

Lampyridae of Ohio, a Survey of the Genus *Pyropyga*

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INTRODUCTION

Lampyridae, also known as fireflies or lightning bugs, are a family of beetles (Insecta: Coleoptera) found worldwide. In Ohio there are 31 recorded species in 9 genera. The genus *Pyropyga* is a diurnal group of fireflies of which three species are found in Ohio; *P. minuta*, *P. decipiens* and *P. nigricans* (Marvin, 1965). This genus is distinguished from other genera of *Lampyridae* by their entirely black or piceous bodies (excluding the prothorax), their small body size (usually 3.0-8.5 mm), and by their generally slender and compressed antennae that are approximately half as long as their body (Green, 1961).

Although adults of *Pyropyga* do not have the ability to flash, the larvae are bioluminescent, and this ability is retained into the pupal stage (Brues, 1946).

Due to changing environmental conditions and land-use patterns, it has been anecdotally stated that biodiversity is in general decline. We initiated a survey to test whether firefly diversity has also declined over the past 50+ years with a focus for this project on the genus *Pyropyga*.



Figure 1: *Pyropyga minuta* (♀) dorsal, lateral, and ventral views. Photos by Jordan Reynolds



Figure 2: *Pyropyga decipiens* (♀) dorsal, lateral, and ventral views. Photos by Jordan Reynolds

METHODS

Collection of Samples:

Three Malaise traps (Fig 4) were set up on The Ohio State University's West campus. Malaise traps have been shown to be successful at collecting Lampyridae specimens (Barrows, 2008). Samples were collected from the traps every week starting in early spring and ending in late autumn, 2016. Once collected, bulk samples were stored in 95% ethanol solutions and stored in the freezer to minimize DNA degradation. Insects were then sorted to order and the beetles were sorted to family. Lampyrid specimens were sorted to genus using morphological keys (Luk *et al.*, 2011). Specimens of *Pyropyga* were sorted to morphospecies.

DNA Extraction, Amplification, and PCR:

A total of 32 specimens were pulled and a single leg was removed from each for use in DNA extraction using a DNeasy QIAGEN tissue kit. The Folmer region of the mitochondrial cytochrome c oxidase (Coi) gene was amplified using PCR with the primers C1-J-1718/C1-N-2329 (Simon *et al.*, 1994). Amplicons were submitted for sequences to GENEWIZ Corp.

DNA and Phylogenetic Analysis:

DNA barcoding was used to aid in identification of *Pyropyga* specimens due to the difficult nature of accurate identification without aedeagus dissection. DNA sequences (each about 600bp long) were trimmed and cleaned using the MEGA X program (Kumar *et al.*, 2018). Sequences were compared with each other, and with reference sequences from the Barcode of Life Database (Clark *et al.*, 2015). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

