

Article

Assessment of Human DNA and Y-DNA Quantity in Blow Fly Larvae Cultured on Human Blood: Evaluation of Utility for Identification and Forensic Reporting in Sexual Offense Cases

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Abstract

Background/Objectives: Forensic entomology remains an underutilized discipline within forensic medicine, particularly in Poland, where it is primarily applied to post-mortem interval (PMI) estimation. Recent studies indicate that insect-derived material may also hold value in the identification of human remains. **Methods:** In this pilot study, we assess whether blow fly larvae fed on human blood retain amplifiable human DNA, including Y-DNA. Larvae were reared on blood obtained from four volunteers and collected at the third instar stage seven days after oviposition. Human DNA quantification, degradation assessment, and STR/Y-STR profiling were performed. **Results:** Despite the deliberately small, exploratory sample size, all larval samples yielded complete and concordant STR and, where applicable, Y-STR profiles matching the respective reference donors. **Conclusions:** These preliminary findings indicate the potential utility of larvae as an alternative biological substrate in forensic contexts, particularly when conventional tissues are unavailable or degraded. However, the results should be interpreted cautiously and require validation in larger, systematically controlled studies before any routine forensic application can be recommended.

Keywords: decomposing bodies; blow fly larvae; human blood; STR; Y-STR; forensic entomology

1. Introduction

The identification of human remains in an advanced stage of decomposition presents a major challenge in forensic medicine. Progressive autolysis, loss of recognizable anatomical features, environmental exposure, and the degradation of genetic material often impede conventional identification methods and require specialized analytical approaches [1–5]. As soft tissues deteriorate, the likelihood of obtaining complete genetic profiles decreases, especially under conditions of high temperature, humidity, or prolonged environmental exposure.

Forensic entomology provides an important complementary tool in such cases. Necrophagous insects, particularly blow flies (*Diptera*: Calliphoridae), rapidly colonize decomposing remains, and their developmental stages are routinely used to estimate the post-mortem interval (PMI) when the time since death exceeds 72 h [6–8]. Blow fly larvae feed on liquefied tissues, re-ingesting material previously digested by their own enzymes, which allows human DNA to accumulate in the larval gut [9,10]. Although DNA degradation may occur, multiple studies have demonstrated that human genetic material recovered



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from larvae can support identification efforts [11]. In certain situations—particularly when conventional soft tissues are absent—larvae may represent the only accessible biological substrate. Previous reports have also documented the recovery of Y-DNA from larvae collected in natural body openings, suggesting potential relevance in cases involving sexual assault [12].

Despite its demonstrated value internationally, forensic entomology remains underutilized in routine casework in Poland. Its integration into forensic investigations is limited, yet the potential of insect-derived material warrants further study, particularly regarding its suitability for genetic analysis.

The aim of this pilot study is to evaluate whether third-instar blow fly larvae reared on human blood retain detectable and amplifiable human DNA, including Y-DNA. We quantified total human DNA, assessed degradation indices, and performed autosomal STR and Y-STR profiling. Resulting profiles were compared with reference samples from four volunteers. The goal is to preliminarily assess whether larvae may serve as an alternative source of genetic material in circumstances where conventional tissues are unavailable or degraded.

2. Materials and Methods

2.1. Materials

The isolation of human DNA from blow fly larvae does not require species identification. In the DNA isolation process, obtaining pure and intact genetic material is crucial, and the species of the larvae is not of primary importance. Third-instar larvae of various species can be used for isolation because DNA isolation procedures are universal and applicable to many types of organisms [13–15].

The research material (for DNA analysis) consisted of third-instar larvae of blow flies obtained from the established culture. The comparative material was venous blood collected from four volunteers (three women and one man) who gave informed consent for the study. All individuals were 24 years old and had higher education. The volunteers also consented to the publication of the research results.

The study received positive approval from the Bioethics Committee of the Pomeranian Medical University in Szczecin. Informed consent was obtained from all study participants. All studies were conducted in accordance with relevant guidelines/regulations.

The electrophoregram datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Experiments on blow flies were conducted in accordance with relevant guidelines and regulations.

2.2. Culture

Four sterile containers were carefully labeled with numbers (samples were anonymized): B1–B4.

