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# DNA Barcoding for Identification of Agriculturally Important Insects

S. K. Jalali, Rakshit Ojha and T. Venkatesan

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## Abstract

Molecular characterization and DNA barcoding is a taxonomic method that uses a short genetic marker in an insect DNA to identify a species, including an unknown species. The DNA barcode method of identification includes, for example, identifying insect species from any developing stage and part; otherwise, identifying insects morphologically generally depends on adult stage and male genitalia. The utility of DNA barcoding for these purposes is subject to debate; however, in insects at least, it is approximately 650 bp of the mitochondrial gene, cytochrome oxidase I (*COX I*). The approximate number of described insect species in India is 59,000; however, the number of barcodes generated from India is 4.6 % of known species, while the corresponding global scenario is about 16 % of described species; a lot of emphasis is required to catch up with the world scenario. In order to speed up taxonomic identification, DNA barcoding is now been considered as an alternative tool for insect biodiversity identification in India and the world. The present chapter deals with the use of barcode in the identification of insects belonging to different orders and families, using the neighbour-joining approach with the bootstrapping method and the Kimura-2 parameter to obtain a clear phylogenetic signal. In a neighbour-joining tree for all sequences, two clades were obtained: the first cluster consisting of hymenopteran insects and the other consisting of other orders. This phylogeny also agrees with the traditional phylogeny of insects. The present results, thus, favour DNA barcoding as a decisive tool in quick and reliable identification of insects.

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## Keywords

*COX I* · DNA barcoding · India · Phylogeny

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## Introduction

Insects are the most abundant of all life on earth and have evolved into a tremendous range of different forms. It took nearly 200 years for

taxonomists to describe 10% of the total number of species estimated. In this context identification of insects has been a monumental task where it calls for the availability of more number of specialists and funding. To catalogue the vast numbers of species, naturalists came up with the idea of classifying living beings on the basis of taxonomy, which is a branch of science that helps us to describe a living being on the basis of morphological features. After 250 years of Darwin and Linnaeus, a new method called DNA barcoding, a tool of DNA-based taxonomy is in current use to identify known and unknown species on the basis of the pattern of nucleotide arrangement in a fragment of DNA of a particular species (Novotny *et al.* 2002). Several researchers have suggested the use of DNA barcoding in taxonomy as a method to achieve rapid species descriptions in the context of the current biodiversity crisis (Herbert *et al.* 2003a, b; Ball and Armstrong 2006). DNA barcoding is the use of a short standardized DNA sequence (in insects, a 658 bp fragment of the mitochondrial cytochrome *c* oxidase (*COX I*) gene) to identify and assign unknown specimens to species besides facilitating the discovery of new species. Wilson (2012) observed that library barcodes gain their value due to an intimate association, through voucher specimens from where they came, with other data, particularly, Linnaean names, collection localities, and morphology in the form of digital images. This tool is widely accepted all over the globe from hard-core taxonomists' to graduate molecular biologists and also well received by governmental and nongovernmental organizations to catalogue all the species on our planet. With the advent of molecular biology and molecular tools, identification of life forms, including insects has become quick, precise, and easy.

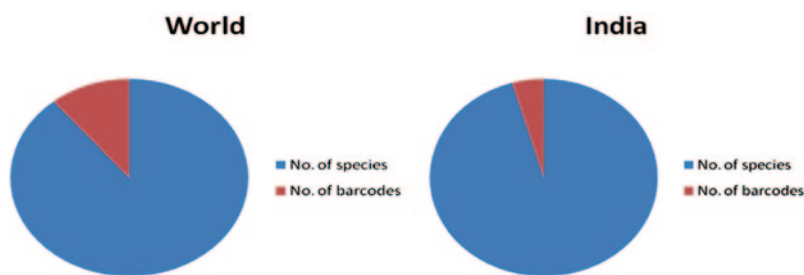
India is one of the world's most biodiverse regions, with a total land area of about 3,287,263 km<sup>2</sup>, covering a variety of ecosystems ranging from deserts to high mountains and tropical to temperate forests. Insects are the most abundant of all life forms on earth. India with about 2% of the global land area is among the top 12 mega biodiversity nations in the world accounting for 7.10% of the world insect fauna. It

is estimated that over 900,000 species of insects are known across the globe with over 60,000 species described from India with nearly as many species remaining to be named. Barcode of Life Datasystem (BOLD) Systems is populated with nearly 142,398 insect species barcodes out of which India has only 2758 barcodes; NBAII had 110 barcodes as on November 2013 (in six different insect orders; Fig. 1, Table 1).

