IDENTIFICATION OF FIREFLIES (COLEOPTERA: LAMPYRIDAE) AT THAYER FARM, OTSEGO COUNTY, NY

BY

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B.S., SUNY College at Oneonta, 2017

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Biology
in the Biology Department of the
State University of New York, College at Oneonta,
Oneonta, New York
2019

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PREFACE

Organismal bioluminescence is an intriguing phenomenon. The ability for something living to glow and produce light seems unnatural. The process of converting chemical energy into light is a popular field of research (e.g., Day et al. 2004). Fireflies (Coleoptera: Lampyridae) are a family of insects that can emit light via unique biochemical pathways. The presence and interaction of the enzyme luciferase with its substrate luciferin in the abdominal fat bodies of fireflies catalyzes the chemical reaction that produces visible light. In the process, luciferase also converts luciferin into oxyluciferin, which is considered the "light emitter" because this transformation aids in the production of bioluminescence (Marques and Esteves da Silva 2009). Oxyluciferin then interacts with luciferin-regenerating enzyme (LRE) to create additional luciferin so the reaction can start over again (Day and Bailey 2003). The repetition of this chemical reaction is necessary for fireflies, as each species uses a different flash pattern in mate recognition: females lay flashing in the grass while males are airborne, signaling to females using species-specific flashing patterns. These patterns differ in flash duration, interval, and color, which are influenced by the amount of luciferin and luciferase produced by the firefly (Demary et al. 2006). Several firefly species are active during the day and do not require bioluminescence. Instead, these diurnal fliers use cuticular pheromones, or chemical signals, to find a mate (Shibue et al. 2004).

Fireflies are a notable group of organisms not only in their varied communication abilities, but in their taxonomic breadth. More than 100 genera and 2,000 species of Lampyridae have been recorded worldwide, and these numbers are expected to increase, as many species are yet to be described (Stanger-Hall et al. 2007). More than 75 of these species are found in the eastern United States and Canada (Faust 2017); however, there is a clear lack of organized knowledge regarding the identity and specific distribution of fireflies in New York. There are

keys available to identify fireflies, but a local, comprehensive, key does not exist, making it difficult to properly classify individuals. Luk et al. (2011) produced the only geographically proximal key; however, their work focuses on the Canadian province of Ontario. Taxonomic identifications are further complicated by species boundaries based on bioluminescence. In the genus *Photuris*, individuals are nearly indistinguishable physically, requiring a known flash pattern for identification. It is difficult to identify species based on their flash patterns alone. For example, species can differ based on subtle changes in the wavelength or frequency of the light emissions. Further confounding identification attempts, female *Photuris*, commonly referred to as "femmes fatales," can mimic the flash patterns of other species to lure in and devour males (Faust 2017, Lloyd 2018).

During the last half century, molecular science has transformed the field of biology. Ground-breaking techniques have allowed us to understand the world on a molecular level, letting us discover information about the structure and function of molecules, including DNA, RNA, and proteins (Davis 2012). Molecular data can be used to elucidate relationships between organisms that may not be discernable by morphological systematics alone. Molecular phylogenies use information such as DNA sequences to help hypothesize relationships and describe evolutionary patterns of related species. DNA barcoding, the mass sequencing of a single gene from multiple species to create a molecular standard, is a tool used for distinguishing species that are physically indistinct (Hebert et al. 2003). Use of phylogenetics, specifically in comparative research, has increased as phylogenies are helpful in resolving species identities and evolutionary uncertainties (Soltis and Soltis 2003).

With increased use of DNA sequence data for a variety of purposes, global online repositories and databases have also become widely used. The genetic sequence database

GenBank contains over 150 million DNA sequences from many organisms but should be used with caution when trying to determine species identifications. Comparing a sequence to one in the database can help confirm a species if they match closely enough, but only if the sequence submitted to GenBank is associated with the correct species. Revisions to the database can be made by the original submitter, but this process tends to be problematic, as it requires the person to recognize and admit fault. Mis-classified sequences can lead to unreliable results (Fritz et al. 2012). Critiques of GenBank and other databases including the Barcode of Life Data systems (BoLD) are common in taxonomic literature. Many discuss the ambiguity of identifications and ways to fix some common issues (e.g., Chang and James 2011, Fritz et al. 2012, Sonet et al. 2013).

Morphology and molecular methods started out as separate analysis techniques, but the integration of phylogenetic results with traditional morphological classifications has gained traction with many biologists. The integrative taxonomy approach, combining both morphological and molecular techniques, is a valuable tool in determining the identity of an organism. It has become prominent in recent literature (e.g., Chang and James 2011, Darienko et al. 2015, Kirichenko et al. 2015, Goulding et al. 2018) and will most likely continue to assist in taxonomic classifications into the future. The two methods that are typically employed by taxonomists are “integration by congruence”, which uses mitochondrial DNA and morphology in species identifications, and “integration by cumulation”, which looks at only mitochondrial DNA or morphological characteristics to identify an individual (Padial et al. 2010). “Integration by cumulation” tends to increase type I error, over-estimating the number of distinct species identified. On the other hand, because molecular databases may not be up to date with recently discovered and/or cryptic species, “integration by congruence” may under-estimate the number

of species that are actually present (increased type II error). It is essential that we focus on the big picture when dealing with taxonomy, keeping in mind that species are not only defined by the visual patterns we observe, but also by the genetic diversity responsible for functional differences (Padial et al. 2010).

Understanding what species are present in an area is imperative for both scientific research and conservation efforts, and species distribution data are necessary for unbiased and realistic conservation policies. In order to plan conservation efforts, biological surveys of species distribution patterns should be performed (Margules et al. 1994). Without understanding the range across which a population exists, it is almost impossible to assess which populations are at risk. Peripheral populations, or populations at the geographic edge of a species range, are known for their lack of genetic diversity. These populations experience less gene flow since they are typically established through founders' effects (Channell 2004). On the other hand, Lesica and Allendorf (1995) made a case for conserving peripheral populations. Central populations adapt to different environmental conditions compared to peripheral populations. Since these populations are genetically distinct from central populations, their ability to adapt to circumstances outside of the norm in our changing environment make them essential in protecting the evolutionary future of populations.

Examining how large-scale environmental changes impact species over time requires first identifying what organisms are currently present and where they exist. For example, we cannot understand the full impacts of increased artificial light and other anthropogenic alterations on fireflies without first knowing the range of species found in specific areas. Ineichen and Rüttimann (2012) found that male fireflies do not have the ability to locate females that establish mating sites under street lamps or other light sources, or males simply avoid illuminated areas

altogether, both lowering the chances of reproduction (Ineichen and Rüttimann, 2012). There is evidence based on species distribution data taken in Florida that the species *Micronaspis floridana* is threatened due to artificial light pollution, increased development, and other human induced modifications (Faust 2017); however, it is unclear which populations are likely to be affected by artificial light in New York.