A 1 cm layer of peat soil was placed at the bottom of each container to maintain moisture, and the peat was moistened with sterile distilled water. Then, 50 g of fresh ground pork was weighed, formed into a ball, and placed on the peat layer. Each meatball was injected with 3 mL of blood collected from study volunteers. The prepared samples were covered with a mesh gauze with openings large enough for adult blow flies to pass through to lay their eggs (Figure 1).



Figure 1. The moment of colonization of the culture by the blow fly (day 1, own source).

Pork muscle tissue was used as the feeding substrate for larval development because it is widely recognized and validated as an appropriate proxy for human soft tissue in entomological research. Numerous experimental studies employ porcine tissue due to its comparable biochemical composition, ethical accessibility, and suitability for standardized laboratory work. In our study, the pork substrate was injected with human blood to ensure a controlled and reproducible source of nutrients for the larvae, thereby reducing variability in feeding material and limiting the influence of uncontrolled decomposition processes. The purpose of this model was not to recreate natural decomposition, but rather to isolate the specific variable of human DNA ingestion and assess its detectability and integrity within the larval gut for downstream molecular analysis.

The samples were placed in the morgue room at a temperature of approximately 20 °C and left for 7 days (in the Department of Forensic Medicine at PUM, next to an open window). The culture was observed daily until the third-instar larvae were obtained (7 days) (Figure 2). The time required to reach this stage is species-specific; for example, for *Calliphora vicina*, it takes 3–5 days from egg laying under favorable temperature conditions. The larval culture was maintained in a standard morgue room with natural airflow, including an open window. This setup was intentionally chosen to approximate real-world forensic conditions rather than to create a strictly controlled laboratory environment. The aim of the study is not to investigate developmental kinetics or species-specific thermal responses but solely to assess the presence, quantity, and integrity of human DNA and Y-DNA in larvae after feeding on human blood. Therefore, minor environmental fluctuations were deliberate, serving as a realistic simulation of typical conditions under which forensic entomological samples are collected. Such variations do not affect the outcome variables, as human DNA quantification and STR/Y-STR profile concordance are known to be highly robust within the range of indoor temperature fluctuations.



Figure 2. Culture—third-instar larvae (day 7, own source).

The obtained third-instar larvae were transferred to sterile Falcon tubes using sterile tweezers, labeled, and euthanized by freezing at -80°C .

In genetic analyses conducted on blow fly larvae, the factor that primarily determines the effectiveness of human DNA isolation is not the insect species but the developmental stage of the larva. Third-Instar Larvae are of particular importance, as their advanced feeding stage and the largest volume of ingested material result in the highest efficiency in providing amplifiable amounts of human DNA. This means that, in the context of forensic applications, obtaining larvae at the correct developmental stage is crucial, whereas species-level differences among Calliphoridae are of secondary relevance to the process of isolating and analyzing human genetic material [11,16].

Larval Instar Identification

The third instar stage was confirmed using standard morphological criteria observed under a stereoscopic microscope (Carl Zeiss Microscopy GmbH, Oberkochen, Germany). Larvae were classified as third instars based on the presence of three distinct posterior spiracular slits, a fully developed peritreme, increased body size, and the characteristic shape and sclerotization of the cephalopharyngeal skeleton, following established entomological guidelines. Additionally, the crop (vole) was clearly visible through the semi-transparent cuticle, which is typical of actively feeding third-instar larvae. These morphological markers are sufficient to reliably determine larval instar irrespective of species.

During sample collection, differences in larval size were qualitatively observed, particularly between individuals originating from female and male donor material. However, larval morphometry was not quantified, as assessing size variation was not part of the experimental design. The study focuses exclusively on evaluating the presence, quantity, and integrity of human DNA and Y-DNA in larvae after feeding. For this reason, no standardized morphometric measurements were collected, and size variation was not included as an analytical variable.

2.3. Preparation of the Material for Research

Dead larvae were thoroughly rinsed (on a rotary stirrer) for 5 min with a ready-made labZAP solution (A&A Biotechnology, Gdańsk, Poland), which removes enzymes with DNase and RNase activity and residual nucleic acids. It also deactivates bacteria and viruses. Next, the larvae were rinsed three times with deionized water and drained on sterile gauze (Figure 3). This procedure was intended to prevent contamination with exogenous DNA.