To catalogue such vast diversity a simple, rapid, and accurate method is the current need. DNA barcoding is a tool that fulfils all the above said criteria to identify a specimen to species level. The rDNA internal transcribed spacers region 2 (ITS-2) (Ashok Kumar *et al.* 2009), cytochrome *c* oxidase subunit 1 (*COX I*), NADH dehydrogenase subunit 1 (*nadh1*), and cytochrome *b* (*cytb*) markers used in recent molecular analysis have substantially increased our understanding of the phylogenetic relationships between insect species. However, cytochrome *c* oxidase subunit 1 (*COX I*) has been used extensively by molecular biologists across the globe to discriminate insect species. In the present chapter, the major focus is on insect pests and parasitoids of agricultural importance in India.

DNA barcoding is an emerging tool, therefore, a reliable database has to be built by performing *COX I* sequencing on specimens previously identified by a taxonomist. Therefore, a prerequisite for genetic investigations in this study will be the technical step of constructing a database of insect pests and natural enemies in India and the world.

Molecular identification and phylogeny using species identification markers using *COX I* of the mitochondrial region is regarded as efficient. The main advantage of DNA barcoding is the rapid acquisition of molecular data (Monaghan *et al.* 2005). Mitochondria are energy-producing organelles, found in nearly every cell in nearly every plant and animal species. The mitochondrial genome in particular has turned out to be exceedingly useful in tracing evolutionary history, as it is present in all eukaryotic organisms, evolves rapidly as compared to nuclear DNA. Nuclear and mitochondrial genomes exhibit different patterns of inheritance (Behura 2006).



**Fig. 1** Number of insect species and barcodes in India and the world

**Table 1** Species barcodes in six different orders submitted to BOLD Systems by NBAIL, Bangalore

Insect order	Number of insects barcoded
Lepidoptera	15
Diptera	14
Hemiptera	4
Coleoptera	26
Neuroptera	1
Hymenoptera	50
<i>Total</i>	<i>110</i>

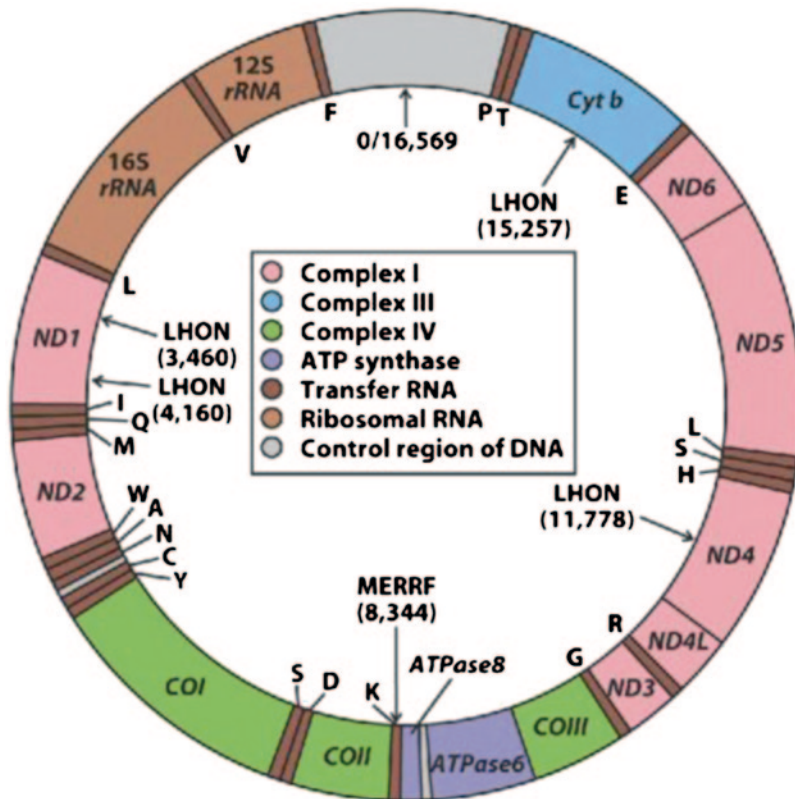
Mitochondrial markers are used for revealing phylogenetic relationships among related groups, because mtDNA is maternally inherited, it evolves fairly rapidly, and most of the nucleotide substitutions occur at neutral sites. With respect to this genetic marker the intra- and inter-phylogenetic relationships have been studied using the sequence data obtained from the *COX I* marker gene amplification. Relative homogeneity is maintained by concerted evolution, where mutations rapidly spread to all members of the gene family even if there are arrays located on different chromosomes (Arnheim 1983; Gerbi 1985; Tautz et al. 2002). However, these advantages are associated with a major drawback; while mitochondrial DNA was considered to be a neutral marker that reflects the history of the species, Ballard and Whitlock (2004) and Bazin et al. (2006) have recently argued that mitochondria are in fact often under strong selection and evolve under unusual evolutionary rules when compared with other genomes. Hurst and Jiggins (2005) suggested that selection can act directly on the mtDNA itself, but it can also arise indirectly from disequilibrium with other maternally transmitted DNA.

