientific research. For example, natural history collections represent a fantastic resource for measuring/predicting changes in population distribution (Elith et al., 2006). Measuring population change/historical biogeography is usually not possible given a lack of historical data with which to compare current populations, this problem is alleviated via the use of natural history collections as a proxy, which for many taxa, contain specimens dating to tens and often hundreds of years (Shaffer et al., 1998, Shaw et al., 2004, Vik et al., 2008, Jeppsson et al., 2010). Natural history collections are also a vital resource in identifying invasive species and even ways to control them (Suarez and Tsutsui, 2008; Lister, 2011). This can be done by finding an invasive species historical distribution and locating potential biological control agents (e.g., predators, viruses, etc.) that have controlled the numbers of that species throughout its evolution, thus, providing a critical historical perspective (Crawford and Hoagland, 2009). Specimens from natural history collections are perhaps best known for their use in species discovery. For example, in a revision of the Australian beetle *Deretaphrus*, Lord and McHugh (2013) examined 1,900 specimens across 44 museums and discovered seven new species. In a similar study of Australian tardigrades, eleven new species were discovered (Claxton, 1998). This type of result is not
unusual, as most of the new species which have been described were described from specimens deposited in natural history collections (Green, 1998; Fontaine et al., 2012). Just last year, 412 new species were described from one natural history collection alone, including two dinosaurs (Chapelle et al., 2019 & Maidment et al., 2019), an extinct bandicoot (Travouillon et al., 2019), several (possibly endangered) plants (ex. Gouvêa, 2019) and 12 polychaete worms (Wiklund et al, 2019).

The historic nature of natural history collections (e.g. series of specimens collected over large periods of time) allows for further comparative studies testing hypotheses, for example those associated with climate change and/or habitat loss. In a study of passerine birds in Israel, Yom-Tov (2001) used natural history collections to measure several species for both body mass and tarsus length and found a trend of decreasing body size from the years 1950 to 1999, coinciding with an increase in average summer temperature of .26°C. Global warming is known to affect many biological aspects of an organism (Hughes, 2000), and as such represents a potential explanation for this trend. Similarly, in a study of stream-inhabiting beetles, it was shown that an increase in size, coupled with a change to a more streamlined body, had occurred during a 60-year period and that a decrease in mean annual temperature was the most predictive variable for this trend (Babin-Fenske et al., 2008). Such studies would be nearly impossible without natural history collections. Further, natural history collections function as the repository of specimens from such studies so study can continue into the future and the associated data from each specimen can be preserved.

**Specimens in Scientific Teaching:**
Specimen-based learning has the potential to be an incredible tool in formal undergraduate education. Specimens bring biology into the classroom and help students appreciate that the concepts they are learning apply to living organisms. This can also help alleviate the problem of attrition due to many students acquiring the misconception that biology is all about memorization (Handelsman et al., 2007). Using specimens and specimen metadata can drive inquiry-based education (Cook et al., 2014), and teach students that biology is really about thinking logically and solving problems, and that discovery is at its core.

Traditionally exposure to biological specimens in formal university education settings is usually limited to upper-level “ology” type courses (e.g. entomology, botany, ornithology). Students in these classrooms learn first-hand the beauty and excitement hidden in the natural world (Wallace, 1869). The problem arises in that these experiences are rarely transferred to introductory level biology courses (Powers et al., 2014) and even those experiences in upper-level “ology” courses rarely focus on the importance of natural history collections and the scientific data that can be harnessed from them.

Lacey et al. (2017) provided modules demonstrating how to incorporate natural history collection data to test hypotheses of adaptation to climate change. Several other studies focusing on formal science education have demonstrated the effectiveness of modules centered around local geography and culture (Castagno and Brayboy, 2008; Kisker et al., 2012; Barnhart, 2014; Lipka et al., 2014). Adding to this research, Anderson et al. (2017) developed a series of modules centered around the collection at the University of Alaska Museum which provide an example of how to utilize local collections in the classroom.

These modules are a wonderful resource for educators who want to include specimen-based active learning in the classroom. From any of the above listed papers, with a little
modification in most cases a learning modules can quickly be assembled. For example, for assembling a module to teach biogeography and given the accessibility of metadata databases, it is fairly straightforward to download a dataset comprising all the county records for a given species. Beforehand, students can be given information about the species and asked to predict a species ideal environment. The dataset can then be incorporated into one of various ecological models so that students can analyze the data and conclude whether their hypothesis is supported or rejected.

