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Identification of human remains DNA in fly larvae

Margaret Odelia Matulatan [1], Lynda Rossyanti [2,*], Ahmad Yudianto [3] and Nily Sulistyorini [3]

[1] *Undergraduate of Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Department of Medical Microbiology and Parasitology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[3] *Departement of Forensic Medicine and Medicolegal, Dr. Soetomo General Hospital/Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.*

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Abstract

Forensic entomology, a branch of forensic science, plays a crucial role in criminal investigations. While it is commonly used to estimate the post-mortem interval (PMI), it is also serves as a valuable tool for identifying human remains through DNA analysis, especially when traditional methods are not feasible due to decomposition or other causes factor. Necrophagous species, which act as vectors of DNA by feeding on and colonizing remains, are central to this process This review highlights the significant role of fly larvae in forensic investigations, focusing on their capacity to carry DNA from human remains. By examining existing methodologies, forensic applications, and key insect species, this review emphasizes highlight the growing importance of insects in crime scene investigations and their broader implications for forensic science.

Keywords: Forensic Entomology; Fly larvae; Human remains; DNA identification; Necrophagous species

1. Introduction

Forensic entomology is a branch of forensic science that associates arthropods and cadavers in criminal investigations [1,2]. In forensic entomology, insects are most commonly used to determine the estimation of the Post Mortem Interval (PMI), which is the time interval that has elapsed after physiological death until the discovery of the corpse or examination by learning the time of colonization (TOC) [1,3] In addition, insects can also help determine the crime scene's location by studying species distribution, identifying trauma locations, and investigating abuse, neglect, or poisoning [1,2,4]. More than that, along with the development of science, insects can be used as a means for DNA identification because insects that consume biological material of both living and deceased animals or human have been recognized as vectors carrying genetic material [4,5].

These insects, which arrive soon after death, can be divided into four groups, necrophagous species that feed on human remains, predators and parasites that feed on necrophagous species, omnivore species that feed on human remains and other insects, and other species that use human remains as an integrated part of their ecosystem. Of these four groups, the most important in forensic entomology are necrophagous (scavengers) and predators/parasites, especially from the orders *Diptera* (flies) and *Coleoptera* (beetles) [2, 6] and the group most commonly used in forensic entomology are necrophagous species, particularly dipterous flies, as they are the first to arrive and colonize the corpse. These dipterous flies, especially the larvae (maggot) are also the most suitable source of human DNA, as they feed on the biological material of the deceased and carry genetic material that can be analyzed for forensic purposes [5, 6, 7, 8].

Given the role of dipterous flies as reliable DNA vectors, their characteristics make them especially valuable in forensic investigations, particularly in cases when the body has been removed after undergoing partial or full decomposition. In

* Corresponding author: Lynda Rossyanti

such cases, larvae can assist in creating a genetic profile to identify the deceased. Moreover, if insects are absent from the body but present in the surrounding area, or if alternative food sources exist nearby, analysing the collected specimens can reveal whether they fed on human remains. This process helps validate or challenge the evidence, eliminating the possibility of accidental contamination or intentional planting of false entomological evidence. Additionally, in cases involving fragmented bodies, insect analysis can help confirm if the remains found in different locations originated from the same source [5, 9,10].

With the increasing use of forensic entomology in criminal investigations, understanding the various applications of this field is essential. This literature review studies previous literature published to explore the role of necrophagous species, particularly dipterous flies, in forensic science, emphasizing fly larvae as vectors for corpse DNA. It aims to examine how these larvae, which feed on decomposing bodies, carry genetic material that can be extracted for identification. This literature review aims to explore the role of dipterous fly larvae as vectors of human DNA in forensic investigations, emphasizing their utility in DNA identification. By summarizing relevant DNA analysis methods and comparing fly larvae with other necrophagous species, the review evaluates their effectiveness in advancing forensic methodologies.

