Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
https://doi.org/10.35229/jaes.1840921

JAES
Year: 11, 2026 (1-7)
AÇEH
Yıl: 11, 2026 (1-7)

REVIEW PAPER **DERLEME MAKALESİ**

DNA Barcoding in Forensic Entomology: A Molecular Tool That Increases Reliability in Estimating the Postmortem Interval

Mahmut KABALAK [1] | **Yavuz TURAN** [2] *
[1]*Hacettepe University, Faculty of Science, Department of Biology, 06800 Ankara, Türkiye*
[2]*Marmara University, Faculty of Science, Department of Biology, 34722 Istanbul, Türkiye*

***Received:* 12.12.2025** ***Accepted:* 27.01.2026**

How to cite: **Kabalak, M., & Turan, Y. (2026).** DNA Barcoding in Forensic Entomology: A Molecular Tool That Increases Reliability in Estimating the Postmortem Interval. *J. Anatolian Environ. Anim. Sci.*, ***11***, 1-7. https://doi.org/10.35229/jaes.1840921

Atıf yapmak için: **Kabalak, M., & Turan, Y. (2026).** Adli Entomolojide DNA Barkodlama: Ölüm Sonrası Zaman Aralığı Tahmininde Güvenirliği Artıran Bir Moleküler Araç. *Anadolu Çev. Hay. Bil. Derg.*, ***11***, 1-7. https://doi.org/10.35229/jaes.1840921

Abstract: Forensic entomology has become an indispensable tool for estimating the postmortem interval (PMI), particularly in advanced stages of decomposition where classical forensic methods are limited. Reliable PMI estimation requires accurate and precise identification of insect evidence. Traditional morphological approaches often fail when dealing with immature stages (eggs, larvae, and pupae), closely related species, or partially damaged specimens. To overcome these limitations, DNA barcoding has been increasingly integrated into forensic entomology over the past two decades. DNA barcoding typically relies on sequencing a standardized fragment of the mitochondrial *cytochrome c oxidase I (coi)* gene and comparing the obtained sequence with reference databases such as BOLD and GenBank to assign species identity. In this review, we summarize the theoretical basis of DNA barcoding in forensic entomology, the genetic markers employed, and the main steps of sampling, DNA extraction, PCR amplification, and sequence analysis. We present application examples from Diptera and Coleoptera and discuss how DNA barcoding contributes to PMI estimation and case reconstruction. Furthermore, we address recent developments such as mini-barcode strategies, Next Generation Sequencing (NGS)/metabarcoding approaches, multi-locus barcoding, and highlight key limitations including database gaps, the barcode gap concept, contamination, and NUMTs. We also evaluate the current situation in Türkiye and propose the establishment of a national barcode reference library for forensically relevant insect taxa. Overall, DNA barcoding emerges as a powerful molecular tool that complements rather than replaces morphology, thereby improving the reliability of species identification and PMI estimation in forensic casework.

Keywords: COI, DNA barcoding, forensic entomology, molecular species identification, postmortem interval.

***Corresponding author's:**
Yavuz TURAN
Marmara University, Faculty of Science,
Department of Biology, 34722 Istanbul, Türkiye
✉: yavuz.turan@marmara.edu.tr

Adli Entomolojide DNA Barkodlama: Ölüm Sonrası Zaman Aralığı Tahmininde Güvenirliği Artıran Bir Moleküler Araç

