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Evaluating the Effectiveness of DNA Barcoding for Insect Identification: A Comprehensive Review

Satoshi Watanabe[1]*, Noriya Masamura[2], Shin-ya Satoh[3], Takashi Hirao[1]

[1]Research and Development Headquarters, House Foods Group Inc., 1-4 Takanodai, Yotsukaido, Chiba, 284-0033, Japan.
[2]Value-added Vegetables Business Development Division, House Foods Group Inc., 6-3 Kioi-cho, Chiyoda-ku, Tokyo, 102-8560, Japan.
[3]House Food Analytical Laboratory Inc., 1-4 Takanodai, Yotsukaido, Chiba, 284-0033, Japan.

***E-mail** ✉ stswata@sogo.t.u-tokyo.ac.jp

ABSTRACT

The limitations of conventional taxonomy have left a large number of arthropods, especially insects, unidentified. Recently, DNA barcoding—an innovative approach that relies on variations within a short DNA sequence—has emerged as a powerful alternative for species identification. This review article aims to evaluate the efficacy of DNA barcoding in modern insect taxonomy and analyze its role in addressing existing taxonomic challenges. This method is recognized for its speed, accuracy, reliability, and broad applicability across various multicellular organisms, including insects. As a critical component in biodiversity research, DNA barcoding bridges the gap between traditional and molecular taxonomic techniques, providing a robust framework for identifying previously unknown, cryptic, or ecologically important species. Furthermore, this technique facilitates the identification of insects in immature stages (e.g., larvae, eggs, and nymphs), which are often indistinguishable through conventional taxonomic methods. Despite its advantages, DNA barcoding has certain limitations. Challenges such as hybridization, speciation events, and contamination by symbiotic organisms such as *Wolbachia* bacteria can compromise the accuracy of the results. Furthermore, the immense diversity of insect species—over one million described species, with millions more yet to be identified—raises concerns about the scalability of barcode-generated data in capturing this vast biodiversity. Given these challenges, an integrative approach that combines DNA barcoding with traditional taxonomic methods appears to be the most effective strategy for the accurate identification of insect species.

Keywords: DNA, Insect, DNA barcoding, Classification, Biodiversity, Identification

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Introduction

The concept of biodiversity was first introduced by Walter Rosen [1] and refers to the variety of life at the genetic, species, and levels of ecosystem within the biosphere [2, 3]. Biodiversity encompasses multiple scientific disciplines, including taxonomy, ecology, evolution, molecular biology, and genetics [4]. Among these, taxonomy—a fundamental branch of natural sciences—plays a crucial role in identifying, describing, and classifying organisms, thereby uncovering biological diversity. Insects, in particular, represent the most different group of organisms on Earth, with over one million documented species and a vast number yet to be identified [5, 6].

Given this immense diversity, relying solely on morphological characteristics for species identification is highly challenging and requires extensive expertise. Furthermore, the number of undiscovered species far exceeds the known ones, highlighting the need for alternative methods to resolve taxonomic challenges [7-9]. One promising approach is to leverage genetic variations that arise from evolutionary processes such as natural selection and genetic drift [10-12]. Unlike morphological traits, DNA sequences provide a stable and reliable means of species identification, as they remain unaffected by environmental factors [13, 14].

The emergence of DNA barcoding has significantly addressed many taxonomic challenges. This technique enables species differentiation by analyzing the nucleotide sequence diversity within a standardized, short genomic fragment [15, 16]. In insects, a portion of the 5' end of the cytochrome oxidase I (COI) gene serves as the standard barcode for species identification [17]. This region has been widely adopted as a rapid and effective alternative to traditional taxonomic methods [18].

In response to these advancements, the International Barcode of Life initiative was established to utilize genomic sequences as molecular markers for species identification, complementing classical taxonomic tools [19, 20]. The rapid progress in biological sciences, combined with the limitations of traditional taxonomy—such as a shortage of taxonomic experts, the overwhelming number of undescribed species, and the time-consuming nature of morphological classification—has underscored the necessity of molecular approaches like DNA barcoding. This review article aims to evaluate the efficiency of DNA barcoding in modern insect classification and analyze its role in addressing existing taxonomic challenges.