Figure 3. The process of rinsing larvae in distilled water (own source).

The cleaned whole larvae were first cut into smaller fragments and subjected to homogenization using a hand homogenizer (Figure 4), maintaining sterile conditions at every stage.

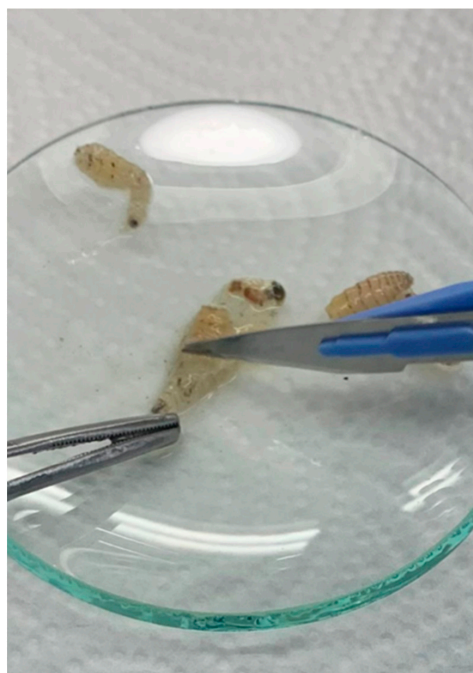


Figure 4. Preparation of larvae for the homogenization process (own source).

2.4. Isolation of DNA from Third-Instar Larvae of Blow Flies

Isolation of DNA from third-instar larvae of blow flies was performed using the Maxwell FSC DNA IQ Casework Kit (Promega Corporation, Madison, WI, USA), following the manufacturer's protocol. Negative controls were included in the extraction process. The Maxwell[®] FSC DNA IQ[™] Casework Kit is specifically designed for efficient extraction of DNA from forensic samples with low DNA yield. In a single instance, 5 larvae were used for isolation.

2.5. Isolation of DNA from Blood Collected

Isolation of DNA from blood collected from volunteers was performed using the Maxwell RSC Blood DNA Kit (Promega), following the manufacturer's protocol. Negative controls were included in the extraction process.

2.6. DNA Concentration Measurement and PCR Inhibition Evaluation

The Quantifiler Trio DNA Quantification Kit (Thermo Fisher Scientific, Waltham, MA, USA) and the Quant Studio 5 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) were used to evaluate the concentration of human DNA and the presence of PCR inhibitors. A CT value of ≥ 31 was adopted as the criterion for the presence of PCR inhibitors, in accordance with the manufacturer's instructions and internal method validation.

2.7. Amplification of STR and Y-STR Markers

Autosomal STR markers were amplified using the GlobalFiler[™] PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA, USA), while Y-STR markers were amplified using the Y-filer[™] Plus PCR Amplification Kit (Thermo Fisher Scientific, Waltham, MA, USA). The reactions were carried out according to the manufacturer's instructions.

DNA degradation was determined by considering locus dropout and peak height ratio. The values were compared to the sum of peak heights at each locus, using a reference value of 100 RFU.

2.8. Analysis of Results

Product detection was performed on a 3500 Genetic Analyzer using the GeneScan[™] 600 LIZ[®] (Thermo Fisher Scientific, Waltham, MA, USA) size standard, following the manufacturer's protocol. The results were then analyzed using GeneMapper ID-X v1.6 software.

Statistical Approach

Due to the intentionally small sample size and the exploratory, proof-of-concept design of the study, no inferential statistical tests were performed. The primary objective was to determine whether human DNA and Y-DNA can be detected in larvae fed on human blood and whether full STR/Y-STR profiles can be obtained, rather than to evaluate population-level variability or interindividual differences.

3. Results

The results of human DNA concentration and DNA degradation are presented in Tables 1 and 2.

Table 1. Results of human DNA concentration isolated from third instar blow fly larvae (examined samples B1–B4).