Öz: Adli entomoloji, özellikle yumuşak dokuların hızla bozulduğu ve klasik adli tıp yöntemlerinin yetersiz kaldığı durumlarda ölüm sonrası zaman aralığı (ÖSZA) tahmininde vazgeçilmez bir araç haline gelmiştir. Ancak böcek gelişim sürelerine dayalı güvenilir bir ÖSZA tahmini için, elde edilen entomolojik örneklerin türlerinin doğru ve kesin biçimde teşhis edilmesi gereklidir. Geleneksel morfolojik teşhis yöntemleri, özellikle yumurta, larva ve pupa gibi ergin olmayan dönemlerde, yakın akraba türlerde ve kısmen tahrip olmuş örneklerde çoğu zaman yetersiz kalmaktadır. Bu sınırlılığı aşmak üzere son 20 yılda DNA barkodlama yaklaşımı adli entomolojiye yaygın biçimde entegre olmuştur. DNA barkodlama, çoğunlukla mitokondriyal *sitokrom c oksidaz I (COI)* geninin standart bir bölgesinin dizilenmesine dayanmakta; elde edilen dizi, BOLD ve GenBank gibi referans veri tabanlarındaki kayıtlarla karşılaştırılarak tür tanısı yapılmaktadır. Bu derlemede, adli entomolojide DNA barkodlamanın kuramsal temeli, kullanılan moleküler genetik belirteçler, örnekleme–DNA izolasyonu–PCR–dizi analizi basamakları, Diptera ve Coleoptera başta olmak üzere çeşitli taksonomik gruplar üzerindeki uygulama örnekleri ve ÖSZA tahminine katkıları ele alınmıştır. Ayrıca mini-barkod stratejileri, Yeni Nesil Sekanslama (YNS)/metabarkodlama yaklaşımları, çoklu belirteç kullanımı, veri tabanı sorunları, barkod boşluğu (barcode gap), kontaminasyon ve NUMT gibi teknik sınırlılıklar tartışılmış; Türkiye'de adli entomoloji bağlamında DNA barkodlamanın mevcut durumu ve ulusal barkod veri tabanı oluşturulmasına yönelik öneriler sunulmuştur. Sonuç olarak DNA barkodlama, morfolojik teşhisi ikame etmekten çok onu tamamlayan, tür tayini ve PMI tahminini daha güvenilir hale getiren güçlü bir moleküler araç olarak öne çıkmaktadır.

Anahtar Kelimeler: Adli entomoloji, COI, DNA barkodlama, moleküler tür tanısı, ölüm sonrası zaman aralığı.

***Sorumlu yazar:**
Yavuz TURAN
Marmara Üniversitesi, Fen Fakültesi, Biyoloji
Bölümü, 34722, İstanbul, Türkiye
✉: yavuz.turan@marmara.edu.tr

INTRODUCTION

Forensic entomology is a broad field in which arthropod science interacts with the judicial system (Lord & Stevenson, 1986). It is an important field within the forensic sciences for estimating postmortem interval (PMI), and postmortem submersion interval (PMSI) (Sharma&Dalal, 2025). It is a subdiscipline that focuses on the use of arthropods—particularly necrophagous insects—as evidence in forensic investigations. The insect species and their developmental stages that arrive at a corpse during different stages of decomposition (Amendt et al., 2007) provide valuable information about the postmortem interval (PMI), thereby complementing classical forensic pathology methods. However, for this information to be used effectively, the correct identification of entomological specimens collected from the crime scene is crucial, as each species has different development rates, thermal thresholds, and ecological preferences.

Traditional species identification relies on morphological characters and the expertise of taxonomists. Although often sufficient for adult specimens, it poses significant limitations for eggs, larvae, and pupae; for distinguishing closely related species; and for poorly preserved or partially damaged samples. In species-rich groups (e.g., Calliphoridae, Sarcophagidae, Fanniidae, Staphylinidae, Cholevidae), identifying larvae or females is particularly challenging for non-specialists.

With advances in molecular biology techniques, DNA barcoding has become the most important complementary tool to morphological identification in forensic entomology. DNA barcoding is based on sequencing a standard region of the mitochondrial *coi* gene and comparing the sequence with reference databases (such as BOLD and GenBank). In this way, specimens that are difficult to identify morphologically can be determined at the species level at all life stages. Comprehensive reviews summarizing the use of DNA barcoding and related molecular approaches in both taxonomy and forensic sciences (Wells & Stevens, 2008; Sagar et al., 2025) outline the general framework of the method. The aim of this review is to discuss the use of DNA barcoding in forensic entomology comprehensively—from its fundamental principles and application examples to its advantages and limitations, as well as the current situation and needs specific to Türkiye.