Published literature on fireflies has not been consistent over time. In the late 1800s, John LeConte was the first to describe many species of fireflies that we see in the United States (LeConte, 1881). About a century later, James Lloyd discovered new lampyrid species and published on the flash behavior of fireflies (Lloyd 1966, 1969). Marc Branham and Michael Greenfield (1996) published a study on female attraction to flash patterns, being the first to examine the natural selection of fireflies. Lloyd (2018) recently published the first extensive guide to North American *Photuris*, introducing new species and helping "fireflyers" identify species by creating elaborate graphics of flash patterns. Research on firefly bioluminescence at the organismal level has been overshadowed by studies performed with bioluminescence at the chemical and cellular levels. The chemical reaction that produces bioluminescence is commonly used in medical research, specifically bioluminescent imaging and tagging in tumor detection and cancer treatment assessment (Rehemtulla et al. 2000). Not only is the literature lacking, but the habitats of bioluminescent organisms, typically dark forests and oceans, are challenging to explore (McInnis 2015).

In order to expand upon our knowledge of fireflies and understand what species are in the area, this study focused on the identification of fireflies at Thayer Farm, a 104-hectare property of the much larger 607-hectare holdings of the State University of New York, College at Oneonta, Biological Field Station (BFS). The history of Thayer Farm goes back to the 1800s. It

was home to many generations of the Thayer family who used the property for agricultural purposes and built structures to support the production of grains, hops, dairy, and wool (Staley 2010). The property was left to the Oneonta Foundation to be used by the State University of New York, College at Oneonta, as a Biological Field Station in 1999. Most structures were remodeled and are currently used for research and education purposes. The fields of the property are partially old-field/hay and are currently surrounded by mixed forests. A group of chain ponds can be found among the fields, which are still maintained.

The spacious property supports a diverse community of fireflies in a very accessible location in Otsego County. Lampyrids were collected and morphologically identified to species based on regional keys developed for Ontario, Canada. To confirm identifications, genetic techniques were implemented and the highly-conserved mitochondrial gene Cytochrome Oxidase I (COI) was used as a barcoding gene for species identifications (Folmer et al. 1994). A Bayesian phylogenetic tree was generated using the Bayesian clustering program MrBayes, giving insight into the evolutionary relationships between the species of fireflies discovered. Species-accumulation curves were made to determine species coverage of the survey. A complete taxon list was then compiled and new species records for Otsego County and New York were documented. In addition, a comprehensive list of fireflies that can be found in New York was created by combining the results of the current study with publicly available species distributions, and a Lampyridae genus and species key for the county was established.

LITERATURE CITED

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology & Evolution. 22(3):148-155.

Branham MA, Greenfield MD. 1996. Flashing males win mate success. Nature. 381:745-746.

Chang CH, James S. 2011. A critique of earthworm molecular phylogenetics. Pedobiologia. 54S:S3-S9.

Channell R. 2004. The conservation value of peripheral populations: the supporting science. Proceedings of the Species at Risk 2004 Pathways to Recovery Conference (ed. by T.D. Hooper). 1-17.

Darienko T, Gustavs L, Eggert A, Wolf W, Pröschold T. 2015. Evaluating the species boundaries of green microalgae (Coccomyxa, Trebouxiophyceae, Chlorophyta) using integrative taxonomy and DNA barcoding with further implications for the species identification in environmental samples. PLOS ONE. 10(6):1-31.

Davis L. 2012. *Basic Methods in Molecular Biology*. Elsevier.

Day JC, Tisi LC, Bailey MJ. 2004. Evolution of beetle bioluminescence: the origin of beetle luciferin. Luminescence. 19(1):8-20.

Day JC, Bailey MJ. 2003. Structure and evolution of the luciferin-regenerating enzyme (LRE) gene from the firefly *Photinus pyralis*. Insect Molecular Biology. 12(4):365-372.

Dayrat B. 2005. Towards integrative taxonomy. Biological Journal of the Linnean Society. 85(3):407-415.

Demary K, Michaelidis CI, Lewis SM. 2006. Firefly courtship: behavioral and morphological predictors of male mating success in *Photinus greeni*. Ethology. 112:485-492.

Faust LF. 2017. Fireflies, glow-worms, and lightning bugs: identification and natural history of the fireflies of the eastern and central United States and Canada. University of Georgia Press.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3(5):294-299.

Fritz U, Vargas-Ramírez M, Široký P. 2012. Phylogenetic position of *Pelusios williamsi* and a critique of current GenBank procedures (Reptilia: Testudines: Pelomedusidae). Amphibia-Reptilia. 33(1):150-154.

Goulding TC, Khalil M, Tan SH, Dayrat B. 2018. Integrative taxonomy of a new and highly-diverse genus of onchidiid slugs from the Coral Triangle (Gastropoda, Pulmonata, Onchidiidae). ZooKeys. (763):1–111.

Ineichen S, Rüttimann B. 2012. Impact of artificial light on the distribution of the common European glow-worm, *Lampyris noctiluca* (Coleoptera: Lampyridae). Lampyrid. 2:31-36.

Kirichenko N, Huemer P, Deutsch H, Triberti P, Rougerie R, Lopez-Vaamonde C. 2015. Integrative taxonomy reveals a new species of *Callisto* (Lepidoptera, Gracillariidae) in the Alps. ZooKeys. (473):157-176.

LeConte JL. 1881. Synopsis of the Lampyridae of the United States. Transactions of the American Entomological Society. 9(1):15-72.

Lesica P, Allendorf FW. 1995. When are peripheral populations valuable for conservation? Conservation Biology. 9(4):753-760.

Lloyd JE. 1966. Studies on the flash communication system in *Photinus* fireflies. Museum of Zoology. 130:1-100.

Lloyd JE. 1969. Flashes, behavior and additional species of Nearctic *Photinus* fireflies (Coleoptera: Lampyridae). The Coleopterists Bulletin. 23(2):29-40.

Lloyd JE. 2018. *A naturalist's long walk among shadows: of North American* Photuris – *patterns, outlines, silhouettes… echoes*. Self-published, Gainesville, FL, USA. 477 pp.

Luk S, Marshall S, Branham M. 2011. The Fireflies of Ontario (Coleoptera: Lampyridae). Canadian Journal of Arthropod Identification. [accessed 2018 Nov 13]. Available from http://www.biology.ualberta.ca/bsc/ejournal/lmb_16/lmb_16.html

Margules CR, Austin MP, Mollison D, Smith F. 1994. Biological models for monitoring species decline: the construction and use of data bases [and discussion]. Philosophical Transactions: Biological Sciences. 344(1307):69-75.

Marques SM, Esteves da Silva JCG. 2009. Firefly bioluminescence: a mechanistic approach of luciferase catalyzed reactions. IUBMB Life. 61(1):6-17.

