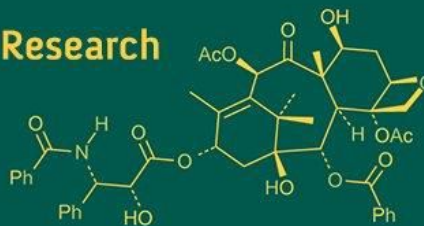


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; 10(1): 559-564
www.biochemjournal.com
 Received: 25-10-2025
 Accepted: 28-11-2025

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Forensic identification of *Chrysomya megacephala* from a hanging cadaver using mitochondrial COI gene sequencing

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1g.7025>

Abstract

Accurate identification of cadaver-associated insects is a critical component of forensic entomology, particularly for reliable estimation of the post-mortem interval (PMI). In the present study, molecular identification of an insect specimen collected from a hanging human body (F1-Hanging) was carried out using mitochondrial cytochrome oxidase I (COI) gene-based DNA barcoding. Genomic DNA extracted from the sample showed a sufficient concentration (116 ng/μl) and high quality, enabling successful PCR amplification and sequencing of the COI region. The amplified COI sequence (653 bp) exhibited 98.93% similarity with *Chrysomya megacephala* reference sequences in the NCBI database based on BLAST analysis. Phylogenetic reconstruction using the Neighbor-Joining method and Jukes-Cantor distance model further confirmed the clustering of the F1-Hanging sample within a well-supported *C. megacephala* clade. The concordant results obtained from sequence alignment, BLAST analysis, and phylogenetic clustering validate the reliability of COI-based molecular identification. The findings highlight the effectiveness of DNA barcoding as a complementary tool to traditional morphological methods, particularly in forensic cases where specimens may be damaged, immature, or morphologically ambiguous, thereby strengthening the application of molecular approaches in forensic entomology.

Keywords: Forensic entomology; DNA barcoding; Cytochrome oxidase I (COI); *Chrysomya megacephala*; Molecular identification; Post-mortem interval

Introduction

Forensic entomology applies entomological evidence to medico-legal investigations, particularly in estimating the post-mortem interval (PMI), reconstructing events surrounding death, and providing corroborative evidence in criminal cases. Traditionally, insect species identification has relied on morphological characteristics, but this approach can be limited when specimens are immature, damaged, or morphologically ambiguous. To address these challenges, molecular methods have increasingly been incorporated into forensic workflows. Sperling, Anderson, and Hickey (1994) [5] pioneered the application of DNA-based identification for insects in PMI estimation, demonstrating that genetic tools could improve accuracy when morphological traits are inadequate.

Among molecular markers, the mitochondrial cytochrome c oxidase subunit I (COI) gene has emerged as a gold standard for DNA barcoding due to its high interspecific variation and low intraspecific divergence, facilitating reliable discrimination among closely related taxa (Hebert *et al.*, 2003) [3]. This COI barcode system has been widely adopted across animal groups and has become central to forensic entomology, enabling accurate species identification even from partial, immature, or degraded specimens (Wells & Stevens, 2008) [7]. Recent research continues to reinforce COI's value: Wang and colleagues (2025) [6] highlighted its ongoing utility in forensic insect identification and explored optimizations that enhance rapid and cost-effective species detection.

Necrophagous blow flies, particularly those of the family Calliphoridae, are among the first colonizers of decomposing remains and have well-studied developmental patterns that make them especially useful in PMI estimation. *Chrysomya megacephala* (Fabricius), the oriental latrine fly, is frequently reported in forensic contexts and often dominates carrion and cadaver insect assemblages across tropical and subtropical regions (Ye *et al.*, 2024) [8].

Its forensic relevance has been underscored by both morphological and molecular studies, and rapid detection methods for this species continue to be developed to enhance on-site identification capabilities.