Fundamentals of DNA Barcoding and the Gene Regions Used:

The Concept of DNA Barcoding: DNA barcoding is a molecular species identification approach that enables the identification of an organism using a short and standardized DNA sequence (Hebert et al., 2003). For animals, the most widely used barcode region is the mitochondrial *cytochrome c oxidase I (coi)* gene. COI provides strong discriminatory power because it exhibits sufficient variation among species (Meyer & Paulay, 2005) while typically showing low intraspecific variation. This forms the basis of the “barcode gap” concept, where a clear distinction between the maximum intraspecific genetic distance and the minimum interspecific distance increases the reliability of species identification using COI.

The barcoding process generally involves the following steps:

(i) Proper preservation of the specimen,
(ii) DNA extraction,
(iii) Amplification of the COI region via PCR,
(iv) Sequencing of the PCR product, and
(v) Comparison of the resulting sequence with reference databases and phylogenetic analysis.

The general principles of DNA barcoding have been comprehensively addressed across a wide range of fields from taxonomy to forensic sciences, and its historical development, technical details, and applications have been extensively reviewed (Hebert et al., 2003; Sagar et al., 2025).

COI and Alternative Molecular Markers: In the forensic entomology literature, the most commonly used marker for barcoding insect species is COI. In most studies, the standard ~650 bp barcode region is amplified using the primer pairs described by Folmer et al. However, in some species complexes, relying solely on COI may be insufficient, as closely related (sibling) species can share highly similar COI sequences (Wells & Stevens, 2008). For this reason, recent studies have also explored other mtDNA genes (such as COII, Cyt-b, and ND genes), nuclear ribosomal regions (particularly ITS2), and various nuclear gene introns and spacer regions for species identification. For example, the ITS2 region has been reported to distinguish certain Sarcophagidae and Calliphoridae species more effectively than COI, showing clear differences even in species pairs that COI cannot discriminate (Park et al., 2018). A multilocus barcoding approach holds significant potential for increasing diagnostic accuracy, especially in problematic species complexes (Dupuis et al., 2012; Liu et al., 2017).

Reference Databases: BOLD and GenBank: The success of DNA barcoding largely depends on reliable reference databases. The two principal platforms are the Barcode of Life Data System (BOLD) and GenBank. BOLD provides standardized formats, metadata, and quality-control criteria for barcode data; each barcode record typically includes the species name, geographic location, collection details, and often a photograph of the specimen. GenBank, on the other hand, is a much broader repository that hosts a wide range of mtDNA and nuclear gene sequences in addition to barcode sequences. However, because submissions are user-generated, heterogeneity in species identification and sequence quality may occur.

The ideal approach is to query the newly obtained COI sequence from a specimen against both BOLD and GenBank, supporting the species identification through high sequence similarity, low e-values, the presence of a barcode gap, and phylogenetic tree–based analyses. Recently, the current status and future directions of DNA and other molecular markers in forensic entomology have been discussed in detail in a review article (Haymer, 2024; Khullar & Singh, 2025).

Application for DNA Barcoding in Forensic Entomology:

Diptera (Calliphoridae, Sarcophagidae, Fanniidae, etc.): Necrophagous flies are the most frequently used group in forensic entomology. Due to their high species richness, COI barcoding provides significant advantages, particularly for species identification of larvae. Species that are morphologically very similar can be clearly differentiated through variations in their COI sequences (Figure 1).

In a study conducted on specimens collected from cadavers in Pakistan, COI barcodes were used to identify species and analyze the population structure of three forensically important dipteran species. High genetic diversity and distinct barcode gaps were reported for *Chrysomya megacephala, C. saffranea,* and *C. rufifacies* (Adnan et al., 2025). Similarly, a study carried out in the Jeddah region of Saudi Arabia generated new forensic Diptera and Coleoptera DNA barcode sequences and demonstrated the potential of these species for PMI estimation (Sharawi, 2025).