McInnis J. 2015. The alluring firefly: nature's lightning bug may hold key to medical breakthroughs. The Guardian. [accessed 2018 Nov 7]. Available from https://www.theguardian.com/environment/2015/aug/19/fireflies-bioluminescence-mating-cancer-tumors

Padial JM, Miralles A, De la Riva I, Vences M. 2010. The integrative future of taxonomy. Frontiers in Zoology. 7(16):1-14.

Rehemtulla A, Stegman LD, Cardozo SJ, Gupta S, Hall DE, Contag CH, Ross BD. 2000. Rapid and quantitative assessment of cancer treatment response using in vivo bioluminescence imaging. Neoplasia. 2(6):491-495.

Shibue K, Goto Y, Kawashima I, Shibue T. 2004. Chemical analysis of surface hydrocarbons in fireflies by direct contact extraction and gas chromatography-mass spectrometry. Analytical Sciences. 20(12):1729-1731.

Soltis DE, Soltis PS. 2003. The role of phylogenetics in comparative genetics. Plant Physiology. 132(4):1790-1800.

Sonet G, Jordaens K, Braet Y, Bourguignon L, Dupont E, Backeljau T, De Meyer M, Desmyter S. 2013. Utility of GenBank and the Barcode of Life Data Systems (BOLD) for the identification of forensically important Diptera from Belgium and France. Zookeys. (365):307-328.

Stanger-Hall KF, Lloyd JE, Hillis DM. 2007. Phylogeny of North American fireflies (Coleoptera: Lampyridae): implications for the evolution of light signals. Molecular Phylogenetics and Evolution. 45(1):33-49.

Staley DP. 2010. Historical archaeology at the Thayer Homestead: Progress report of the 2010 investigations.

Webb CO, Ackerly DD, McPeek MA, Donoghue MJ. 2002. Phylogenies and community ecology. Annual Review of Ecology, Evolution, and Systematics. 33(1):475-505.

SPECIES SURVEY OF LAMPYRIDAE AT SUNY ONEONTA BIOLOGICAL FIELD STATION; INCLUDING NEW STATE AND COUNTY RECORDS FOR OTSEGO COUNTY, NY

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Abstract—

Fireflies are a family of insects that are understudied, especially in their species distribution. Minimal information is documented for the state of New York, and there are no species records for Otsego County. A lack of distributional data makes it difficult for other research to be completed on fireflies, and impedes understanding about which firefly species, if any, require protection. In addition, there are currently no firefly identification keys for the state. The most appropriate key to use was from the Canadian province of Ontario. Fireflies from Thayer Farm, a property of the State University of New York College at Oneonta (SUNY Oneonta) Biological Field Station, were collected and initially identified based on morphology. Molecular techniques were also applied and the DNA barcoding gene, Cytochrome Oxidase I (COI), was used for molecular species identifications. A total of 13 species from 6 different genera were discovered. As no previous county record exists in the literature, each species recovered was documented as a new species record for Otsego County. *Pyractomena palustris* is recorded for the first time as occurring in New York. A collective taxa list for New York was created based on current published literature on fireflies and a local-specific species key was constructed. (Lampyridae, Otsego County, Distribution).

INTRODUCTION

Almost 200 years after lampyrid species were first described, Marc Branham, a pioneer on the interaction of selection and firefly flash patterns reminisced in an interview, "I remember saying: '[Fireflies] are so common and so charismatic, I bet everything is known about them' … I was flabbergasted to see that there was far less than I thought" (McInnis 2015). As humans, we cannot help but be attracted to the glow of fireflies (Coleoptera: Lampyridae) and everyone is bound to have a memory of catching fireflies, but these creatures are still surprisingly understudied.

There are more than 2,000 different species of fireflies found globally (Stanger-Hall et al. 2007) and most species produce a bioluminescent flash pattern that is unique in flash duration, interval, and wavelength (Demary et al. 2006). The patterns assist in species-specific mate recognition. Females typically flash from a stationary position on the ground or in vegetation while males fly searching for the appropriate response signal. Not all species use bioluminescence to guide mate selection. Several diurnal firefly species lack an abdominal lantern and rely on the use of cuticular pheromones to find a mate (South et al. 2008).

Sometimes mate selection can end poorly. Female fireflies, especially in the genus *Photuris*, can mimic the flash pattern of other species to bait fellow fireflies. Males expecting to mate with a female of the same species risk being eaten by these "femmes fatales" (Eisner et al. 1997). The flash of fireflies can also serve as a response to threats. When distressed, some species flash erratically to emphasize toxicity or cease flashing to evade detection, while others induce thanatosis, or produce droplets of hemolymph from their elytra as a defense (Day 2003, Faust 2017, Eisner et al. 1997).

Despite general life history knowledge at the family-level and more in-depth comprehension of select species, other information, including basic topics like species distribution, is often unknown. Distributional knowledge tends to get overlooked, which is problematic. It is necessary to understand the range of a species in order to appropriately protect populations and establish informative conservation policies. Determining where species are located also establishes a basis for species-specific research that relies on presence/absence information, such as how environmental changes impact populations over time. Species-specific distributions of fireflies are rare. Low-resolution ranges are more common but are of little help when trying to perform local research. A consolidated lampyrid taxa list for New York does not exist and there are currently no documented records of firefly species specifically in Otsego County. This work remediates some of those gaps by using integrative taxonomy to confidently identify fireflies present on Thayer Farm at the Biological Field Station in Cooperstown, NY, providing a taxa list for New York fireflies, a local identification key, and new records for New York and Otsego County.

MATERIALS AND METHODS

Study Site and Species Collection

Thayer Farm in Otsego County was the study site for this survey. It is part of the SUNY Oneonta Biological Field Station (BFS) located in Cooperstown, NY (42.73°N, 79.91°W). The 104-hectare property is a part of the of the much larger 607-hectare holdings of the BFS. The farm is partially old-field and partially operational hay fields. Sampling occurred over ~ 6.9 ha, which spanned across the main road of the property, several grassy fields, and along the mixed hardwood forest lines. Lampyrids were haphazardly collected via aerial net on Thayer Farm in

the summers of 2017 and 2018. Individuals were caught in the air and in the grass between 2100 and 0130 hours. To preserve the DNA of each sample, lampyrids were frozen at -80°C.

Morphological and Molecular Identifications

All individuals caught were morphologically identified according to Luk et al. (2011). Fireflies of the genus *Photinus* were identified to species. A subset of individuals from each genus found were used for molecular species identifications. Genomic DNA was extracted from the prothoracic tissue of each sample selected for molecular identification using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). The gene Cytochrome Oxidase I (COI) was amplified in 25 µL reactions containing DNase- and RNase-free water, 1x Hot Master Buffer, 0.5 µM forward and reverse primers (LCO1490 & HCO2198 of Folmer et al. 1994), 0.2 mM dNTPs, 0.04 units Hot Master Taq polymerase (Quantabio), and approximately 30 ng template. Hebert's (2003) thermal cycler protocol was followed. Upon successful amplification, the DNA was purified using AMPure XP Beads (Beckman Coulter Genomics, Brea, CA) and sent to Eurofins Genomics for sequencing (EG, Louisville, KY). Sequences were visualized using Chromas Lite (Technelysium Pty Ltd, Australia), trimmed by hand, and examined for allelic differences. Sequences were then compared to those in GenBank using n-BLAST to provide a molecular species identification. All sequences were submitted to GenBank (accession numbers MK635154-MK635340).