Results and Discussion

DNA barcoding

DNA barcoding is a technique that employs short, standardized segments of the genome as molecular markers to identify species. Just as species vary in their morphology, behavior, and habitat, they also exhibit distinct genetic sequences. This allows for the identification of species using a particular gene or gene fragment, at least in principle. The COI region is widely recognized as the standard genetic marker for DNA barcoding across a broad range of organisms, including insects [18, 21]. This gene segment is short enough to be fully sequenced in a single run, yet sufficiently variable among species to enable differentiation. Specifically, the COI barcode is 648 base pairs (bp) long, and research has demonstrated its effectiveness, with an estimated 98% accuracy in distinguishing various animal species [22, 23].

Selecting the appropriate genomic region for species acknowledgments is a critical step. The chosen sequence must exhibit sufficient differences between closely related species while maintaining conserved regions for the design of PCR primers. Additionally, it should allow for homologous comparisons across different taxa. In most animals, the DNA barcode is derived from a 650 bp fragment near the 5' end of the COI gene, a mitochondrial oxidoreductase [18]. The ultimate goal of DNA barcoding is to establish a universal and efficient method for species identification, facilitating standardized DNA extraction techniques and the development of universal primers that can amplify the target sequence across diverse animal groups.

Once a DNA barcode is obtained, the sequence data can be analyzed using clustering algorithms like the Neighbor-Joining method, or through advanced computational approaches, including machine learning and artificial intelligence. Following this, a reference database is constructed, where each species entry includes its barcode sequence, taxonomic name, collection site, documented specimen data, images, and other relevant biological information. This information is stored within the Barcode of Life Data System (BOLD) [24].

To further expand and standardize DNA barcode databases, the International Barcode of Life Consortium was established in 2005 to coordinate research efforts in this field [24]. However, DNA barcoding is not without challenges. Molecular evolution introduces genetic complexities that can lead to unexpected variations within species [25]. Despite these obstacles, when applied correctly, DNA barcoding simplifies species identification, circumventing the limitations of morphological classification. As this method continues to develop, proponents advocate for its integration into practical identification systems to enhance biological research, particularly in insect classification [26, 27].

The first DNA barcoding conference in 2005 marked the initiation of large-scale barcode sequencing, with 132,000 sequences recorded for 12,700 species. By 2010, the number of defined sequences had grown to 94,000 for 77,000 species. In 2016, an extensive 5,086,577 sequences were documented for arthropod specimens, with 4,572,777 sequences specifically assigned to insects. Among the various genetic markers available, the COI gene

is widely regarded as the most effective for insect identification due to several factors: absence of introns, ease of alignment, limited recombination, and strong primer-binding sites. These attributes make it an ideal molecular marker for species discrimination. The genetic demarcations determined through COI barcoding are highly consistent with morphological and behavioral characteristics observed in traditional taxonomy [18].

A major advantage of DNA barcoding is its ability to match species using mitochondrial DNA (mtDNA) sequences stored in the NCBI database. Furthermore, DNA extracted from any developmental stage—whether egg, larva, nymph, or adult—or even from fragmented or deceased specimens, can yield accurate species identification. This is particularly beneficial for taxa where traditional classification methods rely predominantly on adult morphological traits, making the identification of immature stages more challenging [28].

Advantages of DNA barcoding

Over the years, challenges associated with sequence analysis and interpretation in DNA barcoding have significantly diminished [18]. Advances in technology, data processing, and international collaboration have improved the accuracy, cost-effectiveness, and geographic applicability of this method for species classification. Establishing standardized protocols for barcode generation, sample preparation, sequencing, and database storage has further enhanced its efficiency [18].

