H125 Simplified DNA Barcoding Strategy for Forensically Relevant Blow and Flesh Flies

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Learning Overview: The goal of this presentation is to propose a simplified DNA barcoding strategy for identifying insects commonly encountered in casework at the Harris County Institute of Forensic Sciences (HCIFS).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating a simplified method for DNA barcoding as a tool for medicolegal death investigations.

Accurate insect identification is critical to their use in the estimation of Time Of Colonization (TOC) and Postmortem Interval (PMI) during medicolegal death investigations. Insect specimens are currently identified by evaluating morphologic characteristics as indications of particular taxonomic groups; however, this process is limited because immature life stages typically lack distinguishing morphologies. Identification may be achieved by rearing live specimens; however, this process is time-consuming, labor-intensive, and not always successful.

These deficiencies may be addressed through molecular identification by DNA "barcoding," wherein DNA sequences from unknown samples are matched to references. This technology enables identification of immature specimens, may be performed without specialized forensic entomology training, and requires equipment common to forensic genetics laboratories. DNA barcoding has been demonstrated in numerous entomological surveys of forensically relevant species; however, the technology has not been implemented for medicolegal death investigations. This is due in part to deficiencies in the technology: no single primer set is capable of distinguishing all of the diverse species important to forensic investigations. Instead, multiple primer sets and sequencing reactions are utilized to maximize the species that may be identified.

Proposed here is a simplified DNA barcoding strategy for identifying insects commonly encountered in casework at the HCIFS. The strategy comprises sequencing and phylogenetic analysis of a single barcoding fragment amplified from the mitochondrial COI locus. Using verified reference specimens, this study shows that the DNA barcoding strategy enables statistically supported identification of species previously encountered in the HCIFS medicolegal death investigations, in particular, members of blow fly genera *Lucilia*, *Calliphora*, *Chrysomya*, *Phormia*, and *Cochliomyia*, the flesh fly genus *Blaesoxipha*, and the scuttle fly genus *Megaselia*, and is effective for immature insect specimens, for example, pupal exuvia, larva, and pupa. Identification is demonstrated for immature specimens collected during past HCIFS medicolegal death investigations for which species-level identification was undetermined by morphology. Future work will include elucidating inter-specific and intra-specific sequence variations of local blow and flesh fly populations to provide further statistical support for identifications and validating the DNA barcoding assay for casework application.

DNA Barcoding, Postmortem Interval, Forensic Entomology

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