Sample Number	Biological Sex	Sample Type	Amount of DNA Present (ng/ μ L)	T. Large Autosomal	T. Small Autosomal	Amount of Y-DNA Present (ng/ μ L)	Degradation Index (ID)
B1	Female	blow fly larva	17.0258	23.8143	23.7013	unknown	3.4779
B2	Female	blow fly larva	1.1360	27.3085	28.3593	unknown	1.3959
B3	Female	blow fly larva	0.2053	31.6383	31.3029	unknown	2.3304
B4	Male	blow fly larva	0.7087	28.4398	29.1712	28.4119	1.5567

Table 2. Results of human DNA concentration isolated from blood sample (examined samples P1–P4), reference material.

Sample Number	Biological Sex	Sample Type	Amount of DNA Present (ng/ μ L)	T. Large Autosomal	T. Small Autosomal	Amount of Y-DNA * Present (ng/ μ L)	Degradation Index (ID)
P1	Female	blood	26.0944	19.8400	22.0981	unknown	0.8779
P2	Female	blood	14.5604	20.8229	23.0658	unknown	0.8623
P3	Female	blood	22.2392	19.9872	22.3633	unknown	0.8142
P4	Male	blood	22.3246	20.1972	22.3569	21.8737	0.9223

* Y chromosome DNA concentration (male DNA concentration).

3.1. Descriptive Statistics for Blow Fly Larvae Samples

For the larval samples, we calculated minimum, maximum, and median values for total human DNA quantity and degradation indices to illustrate the variability across the four specimens. Total human DNA quantity (ng/ μ L) values ranged from 0.2053 to 17.0258 ng/ μ L, with a median of 0.9224 ng/ μ L. Degradation index (DI) values ranged from 1.3959 to 3.4779, with a median of 1.9436.

3.2. Descriptive Statistics Summary

To improve quantitative data presentation, we calculated and reported basic descriptive statistics for the total human DNA quantity and degradation indices obtained from the four samples.

For total human DNA quantity (ng/ μ L), the values ranged from 14.5604 to 26.0944 ng/ μ L, with a median of 22.2819 ng/ μ L.

For the degradation index (DI), values ranged from 0.8142 to 0.9223, with a median of 0.8701.

3.3. Interpretation of the Degradation Index

The interpretation of degradation index (DI) values in this study follows thresholds commonly applied in forensic genetics. The criterion of $DI < 10$ as indicative of low to moderate degradation originates from manufacturer validation guidelines of widely used quantification systems and from routine forensic laboratory practice, rather than from a universal biological threshold. These validation studies consistently demonstrate that samples within this range typically yield full or near-full STR profiles.

PCR inhibition was assessed using the internal IPC/ICQ metrics and CT thresholds provided by the quantification system. All samples fell within acceptable ranges, and no additional inhibition testing was required for this proof-of-concept study.

Given the small sample size and exploratory nature of the present work, DI values should be interpreted with caution and are not intended to support population-level conclusions about DNA degradation in larval tissues. Instead, they serve as contextual indicators reflecting the general condition of the recovered DNA.

Importantly, despite variability in DI values across the larval samples, all specimens produced complete and concordant STR and Y-STR profiles when compared with the reference material. This empirical outcome demonstrates that the level of degradation observed in the larvae did not impede downstream genetic analysis and supports the suitability of blow fly larvae as an alternative biological substrate for human DNA profiling.

Sample B1 has the highest DNA concentration at 17.0258 ng/ μ L, indicating a substantial amount of human DNA was isolated from this larva.

Samples B2, B3, and B4 have significantly lower DNA concentrations, with B3 having the lowest at 0.2053 ng/ μ L. The T. Large Autosomal and T. Small Autosomal values represent the quantification of large and small autosomal DNA fragments, respectively. All samples show a similar pattern where T. Large Autosomal and T. Small Autosomal values are close to each other. This suggests consistent amplification efficiency for both large and small DNA fragments within each sample.

The Degradation Index (ID) is a measure of DNA degradation, with higher values indicating more degraded DNA.

Sample B1 shows the highest ID at 3.4779, suggesting significant degradation of DNA. Sample B3 also shows considerable degradation with an ID of 2.3304. Samples B2 and B4 have lower IDs of 1.3959 and 1.5567, respectively, indicating better-preserved DNA compared to B1 and B3.

