



H12 A Simplified DNA Barcoding Strategy for Forensically Relevant Blow, Flesh, and Scuttle Flies

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Learning Overview: The goal of this presentation is to demonstrate 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 building competence for DNA barcoding strategies and demonstrate performance of a simplified method designed for medicolegal death investigations. Future work will include the continued collection of local population data and elucidation of respective interspecific and intraspecific sequence variations. In addition, formal internal validations will be conducted to support casework application.

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 morphological 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 because of 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.

The strategy comprises sequencing and phylogenetic analysis of a single barcoding fragment amplified from the mitochondrial **Cytochrome C Oxidase I (COI)** locus. Using verified reference specimens, the DNA barcoding strategy is shown to enable statistically supported identification of species previously encountered in 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*. The strategy is advantageous over previous methods in the literature in that all target species may be amplified using a single primer set. Identification is demonstrated for larva and pupa collected during past HCIFS medicolegal death investigations for which species-level identification was undetermined by morphology. An ongoing project is the construction of a database of COI sequences from local specimens. This may be used to provide additional statistical analyses, in particular, the interspecific and intraspecific sequence variations, enabling comparisons to local blow, flesh, and scuttle fly populations.

DNA Barcoding, Postmortem Interval, Forensic Entomology