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REVIEW ARTICLE

Advancing Insect Taxonomy and Biodiversity Research: A Comprehensive Review of DNA Barcoding and Non-Destructive DNA Extraction Techniques and Applications

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ABSTRACT: DNA barcoding has revolutionized species identification by utilizing standardized genetic markers, providing a rapid and accurate alternative to traditional taxonomy. Insects, being the most diverse animal group, present unique challenges in classification due to morphological similarities, cryptic species, and life stage variations. This review explores the application of DNA barcoding in insect identification, emphasizing its role in taxonomy, biodiversity assessment, and ecological research. A key focus is placed on non-destructive DNA extraction methods, which enable molecular analysis while preserving specimen integrity—an essential consideration for museum collections and rare species. Recent advancements, including next-generation sequencing (NGS), portable barcoding technologies, and artificial intelligence-driven analyses, are also discussed. Despite its advantages, DNA barcoding faces challenges such as database limitations, methodological inconsistencies, and the presence of nuclear mitochondrial pseudogenes (numts). Addressing these challenges through improved barcode reference libraries, global collaborations, and methodological refinements will further enhance the reliability and efficiency of DNA barcoding in entomology. This review provides a comprehensive overview of the latest developments and future directions in insect DNA barcoding, highlighting its growing significance in biodiversity research and conservation strategies.

Keywords: DNA barcoding, Insect taxonomy, Non-destructive DNA extraction (NDDE), Cytochrome c oxidase I (COI), Internal Transcribed Spacer (ITS), Maturase K (matK), Taxonomic database, Species delimitation.

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1. INTRODUCTION

DNA barcoding is a molecular technique used to identify species based on a short genetic marker in an organism's DNA. The standard barcode for animals is a fragment of the mitochondrial cytochrome c oxidase I (COI) gene, typically around 650 base pairs in length [1]. This technique is particularly effective in distinguishing closely related species due to the conserved nature of COI within species and its variability between species [2]. DNA barcoding is widely applied in taxonomy, ecological studies, and biodiversity

conservation. Insects, being the most diverse group of animals on Earth, present unique challenges in species identification. Morphological identification often requires taxonomic expertise and is hindered by cryptic species, sexual dimorphism, and incomplete specimens [3]. DNA barcoding provides a standardized approach, allowing researchers to quickly and accurately identify species from any developmental stage, including eggs, larvae, and adult specimens.

The concept of DNA barcoding was first introduced by Paul Hebert and colleagues in 2003 as a standardized method for species identification [4]. Since its inception, DNA barcoding has evolved into a global initiative, with projects like the Barcode of Life Data Systems (BOLD) amassing large reference libraries of barcode sequences. The use of DNA barcoding in entomology has grown significantly over the past two decades. Researchers have utilized this technique to explore insect diversity in various ecosystems, from tropical rainforests to temperate grasslands [5]. Additionally, barcoding has contributed to resolving taxonomic ambiguities, recognizing cryptic species, and monitoring invasive insect populations [6].

Traditional insect taxonomy relies heavily on morphological characteristics, which can be difficult to assess due to phenotypic plasticity, sexual dimorphism, and cryptic species complexes [4]. DNA barcoding has emerged as an efficient alternative, overcoming these limitations by allowing identification across all life stages, including eggs and larvae [2]. Beyond taxonomy, DNA barcoding has profound applications in biodiversity assessment. It has been instrumental in identifying and cataloguing insect species in various ecosystems, contributing to conservation efforts by enabling accurate monitoring of declining populations and tracking invasive species [1]. This method has also proven valuable in agricultural and forensic entomology, helping identify pest species and disease vectors efficiently [3].

This review examines the role of DNA barcoding in insect identification, highlighting its advantages, challenges, and recent advancements. Particular emphasis is placed on non-destructive DNA extraction techniques, which preserve specimen integrity—an essential aspect for museum collections and rare species studies. Additionally, we discuss the integration of DNA barcoding with large-scale biodiversity initiatives, regulatory frameworks, and taxonomy. Key challenges such as database gaps, methodological standardization, and the presence of nuclear mitochondrial pseudogenes (numts) are also explored, which can complicate species identification [7]. As sequencing technologies advance, DNA barcoding is expected to further enhance insect taxonomy, biodiversity monitoring, and conservation efforts.

2. PRINCIPLES OF DNA BARCODING

DNA barcoding follows a standardized approach for species identification by utilising short DNA sequences from specific genomic regions. The technique relies on the fundamental principle that genetic divergence between species is greater than within species, allowing for unique identification through sequence comparison. This methodology has been extensively used in insect taxonomy, conservation biology, and biodiversity assessment.

To ensure consistency and reliability, DNA barcoding employs specific genetic markers tailored to different taxa. The choice of barcode marker depends on factors such as mutation rate, sequence conservation, and amplification feasibility. Additionally, an ideal barcode must meet several key criteria, including universality across taxa, high interspecific divergence with low intraspecific variation, and a sequence length that allows efficient amplification and sequencing.

2.1. Genetic Markers in DNA Barcoding: Principles and Applications

DNA barcoding is based on the principle that short, standardized DNA sequences can be used to uniquely identify species by comparing them to a reference library. This technique relies on the assumption that genetic divergence between species is greater than intraspecific variation, allowing for distinct separation of taxa [8]. The effectiveness of DNA barcoding depends on selecting appropriate genetic markers that exhibit sufficient variation to differentiate species while remaining conserved enough for universal amplification.

For animals, the mitochondrial cytochrome c oxidase I (COI) gene has become the standard barcode due to its high mutation rate, maternal inheritance, and lack of recombination, making it ideal for distinguishing closely related species [9]. The reliability of DNA barcoding in insects has been demonstrated in numerous studies, particularly in species-rich orders such as Lepidoptera and Coleoptera, where traditional morphological identification can be challenging [10].

In contrast, nuclear markers such as the Internal Transcribed Spacer (ITS) region are commonly used in fungi, where mitochondrial DNA exhibits less variability [11]. For plants, plastid genes like ribulose-bisphosphate carboxylase (rbcL) and maturase K (matK) are utilized, as mitochondrial DNA evolves too slowly to be informative [12]. In prokaryotic organisms, the 16S ribosomal RNA (16S rRNA) gene serves as a widely accepted barcode, given its highly conserved structure across bacterial and archaeal taxa [13].

Summarizingly, for animals, the barcoding landscape is relatively unified, with the COI gene emerging as a universal standard due to its conserved structure and strong discriminatory power. In contrast, plant barcoding involves a more fragmented approach, as no single locus offers universal resolution across all plant species. Instead, a combinatorial strategy using chloroplast genes (e.g., rbcL, matK) and nuclear regions (e.g., ITS) is employed. This visual distinction underscores the taxon-specific challenges in barcoding, emphasizing the need for tailored molecular markers rather than a one-size-fits-all solution (Figure 1).

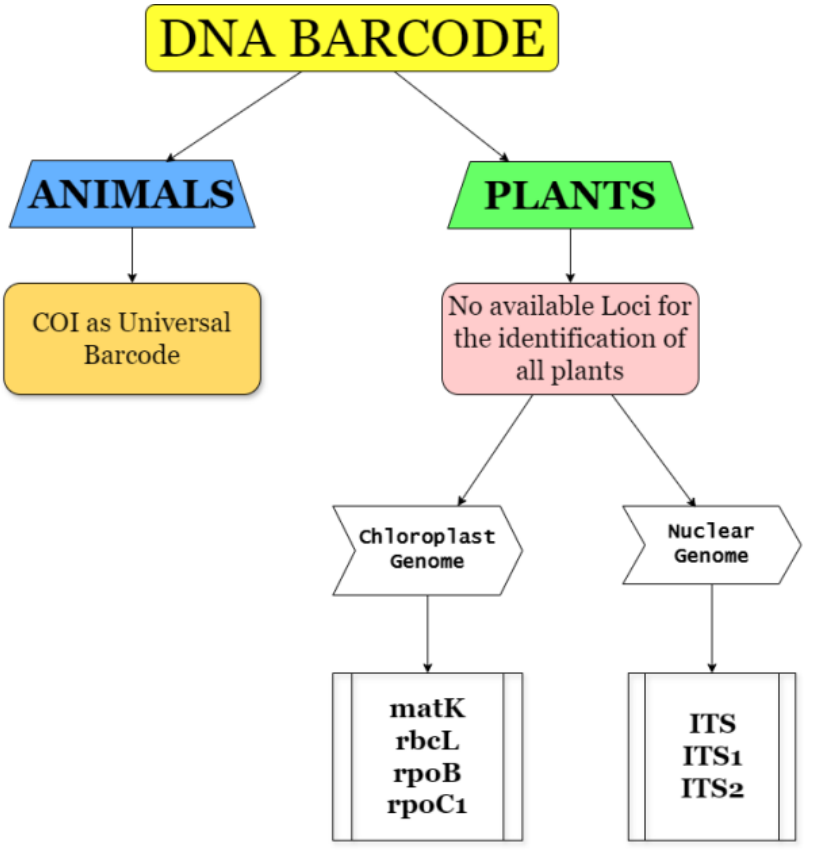

Fig. 1. A visual framework illustrating the DNA barcoding categories for animals and plants.

