Identification of Closely Related Drosophilids

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Abstract

While Drosophila melanogaster is a model organism for genetic research, however the family Drosophilidae includes many similar species, making it necessary for scientists to accurately identify their specimens. Standard invertebrate primers for DNA barcoding have limited accuracy in identifying closely related drosophilids, so this experiment intended to identify drosophilids using morphological methods and DNA barcoding techniques to find the most accurate method of identification. To make species identifications based on morphology, the app Seek by iNaturalist as well as an anatomical guide were used. For DNA barcoding, a general invertebrate primer and several drosophilid-specific primers were used.

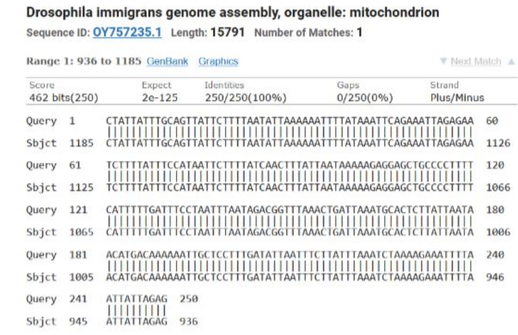
Introduction

- We aimed to gain an understanding of what mode of identification is best to use in the identifying drosophilids, looking at both morphological methods of identifications and DNA barcoding methods
- The morphological methods were the app Seek by iNaturalist and the guide T*he Encyclopedia of North American Drosophilids (Volume 1); Drosophilids of the Midwest and Northeast.*
- To test the accuracy of our DNA barcoding methods we compared a general invertebrate CO1 primer to the drosophilid specific primers Alcohol Dehydrogenase, NADH dehydrogenase and 16s

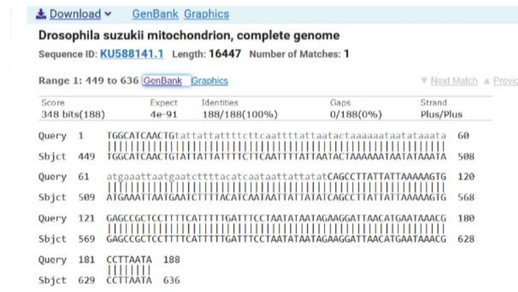
Materials & Methods

- Samples were captured using traps made from plastic bottles, and kept with premade instant drosophila medium
- Specimens were photographed using Seek by iNaturalist; the specimens were then viewed under a dissecting scope and photographed for identification using The Encyclopedia of North American Drosophilids
- DNA was extracted using a silica-based extraction, PCR reactions amplified the CO1 gene, a confirmation of the PCR amplification was done by gel electrophoresis
- Although the bands shown in the gel were extremely faint, samples that seemed to have worked were sent for Sanger sequencing

Sample 3, identified as Drosophila Immigrans



Sample 4: Drosophila Suzukii (100% identity)



Results

Specimen Number	iNaturalist app, first photograph	iNaturalist app, second photograph	Morphological guide	Reasoning for morphological determination
1	Winged insect	Subgenus Sophophora	*Drosophila melanogaster* (f)	thorax color, abdominal patterning
2	Suborder Cyclorrhapha	Winged insect	*Hirtodrosophila duncani* (f) or *Drosophila melanogaster* (f)	Relative thorax to abdomen size ratio, black markings on underside
3	Fly	Subgenus Sophophora	*Drosophila melanogaster* (f) or *Drosophila simulans* (f)	Thorax color, abdominal patterning
4	Suborder Cyclorrhapha	Fly	*Drosophila suzukii* (f) or *Drosophila melanogaster* (m)	Dark ovipositor
5	Winged insect	Acalyptratae (nearest ranked classification: suborder Cyclorrhapha)	*Drosophila suzukii* (m)	Thin dotted wings
6	Winged insect	Suborder Cyclorrhapha	*Drosophila melanogaster* (f)	Light ovipositor, abdominal patterns

Discussion

- iNaturalist identifications showed a high degree of uncertainty and could not distinguish between closely related drosophilids; the morphological guide was easy to use and straightforward, but many species appeared very similar so many identifications were uncertain.
- The barcoding identification technique was mostly unsuccessful. two samples were identified using the NADH dehydrogenase primer. Because the PCR only worked with one primer, the primers could not be compared for accuracy.
- For specimen 3, the barcoding result was not one of the identifications made using the morphological guide. For specimen 4, the result was one of the identifications made using the morphological guide.
- The conclusion can be drawn that there are inaccuracies involved with the use of morphological guides, possibly as a result of human error. For distinguishing very similar species, DNA barcoding is likely the most accurate method.

Sample 4, identified as Drosophila Suzukii

References

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