A well-defined genetic reference system can also help resolve taxonomic uncertainties, particularly in cases involving synonyms, cryptic species, or closely related taxa [29]. For example, DNA barcoding has proven highly effective in distinguishing species within certain arthropod groups, such as scorpions, where many traditionally recognized species may represent complexes of cryptic lineages.

Given its scalability, DNA barcoding is expected to become a universal identification tool for organisms ranging from prokaryotes to higher animals. The selection of appropriate genetic markers that are easily amplifiable and accurately sequenced is crucial for ensuring reliable species identification across diverse biological groups [18].

In the following sections, we examine the applications of DNA barcoding in identifying species within key insect orders, highlighting its role in resolving taxonomic challenges and improving classification accuracy.

Applications of DNA barcoding in the lepidoptera

Lepidoptera, a diverse and visually striking order of insects, has long been the focus of extensive taxonomic and systematic research. To date, around 165,000 species of Lepidoptera have been formally described, accounting for nearly 10% of all documented animal species worldwide, which currently total approximately 1.5 million [30]. However, a significant number—ranging from 135,000 to 150,000 species—remain unidentified and await formal classification. The process of describing these species is hindered by a shortage of taxonomists and the complexity of distinguishing species within this order due to high levels of morphological convergence. As a result, many species remain undescribed, and some can only be estimated.

Lepidoptera has played a pivotal role in advancing DNA barcoding as a molecular tool for species identification. The pioneering work of Hebert *et al*. [18] demonstrated the power of COI-based DNA barcoding by successfully distinguishing North American moth species. Studies have since confirmed that molecular techniques, particularly DNA barcoding, offer a reliable means of species identification. This method facilitates the association of different life stages within Lepidoptera, as well as the distinction between sexually dimorphic species, a common challenge in this order [31].

Butterflies and larger moths are often used as bioindicators for environmental changes, habitat quality assessments, and climate change studies [32, 33]. However, their usefulness in ecological evaluations is often limited due to insufficient taxonomic data. In this context, DNA barcoding provides a novel and more effective approach, enhancing the accuracy and comparability of ecological assessments. By cataloging species through DNA sequences rather than morphological features, researchers can better correlate species traits with environmental conditions, track population distributions, and even uncover cryptic species.

A notable example of DNA barcoding's effectiveness is the case of *Astraptes fulgerator*, a species previously considered a single taxonomic entity. A study conducted in Costa Rica analyzed 484 specimens from a single region and revealed, through DNA barcoding, that *Astraptes fulgerator* was a complex of closely related sister species. This molecular evidence was further validated through morphological analysis of both adult specimens and larval forms, confirming the accuracy of the barcoding results.

Building on these findings, Hebert *et al*. [34] proposed that at least ten distinct species existed within what was originally classified as *Astraptes fulgerator*. This conclusion was based on both COI gene sequence variation and

the correlation between host plant preferences and insect morphology. A subsequent study by Brower [35] re-evaluated the same genetic dataset and arrived at a similar conclusion, suggesting that multiple species were hidden within the single taxonomic name. Further analyses by additional researchers have reinforced these findings, confirming the presence of at least ten distinct species within the *Astraptes fulgerator* complex.

While further investigation and refinement of DNA barcoding techniques are necessary for widespread taxonomic application, this case study illustrates the potential and effectiveness of DNA barcoding when integrated into comprehensive molecular databases for species identification and classification.

Applications of DNA barcoding in the diptera

Diptera, one of the most diverse insect orders, consists of approximately 150,000 described species [36]. This group includes some of the most medically significant insects, such as tsetse flies and mosquitoes, which serve as vectors for diseases like sleeping sickness, malaria, and filariasis, posing major health risks to both humans and livestock.

Long before the advent of DNA barcoding, molecular diagnostic methods such as Allozyme electrophoresis (Beebe DNA hybridization) [37] and restriction fragment length polymorphism (RFLP) [38] were employed to differentiate mosquito species. More recently, sequencing-based approaches have gained widespread use for identifying species within this order. These methods have traditionally focused on nuclear and ribosomal genes rather than COI [39]. However, recent studies in Canada [40] and India [41] have demonstrated that the COI barcode marker is highly effective for species identification within mosquito populations.

