Alqalam Journal of Medical and Applied Sciences. 2025;8(2):739-743
https://doi.org/10.54361/ajmas.258228

Original article

Identification of Human Remains by DNA Analysis of the Gastrointestinal Contents of Fly Larvae (maggots)

Waed Almegri[1], Nawal Mahfoud[1*], Jalal Alshrayqi[2], Adel Kaweldi[2]

[1]*Department of Zoology, Entomology Unit, Faculty of Science, University of Tripoli, Libya*
[2]*Criminal Investigation Department, Tripoli, Libya.*
Corresponding Email: *nawalmahfud9@gmail.com*

Abstract
Forensic science integrates diverse scientific disciplines to uncover facts related to unexplained events of legal significance. It is a vital component of modern investigations, involving the careful examination of physical evidence from crime scenes or suspects. Forensic biology, a key subfield, focuses on analyzing biological evidence to provide objective data that aids legal decisions and promotes justice. With ongoing scientific and technological advancements, forensic biology has emerged as crucial tool in resolving complex criminal cases. Dipteran fly larvae (maggots) are frequently collected from corpses as part of criminal investigations. Previous studies have demonstrated that DNA analysis of the gastrointestinal contents of maggots can be used to identify victims. Although this method has not yet been practically applied in criminal investigations, its practical utility remains to be validated and assessed. In a documented case, a highly decomposed body was discovered with fly larvae colonizing the facial and neck regions, rendering visual identification impossible. Short Tandem Repeat (STR) analysis was performed on the gastrointestinal contents of the collected larvae, and the resulting genetic profile was compared with that of a presumed father. The analysis revealed a paternity probability of 99%. Accordingly, the comparative DNA test enabled the accurate identification of the human remains. This case represents the first documented instance of victim identification through DNA analysis extracted from the digestive tract of larvae in a criminal investigation. The findings highlight the potential of forensic entomology in advancing forensic identification techniques and expanding its application in future criminal investigations
Keywords. Maggots, Gastrointestinal Contents, STR, DNA Analysis.

Introduction

The identification of human remains constitutes a fundamental component of forensic investigations and becomes increasingly critical in cases involving advanced decomposition or fragmentation of the body. One promising approach involves the analysis of DNA extracted from the gastrointestinal (GI) contents of fly larvae that have fed on human remains [1]. Fly larvae, commonly known as maggots, are often the first colonizers of human remains and can provide a valuable source of genetic material. When human remains are exposed, various species of flies are attracted to the body and deposit their eggs [2] . The newly hatched larvae (maggots) feed on decomposing organic tissues, consuming the body's internal organs and fluids [3]. The larvae's GI tract contains a significant amount of genetic material from the human remains, which can be extracted and analyzed. Researchers have developed techniques to extract high-quality DNA from the GI contents of fly larvae feeding on human remains [4]. The extracted DNA can be amplified using polymerase chain reaction (PCR) and analyzed for genetic markers, such as short tandem repeats (STRs), which are used for human identification[5] ،[6] . Studies have shown that the DNA extracted from fly larvae' GI contents is often well-preserved, even in cases of advanced decomposition or fragmentation of the body. Advantages and Limitations The use of fly larvae GI contents for DNA analysis offers several advantages. It provides a reliable source of genetic material, particularly in cases where conventional methods are unavailable or the quality of biological samples is compromised [2]. Additionally, the GI contents can be easily collected and analyzed, making the process relatively non-invasive. However, the quality and quantity of the DNA extracted can be influenced by factors such as the stage of decomposition, environmental conditions, and the species of fly involved [7]. Proper collection and preservation of the larval samples are crucial to maintain the quality and integrity of the genetic material.

The use of fly larvae, also known as maggots, in forensic investigations has gained significant attention due to their potential to provide valuable information for the identification of human remains. Fly larvae, particularly those found in decomposing bodies, can ingest and retain DNA from the host's tissues, It can subsequently be extracted and analyzed for identification purposes. Previous research has demonstrated that DNA analysis of the gastrointestinal contents of maggots can be used to reveal the identity of a victim. However, this method has not been widely adopted in forensic investigations, and its practical applicability remains under continuous evaluation [3].