Sampling Coverage, Taxa Lists, and New Records

Species-accumulation curves were created for genera with 3 or more species to ensure the maximum number of species were recovered and identified using the "vegan" package in R

(Version 1.1.463). Molecular species identifications were randomly sampled to determine how many individuals should be collected in order to retrieve the maximum number of species present. A curve for all nocturnal species was created as well. A complete firefly taxon list for New York was compiled from the data from this study and a review of the literature. Most of the distributional data from the literature came from Faust (2017), Lloyd (1966, 1969), and Green (1961). New species records for Otsego County and New York were documented.

Phylogenetic Analyses

A Bayesian phylogenetic tree was created using MrBayes (v3.2.6, Huelsenbeck and Ronquist 2001, 2003) after jModelTest (version 2.1.3, Posada and Crandall 1998) was used to select the most appropriate mutational model (invgamma). The consensus tree was created using 5 chains (4 hot, 1 cold) to evaluate 1,000,000 simulated trees each with every 100^{th} tree sampled and a burn-in value of 2,000 generations. The burn-in value was chosen as a generation beyond the point at which posterior likelihoods reached stationarity (Ronquist et al. 2005). A species of net-wing beetles that belong to the family Lycidae was used as an outgroup (Accession number = MG060334.1), as it was the closest related family to Lampyridae within the subfamily Elateroidea. The phylogenetic tree viewer FigTree was used to create a tree of the inferred relationships based on the molecular data (Morariu et al. 2008).

RESULTS

Morphological and Molecular Identifications

A total of 383 fireflies were caught and morphologically identified to genus (Table 1). We found 13 firefly species on the Thayer Farm property (Table 4). Most individuals belonged

to the genus *Photinus*, and a total of 6 genera were discovered (Table 1). Initial morphological identifications revealed 7 species of the genus *Photinus*. The number of species identified in that genus was reduced to 4 species after both molecular and morphological results were examined (Table 3). The genus *Pyropyga* was not used in molecular identifications, as we were unable to obtain usable DNA from any of the individuals despite attempting extractions from the prothorax, head, legs, elytra, head+legs, head+thorax, and whole body.

Sampling Coverage, Taxon Lists, and New Records

The species-accumulation curve for the genus *Photuris* plateaued at 4 species after approximately 12 individuals (Figure 2). The curve for *Photinus* nearly, but did not completely plateau, after sampling 140 individuals (Figure 1). There was uncertainty in both simulations; however, the estimated number of species in the genus *Photinus* showed much more ambiguity. The accumulation curve for the 10 nocturnal species did not plateau after sampling 186 individuals (Figure 3).

The review of the literature revealed a total of 30 documented species in New York (Table 2). The following species are documented here for the first time for Otsego County: *Photinus ignitus* Fall, 1927, *Photinus macdermotti* Lloyd, 1966, *Photinus carolinus* Green, 1956, *Photinus obscurellus* LeConte, 1851, *Photuris quadrifulgens* Barber, 1951, *Photuris lucicrescens* Barber, 1951, *Photuris tremulans* Barber, 1951, *Photuris pennsylvanica* (De Geer, 1774), *Pyractomena angulata* (Say, 1825), *Pyractomena palustris* Green, 1957, *Lucidota atra* (G. Oliver, 1790), *Ellychnia corrusca* (Linnaeus, 1767), and *Pyropyga sp(p)* (Harris, 1836), (Say, 1823). *Pyractomena palustris* Green, 1957 had not been previously documented in New York.

Phylogenetic Analyses

The consensus Bayesian tree (Figure 5) was separated into two major clades (PP= 100). One clade included most of the genera: *Photinus*, *Ellychnia*, *Pyractomena*, and *Lucidota*, while the other contained species of the genus *Photuris*. *Photinus macdermotti* and *Photinus ignitus* were found to be sister taxa (PP= 100). Relationships between *Lucidota* haplotypes, *Ellychnia* haplotypes, and *Pyractomena* species were strong (PP= 100), while the weakest relationships included the group of all *Photuris* species except *Photuris pennsylvanica* (PP=50) and the grouping of *Pyractomena* with *Photinus* and *Ellychnia* with only 57% of trees supporting that linage.

DISCUSSION

We present the first lampyrid species records for Otsego County, for at least 13 different species. The species *Pyractomena palustris* was also documented as a new species record for the state of New York, bringing the total number of species for the state to 31. Otsego County and New York now have firefly species taxa lists, as this information was previously lacking. Since there was no local species key, the number and identity of species discovered morphologically was much different than what was found molecularly. *Photinus macdermotti* was not morphologically identified because it was not present on the *Photinus* species key used, but it appeared in more than half of the COI species identifications that were "morphologically" *Photinus ignitus*. The lack of regional species keys and distributional data made it difficult to understand what species were present and made it nearly impossible to begin other research on lampyrids.

The combination of morphological and molecular taxonomy proved to be the most appropriate and accurate way to identify species, as we ran into issues using both methods separately. Visually determining different firefly genera is straightforward, but species morphology can be confusing, as there are many characteristics that vary only slightly and required significant practice to discern. If we used morphology alone, we would have overestimated the number of species present in the genus *Photinus* by 3 species, and entirely missed another common species (*Photinus macdermotti*). There were issues with our molecular identifications as well. GenBank was untrustworthy at times and gave us reason to believe that some sequences previously submitted to the database were incorrectly identified. Several times, we discovered two species that were 98-99% identical to our sequence, which was within the 2% sequence divergence used to delineate species boundaries in insects. One way to clarify this issue was re-examining morphological characters. For example, a few of our sequences were 99% identical to both *Photinus ignitus* and *Photinus indictus*. The species descriptions revealed one major physical difference: *Photinus ignitus* has a lantern, while *Photinus indictus* does not. This discrepancy was enough evidence to prove that the individual in question was *Photinus ignitus*, and ultimately made us more skeptical of GenBank identifications. Most of the time, looking at morphological distinctions solved the problem; however, for species of the genus *Photuris*, we were not able to use this method because species cannot be delineated based on morphology.

