



Original Article

Estimation of Post Mortem Interval from Molecular identification of Necrophagous Insects on Human Corpses

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ABSTRACT

Background: Estimation of post-mortem interval (PMI) is a crucial component of medico-legal investigation. Conventional methods based on post-mortem changes become unreliable with advancing decomposition. Forensic entomology provides an objective and scientifically robust alternative; however, entomological data are region-specific and limited from the Indian subcontinent, particularly Punjab.

Objectives: To identify necrophagous insect species infesting human corpses in south-west Punjab using molecular techniques, to estimate minimum PMI based on insect succession and development, and to evaluate the accuracy of entomological PMI in comparison with conventional methods.

Materials and Methods: A descriptive observational study was conducted on 26 medico-legal autopsies at Guru Gobind Singh Medical College & Hospital, Faridkot, between January 2023 and June 2024. Insect specimens were collected from bodies and divided into two sets: one for rearing to adulthood for life-cycle-based PMI estimation and another for molecular identification using PCR amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Entomological PMI was compared with PMI derived from police records, eyewitness accounts, and decomposition changes.

Results: Males constituted 88.46% of cases, predominantly aged 20–50 years. Most cases (88.46%) occurred between May and October, and 61.54% of bodies were recovered from outdoor locations. The bloat stage of decomposition was most common (73.08%). Molecular Identification of species included *Chrysomyamegacephala*, *Sarcophagacarnaria*, *Muscadomestica*, *Luciliacuprina*, and *Dermestesmaculatus*. Entomological PMI correlated with known PMI in 96.15% of cases, with discrepancies mainly in drowning cases.

Conclusion: Forensic entomology supported by molecular identification is a reliable tool for estimating minimum PMI in decomposed bodies in south-west Punjab. Development of regional entomological databases and incorporation of entomology into routine medico-legal practice in India are strongly recommended.

Keywords: Forensic entomology; Post-mortem interval; Insect succession; COI gene; Molecular identification.

INTRODUCTION

Determination of time since death is a cornerstone of medico-legal investigation, directly influencing reconstruction of events, verification of alibis, and administration of justice. Conventional methods of PMI estimation—such as algor mortis, livor mortis, rigor mortis, and putrefactive changes—are time-dependent and become increasingly unreliable as decomposition progresses.¹ Environmental variables, body habitus, cause of death, and post-mortem handling further compromise their accuracy.²

Forensic entomology is defined as the application of insect biology to legal investigations.³ Necrophagous insects colonise decomposing remains in a predictable manner, and their development is primarily governed by temperature and ecological conditions. By identifying insect species and determining their developmental stages, a minimum PMI can be estimated with considerable precision.⁴

The scientific foundation of forensic entomology dates back to the nineteenth century, with Bergeret's landmark application in 1855 and Mégnin's formulation of insect succession waves in 1894.^{5,6} Subsequent experimental and case-based studies across Europe, North America, and Australia validated insect succession and developmental data as reliable indicators of PMI.^{7,8,9}

However, insect fauna, succession patterns, and developmental rates vary with geography and climate. Data generated in one bio-geoclimatic zone cannot be directly extrapolated to another.¹⁰ India, with its vast climatic diversity, lacks region-specific human-based forensic entomology studies. Punjab, despite its extreme seasonal variations, remains under-represented in entomological literature.

Morphological identification of insects is often hindered by damaged specimens, immature stages, or close inter-species similarity. Molecular identification using mitochondrial DNA markers, particularly the cytochrome c oxidase subunit I (COI) gene, has emerged as a robust adjunct for accurate species identification.^{11,12}

The present study was undertaken to generate region-specific forensic entomological data from south-west Punjab using human autopsy material, to validate molecular identification of necrophagous insects, and to assess the reliability of entomological PMI in medico-legal practice.

Review of Literature

Forensic entomology has evolved from anecdotal observations to a well-established forensic discipline. Sung Tz'u's *Washing Away of Wrongs* in the 13th century represents the earliest documented use of insects in death investigation.¹³ Redi's experiments in 1668 disproved spontaneous generation, establishing that maggots arise from fly eggs.¹⁴

Bergeret's application of insect development to estimate PMI in an infanticide case in 1855 marked the first medico-legal use of forensic entomology.⁵ Mégnin later proposed eight successive waves of insects colonising exposed corpses, forming the conceptual basis of insect succession.⁶

Twentieth-century research expanded empirical evidence. Motter analysed insect fauna from 150 exhumations, highlighting the relationship between burial conditions and insect colonisation.¹⁵ Reed and Payne conducted controlled carcass studies demonstrating predictable succession patterns and the accelerating effect of insect activity on decomposition.^{16,17}

Rodriguez's human cadaver studies established a direct relationship between decomposition stages and insect succession.¹⁸ O'Flynn provided region-specific developmental data for blowflies in Australia, emphasising climatic influence on PMI estimation.⁹

In India, Aggarwal studied 54 cases in Punjab and demonstrated improved PMI accuracy when entomological evidence was combined with conventional findings.¹⁹ Subsequent studies confirmed seasonal variation in insect activity and accelerated larval development during summer months.²⁰

Recent advances focus on molecular identification. COI gene sequencing has proven effective in distinguishing morphologically similar species and damaged specimens.^{21,22,23} However, genetic databases remain incomplete for Indian insect populations, underscoring the need for regional molecular data.