While morphological data are usually time-consuming and need specialists, DNA barcoding techniques are a uniform and practical method of species identification of insects and can be used for the identification of all developmental stages of insects, their food webs and biotypes and this may not be possible with morphology-based taxonomy. Tree-based taxon clustering as well as statistical taxon separation analysis indicates that molecular evidence does coincide with morphological hypotheses. Hence, species identification based on DNA sequence analysis proved to be feasible for the analysed taxa. DNA barcoding has the potential of being a valuable tool to biologists. It has helped to evolve many advanced tools as species diagnostics like species-specific primers developed for tea mosquito bugs (Rebijith et al. 2012) and mini barcodes for archival specimens.

### Insect Mitochondrial Genome

The arrangement of genes in mt genomes has been studied in more insects than in any other group of invertebrates. So far, 15 species of insects have had their mt genomes sequenced completely; the mitochondria of insects contain their own double-stranded circular genomes (Fig. 2), which range from 14,503 bp (Beckenbach and Joy 2009) to 19,517 bp in size (Lewis et al. 1995).

The arrangement of genes in the mt genomes of insects studied so far are conserved since all species, except the wallaby louse, have the same arrangement of protein-coding and rRNA genes and most tRNA genes. Only the positions of a few tRNA genes differ, in particular, those in



**Fig. 2** Organization of insect mitochondrial genome (Source: [http://chimerasthebooks.blogspot.in/2011\\_12\\_01\\_archive.html](http://chimerasthebooks.blogspot.in/2011_12_01_archive.html))

“hot spot” regions (Dowton and Austin 1999), e.g., near the control region, and in the two clusters of tRNA genes, *trnK-trnD* and *trnA-trnR-trnNtrnSI-trnE-trnF*. The most common arrangement of the 37 genes in the mt genome, which is present in the fruit fly *Drosophila yakuba*, the bug *T. dimidiata*, and many other species, is inferred to be ancestral for insects (Boore and Brown 1998; Crease 1999).

### Agriculturally Important Insects

Insects have been used in landmark studies in biomechanics, climate change, developmental biology, ecology, evolution, genetics, paleolimnology, and physiology. Because of their diversity and many roles, they are familiar to the general public. However, their conservation is a challenge. The goal of this chapter is to docu-

ment agriculturally dominant insect pests, their natural enemies, pollinators, and veterinary insect pests.

### Importance of DNA Barcoding in Agricultural Entomology

Insect pests are a major concern for farmers across the world and more than 10,000 species of insects have been recorded damaging crops (Dhaliwal et al. 2007). Sometimes the yield loss by insects reaches as high as 60–70% and it is reported that Indian agriculture is currently suffering an annual loss of about ₹ 86.39 million due to insect pests (Dhaliwal et al. 2007). An automated DNA-based system will free taxonomists from routine identifications, allowing them to direct their efforts to new collections, descriptions, and assessments of taxonomic relationships. In 2003, Paul D.N.