Molecular identification methods not only confirm species identity through sequence similarity but also through phylogenetic context, allowing forensic investigators to place unknown samples within a broader evolutionary framework. Concordance between BLAST database matches, sequence alignments, and phylogenetic clustering significantly strengthens species-level assignments, as emphasized in recent reviews of molecular applications in forensic entomology (Scieuzo *et al.*, 2025) ^[4]. Collectively, these advances underscore the importance of integrating COI barcoding with robust analytical frameworks to improve the precision and reliability of forensic insect identifications.

In this study, we applied an integrated molecular approach to identify an insect specimen collected from a hanging human body (F1-Hanging). By evaluating DNA quality, amplifying and sequencing the mitochondrial COI gene, and confirming species identity through BLAST similarity and phylogenetic clustering, we demonstrate the applicability of COI-based barcoding for forensic entomological investigations under real-world conditions.

2. Materials and Methods

2.1 Study Area

The present study was conducted at Jagdalpur District, under the Department of Biosciences, MATS University. Jagdalpur is located in the Bastar region of Chhattisgarh and is characterized by a tropical climate with distinct seasonal variations, which support a rich diversity of necrophagous insect fauna. Laboratory-based molecular work, including DNA extraction, PCR amplification, sequencing, and bioinformatic analyses, was carried out in the molecular biology facilities and information were provided of Department of Biosciences, MATS University, Raipur.

2.2 Sample Collection and Preservation

Cadaver-associated insect specimens were collected from a hanging human body (designated as Sample F1-Hanging) following standard forensic entomology collection protocols. The specimens were handled using sterile forceps to avoid cross-contamination. Collected samples were preserved immediately in 95-100% ethanol and stored at -20 °C until further molecular analysis. Proper labeling and documentation were maintained throughout the sampling and storage process.

2.3 DNA Extraction

Genomic DNA was extracted from individual insect specimens using a standard insect DNA extraction protocol. A small portion of tissue was homogenized, and DNA was isolated following cell lysis, protein digestion, and purification steps. The extracted DNA was finally eluted in nuclease-free water and stored at -20 °C for downstream applications.

2.4 DNA Quantification

The concentration and quality of the extracted DNA were assessed using spectrophotometric analysis. DNA concentration was recorded in ng/μl, and samples showing sufficient yield and purity were selected for PCR

amplification. Only DNA extracts free from visible degradation and with adequate concentration were used for molecular identification.

2.5 PCR Amplification of COI Gene

The mitochondrial cytochrome oxidase I (COI) gene was amplified using mitochondrial-specific primers (mtCOI-5' and mtCOI-3'). PCR reactions were carried out in a total volume of 50 μl containing DNA template, primers, dNTPs, Taq DNA polymerase, assay buffer, and nuclease-free water. The PCR cycling conditions included an initial denaturation at 94 °C, followed by 35 cycles of denaturation, annealing at 40 °C, extension at 72 °C, and a final extension step. Amplification success was confirmed by agarose gel electrophoresis.

2.6 DNA Sequencing

Successfully amplified PCR products were purified and subjected to Sanger sequencing in both forward and reverse directions. High-quality consensus sequences were generated after trimming low-quality ends and resolving ambiguous bases.

2.7 Sequence Alignment and BLAST Analysis

The obtained COI sequences were aligned using standard alignment software to ensure correct reading frames and sequence integrity. Species identification was performed using the BLASTN tool against the NCBI nucleotide database. Sequence similarity, query coverage, alignment score, and E-values were considered for accurate species-level identification.

2.8 Phylogenetic Analysis

Phylogenetic analysis was conducted using reference COI sequences of closely related taxa retrieved from GenBank. Evolutionary distances were calculated using the Jukes-Cantor model, and phylogenetic trees were constructed using the Neighbor-Joining method. Bootstrap analysis was performed to assess the robustness of the tree topology and clustering patterns.

2.9 Data Interpretation

Species identification was confirmed based on concordance among BLAST similarity results, sequence alignment, and phylogenetic clustering. This integrated molecular approach ensured reliable identification of the cadaver-associated insect specimen for forensic entomological interpretation.