Further evidence supporting the effectiveness of DNA barcoding in Diptera comes from Foley *et al*.'s [42] molecular phylogenetic study of Anopheles arotulipes in Australia. This research analyzed four nuclear and mitochondrial gene loci (COL, COIL TS2, EF-la) and found that even though a shorter COI fragment (258 bp) was used instead of the standard 658 bp barcode region, 11 out of 17 sister species exhibited distinct COI sequences. Based on these findings, the researchers concluded that DNA barcoding holds great potential for species recognition within the genus Arotulipes.

A key application of DNA-based identification in Diptera is its role in forensic investigations, particularly in cases involving decomposition. Several species in the families Calliphoridae and Sarcophagidae—commonly known as blowflies and flesh flies—lay their eggs on decomposing remains shortly after death. Since each species follows a specific developmental timeline from egg to adult, forensic entomologists can estimate the postmortem interval (PMI) based on the species present in different life stages [43]. Accurate species identification is crucial for PMI determination, but traditional methods require waiting for larvae to mature into adult flies, which is time-consuming and delays forensic analysis [44]. To overcome this issue, researchers specializing in forensic entomology have explored DNA sequencing, particularly COI-based barcoding, as a faster and more precise method for species identification in criminal investigations [45].

Another major application of DNA sequencing in Diptera relates to species identification in agriculturally significant pests, particularly those in the Agromyzidae family. These insects are notorious for causing extensive crop damage, especially during seasonal population surges. A study conducted in the Philippines examined 258 insects from three species within this family, revealing that invasive populations exhibited fewer mitochondrial haplotypes compared to native populations [46]. Additionally, genetic variation was observed even within a single species, often showing significant divergence [47]. This pattern suggests that these populations experienced genetic bottlenecks, a phenomenon associated with molecular markers like mitochondrial DNA, which is haploid and maternally inherited. The sequencing data not only provided insight into population genetics but also proved highly effective in species identification, demonstrating accuracy comparable to traditional methods.

Applications of DNA barcoding in the coleoptera

The Coleoptera order is recognized for its immense species diversity, with approximately 350,000 species identified so far. Extensive research has been conducted to explore the use of DNA barcoding in beetle species classification. In one study, COI 3' end and 285rRNA nuclear genes were utilized to distinguish *Canthon* sp. (from the Scarabaeidae family) and certain species of water beetles from the Hydrophilidae family.

Findings from this research demonstrated that COI sequences provided a reliable representation of species boundaries within these beetle groups, reinforcing the credibility of this marker for species identification. Another study applying DNA barcoding sequenced four different DNA markers across 118 samples of Copelatus (a genus within the Dytiscidae family) collected from 20 oceanic islands [48]. This research presented a unique challenge

due to the presence of multiple evolutionary lineages that had never been studied before. The findings revealed numerous previously unrecognized species with highly intricate genetic backgrounds.

To classify beetles within Coleoptera, researchers employed both DNA sequencing-based approaches and traditional morphological methods, such as examining male genitalia morphology. However, inconsistencies emerged between the classification patterns derived from these 2 approaches. The authors of the study suggested that integrating morphological taxonomy with the Linnaean nomenclature system would provide a more accurate depiction of evolutionary relationships among beetles. Morphological classification, despite being a conventional approach, is time-intensive and demands specialized expertise to distinguish species-level characteristics [48]. This reliance on morphology can also be problematic due to challenges in accessing type specimens and potential ambiguities in species descriptions.

A case highlighting such challenges was encountered by Monaghan *et al*. [48] when examining five previously identified Copelatus species from Fiji. Their study found that phylogenetic analysis of DNA sequences offers a comprehensive overview of evolutionary histories, and once sequences are added to genetic databases, they become readily accessible for further analysis by other researchers. Monaghan *et al*. [48] proposed that DNA sequences alone could serve as a standalone taxonomic system, potentially reducing the necessity for traditional Linnaean classification methods. This study underscores that while standard morphological techniques are insufficient or too labor-intensive, DNA barcoding can efficiently facilitate species classification on a global scale.