In the case of reference material, an ID value below unity is expected, indicating that the material is not degraded. Such results were obtained for the blood samples (P1–P2) in Table 2.

As a result of amplifying autosomal markers using the Global Filer (Thermo Fisher Scientific, Waltham, MA, USA), a human identification system, three complete profiles were obtained, suitable for further comparative studies (Table 3). These profiles included two female profiles and one male profile. Sample B3 yielded a partial profile in three loci (Table 3). The average DNA concentration in this sample was 0.2053 ng/ μ L, with an ID of 2.3304 (Table 1). Such a result could be attributed to the presence of potential PCR inhibitors and DNA degradation.

Table 3. Amplification of STR markers—blow fly larvae.

Locus	B1	B2	B3	B4
D3S1358	15,17	15	-	16,17
vWA	15,17	17,18	-	15,17
D16S539	12,13	9,12	-	11,12
CSF1PO	10	10,13	-	10,12
TPOX	8,10	9,11	-	9,10
INS/DEL	-	-	-	2
AMELO	XX	XX	-	XY
D8S1179	13,14	13,15	14,15	13,14
D21S11	30.2,32.2	29,30.2	-	29,34.2
D18S51	14,16	12,17	-	17,19
DYS391	-	-	-	10
D2S441	10,14	11.3,14	10,14	11.3,14
D19S433	14,15	15,16	11,13	13
TH01	6,9.3	6,9.3	-	7,9.3
FGA	19,22	20,22	-	22,23

Table 3. *Cont.*

Locus	B1	B2	B3	B4
D22S1045	16	11,16	-	15,17
D5S818	12,13	12,13	-	10,14
D13S317	10,11	11,12	-	8,12
D7S820	8,13	8,12	-	9,12
SE33	24.2,28.2	14, 28.2	-	12,22
D10S1248	14	13,14	-	13,14
D1S1656	17.3,18	11,12	-	13,14
D12S391	20,23	18,23	-	15,18
D2S1338	17,20	24,25	-	21,23

In the case of the reference material (blood samples), four complete DNA profiles were obtained (three female profiles and one male profile) as shown in Table 4. Sample P3 matched sample B3 in three loci (Table 3).

Table 4. Amplification of STR markers—blood sample.

Locus	P1	P2	P3	P4
D3S1358	15,17	15	15,17	16,17
vWA	15,17	17,18	16,17	15,17
D16S539	12,13	9,12	11,13	11,12
CSF1PO	10	10,13	10,11	10,12
TPOX	8,10	9,11	10	9,10
INS/DEL	-	-	-	2
AMELO	XX	XX	XX	XY
D8S1179	13,14	13,15	14,15	13,14
D21S11	30.2,32.2	29,30.2	31.2, 33.2	29,34.2
D18S51	14,16	12,17	12,16	17,19
DYS391	-	-	-	10
D2S441	10,14	11.3,14	10,14	11.3,14
D19S433	14,15	15,16	11,13	13
TH01	6,9.3	6,9.3	6,9.3	7,9.3
FGA	19,22	20,22	19,24	22,23
D22S1045	16	11,16	11,16	15,17
D5S818	12,13	12,13	9	10,14
D13S317	10,11	11,12	11,12	8,12
D7S820	8,13	8,12	10,11	9,12
SE33	24.2,28.2	14, 28.2	19,23.2	12,22
D10S1248	14	13,14	14,16	13,14
D1S1656	17.3,18	11,12	15,18.3	13,14
D12S391	20,23	18,23	17,20	15,18
D2S1338	17,20	24,25	17,18	21,23

The analysis of Y-STR markers was conducted on only one tested sample. Among the study volunteers, there was only one man. In the analysis of Y-STR markers using the Y-Filer Plus system (ThermoFisher Scientific), a male profile was exclusively obtained for sample P4 (Table 5). This profile was identical to the profile obtained from the tested sample B4 (Table 5).

Table 5. Amplification of Y-STR markers—blood sample (P) and blow fly larvae (B).