Based on the species-accumulation curves, we appear to have sufficiently sampled *Photuris* species that are at Thayer Farm. It is likely that we sampled the common species of *Photinus* in the area, but there is a chance other rare species are present on the property, since the estimated species-accumulation did not reach a plateau. We were unable to produce plots for the

other genera, as we only observed one or two species on Thayer Farm. If we collected more individuals from these genera, we may have encountered more species; however, it is evident that these genera are much less common than *Photinus* and *Photuris* fireflies. The nocturnal species-accumulation curve resulted in a similar curve to *Photinus*; a phenomenon likely driven by the large number of *Photinus* in our samples.

The Bayesian phylogeny provided support for the monophyly of most genera, and *Photuris* was clearly distinct from the other taxa recovered from the BFS. The weak support for internal branches in the *Photuris* groupings (PP= 50, 52, 63, 75) was not unexpected, as the nucleotide diversity between species in this genus were sometimes below the 2% species boundary.

In the remainder of the tree, several polytomies arose, which signified that there was not enough information present to evolutionarily separate species in many of the hypothesized groupings. This occurred with several haplotypes of *Photuris* (*P. quadrifulgens* 01, 02, 03, *P. lucicrescens* 01, 02, 04, 07), *Pyractomena* (*P. angulata* 01, 03, 04) and *Lucidota* (*L. atra* 01, 02, 03). There are different biological reasons evolutionary divergences occur. Actual morphological differences may define lineages as two different species, or species could diverge because of strong selection pressures, geographic isolation, or a plethora of genetic diversity seen within that species. Since the highly conserved gene COI was used, it may fail to detect evolutionarily recent divergences, even when clear morphological or ecological differences exist. Integrating both molecular and morphological techniques is one way to resolve this ambiguity; however, it is likely that morphological traits (e.g., flash patterns) drove the divergence of lampyrid groups.

Our phylogenetic tree strongly supported the paraphyly of *Photinus*, first reported by Stanger et al. (2007). The diurnal genus *Ellychnia* appeared in the middle of the *Photinus* species group, suggesting that either one clade of *Photinus* needs assignment to a new genus or the *Ellychnia* group should be reassigned into *Photinus*. Our phylogeny included multiple species of *Photinus* that were not present in Stanger et al.'s (2007) study, thus providing stronger support for their finding. Similarly, corroborating Stanger et al. (2007), it is clear that based on this phylogeny, the diurnal habit arose multiple times in lampyrids.

Integrative taxonomy allowed us to properly identify almost all of the fireflies encountered in this survey. The species lists compiled for the county and the state should aid others who work with lampyrids. The distribution of existing firefly species and species that have yet to be described needs to be understood in order to facilitate and complete other research on lampyrid populations (e.g., impacts of artificial light, anthropogenic modifications, and climate change).

ACKNOWLEDGEMENTS

We would like to thank the State University of New York College Senate Committee on Research for the grant that was used to help fund this research, Donna Vogler and Daniel Stich for all of their help on this project, and everyone who assisted in sampling and lab work, including E. Berezowski, C. Cannon, H. Dower, L. Hayes, N. Pedisich, E. Proutey, A. Rothfuss, A. Szymanski, and the SUNY College at Oneonta BIOL344: *Field Entomology* class of 2017.

LITERATURE CITED

Day JC, Tisi LC, Bailey MJ. 2004. Evolution of beetle bioluminescence: the origin of beetle luciferin. Luminescence. 19(1):8-20.

Demary K, Michaelidis CI, Lewis SM. 2006. Firefly courtship: behavioral and morphological predictors of male mating success in *Photinus greeni*. Ethology. 112:485-492.

Eisner T, Goetz MA, Hill DE, Smedley SR, Meinwald J. 1997. Firefly “femmes fatales” acquire defensive steroids (lucibufagins) from their firefly prey. Proceedings of the National Academy of Sciences. 94(18):9723-9728.

Faust LF. 2017. Fireflies, glow-worms, and lightning bugs: identification and natural history of the fireflies of the eastern and central United States and Canada. University of Georgia Press.

Green JW. 1961. Revision of the species *Pyropyga* (Lampyridae). The Coleopterists Bulletin. 15:65-74.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences. 270(1512):313-321.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17(8):754-755.

Ineichen S, Rüttimann B. 2012. Impact of artificial light on the distribution of the common European glow-worm, *Lampyris noctiluca* (Coleoptera: Lampyridae). Lampyrid. 2:31-36.

Lloyd JE. 1966. Studies on the flash communication system in *Photinus* fireflies. Museum of Zoology. 130:1-100.

Lloyd JE. 1969. Flashes, behavior and additional species of Nearctic *Photinus* fireflies (Coleoptera: Lampyridae). The Coleopterists Bulletin. 23(2):29-40.

Luk S, Marshall S, Branham M. 2011. The Fireflies of Ontario (Coleoptera: Lampyridae). Canadian Journal of Arthropod Identification. [accessed 2018 Nov 13]. Available from http://www.biology.ualberta.ca/bsc/ejournal/lmb_16/lmb_16.html

McInnis J. 2015. The alluring firefly: nature’s lightning bug may hold key to medical breakthroughs. The Guardian. [accessed 2018 Nov 7]. Available from https://www.theguardian.com/environment/2015/aug/19/fireflies-bioluminescence-mating-cancer-tumors

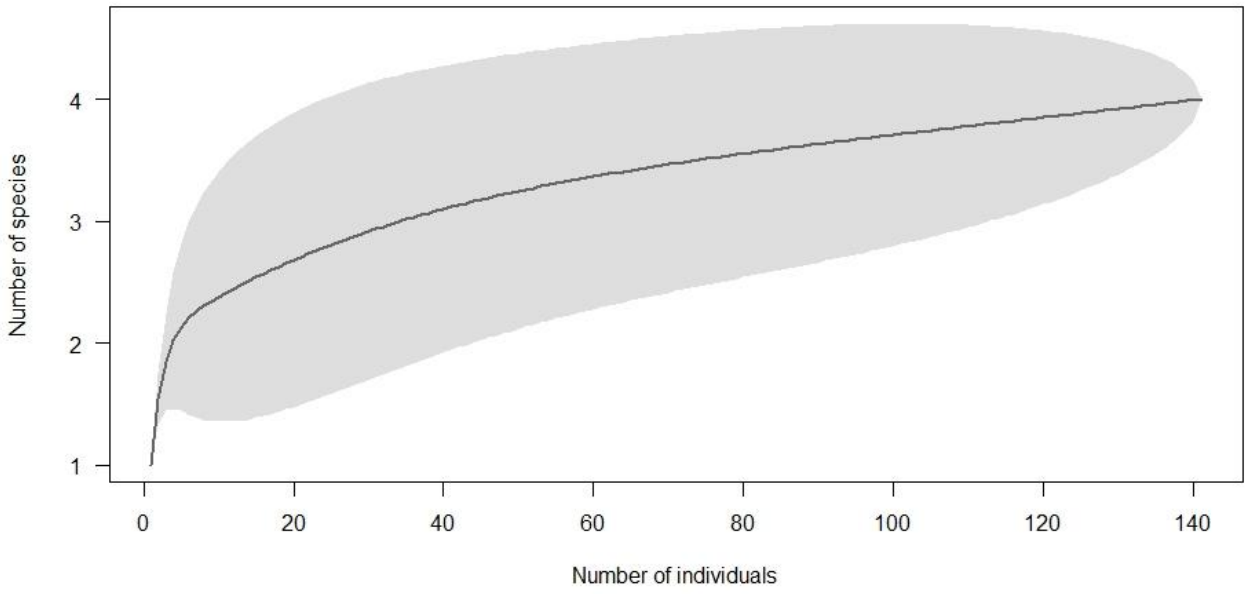
Morariu VI, Srinivasan BV, Raykar VC, Duraiswami R, Davis LS. 2009. Automatic online tuning for fast Gaussian summation. In: Koller D, Schuurmans D, Bengio Y, Bottou L, editors. Advances in Neural Information Processing Systems 21. Curran Associates, Inc. p. 1113-1120. [accessed 2018 Nov 12]. http://papers.nips.cc/paper/3420-automatic-online-tuning-for-fast-gaussian-summation.pdf