Hebert from the University of Guelph, Ontario, Canada, proposed the compilation of a public library of DNA barcodes that would be linked to named specimens. This library would “provide a new master key for identifying species, whose power will rise with increased taxon coverage and with faster, cheaper sequencing.” The goal of a DNA barcoding library is the construction of an enormous, online, freely available sequence database. Participants in the DNA barcode initiative come in many configurations, including consortia, databases, networks, labs, and projects that range in size from local to global. An extensive survey is required to catalogue all the insect pests for better understanding of their habits and habitat so that proper measures can be used to control them. In recent years a new taxonomic approach called “DNA barcoding” has been proposed to aid the determination of species. This method suggests that large-scale surveys of DNA variation would accelerate studies on ecology, biodiversity, and conservation planning of poorly studied ecosystems and groups of organisms. Recently, several museums, herbaria, universities, biodiversity inventory agencies, and commercial experts have created the international consortium Barcode of Life (CBOL). The use of DNA sequence for species recognition, assessment, and taxonomic description, these include taxonomic accuracy, low cost, ease of application in diverse contexts (including by non-specialists), portability, routine and immediate access to information, and utility across a broad phylogenetic and taxonomic spectrum of organisms, including many species new to science.

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## Materials and Methods

### Collection and Identification

Insects were collected from various ecosystems across India. At each site, insects were collected by brush and cotton wool pad and sweeping net methods and transferred to collection tubes containing 95% alcohol. The specimens were identified to species level and distributed into

their respective families to obtain a clear phylogenetic signal (Table 2) immediately upon their collection.

The specimens, thus collected and morphologically identified, were used for *COX 1* barcoding at the National Bureau of Agriculturally Important Insects (NBAIL) Bangalore, India.

### Genetic Analysis

The DNA was extracted from somatic tissue rich in mitochondria (e.g., thorax and upper abdominal region) using Qiagen DNeasy® Kit, following the manufacturers' protocols. The remaining parts of each of the insects and respective individuals were kept as voucher specimens at NBAIL. The extracts were subjected to PCR amplification of a 658 bp region near the 5' terminus of the *COX 1* gene following standard protocols. Primers used were forward primer: (LCO 1490 5'-GGTCAACAAATCATAAAGATATTG G-3') and reverse primer: (HCO 2198 5'-TAACTTCAGGGTGACCAAAAAATCA-3').

PCR reactions were carried out in 96-well plates with 50 µl reaction volume containing GeNei™ Taq buffer 5 µl, 1 µl of GeNei™ 10 mM dNTP mix, 2.5 µl of (20 pmol/µl) forward primer, 2.5 µl of (20 pmol/µl) reverse primer, 1 µl of GeNei™ Taq DNA polymerase (1 U/µl), DNA (50 ng/µl) 2 µl, and sterile water 36 µl. Thermocycling consisted of an initial denaturation of 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min. PCR was performed using a C1000™ Thermal Cycler. The amplified product was analysed on a 1.5% agarose gel electrophoresis as described by Sambrook and Russell (2001). The amplified products were sent to commercial sequencing company, M/s Eurofins Pvt Ltd. India. Each species was bidirectionally sequenced and checked for quality by Bioedit 7.0.2 software and homology, insertions and deletions, stop codons, and frameshifts by using NCBI BLAST. All sequences were uploaded to GenBank and the BOLD (<http://www.boldsystems.org>).