Locus	B4	P4
DYS576	17	17
DYS389I	13	13
DYS635	22	22
DYS389II	30	30
DYS627	21	21
DYS460	10	10
DYS458	16	16
DYS19	17	17
Y GATAH4	11	11
DYS448	20	20
DYS391	10	10
DYS456	14	14
DYS390	21	21
DYS438	11	11
DYS392	11	11
DYS518	41	41
DYS570	17	17
DYS437	14	14
DYS385	18,19	18,19
DYS449	32	32
DYS393	14	14
DYS439	13	13
DYS481	26	26
DYF387S1	37,19	37,39
DYS533	11	11

4. Discussion

During the experiment, it was observed that the flies were reluctant to colonize the samples. The low degree of colonization observed in our experimental setup suggests that rearing blow fly larvae directly on human blood may present practical challenges. This outcome should be interpreted with caution, as the feeding conditions used in the study were artificial and may not fully reflect natural behavior. Human blood, unlike decomposing soft tissue, can dry or coagulate rapidly, potentially reducing its accessibility as a sustained food source. In natural settings, blow fly larvae feed on moist, decomposing tissues with a different physical structure and more stable nutrient availability. Additionally, maintaining suitable temperature and humidity is essential for successful larval development, and

deviations from optimal conditions or microbial growth within the substrate may have affected larval feeding efficiency in this model [17–19].

Our analyses are pilot studies, and although they were conducted on a small number of samples, they demonstrate that blow fly larvae show the potential to retain human DNA. Blow flies are among the first insects to visit locations with human remains, especially in advanced stages of decomposition. They can colonize the body as well as fragments of tissues or biological traces such as blood, thereby consuming human DNA [7,8]. By feeding blow fly larvae human blood, we demonstrate that human genetic material can be recovered from larval tissues under controlled conditions. These preliminary findings indicate the potential for obtaining human DNA from larvae collected at crime scenes or from locations where human remains are discovered, although further validation in real-case environments is required. This observation is speculative and requires controlled studies. This is important for better understanding how flies behave in natural environments and what traces they might leave at a crime scene. These analyses also involve developing and testing new methods for detecting and analyzing biological traces. To our knowledge, there are few studies describing STR analysis using blow flies. Comparable experimental studies have not yet been reported from Poland. However, international research has examined the time window for obtaining successful STR profiles from third-instar larvae collected from decomposing human remains, as well as the degradation dynamics of human DNA in larvae fed on animal tissue. These studies consistently demonstrate that larvae can retain human DNA, although its concentration decreases progressively with the time elapsed since feeding [12]. In our study, we provide an assessment of the amount of DNA recovered from the cultivation of human blood. Both studies agree that blow fly larvae serve as a source of human material. Up until now, the analysis has focused on the gut contents of lice [20], mosquitoes [21], and necrophagous flies [11] and their potential in forensic analysis.

In the case of DNA concentration isolated from the larvae, the highest value recorded was 17.0258 ng/ μ L, while the lowest was 0.2053 ng/ μ L. These values allowed us to obtain three complete genetic profiles suitable for comparative studies. A partial genetic profile was obtained for sample B3 (Table 3). Out of 24 loci, only 3 loci were amplified in this sample. However, this sample had the lowest DNA concentration among those tested. Additionally, it was observed in the culture that larvae raised on this sample were significantly smaller. The differences in DNA concentration between samples could reflect variations in larval size, developmental stage, or differences in DNA extraction efficiency; however, no biological interpretations can be drawn from these preliminary observations. Higher DNA concentrations (like in B1) are generally advantageous for downstream genetic analysis, providing more material for accurate identification and profiling. Lower concentrations (such as in B3 and B4) might still be usable but could pose challenges in achieving reliable genetic profiles, potentially requiring more sensitive techniques or higher sample volumes. In forensic applications, the quantity and quality of DNA extracted are critical for accurate identification and profiling [9,11,12]. The presence of measurable DNA in all samples suggests that extraction protocols were generally effective, but the varying concentrations highlight the need for consistency and careful interpretation in forensic settings. Differences in the amount of DNA between larvae isolated from individuals of male and female sexes may prompt further research into whether sex influences DNA quantity, potentially impacting the reliability of genetic profiling. Understanding if there are consistent differences in DNA yield based on sex could have significant implications for forensic and genetic studies. Higher DNA concentrations generally enhance the robustness and accuracy of genetic profiles, making them more reliable for identification purposes. Conversely, lower DNA yields might necessitate more sensitive techniques or larger sample

volumes to achieve comparable results. Research into sex-based variations in DNA quantity could involve investigating biological factors such as hormone levels, metabolic differences, or cell turnover rates, which may influence DNA extraction efficiency. Additionally, considering the potential impact of these variations on the accuracy and reproducibility of genetic profiles is crucial for forensic applications, where precision and reliability are paramount. Such studies are needed on a significantly larger sample size. The presence of measurable DNA in all samples suggests that extraction protocols were generally effective, but the varying concentrations highlight the need for consistency and careful interpretation in forensic settings.

