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Investigating the Efficiency of DNA Barcoding in Insect Classification: A Review Study

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ABSTRACT

The limitations of the usual and traditional taxonomy have caused a large number of arthropods, especially insects, have not yet been identified. In recent years, DNA barcoding, which is based on the variation of a short sequence of DNA, has provided a new alternative method for species identification. This method is innovative, fast, accurate, reliable, and applicable to a wide range of multicellular animals including insects. The DNA barcoding method is an important branch of biodiversity science that fills the gap between molecular and traditional methods for species identification. This method has provided a suitable framework for identifying many unknown species, important species, and hidden species. In addition, it has provided the basis for the identification of different species of insects based on their immature stages (including eggs, larvae, and nymphs) that cannot be identified by traditional methods. With all these positive points in using the barcode method, this method suffers from some limitations. Issues such as speciation, hybridization, and excessive contamination of insects with symbiotic species such as Wolbachia bacteria, which make the results of this method problematic, cause limitations for this method. Most importantly, the reliability of the mentioned method has been questioned considering that more than 1 million insects have been identified and millions of other species have not yet been identified. This high amount of diversity of insect species has caused the amount of data obtained from the barcoding method cannot respond to the high biodiversity of insects. According to the stated contents, it seems that to identify the species, a combination of molecular methods such as barcoding and traditional methods should be used to identify living animals, especially insects.

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

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INTRODUCTION

The term biodiversity was first proposed by Walter Rosen [1]. Biodiversity is defined as diversity at the gene, species, and ecosystem levels of the biosphere [2, 3]. Biodiversity includes various fields of natural sciences including taxonomy, molecular biology, natural geography, ecology, evolution, and genetics [4]. Meanwhile, the science of taxonomy, which focuses on the discovery, description, and classification of living organisms, is a basic branch of natural science that is used to reveal the biological diversity of organisms. Among living organisms, insects are the most diverse group of organisms on the planet with more than one million described species and more than 80 unknown species [5, 6].

With this amount of diversity, the determination of species limits in insects through morphological characteristics is very complex and usually requires very high specialized knowledge. On the

other hand, there are still many new species to be identified, described, and named, and the number of undescribed species is far more than the number of known cases [7-9]. Therefore, new approaches are needed to overcome these taxonomic problems [10, 11]. One of the existing approaches to overcome the problems associated with traditional methods is to take advantage of the genetic changes that have occurred between different groups as a result of processes such as genetic drift or natural selection [12]. However, nucleic acid analysis provides the most reliable framework for species determination, because DNA characteristics are not directly influenced by environmental factors [13, 14].

In recent years, with the advent of the DNA barcoding approach, many problems related to taxonomy have been solved. The DNA barcoding makes it possible to distinguish species by using the nucleotide diversity of a small and standard piece of the genome [15, 16]. In most organisms, such as insects, a part of the 5′ end of a Cytochrome oxidase subunit I (COI) gene subunit is used as standard DNA barcoding for species identification [17]. In the case of insects, this gene region is a rapid alternative method for species identification that has been proposed [18].

In recent years, an international movement called the Barcode of Life has been launched to use DNA sequences in parts of the genome as a marker. Molecules have been developed to recognize the living species of the earth along with taxonomic tools [19, 20]. In addition, due to the rapid development of biological sciences, the lack of facilities associated with traditional taxonomic methods such as the lack of experts, the large number of undescribed species, the time-consuming and low efficiency of traditional methods, the need for molecular data and tools such as DNA barcoding are necessary and necessary. In this review article, it is also tried to the effectiveness of DNA barcoding in the new classification should be discussed and analyzed.

DNA barcoding

DNA barcoding is the application of short standardized genomic fragments as biomarkers for species identification. Just as species differ in their morphology, environment, and behavior, they also differ in their DNA sequences, so a specific gene or a specific gene fragment can, at least theoretically, be used to identify a species. Today, for the DNA barcoding of most organisms, the COI region is used as a specific and standard region for species identification and separation [18, 21]. The region of this gene that is considered to determine the DNA barcoding is so short that the sequence of its nucleic acid base pairs can be decoded with a single reading with the DNA barcoding reader. This very small area is so diverse in different species that the species can be distinguished based on it. The length of the COI identifier line is only 648 bp. To test this small DNA tag and be sure of its ability to distinguish species, the researchers tested the COI ID line belonging to different groups and concluded that the COI ID line alone has a capacity of about 98%. Isolate and identify animal species in different forms [22, 23].