3. Results and Discussion

3.1 DNA Quantification

The extracted DNA concentration for the F1-Hanging sample was 116 ng/μl (Table 1). This concentration is well within the optimal range required for downstream molecular applications, including high-fidelity PCR amplification and Sanger sequencing. The yield indicates that the DNA extraction protocol was effective and produced DNA with minimal degradation or inhibitory contaminants.

Adequate DNA quality and quantity are essential in forensic entomology for accurate species identification, particularly for mitochondrial COI gene amplification (Sperling, Anderson, & Hickey, 1994) ^[5]. The obtained concentration confirms the suitability of the F1-Hanging sample for reliable molecular identification and supports the effectiveness of the extraction methodology employed.

3.2 PCR Amplification and COI Sequencing

Both cadaver-associated insect samples successfully amplified the mitochondrial cytochrome oxidase I (COI) gene under the described PCR conditions (Table 4). The obtained sequence lengths were 656 bp for F1-Cow and 653 bp for F1-Hanging (Table 5). Successful amplification and sequencing indicate that the extracted DNA was sufficiently intact and free from major PCR inhibitors, thereby allowing reliable DNA barcoding. Amplification of the COI gene was achieved using mitochondrial-specific primers (mtCOI-5' and mtCOI-3'), which generated the expected ~1.5 kb PCR product. The PCR cycling conditions—initial denaturation at 94 °C, followed by 35 cycles with annealing at 40 °C and extension at 72 °C—were effective for high-fidelity amplification. These optimized conditions ensured consistent and specific amplification of the target mitochondrial region.

The successful PCR outcomes demonstrate that (i) the DNA quality was adequate with no significant degradation, (ii) the primers exhibited high specificity toward the mitochondrial COI region, and (iii) no PCR inhibition occurred, which is particularly important when working with forensic samples that may contain environmental contaminants. The presence of amplifiable mitochondrial DNA in both samples confirms their suitability for accurate molecular identification, supporting the reliability of DNA-based methods in forensic entomology (Wells & Stevens, 2008) [7].

3.3 Sequence Alignment and Species Identification: Sample F1-Hanging

The mitochondrial COI gene sequence obtained from Sample F1-Hanging was 653 bp in length. Sequence alignment and BLAST analysis revealed 98.93% similarity with *Chrysomya megacephala* (GenBank Accession No. JX430023.1), indicating a strong genetic match and confirming the species identity. The high percentage similarity reflects minimal nucleotide variation between the sample and the reference sequence, supporting accurate species-level identification.

Phylogenetic analysis further validated the BLAST results, as the F1-Hanging sequence clustered closely with other authenticated *C. megacephala* reference sequences within the same clade. This consistent clustering demonstrates the reliability of mitochondrial COI sequencing for molecular identification of cadaver-associated insects in forensic investigations.

The aligned COI nucleotide sequence of Sample F1-Hanging is presented below, representing a high-quality sequence suitable for DNA barcoding and comparative analysis:

```
ATTGGTACTTTATATTTTCATTTTCGGAGCTTCGGAATAGTAGGAACCTCA
TTAAGTATTTTAATTCGAGCTGAATTAGGACACCCTGGAGCATTAAATGGGA
GACGACCAAAATTATAATGTAATTGTAACAGCTCACGCTTTTATTATAATT
TTCTTTATAGTAATGCCAATTATAATTGGAGGATTGGAAATGTTCTTTTA
ATGTTAGGAGCTCCAGATATAGCTTTCCACGAATAAATAATATAAGTTTC
CTTTACCTCCTGCATTAACCTTTATTATTAGTAAGTAGTATAGTAGAAAAT
GGGGCTGGAACAGGAAGTGTACCCACCTTTATCTTCTAATATTGCTCAT
GGAGGAGCATCAGTTGATTAGCTATTTCTCTTACACTAGCAGGAATT
TCTTCAATTTTAGGAGCTGTAATTTTATTACAAGTGAATTAATATACGA
TCTACAGGAATTACATTTGATCGAATACCTTTATTGTATCTGTAGTTAGGT
TACTGCTCTATTATTATTATTCTTTACCAGTCTAGCTGGAGCTATTACTA
TATTATTAAGTACCGAAATCTAAATACTTCATTCTTTGACCCAGCAGGAG
GAGGAGATCCTATTTTATACCAACATTTATTTTITTTT
```

Fig 1: Sequence alignment of mitochondrial COI gene from Sample F1-Hanging.