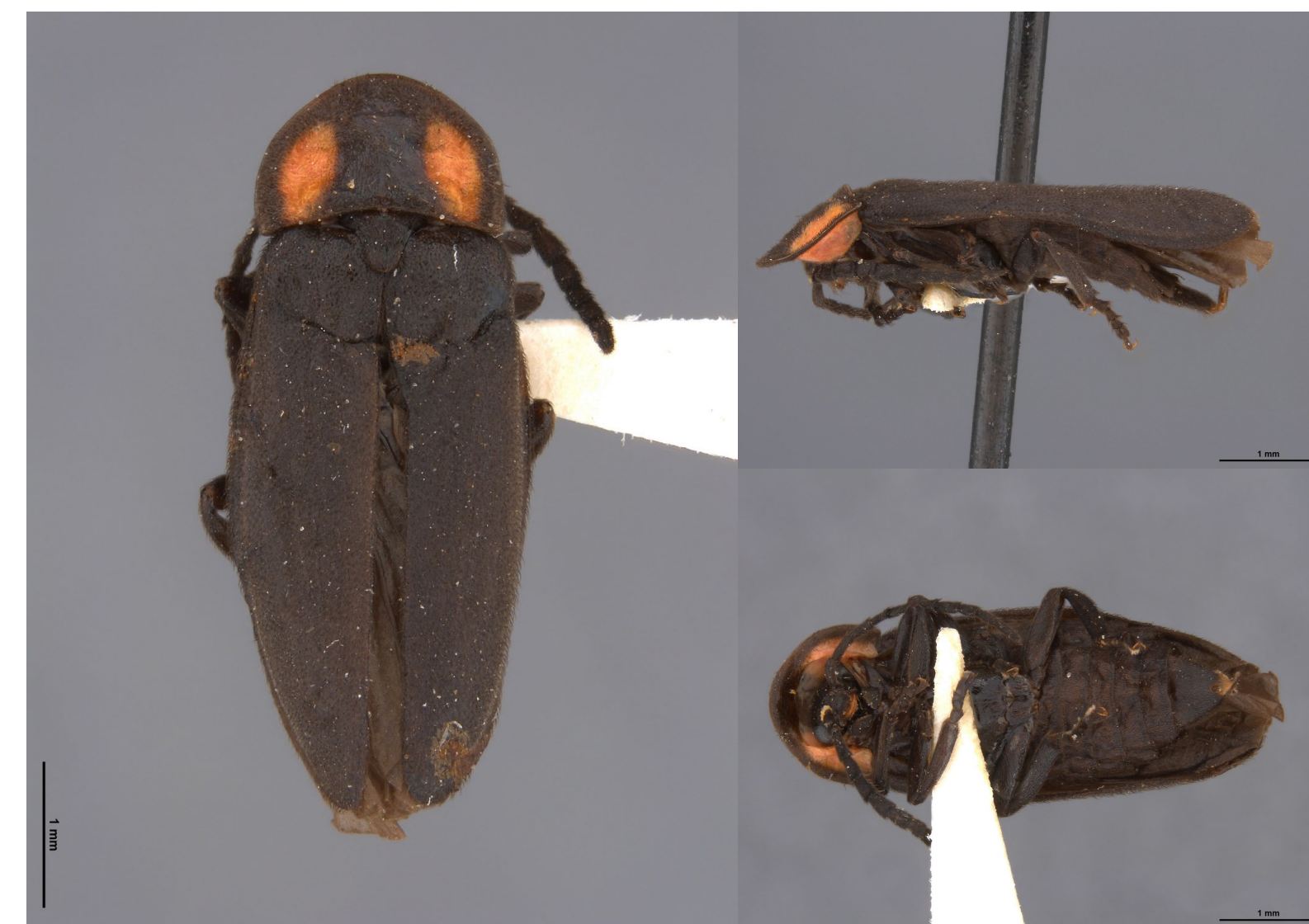


Figure 3: *Pyropyga nigricans* (♀) dorsal, lateral, and ventral views. Photos by Jordan Reynolds



Figure 4: Malaise trap 3 at the Carmack woods, near pond. Photo by M. Drake.

RESULTS

Of the three species of *Pyropyga* recorded in Ohio, only two were found: *P. minuta* and *P. decipiens*. Substantially more *P. decipiens* were collected (192) than *P. minuta* (27). Two additional specimens were separated on the basis of morphology, one of which was identified as *P. decipiens* on the basis of DNA evidence.

Of the 32 *Pyropyga* specimens that went through PCR only 19 specimens had successful DNA amplification, 17 of which were determined as *P. minuta* and 2 as *P. decipiens*.