In a comprehensive DNA barcoding study on European Fanniidae species, COI barcode regions were sequenced for 27 species, and COI data were produced for the first time for 13 of these (Grzywacz et al., 2017). The study showed that both the full-length (~658 bp) barcode and the ~130 bp mini-barcode region provided very high accuracy in specimen identification; however, incorrectly labeled records in BOLD and GenBank were found to negatively affect barcode analyses.

Research conducted on necrophagous fly species in South Korea provides a good example of how regional molecular barcoding studies can be structured. In these studies, COI and ITS2 sequences were generated for species collected from human cadavers and shared with the scientific community through conference presentations and posters (Park et al., 2018).

Coleoptera and Other Necrobiont Groups: Although Diptera are the most commonly used insects in forensic investigations, Coleoptera (e.g., Dermestidae, Staphylinidae, Histeridae, Silphidae, Nitidulidae, Cleridae, Cholevidae) can provide important information, particularly during the later stages of decomposition and skeletalization. However, morphological identification of these groups is limited due to the scarcity of experts and the difficulty of identifying larvae.

In a DNA barcoding study conducted on Cholevidae (small necrobiont staphylinoid beetles), COI sequences were obtained for numerous species, demonstrating that the barcode gap concept is strongly applicable in this family as well (Schilthuizen et al., 2011). Intraspecific distances were found to be low, whereas interspecific distances were markedly high, allowing most species to be clearly distinguished based on their COI sequences. Furthermore, the activity of this taxon particularly during cold seasons suggests that it may provide complementary information for PMI estimation in deaths occurring in winter months.

In a faunistic study conducted on pig carcasses in Beytepe, Ankara, a total of 40 species belonging to Staphylinidae, Histeridae, Dermestidae, Silphidae, Nitidulidae, and Cleridae were recorded; 22 of these were evaluated as “forensically important species” due to their regular occurrence during the decomposition process (Özdemir & Sert, 2009). The study demonstrated that Silphidae generally appear first, followed by Staphylinidae and Histeridae, while Dermestidae, Nitidulidae, and Cleridae become dominant in later stages of decomposition. This successional pattern is consistent with classical models described in many international studies.

Species Identification, PMI, and Crime Scene Reconstruction: The contributions of DNA barcoding to forensic entomology can be summarized under three main categories. The first is species identification. For non-adult stages, damaged specimens, or cryptic species, species determination using the COI barcode has become far more reliable than morphological methods. This greatly reduces the risk of incorrect minimum PMI (min PMI) estimates caused by misidentification (Wells & Stevens, 2008).A substantial body of research has shown that DNA barcodes based on the mitochondrial *cytochrome oxidase I (coi)* gene and the nuclear internal transcribed spacer 2 (ITS2) region constitute effective and reliable molecular markers for the accurate identification of mosquito species and the resolution of cryptic species complexes (Hebert et al., 2004; Kumar et al., 2007; Günay et al., 2015; Yavaşoğlu et al., 2016).

The second contribution is PMI estimation. DNA barcoding does not calculate the PMI directly; however, by ensuring accurate species identification, it enables the correct use of developmental data and thermal thresholds specific to that species. Calculations based on an wrong species can shift the min PMI by days or even weeks. The integration of regional population data with DNA barcodes, as demonstrated in the Jeddah study, enhances the reliability of PMI interpretations (Sharawi, 2025).

The third contribution concerns crime scene reconstruction and body relocation. When significant genetic differences exist among geographic populations of the same species—based on COI or other markers—the genetic profile of insects found on a body can provide clues regarding whether the body was moved from the place of death and from which geographic region it may have originated. This is particularly valuable for regionally endemic species or populations restricted to specific altitude or climate zones.

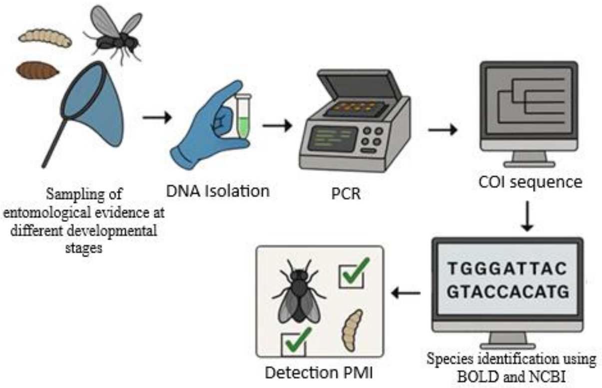

Figure 1. Stages of DNA barcoding application in forensic entomology.