3.4 Phylogenetic Analysis

Phylogenetic analysis of the mitochondrial COI sequence from Sample F1-Hanging was performed using the Neighbor-Joining method based on the Jukes-Cantor corrected distance model. The resulting phylogenetic tree clearly placed the F1-Hanging sequence within a well-supported clade of *Chrysomya megacephala* reference sequences retrieved from the NCBI database (Fig. 2).

The F1-Hanging sample clustered closely with multiple authenticated *C. megacephala* isolates and voucher sequences, indicating low evolutionary divergence and high genetic similarity typical of conspecific COI barcoding data. The node grouping the F1-Hanging sequence with reference sequences was supported by strong bootstrap values, demonstrating the robustness and reliability of the phylogenetic placement.

The absence of anomalous branching or distant clustering suggests that the amplified COI sequence was free from contamination and accurately represents the target species. Overall, the phylogenetic results conclusively confirm that the insect specimen collected from the hanging body belonged to *Chrysomya megacephala*, validating the BLAST-based identification and supporting the use of mitochondrial COI sequencing for forensic species determination.

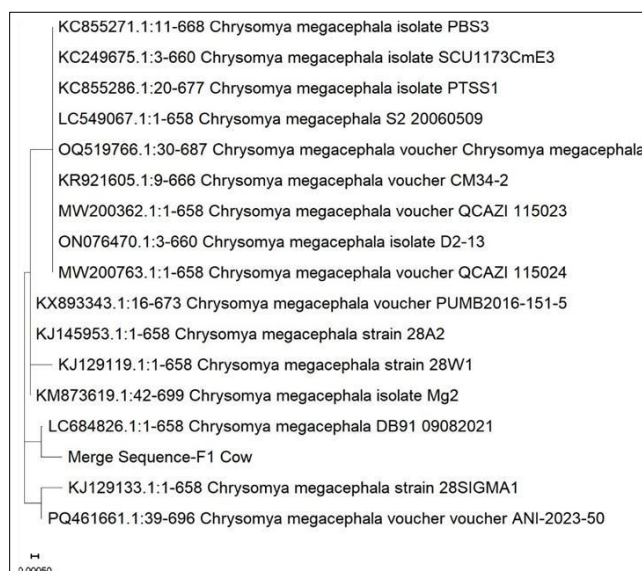


Fig 2: Phylogenetic tree showing the clustering of Sample F1-Hanging with reference *Chrysomya megacephala* sequences based on mitochondrial COI gene analysis.

3.5 BLAST Analysis

BLASTN analysis of the mitochondrial COI sequence obtained from Sample F1-Hanging produced highly significant alignments with *Chrysomya megacephala* sequences available in the NCBI nucleotide database. The query sequence (653 bp) showed 98.93% sequence identity with multiple *C. megacephala* reference entries, accompanied by high alignment scores, 100% query coverage, and an E-value of 0.0, indicating an extremely reliable match.

All top-ranked BLAST hits corresponded exclusively to *C. megacephala*, with no competing species appearing among the highest similarity results. The minimal number of nucleotide mismatches observed further supports the accuracy of the sequence and confirms the absence of

contamination or sequencing artifacts. These BLAST parameters meet and exceed the accepted similarity thresholds for species-level identification of forensic insects using COI barcoding (Sperling *et al.*, 1994)^[5]. Overall, the BLAST results conclusively identify the F1-Hanging specimen as *Chrysomya megacephala*, validating

the molecular findings obtained through sequence alignment and phylogenetic analysis and confirming the reliability of COI-based DNA barcoding for forensic entomological investigations.