Aims and Objectives

1. To establish molecular identification of necrophagous insects infesting human corpses in south-west Punjab.
2. To estimate minimum PMI using insect succession and developmental stages.
3. To compare entomological PMI with PMI derived from conventional methods.
4. To assess the applicability of forensic entomology in routine medico-legal practice in India.

MATERIALS AND METHODS

Study Design and Setting

A descriptive observational study was conducted at the Mortuary, Department of Forensic Medicine and Toxicology, and the Molecular Research Unit (MRU), Guru Gobind Singh Medical College & Hospital, Faridkot, Punjab.

Study Period and Sample Size

The study spanned 18 months (January 2023–June 2024). Twenty-six medico-legal autopsy cases fulfilling inclusion criteria were analysed.

Inclusion and Exclusion Criteria

Cases showing insect infestation with reasonably confirmed PMI were included. Bodies without insect evidence or unreliable PMI were excluded.

Collection of Entomological Evidence

Eggs, larvae, pupae, and adult insects were collected from natural orifices, wounds, and maggot masses using sterile forceps. Two sets were prepared: (1) live specimens for rearing and (2) preserved specimens for molecular analysis. Larvae were killed by hot-water immersion before preservation in 70% ethanol.

Estimation of PMI

Minimum PMI was calculated based on the most advanced developmental stage, rearing observations, and ambient temperature data. Entomological PMI was compared with PMI estimated from police records and decomposition changes.

Molecular Identification

DNA extraction was performed using the NucleoSpin® Tissue Kit. PCR amplification targeted the mitochondrial COI gene. Sequencing results were analysed using MEGA-X software and compared with reference databases.

Ethical Considerations

The study was approved by the Institutional Ethics Committee. Consent was obtained from relatives through standard medico-legal procedures.

RESULTS

Demographic and Environmental Profile

Out of 26 cases, 23 (88.46%) were males. The predominant age group was 20–50 years, with 61.54% identified. Most cases occurred during summer and monsoon months (88.46%). Outdoor recoveries constituted 61.54% and where partially appears worn in 80.77%.

Table 1. Age & Sex distribution of cases observed

GENDER	FREQUENCY	PERCENT%
Female	3	11.54
Male	23	88.46
Other	0	0
Total	26	100
AGE	FREQUENCY	PERCENTAGE%
0-10	0	0.00
10-20	1	3.85
20-30	6	23.08
30-40	5	19.23
40-50	3	11.54
60-70	2	7.69
>70	1	3.85
Unknown	6	23.08
Total	26	100.00

Table 2. Distribution of cases across seasons

SEASON	No. of Cases	PERCENTAGE%
Winter (Dec-Feb)	1	3.85
Spring (March-April)	2	7.69
Summers (May-June)	12	46.15
Rainy/Monsoon (July- Sept)	10	38.46
Autumn (Oct-Nov)	1	3.85
Total	26	100

Table 3. Distribution of cases according to the state of Apparels over the bodies

APPARELS COVERAGE	FREQUENCY	PERCENTAGE
Entire	1	3.85
Partial	21	80.77
Nude	4	15.38
Total	26	100

Decomposition Stage

Nineteen bodies (73.08%) were in the bloat stage, and seven (26.92%) in active decay. 13 (50.00%) had larvae, 7 (26.92%) had eggs, and 6 (23.08%) had adult flies.

Table 4. Distribution of cases according to their decomposition stage

STAGE OF DECOMPOSITION	No. of Cases	PERCENTAGE %
Fresh	0	0
Bloat	19	73.08
Decay	7	26.92
Skeletonization	0	0
Saponification	0	0
Mummification	0	0
Total	26	100

Table 5. Stage of insect found on bodies

STAGE OF INSECT	No. Of Cases	PERCENTAGE %
Egg	7	26.92
Larvae	13	50.00
Fly	6	23.08
Total	26	100.00

Insect Species Identified morphologically and molecularly

The most common species was *Chrysomyamegacephala* (61.54%), followed by *Muscadomestica* (19.23%), *Sarcophagacaritaria* (15.38%), *Luciliacuprina* (3.85%), and *Dermestesmaculatus* (3.85%) which were confirmed by Molecular identification was done by amplifying COI region of these flies.