**Table 2** Distribution of insect species into their respective subfamilies and tribes on the basis of classification

Order	Family	Insect	Genbank accession numbers	Barcode ID
Hemiptera	Agromyzidae	<i>Phytomyza orobanchia</i>	KC732453	AGIMP017–13
	Aphididae	<i>Xylocoris flavipes</i>	KF365462	Not available
	Anthocoridae	<i>Blaptostethus pallescens</i>	KF365463	Not available
Diptera	Tephritidae	<i>Acroceratitis histrionica</i>	KF471502	Not available
		<i>Bactrocera correcta</i>	KF289766	AGIMP022–13
		<i>Bactrocera dorsalis</i>	KF289767	AGIMP023–13
		<i>Bactrocera zonata</i>	KF289768	AGIMP024–13
Lepidoptera	Pyrallidae	<i>Chilo auricilius</i>	KC306949	AGIMP003–12
		<i>Chilo partellus</i>	KC911712	AGIMP007–13
		<i>Chilo sacchariphagus indicus</i>	KC306951	AGIMP005–12
		<i>Conogethes punctiferalis</i>	KF114864	AGIMP012–13
		<i>Galleria mellonella</i>	KF289770	AGIMP026–13
		<i>Polyocha depressella</i>	KC306950	AGIMP004–12
		<i>Scirpophaga excerptalis</i>	KC306948	AGIMP002–12
	Noctuidae	<i>Bombyx mori</i>	JX025640	BMSW002–12
		<i>Corcyra cephalonica</i>	KF289769	AGIMP025–13
		<i>Helicoverpa armigera</i>	KC911713	AGIMP008–13
	Plutellidae	<i>Plutella xylostella</i>	KC911716	AGIMP011–13
	Galleriidae	<i>Sesamia inferens</i>	KC911715	AGIMP010–13
	Bombycidae	<i>Spodoptera litura</i>	KC911714	AGIMP009–13
Coleoptera	Scolytidae	<i>Euwallacea fornicates</i>	KC590061	AGIMP027–13
	Anobiidae	<i>Stegobium panecium</i>	KF471501	Not available
Hymenoptera	Formicidae	<i>Anoplolepis gracilipes</i>	JN987860	ANIND016–11
		<i>Aphaenogaster beccarii</i>	JN886031	ANIND005–11
		<i>Camponotus compressus</i>	JN886027	ANIND001–11
		<i>Camponotus compressus</i> GR-17	JN987857	ANIND013–11
		<i>Camponotus irritance</i>	JN886033	ANIND007–11
		<i>Camponotus parius</i>	JN886032	ANIND006–11
	Braconidae	<i>Chelonus blackburni</i>	KF365461	Not available
	Formicidae	<i>Leptogenys chinensis</i>	JN886030	ANIND004–11
		<i>Monomorium scabriceps</i>	JN987858	ANIND014–11
		<i>Myrmicaria brunnea</i>	JN886029	ANIND003–11
		<i>Oecophylla samaragdina</i>	JN886035	ANIND009–11
		<i>Paratrechina longicornis</i>	JN886034	ANIND008–11
		<i>Pheidologeton diversus</i>	JN987859	ANIND015–11
		<i>Plagiolepis</i> sp.	JN886037	ANIND011–11
		<i>Solenopsis geminate</i>	JN886028	ANIND002–11
		<i>Tapinoma melanocephalum</i>	JN886036	ANIND010–11
		<i>Technomyrmex albipes</i>	JN886038	ANIND012–11
	Trichogrammatidae	<i>Trichogramma achaeae</i>	KF234139	AGIMP021–13
		<i>Trichogramma chilonis</i>	KF234137	AGIMP019–13
		<i>Trichogramma japonicum</i>	KF234138	AGIMP020–13



## Data Analysis

The pairwise analysis of 43 sequences was conducted using neighbour-joining bootstrap method and Kimura-2 parameter in MEGA5. The number of base substitutions per site was analysed between all sequences. Codon positions included were 1st+2nd+3rd+non-coding. All positions containing gaps and missing data were eliminated from the dataset. The A, T, G, C, AT, and GC content of all sequences was obtained using a computer program designed in the bioinformatics lab at NBAII, Bangalore, India. The AT% at three codon positions was calculated using the same program.

Sequences were aligned using the Mega 5 software package. Residue and pairwise distances were estimated using the Clustal W tool of MEGA 5 software with default settings of gap opening penalty ten, and a gap-extension 0.1 in pairwise and 0.05 in multiple alignments, sequence divergences were calculated and an NJ tree of distances was created to provide a graphic representation of the among-species divergences (Tamura et al. 2011). Sequences and other specimen information are available in the project “Ants of India,” project code: ANIND in the campaign section “Ants of the World” and in “General Projects, Agriculturally Important Insects of India,” project code: AGIMP in BOLD Systems at website ([www.barcodinglife.org](http://www.barcodinglife.org)). Sequences and other specimen information are available at BOLD Systems ([www.barcodinglife.org](http://www.barcodinglife.org)) and GenBank.

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## Results and Discussion

All mt CO1 sequences were submitted to the NCBI-GenBank under accession numbers provided in Table 3, PCR products from different ant species were easily produced and checked for low-quality bases at the ends.

