

DNA Barcoding of Insects and Its Direct Application for Plant Protection

Peter Quandahor^{1*}, Iddrisu Yahaya¹, Francis Kusi¹, Issah Sugri¹, Julius Yirzagla¹, Abdul Karim Alhassan¹, Jerry A. Nboyine¹, George Y. Mahama¹, Godwin Opoku¹, Mohammed Mujitaba Dawuda², Asieku Yahaya¹, Theophilus Kwabla Tengey¹, Rofela Combey³, John Abraham³

¹CSIR-Savanna Agricultural Research Institute, Tamale, Ghana

²Faculty of Agriculture, Food and Consumer Science, University for Development Studies, Tamale, Ghana

³Department of Conservational Biology and Entomology, School of Biological Sciences, University of Cape Coast, Ghana

Email: *quandooh@yahoo.com

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Abstract

The introduction of invasive insect pests across national borders has become a major concern in crop production. Accordingly, national plant protection organizations are challenge to reinforce their monitoring strategies, which are hampered by the weight and size of inspection equipment, as well as the taxonomic extensiveness of interrupted species. Moreover, some insect pests that impede farmer productivity and profitability are difficult for researchers to address on time due to a lack of appropriate plant protection measures. Farmers' reliance on synthetic pesticides and biocontrol agents has resulted in major economic and environmental ramifications. DNA barcoding is a novel technology that has the potential to improve Integrated Pest Management decision-making, which is dependent on the ability to correctly identify pest and beneficial organisms. This is due to some natural traits such as phenology or pesticide susceptibility browbeaten by IPM strategies to avert pest establishment. Specifically, Deoxyribonucleic acid (DNA) sequence information was applied effectively for the identification of some micro-organisms. This technology, DNA barcoding, allows for the identification of insect species by using short, standardized gene sequences. DNA barcoding is basically based on repeatable and accessible technique that allows for the mechanisation or automation of species discrimination. This technique bridges the taxonomic bio-security gap and meets the International Plant Protection Convention diagnostic standards for insect identification. This review therefore discusses DNA barcoding as a technique for insect pests' identification and its potential application for crop protection.

Keywords

DNA Barcoding, Integrated Pest Management, Taxonomy, Biosecurity, Crop

1. Introduction

Timely and appropriate identification of pest and beneficial arthropods is paramount for crop protection. Accurate and timely species-level identifications of insect pests are required to develop an efficient Integrated Pest Management strategy for farmers in order to improve crop yield. The government demands precise species identification to protect crops from invasive pests and to address the International Plant Protection Convention's international concern [1]. Researchers, on the other hand, demand precise and fast species identification to facilitate successful scientific communication, agricultural extension research, and communication between farmers and government. Despite this, taxonomists face difficulties in classifying insect specimens to the species level due to the number, diversity, puzzling lifestyle, unique morphological life-stage traits, and diversity of arthropods. Conventional, insect species are classified based on their diagnostic characteristics or taxonomic keys, which is extensive, time-consuming, and labour-intensive. As a result, only taxonomists are capable of carefully and analytically identifying and classifying the vast majority of known species [2]. In recent years, the science of discovering, identifying, classifying, and naming organisms, particularly insects, has been thought to be diminishing. This taxonomic restriction posed a significant challenge in crop protection [3]. As a result, an alternate insect identification technique that allows both expert and non-expert taxonomists to easily identify insect species is critical for insect pest management. This will reduce the consistent threat that insect pests pose to agricultural productivity while also increasing yield to meet global food security [4] [5].

Through the confluence of concept and technology, genetic information considerably contributes to species identification. In recent years, the availability of trustworthy and verifiable information through molecular and systematic research has aided in the identification, appraisal, and comprehension of insect variety. This innovation makes a significant contribution to species recognition, identification, and categorization. Deoxyribonucleic acid (DNA) sequence information, in particular, has been used successfully for the identification of several microorganisms [6]. This technology, DNA barcoding, allows for the identification of insect species by using short, standardised gene sequences [7]. The sequential construction of identified species barcoding profiles generated a pool for the identification of unknown species using DNA sequences. DNA barcoding is fundamentally based on a repeatable and accessible technique that allows for the mechanisation or automation of species discrimination. This technique bridges the taxonomic bio-security gap and passes the diagnostic standards for insect identification established by the International Plant Protection Convention [8]. The ability to monitor and manage insect pest species is heavily reliant

on the timely and accurate identification of dangerous and beneficial insects within the vast insect diversity. This could also help to enhance successful protection techniques like quarantine or eradication that require less work and time [8]. The use of DNA barcoding as a technique for species identification is intended to speed up insect diagnostics for proper monitoring and categorization through standardised and mainly automated techniques. Therefore, the purpose of this study is to review on the detection of invasive insect pests using DNA barcoding and its possible application for crop protection.

