Reviving Classical Techniques- Staining and its Uses in Modern Taxonomy

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INTRODUCTION

The taxonomy of fireflies and glow-worms (Coleoptera: Lampyridae) is complicated by overlapping diagnoses based on few fast-evolving, inconsistent characters (Roza et al 2022). The paucity of data on most informative structures, such as terminalia and genitalia (Vaz et al 2020), are often transparent, which hinders trait visualization and thus comparisons. Advanced digital microscopy helps to overcome this issue by tinkering with camera parameters (e.g. exposure, gamma). However, the ability of these software to improve visualization is limited, and this equipment can be prohibitively expensive.

Biological staining methods are a well-established set of affordable tools to aid visualizing small structures of different surface structure. Yet, it has fallen out of fashion in the wake of recent advancements, despite its value and practicality in biological imaging. My research aims to revise existing methods for use in Lampyridae taxonomy, as well as to develop new techniques to facilitate and make Lampyrid taxonomy more affordable.

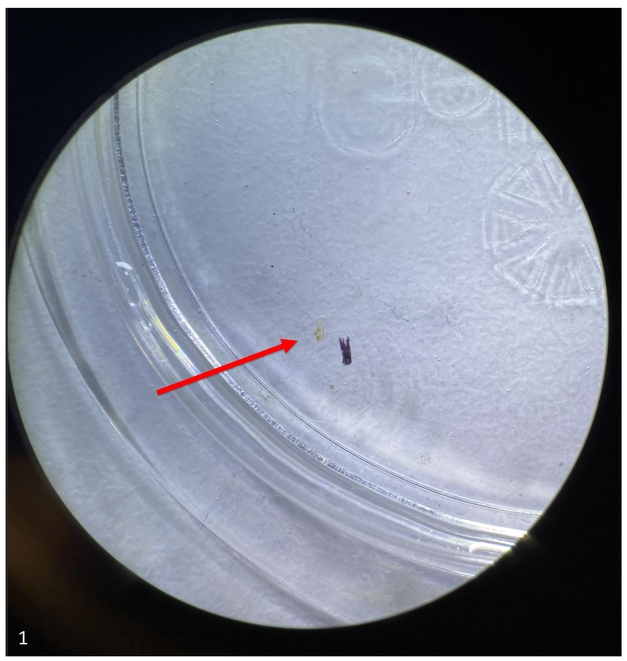


Figure 1: *An unstained Luciola pedemontana aedeagus (indicated with a red arrow) placed next to a stained L. mingrelica aedeagus viewed through the microscope*

METHODS and PRELIMINARY RESULTS

Chlorazol Black E Stain, Acid Fuchsin Anti-stain

Solutions Needed

Chlorazol Black E: 5% m/v dissolved in 70% EtOH

Acid Fuchsin Concentrate: 2% m/v dissolved in tap water

Acid Fuchsin Dilution: 1:10 dilution of concentrate with tap water

Steps:

1) Place structures on a dry plate
2) Add 1 drop of Chlorazol Black E, let sit for **no more** than 10s
3) Rinse thoroughly with water
4) Place into dilute Acid Fuchsin solution 15 minutes for small structures or up to 24 hours for larger structures
5) Rinse thoroughly with water

Stains vs Anti-stains: What are they and why do we use this technique?

Stains and anti-stains are functionally the same, they are used in tandem to create **contrast** between **different types of structures** (ex: to show the boundaries between sclerotized plates and membranes). An anti-stain is simply the **second stain used in a procedure**. For this procedure, **Acid Fuchsin** is considered the anti-stain.

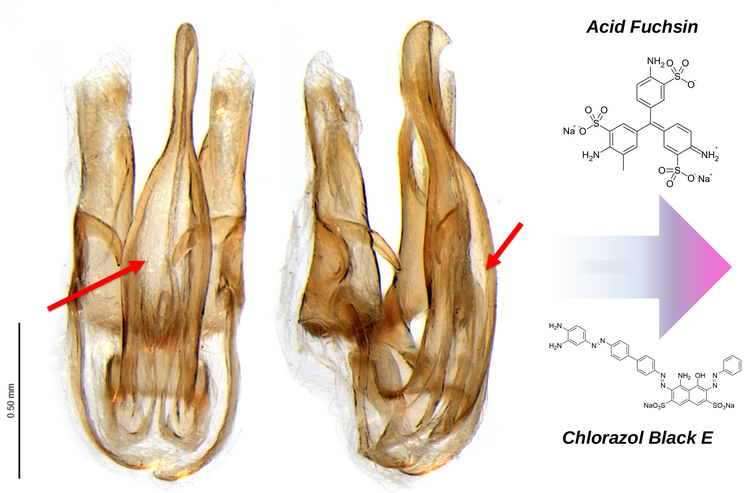


Figure 2: *A Luciola pedemontana aedeagus before and after staining. The bottom right image denotes the phallus separated from the parameres post-staining. Membranous areas defining the ventral plate become more more clearly defined (indicated with red arrows). Scale bars: 0.5mm*

