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A survey of Lampyridae biodiversity, with a focus on *Lucidota*

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INTRODUCTION

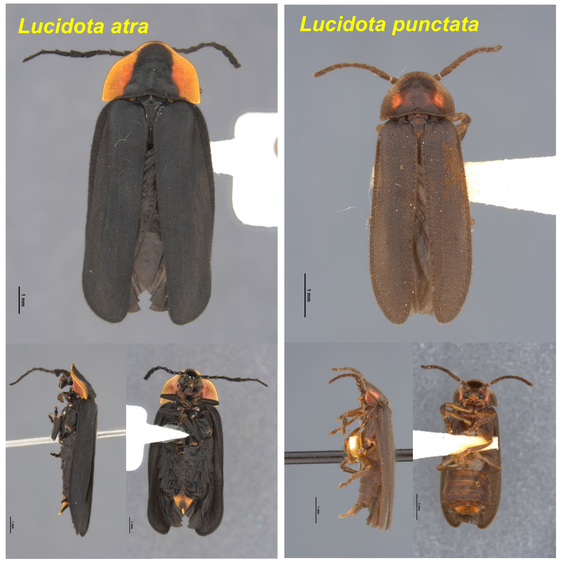
Lampyridae (Coleoptera) is a diverse family of beetles commonly referred to as fireflies or lightening bugs. The last lampyrid diversity survey in Ohio, conducted over 50 years ago (Marvin, 1965), reported 31 species in 9 genera for the state. Recent studies suggest that insect diversity has declined significantly over the last few decades due to compounding human involvement in the environment (Brooks *et al*., 2012). We hypothesize that diversity of lampyrids has decreased as well. To test this hypothesis, a new survey of lampyrids was started in Central Ohio.

The few taxonomic keys available for North American fireflies are applicable only to males; females and larvae often cannot be identified on the basis of morphology.

DNA barcoding offers an alternative means of identification that can be applied to all life stages.

This study focuses on *Lucidota*, a group of poorly studies diurnal fireflies that do not produce light as adult. Member of the genus *Lucidota* can be separated from other local fireflies by the strongly flattened, serrated antennae of the adults. There are three widespread species of *Lucidota* in the United States, but only two have been recorded for Ohio: ***L. atra* (Olivier)** and ***L. punctata* (LeConte)** (Marvin, 1965).

In this study, both morphology and DNA barcoding were used to identify *Lucidota* specimens to species level and to explore the possibility of the existence of cryptic species within the genus.



Dorsal, ventral, and lateral views of female voucher specimens of *Lucidota*.

METHODS

Specimen sampling

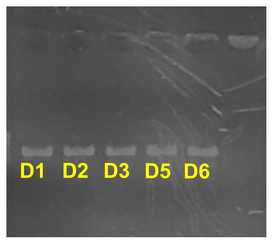
Malaise traps are an effective collecting method for lampyrids (Barrows *et al*., 2008). Three traps (MT1, MT2 and MT3) were set in different habitats around Ohio State's West campus and serviced weekly from spring to fall 2016. Samples were collected in 95% ethanol and kept in freezers (-10ºC). Firefly specimens were identified to species level using available literature (Luk *et al*., 2011; Faust, 2017).

Malaise trap (MT2) near a small stream in the woods, OSU Waterman Farm.

Bulk sample in 95% ethanol.

DNA extraction, PCR, and sequencing

15 specimens were selected for DNA extraction and given a unique identification number. The right hind leg was used for DNA extraction with DNeasy QIAGEN tissue kit. A ~600-bp segment of the cytochrome oxidase I (cox1) gene was amplified. Amplification was performed in a 25 µL reaction solution, using primers C1-J-1718 and C1-N-2329 (Simon *et al.*, 1994). PCR protocol included an initial 95°C for 2 minutes and 35 cycles at 94°C for 30 seconds, 49°C for 30 seconds, and 72°C for 45 seconds, with a final extension period of 5 minutes at 72°C. PCR products were verified by agarose gel electrophoresis.