2. DNA Barcoding

The molecular barcoding model identifies species quickly and accurately by implication and sequencing a short, homogeneous section of its genome, specifically the mitochondrial gene, cytochrome c oxidase subunit 1 (COI) [7]. Because it relies on DNA rather than morphology, this approach can identify insects at any stage of development, from egg to adult. The technique will also apply established protocols, allowing a broad variety of understanding of classical morphological taxonomy. In crop protection, DNA barcoding has the capacity to identify invasion species in real time through regular monitoring, giving authorities the opportunity to establish control and prevention tactics before a population of new invading species becomes uncontrollable [9] [10].

The DNA barcoding approach also provides a standard ID system for commercialised biocontrol agents, ensuring that the correct species are made available and that agents sold under the same species name are unquestionably the same species [11]. In order to establish a DNA barcoding system, tissue samples of insects are gathered and utilised to create a DNA sequence, while photos of the original specimen are acquired and saved as a morphological voucher. The image and all assurance information, such as identification and collecting data, are properly secured in a proprietary database with the DNA barcode sequence.

As a result, a reference library of COI barcode sequences matched to known species names and photos is generated, and any unique COI sequence from an unknown specimen may be checked against the database, and species identification can be accomplished quickly if a match is found.

A crucial component of constructing a DNA barcoding system is a reference database of known cytochrome c oxidase subunit 1 sequences. The Barcode of Life Data Systems, or BOLD [12], a web-based platform, provides a universal platform that serves as a repository for DNA barcode data as well as an online workbench with data analysis tools. The long-term viability and effectiveness of a DNA barcoding organisation may be dependent on rigorous standardisation of both methodology and data handling. Furthermore, the cytochrome c oxidase subunit 1 sequence, total validated barcode records may require comprehensive taxonomy of the specimen from which they derive, photographs, GPS-coordinate data indicating their specific geographical locations, and a traceable ID linking the record to a voucher specimen kept in a convenient repository or museum. The frozen DNA extract is also appropriately stored for future research. The

DNA barcoding methodology is not intended to be the sole method for species identification; after standardised COI reference sequences have been gathered, submitted, and approved, supplementary nuclear gene(s) for specific taxa can be joined to strengthen a global identification system. DNA barcoding appears to support standard morphology-based and nuclear gene-based identification methodologies in showing the extraordinary accuracy and effectiveness of arthropod species identification [13]. **Figure 1** depicts the DNA barcoding analytical chain.