The figure also reflects the evolutionary constraints shaping marker choice—such as slow mitochondrial evolution in plants versus its higher variability in animals.

By utilizing standardized genetic markers across different domains of life, DNA barcoding enables taxonomic resolution and biodiversity assessment on a global scale. This approach not only facilitates species identification but also contributes to phylogenetic studies, conservation efforts, and ecological monitoring [14].

2.2. Standard DNA Barcode Regions for Different Taxa

Single-locus DNA barcodes often lack sufficient variation, whereas fully annotated super barcodes are costly and require advanced technical expertise, posing challenges for laboratories without specialized knowledge. To address this, researchers have introduced "specific barcodes," which serve as an intermediate approach by utilizing DNA fragments with a sufficiently high mutation rate to enable species identification within a defined taxonomic group. Because these barcodes are derived directly from the plastid genome sequences of a target family or genus, universal primers can be easily designed, reducing technical challenges such as low PCR efficiency and the need for extensive optimization. DNA barcoding strategies vary across taxa to maximize identification accuracy, with various barcode categories designated for both plants and animals. The choice of barcode marker depends on factors such as evolutionary rate, sequence conservation, and amplification feasibility. Below are the primary barcode regions used for various groups of organisms as mentioned below.

2.2.1. Animals

The mitochondrial cytochrome c oxidase subunit I (COI) gene is the most widely used barcode for animals due to its rapid mutation rate, which allows species-level differentiation [15]. COI is highly conserved across metazoans and contains regions suitable for universal primer binding, facilitating large-scale biodiversity studies. Research on true bugs (Hemiptera) has demonstrated that COI barcoding achieves a high success rate in species identification but also reveals cryptic diversity requiring further taxonomic revision [8].

2.2.2. Plants

Some well-established and functionally significant plant DNA barcodes include both chloroplast-derived and nuclear DNA sequences, each selected based on their reliability, universality, and ability to resolve species-level distinctions in angiosperms. Among these, the Internal Transcribed Spacer (ITS) region, located in the nuclear genome, consists

of ITS1, 5.8S, and ITS2, flanked by conserved 18S and 25S rRNA genes. Owing to its high variability and consistent amplification, ITS has proven highly effective for resolving species boundaries across various plant groups. In the chloroplast genome, the rbcL gene, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, is universally used in plant barcoding due to its broad coverage, stable sequence, and high amplification success, though its resolution at the species level is moderate. Complementing rbcL, the matK gene, also part of the chloroplast genome, encodes maturase K and offers higher nucleotide substitution rates, making it suitable for distinguishing closely related taxa. Another non-coding but widely adopted chloroplast region is the trnH-psbA intergenic spacer, situated between the trnH and psbA genes. Known for its substantial interspecific variability, this spacer region enhances species discrimination when used alongside coding barcodes, despite occasional alignment difficulties due to sequence length variation (Figure 2). Together, these markers form a robust molecular toolkit for plant identification and biodiversity assessment [16].

2.2.3. Fungi

The Internal Transcribed Spacer (ITS) region is the standard barcode for fungi, as it demonstrates substantial sequence variation between species while maintaining conserved primer-binding sites. ITS is particularly useful in distinguishing closely related fungal species and has been widely adopted in mycological research [17].

2.2.4. Bacteria & Archaea

The 16S ribosomal RNA (16S rRNA) gene serves as the primary barcode for bacterial and archaeal identification. This gene is highly conserved across prokaryotes but contains hypervariable regions that allow differentiation at the genus and species levels. Due to its slow evolutionary rate, 16S rRNA is often used in microbial ecology and phylogenetic studies [10].

By selecting appropriate barcode regions for each taxonomic group, researchers can achieve accurate species identification while maintaining methodological consistency across diverse lineages.

2.3 Mitochondrial Genome Map Highlighting Key Genetic Regions for DNA Barcoding

The mitochondrial genome highlights the location of the cytochrome c oxidase subunit I (COI) gene, a pivotal region in DNA barcoding for animal species. Situated among other protein-coding genes such as cytochrome b (Cyt b), NADH dehydrogenase subunits, and ATPase genes, COI is uniquely valuable for its balance of sequence conservation and variation (Figure 3).

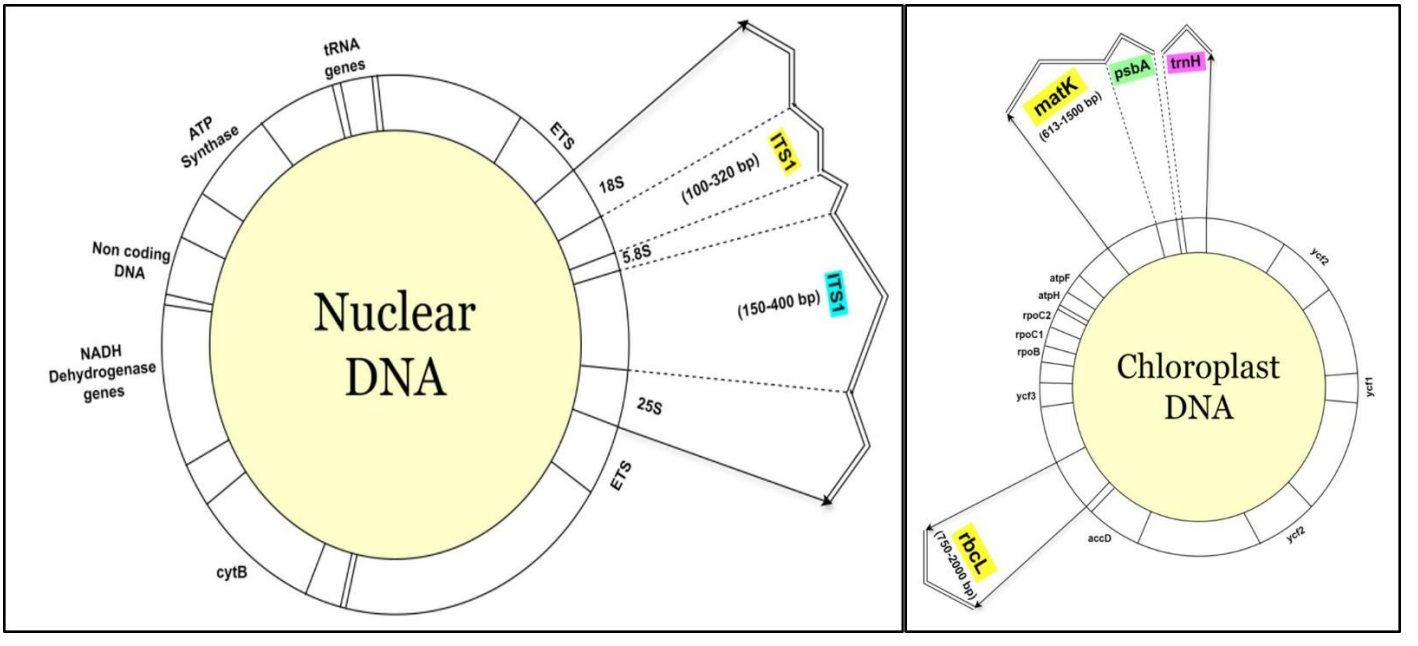

Fig. 2. A schematic cell layout and representative gene sequences, not drawn to actual size or scale. Gene regions have been enlarged for clearer visualization. [**Gene abbreviations:** accD – acetyl-CoA carboxylase, atpF – ATP synthase subunit b, atpH – ATP synthase subunit delta, bp – base pair, ETS – external transcribed spacer, ITS1/ITS2 – internal transcribed spacers 1 and 2, matK – maturase K, psbA – photosystem II D1 precursor, R – reverse, rbcL – ribulose-1,5-bisphosphate carboxylase/oxygenase, rpoB – RNA polymerase beta subunit, rpoC1/rpoC2 – RNA polymerase beta prime subunits 1 and 2, SSC – small single copy, trnH – tRNA-His gene, ycf – hypothetical chloroplast open reading frame.]