Integration with Faunistic and Succession Studies from Türkiye: In Türkiye, several key studies conducted over the past two decades-particularly in the Ankara and İstanbul regions-stand out in the context of forensic entomology. In a study conducted on 12 pig carcasses in Beytepe, four Calliphoridae species were identified; the seasonal activity windows and temperature ranges of *Lucilia sericata*, *Chrysomya albiceps*, *Calliphora vomitoria*, and *Calliphora vicina* were reported in detail (Şabanoğlu & Sert, 2010). In a second study carried out at the same site, 40 Coleoptera species were recorded, 22 of which were shown to occur regularly throughout the decomposition process (Özdemir & Sert, 2009).

In another study conducted on a decomposing dog carcass in Hacettepe Beytepe, species belonging to Calliphoridae, Sarcophagidae, and Muscidae (Diptera), as well as Staphylinidae, Histeridae, Dermestidae, Nitidulidae, and Cleridae (Coleoptera), were recorded, resulting in a total of 21 identified species (Sert et al., 2012). This was the first faunistic forensic entomology study conducted on a dog carcass in Türkiye.

The first forensic entomology study conducted in Edirne Province was carried out by Bana & Beyarslan (2012). In this study, pig carcasses and internal organs of large livestock were used to identify Coleoptera species attracted to carrion. As a result of the study, a total of 11 species belonging to three genera and seven families within the order Coleoptera were recorded from three pig carcasses and two sets of large livestock internal organs (lung, liver, heart, and kidney).

In 2017, Coleoptera species attracted to pig carcasses were identified in Eskişehir Province. This study also evaluated the succession of Coleoptera species and compared urban and rural species composition. The study was conducted using a total of 24 pig carcasses. At the end of the study, 80 species belonging to five families were identified. In addition, species representing new carrion records for Türkiye were also reported (Altunsoy et al. 2017).

Karataş&Darılmaz (2018) investigated species attracted to two pig carcasses in Aksaray Province over a 36-day period in an aquatic habitat. As a result, from the first experimental animal, nine species belonging to five families of the order Diptera were identified, along with five species and one subspecies from four families of the order Coleoptera. In addition, one species belonging to the family Gerridae (Hemiptera) was recorded. From the second experimental animal, seven species belonging to three families of Diptera, four species from three families of Coleoptera, and one species from the order Odonata were identified. This study represents the first research in Türkiye's forensic entomology literature to document the importance of aquatic insects.

The most recent study was carried out in the Maltepe district of İstanbul, at Marmara University Başıbüyük Campus, using four rabbit carcasses to determine the forensic insect fauna and succession patterns (Mutlu et al., 2024). A total of 33 species belonging to four orders were identified: 11 Diptera, 20 Coleoptera, one Hymenoptera, and one Hemiptera. Detailed tables presented the annual succession patterns of species such as *Calliphora vicina*, *C. vomitoria*, *Lucilia sericata*, *L. cuprina*, *L. caesar*, *L. ampullacea*, *Chrysomya albiceps*, *Dermestes undulatus*, *D. frischii*, *Thanatophilus rugosus*, *Necrobia rufipes*, and *N. violacea*.

When these studies from Ankara and İstanbul are evaluated together, a robust ecological and successional framework emerges, indicating which forensically important Diptera and Coleoptera species occur on which type of carcass, during which seasons and temperature ranges, and at which stages of decomposition in Türkiye. DNA barcoding will complement this framework at the molecular level by enabling accurate species identification at all life stages, thereby allowing these succession data to be applied more confidently to case-specific PMI interpretations.

