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The Role of DNA Barcoding in Modern Forensic Entomology: A Comprehensive Review

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Abstract

Forensic entomology relies on the accurate identification of insects and other arthropods recovered from crime scenes, decomposing remains, and skeletal recoveries to estimate the post-mortem interval (PMI), reconstruct events surrounding death, and, in some cases, associate suspects or victims with a scene. For decades, this identification depended almost entirely on morphological taxonomy, a method that performs poorly on damaged specimens, immature life stages such as eggs and early-instar larvae, and cryptic or sibling species that are morphologically indistinguishable. Over the past two decades, DNA barcoding, most commonly built around a standardized fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, has emerged as a robust molecular complement and, in many casework scenarios, a superior alternative to morphology-based identification. This review synthesizes the theoretical foundation of DNA barcoding, the practical workflow from sample collection through sequence-based species assignment, and its documented applications in forensic entomology, drawing on peer-reviewed research largely published between 2016 and 2026. Particular attention is given to regional case studies from India; emerging technologies including mini-barcoding, next-generation sequencing (NGS)-based metabarcoding, multi-locus approaches, and machine-learning-assisted identification; and persistent challenges such as reference database gaps, the barcode gap problem, nuclear mitochondrial pseudogenes (NUMTs), contamination risk, and courtroom admissibility. The review concludes that DNA barcoding has matured into an indispensable, though not standalone, pillar of modern forensic entomology, and it outlines priority areas — regional reference libraries, standardized protocols, and integration with artificial intelligence — for strengthening its evidentiary reliability going forward.

Keywords: DNA barcoding; forensic entomology; cytochrome c oxidase I (COI); post-mortem interval; Calliphoridae; metabarcoding; BOLD; GenBank; species identification

1. Introduction

Insects and other arthropods colonize a corpse in a broadly predictable succession that begins within minutes to hours of death and continues through the various stages of decomposition. Because the developmental biology of necrophagous species — particularly blow flies (Diptera: Calliphoridae), flesh flies (Sarcophagidae), and certain beetles (Coleoptera) — is well characterized for many species, the age of the oldest insects recovered from a body can be used to estimate a minimum post-mortem interval (PMImin). This principle underlies the discipline of forensic entomology and has been used in death investigations for well over a century.

The reliability of any PMI estimate derived from insect evidence depends entirely on correct species identification, since closely related necrophagous species can differ substantially in development rate, temperature thresholds, and geographic distribution. Traditional identification keys rely on morphological characters of adults, which are frequently unavailable at a crime scene where only eggs, larvae, puparial cases, or fragmentary remains are recovered. Even where adults are present, several forensically important genera — including Chrysomya, Lucilia, and Sarcophaga — contain sibling species that are essentially indistinguishable using external morphology alone.

DNA barcoding addresses this gap directly. Introduced as a general framework for species-level identification using a short, standardized gene region, DNA barcoding in animals is built almost universally around a roughly 650 base-pair segment of the mitochondrial COI gene. Because COI shows sufficient interspecific variation coupled with comparatively low intraspecific variation across most animal taxa, a query sequence can typically be matched against a well-populated reference library — principally the Barcode of Life Data System (BOLD) and the National Center for Biotechnology Information's GenBank — to assign species identity with high confidence, regardless of the developmental stage or physical condition of the specimen.

This review consolidates recent research, largely from the last five to ten years, on the application of DNA barcoding within forensic entomology. It covers the underlying molecular principles, the standard laboratory-to-database workflow, documented case applications across several countries, the newer generation of sequencing-based approaches that extend barcoding to degraded and mixed samples, and the methodological and legal challenges that continue to shape how confidently barcode-based identifications can be relied upon in criminal investigations and courts.

2. Principles of DNA Barcoding

2.1 The COI Gene as a Universal Marker

The mitochondrial COI gene is favored as a barcoding marker for several converging reasons: it is present in essentially all animal cells in high copy number, which improves amplification success from small or degraded samples; it evolves quickly enough at the third codon position to accumulate species-diagnostic variation while remaining functionally conserved enough to be amplified with a small set of largely universal primers; and it lacks the introns and heteroplasmy complications associated with some nuclear markers. A recent comprehensive review of DNA barcoding in insect classification reinforced that this combination of properties has made COI-based identification fast, reliable, and broadly applicable across insect orders, including for immature life stages such as eggs, larvae, and nymphs that cannot be resolved through classical taxonomic keys.