In a case report, a body was recovered in an advanced stage of decomposition, exhibiting extensive colonization of the facial and cervical skin by fly larvae. Due to the condition of the body, traditional identification methods were not feasible. Short tandem repeat (STR) typing was performed using the

Received*: 10-02-2025 -* ***Accepted****: 18-04-2025 -* ***Published****: 29-04-2025*

gastrointestinal contents of the maggots collected from the victim, and these were compared to STR profiles obtained from the alleged father.
The probability of paternity was found to be 99.99%, enabling the conclusive identification of the remains. This was the first reported case of using this method to identify a victim in a criminal case [8] .Other studies have explored the use of mitochondrial DNA and STR analyses of maggot crop contents for human DNA identification, demonstrating the potential of this approach[9] . The impact of specimen preservation techniques on DNA analysis has also been investigated. [10] ,[2]. For instance, the best results in preserving DNA within the crop contents of larvae were obtained by storing the larvae without any preservative at a temperature of -70°C. This method facilitated the successful amplification of both mitochondrial DNA (mtDNA) and short tandem repeat (STR) loci [6]. Effective preservation methods also included storing maggots in 70% or 95% ethanol at 4°C or 24°C. These ethanol-based preservation techniques enabled successful mtDNA and STR amplification from the maggot crop contents. In contrast, preservation methods using formalin-containing solutions, such as Kahle's solution and formaldehyde, were found to reduce DNA recovery from maggot crop contents, likely due to DNA degradation[6] . However, surface sterilization of maggots using bleach did not interfere with the ability to extract mitochondrial DNA, suggesting that surface decontamination can be performed without compromising DNA analysis. This study aims to evaluate the effectiveness of obtaining human DNA for STR analysis from fly larvae that have fed on decomposing human corpses at crime scenes. By investigating the potential of maggots as a forensic tool, this research seeks to contribute to the development of alternative methods for human identification in forensic investigations ,[9] [11].

Material and methods

This experimental research was conducted according to ethical rules in the laboratories of the Criminal Investigation Department, Tripoli, Libya.

Maggot and tissue sample collection

The larvae were collected by hand from a decomposed body discovered inside a car at a crime scene. The corpse was with feet and hands missing. Fly larvae extensively colonized the face neck. Due to the advanced decomposition of the body, investigators were unable to determine either gender or approximate age. No physical evidence was found near the remains, except the car where the body was found. the body was so badly decomposed that he was unable to identify it by facial or other physical features. The species of maggots were identified using morphological characteristics. Near-boiling water was used to kill the maggots before they were stored in 70% ethanol at room temperature for later analysis. Three maggots were collected from the victim and were identified by morphological observation as specimens of flesh flies (Diptera: Sarcophagidae). All of the maggots were individually separated into sterile 1.5-mL microcentrifuge tubes and preserved in 70% ethanol at 4°C [12].

Maggot dissection and crop extraction

Maggot samples, Phosphate-buffered saline (PBS). Sterile scalpel or scissors, 1.5 mL microcentrifuge tubes, Vortex mixer, Centrifuge, Phenol-chloroform-isoamyl alcohol (25:24:1). Chloroform, Absolute ethanol, 70% ethanol, TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) [10] .

Sample Preparation

Gently rinse the maggot samples with PBS to remove any surface contaminants. Using a sterile scalpel or scissors, dissect the maggots and collect the gastrointestinal contents into a 1.5 mL microcentrifuge tube.
Cell Lysis was done by adding 500 µL of PBS to the microcentrifuge tube containing the gastrointestinal contents. Then vortex the sample for 30 seconds to homogenize the contents. Add 50 µL of 10% SDS (sodium dodecyl sulfate) and 10 µL of proteinase K (20 mg/mL), Incubate the sample at 55°C for 2-3 hours, or until the tissue is completely lysed. Phenol-Chloroform Extraction: Add an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) to the lysed sample, Vortex the sample for 30 seconds to mix the organic and aqueous phases, Centrifuge the sample at 12,000 × g for 10 minutes to separate the phases. Transfer the upper (aqueous) phase to a new 1.5 mL microcentrifuge tube, to avoid mixing the contents [13] ، [14].

Extraction

Add an equal volume of chloroform to the aqueous phase, Vortex the sample for 30 seconds, and centrifuge at 12,000 × g for 10 minutes. Transfer the upper (aqueous) phase to a new 1.5 mL microcentrifuge tube.