Current Molecular Approaches:

Mini-barcode Strategies: In forensic samples, DNA is often degraded due to environmental factors (heat, humidity, and UV), chemical substances (drugs, preservatives), and long-term storage. Under such conditions, amplification of the full-length (~650 bp) COI region may fail. For this reason, the mini-barcode approach was developed. A mini-barcode targets a shorter region (100–300 bp) of the COI gene (Hajibabaei et al., 2006); being shorter, it has a higher likelihood of successful amplification even from degraded DNA.

In a mini-barcode study conducted on Neotropical necrophagous Calliphoridae species, a ~300 bp COI fragment was used to analyze numerous specimens, and the vast majority of species were successfully distinguished at the genus or species level (López-Rubio et al., 2017). The method was shown to work even on older samples obtained from museum collections, enabling the reanalysis of both historical collections and old forensic specimens.

In a large-scale study of European Fanniidae species, a ~130 bp COI mini-barcode was shown to provide identification accuracy comparable to the full-length barcode (Grzywacz et al., 2017). The same study emphasized that mislabeled records in BOLD and GenBank can disrupt the barcode gap and lead to incorrect matches, underscoring the necessity of a high-quality and reliable reference library even for mini-barcode analyses.

NGS and Metabarcoding: Classical Sanger-based barcoding requires separate PCR and sequencing for each specimen. When hundreds of larvae are collected from a crime scene, this approach can become limiting in terms of both time and cost. Next-generation sequencing (NGS)–based metabarcoding (Taberlet et al., 2012; Yu et al., 2012) provides an important solution to this problem.

In NGS-based studies, mixed insect communities (bulk samples) can be barcoded in a single analysis. For example, in a pilot study conducted on samples obtained from numerous human corpses from which necrobiont insects had been collected, a reference library was first generated using classical DNA barcoding. Subsequently, bulk DNA samples were sequenced with NGS, and more than 30 species/BINs (Barcode Index Number) were detected in a single run using the BOLD database (Chimeno et al., 2019)

This approach offers significant advantages, particularly for the rapid screening of large numbers of samples, quick characterization of species composition, and the detection of even rare species. The PCR-HRM (high-resolution melting) combined with COI barcoding approach proposed by Wang et al. (2025) also stands out as a fast and low-cost tool for species identification.

Multi-marker Approaches and Complete Mitochondrial Genomes: In some cases, COI alone may be insufficient for species discrimination. In such situations, multi-locus barcoding is performed using combinations such as COI + Cyt-b, COI + ITS2, or COI + COII, allowing problematic species pairs or complexes to be distinguished more clearly (Wells & Stevens, 2008; Park et al., 2018). With the rapid decline in sequencing costs, some researchers now recommend sequencing not only the COI gene but the entire mitochondrial genome, thereby maximizing the information available for species identification as well as population and phylogenetic analyses (Haymer, 2024).

Molecular Barcoding and Evidence Security: DNA barcoding is not only used for identifying insect species but has also begun to be applied to the security of forensic DNA samples. Molecular barcoding approaches developed in forensic genetics involve adding artificial DNA tags to blood, tissue, or other biological samples to detect any subsequent tampering or manipulation (Eenhakkattu Mana et al., 2025). Although these methods have not yet been applied directly to entomological evidence, it is likely that similar security barcodes could be incorporated into the chain-of-custody processes for insect samples in the future.

Evaluation of DNA Barcoding in Forensic Entomology:

Perspectives from Reviews and Case Studies: Recent comprehensive reviews summarize insect-based forensic approaches by outlining their historical development, fundamental concepts, and practical applications (Alam et al., 2024; Scieuzo et al., 2025; Khullar & Singh, 2025). These works highlight the importance of decomposition stages and succession patterns, insect biology and life cycles, ecological and biogeographic knowledge, and the integration of molecular data in forensic entomology.

Current case studies and field investigations demonstrate that DNA barcoding is being increasingly applied in both faunistic and practical forensic contexts (Adnan et al., 2025; Abu El-Hassan et al., 2025; Sharawi, 2025; Mutlu et al., 2024). Data from countries such as Korea, Pakistan, Saudi Arabia, and Türkiye underscore the importance of establishing regional barcode libraries (Ceylan, 2020).