2.2 Reference Databases: BOLD and GenBank

A barcode sequence is only as useful as the reference library against which it is compared. BOLD and GenBank remain the two principal repositories used for forensic entomological identification. Institutional efforts to build dedicated, forensically curated reference libraries have expanded significantly in recent years; for example, a collaboration between the Bavarian State Collection of Zoology and the Bavarian State Criminal Police Office produced a curated reference library of several hundred high-quality COI sequences covering dozens of arthropod species of forensic relevance, which was subsequently used as a backbone for high-throughput sequencing analysis of bulk arthropod samples recovered from human remains. Regional efforts of this kind are increasingly recognized as a prerequisite for reliable forensic barcoding, since global databases such as GenBank and BOLD remain unevenly populated across geographic regions and taxa.

3. Methodology: From Crime Scene to Species Assignment

The forensic DNA barcoding workflow follows a broadly consistent sequence of steps regardless of the specific case or laboratory, moving from careful field collection through laboratory processing to database-driven species assignment.

3.1 Sample Collection and Preservation

Entomological evidence — eggs, larvae of varying instars, puparia, and adult specimens must be collected using standardized protocols that preserve both morphological and molecular integrity. Guidance documents such as those issued by the U.S. National Institute of Standards and Technology's Organization of Scientific Area Committees emphasize proper preservation (typically in ethanol) to prevent DNA degradation prior to laboratory analysis, alongside thorough scene documentation of larval mass location, substrate, and microclimate.

3.2 DNA Extraction and PCR Amplification

Genomic DNA is typically extracted using commercial silica-column kits or rapid enzymatic lysis methods. Non-destructive extraction protocols that preserve the physical specimen for parallel morphological examination have gained particular importance in forensic contexts, since evidentiary specimens are often irreplaceable. Following extraction, the COI barcode region is amplified using PCR with primer pairs selected to balance universality against amplification efficiency; the resulting amplicon is then purified and prepared for sequencing.

3.3 Sequencing and Species Assignment

Sanger sequencing of the amplified COI fragment remains the standard approach for single-specimen barcoding. The resulting chromatogram is edited, trimmed, and the consensus sequence is queried against BOLD or GenBank. A match is typically considered reliable when intraspecific genetic divergence (commonly calculated using the Kimura two-parameter, K2P, model) remains low — often cited in the range of 0–2% — while divergence from the nearest congeneric species is substantially higher, a separation referred to as the 'barcode gap.'

3.4 Species Delimitation Approaches

Where taxonomic boundaries are ambiguous or where cryptic diversity is suspected, researchers increasingly apply formal species delimitation algorithms alongside simple sequence matching. These include the distance-based Barcode Index Number (BIN) system used by BOLD, Assemble Species by Automatic Partitioning (ASAP), the Poisson Tree Processes model (PTP, including its Bayesian variant bPTP), and the Generalized Mixed Yule Coalescent (GMYC) approach. Comparative studies applying several of these methods in parallel — for example, in a large-scale molecular survey of blow flies from eastern India — have shown that BIN, ASAP, PTP, and GMYC can produce broadly congruent species boundaries, lending confidence to COI-based identifications even in taxonomically complex genera such as *Chrysomya*.

Table 1. Comparison of DNA-based molecular techniques used in forensic entomology.

Technique	**Genetic Marker(s)**	**Primary Forensic Use**	**Key Limitation**
Standard DNA barcoding	Full-length COI (~658 bp, 5' region)	Species ID of adults, larvae, pupae, fragments	Fails on degraded/short DNA
Mini-barcoding	Short COI fragments (100–250 bp)	Degraded, charred, or trace samples	Reduced resolution in some taxa
DNA metabarcoding (NGS-based)	COI/16S amplicons, high-throughput sequencing	Bulk/mixed insect samples, community profiling	PCR bias, NUMTs, bioinformatic complexity
Multi-locus barcoding	COI + 16S/18S/ITS2 combined	Resolving cryptic or closely related species	Higher cost, more reference data needed
Gene expression / omics profiling	mRNA transcripts, proteomic/metabolomic markers	Refining PMI within a known species	Not a species-ID tool; needs standardization