2. Material and methods

To investigate the use of fly larvae in the identification of human remains DNA, Google Scholar was used as the primary database to collect relevant literature. Keyword-based search terms in one sentence “DNA identification, fly larvae, forensic entomology, human remains” were used in the search. To explore the detailed concept of fly larvae as DNA vectors, additional terms such as “fly larvae,” “human remains,” “forensic entomology,” “DNA identification,” and “necrophagous species” were also used. The articles reviewed varied in focus. Some focused on the DNA identification of human remains through fly larvae, while others examined the broader applications of forensic entomology. By combining the findings of the relevant studies, this literature review aims to present an in-depth analysis of how the identification of human remains DNA in fly larvae can be applied in forensic investigations.

3. Results

The search results on Google Scholar for Identification of Human Remains DNA in Fly Larvae information, a total of 3840 articles were retrieved using “DNA identification, fly larvae, forensic entomology, human remains”. All articles were then reviewed to synthesize the overview of this study. A total of 35 articles were selected for this literature review, providing a comprehensive understanding of the topic. Some of the articles focused specifically on the role of fly larvae in the DNA identification of human remains, while others explored the broader applications of forensic entomology in criminal investigations. Several studies highlighted the importance of necrophagous species, particularly dipterous flies, in carrying genetic material from human remains. This review also discusses the potential of fly larvae as vectors for human DNA and their significance in advancing forensic methodologies.

4. Discussion

4.1. The Role of Fly Larvae as Vectors for Human Remains DNA

Necrophagous species are insects that derive their nourishment from decomposing bodies, with dipterous flies being among the most commonly studied and utilized in forensic entomology. These flies feed and breed on human remains, which allows them to act as vectors for DNA from human remains. For instance, a 2013 case report in Italy [5] described a burned corpse that could not be identified. In this case, larvae from *Calliphoridae* and *Sarcophagidae* flies were used for DNA identification, and the results indicated a paternity probability of 99.685%. Similarly, in 2011, a case in southern China [10] involved a dismembered body, with the head found 500 meters away from the torso, larvae from Aldrichina graham were collected and used for DNA identification to determine whether the body parts were linked using STR profiles, result confirmed that the parts were from the same individual. Larvae also provide a quick and reliable means of extracting DNA from decomposed human remains. Moreover, larvae are less likely to cause accidents or trauma to DNA analysts when compared to more challenging specimens, such as teeth or fingernails, which may not always yield usable DNA. These findings highlight larvae's importance as DNA vectors in forensic investigations[11].

In particular, larvae can be invaluable in the following scenarios:

- When a body cannot be identified due to decomposition or burn damage [5]
- In cases involving fragmented remains, insect analysis can verify if body parts found in different locations originate from the same individual [11]
- To aid in identifying a missing person when maggots are found at a crime scene, even in the absence of a body [12]

Thus, larvae play a crucial role in forensic entomology by facilitating DNA identification, especially when traditional identification methods are not possible.

4.1.1. Important Fly Species

In forensic entomology, necrophagous fly species, particularly from the *Calliphoridae* (blow flies), *Sarcophagidae* (flesh flies), and *Muscidae* (house flies) families, are key to estimating the postmortem interval (PMI) and identifying human remains. These species are typically the first to colonize a decomposing body, arriving within minutes to hours after death due to their attraction to the volatile organic compounds released during decomposition [13]. Among them, *Calliphora vicina, Chrysomya megacephala,* and *Lucilia sericata* are commonly found to be the first to colonize, with their larvae providing critical data for forensic investigations. As the larvae develop rapidly on carrion, they facilitate accurate PMI estimations and enable reliable DNA analysis for identifying human remains [14].

However, environmental factors can alter the expected colonization pattern. In certain ecological settings, less common species may take precedence over the typical necrophagous species. For example, Vasconcelos et al. [15] documented that in a rainforest environment in Brazil, *Hemilucilia segmentaria* and *Ophyra chalcogaster* were among the early colonizers, whereas the more common species like *Calliphora spp.* were less prominent. Similarly, in a mountainous region of Thailand, Monum et al. [16] reported that *Chrysomya pinguis* and *Lucilia porphyrina* were the first to colonize human remains, thriving under specific climatic conditions during the winter.