Natural history collections provide an opportunity to use specimens to increase awareness of diversity as they provide a way to interact with biodiversity on a personal level (Efthim, 2006; Kimble, 2014; Pickering et al. 2012; Monfils et al., 2017). As one of the more diverse groups of life, insects offer the greatest opportunity to teach students about biodiversity (Wilson, 1992; Mora, 2011). When most students think of insects, they may only think of the most common varieties: butterflies, ants, bees, cockroaches, mosquitos. With more than one million described species, and perhaps as many as 30 million species total (Erwin, 1982), there is much more for students to learn about the world around them, and insects are a fantastic place to start this inquiry. Insects also make up the majority of the holdings in many of the world’s natural history collections (Holmes et al., 2016). This is due in part to the general ease of collecting insects. Usually, only a hand net and an eye for the obscure is needed. In addition to this is the ease of collecting several specimens, sometimes several hundred at a time. The small size of the specimens makes storage, and more importantly transport, of thousands of specimens relatively easy. In addition to the physical specimens, and perhaps more important/useful in the classroom, is the data associated with each specimen such as the label. Label data will ideally contain general locality information, and for more recently collected specimens specific GPS
coordinates. Labels should also reference the date of collection, the collector as well as any special collecting techniques or host plant information.

This metadata associated with each specimen from natural history collections is perhaps the most meaningful to educators. In many instances, use of actual specimens is too difficult. Travel to a collection can take too long, and loan of necessary specimens can exceed the ability of local collection managers. In these cases, the metadata can still be used. For example, in several databases, housed across the world, there are thousands of data points that instructors can use in bringing the scientific process into the classroom. See for example the databases iDigBio (https://www.idigbio.org/) and SCAN (https://scan-bugs.org/portal/). Through these databases, anyone can download datasets containing the records of hundreds of firefly sightings recorded in several major natural history collections across the United States. Included in this data usually are observations of locality, date, time of day, and in many cases, other useful information such as type of vegetation and/or habitat. This information can be curated and brought into the undergraduate classroom to investigate patterns of firefly distribution. Students can be challenged to develop hypotheses that would explain the data, and to draw predictions from these hypotheses. Other, more locally-focused, independent databases (i.e. https://nhmu.utah.edu/fireflies, based largely on museum specimens) can then be consulted. Using such resources, students can explore the scientific method in a meaningful, personalized way by testing predictions and revising hypotheses. Utilizing the metadata with these collections can bring scientific inquiry into the classroom in a way that cannot be done otherwise.

Following this line of thinking we seek to add to the current literature an online learning module with two objectives, both aimed at undergraduate students in non-majors introductory biology courses. The first of these is to increase student perception of the importance of natural
history collections. Our second objective is to increase pro-environmental thinking as assessed by the Revised-New Environmental Paradigm scale (Dunlap and Van Liere, 1978, Dunlap et al., 2000). As stated above, a current major line of study within natural history collections is that of climate change, but other issues can also be explored using museum metadata such as changes in species distribution due to such factors as environmental degradation and/or pollution.
MATERIALS AND METHODS

Undergraduate students in two introductory biology courses for non-majors were selected for this study (~350 total). Students were administered a pre-assessment composed of the 15 R-NEP scale questions with two additional likert-scale questions: 1: Natural history collections are important for scientific research and 2: Natural history collections are important for the general public. The student then participated in a short online module (described in detail below) followed by a post-assessment. The post assessment was comprised of the same 17 questions above; however, we added several questions after seeing the responses to the pre-assessment. Two free-response questions were added to ascertain more detail about specific responses: 1: Concerning statement 12, how do you define “rule”? and 2: Concerning statements 16 and 17, please define a “Natural history collection” to the best of your understanding. Because this module centered on fireflies in the Utah region, four more questions were added to investigate if our choice of organism had an effect: 1: On a scale of 1 (no concern) to 10 (extreme concern) how concerned are you with the extinction of fireflies? 2) On a scale of 1 (no concern) to 10 (extreme concern) how concerned are you with the extinction of pandas? 3) On a scale of 1 (no concern) to 10 (extreme concern) how concerned are you with the extinction of moss? and 4) In your opinion, how would you rank the above three organisms (fireflies, pandas, and moss) in the importance of preventing them from going extinct? The 17 likert-scale questions were compared pre- and post- using a paired sample T test implemented in SPSS. Additionally, the 15 R-NEP scale questions were divided into six groups, the total score as well as the five facets of an ecological worldview (Dunlap et al. 2000): the reality of limits to growth, antianthropocentrism, the fragility of nature’s balance, rejection of exemptionalism, and the possibility of an ecocrisis.