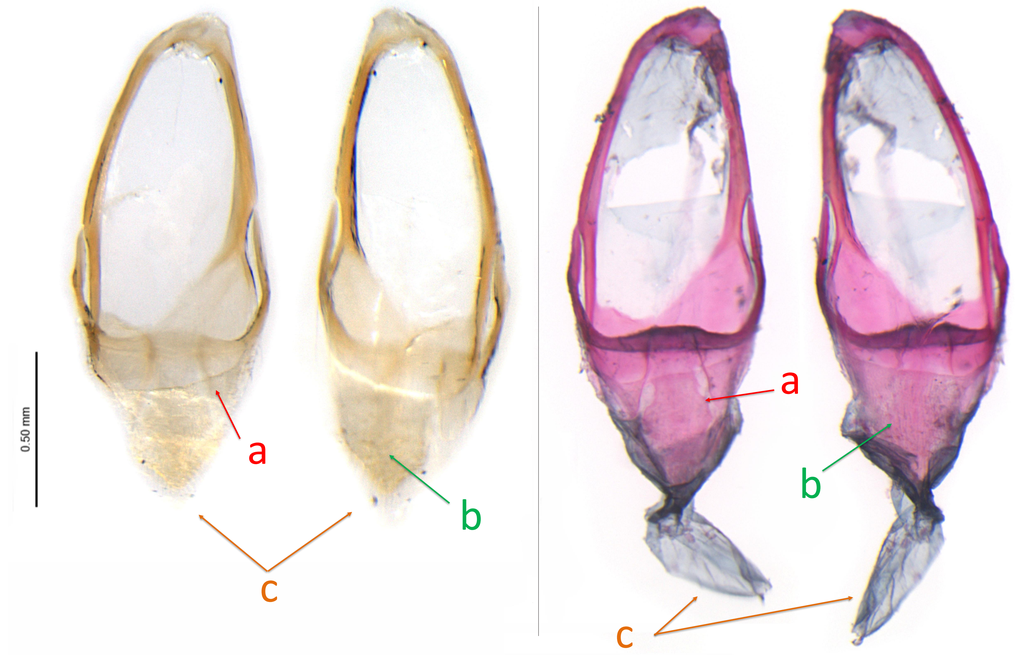
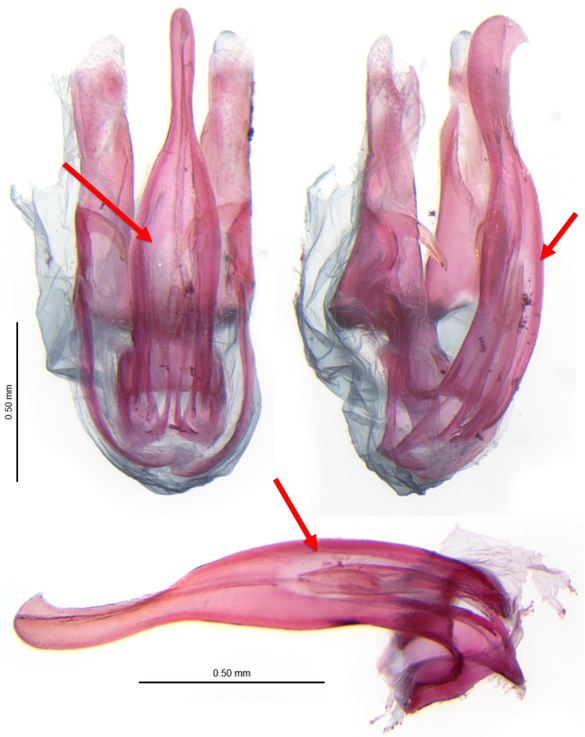


Figure 3: *Luciola italica aedeagal sheath before and after applying stains. Major differences are highlighted including a membranous, disjunct area of the syntergite (a), texture and sensilla on sternum IX (b), and distinct connective membranes attached at the posterior margin (c). Scale bar: 0.5mm*



FUTURE WORK

- Giemsa Procedure
- Methylene Blue
- Calcofluor White
- Congo Red
- Highlighters
- Rit fabric dye

References

Roza, A. S., Mermudes, J. R. M., & Silveira, L. F. L. da. (2022). A New Genus and Two New Species of Fireflies from South America (Lampyridae: Lampyrinae: Photinini). *Diversity*, *14*(11), Article 11

Vaz, S.; Mermudes, J.R.M.; Paiva, P.C.; Silveira, L.F.L. Systematic review and phylogeny of the firefly genus Dilychnia (Lampyridae: Lampyrinae), with notes on geographical range. Zool. J. Linn. Soc. 2020, 190, 844–888.

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