Furthermore, Zhang et al. [14] highlighted that 91 Dipteran species were mentioned 640 times across forensic case reports, with *Calliphora vicina* (14.5%), *Chrysomya megacephala* (12.0%), and *Lucilia sericata* (11.0%) being the top three species whose developmental data were most frequently applied. This underscores the importance of considering local environmental conditions when identifying the species that are most likely to be the first to colonize a cadaver. Although species like *Calliphora vicina* and *Lucilia sericata* are often the first colonizers, the species that dominate early stages of decomposition can vary based on habitat, temperature, and other environmental factors.

The location of a cadaver, whether in an urban, rural, or concealed environment, can also influence which species arrive first. Zabala et al. [17] found that *Calliphora vicina* was more abundant in urban areas, while *Calliphora vomitoria* was typically found in rural habitats. Indoor environments have been shown to attract fewer species than outdoor locations, with *Calliphora vicina* often being the dominant species in these settings. Reibe and Madea [18] observed that indoor colonization was often delayed, and only *Calliphora vicina* infested pig carcasses indoors. In contrast, outdoor environments supported a more diverse community of flies, including *Lucilia sericata, Lucilia caesar,* and *Lucilia illustris,* indicating that local environmental factors play a key role in determining which species colonize a body first. This pattern was also supported by other studies, such as Centeno et al. [19], who found more species on sheltered carrions during winter experiments, and Caine´ et al. [20], who observed greater species diversity on outdoor cadavers in Portugal. These findings underscore the importance of considering environmental conditions when analyzing insect evidence in forensic investigations.

These studies emphasize that while *Calliphoridae* and other common species are generally the first to colonize a cadaver, environmental factors such as habitat type, temperature, and geographical location can significantly influence which species arrive first. Thus, forensic entomologists must consider these factors when interpreting insect evidence to ensure accurate conclusions about the species involved in the decomposition process.

4.2. DNA Identification

DNA identification is a fundamental process used to analyze and characterize the DNA of individuals or specific organisms. This method involves several key steps: DNA extraction, quantification, amplification, and detection of amplified DNA products [21, 22]. The initial step, DNA extraction, isolates DNA from cells to produce a usable sample. Various techniques are employed to ensure the effective separation of DNA from other cellular components. Once the DNA is extracted, amplification is performed using Polymerase Chain Reaction (PCR), a technique that generates multiple copies of specific DNA segments. PCR operates through three main phases: denaturation (92-95°C), primer

annealing (50-70°C), and extension (72°C). These steps enable the production of sufficient DNA for detailed examination [22, 23].

Several methods are available for analysing the amplified DNA, including Y chromosome analysis, mitochondrial DNA analysis, autosomal Single Nucleotide Polymorphism (SNP) typing, and Autosomal Short Tandem Repeat (STR) profiling. Among these, STR profiling is considered the gold standard in forensic science due to its high accuracy and efficiency [21, 24]. STR sequences, which consist of short repeating DNA units (2-6 base pairs), represent approximately 3% of the human genome. These sequences are highly polymorphic, with variations in the number of repeats among individuals, making them highly reliable for identification purposes [24, 25]. Even with a limited number of loci (5-6), STR analysis can achieve significant differentiation. For example, using FBI-determined allele frequencies, the probability of two unrelated Caucasians having identical STR profiles often referred to as "DNA fingerprints" is estimated at 1 in 575 trillion [24, 26]. STR analysis is highly efficient, requiring only a small quantity of DNA (0.5 to 1 ng), making it particularly useful for degraded samples [26, 27, 28]. To ensure standardization, the FBI has established the CODIS system, which uses 13 core STR loci for forensic applications. National DNA databases further enhance forensic investigations by enabling the comparison of crime scene DNA with potential suspects. These factors highlight the critical role of STR analysis in solving criminal cases and advancing forensic science [24, 25, 26, 27, 28].