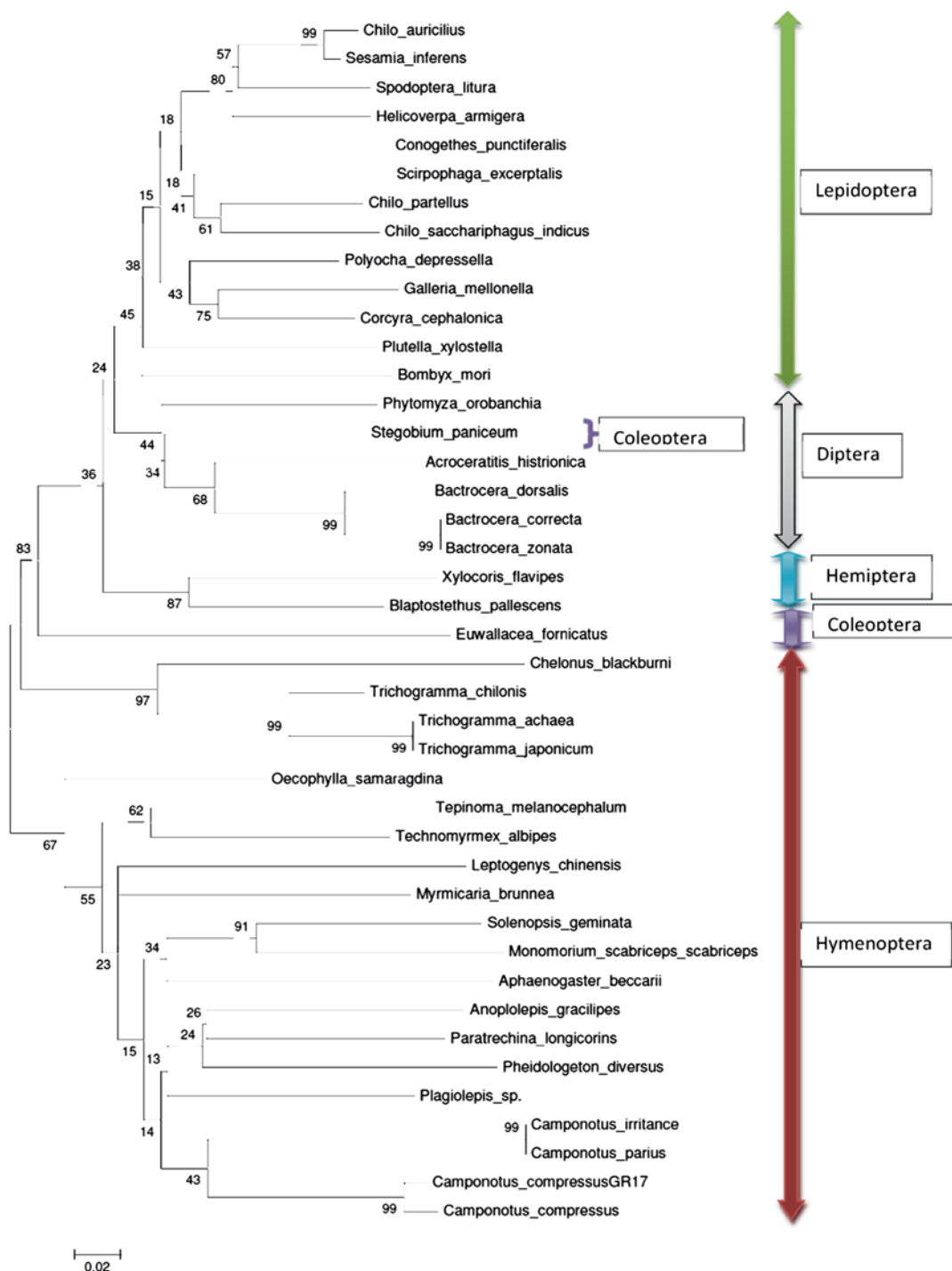
The sequences were edited accordingly and aligned using Bioedit 7.0.2 software. The visualized PCR product contained no double bands on agarose gel, thus indicating that sequences

obtained were mitochondrial DNA and not nuclear pseudogenes. The CO1 region in almost all the samples was in the range of 500–658 bp. A total of 42 insect species were studied; there were a total of 540 positions in the final datasets (software generated) according to the full K2P/NJ tree (Fig. 3). All 42 species could be differentiated by CO1 barcoding. Most of the amplified sequences were up to 658 bp in length. In general, the lengths suggest that nuclear DNA sequences originally from mitochondrial DNA sequences (NUMTs) were not sequenced because NUMTs are generally smaller than 600 bp. Although the *COX 1* region is highly conserved, there were differences in the length and sequence of the regions flanking *COX 1*. Previous phylogenetic studies have showed the utility of *COX 1* for the identification of genetic variability. Insect species were also subjected for analysis of nucleotide composition in all the different specimens, AT, GC%, and AT content at the first, second, and third codon positions were calculated, the difference was attributed to the AT content of the 3rd codon, AT<sub>3</sub>, (25.58) which ranged from 32.07–5.78%. The AT content at the first and second codon positions was nearly invariant (Table 3). As expected, AT content was significantly found higher by 69.07% than the GC content of 30.92%. Average genetic distances among the different groups of insects used in this study showed higher values at the 3rd codon position, indicating that detailed study of the 3rd codon position for insects might reveal possible evolutionary information among this closely related group of organisms. Sequences were heavily AT-biased due to this 3rd codon position, which is expected in insect mtDNA. A phylogeny tree constructed using the N–J method revealed two clusters (Fig. 3); two clades were obtained, the first cluster consisting of orders lepidoptera, diptera, hemiptera, and coleoptera, whereas another clade showing relationship between hymenopteran insects. All the sequences obtained were submitted to GenBank. Sequence and barcode information is also available at BOLD Systems ([www.barcodinglife.org](http://www.barcodinglife.org)) in the General Project section as Agriculturally