Module
The online module was designed with specimen data from the Monte L. Bean Science Museum (MLBM) at Brigham Young University. Fireflies were chosen as the module organism due to several factors: fireflies are the main area of expertise of two of the authors (GJM & SMB), they are a gender-neutral insect (i.e., they are equally attractive to or disliked by both female and male students), fireflies are charismatic, well known animals rarely encountered in Utah, and several populations under study by the authors are at risk of extirpation or have already been extirpated.

The module begins with a brief description of the MLBM and the primary purpose of its natural history collections (i.e. to advance scientific knowledge, address societal issues, and to increase scientific literacy). Students are instructed about the major holdings of the MLBM and taught that one of the major strengths of the MLBM is the insect collection with over 2 million specimens. Students are then given a brief background on fireflies with facts such as: fireflies are beetles, many species rely on bioluminescence (use of light for communication) for sexual reproduction, and that because of this, fireflies are highly susceptible to light pollution.

Students are then given an excel spreadsheet of curated data from MLBM specimens and told to examine the data to determine where in Utah and at what time of year fireflies have traditionally been found. Students are then given instructions on how to use the website simplemappr.net to achieve these goals. Students were then given a short, formative assessment to determine if they could correctly interpret the data. This assessment consisted of one multiple choice question:

If you wanted to go and see fireflies in Utah, where and when would you go?

A. Lake Bottom Canal, Provo; last week of June
B. Goshen Marshes; First week of June
C. Spring Lake, Payson; Last week of February
D. Dugway; First week of July.

All location responses except Dugway were listed on the excel file, and all times of year responses except February were also included in the excel file. This was designed to lead students to select either A. or B. If students choose A., then they are congratulated on selecting a correct answer. However, students are then instructed that although fireflies have been encountered at this location before, fireflies have not been seen there for several years. Students are presented with images of what the location looks like now where they can observe that locality is now bordered by a freeway with several billboards and extensive lighting. Students are then asked to hypothesize why fireflies are no longer found at this locality. They are then directed to the slides for option B. If students originally choose option B. they are congratulated for choosing a correct answer and presented with a short youtube clip of fireflies in Utah. They are then directed to the slides for option A. After seeing both options, students are asked to compare the two habitats. Students are then asked to think about the unintended effects on nature of certain human needs (i.e. building a well-lit freeway for safety reasons that at the same time increases light pollution). After a series of thought questions and statements about how the only way we know certain populations existed is through natural history collections, students are left with the final question: When human development is at odds with the natural world, what, if any, is our responsibility to protect it?
RESULTS

The pre-assessment resulted in 326 responses, while the post-assessment had 294 responses. After curating the data for students who took both the pre- and post-assessments, we had a sample size of 267. Analysis of the responses from the 267 students showed no significant differences looking at the total score or any of the five facets of the R-NEP with the exception of the facet “possibility of an ecocrisis” ($P=.049$). When looking at individual items, there was a significant difference ($P<.05$) for the following items: question 3 ($P=.012$), question 5 ($P=.005$), and question 7 ($P=.032$). However, it should be noted that none of these questions showed any practical difference between pre- and post-assessments. Where we saw the highest differences were in the additional questions 16 ($P<.001$) and 17 ($P<.001$). Not only did we see statistically significant changes in these two items, we also saw practical changes, especially in the composition of answers (figs. 1 and 2). Pre-intervention for question 16 (Natural history collections are important for scientific research), 83 students strongly agreed, 126 somewhat agreed, 52 neither agreed nor disagreed, one somewhat disagreed, and three strongly disagreed. Post-intervention, 129 students strongly agreed, while only 98 somewhat agreed, and 27 neither agreed nor disagreed, while seven somewhat disagreed, and three strongly disagreed. This pattern was the same for question 17 (Natural history collections are important for the general public). Pre-intervention 67 students answered strongly agree (117 somewhat agree and 71 neither agree nor disagree, six somewhat disagreed and four strongly disagreed). Post-intervention 109 students answered strongly agree (98 somewhat agree and 46 neither agree nor disagree, eight somewhat disagreed and three strongly disagreed).
DISCUSSION