4. Applications in Forensic Entomology

4.1 Species-Level Identification of Diptera

Blow flies (Calliphoridae) are the most extensively barcoded forensic taxon because they are typically the first colonizers of a corpse and therefore the most informative for minimum PMI estimation. A 2025 molecular survey covering 17 calliphorid species across four genera — Calliphora, Chrysomya, Lucilia, and Hemipyrellia — collected from four geo-climatic zones of West Bengal, India, found that COI barcoding successfully confirmed morphological identifications through low intraspecific and moderate-to-high interspecific genetic divergence, while also revealing previously undocumented genetic diversity within Indian populations of Chrysomya megacephala, Chrysomya saffranea, and Chrysomya rufifacies. An earlier PubMed-indexed Indian study similarly established COI as a reliable marker for DNA-based

identification of Indian blow flies, though it also flagged paraphyly between Chrysomya megacephala and Chrysomya chani, illustrating the resolution limits that even COI barcoding can encounter within very closely related lineages.

4.2 Coleoptera and Other Forensically Relevant Arthropods

While Diptera dominate the early stages of decomposition, beetles become increasingly important during later decompositional stages, including dry and skeletal remains. Reviews of molecular identification in forensic entomology have documented COI-based identification of carrion beetles and, in related work, of carrion-breeding scuttle flies (Diptera: Phoridae), extending molecular identification capability beyond the classical blow fly focus into a broader range of necrophagous arthropods relevant to later PMI intervals.

4.3 Postmortem Interval (PMI) Estimation

Because developmental rate models are species-specific, an incorrect species identification can propagate directly into a materially inaccurate PMI estimate. Recent reviews emphasize that DNA barcoding functions to refine, rather than replace, entomological PMI methodology: it resolves the identification step so that the correct species-specific growth model can be applied to larval length, instar stage, or accumulated degree-day data recovered from the scene. Broader reviews of molecular biology's contribution to forensic entomology have additionally highlighted complementary approaches — including gene expression profiling and omics-based techniques such as transcriptomic and metabolomic biomarkers — that are being explored to narrow PMI estimates within an already-identified species, particularly for the later, less morphology-dependent stages of decomposition.

4.4 Case Studies and Regional Research

Beyond the pure species-identification role, DNA barcoding and related molecular approaches have been utilized in actual criminal casework. Review literature on the molecular contribution to forensic entomology references documented cases in which insect-derived molecular evidence assisted in victim identification, PMI estimation, or the detection of toxic substances via entomotoxicological analysis, underscoring that barcoding now operates as part of an integrated molecular toolkit rather than an isolated technique. Reviews addressing entomotoxicology specifically describe how insects developing on a drug-affected corpse can serve as bioindicators, with molecular and omics-based methods — including machine-learning-assisted analysis of biological changes — increasingly used to interpret those toxicological signals alongside standard barcode-based species identification.

4.5 Human DNA Recovery and Non-Destructive Analysis

A further forensic application involves recovering human DNA from the gut contents or body surfaces of necrophagous insects that have fed on a corpse, which can assist in victim identification when direct tissue sampling is not possible. Non-destructive DNA extraction protocols — for instance, methods developed for fly larvae that preserve external morphological features for parallel taxonomic examination — support this dual use of a single specimen for both species-level barcoding and, where applicable, recovery of associated human genetic material.

5. Emerging Technologies

5.1 Mini-Barcoding

Standard COI barcodes of roughly 650 base pairs frequently fail to amplify from degraded, charred, or otherwise compromised forensic samples. Mini-barcodes — shorter diagnostic fragments typically in the 100–250 base-pair range — have been shown across multiple taxa to amplify more reliably from degraded template DNA while retaining most of the species-discriminatory power of the full-length barcode, making them increasingly attractive for casework involving old, heat-damaged, or trace insect material.

5.2 Next-Generation Sequencing and Metabarcoding

DNA metabarcoding pairs high-throughput sequencing (HTS) with COI or other marker amplification to identify multiple species simultaneously from a single bulk or mixed sample, rather than requiring one specimen to be processed at a time. This is directly relevant to forensic entomology, where a single scene commonly yields a mixed sample of arthropods spanning several taxa and developmental stages. The Bavarian reference-library study demonstrated this application concretely, using its curated COI database as the identification backbone for a high-throughput sequencing analysis of bulk arthropod samples collected from human corpses, successfully identifying dozens of distinct taxa from a single mixed sample.

Broader methodological reviews of metabarcoding highlight its capacity to dramatically increase throughput and taxonomic breadth compared with specimen-by-specimen Sanger sequencing, while also cataloguing the accompanying methodological challenges, discussed further below.