**Table 3** AT% at the first, second, and third codon positions and AT and GC% of 42 different insect species

Insect species	First	Second	Third	AT%	GC%
<i>Acroceratitis histrionic</i>	17.21	19.11	30.80	67.14	32.85
<i>Anoplolepis gracilipes</i>	20.96	27.58	19.51	68.1	31.9
<i>Aphaenogaster beccarii</i>	20.44	24.91	20.27	65.6	34.4
<i>Bactrocera correcta</i>	16.40	18.75	27.50	62.65	37.34
<i>Bactrocera dorsalis</i>	17.18	18.75	27.96	63.90	36.09
<i>Bactrocera zonata</i>	17.03	14.68	5.78	62.5	37.5
<i>Blaptostethus pallascens</i>	19.47	18.53	30.99	69.00	30.99
<i>Bombyx mori</i>	25.90	18.88	24.88	69.66	30.33
<i>Camponotus compressus</i>	30.74	22.26	20.49	73.5	26.5
<i>Camponotus compressus</i> GR17	30.39	21.58	20.51	72.5	27.5
<i>Camponotus irritans</i>	20.49	24.95	19.83	65.3	34.7
<i>Camponotus parius</i>	25.22	20.51	20.36	65.5	34.5
<i>Chelonus blackburni</i>	24.18	21.59	30.35	76.13	23.86
<i>Chilo auricilius</i>	20.45	19.14	31.26	70.86	29.13
<i>Chilo partellus</i>	20.63	19.72	30.19	70.56	29.43
<i>Chilo sacchariphagus indicus</i>	20.13	19.31	30.27	69.72	30.27
<i>Conogethes punctiferalis</i>	18.84	18.99	30.69	68.54	31.45
<i>Corcyra cephalonica</i>	19.68	19.06	31.09	69.84	30.15
<i>Eurwallacea fornicates</i>	17.62	19.14	27.81	64.58	35.41
<i>Galleria mellonella</i>	19.37	19.06	31.25	69.68	30.31
<i>Helicoverpa armigera</i>	21.09	19.27	30.19	70.56	29.43
<i>Leptogenys chinensis</i>	21.32	30.90	20.82	73.1	26.9
<i>Monomorium scabriceps</i>	25.07	19.90	20.51	65.5	34.5
<i>Myrmecaria brunnea</i>	20.66	26.11	20.00	66.8	33.2
<i>Oecophylla smaragdina</i>	33.13	22.18	20.97	76.3	23.7
<i>Paratrechina longicornis</i>	28.57	20.66	21.12	70.4	29.6
<i>Pheidologeton diversus</i>	24.20	19.78	20.49	64.5	35.5
<i>Phytomyza orobanchia</i>	19.30	30.24	18.23	67.78	32.21
<i>Plagiolepis</i> sp.	28.11	20.82	21.12	70.1	29.9
<i>Plutella xylostella</i>	20.63	19.27	29.74	69.65	30.34
<i>Polyocha depressella</i>	20.62	19.47	32.07	72.17	27.82
<i>Scirpophaga excerptalis</i>	19.63	19.47	30.60	69.72	30.27
<i>Sesamia inferens</i>	20.18	19.11	30.34	69.65	30.34
<i>Solenopsis geminate</i>	25.22	19.75	20.66	65.7	34.3
<i>Spodoptera litura</i>	20.96	19.46	29.61	70.04	29.95
<i>Stegobium paniceum</i>	19.56	18.93	31.29	69.79	30.20
<i>Tapinoma melanocephalum</i>	24.46	20.82	20.51	65.8	34.2
<i>Technomyrmex albipes</i>	26.89	20.51	20.82	68.2	31.8
<i>Trichogramma achaeae</i>	23.41	20.48	31.54	75.44	24.55
<i>Trichogramma chilonis</i>	23.08	20.00	32.03	75.12	24.87
<i>Trichogramma japonicum</i>	23.18	20.45	31.56	75.20	24.79
<i>Xylocoris flavipes</i>	17.55	18.19	28.50	64.25	35.74
Mean	22.12	20.76	25.58	69.07	30.92