Choosing a specific genomic region to be used as a code for species identification is very important. This region should be different between organisms the comparison must be homologous and have a degree of evolution that shows adequate and significant variation between closely related species, and the regions of interest have enough conserved sequences to allow a PCR primer set to target the gene region. In addition, the information obtained from the said sequence should create a strong alignment so that the sequences can be compared. In the animal family, attention is directed to a region of 650 base pairs, which is near the end of the 5th gene sequence. Cytochrome subunit 1 is the mitochondrial oxidoreductase (COI) [18]. It should be noted that the strategic goal of DNA barcoding is to use a uniform and inclusive method for species identification and to achieve DNA extraction procedures and identical primers for multiplying the desired sequence in a large range of animal groups is one of the axes of research and scientific studies.

Line ID sequence data can usually be analyzed and interpreted using one of the clustering methods such as the Neighbor-joining method. More complex methods including various statistical algorithms and artificial intelligence are being tested and will gradually open their place. The third step after determining the identification line is to create a library of these parts as a reference, where the identity of the species in it has already been confirmed. The method of creating a library is very simple, as after extracting DNA from each sample and

multiplying the ID line region, the obtained information is recorded in the database under the title "Barcode of Life Data" [24]. Each of the entries in this library includes the species name, sequence of identification lines, sample collection location, link to documented sample, photo, and other biological data.

The Lifeline Consortium was established in 2005 to help expand this database by harmonizing and integrating various studies in this field [24]. In practice, it should not be expected that the use of encryption methods will be able to identify species easily. DNA sequences have been exposed to various complexities of molecular evolution and can show significant variation within species [25]. However, if DNA coding is successful, it certainly can be used in the identification of samples through correct barcoded sequencing and avoid the complexities of morphological identifications, and its proponents will eventually what is more encouraging to establish a practical system based on the mentioned method to identify living organisms including insects [26, 27].

In 2005, in the first meeting related to line identification, 132,000 sequences were recorded for 12,700 species. In 2010, about 94,000 sequences were defined for 77,000 species. In 2016, 5086,577 sequences were recorded for arthropod specimens, among which 4572,777 were related to insects. The COI gene in insects, because they lack introns, create alignments or simplexes, are subject to limited recombination, and have strong sites for primers, making them an ideal marker for species identification. Demarcations determined by this molecular marker are strongly aligned with the results of morphological studies and behavioral characteristics of the species based on which the mentioned species have been identified in the traditional way [18]. The important advantage of this molecular sequencing-based approach in species determination is that species can be matched and identified based on mitochondrial DNAs stored in the NCBI database. In addition, DNAs extracted from any life stage of an organism such as the egg, larva, or adult stage of an insect, or from dead parts, give the same result in species identification. Whereas, conventional insect identifications (at least for fully metamorphosed insects) are often based on characteristics of the adult insect [28].

Advantages of using DNA barcoding

The problems related to the analysis and analysis related to the identification line during the years after its introduction have disappeared [18]. Also, as a result of using the mentioned method, the costs associated with it have been reduced and its efficiency has been proven in different geographical areas and the classification of living organisms. In this approach, it is possible to conduct better studies if standard guidelines are used, the cooperation of different researchers to identify samples, prepare and analyze sequences, and store barcoded samples [18].

A standard identification line that can be used in species identification can also significantly reduce potential taxonomic problems caused by the presence of synonyms and hidden or congeneric species in the studied groups [29]. For example, using the identification line method is very effective for many animal species, especially scorpions. Many decisions about the classification of these arthropods are made because probably many basic species include a large number of cryptic species. Considering that this method is expected to be used on a large scale for all groups of living organisms from prokaryotes to higher animals, as a result of the use of suitable genes that can be easily reproduced and sequenced and sufficient accuracy in it is very important to identify a wide range of living organisms [18]. In the continuation of this article, the use of DNA barcoding in the identification of species of some important orders of insects is investigated.

Applications of DNA barcoding in the Lepidoptera

The order Lepidoptera is a diverse and attractive group of insects that have received special taxonomic and systematic attention. Approximately 165,000 species of Lepidoptera have been described, representing about 10% of the 1.5 million animal species known [30]. The remaining 150,000 to 135,000 species of Lepidoptera are awaiting description and identification. The lack of taxonomists, and the existence of problems to identify the species of the mentioned order due to the abundant convergence of species, most of the species are not described and some of them can only be estimated.

The order Lepidoptera has been a model for current DNA barcoding studies since Hebert *et al*.

[18] used North American mice to demonstrate the ability of COI to distinguish between samples of different species. The conducted studies show that molecular methods and DNA barcoding can be used for their diagnosis. It makes it possible to find the relationship between the different life stages of Lepidoptera and also the species that are sexually male and female in a form that is very abundant in this order [31].