Ethanol Precipitation

Add 2.5 volumes of absolute ethanol to the aqueous phase, and gently mix the sample by inverting the tube several times. Incubate the sample at -20°C for at least 30 minutes to allow the DNA to precipitate. Centrifuge the sample at 12,000 × g for 15 minutes at 4°C to pellet the DNA. Carefully remove and discard the supernatant, being careful not to disturb the DNA pellet.

Received*: 10-02-2025 -* ***Accepted****: 18-04-2025 -* ***Published****: 29-04-2025*

https://doi.org/10.54361/ajmas.258228

Add 500 µL of 70% ethanol to the DNA pellet and gently mix by inverting the tube, Centrifuge the sample at 12,000 × g for 5 minutes at 4°C to wash the DNA pellet, carefully remove and discard the supernatant, being careful not to disturb the DNA pellet, Air-dry the DNA pellet for 5-10 minutes, or until the ethanol has evaporated, Resuspend the DNA pellet in 50-100 µL of TE buffer.
Store the extracted DNA at -20°C or -80°C for long-term storage. This protocol provides a standard method for extracting DNA from the gastrointestinal contents of maggots using the phenol-chloroform extraction and ethanol precipitation technique. The extracted DNA can then be used for various downstream applications, such as PCR amplification and sequencing for identification purposes.
Polymerase chain reaction (PCR) was carried out with DNA extracts from the crop contents and the buccal sample using the commercially available PowerPlex® 18E System (Promega) according to the manufacturer's recommended protocol. Capillary electrophoresis was performed in an ABI 3500 genetic analyzer (Applied Biosystems). Samples were run on a capillary containing POP-4 polymer, and the GeneScan 500 LIZ standard was used for sizing alleles [15,16].

Results

Data were analyzed with Gene Mapper ID analysis software version 1.7 (Applied Biosystems). The genetic profiles generated from the samples are presented in Table 1. The results obtained from the larvae were verified by DNA analysis of the bones coming from the remains, where a comparison between the bone sample and the larva sample was compatible. The amelogenin locus revealed that the maggots were raised on the remains of a female person. Further, this larva sample gave rise to 99 % loci, each of which shared at least one allele with the corresponding STR loci generated from the alleged father. The probability of paternity Paternity test is presumed when genetic tests establish a probability of paternity of 99%. Thus, the comparative DNA test enabled conclusive identification of the remains.

Table 1. Comparative Analysis of Short Tandem Repeat (STR) Profiles from DNA Recovered from Larvae, Bone, and Alleged Father in Forensic Identification

Locus	Victim (Maggots' Samle)		Victim (Bone Samle)		Alleged Father	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D3S1358	16	18	16	18	16	16
TH01	6	8	6	8	6	7
D21S11	30	32.2	30	32.2	28	30
D18S51	14	17	14	17	13	17
Penta E	8	10	8	10	8	12
D5S3818	12	12	12	12	12	12
D13S317	8	11	8	11	11	11
D7S820	10	12	10	12	12	12
D16S539	11	12	11	12	10	11
CSF1PO	9	11	9	11	9	13
Penta D	11	13	11	13	13	13
Amelogenin	X	X	X	X	X	Y
VWA	17	19	17	19	16	17
D8S1179	11	13	11	13	13	15
TPOX	8	11	8	11	8	12
FGA	22	26	22	26	20	26
D19S433	13	15	13	15	13	13
D2S1338	17	17	17	17	17	21

Discussion

DNA genotyping is a highly significant analytical tool in forensic science due to its exceptional levels of accuracy and reliability. This has led to its widespread adoption in the identification of human remains, particularly in cases where conventional identification methods are not feasible. The ideal source material for genotyping should not only be abundant and easy to collect, but also allow the generation of good-quality DNA extracts However, meeting these criteria may be challenging in certain cases, such as with victims whose bodies were subjected to severe trauma or extensive burning it is sometimes difficult to obtain biologic material suitable for genetic analysis.
In this research, we report the identification of a badly decomposed body utilizing the genetic analysis of the gut contents of fly larvae raised therein and their comparison with the genetic profiles of a presumptive relative. STR profiles obtained from the maggots were complete; a total of 18 genetic loci were successfully amplified, which was sufficient to conduct a comparative DNA analysis with the alleged father, enabling a conclusive identification of the remains. It is evident that forensic biological technologies have become an

Received: *10-02-2025* - ***Accepted***: *18-04-2025* - ***Published***: *29-04-2025*

integral part of criminal investigations and are expected to continue playing a vital role in this field. However, the rapid and continuous advancement of these technologies necessitates the constant updating of relevant knowledge by investigative agencies, forensic science professionals, and biologists [17].