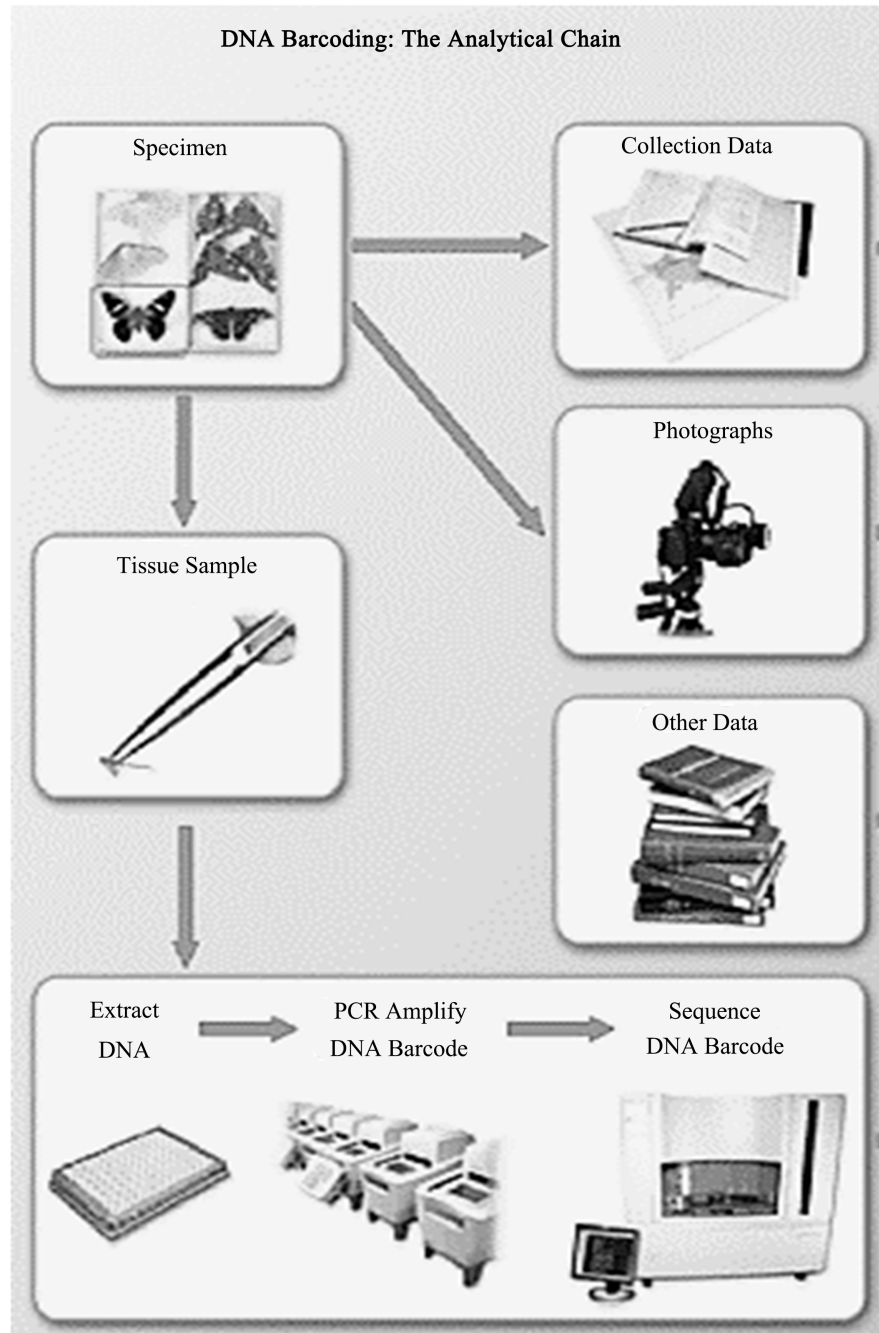


Figure 1. Overview of DNA Barcoding technique [14].

3. Direct Applications of DNA Barcoding for Plant Protection

3.1. Monitoring Biosecurity

The spread of invasive insect pests across national borders has become a major concern in crop production. Accordingly, national plant protection organizations are challenge to reinforce their monitoring strategies, which are challenged due to the weight and size of equipment that are involved for inspection and the taxonomic extensiveness of interrupted organisms. Previous research indicates that only 40% of 565,046 non-indigenous pest species were detected over a 17-year period [15]. This was due to time constrain, luck of funding and experts or taxonomist to facilitate the species identification rate. Moreover, the accuracy and precision of the species identification data is for the boundary interception is not known. Jinbo *et al.* [16] examined the data on the amount of insect barcodes in BOLD and reported a total of 664,924 insect specimens barcoded and 79,320 insect species barcoded (Table 1).

There is still knowledge gap concerning arthropod species identification; nevertheless, 7% to 33% error rate at the genus level has been detected for locally inhibited samples of benthic arthropods [15]. DNA barcoding strategy is therefore imperative to monitor interrupted arthropods related to internationally imported produces. Apparently, advance monitoring program is required in this gap could be bridged by DNA barcoding technique. For instance, DNA barcoding under laboratory protocols are extremely workable [17], which could mitigate the spread of invasive species. This technique also decreases the necessity for several species-specific molecular tests [18]. This is due to the ability of the DNA barcoding to replace the preset 17 different molecular methods for the discrimination of the economically important species [19]. Moreover, DNA barcoding could possibly decrease the errors produced by some species diagnosis, as to delivers enough taxonomic data compared with most alternative molecular species identification techniques [20]. DNA barcoding has been suggested the most efficient process for identification and discrimination of a number of insects such as of *Liriomyza* (Agromyzidae), *Lymantriidae*, *Spodoptera* (Noctuidae), *Tephritidae*, *Thysanoptera*, and *siricid* wood wasps [21]. Hence, it is recommended as a potential diagnostic protocol for pest control as published under the IPPC ISPM No. 27 [22]. Although the step of publication for internationally accepted diagnostic protocols of any kind remains worryingly slow, broad methodological embracing of barcoding could help to facilitate the process.

3.2. Integrated Pest Management Decision Making

Several studies show that DNA barcoding has the potential to aid Integrated Pest Management decision-making, which pivots on the capacity to correctly identify pest and beneficial organisms [22] [23] [24]. This is due to some natural traits such as phenology or pesticide susceptibility browbeaten by IPM tactics to avert pest establishment. For instance, *Aphis pomi* and *Aphis spiraecola* are sympatric

Table 1. Current status of DNA barcoding library of insects in the BOLD system.