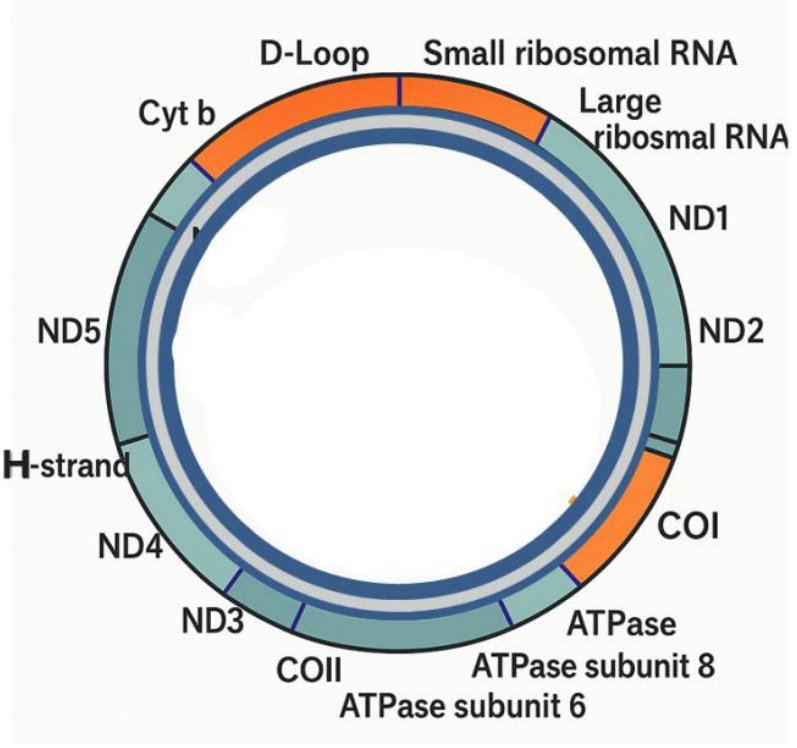

Fig. 3. Mitochondrial genome map showing the position of the cytochrome c oxidase subunit I (COI) gene, commonly used as a standard animal barcode due to its conserved nature and utility in species-level identification.

Its specific placement allows for the design of robust universal primers, making it an ideal candidate for efficient species discrimination in biodiversity assessments. Unlike more variable or functionally constrained regions, COI provides consistent amplification and alignment potential across a wide range of taxa, underscoring its central role in large-scale genetic identification efforts [8].

3. PROCEDURES AND PROTOCOLS IN DNA BARCODING

DNA barcoding involves a series of well-defined laboratory and analytical steps, from collecting specimens to analysing barcode sequences for species identification (Figure 4). These standardized procedures ensure accuracy, reproducibility, and reliability in species determination. Proper handling of specimens during collection and storage is critical to maintaining DNA integrity, while optimized extraction, amplification, and sequencing techniques enhance data quality. Finally, bioinformatics tools play a crucial role in interpreting sequence data and assigning species identities. The following sections outline the essential steps involved in DNA barcoding, from field collection to data analysis.

3.1. Sample Collection and Preservation

Proper sample collection and preservation are crucial for successful DNA barcoding, as DNA integrity significantly impacts sequencing quality and accuracy. Insects can be collected using various techniques depending on their habitat and behaviour. Common field collection methods include sweep netting, pitfall traps, malaise traps, and light traps, which help capture a wide range of insect species efficiently [18].

Specimen storage plays a critical role in preserving DNA integrity. Ethanol preservation (preferably 95–100%) is the most widely used method, as it effectively prevents DNA degradation while maintaining specimen morphology [19]. Other methods include silica gel desiccation, deep freezing at -80°C, and liquid nitrogen storage, which are particularly useful for long-term preservation [20]. For museum specimens and rare species, non-destructive sampling methods such as leg clipping and external tissue swabbing allow DNA extraction while preserving the original specimen.

3.2. DNA Extraction Methods

DNA extraction is the first step in obtaining genetic material for barcoding and can be performed using either traditional destructive or non-destructive methods. Destructive methods, including whole-body maceration and tissue digestion, yield high-quality DNA but render the specimen unusable for further morphological studies [21]. Non-destructive techniques, such as external soaking in lysis buffers, enzymatic digestion, and tissue swabbing, are increasingly preferred for museum specimens and rare insects, as they

preserve morphological characteristics while extracting sufficient DNA [22].

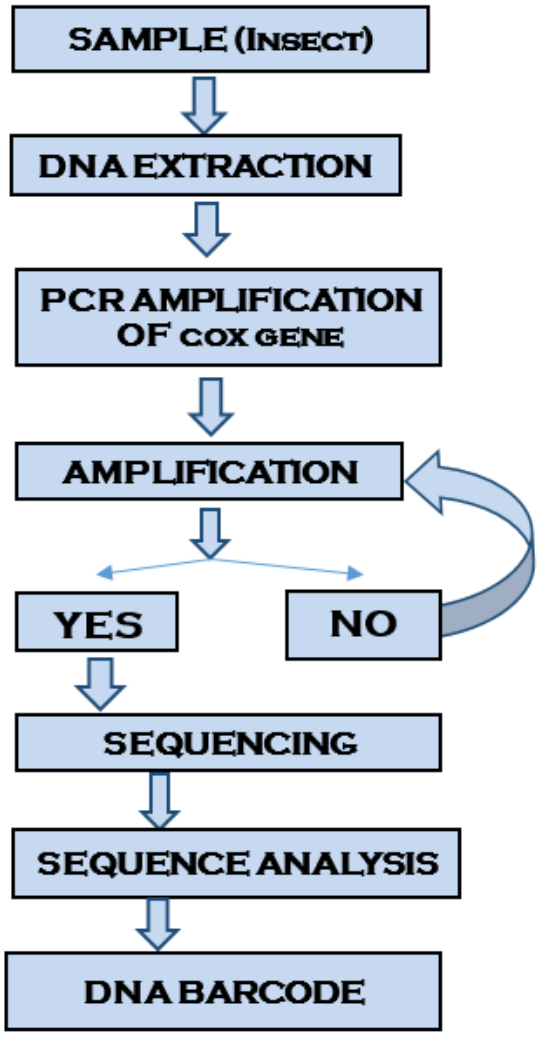

Fig. 4. Steps involved in the DNA Barcoding in Insects.

3.3. PCR Amplification

Polymerase Chain Reaction (PCR) is a critical step in DNA barcoding, where specific barcode regions are amplified for sequencing. Primer selection is crucial, with Folmer primers (LCO1490 and HCO2198) being the most commonly used for COI amplification in insects [11]. PCR conditions, including annealing temperature and cycle number, must be optimized to ensure successful amplification while minimizing non-specific products and nuclear mitochondrial pseudogene (numt) co-amplification, which can lead to erroneous results [7].

3.4. Sequencing Techniques

Once amplified, the barcode region is sequenced using one of two primary approaches: Sanger sequencing or Next-Generation Sequencing (NGS). Sanger sequencing, a widely used method, provides high-accuracy single-gene sequences but is limited in throughput and cost-effectiveness for large-scale studies [23]. NGS approaches, such as Illumina and Oxford Nanopore sequencing, offer higher throughput, allowing for DNA metabarcoding applications in environmental samples and large-scale biodiversity assessments [24].

3.5. Data Analysis & Interpretation

Post-sequencing, the obtained DNA sequences must be analyzed for species identification. Sequence alignment is performed using tools like MUSCLE or ClustalW to ensure high-quality data for phylogenetic analysis [25]. Phylogenetic trees are constructed using methods like Maximum Likelihood or Bayesian Inference to visualize evolutionary relationships among species [18].

Species assignment relies on comparing sequences against established databases such as the Barcode of Life Data Systems (BOLD) and GenBank. BLAST searches provide similarity-based identification, while BOLD offers curated species reference data with barcode gap analysis for improved accuracy. Advances in machine learning and AI-driven classification tools are further improving the accuracy and efficiency of DNA barcode-based species identification [26]. By following these standardized procedures and utilizing advanced sequencing and bioinformatics tools, DNA barcoding continues to enhance species identification, biodiversity assessment, and conservation biology.