In recent years, DNA analysis from the gastrointestinal contents of insects feeding on decomposing bodies has emerged as a valuable tool in forensic investigations, particularly when traditional biological tissues are severely degraded. Insects such as blowfly larvae (Calliphora vicina) ingest human DNA while feeding, making their digestive systems an alternative source for genetic material. Studies have shown that DNA extracted from these insects can remain stable for a reasonable period [16,18], allowing for successful genetic profiling even during advanced decomposition stages. However, careful interpretation is necessary, as environmental factors such as temperature, insect species, and the duration of DNA exposure can influence the quality of the extracted material. This innovative approach significantly enhances the ability of forensic scientists to identify human remains when conventional sampling methods are not feasible [19], [20].

Conclusion

This is the first reported case of analysis of human DNA isolated from the gastrointestinal tract of Diptera fly larvae (maggots) used to identify a victim in a criminal case. In cases where fly larvae are found in association with human remains, investigators may rely on this approach as an alternative method for DNA extraction when recovery from conventional sources is not feasible. Moreover, forensic biology encompasses a suite of scientific disciplines and underlying principles devoted to the analysis of diverse forms of biological evidence, leveraging DNA-sequencing methodologies and other molecular-biotechnology techniques. This progress has facilitated the effective application of DNA evidence both to identify crime perpetrators and to exonerate innocent individuals before they are ever implicated.

Author Contributions

All authors contributed to the study's conception and design. All authors are equal in material preparation, data collection, and analysis. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

All authors for their collective work to reach the final form of the manuscript. And we appreciate anonymous reviewers' helpful comments and revisions.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Gagliano-Candela R, Aventaggiato L. The detection of toxic substances in entomological specimens. Int J Legal Med. 2001;114(4-5):197-203.
2. Wells JD, Stevens JR. Application of DNA-based methods in forensic entomology. Annu Rev Entomol. 2008;53:103-20.
3. Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJ. Best practice in forensic entomology—standards and guidelines. Int J Legal Med. 2007;121(2):90-104.
4. Bruns DE, Ashwood ER, Burtis CA. Fundamentals of Molecular Diagnostics. St. Louis, MO: Saunders-Elsevier; 2007:167.
5. Drábek J. Validation of software for calculating the likelihood ratio for parentage and kinship. Forensic Sci Int Genet. 2009;3(2):112-8.
6. Rubi-Castellanos R, Anaya-Palafox M, Mena-Rojas E, Bautista-España D, Muñoz-Valle JF, Rangel-Villalobos H. Genetic data of 15 autosomal STRs (Identifiler kit) of three Mexican Mestizo population samples from the States of Jalisco (West), Puebla (Center), and Yucatan (Southeast). Forensic Sci Int Genet. 2009;3(3):71-6.
7. Cerda-Flores RM, Budowle B, Jin L, Barton SA, Deka R, Chakraborty R. Maximum likelihood estimates of admixture in Northeastern Mexico using 13 short tandem repeat loci. Am J Hum Biol. 2002;14(4):429-39.
8. Amendt J, Richards CS, Campobasso CP, Zehner R, Hall MJ. Forensic entomology: applications and limitations. Forensic Sci Med Pathol. 2011;7(4):379-92.
9. Dillon N, Austin AD, Bartowsky E. Comparison of preservation techniques for DNA extraction from hymenopterous insects. Insect Mol Biol. 1996;5(1):21-4.
10. Campobasso CP, Linville JG, Wells JD, Introna F. Forensic genetic analysis of insect gut contents. Am J Forensic Med Pathol. 2005;26(2):161-5.
11. Linville JG, Hayes J, Wells JD. Mitochondrial DNA and STR analyses of maggot crop contents: effect of specimen preservation technique. J Forensic Sci. 2004;49(2):341-4.
12. Smith KGV. A Manual of Forensic Entomology. London: British Museum (Natural History); 1986:1-205.
13. Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques. 1991;10(4):506-13.
14. Sambrook J, Russel DW. Molecular Cloning: A Laboratory Manual. 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 2001.

