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Cuticular Hydrocarbons Profiling of 1st Instar Larvae for Species Identification of Forensically Important Phorid Flies

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Structured Abstract

Background: Precise identification of immature fly larvae is vital in forensic entomology for calculating the minimum post-mortem interval (PMI_{min}). This study focuses on evaluating the cuticular hydrocarbon (CHC) profiles of 1st instar larvae from two forensically significant fly species, *Spiniphora genitalis* and *Megaselia scalaris*. CHC profiling offers a quick, economically viable option to traditional morphological and DNA-based approaches. However, the reliability of CHC analysis in discriminating closely related species throughout early developmental stages remains understudied.

Methods: The larvae of *Spiniphora genitalis* and *Megaselia scalaris* were reared under controlled laboratory settings to achieve consistent sampling. CHC chemicals were isolated from larvae using hexane. Hydrocarbon compounds were detected via Gas Chromatography-Mass Spectrometry (GC-MS), and Principal Component Analysis (PCA) was used to assess their abilities to differentiate across species.

Results: The GC-MS study demonstrated discrete CHC profiles for each species, consisting of distinguish hydrocarbons. Species-specific hydrocarbons, notably Octadecane, 1-iodo-, Heptadecane, 9-octyl-, 10-Methylnonadecane, Cyclopentasiloxane, decamethyl-, Cyclohexasiloxane, dodecamethyl-, and Dotriacontane, i-iodo- were specific to *S. genitalis*. Meanwhile, hydrocarbon compounds that were identified as specific to *M. scalaris* were Pentadecane, Dodecane, Hexadecane, Cyclohexadecane, and Tetradecane. Nevertheless, overlapping chemical markers constrained the distinction between *S. genitalis* and *M. scalaris* at the 1st instar stage. PCA exhibited an imperfect grouping of the two species, with considerable overlap along the major component. These findings underscore the difficulties of relying solely on CHC profiling for species identification in early larval stages. Despite these constraints, CHC analysis demonstrated cost-effectiveness and rapidity, indicating its potential as an additional tool for forensic investigations.

Conclusion: In conclusion, the findings of this study indicated that CHC profiling offers promising although restricted utility for distinguishing closely related species during the 1st instar stage. Although distinctive hydrocarbons were identified, overlapping profiles diminished precision in PCA. Future research must incorporate other developmental phases, a wider species analysis, and examine the influence of environmental factors on the CHC profiles of 1st instar larvae to ensure reliable and consistent identification across diverse forensic contexts.

Keywords: Forensic Entomology, Cuticular Hydrocarbons, Gas Chromatography-Mass Spectrometry, *Megaselia scalaris*, *Spiniphora genitalis*

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