RID: MK3VJXW3013
Job Title: F1-Hanging
Program: BLASTN
Database: core_nt Core nucleotide BLAST database
Query #1: Query ID: lc|Query_5145533 Length: 653

Sequences producing significant alignments:

Description	Scientific Name	Common Name	Taxid	Max Score	Total Query Score	E	Per. Value	Acc. Ident	Len	Accession
Chrysomya megacephala isolate MH1 cytochrome oxidase subunit I...	Chrysomya me...	oriental lat...	115424	1170	1170	100%	0.0	98.93	655	JX430023.1
Chrysomya megacephala strain DI212 isolate Zaozhuang 3...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075797.1
Chrysomya megacephala strain DI212 isolate Tainan 3...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778900.1
Chrysomya megacephala strain DI212 isolate Zaozhuang 2...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075798.1
Chrysomya megacephala strain DI212 isolate Kunming 13...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075785.1
Chrysomya megacephala strain DI212 isolate Dezhou 2...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075815.1
Chrysomya megacephala voucher ANI-2023-50 cytochrome c...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1510	PQ461661.1
Chrysomya megacephala isolate 3 cytochrome oxidase subunit I...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	702	FJ614816.1
Chrysomya megacephala strain DI212 isolate Xinzhou 3...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778908.1
Chrysomya megacephala isolate ks2 cytochrome oxidase subunit I...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1249	JX027563.1
Chrysomya megacephala strain DI212 isolate Baotou 1...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778858.1
Chrysomya megacephala isolate II cytochrome oxidase subunit I...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	686	MF598733.1
Chrysomya megacephala strain DI212 isolate Binzhou 2...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075818.1
Chrysomya megacephala strain DI212 isolate Heze 3 mitochondrio...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075811.1
Chrysomya megacephala voucher Chrys cytochrome oxidase subunit...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	2383	AY909053.1
Chrysomya megacephala strain DI212 isolate Chifeng 4...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778865.1
Chrysomya megacephala strain DI212 isolate hezhou...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075782.1
Chrysomya megacephala isolate CSU160701CB3 cytochrome oxidase...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1278	KY001897.1
Chrysomya megacephala strain DI212 isolate Kunming 10...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075788.1
Chrysomya megacephala strain DI212 isolate Guiyang 3...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778872.1
Chrysomya megacephala strain DI212 isolate zunyi17 mitochondrio...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075776.1
Chrysomya megacephala strain DI212 isolate Yantai 1...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075802.1
Chrysomya megacephala strain DI212 isolate Kunming 3...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778892.1
Chrysomya megacephala strain DI212 isolate Suizhou 4...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778897.1
Chrysomya megacephala strain DI212 isolate Kunming 11...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075787.1
Chrysomya megacephala strain DI212 isolate Kunming 12...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075786.1
Chrysomya megacephala strain DI212 isolate Heze 1 mitochondrio...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075813.1
Chrysomya megacephala strain DI212 isolate Tainan 4...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778901.1
Chrysomya megacephala strain DI212 isolate Liaohe 1...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075809.1
Chrysomya megacephala strain DI212 isolate Yantai 2...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075801.1
Chrysomya cabrerai voucher WU2009-017-2 cytochrome oxidase...	Chrysomya ca...	NA	604328	1088	1088	99%	0.0	96.70	1509	FJ195378.1
Chrysomya megacephala strain DI212 isolate Kunming 4...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MH778893.1
Chrysomya chani voucher PUBM2016-151-25 cytochrome oxidase...	Chrysomya chani	NA	604321	1088	1088	99%	0.0	96.70	1423	KX893340.1
Chrysomya megacephala voucher ANI-2023-57 cytochrome c...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1510	PQ461664.1
Chrysomya megacephala isolate 2 cytochrome oxidase subunit I...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	702	FJ614817.1
Chrysomya megacephala isolate CSU148801CB2 cytochrome oxidase...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1299	KY001901.1
Chrysomya megacephala haplotype VIII cytochrome c oxidase...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1509	KM434362.1
Chrysomya megacephala voucher UM KAVITHA C29 UT cytochrome...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	2272	JN229003.1
Chrysomya megacephala haplotype IX cytochrome c oxidase subuni...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1509	KM434363.1
Chrysomya megacephala strain DI212 isolate zunyi9 mitochondrio...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	14932	MK075774.1
Chrysomya megacephala isolate CSU120801CB3 cytochrome oxidase...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	1298	KY001902.1
Chrysomya megacephala voucher UM KAVITHA C7 06i cytochrome...	Chrysomya me...	oriental lat...	115424	1088	1088	99%	0.0	96.70	2247	JN228996.1