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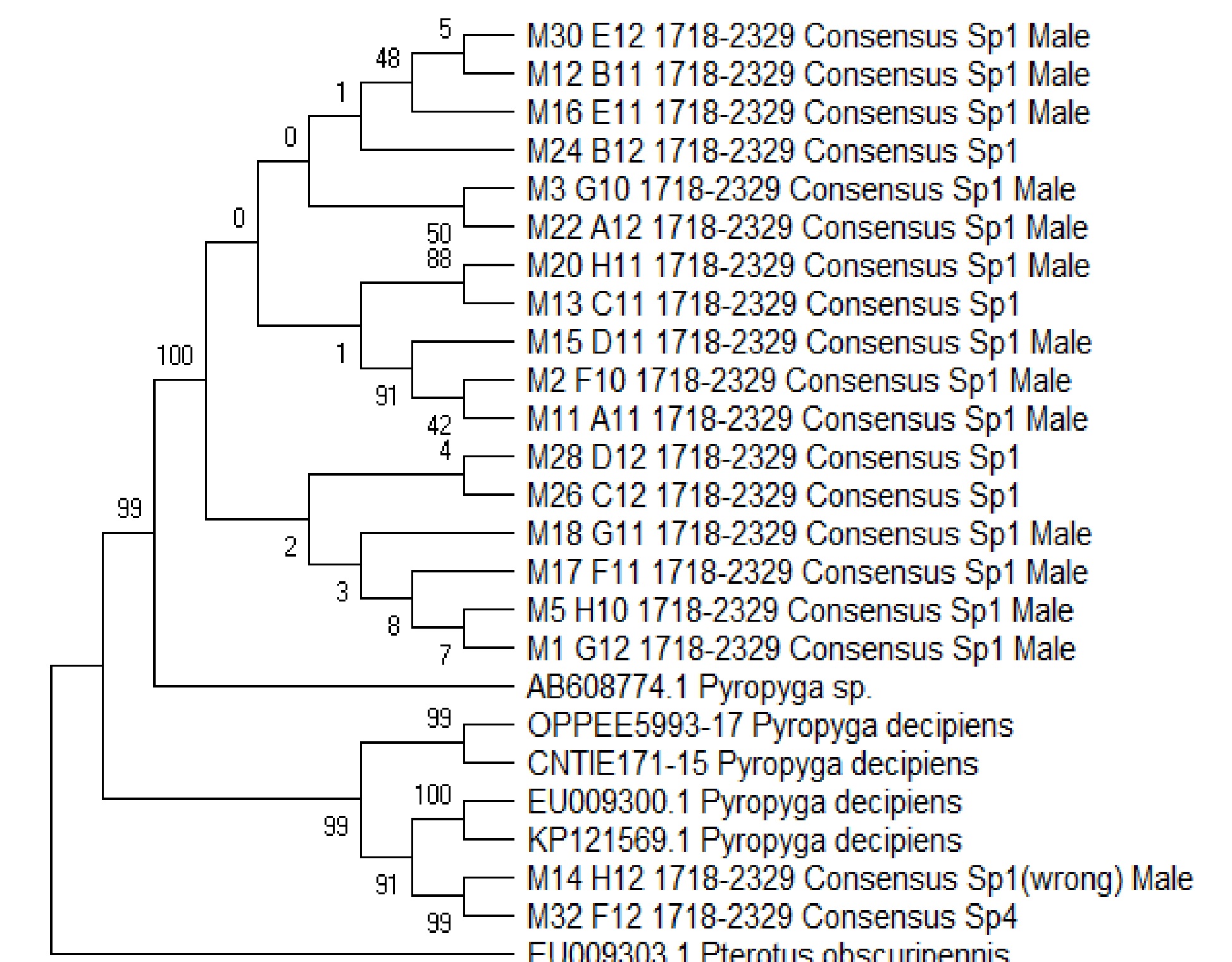


Figure 5: Phylogenetic tree of *Pyropyga* and one *Pterotus*. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Bootstrap values and branching display a distinct grouping of specimens identified as *P. minuta* and *P. decipiens* showing they likely belong to the same species.

CONCLUSIONS

- The majority of *Pyropyga* morphologically sorted to *P. minuta* were genetically similar to each other with an average similarity of 98.2%
- Two *Pyropyga* were found to be genetically similar to *P. decipiens* with an average similarity of 91.2%
- Specimens of *P. nigricans* were not recovered

Analysis will be extended to samples collected in 2017-2018 and new collecting sites will be added in order to further sample *Pyropyga* diversity across Ohio. Two of the three species of *Pyropyga* recorded from Central Ohio over 50 years ago were recovered in this work. The status of the third species is uncertain. *P. decipiens* were the largest majority of *Pyropyga* specimens collected in both this study and the study done in 1965.

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