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 19(12):1572-1574.

South A, LeVan K, Leombruni L, Orians CM, Lewis SM. 2008. Examining the role of cuticular hydrocarbons in firefly species recognition. Ethology. 114(9):916-924.

Stanger-Hall KF, Lloyd JE, Hillis DM. 2007. Phylogeny of North American fireflies (Coleoptera: Lampyridae): implications for the evolution of light signals. Molecular Phylogenetics and Evolution. 45(1):33-49.

List of Figures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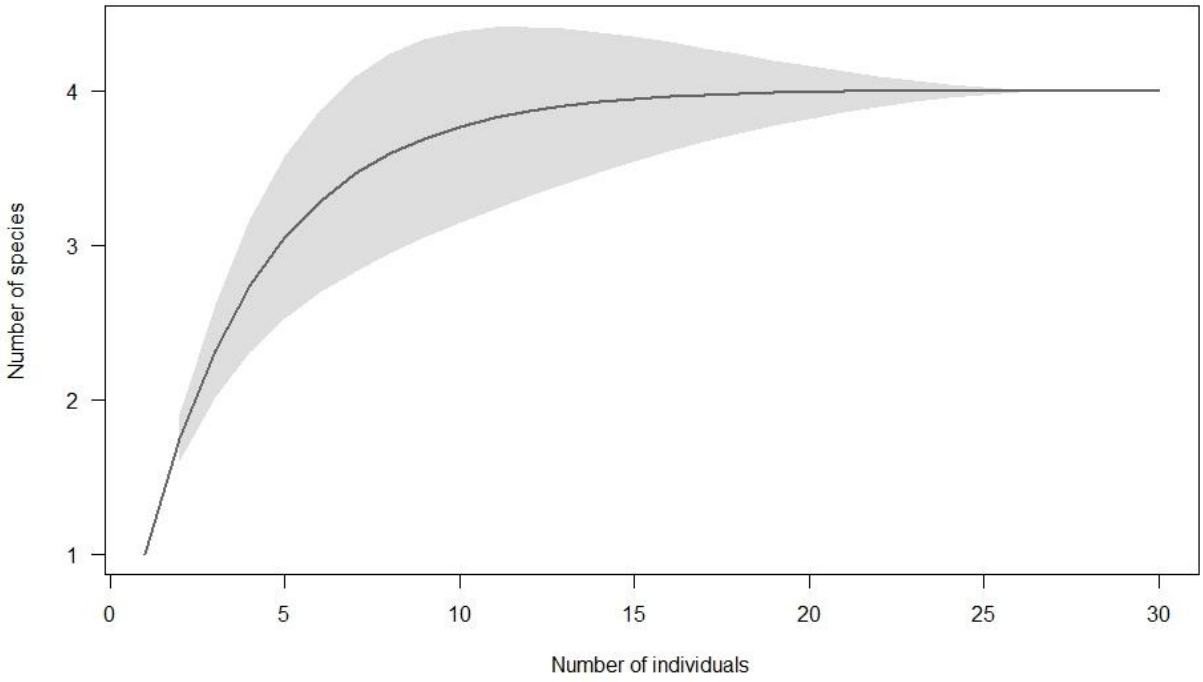
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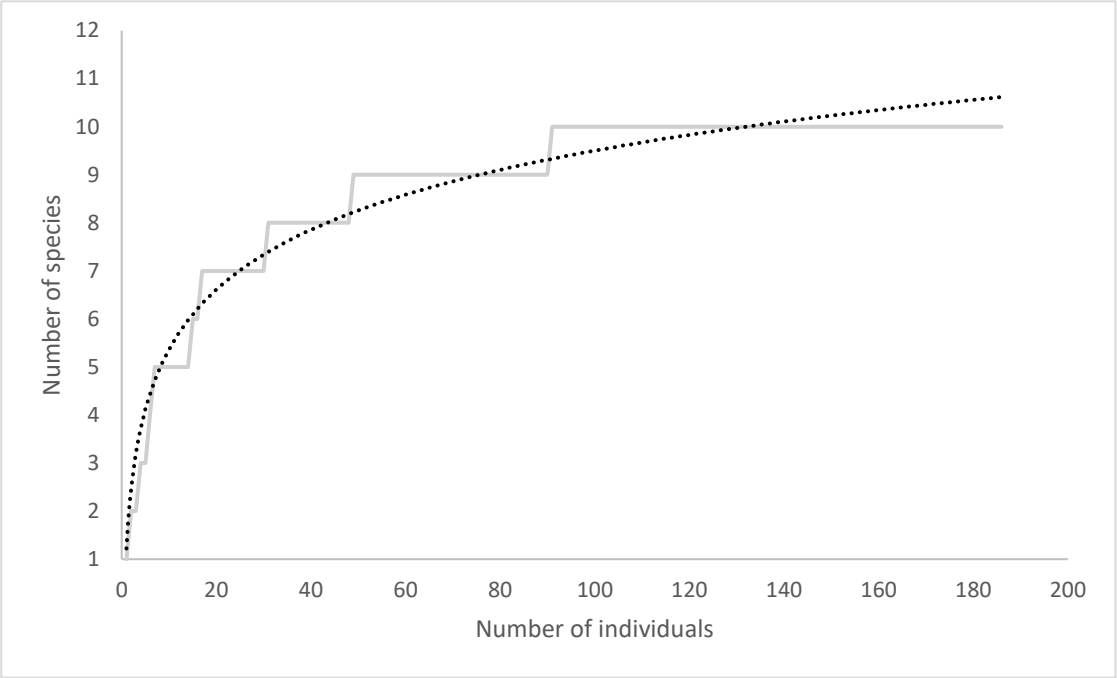
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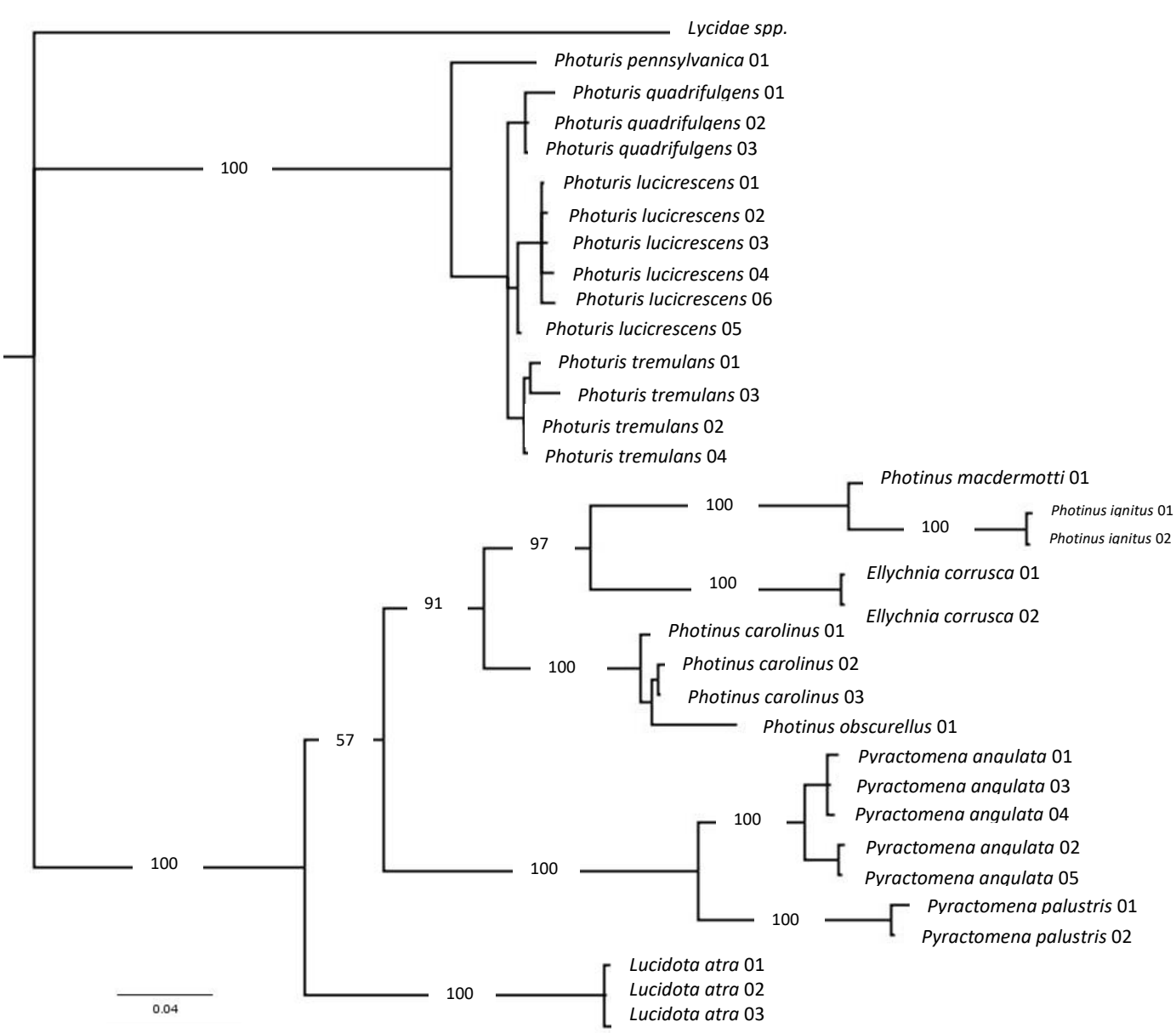
Lycidae spp.
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Photuris quadrifulgens 01
Photuris quadrifulgens 02
Photuris quadrifulgens 03
Photuris lucicrescens 01
Photuris lucicrescens 02
Photuris lucicrescens 03
Photuris lucicrescens 04
Photuris lucicrescens 06
Photuris lucicrescens 05
Photuris tremulans 01
Photuris tremulans 03
Photuris tremulans 02
Photuris tremulans 04
Photinus macdermotti 01
Photinus ignitus 01
Photinus ignitus 02
Ellychnia corrusca 01
Ellychnia corrusca 02
Photinus carolinus 01
Photinus carolinus 02
Photinus carolinus 03
Photinus obscurellus 01
Pyractomena angulata 01
Pyractomena angulata 03
Pyractomena angulata 04
Pyractomena angulata 02
Pyractomena angulata 05
Pyractomena palustris 01
Pyractomena palustris 02
Lucidota atra 01
Lucidota atra 02
Lucidota atra 03
100
100
97
100
100
91
100
57
100
100
100
100
100
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Table 1. Lampyrid genera and number of individuals surveyed at Thayer Farm, Richfield Springs, NY. Individuals were separated to genus based on morphology. Individuals of the genus *Pyropyga* were collected in the summer of 2018.