Large moths and butterflies were used to indicate environmental quality (such as habitat destruction), habitat diversity classification, and climate change indicators [32, 33]. However, due to insufficient taxonomic information about them, their role as an important group in environmental assessments is limited and weak. In such cases, DNA barcoding can offer a new horizon of efficiency and comparability with ecological assessments. By registering DNA barcodes, instead of using morphological traits, it is possible to create a better connection between the characteristics of different species of scale insects and the characteristics of the place and time where the insect is located, and even identify and introduce the hidden species in their populations. did for example, in the case of the *Astraptes fulgerator* species, the DNA barcoding method was used to identify the target species and hidden species within the target insect population, so that the coding of 484 samples from a region in Costa Rica showed that the *Astraptes fuigerator* is a group of sister species and even the morphological studies of the adult insect and the morphology of the obtained larvae confirm the results of the desired molecular method.

Hebert *et al*. [34] suggested the existence of ten species within the species based on the similarities between the COI gene sequences and the relationship between the morphological traits of the target insect and the host plant it feeds on. Also, Brower [35] analyzed and studied the information obtained from the DNA of the mentioned insect differently and concluded that the sample of *Astraptes fuigerator* includes several species. Even some other researchers by reanalyzing the same genetic data confirmed the existence of 10 new species. Although the use of the DNA barcoding method in species determination requires more research and research, the above example shows its ability and efficiency when it is used as an integrated database.

Applications of DNA barcoding in the Diptera

The order Diptera is another extremely diverse order of insects, with approximately 150,000 described species [36]. Among insects, members of the order Diptera, with important groups such as mosquitoes and tsetse flies, which act as agents of transmission of many diseases such as malaria, sleeping sickness, and filariasis, have had the most negative impact on human and livestock health.

Even before the creation of DNA encoding technology, molecular diagnostic tools such as the Allozyme electrophoresis method (Beebe DNA hybridization) [37] and Restriction Fragment Length Polymorphism (RFLP) [38] have been used to identify mosquito species. Today, sequencing-based methods have also been widely used to identify species of the desired order, although the main focus of these methods is on nuclear and ribosomal genes rather than COI [39], although several recent studies have shown that the standard COI barcode marker can be used effectively in the evaluation of mosquito groups for identification at the species level in Canada [40] and India [41] should be used.

In other studies such as Foley *et al.*'s [42] study on the molecular phylogeny of *Anopheles arotulipes* in Australia based on four different nuclear and mitochondrial gene loci (COL, COIL TS2, EF-la). The results show that despite the use of a shorter COI fragment (258 bp) compared to the standard barcode region (658 bp), 11 species out of 17 sister species have unique COI sequences, and accordingly, the aforementioned researchers. They concluded that DNA coding may be a promising method for species recognition within the genus *Aroulipes*.

One of the applications of molecular methods based on DNA is in identifying species of the bivalve order that are important in forensic medicine. A variety of species belonging to the Calliphoridae family and Sarcophagidae flesh flies lay eggs in decaying corpses shortly after death, and since each species has a specific time frame for development from the egg stage to a complete insect, therefore the presence of any species in the developmental stages on decaying corpses can provide a clue to the PMI or time

elapsed since the death of the decomposing organism [43]. For the correct estimation of PMI, it is necessary to accurately identify the target species. Considering that whole insects or target flies are necessary for definitive identification, therefore, the larvae of these flies must be removed from the corpses beforehand. Collected and reared until becoming a complete insect, which requires a lot of time and delay in species identification [44]. For this reason, entomologists specializing in criminology and medicine solve this problem. As a result, there is a lot of research in this field that studies how to accurately identify forensically important fly species using DNA sequences, mainly COI [45].

Among other studies that can be mentioned in the field of using sequencing in the field of species identification, it is related to the Agromyzidae family, which are economically important agricultural pests, because these insects during periodic flooding Their populations can destroy the entire crop. COI sequences obtained from 258 insects belonging to three species belonging to the family Agromyzidae in the Philippines showed that fewer mitochondrial haplotypes were found in the invasive populations compared to the native ranges of these species [46] and those that were observed even within the same species were often highly divergent [47]. This pattern indicates the occurrence of genetic bottlenecks for a population that is related to a molecular marker such as mitochondrial DNA that has both haploid and maternal inheritance. Analysis of the data from the sequencing of the markers in question was able to identify all samples of the case Identify the review as traditional methods.

Applications of DNA barcoding in the Coleoptera

In the Coleoptera order, species diversity is very high and so far 350,000 species of this order have been described. Many researches have been conducted in the field of using the DNA line identification method with DNA-based methods. In a study, COI 3 end and 285rRNA nuclear genes were used to identify species of beetles of the genus *Canthon* sp. from the family and some individuals of water beetles of the Hydrophilidae family.

The results showed that the COI sequence provides an almost accurate picture of species boundaries in these two groups of cockroaches, and this result can give credibility to the use of the said sequence in species identification. In the investigation of the DNA barcoding method, four DNA markers were sequenced for 118 samples from the 20 islands of water beetles belonging to the genus *Copelatus* of the Dyticidae family collected from the Islands [48]. This effort was considered a particularly challenging test case for cryptography, as many lineages on oceanic islands were studied for the first time, leading to the identification of many new divergent species with very complex genetic histories. Finally, the Coleoptera order was classified by using two approaches contiguous DNA sequences and conventional morphological methods (such as the morphology of male genitalia). Although the classification patterns were inconsistent using these two approaches.