4.2.1. STR Analysis in Forensic Entomology

Short Tandem Repeat (STR) analysis has been widely applied in forensic entomology to identify human DNA from fly larvae, showcasing its value in forensic investigations. Chávez-Briones et al. [5], for instance, analysed the gastrointestinal contents of fly larvae to detect human remains, demonstrating the potential of this method. Similarly, Li et al. [10] utilized mitochondrial DNA and STR analysis on maggot crop contents in a forensic case from central-southern China, further highlighting its utility. Zehner et al. [29] advanced this research by focusing on STR typing of human DNA extracted from fly larvae that had fed on decomposing bodies, offering important insights into the role of insects in forensic science. Di Luise et al. [30] also contributed by successfully genotyping human nuclear DNA recovered from the gut of fly larvae. In addition, Oliveira et al. [31] obtained human autosomal DNA and X chromosome STR profiles from *Chrysomya albiceps* larvae, while Chamoun et al. [32] identified Y-STR DNA from larvae fed on a mixture of human semen and ground beef. Lastly, Njau et al. [33] examined the timeframe for effective STR analysis of human DNA from maggots fed on decomposing bodies, shedding light on the temporal dynamics of DNA recovery. Together, these studies underscore the practicality and versatility of STR analysis in forensic entomology, particularly in identifying human remains from fly larvae.

4.2.2. Challenges in DNA Recovery

Although numerous studies have successfully demonstrated the application of STR analysis for extracting human DNA from fly larvae, these outcomes are not always consistent. Various factors can impact the reliability of DNA recovery, as some experiments have failed to detect human DNA in larvae. For example, Sanavio et al. [34] reviewed cases that highlighted the difficulties in extracting "mummified" human DNA from larvae. They proposed that these failures were not solely due to mummification and putrefaction but also to significant post-mortem alterations affecting the corpse's viscera and DNA. Their findings suggested that digestion and degradation of already compromised tissues occur rapidly in the larvae's digestive system, thereby limiting the window for successful DNA recovery.

Similarly, Njau et al. [33] observed that while STR profiles could be generated from maggots fed on decomposing bodies, some alleles were not amplifiable, particularly when the maggots were starved for four days or fed non-human tissues such as beef. These findings underscore the difficulties in consistently recovering human DNA from larvae under varying conditions.

Nonetheless, there is evidence that human DNA can still be retrieved under specific circumstances. For instance, Piunno et al. [35] demonstrated that viable genetic material suitable for human identification could be recovered from the larvae’s gut even after a two-hour fasting period. This suggests a brief window of opportunity for effective DNA extraction shortly after larvae have ingested human tissue.

These studies highlight the complexities of recovering human DNA from larvae. While STR analysis has proven to be a powerful tool in many cases, factors such as environmental conditions, post-mortem changes, and the rapid digestive processes in larvae present significant challenges, making it difficult to achieve consistent results.

5. Conclusion

Fly larvae play an essential role in forensic entomology by serving as reliable vectors for human DNA. Their ability to feed on decomposing remains allows for the extraction and analysis of genetic material, which is particularly valuable in cases where traditional identification methods, such as dental records or fingerprints, are unavailable due to decomposition, burns, or fragmentation. STR analysis of DNA extracted from larvae has proven effective in confirming relationships, linking fragmented body parts, and identifying individuals.

Despite challenges such as environmental factors and post-mortem changes, advancements in DNA identification techniques, including the use of fly larvae, have enhanced the accuracy and reliability of forensic investigations. Future studies should explore optimizing DNA recovery methods from larvae and the impact of environmental factors on DNA quality, aiming to further enhance forensic entomology's capabilities.

Compliance with ethical standards

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Disclosure of conflict of interest

All of the authors declare no conflict of interest to disclose.

Statement of ethical approval

This study is a literature review collecting previous studies therefore no ethical approval required in this study.

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