Electrophoresis gel of *Lucidota* vouchers.

Extracting DNA from voucher specimens.

Successful PCR products were sequenced from both strands with the same primers used for PCR amplification by Genewiz (USA). Resulting DNA sequences were trimmed and manually assembled using the BioEdit software (Hall, 1999).

METHODS, cont.

DNA analysis

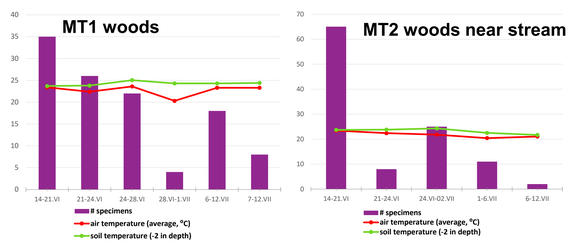
BLAST searches (Altschul *et al.*, 1990) using the NCBI GenBank database were performed for each DNA sequence. The criteria used for identification were: sequence coverage >80%, similarity to species level 98-100% and similarity to genus level 94-98%.

The relationships among sequences were then inferred as a phylogenetic tree in MEGA X software package (Kumar *et al.*, 2018) using the maximum-likelihood algorithm and the Tamura-Nei model. The sequences of *Pterotus obscuripennis* (EU009303), *Pyropyga* (OSUC 247419) and *Photinus* (OSUC 522006) were used as outgroups in the analysis.

RESULTS

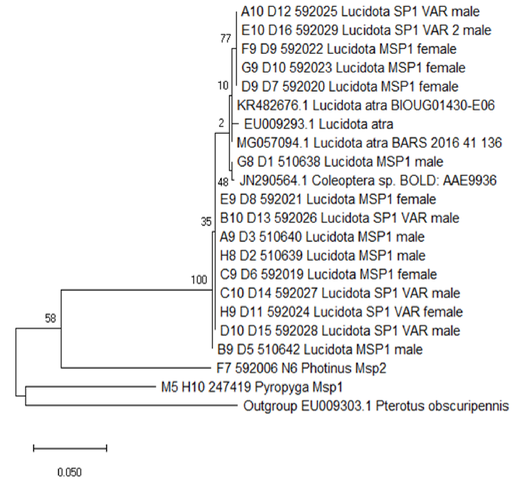
323 specimens of *Lucidota* were collected in 2016 and all were identified as *L. atra* based on morphology. The survey also provided information on the seasonal distribution and abundance of *L. atra* in Central Ohio. MT1 and MT2 collected nearly equal numbers of specimens (113 and 111, respectively), followed closely by MT3 (99).

Results suggest that *Lucidota* flight period starts abruptly in early June and tapers off as the summer progresses.



Number of specimens of *Lucidota* collected per week in each of the three Malaise traps used in the study. **MT1** is surrounded by woods. **MT2** is set in woods near a small stream. **MT3** is set in the Carmack woods near a pond.

Samples OSUC 510638, OSUC 510642, OSUC 592019, and OSUC 592024 are 99% identical to *L. atra* sequences KR485606 (published) and KR482676 (published). In addition, OSUC 510639, OSUC 510640, OSUC 592020-592023, and OSUC 592025-592029 are identical to KR482676 (published) and KR485606 (published).



Phylogenetic tree of *Lucidota*. The bootstrap values and branch length show that *Lucidota* vouchers are closely related and likely belong to the same species, identified as *L. atra*.

CONCLUSIONS

- All *Lucidota* specimens collected were members of the species *L. atra* (avg. 99.7% similar in COI sequences).
- Survey sample did not include *L. punctata*.
- There were no cryptic species present in the sampled population.

More data is needed in order to determine whether *L. punctata* persists in Central Ohio. To address this need, samples collected in 2017 and 2018 will be sorted and new samples will be collected in the same area in the 2019. Malaise traps will be set up earlier in the season (April-May) and will be combined with flight intercept traps.

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