**Fig. 3** Phylogenetic tree for 42 insect species (5 orders) depicting genetic relationships derived from CO1 sequences. Note: Bootstrap consensus tree generated by Bootstrap Test Phylogeny using neighbour-joining (N-J) method of MEGA 5 Software. All the 43 species are from 5 orders, which are distributed into two main clades that are 61 % similar

Important Insects in India (AGIMP) and in Ants of the World section as Ants of India (ANIND).

### Limitations of DNA Barcoding

A short standardized DNA sequence originating a fragment of the mitochondrial gene has emerged as the standard barcode region for animals for unknown species and an aid in the discovery of new species. Mitochondrial DNA genes are maternally inherited which sometimes may result in interspecific hybridization or endosymbiont infections that generate transfer of mitochondrial genes outside the species, therefore DNA barcoding requires an expertise at the analysis level, and one must be trained on the analytical part of it. The proper knowledge about different kinds of bioinformatic tools enables one to analyse DNA sequence. Pseudogenes commonly known as nuclear mitochondrial DNA (NUMTs), originating from mitochondrial nucleus is one of the major obstacles in discriminating species on the mitochondrial DNA basis. Their integration into the nuclear genome was originally associated with transposable elements or short dispersed repeats, but close examination of many different NUMTs loci reveals a lack of common features at integration sites (Bensasson et al. 2001). Whitworth et al. (2007) observed that the patterns of mitochondrial variability can be confounded by the spread of maternally transmitted bacteria that cosegregate with mitochondria. They further reported that here, the performance of barcoding in a sample comprising 12 species of the blow fly genus *Protophormia*, known to be infected with the endosymbiotic bacteria *Wolbachia* showed very limited success: assignment of unknown individuals to species is impossible for 60% of the species, while using the technique to identify new species would underestimate the species number in the genus by 75%. In another study, Smith et al. (2012) analysed >2 million insect *COX I* trace files on the BOLD and reported that *Wolbachia COX I* was present in 0.16% of the cases. It is possible to generate *Wolbachia COX I* using standard insect primers; however, that amplicon was never confused with the *COX*

*I* of the host. *Wolbachia* alleles recovered were predominantly Super group A and were broadly distributed geographically and phylogenetically and it was concluded that the presence of the *Wolbachia* DNA in total genomic extracts made from insects is unlikely to compromise the accuracy of the DNA barcode library and suggested that regular assays for *Wolbachia* presence and type can, and should, be adopted by large-scale insect barcoding initiatives.

### Future Perspectives

DNA barcoding will greatly facilitate and complement taxonomic studies; the sequencing data coupled with traditional taxonomy is a model that can be applied on various disciplines and will allow analytical needs to be scaled to match the enormity of the current biodiversity crisis. It will help in the identification and conservation of the evolutionary processes that generate and preserve biodiversity. For groups in which identification can be difficult, the potential utility of DNA barcoding is immense. In this study, we showed that DNA barcoding allows the rapid identification of important functional units of hyper diverse arthropods in the rapid manner needed by conservation groups responding to habitat destruction and degradation. Insect diversity, measured via DNA barcoding in collaboration with taxonomists, should provide the essential fine-scale maps for assessing biodiversity at a scale at which conservation decisions are made. Our results reveal that *COX I* barcoding will permit the unambiguous identification of insect species of India. Taxonomists, equipped with modern tools and collaborations, have a chance to move systematically to the forefront of conservation. DNA barcoding is not a perfect approach, but it has immense impact on the scientific community, becoming a widely used approach, characterized by many relevant aspects of uniformity and generalization. A critical knowledge of the method is essential for a proper use of it. In recent past many online resources help researchers to upload and retrieve DNA sequence and specimen data across insect orders for phylogenetic and barcoding studies.

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