Genus	Individuals
Photinus	205
Photuris	90
Pyractomena	14
Ellychnia	6
Lucidota	4
Pyropyga	64

Table 2. List of firefly species found in New York. The general range of each species is documented in relation to New York.

Species	Range	Reference
Ellychnia corrusca	Widespread across eastern U.S.	Faust 2017
Lucidota atra	Widespread across eastern U.S.	Faust 2017
Lucidota punctata	Widespread across eastern U.S.	Faust 2017
Photinus ardens	Oneida, NY	Lloyd 1966
Photinus carolinus	Allegheny region of NY	Faust 2017
Photinus consimilis	Eastern U.S.	Faust 2017
Photinus consanguineus complex (*consanguineus, macdermotti, greeni*)	Southern NY	Lloyd 1969
Photinus curtatus	Central NY	Lloyd 1966
Photinus ignitus	Northeastern U.S.	Faust 2017
Photinus indictus	States bordering Great Lakes	Faust 2017
Photinus macdermotti	Eastern U.S.	Faust 2017
Photinus marginellus	Northeast states	Faust 2017
Photinus obscurellus	Northeast states	Faust 2017
Photinus pyralis	Widespread across eastern U.S.	Faust 2017
Photinus sabulosus	Eastern U.S.	Faust 2017
Photinus scintillans	Southeastern NY	Faust 2017
Photuris "Chinese Lanterns"	Southern NY	Faust 2017
Photuris hebes	Eastern U.S.	Faust 2017
Photuris lucicrescens	Eastern U.S.	Faust 2017
Photuris pennsylvanica	Mid-Atlantic states	Faust 2017
Photuris "Primitive Unnamed" -species of *versicolor* complex	Found where other *Photuris* are	Faust 2017
Photuris tremulans	Widespread across eastern U.S.	Faust 2017
Photuris quadrifulgens	Widespread across eastern U.S.	Faust 2017
Pyractomena angulata	Every state east of the Mississippi River	Faust 2017

Pyractomena borealis	Every state east of the Mississippi River	Faust 2017
Pyractomena linearis	States bordering Great Lakes	Faust 2017
Pyractomena lucifera	Eastern U.S.	Faust 2017
Pyractomena marginalis	Eastern U.S.	Faust 2017
Pyractomena palustris	Otsego County, NY	Current study
Pyropyga decipiens	Northern species, northeastern states	Faust 2017
Pyropyga nigricans	All but southeastern U.S., uncommon east of Mississippi River	Green 1961

Table 3. List of New York lampyrid species that were morphologically and molecularly discovered in Otsego County.