Applications of DNA barcoding in the hymenoptera

The Hymenoptera order ranks as the fourth-largest insect order, following Lepidoptera, Coleoptera, and Diptera, with approximately 135,000 recognized species [36]. However, due to the high likelihood of cryptic species within this order, the actual species count is expected to be significantly greater than current estimates [7].

Ants play a crucial ecological role across various ecosystems worldwide, acting as a dominant group among arthropods. They contribute to nutrient cycling, modify soil composition, and influence plant growth and distribution through their interactions with microbial communities. In Madagascar, the ant fauna is particularly diverse, with an estimated 1,000 species, 96% of which are considered endemic. However, only one-quarter of this estimated diversity has been formally described, creating challenges in biogeographical research, conservation efforts, and understanding their role in ecosystem dynamics.

To assess whether DNA barcoding could serve as an effective alternative to traditional morphological identification, a study was conducted in which 280 ant samples were collected from four different locations. These specimens were first classified using morphological techniques and subsequently sequenced based on their COI gene [28]. Using the obtained sequence data, the samples were categorized into MOTU (molecular operational taxonomic units) and compared with species identified through morphological characteristics. Although there were some discrepancies between molecular and morphological classification, a strong correlation between the two was evident in several cases.

The results suggested that morphological methods often categorize species more conservatively than molecular techniques, grouping multiple cryptic species under a single taxonomic entity. However, when comparing species richness patterns across the 4 locations, no significant differences were observed between MOTUs and morphologically identified species. Furthermore, adjusting MOTU definitions to 2% and 3% sequence divergence altered the absolute number of identified units but did not significantly impact the overall diversity trends detected in the study.

These findings suggest that DNA barcoding can serve as an effective alternative for species identification, particularly when using MOTUs in place of conventional morphological methods. While MOTUs do not always correspond directly to traditional taxonomic classifications, they tend to reveal similar biodiversity patterns. This highlights the potential of DNA-based classification approaches, which, compared to morphological methods, offer faster, more scalable, and comprehensive species identification across broader geographical regions and diverse taxonomic groups.

Conclusion

DNA barcoding has demonstrated significant advantages across various fields, particularly in large-scale species identification for ecological and biodiversity research. This method facilitates the recognition of new species, aids in detecting cryptic species, and enhances classification efficiency. While molecular identification techniques are

not entirely new, DNA barcoding accelerates the collection of genetic data and improves the accuracy of species classification, benefiting from advancements in technology [49].

It is important to note that DNA barcoding does not aim to replace traditional taxonomic methods but rather to complement and refine them. By integrating molecular techniques with conventional classification, researchers can reduce time and costs, streamline the identification process, and improve the accuracy of taxonomic studies. Unlike morphological approaches, which often rely on fully developed specimens, DNA barcoding enables species identification from partial samples, such as leaf fragments, stems, or even non-adult insect stages. This expands its applications beyond traditional taxonomy, making it useful for species identification in various sectors. Among its many applications, DNA barcoding plays a crucial role in forensic science, public health, and agriculture. It allows for the identification of mosquito species carrying infectious diseases, verification of meat sources in restaurants, detection of agricultural pests, and authentication of food supplements and herbal medicines. Additionally, it is instrumental in identifying fungal pathogens, like Plasmodium, which causes malaria, as well as analyzing museum specimens and verifying livestock feed composition. Just as aerial photography has largely replaced ground surveys in environmental assessments, DNA barcoding offers a rapid and cost-effective preliminary step for species identification and discovery.

Although further research and refinement are required to fully integrate DNA barcoding into species classification, this approach presents a promising and innovative pathway for advancing taxonomy and biodiversity studies, particularly in the field of entomology [21].

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