Fig 2: Blast Data - Sample F1-Hanging

3.6 Phylogenetic Clustering

Although the phylogenetic tree data are not reproduced in tabular form, the phylogenetic analysis clearly confirmed that the mitochondrial COI sequence of Sample F1-Hanging clustered tightly with authenticated *Chrysomya megacephala* reference sequences. The clustering pattern was consistent with the BLAST and sequence alignment results, demonstrating strong genetic affinity between the F1-Hanging sample and confirmed *C. megacephala* entries.

4. Discussion

The present study successfully demonstrated the utility of mitochondrial cytochrome oxidase I (COI) gene sequencing for the accurate species-level identification of a cadaver-associated insect from a hanging body (Sample F1-Hanging). The extracted DNA concentration (116 ng/μL) was adequate for downstream molecular analysis, indicating that the extraction protocol preserved DNA integrity and effectively minimized potential inhibitors. High-quality DNA is fundamental for reliable polymerase chain reaction (PCR) amplification and sequencing, particularly in forensic entomology where precise species identification is critical (Sperling, Anderson, & Hickey, 1994)^[5].

The PCR amplification and sequencing of the COI region yielded high quality sequences (~653 bp) that could be reliably compared with reference databases. Mitochondrial COI has been widely accepted as the standard marker for DNA barcoding of animal taxa due to its high interspecific divergence and low intraspecific variation, making it particularly effective in distinguishing closely related species in forensic contexts (Wells & Stevens, 2008; Hebert *et al.*, 2003)^[7, 3].

BLAST analysis of the F1-Hanging COI sequence showed 98.93% identity with *Chrysomya megacephala* reference sequences, with high query coverage and zero E-value, confirming the accuracy of the match. The absence of top hits from alternative taxa and the minimal nucleotide mismatches observed support the conclusion that the sequence represents *C. megacephala* without evidence of contamination or sequencing errors. Such high levels of COI similarity are consistent with accepted thresholds for species-level identification in forensic entomology (Yusseff-Vanegas & Agnarsson, 2017)^[9].

Phylogenetic analyses further corroborated the BLAST and alignment results. The F1-Hanging sequence clustered strongly within a clade of authenticated *C. megacephala*

reference sequences with robust bootstrap support, reflecting low evolutionary divergence typical of conspecific samples. Congruence among BLAST similarity, phylogenetic clustering, and sequence alignment enhances confidence in the molecular identification, which is essential given that morphological identification can be challenging when specimens are immature, damaged, or lacking diagnostic features. Indeed, molecular approaches such as COI barcoding provide a standardized and reproducible mechanism for species determination in forensic casework (Scieuzo *et al.*, 2025) [4].

The identification of *C. megacephala* has significant forensic implications. Members of the family Calliphoridae, including *C. megacephala*, are among the primary colonizers of decomposing remains and are extensively used in estimating the post-mortem interval (PMI) because their predictable colonization patterns and life history traits can serve as a biological clock in decomposition studies. This species' widespread distribution, rapid colonization behavior, and well-characterized developmental stages make it a valuable indicator in medico-legal investigations (*Chrysomya megacephala*, 2025) [1].