Species	Morphological ID	Molecular (N)	Molecular ID(Haplotype)	Accession numbers
Ellychnia corrusca	6	6	3(2)	MK635338-MK635340
Lucidota atra	4	4	4(3)	MK635334-MK635337
Lucidota punctate	0	0	0	
Photinus ardens	2	2	0	
Photinus carolinus	0	0	6(3)	MK635284-MK635289
Photinus consimilis	23	23	0	
Photinus consanguineus complex (*consanguineus, macdermotti, greeni*)	12	12	0	
Photinus curtatus	1	1	0	
Photinus ignitus	164	54	63(2)	MK635224-MK635283
Photinus indictus	0	0	0	
Photinus macdermotti	0	0	71(1)	MK635154-MK635223
Photinus marginellus	0	0	0	
Photinus obscurellus	1	1	1(1)	MK635290
Photinus pyralis	1	1	0	
Photinus sabulosus	2	2	0	
Photinus scintillans	0	0	0	
Photuris "Chinese Lanterns"	0	0	0	
Photuris hebes	0	0	0	
Photuris lucicrescens	0	0	6(6)	MK635295 MK635300 MK635302 MK635308 MK635316 MK635320
Photuris pennsylvanica	0	0	9(1)	MK635293 MK635297 MK635304-MK635306 MK635310 MK635312 MK635315 MK635319
Photuris "Primitive Unnamed" -species of *versicolor* complex	0	0	0	

Photuris tremulans	0	0	10(4)	MK635294 MK635298 MK635299 MK635301 MK635303 MK635307 MK635309 MK635311 MK635313 MK635317
Photuris quadrifulgens	0	0	5(3)	MK635291 MK635292 MK635296 MK635314 MK635318
Pyractomena angulata	0	0	6(5)	MK635321-MK635323 MK635326 MK635329 MK635332
Pyractomena borealis	0	0	0	
Pyractomena linearis	0	0	0	
Pyractomena lucifera	0	0	0	
Pyractomena marginalis	0	0	0	
*Pyractomena palustris**	0	0	7(2)	MK635324 MK635325 MK635327 MK635328 MK635330 MK635331 MK635333
Pyropyga decipiens	**	0	0	
Pyropyga nigricans	**	0	0	

*Species not previously documented in New York but was recovered in Otsego County.

**Genus morphologically identified but was unable to be molecularly verified to species.

Table 4. List of Lampyridae on Thayer Farm Property; SUNY Oneonta Biological Field Station.

Species	**Authority**
Photinus ignitus	Fall, 1927
Photinus macdermotti	Lloyd, 1966
Photinus carolinus	Green, 1956
Photinus obscurellus	LeConte, 1851
Photuris quadrifulgens	Barber 1951
Photuris lucicrescens	Barber, 1951
Photuris tremulans	Barber, 1951
Photuris pennsylvanica	(De Geer, 1774)
Pyractomena angulata	(Say, 1825)
Pyractomena palustris	Green, 1957
Lucidota atra	(G. Olivier, 1790)
Ellychnia corrusca	(Linnaeus, 1767)
Pyropyga spp.	*Pyropyga decipiens* (Harris, 1836) *Pyropyga nigricans* (Say, 1823)

APPENDIX 1

Species key with morphology and flash patterns for Otsego County

GENERA

1. Lantern present; or if absent, yellow stripe on inner margin of wings ……..... 2

   1’. Lantern absent (right) ……..…… 4

2. Large in size (+13 mm) with median stripe on each elytron ……..… *Photuris*

   2’. Small to medium in size (<10 mm) ……...……... 3

3. Dark mark on pronotum is prominent in the center ……... *Photinus*

   3’. Pronotum center and edges are dark; clear median keel ……..…… *Pyractomena*

4. Tiny in size (5-8 mm) ……... *Pyropyga*

4'. Medium to large (>8 mm) ……..…….. 5

5. Distinctively large and serrated antennae ……. *Lucidota*

5'. Normal antennae; yellow and pink parentheses on dark pronotum …… *Ellychnia*

SPECIES

Photinus

1. Light/pale scutellum; narrow body (~3x longer than wide) …… *P. ignitus* (yellow flash once every 5 seconds)

1'. Dark scutellum ……..…… 2

2. Broad body (~2.4x longer than wide) …… *P. macdermotti*
(two yellow flashes that are two seconds apart, then 4 seconds of dark)

2'. Different pronotum pattern (typical dark patch down middle with additional characteristic) ……..… 3

3. Small dark patches on edge of pronotum ……. *P. carolinus*
(Repeating yellow flash, ~6 every half second, then 6-9 seconds of dark)

3'. Maroon, rather than pink, side patches on pronotum …… *P. obscurellus*
(3 quick yellow flashes every 5-7 seconds)

Pyractomena

1. Wide pale margins on elytra …… *P. angulata*
(orange diagonal sputtering flash every 2-4 seconds)

1’. Pale brown elytra with 2 paler ribs down middle …… *P. palustris* (orange crescendo dive every 3 seconds)

Photuris (Identification of species requires flash pattern)

- 2 different flashes; 2 to 5 green flashes every 4 seconds (more common), or half a second green sparkler flash once every 4 seconds …… *P. quadrifulgens*
- Green upward crescendo flash (2 seconds long) every 5 seconds; females aggressively mimic others …… *P. lucicrescens*
- Green, vibrating, 1 second flash every 4 seconds ……. *P. tremulans*
- Dot-dash flash lasting 1-3 seconds, yellow-green (sometimes dot without the dash, and vice versa) …… *P. pennsylvanica*

Lucidota

Long, flatly serrated antennae ……..…… *L. atra*

Ellychnia

Yellow/pink pronotum parentheses; elytra covered in yellowish hairs …… *E. corrusca*

Pyropyga

1. Dark border usually present on pronotum …… *P. nigricans*

1’. Pronotum edges are paler ……..… *P. decipiens*

We would like to thank Lynn Frierson Faust (Fireflies, Glow-worms, and Lightning Bugs), Jay Cossey (PhotographsFromNature.com), Hadel Go, Patrick Coin, and Tom Murray (BugGuide.net) for allowing us to use their photographs in this work.