4. ADVANTAGES AND LIMITATIONS OF DNA BARCODING

4.1. Advantages of DNA Barcoding

DNA barcoding has revolutionized species identification and biodiversity studies by providing a standardized, efficient, and scalable method for distinguishing organisms at the molecular level. Its advantages extend across multiple domains, including taxonomy, ecological monitoring, agriculture, and conservation biology [3].

4.1.1. Rapid and Accurate Species Identification

One of the primary advantages of DNA barcoding is its ability to identify species rapidly and accurately, even in cases where traditional morphological identification is challenging. This is particularly useful for insects, where cryptic species, sexual dimorphism, and incomplete specimens complicate taxonomy [27]. By using a standardized genetic marker, typically the mitochondrial cytochrome c oxidase subunit I (COI) gene, DNA barcoding enables reliable differentiation between species, even at early

developmental stages [2]. It has been shown to provide species-level resolution in 95-97% of cases, making it highly effective for distinguishing morphologically similar or cryptic species (Consortium for the Barcode of Life - CBOL). This has greatly improved taxonomic classification and has been instrumental in biodiversity research and conservation efforts. Furthermore, DNA barcoding reduces reliance on taxonomic expertise, allowing non-specialists to accurately identify species, making it an invaluable tool in environmental monitoring and regulatory frameworks. It also aids in preventing mislabelling of species and clarifying cases of synonymy, sexual dimorphism, and life-stage variation within species (Consortium for the Barcode of Life - CBOL).

4.1.2. High-Throughput Processing

The scalability of DNA barcoding makes it highly suitable for large-scale biodiversity assessments. With the advent of Next-Generation Sequencing (NGS) technologies, researchers can now process thousands of specimens simultaneously, streamlining species identification across diverse ecosystems [5]. This high-throughput capability is particularly valuable in monitoring insect diversity in ecological studies, identifying invasive species, and cataloguing new or cryptic taxa [28]. The efficiency of DNA barcoding also extends to the detection of new cryptic species and sibling species, which might otherwise go undetected using traditional morphological methods (Consortium for the Barcode of Life - CBOL). Additionally, the automation of sequencing and data analysis has minimized the reliance on expert taxonomists, making species identification more accessible and efficient. Large-scale barcode databases can be used in conjunction with automated classification tools, further expediting species identification and reducing human error [29].

4.1.3. Non-Invasive Sampling

Traditional morphological identification methods often require destructive sampling, which is impractical for rare or fragile specimens. DNA barcoding, on the other hand, allows for non-destructive or minimally invasive sampling techniques, such as leg or wing clipping, tissue swabbing, or environmental DNA (eDNA) analysis [11]. These methods preserve specimen integrity while still yielding high-quality DNA for sequencing. Non-invasive approaches have proven particularly useful for museum collections, forensic entomology, and conservation biology, where maintaining specimen morphology is crucial [30]. In forensic science, DNA barcoding has helped identify insect species in criminal investigations and wildlife trade enforcement, further demonstrating its broad applications beyond traditional taxonomy. Additionally, DNA barcoding is a valuable tool in detecting market substitutions, such as in the herbal and seafood industries, where it helps identify contaminants and prevent mislabelling (Consortium for the Barcode of Life - CBOL).

4.1.4. Data Sharing and Collaboration

A key advantage of DNA barcoding is the establishment of global, publicly accessible reference databases such as the Barcode of Life Data Systems (BOLD) and GenBank [31]. These repositories facilitate international collaboration by providing researchers with a comprehensive library of barcode sequences for comparative analysis. The integration of bioinformatics tools, artificial intelligence, and machine learning algorithms has further enhanced the accuracy and efficiency of DNA-based species identification [32]. By promoting open data sharing, DNA barcoding contributes to the rapid advancement of taxonomy, ecological monitoring, and regulatory frameworks for species conservation and management. Additionally, these databases enable the early detection of invasive species and the development of biosecurity strategies, ensuring proactive measures can be taken to mitigate ecological disruptions. Moreover, the increasing use of DNA barcoding in commercial sectors, such as in detecting seafood and herbal product contamination, highlights its broad relevance beyond scientific research.

4.1.5. Contributions to Conservation and Biodiversity Monitoring

DNA barcoding is also essential for conservation biology, offering insights into species diversification, historical habitat fragmentation, and identifying priority areas for conservation (Consortium for the Barcode of Life - CBOL). It optimizes biodiversity assessments by uncovering hidden biological diversity, which may otherwise remain undetected using traditional methods. The technology is instrumental in monitoring species distribution, genetic diversity, and population structures across large geographic areas, contributing to more effective local and global conservation strategies. The ability of DNA barcoding to distinguish cryptic species and provide molecular-based identification in challenging cases, such as with immature stages or damaged specimens, has made it a crucial tool in conservation efforts.

In summary, DNA barcoding offers a powerful, standardized, and efficient tool for species identification, biodiversity assessment, and ecological research. Its rapid processing, non-invasive sampling techniques, and global data-sharing capabilities make it a cornerstone of modern taxonomy and conservation biology. As sequencing technologies advance and bioinformatics tools improve, the role of DNA barcoding in global biodiversity monitoring and conservation efforts is expected to expand significantly, ensuring its continued relevance in scientific research and practical applications.

4.2. Limitations and Challenges of DNA Barcoding

Despite its significant contributions to taxonomy, biodiversity assessment, and ecological monitoring, DNA barcoding faces several limitations and challenges. These challenges range from technical difficulties in marker selection and primer design to broader issues such as database completeness and species misidentification. Additionally, inherent biological constraints, such as mitochondrial inheritance and evolutionary phenomena, can complicate barcode-based species identification. Addressing these limitations is crucial to enhancing the accuracy and reliability of DNA barcoding.

4.2.1. Marker Selection and Primer Design

The success of DNA barcoding depends on the use of standardized genetic markers that provide high interspecific divergence while maintaining low intraspecific variation. The mitochondrial cytochrome c oxidase subunit I (COI) gene is the most widely used barcode for animals, including insects, due to its relatively high mutation rate and ease of amplification [11]. However, challenges arise when selecting universal primers that work efficiently across diverse insect taxa. Some species exhibit primer mismatches due to mutations at primer-binding sites, leading to amplification failures or biased results [33].

Another major issue is the presence of nuclear mitochondrial pseudogenes (numts), which are non-functional copies of mitochondrial genes integrated into the nuclear genome. These pseudogenes can be co-amplified along with mitochondrial DNA, leading to erroneous species identifications [34]. The presence of numts is particularly problematic because they can be mistaken for true mitochondrial sequences, distorting taxonomic conclusions. Although sequence-checking protocols such as those implemented in the Barcode of Life Data System (BOLD) help detect these pseudogenes, recently integrated numts can be difficult to distinguish from functional mitochondrial sequences.

Moreover, different rates of genome evolution across taxa affect the utility of COI as a universal barcode. Some groups, such as cnidarians and sponges, exhibit low COI variability, making it challenging to distinguish closely related species. In such cases, alternative mitochondrial or nuclear markers, such as the internal transcribed spacer (ITS) region, are required [11].

4.2.2. DNA Degradation and Contamination

The quality of DNA is a critical factor affecting the success of barcoding studies. DNA degradation occurs due to environmental exposure, specimen age, and improper storage conditions. Museum specimens, for example, often yield fragmented DNA due to prolonged preservation in suboptimal conditions [6]. This limits the ability to generate full-length barcodes and may require the use of mini-barcodes—shorter sequences designed to amplify degraded DNA.

Additionally, contamination is a major concern, particularly in studies involving environmental DNA (eDNA) or bulk insect samples. Cross-contamination during DNA extraction, PCR amplification, or sequencing workflows can lead to false positives, misidentifications, or chimeric sequences [35]. Strict laboratory protocols, negative controls, and bioinformatics filters are necessary to minimize such errors.

Another factor that can distort barcoding results is endosymbiont infections. Bacteria such as Wolbachia can alter mitochondrial sequences, leading to inaccurate species assignments. Studies on parasitoid wasps have shown that Wolbachia-induced mitochondrial introgression can distort DNA barcode-based taxonomic delineations [36]. This is particularly problematic in insects, where symbiont-induced distortions can lead to incorrect species delimitations.

4.2.3. Intraspecific Variation and Hybridization

One of the core assumptions of DNA barcoding is that interspecific genetic variation exceeds intraspecific variation. However, in some insect species, high intraspecific divergence can lead to incorrect species assignments. This is particularly problematic in groups with rapid evolutionary diversification or strong geographic structuring [6].