While we did see gains in the number of students agreeing with the importance of natural history collections to both science and the general public, we did not see the gains we expected in pro-environmental thinking as assessed by the R-NEP. There are several possible factors that can explain this. A large portion of the online module was dedicated to explaining what natural history collections are. Perhaps the animal biology portion did not make a clear enough case that fireflies in Utah are at critical risk of extinction. It is also possible that the example organism (fireflies) are not seen as a vital component of the ecosystem. At the end of the post-assessment students were asked to rank various organisms. However, the data seem to tell two different stories demonstrating that our students may care less about fireflies than we previously thought. When asked to quantify the level of concern for each organism pandas receiving the highest support (average: 6.56), then moss (5.88), leaving fireflies (5.58) with the lowest level of concern. This pattern was surprising to us. Students expanded their reasoning in the free response section where they were asked to rank the three organisms. Many students placed moss as the most important organism to save (based on their stated assumption that mosses are more important to the ecosystem) and fireflies as the least important. It would be interesting if more/different examples of this pattern of extirpation in Utah could affect more of a change in the R-NEP. It is also possible that utilizing specimens from a more global organism could affect a desired change.

While no gain was shown in pro-environmental thinking, we did see substantial gains in the “importance” students placed on natural history collections. What is additionally encouraging is the fact that our students started relatively high in agreeance (table 1). It is also worth noting that while we did not see a large practical change in the average level of agreement (shift in
average for question 16 of 0.215 and question 17 of 0.242), we did see a major shift in the composition of each response group. For example, in question 16 students were asked their agreement with the statement “Natural history collections are important for scientific research.” At the beginning of the study the majority of the students answered either “somewhat agree” or “neither agree nor disagree.” Post-intervention both of these response groups were diminished with a marked increase in the number of students answering “strongly agree” (Fig. 1). This same pattern was shown in question 17 “Natural history collections are important for the general public.” Although the general pattern in gains was the same between both questions, we did see a smaller component of the students who strongly agreed with question 17 as opposed to those who strongly agreed with question 16 (129 students post-intervention in question 16 vs. 109 students post-intervention in question 17).
CONCLUSIONS

We’ve argued for the value of using data from natural history collections in the classroom as a means to increase student appreciation for both collections and the environment. As stated above, the diversity of life preserved in natural history collections is used in various scientific pursuits and inherent value of natural history collections cannot be quantified. Not only are collections used in the discovery of life (a fact the general public might not be aware of), these collections are also actively being used in a wide variety of studies that are important to human origins and existence as well general earth health, including the maintenance and protection of biodiversity in today's rapidly changing world. If we can affect even a small change in attitudes towards the importance of these collections, then as biologists we have the responsibility to do what we can to ensure their future protection.
ACKNOWLEDGEMENTS

The authors are extremely grateful to Dr. Michael Whiting (curator) and Dr. Shawn Clark (collections manager) at the Monte L. Bean Science Museum for access to the firefly specimens from which data were gathered. We also thank Laura Sutherland (BYU) for assistance with SPSS.
LITERATURE CITED


Crawford PHC, Hoagland BW (2009) Can herbarium records be used to map alien species invasion and native species expansion over the past 100 years? J Biogeogr 36: 651–661.


Symbiota Collections of Arthropods Network (SCAN): A Data Portal Built to Visualize, Manipulate, and Export Species Occurrences.


### Table 1: Summary statistics of paired differences for paired samples test

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<th>Upper</th>
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CHAPTER 4 FIGURES

Figure 1: Scaled bar graph of responses for question 16.

Figure 2: Scaled bar graph of responses for question 17.
## APPENDIX

Supplemental material for chapter 1

Supp. Table 1. Genomic resources used to develop Anchored Hybrid Enrichment probe kit design.

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Supp. Fig. 1. Maximum Likelihood reconstruction of trimmed dataset aligned in MAFFT; log-likelihood = -10921284.680. Star at node depicts Lampyridae. Numbers at nodes indicate UFboot values. Unlabeled nodes are 100%.
Supp. Fig. 2. Coalescent reconstruction (ASTRAL) of full, untrimmed dataset aligned in MAFFT. Star at node depicts Lampyridae. Numbers at nodes indicate localized posterior probability.
Supp. Fig. 3. Coalescent reconstruction (ASTRAL) of trimmed dataset aligned in MAFFT. Star at node depicts Lamyridae. Numbers at nodes indicate localized posterior probability.
Supplemental material for chapter 3

Supp. Table 1: Assembly summary statistics for transcriptomes new to this study (Code=CO_###) as well as for those previously published.

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Supp. Table 2: Presence absence data genes sampled in the phototransduction pathway. Gray box indicates presence. Orange box indicates presence of duplication in *Photinus carolinus* only.
Supp. Table 3: Summary of positive selection analyses carried out in PAML and BAi-L-Phy. P value for lrt significance is .05. Level of support for Bayes Factor is after Kass and Raferty (1995).

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