5.3 Multi-Locus and Omics-Based Approaches

Because COI alone occasionally fails to resolve very recently diverged or hybridizing species, researchers increasingly supplement it with additional loci — such as 16S, 18S, or nuclear ITS2 regions — in multi-locus barcoding schemes. In parallel, omics-based techniques, including genomics, transcriptomics, and proteomics applied to necrophagous insects, are being explored as a complementary layer that moves beyond species identification toward more precise estimation of developmental age and, by extension, PMI.

5.4 Artificial Intelligence and Machine Learning Integration

Recent work has begun to combine molecular barcode data with computational approaches. Machine-learning models have been applied to detect out-of-distribution samples — specimens belonging to species absent from the reference database — a persistent weak point of pure sequence-matching approaches. Separately, deep convolutional neural networks have been used to identify forensically important fly larvae directly from images of morphological structures such as posterior spiracles, and entomotoxicology reviews describe artificial-intelligence-assisted integration of metabolomic, proteomic, and spectroscopic data to improve PMI estimation accuracy. While these approaches are not DNA barcoding in the strict sense, they represent a convergent trend toward hybrid molecular-computational identification pipelines in forensic entomology.

6. Challenges and Limitations

6.1 Database Gaps and Geographic Bias

The reliability of any barcode match is bounded by the completeness of the reference database. Multiple regional studies explicitly note that global repositories such as BOLD and GenBank remain unevenly populated, with certain geographic regions, taxa, and life stages severely underrepresented.

6.2 The Barcode Gap Problem and Cryptic Species

Reliable species assignment assumes a clear separation between intraspecific and interspecific genetic distances — the so-called barcode gap. In practice, this gap can narrow or disappear in recently diverged species complexes, as observed in the paraphyly documented between Chrysomya megacephala and Chrysomya chani. Evaluations of barcoding reference databases in other contexts have similarly documented quality issues including over- or under-represented species, short or ambiguous sequences, and cases of high intraspecific versus low interspecific divergence, all of which can compromise identification confidence in casework-sensitive applications.

6.3 NUMTs and Contamination

Nuclear mitochondrial DNA segments, or NUMTs — copies of mitochondrial sequence that have become inserted into the nuclear genome over evolutionary time — can co-amplify alongside the true mitochondrial target and be mistaken for genuine barcode sequences, potentially inflating apparent species diversity or producing spurious identifications. This risk is heightened in metabarcoding workflows, where failed or biased amplification of particular taxa within a bulk sample may go unnoticed because other taxa in the mixture still yield sequence data. Reviews of non-human forensic species identification highlight NUMTs, alongside population substructure and the inherent limitation of mitochondrial markers in detecting hybrid individuals, as persistent sources of interpretive uncertainty.

6.4 Standardization, Cost, and Legal Admissibility

Beyond the purely molecular challenges, forensic entomology reviews increasingly emphasize the need for standardized protocols covering evidence collection, chain of custody, laboratory processing, and result interpretation, so that DNA barcoding evidence can withstand legal scrutiny. Standards bodies such as NIST's Organization of Scientific Area Committees have begun issuing formal guidance on collecting and preserving entomological evidence for exactly this reason. Cost and access to sequencing infrastructure also remain practical barriers in many jurisdictions, particularly where regional reference libraries are still under development.

7. Conclusion

DNA barcoding, anchored primarily in the mitochondrial COI gene, has fundamentally strengthened the evidentiary reliability of forensic entomology over the past decade. By enabling accurate species identification across all developmental stages and even from damaged or fragmentary specimens, it directly addresses the central limitation of morphology-only taxonomy and, by extension, improves the accuracy of postmortem interval estimation and broader case reconstruction. Regional studies from India collectively demonstrate both the maturity of the technique and the continuing need for locally curated reference databases. Emerging tools — mini-barcoding, NGS-based metabarcoding, multi-locus schemes, and AI-assisted classification — are extending barcoding's reach to increasingly degraded, mixed, and complex forensic samples. At the same time, unresolved challenges around database completeness, the barcode gap, NUMTs, contamination, and legal standardization mean that DNA barcoding is best understood as a powerful complement to, rather than a wholesale replacement for, traditional morphological and entomological expertise. Continued investment in regional reference libraries, standardized forensic protocols, and integrative molecular-computational pipelines will be central to fully realizing DNA barcoding's potential in modern forensic entomology.

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