Table 6. Species of insect found on bodies

SPECIES	No. of Cases	PERCENTAGE %
<i>Chrysomyamegacephala</i>	16	61.54
<i>Sarcophagacaritaria</i>	4	15.38
<i>MuscaDomestica</i>	5	19.23
<i>Luciliacuprina</i>	1	3.85
<i>Sarcophaga caritaria</i> & <i>Dermestesmaculatus</i>	1	3.85
Total	26	100.00

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Sarcophaga kamyi isolate Bk1A_SWK cytochrome oxidase subunit I (COI) gene, partial cds: rRNA-L eu (tr...	Sarcophaga kar...	1110	1110	94%	0.0	97.98%	2303	JF500464.1
Sarcophaga peregrina isolate bp2cb1 cytochrome c oxidase subunit I (COX1) gene, partial cds: mitochondrial	Sarcophaga cer...	1110	1110	94%	0.0	97.98%	680	PP784528.1
Sarcophaga kamyi isolate bk1ob1 cytochrome c oxidase subunit I (COX1) gene, partial cds: mitochondrial	Sarcophaga kar...	1110	1110	94%	0.0	97.98%	689	PP784491.1

Fig.1 Sarcophagacaritaria

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Dermestes maculatus voucher DFBUR0N14 cytochrome oxidase subunit I (COI) gene, partial cds: mitoch...	Dermestes mac...	320	320	48%	3e-82	86.94%	659	MN253542.1
Dermestes maculatus isolate Dma2 cytochrome c oxidase subunit I (COI) gene, complete cds: mitochondrial	Dermestes mac...	320	320	48%	3e-82	86.94%	1545	MK332618.1
Dermestes maculatus isolate Dma8 cytochrome c oxidase subunit I (COI) gene, complete cds: mitochondrial	Dermestes mac...	320	320	48%	3e-82	86.94%	1545	MK332617.1
Dermestes maculatus isolate Dma2 cytochrome c oxidase subunit I (COI) gene, complete cds: mitochondrial	Dermestes mac...	320	320	48%	3e-82	86.94%	1545	MH038473.1

Fig.2 DermestesMaculatus

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lucilia cuprina cytochrome oxidase subunit I (COI) gene, partial cds: mitochondrial	Lucilia cuprina	1136	1136	86%	0.0	98.61%	875	GQ912607.1
Lucilia cuprina isolate GBP cytochrome c oxidase subunit I (COX1) gene, partial cds: mitochondrial	Lucilia cuprina	1136	1136	86%	0.0	98.60%	666	MW222990.1
Lucilia cuprina isolate GBL cytochrome c oxidase subunit I (COX1) gene, partial cds: mitochondrial	Lucilia cuprina	1136	1136	86%	0.0	98.60%	666	MW222993.1

Fig3: Luciliacuprina

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chrysomya megacephala voucher CM01 cytochrome oxidase subunit I (COI) gene, partial cds: mitochondrial	Chrysomya meg...	1168	1168	90%	0.0	99.84%	693	KT894991.1
Chrysomya megacephala voucher F09 cytochrome oxidase subunit I (COI) gene, partial cds: rRNA:Leu ge...	Chrysomya meg...	1164	1164	90%	0.0	99.84%	2303	JN014844.1
Chrysomya megacephala isolate JN1 mitochondrion, complete genome	Chrysomya meg...	1184	1184	90%	0.0	99.84%	14932	MK075910.1
Chrysomya megacephala strain D1212 isolate Zhoukou 6 mitochondrion, complete genome	Chrysomya meg...	1164	1164	90%	0.0	99.84%	14932	MK075772.1

Fig

4:Chrysomyamegacephala

Correlation of PMI

Entomological PMI matched known PMI in 25 cases (96.15%). One mismatch occurred in a drowning case.

DISCUSSION

The predominance of males and younger adults mirrors findings from Indian and international studies.^{19,20} Seasonal clustering during warmer months reflects enhanced insect activity and accelerated larval development.²⁴

The dominance of *Chrysomyamegacephala* is consistent with tropical forensic entomology literature.²⁵ Molecular confirmation using COI gene region of these flies, which shows 98-99% similarity with the nucleotide sequences in GEN BANK submitted by former researchers after the Basic Local Alignmentsearch Tool (BLAST)

High concordance between entomological and known PMI confirms the reliability of forensic entomology. Discrepancies in aquatic deaths highlight delayed insect access as a limiting factor.²⁶

CONCLUSION

Forensic entomology supported by molecular identification is a reliable and practical method for estimating minimum PMI in decomposed bodies in south west Punjab. Development of regional entomological and genetic databases and integration of entomology into routine forensic practice in India are strongly advocated.

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