Received*: 10-02-2025 -* ***Accepted****: 18-04-2025 -* ***Published****: 29-04-2025*

15. Smith LJ, Braylan RC, Nutkis JE, Edmundson KB, Downing JR, Wakeland EK. Extraction of cellular DNA from human cells and tissues fixed in ethanol. Anal Biochem. 1987;160(1):135-8.
16. Wells JD, Introna F Jr, Di Vella G, Campobasso CP, Hayes J, Sperling FA. Human and insect mitochondrial DNA analysis from maggots. J Forensic Sci. 2001;46(3):685-7.
17. Puri A, Arya N. Introduction: Forensic Biology. In: Puri A, Mahalakshmi N, Chauhan T, Mishra A, Bhatnagar P, eds. Fundamentals of Forensic Biology. Singapore: Springer; 2024.
18. Zehner R, Amendt J, Krettek R. STR typing of human DNA from fly larvae fed on decomposing bodies. J Forensic Sci. 2004;49(2):337-40.
19. Campobasso CP, Di Vella G, Introna F. Human DNA typing from insects: a review. Int J Legal Med. 2001;114(4-5):202-5. DOI: 10.1007/s004140000192.
20. Singh D, Ballantyne KN. Forensic DNA profiling of blowfly gut contents: a systematic review. Int J Legal Med. 2018;132(3):715-25. DOI: 10.1007/s00414-017-1672-4.

الملخص

علم الطب الشرعي هو تراكم وتطبيق مجال واسع من المعرفة المُجمعة، مُستعار من عدد كبير من التخصصات العلمية، للكشف عن حقائق حول مواقف غامضة تتعلق بأحداث غير مُفسرة، ذات صلة بالنظام القانوني للمجتمع. يُعدّ التحقيق العلمي، وتطبيقه، أو ممارسة الفحص العلمي للأدلة المادية المُجمعة من مسرح الجريمة أو من شخص ذي صلة بالجريمة، أحد أهم جوانب التحقيق وأكثرها ضرورةً في الوقت الحاضر. أما علم الأحياء الجنائي، فهو التحليل العلمي للأدلة البيولوجية لتوفير معلومات موضوعية حول المسائل القانونية. وعلى مر السنين، تطور تطبيق الأساليب العلمية في التحقيق في الجرائم إلى مجال تخصصي متكامل، مثل علم الأحياء الجنائي. وكثيرًا ما تُجمع يرقات ذباب ثنائيات الجناح (اليرقات) من الجثث كجزء من التحقيقات الجنائية. وقد أثبتت الدراسات السابقة إمكانية استخدام تحليل الحمض النووي لمحتويات الجهاز الهضمي لليرقات لتحديد هوية الضحايا. ومع ذلك، لم يُطبّق هذا النهج بعد في التحقيقات الجنائية، لذا لا تزال فائدته العملية غير مُختبرة. في حالة مُبلّغ عنها، عُثر على جثة متحللة، وقد استعمرت يرقات الذباب وجهها ورقبتها. حالت حالة الجثة دون تحديد هويتها. أُجري فحص التكرارات القصيرة المترادف (STR) على محتويات الجهاز الهضمي لليرقات المأخوذة من الضحية، ومقارنتها بملف التكرارات القصيرة المترادف (STR) لأب مُشتبه به. وُجد أن احتمال الأبوة 99%. وهكذا، مكّن اختبار الحمض النووي المُقارن هذا من تحديد هوية الرفات. تُمثل هذه الحالة أول حالة مُوثّقة لتحديد هوية الضحية من خلال تحليل الحمض النووي المُستخرج من الجهاز الهضمي لليرقات في تحقيق جنائي. تُسلّط هذه النتائج الضوء على إمكانات علم الحشرات الجنائي في تطوير تقنيات تحديد الهوية الجنائية وتوسيع نطاق تطبيقه في التحقيقات الجنائية المستقبلية.

Received*: 10-02-2025 -* ***Accepted****: 18-04-2025 -* ***Published****: 29-04-2025*