Situation Assessment for Türkiye: Forensic entomology in Türkiye is still in a developmental stage; the number of forensic entomologists is limited, and systematic use of insect evidence in casework remains relatively rare. Nevertheless, carrion experiments conducted around Ankara and Istanbul using different carcass types (pig, dog, and rabbit) and habitats have provided an important foundation by documenting Diptera and Coleoptera succession patterns (Özdemir & Sert, 2009; Şabanoğlu & Sert, 2010; Sert et al., 2012; Mutlu et al., 2024).

In addition, a master's thesis focusing on COI barcodes of forensically important Diptera species in Türkiye generated sequence data from numerous species based on Turkish populations; these sequences both validated morphological identifications and were added as new records to international databases (Ceylan, 2020). Such efforts form the basis for a national reference library that can be used in future forensic investigations. The molecular markers used in DNA barcoding applications in forensic entomology and the corresponding references are presented in Table 1.

Recommendations for Türkiye: The primary objective for Türkiye should be the establishment of a national DNA barcode database for forensically important species, particularly necrophagous Diptera and Coleoptera. For this purpose, country-wide sampling should be conducted to generate COI and, when necessary, additional marker sequences, which should then be submitted both to BOLD/GenBank and to a national database.

Second, the necessary equipment, kits, and software infrastructure for DNA barcoding should be established in the laboratories of the Council of Forensic Medicine and universities, and personnel trained in entomology and molecular biology should be employed. Reviews of molecular techniques by Khullar and Singh (2025) and recent studies such as Haymer (2024) provide guidance on which methods and markers should shape this infrastructure.

Third, national standard protocols should be developed for sample collection at the crime scene, preservation, laboratory procedures, and data analysis. Methodological recommendations for rapid NGS-based species identification (Wang et al., 2025) can be integrated into these protocols. Regular communication and training programs should be organized among forensic physicians, entomologists, molecular biologists, and law enforcement personnel.

Finally, forensic entomology case studies from Türkiye should be published in the international literature together with DNA barcode data, thereby increasing visibility at both national and regional scales.

Table 1. Molecular markers used in forensic entomology.

Markers	Taxon	Reference
COI	20 species belonging to *Catops, Fissocatops, Apocatops, Choleva, Nargus, Ptomaphagus, Sciodrepoides* genera	Schilthuizen et al., 2011
COI	Calliphoridae species	López-Rubio et al., 2017
COI	Fanniidae species	Grzywacz et al., 2017
ITS2	11 Calliphoridae, five Sarcophagidae species	Park et al., 2018
COI-5P	88 Arthropoda species	Chimeno et al., 2019
COI	12 species belonging to Diptera	Ceylan, 2020
COI	*Sarcophaga ruficornis*	Abu El-Hassan et al., 2025
COI	*Chrysomya megacephala, C. saffranea, C. rufifacies*	Adnan et al., 2025
COI	Phoridae sp., *Physiphora alceae, Philonthus discoideus, Physiphora demandata*	Sharawi, 2025
COX1 and COX2	10 common necrophilous fly species	Wang et al., 2025

CONCLUSION

DNA barcoding makes it possible to identify insect species in forensic investigations rapidly, reliably, and across all life stages, thereby contributing significantly to PMI estimations and crime scene reconstruction. Although it does not completely eliminate the need for expertise in morphological identification, it complements it and provides an objective molecular tool that reduces errors in many cases.

With recent advancements such as mini-barcode strategies, NGS/metabarcoding, multi-locus approaches, and AI-assisted data analysis, the role of DNA barcoding in forensic entomology is expected to become even stronger in the coming years. For Türkiye, the establishment of a national barcode database, the strengthening of laboratory infrastructure, and interdisciplinary collaboration can accelerate the integration of DNA barcoding into forensic procedures. In this way, the contribution of entomological evidence to forensic investigations can be maximized both scientifically and practically.

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