Insect order	Number of specimen barcoded	Number of species barcoded
Diplura	8	4
Archaeognatha	4	24
Thysanura	11	3
Ephemeroptera	7192	513
Odonata	3521	219
Dictyoptera	4	2
Blattaria	494	60
Isoptera	467	134
Mantodea	228	140
Dermaptera	49	6
Plecoptera	3221	400
Orthoptera	3395	654
Phasmida	87	25
Embioptera	19	11
Grylloblattodea	1	1
Mantophasmatodea	2	1
Psocoptera	70	3
Phthiraptera	527	85
Thysanoptera	880	103
Hemiptera	14,518	2129
Neuroptera	769	99
Megaloptera	829	103
Raphidioptera	10	5
Coleoptera	18,926	4428
Strepsiptera	9	7
Mecoptera	32	26
Siphonaptera	75	11
Diptera	61,140	6182
Trichoptera	24,003	3457
Lepidoptera	433,843	47,732
Hymenoptera	91,024	12,247

*Jimbo *et al.* [15].

pests of apples, and can be difficult to discriminate morphologically [23] [24]. These pests management involves pesticides application, yet such species easily develop biotype against it [25]. Moreover, *Aphis pomi* and *Aphis spiraecola* species use a diverse hibernating host that influence management selection and timing. DNA barcoding readily distinguishes these two pests and so could be easily assimilated into IPM programs for this particular pest complex [22]. It is reported that leaf miners (*Liriomyza* spp), which is a group consisting of at least 376 species are difficult to diagnose though morphological characteristics. These species vary in fecundity and susceptibility to various chemical and biological management practices [26]. These biological variances are speculated to have led to the translation of *Liriomyza sativae* (Blanchard) by *Liriomyza trifolii* (Burgess) in China and California, and of *L. trifolii* by *L. sativae* in Japan [27]. Specifying the possibility of pest complex instability as a result of pest control practices, DNA barcoding appears to have the potential to improve management decisions, as DNA barcoding efficiently detects the four most common *Liriomyza* pest species [10]. When visually identical pest species have different economic injury thresholds, phenology, and susceptibility to various management practices, DNA barcoding will be extremely useful in IPM.

3.3. Integrative Taxonomy of Pest and Beneficial Organisms

DNA barcoding has demonstrated significant promise in highlighting examples of cryptic diversity and assisting in taxonomy revisions [28]. Even though the theory of describing species uniquely based on confirmation from DNA barcode data is highly debated, the use of DNA barcoding to supplement traditional morphological taxonomy is extensively acknowledged [29] [30]. As a result, DNA barcoding is well suited to assisting in the integrative taxonomy of pest and beneficial organisms. DNA barcoding has aided in the development of species hypotheses for a number of insect taxa, which may ultimately lead to enhanced pest management. *Frankliniella occidentalis* (Pergande), a western flower thrips, is thought to be made up of two sympatric species in California and New Zealand [31]. This partition may explain some of the morphological and behavioural variety exhibited in *F. occidentalis*, a species with 18 known synonyms. Similarly, strong evidence from mating studies and DNA barcode data is calling the status of the species *Bemisia tabaci* into question. *B. tabaci* is composed of 24 cryptic species, the majority of which correspond to previously described biotypes and some of which differ in management-relevant characteristics such as pesticide susceptibility and ability to spread plant viruses [32]. Notably, DNA barcoding successfully identifies *B. tabaci* biotypes that are important for plant development. It has also helped identify two cryptic species of the fruit pest *Zaprionus indianus* (Drosophilidae) [33]. *Binodoxys koreanus* (Braconidae), a cryptic species of soybean aphid parasitoid from Korea, was described using a single fixed nucleotide variation within the DNA barcode region, as well as behavioural and morphological data. *Antispila oinophylla*, a species of invasive leaf mining moth originating in North America, was also identified via barcoding

[34]. Although there are a variety of analytical techniques available to explore the presence of cryptic species, they are normally only used to test an existing hypothesis. Because barcoding can help identify known species and signal the presence of potential new ones, it can be used to test existing species hypotheses while also generating new taxonomic hypotheses for testing within an integrative taxonomic framework [34].

4. Conclusion

Deoxyribonucleic acid (DNA) barcoding provides prospective protocols to enhance pest identification and their management practices. This will play a major role in understanding the global movement of insect species in a detail level. It has the potential to help decision-making in Integrated Pest Management, which is dependent on the ability to correctly identify pest and beneficial organisms. This is because some natural features, such as phenology or pesticide susceptibility, have been browbeaten by IPM strategies to prevent pest establishment. The DNA barcoding technology bridges a taxonomic bio-security gap while also meeting International Plant Protection Convention diagnostic standards for insect identification. This will reduce the consistent threat insect pests pose to agricultural productivity while also increasing yield to meet global food security. Although the DNA barcoding approach is more efficient for pest identification, new reference libraries for worldwide pest species and IPPC procedure development are required to improve the efficiency and volume of pest interceptions identifications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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