Hybridization further complicates species identification. Closely related insect species may exhibit mitochondrial introgression, where one species acquires mitochondrial DNA from another through hybridization. This can blur species boundaries, making it difficult to distinguish between hybrid individuals and true species [34]. For instance, studies on Orthoptera have revealed cases where multiple species share identical barcodes, likely due to incomplete lineage sorting or historical hybridization events.

To address this issue, researchers advocate for integrative taxonomy approaches that combine DNA barcoding with morphological and ecological data. The use of multiple genetic markers, including nuclear genes, can also help resolve taxonomic ambiguities in cases of high intraspecific variation [36].

4.2.4. Database Completeness and Standardization

The effectiveness of DNA barcoding depends on the availability of comprehensive and well-curated reference databases. The Barcode of Life Data Systems (BOLD) and GenBank house extensive DNA barcode libraries, but significant gaps remain, particularly for insect taxa from under-sampled regions [14]. Inaccurate or incomplete reference sequences can lead to misidentifications, especially in large-scale biodiversity assessments. Studies have highlighted cases where barcode sequences were erroneously assigned to incorrect taxa due to sequencing errors,

mislabeling, or contamination [35]. Improving data validation and curation efforts, along with community-driven initiatives to expand barcode coverage, is essential for enhancing database reliability.

Standardization of barcode protocols across different research groups remains another challenge. Variations in DNA extraction methods, PCR conditions, and sequencing platforms can lead to inconsistencies in barcode data [33]. Establishing universal guidelines for DNA barcoding, along with adopting quality control measures, can improve reproducibility and data consistency. While DNA barcoding has transformed species identification and biodiversity monitoring, several challenges must be addressed to enhance its accuracy and reliability. Issues related to marker selection, DNA degradation, intraspecific variation, and database completeness highlight the need for continuous improvements in methodology and data management. Integrative approaches that combine DNA barcoding with other taxonomic tools will be crucial in overcoming these limitations and advancing the field of insect molecular taxonomy.

5. DNA BARCODING IN INSECTS

Insects represent the most diverse group of animals, accounting for more than 80% of known species on Earth. They play essential roles in ecosystems, such as pollination, decomposition, and serving as primary consumers in food webs. Despite their ecological importance, insect biodiversity remains poorly documented, particularly in tropical regions where the highest diversity is found [6]. Traditional morphological taxonomy often struggles with insect identification due to their vast diversity, phenotypic plasticity, and the presence of cryptic species. Many insect species exhibit sexual dimorphism, polymorphism, and high morphological similarity among related taxa, making classification challenging [1]. Moreover, identifying immature stages (eggs, larvae, pupae) using traditional methods is often impossible due to their undeveloped morphological traits. DNA barcoding provides a standardized and efficient solution to these challenges. By using a short, conserved genetic marker, such as the cytochrome c oxidase subunit I (COI) gene, researchers can accurately classify species across all life stages [3]. The increasing application of DNA barcoding has significantly enhanced biodiversity assessments, particularly in regions where taxonomic expertise is limited.

5.1. DNA Barcoding in Insect Taxonomy

DNA barcoding has revolutionized insect taxonomy by enabling rapid species identification, resolving cryptic species complexes, and reconstructing phylogenetic relationships [28]. Unlike traditional taxonomy, which often relies on subjective morphological characters, DNA barcoding offers an objective and reproducible method for species delimitation. Extensive barcoding efforts across diverse insect groups have significantly expanded reference databases and improved taxonomic resolution, offering valuable insights into species diversity and evolutionary relationships (Table 1).

5.1.1. Key applications of DNA barcoding in insect taxonomy include

Identification of cryptic species: Many insect taxa contain morphologically indistinguishable species that were previously classified as single species. DNA barcoding has helped uncover cryptic diversity in groups such as butterflies (Lepidoptera) and parasitic wasps (Hymenoptera) [29].

Clarifying species boundaries: Traditional taxonomy often struggles with species delimitation, especially in cases where hybridization, mitochondrial introgression, or incomplete lineage sorting occurs. DNA barcoding provides a genetic-based classification, complementing morphological and ecological data [37].

Phylogenetic studies: DNA barcoding contributes to phylogenetic reconstructions by providing molecular data that help infer evolutionary relationships among insect taxa [38].

5.2. Case Studies

Several studies have highlighted the effectiveness of DNA barcoding in identifying and classifying insects. A large-scale DNA barcoding study conducted in Jinnah Garden, Lahore, Pakistan, analyzed 10,792 arthropod specimens, successfully generating DNA barcodes for 88% of the samples. The study revealed significant insect diversity and underscored the need for improved reference libraries to enhance taxonomic accuracy [39].

DNA barcoding was used to analyze beetles (Coleoptera) in the Jammu region of India. The study successfully generated barcodes for multiple beetle species and used neighbor-joining phylogenetic trees to confirm taxonomic relationships. The findings demonstrated the reliability of barcoding for insect biodiversity monitoring and conservation efforts [38].

DNA barcoding revealed previously unrecognized cryptic species in sootywing and cloudywing butterflies (Lepidoptera: Hesperiidae). The study found that genetic divergences of 2–3% suggested potential new species, highlighting the importance of genetic data in refining insect classification [29]. DNA barcoding has proven invaluable for identifying pest species at various life stages, including larvae and eggs, which are often impossible to distinguish morphologically. This has facilitated the development of early pest detection systems for agricultural management [3].

5.3. Insect-Specific Challenges and Considerations

While DNA barcoding has transformed insect taxonomy, its application presents many unique challenges. Insects exhibit extreme diversity, with an estimated 5.5 million species, many of which remain undescribed. This high diversity complicates database completeness and reference sequence availability [40]. Standard COI primers often fail to amplify certain insect groups due to genetic divergence at primer binding sites. Developing group-specific primers and optimizing PCR conditions are critical to improving amplification success [33]. Hybridization between closely related species can result in mitochondrial DNA introgression, leading to misleading barcode assignments. Additional nuclear markers, such as ITS or 28S rRNA, can complement COI-based identifications [41]. DNA barcoding has revolutionized insect taxonomy by providing a fast, accurate, and scalable approach to species identification. While challenges remain, ongoing improvements in database completeness, primer design, and multi-marker approaches will enhance its effectiveness in biodiversity conservation and ecological research

6. NON-DESTRUCTIVE DNA EXTRACTION METHODS FROM INSECTS

6.1. Introduction to Non-Destructive DNA Extraction Methods (NDDE)

DNA extraction is a crucial step in molecular taxonomy and genetic studies. However, traditional extraction techniques often involve destructive methods that require maceration of whole specimens, leading to the loss of valuable morphological features. This poses a significant challenge, particularly for rare, endangered, or museum-preserved insect specimens, where specimen integrity is critical for taxonomic validation [53].

Table 1. A brief account of insects that have been barcoded so far.

Insect	Classification	Ref.
Fly Larvae	Diptera: Muscidae	[42]
Aphid	Hemiptera: Aphididae	[43]
Scirtothrips	Thysanoptera	[44]
PulexIrritans ***Phlebotomus sp.*** ***Aedes aegypti*** ***Geranomyia sp.*** ***Chrysopilusbalbii*** ***Aphrophilachilena***	Siphonaptera: Pulicidae Diptera: Psychodidae Diptera: Culicidae Diptera: Limoniidae Diptera: Rhagionidae Diptera: Limoniidae	[45]
Thrips	Thysanoptera	[46]
Encarsiaformosa	Hymenoptera: Aphelinidae	[47]
Chironomid Pupal Exuviae	Diptera: Chironomidae	[48]
Culicoides	Diptera: Ceratopogonidae	[49]
***Bactericeracockerelli (*tomato potato psyllid)** ***Diuraphisnoxia (*Russian wheat aphid)**	Hemiptera	[50]
	Coleoptera: Dermestidae,Buprestidae and Cerambycidae Hemiptera: Aphididae Diptera: Tephritidae Prostigmata: Eryophyidae	[51]
Spider	Araneae	[52]

NDDE techniques have been developed to obtain genetic material while preserving specimens for future morphological analysis. These methods play a vital role in taxonomic research, museum collections, forensic entomology, and biodiversity monitoring [54]. NDDE approaches primarily rely on minimal tissue removal, exoskeleton-based DNA extraction, or non-invasive enzymatic treatments, ensuring specimen preservation while maintaining DNA quality for sequencing and PCR applications [55].

6.2. Non-Destructive DNA Extraction Methods

Several NDDE methods have been developed to obtain genetic material from insect specimens while preserving their morphological integrity. The selection of an appropriate method depends on factors such as specimen size, preservation state, and taxonomic classification. Below are some widely used NDDE techniques, each with its own advantages and limitations.

