

# DNA barcoding: A new age tool for studying insect diversity

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## INTRODUCTION

Ronald Ross, a Scottish doctor practicing in India, discovered in 1897 that only *Anopheles* mosquitoes transmit the Plasmodium parasite that causes malaria in humans. For this study, he was awarded the 1902 Nobel Prize in Medicine. Unfortunately, despite ongoing attempts to eradicate the disease, malaria still claims more than 1 million lives annually, mostly in tropical Africa and Asia. Ross had hoped that this understanding would lead to malaria control swiftly. The most significant human malaria vector, *Anopheles gambiae*, is a member of a complex of morphologically similar sister species that exhibit significantly different habitat preferences, behavioral patterns, and transmission capacities. Although these species cannot be distinguished morphologically, they can be easily distinguished on the basis of DNA sequences. This story's plain lesson is that cryptic biological variety is important. Numerous types of life exist, many of which are small, challenging to study, and even more challenging to distinguish. Insects are the most varied group of creatures on the world, with more than 1 million recognized

species and millions more either awaiting description or simply undiscovered. They have a variety of effects on human society, both good and bad. Because of their richness, groups like insects pose significant problems for the taxonomic enterprise. By using conventional, morphological methods, it is difficult and frequently requires specialized knowledge to identify species. New species are increasingly frequently identified, described, and named, yet there are still many undescribed insect species that outnumber these. Here lies the importance of DNA barcoding.

### What is DNA Barcoding?

Just as species show differences in their morphology, ecology, and behavior, they also show differences in their DNA sequences (Wilson *et al.*, 2017). “DNA barcoding,” used in a broad sense, refers to the use of short, standardized DNA sequences as markers for the recognition of species. When used more precisely, “DNA barcoding” refers to the technique of sequencing a short fragment of the DNA sequence of the mitochondrial cytochrome c oxidase subunit I (COI) gene, the animal “DNA barcode,” from a taxonomically unknown specimen and performing comparisons with a library of DNA barcodes from taxonomically known specimens to establish a taxonomic identification.

### Benefits of DNA Barcoding

- ✚ identification of new species.
- ✚ can be linked to readily observable morphological characters.
- ✚ can provide an avenue to encourage new participants into taxonomy.
- ✚ Benefit to applied taxonomic research areas
- ✚ enables non-taxonomists to identify specimens.
- ✚ can identify pests in any life stage
- ✚ Identifying Disease Vectors: Combating Diseases

### Applications of DNA Barcoding

- Quickly and cheaply recognize known species
- Identifying plant leaves even when flowers or fruit are not available
- Identifying insect larvae e.g., skipper butterfly is ten distinct species
- Identifying the diet of an animal, based on its stomach contents
- Identifying products in commerce e.g., herbal supplements, wood, or skins

With the application to insects, and the endeavour to build a systematic database of 'DNA barcodes linked to data about the species they represent, the barcoding movement has begun to gather real momentum in the past few years (Hebert *et al.* 2003). We, therefore, move to discuss some specific cases in which barcoding has been applied to particular insect groups, and examine how it has advanced our knowledge of biodiversity

### DNA barcodes Records and insect biodiversity

Order	No. of DNA Barcode Record
Lepidoptera	8,10,000
Diptera	7,17,433
Hymenoptera	3,16,747
Coleoptera	1,35,376
Hemiptera	97,975
Trichoptera	38,054

**Order: Lepidoptera:** An interesting and charismatic group of insects is the

Lepidoptera. Lepidoptera are now the standard model group for investigations on barcoding. Lepidoptera's many life stages and the sexes of sexually dimorphic species can now be linked thanks to barcodes. (Janzen *et al.* 2005). Work on the Neotropical skipper *Astrartes fulgurator* provides a prime example of the way in which DNA barcoding can aid species discovery, especially when coupled with morphological and ecological studies. Barcoding of 484 specimens from Costa Rica revealed that the *A. fulgurator* group comprises a complex of sister species, confirming and extending earlier suspicions gained through studies of adult morphology and larval morphologies.

**Order: Diptera:** With species like mosquitoes and tse tse serving as the carriers of various serious diseases like malaria, sleeping sickness, and filariasis, this group of insects are the ones that have the biggest detrimental effects on human health and cattle. Foley *et al.* (2007) constructed a molecular phylogeny of the Australian *Anopheles annulipes* species complex based on four different loci, both nuclear and mitochondrial (COI, COII, ITS2, and EF-1 $\alpha$ ). Despite using a shorter fragment of COI (258 bp) than the standard barcode region (658 bp), it was found in this study that 11 of the 17 sibling species (65%) had unique COI sequences, and the authors concluded that 'DNA barcoding holds some promise for diagnosing species within the Annulipes Complex, and perhaps for other anophelines'. COI sequences were generated from 258 individuals belonging to three species of invasive leafminers in the Philippines: *Liriomyza huidobrensis*, *L. trifolii*, and *L. sativae*.

**Order: Coleoptera:** Beetles are said to make up one in every five animals on the earth. As a result, the Coleoptera exemplify

a class where variety has outweighed taxonomic enterprise. Even though 350,000 species of beetles have been identified (including many pest species that are economically significant), the actual number of species may range from 5-8 million. Major barcoding research with beetles has concentrated on the use of DNA-based techniques for species discovery because there are so many undiscovered species. Monaghan *et al.* (2005) used the 3 end of COI and the nuclear gene 28S rRNA to identify clusters of beetles in dung beetles of the genus *Canthon* and water beetles of the family Hydrophilidae. An exact match of nuclear genotypes and mitochondrial clusters suggested that the mtDNA groupings were not misleading due to introgression, and the clusters likely correlated with previously described or undescribed species. The results indicated that COI provides a largely accurate picture of species boundaries in these two beetle groups and provides validation for its use in species discovery.

**Order: Hymenoptera:** After Coleoptera, Lepidoptera, and Diptera, the Hymenoptera are the fourth biggest insect order with around 125,000 known species. Given the amount of cryptic species thought to exist, the true richness of its species may even exceed that of the "big three.". In a recent case study conducted by Smith *et al.* 2005 in Madagascar A total of 280 specimens from four localities were collected and independently identified to morphospecies and sequenced for COI. The specimens were classified both into MOTUs based on their sequence data and morphospecies based on their morphological traits, allowing the two methods to be directly compared. A total of 90 morphospecies, 117 3% MOTU and 126 2% MOTU were found. Morphological species designations, therefore, tended to

lump specimens that were split by the molecular approach. As in many cases, molecular markers detect cryptic taxa that are difficult or impossible to detect by morphology alone.

**Conclusion:** As a vital tool for species identification and evolutionary research, DNA barcoding has evolved and become well-established. In addition, it has helped safeguard endangered species, identify pests and disease carriers in agriculture, track product adulteration, and preserve the environment.

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