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mtDNA polymorphisms for the differentiation of forensically relevant Calliphoridae species

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Abstract

Background: Forensic entomology estimates the minimum post-mortem interval (minPMI) by identifying insect species colonising remains. Within Calliphoridae, closely related taxa (e.g., *Lucilia*, *Calliphora*, *Chrysomya*) exhibit overlapping distributions and limited morphological differentiation, particularly in immature or degraded samples, increasing the risk of misidentification and affecting minPMI estimates [1]. Molecular markers such as COI and CytB are widely used, but sequencing remains time-consuming and costly for routine casework. **Objective:** To identify polymorphic sites in COI and CytB genes across selected Calliphoridae species and select candidate SNPs for developing a rapid, cost-effective SNaPshot assay for species identification. **Methods:** More than 20 sequences per species (*L. sericata*, *L. cuprina*, *L. caesar*, *L. illustris*, *C. vicina*, *C. vomitoria*, *C. albiceps*, *C. megacephala*) were retrieved from GenBank. Sequences were aligned in Geneious and analysed phylogenetically in MEGA12. Multiple sequences per species ensured consistency. Polymorphisms were screened and filtered to retain sites conserved within species and variable between species as candidate SNPs. **Results:** COI showed consistent interspecific variation. The highest polymorphism occurred in *C. albiceps/C. megacephala* (27 sites), followed by *C. vicina/C. vomitoria* (21). Moderate variation was observed in *L. caesar/L. illustris* (5), and minimal in *L. sericata/L. cuprina* (3). These sites were species-conserved and interspecifically variable, supporting their diagnostic use. CytB showed limited variability, with informative sites only in *C. vicina/C. vomitoria* (10), and none in other pairs. **Conclusions:** COI provides robust diagnostic polymorphisms for Calliphoridae species identification, while CytB offers complementary resolution in specific taxa. These results support the development of a targeted SNP panel for rapid, accurate, and cost-effective forensic identification. Additional markers (e.g., ITS2, 28S rRNA, NAD4, NAD6) may improve resolution in closely related species [2,3]. Reliance on public sequences may underrepresent intraspecific variability, highlighting the need for validation with new data. Future work includes specimen collection, DNA sequencing, SNP validation, and design of species-specific tailed primers for SNaPshot, establishing a scalable framework to improve identification accuracy and efficiency in forensic entomology.

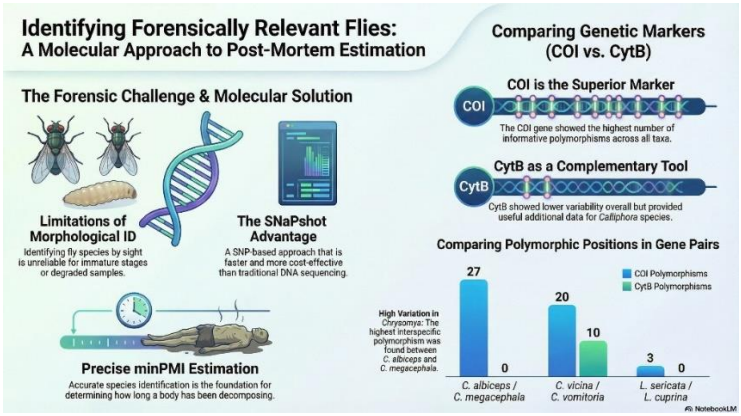

Figure 1. COI and CytB polymorphisms as markers for molecular identification of forensically relevant blowflies.

Keywords: Calliphoridae; forensic entomology; SNP

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