6.2.1. Leg or Wing Clipping

Leg or wing clipping is one of the most common NDDE methods, involving the removal of a small portion of an insect's appendage (e.g., leg, wing, or antenna) for DNA extraction. This approach is particularly effective for hard-bodied insects, including beetles (Coleoptera), butterflies (Lepidoptera), and ants (Formicidae), where the excised body part does not significantly affect specimen integrity [56]. One of the main advantages of this method is that it minimally impacts the overall morphology of the specimen, allowing for future taxonomic examination. However, a key limitation is that DNA yield may be lower compared to whole-body extraction methods, and the technique is not suitable for soft-bodied larvae or fragile specimens.

6.2.2. Non-Lethal Sampling (Buccal Swabbing and Hemolymph Extraction)

Non-lethal sampling techniques are entirely non-destructive and allow for DNA collection from live specimens without causing harm. Two commonly used approaches include:

Buccal swabbing: This method involves using a sterile swab to collect oral epithelial cells from an insect's mouthparts.

Hemolymph extraction: A micropipette is used to extract hemolymph (insect blood) from leg joints or the thorax, which contains sufficient nuclear and mitochondrial DNA for genetic analysis [57].

These techniques provide high-quality DNA suitable for PCR and sequencing without affecting insect viability. However, they require skilled handling to prevent specimen stress, and they may not be feasible for extremely small insects where sufficient material cannot be collected.

6.2.3. DNA Extraction from Insect Exoskeletons

Exoskeleton-based DNA extraction is particularly useful for soft-bodied insects, such as aphids, fly larvae, and thrips, where destructive methods would result in the complete loss of taxonomically relevant structures. Instead of extracting DNA from internal tissues, this method uses washing or buffer-based techniques to recover DNA from the insect's exoskeleton [54]. The primary advantage of this technique is that it fully preserves external morphology, making it ideal for specimens in museum collections. However, its limitations include lower DNA yields compared to internal tissue extraction, and the process requires optimized buffers to enhance DNA recovery efficiency.

6.3. Comparison of Efficiency with Traditional Extraction Methods

The effectiveness of NDDE methods is typically evaluated based on DNA yield, purity, and PCR success rates compared to traditional destructive extraction techniques. While NDDE methods preserve specimen morphology, they often produce lower DNA concentrations due to the limited amount of genetic material available for extraction. However, recent advancements in enzymatic buffers and optimized lysis protocols have improved DNA recovery, making NDDE a viable alternative for various molecular studies [58].

6.3.1. DNA Yield and Purity Differences

Comparative studies on microhymenoptera DNA extraction have demonstrated the effectiveness of NDDE methods in amplifying mitochondrial COI sequences. A study assessing different extraction techniques reported the following COI amplification success rates:

- 100% success rate for destructive whole-body maceration methods.
- 88% success rate for enzyme-based NDDE methods, which utilize mild enzymatic digestion.
- 77% success rate for calcium chloride buffer-based NDDE methods, which rely on chemical lysis [55].

While DNA purity remains comparable between destructive and non-destructive methods, lower DNA concentrations in NDDE techniques may pose challenges for downstream applications.

6.3.2. Suitability for PCR and Sequencing

Despite their lower DNA yield, NDDE methods have been successfully applied in PCR-based species identification and Sanger sequencing. However, next-generation sequencing (NGS) applications, which require higher DNA concentrations and longer fragment lengths, still rely on traditional destructive extraction for optimal results [56].

6.4. Recent Advances in Non-Destructive DNA Extraction

Recent innovations in NDDE have significantly improved DNA yield and quality, allowing for more effective molecular analysis while preserving specimen integrity. Two major advancements—microfluidic technologies and single-cell sequencing—are revolutionizing NDDE approaches.

6.4.1. Use of Microfluidic Technologies

Microfluidic systems, also known as lab-on-chip technologies, have enhanced DNA extraction efficiency by minimizing sample volume requirements and allowing precise reagent control. These miniaturized devices reduce contamination risks, improve DNA recovery rates, and enhance overall workflow efficiency **[**59**].** One of the key advantages of microfluidic technologies is their ability to reduce the risk of contamination by operating in a controlled, enclosed environment. Additionally, they lower reagent costs by using minimal volumes and enable faster processing times due to automation and parallelization. However, despite these benefits, microfluidic systems require specialized equipment, which limits their accessibility, particularly for field studies. Their adoption is further hindered by the technical expertise needed to design and operate such devices, making them less practical for researchers working outside well-equipped laboratories.

6.4.2. Advances in Single-Cell Sequencing for Tiny Specimens

Single-cell sequencing has transformed NDDE approaches, particularly for minute insect specimens, such as mites, thrips, and parasitoid wasps. Using ultra-low input DNA protocols, researchers can extract high-quality genetic material while keeping the specimens intact for further morphological analysis [60]. This method allows for DNA analysis of micro-insects that were previously difficult to study and is highly compatible with high-throughput sequencing techniques, enabling large-scale genetic studies. However, the high cost of single-cell sequencing remains a major limitation, making it less feasible for routine applications. Additionally, the approach requires advanced bioinformatics expertise for data processing, which can be a barrier for researchers without access to specialized computational resources and training.

6.5. Kits used for DNA Extraction

The integration of NDDE methods into molecular taxonomy has significantly improved the ability to obtain genetic data without compromising specimen integrity. Traditional DNA extraction techniques, which often rely on whole-body maceration or destructive tissue sampling, limit the applicability of molecular studies for rare, museum-preserved, or endangered specimens. Taxonomists and molecular researchers face a critical trade-off between preserving morphological traits and acquiring high-quality DNA for sequencing. Extracting DNA from a single leg or wing fragment may not provide a sufficient yield, particularly for small-bodied insects, making it essential to refine non-destructive protocols for broader applicability.

Additionally, museum specimens, ancient organisms, and endangered species require special handling to prevent damage to irreplaceable collections. Developing non-destructive DNA barcoding approaches is crucial to enhancing biodiversity documentation, phylogenetic research, and species conservation while ensuring specimen longevity. Various commercial DNA extraction kits, such as the Qiagen DNeasy Blood and Tissue Kit, have been optimized for non-destructive applications. These kits are selected after getting a clear understanding of their respective efficacies and limitations and the criteria based on which they are slected should be justified based on their advantages, limitations, and ideal applications (Table 2). The process typically begins with the collection of a biological specimen, followed by DNA extraction using commercially available kits, ensuring consistent yield and purity. The extracted DNA is then subjected to PCR amplification using high-fidelity polymerases, and the resulting amplicons are verified via agarose gel electrophoresis. After cleanup of excess reagents, sequencing is performed, and the obtained data is edited to remove primer regions before comparison with publicly accessible barcode databases for species assignment (Figure 5). The silica-based purification approaches enable high-throughput processing and have been particularly effective in extracting DNA from archived and fragile specimens.

6.6. Protocol for Non-Destructive DNA Extraction from Insects

NDDE methods have become increasingly essential in molecular taxonomy, particularly for museum specimens, rare species, and conservation studies. NDDE is an innovative technique that preserves specimen integrity while enabling high-quality genetic analysis. The use of enzyme-based lysis, silica-column purification, and commercial extraction kits such as Qiagen DNeasy has significantly enhanced the efficiency of these methods. Unlike traditional DNA extraction techniques that require maceration or destruction of the specimen, NDDE methods allow for genetic analysis while maintaining the specimen's morphological integrity [53]. These methods are particularly beneficial for soft-bodied insects and small taxa, where destructive extraction may lead to the complete loss of

important taxonomic features [56].

Preparation before Extraction: Ensure that a set of standard images of the specimens is taken before DNA extraction to preserve taxonomic records. Confirm that essential laboratory equipment such as a hot bath or oven, centrifuge, racks, and vortex mixer are available. If the specimens are stored in ethanol at low temperatures, allow them to reach room temperature before proceeding with extraction.

Inspect the DNA extraction kit: Ensure that precipitates in buffers ATL and AL are fully dissolved. Confirm that ethanol has been added to buffers AW1 and AW2. Check the availability of spin columns, collecting tubes, and micro-centrifuge tubes for the entire set of specimens.