Recent studies, which are quite intriguing, have focused on analyzing DNA from blow fly larvae to determine phenotypic traits of living individuals. Researchers evaluated forensic DNA phenotyping, predicting human appearance. Analyses were conducted on living individuals, similar to our study. Scientists utilized the larvae's ability to clean wounds [22].

Undoubtedly, the gut contents of blow fly larvae can provide information on whether they were fed human tissue [23,24]. Furthermore, such analyses can assist in linking the development stages of insects up to the time of death, aiding in the determination of the post-mortem interval (PMI). In the case of DNA analysis, it can be determined whether the insects fed on human remains or biological material, which helps establish a connection between the insect and the victim.

A fascinating aspect is the recovery of human Y-STR from blow fly larvae. This is crucial evidence in forensic investigations, especially in cases involving sexual offenses. Many women worldwide fall victim to rape, and their bodies are often found in an advanced state of decomposition. In such cases, vaginal secretions, which are crucial for semen analysis, are often difficult to obtain. This results in the loss of valuable evidence that could convict the perpetrator. Researchers have drawn interesting conclusions based on their studies, where they cultivated larvae on a mixture of meat and semen. They successfully established the Y-STR profile [25]. Other researchers conducted similar studies using an animal model (female pig, *Sus scrofa*) that was euthanized in a wooded area with three gunshot wounds. Subsequently, semen was introduced into the anus and vagina to simulate rape and murder. The larvae that colonized these areas allowed for the recovery of Y-STR profiles [25,26]. We can say that the referenced studies align with ours. We also recovered a full Y-STR profile identical to the reference material (Table 5).

In summary, the data presented here illustrate the variability in DNA concentrations obtained from blow fly larvae and highlight factors such as larval size, developmental stage, and extraction efficiency that may influence DNA recovery. These observations underline the importance of careful interpretation and optimization of protocols when working with insect-derived material in forensic genetics. While preliminary, this study contributes to the growing body of research exploring the potential of necrophagous larvae as alternative sources of human DNA in situations where conventional tissues are unavailable or degraded. Further work on larger sample sets and under more realistic forensic conditions is required to better understand the applicability and limitations of this approach.

Forensic entomology integrates elements of biology, ecology, and forensic science and is increasingly recognized as a valuable complementary tool in selected types of criminal investigations. Its established applications, particularly in post-mortem interval estimation, have supported casework in many jurisdictions. As research in this field advances, the potential use of insect-derived material in forensic analyses may continue to expand, although further validation and standardization are needed before broader implementation.

5. Conclusions

Our findings show that larvae cultured on human blood retained detectable amounts of human DNA, and in three of the four samples (B1, B2, and B4), the DNA quantity and integrity were sufficient to generate complete STR and Y-STR profiles concordant with the reference material. Although sample B3 yielded only a partial profile, the loci that amplified matched the corresponding reference profile. These results are preliminary and based on a small number of samples; therefore, validation on a larger dataset and under more diverse conditions is necessary before drawing broader conclusions about reliability or forensic applicability.

Forensic entomology is well established in many countries as a complementary tool in legal investigations. In Poland, however, its routine application remains limited. Increasing awareness among investigators and forensic practitioners regarding the collection, potential utility, and limitations of entomological material may support more informed integration of this evidence type into forensic assessments.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (Bioethics Committee of the Pomeranian Medical University in Szczecin, approval no. KB-006/45/2024, approval date: 26 June 2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The data supporting the findings of this study are available upon request from the corresponding author, in accordance with the MDPI Research Data Policies (<https://www.mdpi.com/ethics>, accessed on 13 November 2025).

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