Molecular identification through COI barcoding also overcomes limitations of traditional morphological identification, which can be hindered by cryptic species, phenotypic variation, and the absence of key morphological characters in immature life stages. Employing standardized

DNA barcoding protocols enhances the precision and reliability of taxonomic assessment, which is crucial for accurate PMI estimation and forensic interpretation (Grundy, 2025) [2]. Overall, the integration of DNA extraction, PCR amplification, sequencing, BLAST comparison, and phylogenetic confirmation demonstrates a robust molecular workflow that strengthens the evidential value of insect specimens in forensic cases. The results emphasize the importance of molecular tools in forensic entomology, particularly when traditional methods are insufficient or ambiguous.

Table 1: DNA Quantification of Extracted Samples

S. No.	Sample ID	DNA Concentration (ng/μl)
1	F1-Hanging	116

Table 2: PCR reaction mix for COI gene amplification (Total Volume: 50 μL)

Component	Volume (μL)
DNA Template	1
16s Forward Primer	2
16s Reverse Primer	2
dNTPs (2.5 mM each)	4
10X Taq DNA Polymerase Assay Buffer	10
Taq DNA Polymerase Enzyme (3 U/ml)	1
Nuclease-free Water	30
Total Volume	50 μL

Table 3: Primer Details for COI Amplification

S. No.	Primer Name	Sequence (5' → 3')	Tm (°C)	GC%
1	mtCOI-5'	TAAAGTTCAGGGTGACCAAAAAATCA	64	35

Table 4: PCR Cycling Conditions for COI Gene

Step	Temperature (°C)	Time	Cycles
Initial Denaturation	94°C	5 min	1
Denaturation	94°C	1 min	35 cycles
Annealing	40°C	1 min	35 cycles
Extension	72°C	1 min	35 cycles
Final Extension	72°C	7 min	1

Table 5: Sequence Similarity (BLAST Results) for Species Identification

Sample ID	Sequence Length (bp)	% Similarity	Identified Species	Reference Accession No.
F1-Hanging	653	98.93%	<i>Chrysomya megacephala</i>	JX430023.1

Table 6: Summary of Molecular Identification

Parameter	F1-Hanging
DNA Yield	116 ng/μl
COI Sequence Length	653 bp
BLAST % Similarity	98.93%
Identified Species	<i>C. megacephala</i>
Phylogenetic Tree Clustering	Same clade

4. Conclusion

The study confirms that mitochondrial COI gene-based DNA barcoding is a robust and reliable approach for the species-level identification of cadaver-associated insects in forensic investigations. The F1-Hanging sample yielded high-quality DNA in sufficient quantity, allowing successful PCR amplification and sequencing without evidence of degradation or inhibition. Sequence alignment, BLAST analysis, and phylogenetic clustering consistently identified the specimen as *Chrysomya megacephala*, with high

sequence similarity and strong phylogenetic support, thereby validating the accuracy of the molecular workflow employed. The confirmed presence of this forensically important blowfly species, known for its early colonization behavior, underscores the relevance of molecular identification in improving the accuracy of post-mortem interval estimation, particularly in cases where morphological identification is limited or unreliable.

5. Acknowledgement

The authors sincerely thank MATS University, Raipur (C.G.), for providing laboratory facilities and academic support throughout this study. We are grateful to the Department of Bioscience for technical assistance during sample processing and molecular work. Special thanks to the forensic authorities who facilitated sample access and field support. The first author, Anupama Meshram, expresses deep appreciation to Dr. Aparna Jha for her

valuable guidance and encouragement. We also acknowledge all colleagues and family members for their support during this research.

6. Conflict of Study

The author hereby declares that there is no conflict of interest or financial relationship that could have influenced the outcomes of this research work.

7. Future Scope

This study highlights the potential of COI gene sequencing for accurate identification of cadaveric insects. Future research should focus on developing region-specific DNA barcode libraries, generating local developmental datasets for PMI estimation and examining insect behaviour under diverse environmental conditions.

Expanding molecular markers, integrating next-generation sequencing and enhancing collaboration with forensic agencies will further strengthen the application of forensic entomology in medico-legal investigations.

8. References

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