6.6.2. Stepwise Non-Destructive DNA Extraction Procedure

Day 1: Incubation and Enzymatic Lysis

The first step involves labeling and sterilizing three sets of microcentrifuge tubes by exposing them to UV light to minimize contamination. A hot bath or oven should be preheated to 56°C. Next, 90 µL of buffer ATL is added to each microcentrifuge tube. If the specimen is stored in ethanol, excess liquid should be gently blotted using a Kimwipe to prevent buffer precipitation, and the entire specimen should be placed into the microcentrifuge tube containing buffer ATL. For very small specimens, this step should be performed under a stereomicroscope. It is important to avoid crystallization of buffer ATL, as this may affect DNA yield. Then, 10 µL of proteinase K is added to each tube, making a final volume of 100 µL. The specimen must be fully submerged in the solution; if it adheres to the tube wall, the tube should be gently tapped on a bench surface. Centrifugation should be avoided, especially for fragile or weakly sclerotized insects. The tubes are then incubated at 56°C overnight (8–12 hours) for enzymatic digestion.

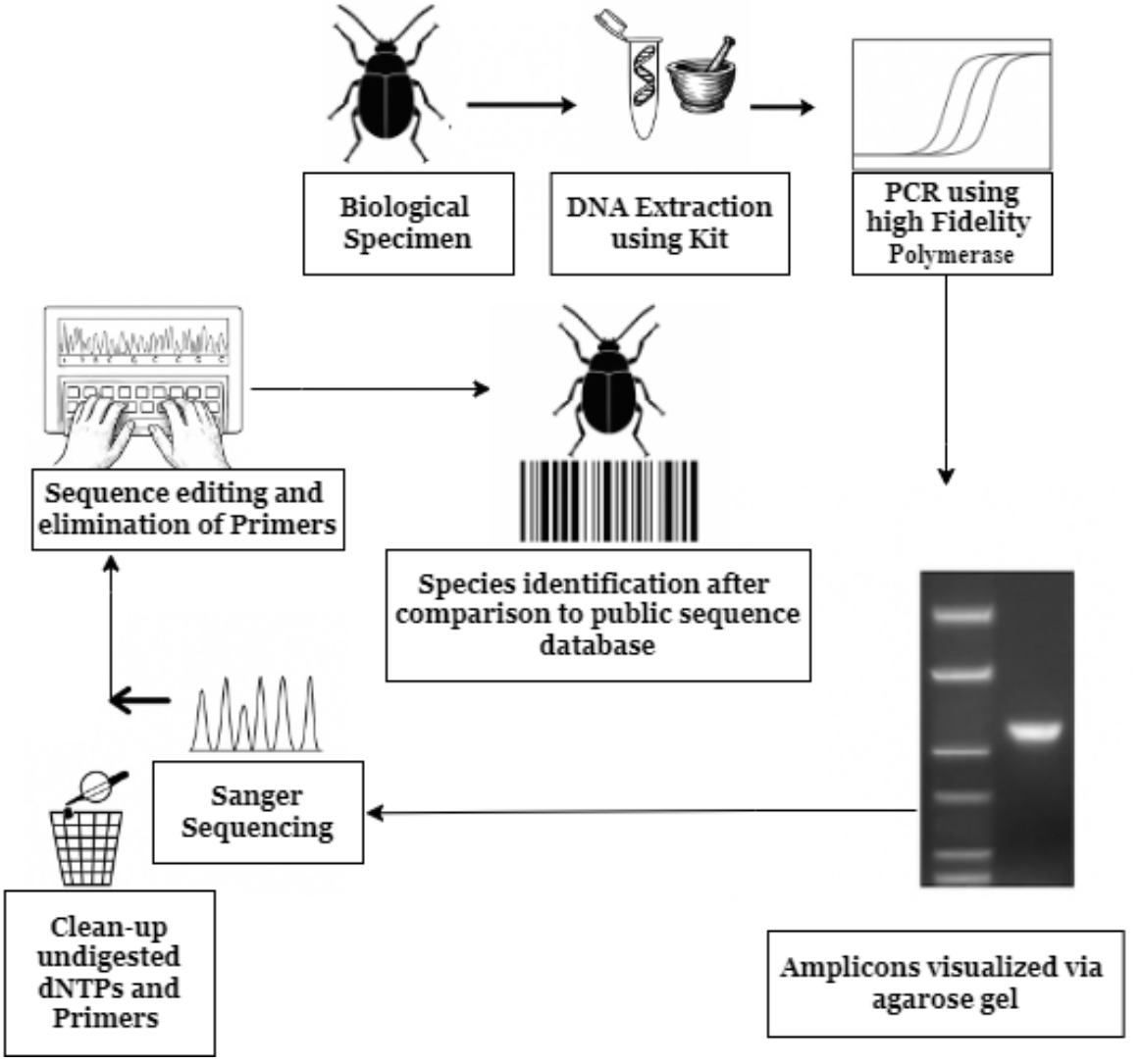

Fig. 5. Schematic of DNA barcoding workflow using kit-based extraction from insect specimens, followed by high-fidelity PCR, sequencing, and species identification via database comparison.

Table 2. The Commercial Kits used for the extraction of DNA from different types of animal tissues.

Commercial Kit and Supplier	Starting Material and Purification Method	Advantages
Qiagen DNeasy Blood and Tissue Kit (QIAGEN)	Animal tissues and blood; Silica-based technology	Optimized protocols for a range of tissues, 96-well high-throughput formats.
QIAamp DNA Stool Mini Kit (QIAGEN)	Fresh or frozen stool; Silica-based technology	No organic extraction or alcohol precipitation, complete removal of contaminants and inhibitors.
TIANamp Marine Animals DNA Extraction Kit (TIANGEN Biotech)	Tissues of fish, shrimp, shellfish, crab, etc.; Silica-based technology	Specially developed for marine animals, no organic extraction.
NucleoSpin® DNA RapidLyse Kit (Macherey-Nagel)	Fresh, frozen, dried or ethanol preserved animal organs, eukaryotic cells, tail and ear clippings; Silica-based technology.	Powerful lysis to efficiently release genomic DNA, superior genomic DNA yields.
NucleoSpin® DNA Insect (Macherey-Nagel)	Fresh, frozen, dried or ethanol preserved insect or crustacean; Silica-based technology and NucleoSpin® Bead Tubes Type D.	NucleoSpin® Bead Tubes for efficient lysis of an exoskeleton.
NucleoSpin® DNA Lipid Tissue (Macherey-Nagel)	Fresh or frozen, lipid-rich tissue (e.g., brain, adipose tissue, fatty fish tissue); Silica-based technology and NucleoSpin® Bead Tubes Type D.	Special buffer for efficient removal of lipids, NucleoSpin® Bead Tubes for efficient lysis.
Non-organic DNA Extraction Kit (Merck Millipore)	Whole blood, body fluid, bone marrow, mononuclear cells, solid tissues; Salting out precipitation.	A simple and non-toxic way to isolate high molecular weight genomic DNA.
OmniPrep™ kit (G-Biosciences)	Tissues from different species including animal, plant, bacteria, yeast and fungus; Unique precipitation reagents.	High yield of ~100 kb genomic DNA, no organic extraction.

Day 2: DNA Isolation and Purification

A second set of sterile Eppendorf tubes should be prepared, and a set of spin columns from the extraction kit should be labeled. Using a pipette set to 150 µL, the buffered extract is carefully transferred to a new Eppendorf tube, ensuring the specimen remains in its original tube. To preserve specimen integrity, it should be rinsed with distilled water and left submerged while completing the remaining steps. The Eppendorf tubes containing DNA extracts are vortexed for 15 seconds, followed by the addition of 100 µL of buffer AL and another vortexing step. Then, 100 µL of absolute ethanol is added, and the mixture is vortexed again. Next, 350–400 µL of the solution is transferred to a DNeasy Mini spin column placed in a collection tube and centrifuged at 8,000 rpm for 1 minute. The flow-through is discarded, and the spin column is placed into a new collection tube. Then, 500 µL of buffer AW1 is added to each spin column, followed by centrifugation at 8,000 rpm for 1 minute, and the flow-through is discarded. This is repeated with 500 µL of buffer AW2, centrifuging at 14,000 rpm for 3 minutes, and the flow-through is carefully discarded. The spin column is then transferred to a labeled 1.5 mL microcentrifuge tube. Next, 100 µL of buffer AE is added and allowed to incubate at room temperature for 1–2 minutes. The tube is centrifuged at 8,000 rpm for 1 minute, and this step may be repeated for efficient DNA elution. For small specimens, excess AE buffer should be avoided to prevent excessive dilution. Finally, the extracted DNA is stored at –20°C for future PCR and sequencing applications.