The authors argued that if the morphological approach was combined with a Linnaean nomenclature system, the evolutionary understanding of the various lineages would be more valid. The morphological method is time-consuming and requires specialized knowledge of the differences in traits related to classification at the species level [48]. Therefore, the subsequent identification of the species using morphology can be problematic due to important descriptions and the existence of problems in obtaining type samples.

A situation encountered by Monaghan *et al*. [48] with five previously described *Copelatus* species from Fiji. The sequencing approach combined with phylogenetic analysis provides a comprehensive summary of the evolutionary history, and once the sequences are submitted to the database, the data is readily available, and subsequent analyses can be performed by anyone. Monaghan *et al*. [48] suggested that DNA sequences themselves can form a system of taxonomic grouping and association without the need for a formal Linnaean classification system. This study shows that when standard morphological methods are incomplete or too time-consuming, DNA sequencing can do the job of classifying existing global species.

Applications of DNA barcoding in the Hymenoptera

Hymenoptera is the fourth largest order with approximately 135,000 described species of insects after Coleoptera, Lepidoptera, and

Diptera [36]. Given that there are believed to be significant cryptic species in this order, the true species richness of this order may exceed expectations [7].

Ants in many ecosystems of the world consist of a major group of arthropods. They are important in the recycling of nutrients, their activities in the soil cause the production of very diverse food microbiomes that affect the sequence of growth and distribution of plants. In Madagascar, these insects contain an extremely diverse fauna, which is currently estimated to include 1000 species, and 96% of them are believed to be endemic, but only 25% of this estimated collection has been described, which is a major obstacle to biogeographical studies, their conservation status and their role in ecosystem processes. Therefore, in a study, the question of whether DNA barcoding can act as an effective alternative for the morphological identification of species slowly or not was investigated. In this regard, 280 samples were collected from four locations, identified based on the morphological method, and sequenced in terms of COI [28]. The samples were classified into MOTU units based on the mentioned sequence data and classified into morphological species according to morphological characteristics, then the results of both classification methods were compared. Although there was no high agreement between the results of the molecular and morphological methods of the arrays, strong correlations between the two were observed to some extent. Based on this, it can be concluded that the morphological method tends to limit the species identified by the molecular method to one or more specific species. However, examining the patterns of array richness across these four locations, no significant differences were observed between the data provided by MOTUs and morph species. In addition, MOTUs were defined based on divergence of 2 and 3, which only changed the absolute number of determined arrays and did not cause a significant difference in terms of the overall patterns of diversity observed. This finding shows that they can be an effective alternative for determining AMOTU species using conventional methods, such as morphological methods. Although MOTUs do not necessarily specify the same taxonomic groupings, they will specify the same general taxonomic patterns. Such results emphasize the fact that studies based on the use of DNA sequence arrays, which determine taxonomic and morphological arrays as a result, can be very useful compared to the laborious and time-consuming morphological method. Accurate assessments of species identification between much larger geographic areas and more taxonomic groups are provided by these methods.

CONCLUSION

Among the various applications of DNA barcoding, we can mention the efficiency of this method in the large-scale identification of living organisms in ecological and biodiversity studies, the possibility of identifying and describing potential new species, and detecting hidden species. Although these methods are not considered new, however, the DNA barcoding method can speed up the process of collecting molecular data and increase the efficiency of species classification due to technical advances [49].

The solution of using DNA barcoding is not only to abandon the traditional methods of classification but to direct the studies related to the classification of different organisms, which in itself saves time, increases the efficiency of identifying and describing different animal groups, and reduces costs associated with traditional classification. It should be noted that the barcode method of living organisms has many wider applications than the traditional methods. Among these things, it is possible to identify plant species using only a part of the leaf, stem, or other parts, without the need for flowers or fruits, identify insects using non-adult stages and without the need for non-adult samples, identify commercial products such as food supplements, herbal medicines, etc., speeding up biodiversity studies and identifying millions of unknown species as quickly as possible, identifying mosquitoes that carry infectious diseases, identifying the type of meat in Restaurants, identifying the types of agricultural and horticultural pests, detecting fungal diseases caused by single cells such as Plasmodium, which is the cause of malaria, identifying samples that exist in museums, ensuring the type of livestock feeding, etc. As the speed and cost of aerial photography have replaced ground surveys, DNA

line identification can be a quick and cheap first step in species discovery. Although this method requires considerable time to be completed, it can promise a new approach to the identification and classification of organisms, including insects [21].

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