6.6.3. Preserving the Voucher Specimen Post-Extraction

To ensure specimen integrity, the residual ATL and proteinase K solution must be removed before reintroducing the insect

into ethanol. The specimen should be examined under a stereomicroscope and gently transferred into a series of ethanol washes, starting with 30% ethanol, followed by 50% ethanol, 75% ethanol, 90% ethanol, and finally 100% ethanol. Depending on the specimen's structural integrity, it may be mounted on a card or slide, particularly for weakly sclerotized insects.

6.6.4 PCR Amplification of the COI Gene

The cytochrome c oxidase I (COI) gene is a mitochondrial marker commonly used in DNA barcoding due to its high species resolution, conserved primer-binding sites, and universality across animal taxa. Amplification of COI sequences follows a standardized protocol.

Primer Selection and Amplification Efficiency

Universal COI barcode primers are commonly used for insects. If universal primers fail, taxon-specific primers or minibarcodes may be necessary for difficult-to-amplify species. Bioinformatics tools such as Primer Premier, Oligo, and Whitehead can assist in designing new primers for specific insect taxa.

6.6.5 PCR Components and Optimization

A standard PCR reaction mix includes heat-stable DNA polymerase, such as Taq polymerase, a dNTP mixture for nucleotide incorporation, and a buffer system to optimize enzyme activity. The $MgCl_2$ concentration may need adjustments based on DNA template quality. Thermocycling conditions should be tailored to annealing temperature and extension times for improved amplification success.

7. ADVANCES IN DNA BARCODING TECHNOLOGY

DNA barcoding has rapidly evolved as a powerful tool in taxonomy, biodiversity assessment, and ecological research. Recent technological advancements have enhanced its accuracy, efficiency, and applicability, allowing researchers to explore novel methodologies for species identification and environmental monitoring. The integration of Next-Generation Sequencing (NGS), portable sequencing technologies, artificial intelligence (AI)-driven analysis, and improvements in barcode reference databases has expanded the scope of DNA barcoding beyond conventional taxonomic studies, facilitating large-scale biodiversity projects and global conservation initiatives [61].

7.1. Integration with Next-Generation Sequencing (NGS)

The advent of NGS technologies has revolutionized DNA barcoding by enabling high-throughput sequencing, allowing the simultaneous processing of thousands of specimens. NGS-based metabarcoding approaches have become instrumental in studying insect biodiversity, as they facilitate the rapid identification of species from bulk samples, including environmental DNA (eDNA) extracted from soil, water, and air [6]. This approach is particularly useful for monitoring insect populations in changing ecosystems, assessing the impact of habitat loss, and detecting invasive species at an early stage. Furthermore, NGS has allowed for more comprehensive reference database expansions by enabling the sequencing of multiple genes in parallel, thus improving taxonomic resolution [62].

7.2. Portable DNA Barcoding

Recent advancements in portable sequencing technologies have significantly improved field-based DNA barcoding applications. Devices such as Oxford Nanopore Technologies' MinION sequencer have enabled researchers to conduct real-time DNA sequencing in remote locations, eliminating the need for transporting specimens to centralized laboratories [63]. These portable sequencing platforms have been successfully deployed in ecological surveys, conservation programs, and biosecurity monitoring efforts, allowing for the rapid detection of pest species and biodiversity assessments in the field. The ability to perform DNA barcoding in situ has profound implications for ecological research, as it enhances the efficiency of species identification and supports time-sensitive decision-making processes in environmental management [3].

7.3. Artificial Intelligence and Machine Learning in Barcoding Analysis

The integration of AI and machine learning algorithms in DNA barcoding has significantly enhanced species identification accuracy and data processing efficiency. AI-driven sequence analysis tools enable automated species identification by comparing DNA barcodes against extensive reference databases, reducing human error and expediting taxonomic classification [35]. Additionally, computational tools leveraging deep learning have been developed to detect cryptic species, identify hybridization events, and improve taxonomic resolution through enhanced phylogenetic modeling [11]. These AI-powered innovations are paving the way for a more automated, scalable, and accurate approach to biodiversity monitoring and species discovery.

7.4. Improving Barcode Reference Databases

The effectiveness of DNA barcoding is contingent upon the availability of comprehensive and well-curated barcode reference libraries. Efforts to expand databases such as the Barcode of Life Data Systems (BOLD) and GenBank have

significantly improved the taxonomic coverage of insect species. However, gaps in barcode representation still exist, particularly for underrepresented taxa and geographically isolated populations [64]. Community-driven initiatives and collaborative research networks are essential for addressing these gaps through continuous data validation, species verification, and curated additions of high-quality sequences. The integration of metadata, including ecological and morphological data, further enhances the utility of barcode reference libraries in taxonomic and conservation research.

7.5. Developing Universal Barcoding Markers for Insects and Global Collaborations

One of the persistent challenges in insect DNA barcoding is the identification of universal barcode markers that provide high resolution across diverse insect orders. While the COI gene remains the standard marker for most insect species, alternative genetic loci such as the nuclear Internal Transcribed Spacer (ITS) region and mitochondrial 16S rRNA are being explored to complement COI-based identifications [14]. Standardizing barcoding protocols through global collaborations and data-sharing initiatives is crucial for achieving consistency and reproducibility across research studies. International efforts such as the iBOL (International Barcode of Life) initiative have played a pivotal role in fostering cross-border cooperation and establishing best practices for DNA barcoding applications worldwide.

7.6. Enhancing Non-Destructive DNA Extraction Methods

Advancements in NDDE methods have enabled researchers to obtain high-quality genetic material without compromising specimen integrity. Microfluidic technologies and low-impact sampling techniques have been developed to improve DNA recovery from museum specimens, rare taxa, and fragile insects [65]. These innovations ensure that barcode analysis can be conducted while preserving morphological features, making DNA barcoding a more viable tool for historical specimen studies and long-term biodiversity monitoring. Furthermore, the refinement of single-cell sequencing approaches has enhanced the sensitivity of barcoding methods, allowing for the analysis of minute insect specimens and degraded DNA samples.

8. CONCLUSION

DNA barcoding has transformed insect taxonomy and biodiversity research by providing a standardized, rapid, and accurate approach to species identification. This review has highlighted the fundamental role of DNA barcoding in resolving taxonomic ambiguities, identifying cryptic species, and enhancing biodiversity assessment. The technique has proven invaluable in ecological monitoring, conservation biology, and forensic entomology, offering a molecular alternative to traditional morphology-based classification. Additionally, the integration of NDDE methods has expanded the applicability of DNA barcoding, ensuring that rare and museum specimens can be analyzed without compromising their structural integrity.

The significance of DNA barcoding in entomology extends beyond species identification. Its applications in large-scale biodiversity initiatives and regulatory frameworks demonstrate its potential as a crucial tool for environmental monitoring and pest management. The advancements in next-generation sequencing (NGS) and portable barcoding technologies have further enhanced its efficiency, enabling large-scale species assessments and real-time sequencing in the field. Additionally, artificial intelligence and machine learning are now being integrated to automate and improve the accuracy of barcode-based taxonomic classification, making the process even more robust.

Despite its numerous advantages, DNA barcoding still faces several challenges that must be addressed to maximize its potential. Issues such as incomplete barcode reference databases, methodological inconsistencies, and the presence of nuclear mitochondrial pseudogenes (numts) continue to hinder species identification. Ensuring global collaboration and data sharing through initiatives like the Barcode of Life Data Systems (BOLD) and GenBank will be crucial in overcoming these limitations. Moreover, developing universal barcoding markers tailored to diverse insect orders will improve taxonomic resolution and standardization across studies.

Looking ahead, advancements in sequencing technology, computational analysis, and non-destructive extraction techniques will continue to refine DNA barcoding methodologies. The integration of environmental DNA (eDNA) approaches will further expand its utility in ecosystem monitoring, allowing researchers to assess insect diversity from soil, water, and air samples. As barcoding databases grow and methodologies become more standardized, DNA barcoding will remain an indispensable tool for entomologists, conservationists, and policymakers worldwide.

To summarize, DNA barcoding has revolutionized insect taxonomy and biodiversity research, offering a powerful tool for species identification, conservation, and ecological monitoring. While challenges remain, continued advancements in sequencing technology, data integration, and global collaboration will further enhance its efficiency and reliability. By addressing current limitations and fostering interdisciplinary research, DNA barcoding will play a critical role in shaping the future of entomological studies and biodiversity conservation efforts.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

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Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally to this work.

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