



DNA barcoding of insects from India: Current status and future perspectives

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Abstract

Insect fauna occupy the largest proportion of animal biodiversity on earth, but the assessment or quantification in terms of species diversity is far from complete. Several recent studies have demonstrated the rapid pace at which insect population decline is occurring. There is an urgent need to document and quantify the diversity of insect fauna for a proper understanding of terrestrial ecosystems. This can be achieved by using modern technology to identify species much faster than relying on traditional methods alone. In line with this, the molecular approach through DNA barcoding coupled with morphological identification needs to be focused and accelerated. The present paper describes the current status of barcoding of insect species in India along with the gaps that need to be remedied. This analysis shows that barcoded specimens cover a very meagre proportion of less than 3.73% of the known taxa/described species and the most represented orders are Lepidoptera and Hemiptera followed by Diptera and Coleoptera. There is a need to expedite insect species discovery and documentation in a collaborative mode between traditional taxonomists and molecular biologists, to accomplish the DNA barcoding of all known insect taxa from India.

Keywords Cytochrome oxidase I · Barcode · Arthropods · Biodiversity · Hexapoda · Molecular biology

Introduction

The identification of insect species is important for addressing the fundamental questions in ecology, evolution, conservation biology and agro-biodiversity. Monitoring,

conservation and the proper utilization of biodiversity is also dependent on the proper diagnosis of insect species. With a history of more than two and a half centuries, the Linnaean classification system has contributed immensely to taxonomy, however morphology-based taxonomy has

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some limitations (E.g., difficulty in resolving cryptic species complexes, understanding sexual dimorphism, immature stages identification, etc.) which hinder its progress [1, 2]. The lack of experts in the morphotaxonomy of some insect groups places further limits on this traditional approach. Hence, the majority of species remain unknown to science, some are only known from their sole descriptions and type specimens, while some type materials have been either lost or are unavailable. The amount of material awaiting identification and description in the collections of museums has been substantial for centuries [3]. Since its proposal in 2003, DNA barcoding is a useful tool to quickly identify species and is used as a complementary approach to traditional taxonomy in biodiversity studies [4]. DNA barcoding, the method of characterizing species using one or a few conserved and standardized regions of DNA, has become an effective tool in species identification or delineation [5].

DNA barcoding finds its application in cryptic species identification [6], identification of immature life stages [7], rapid identification in quarantine [8], host-parasitoid interactions [9], herbivore diet analysis [10], disentangling food webs [11], understanding pollinator communities and their interactions using environmental DNA barcodes [12], identification of species from different environments [13] and in seasonal diversity shifts [14]. To support this range of applications, high-quality well curated reference libraries, based on voucher specimens are necessary [5]. To achieve a complete reference database of sequences for all the species from a particular country is challenging because of the huge diversity of life forms that needs to be tackled, and lack of taxonomic expertise for these organisms. However, many countries have developed large scale DNA barcode reference datasets, combined with traditional taxonomic expertise, for specific group of organisms including insects [15–19].

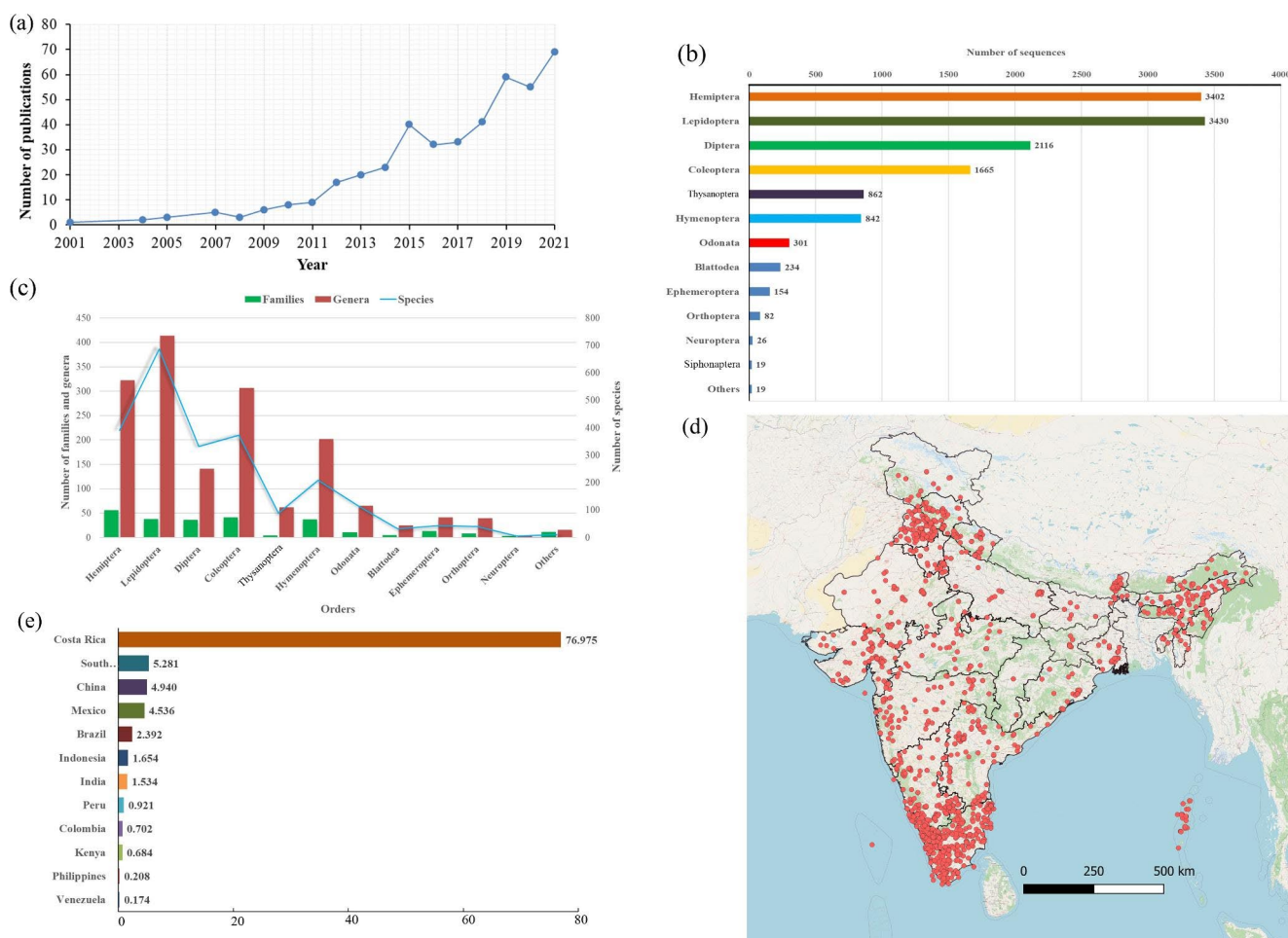


Fig. 1 Overview of DNA barcoding of Insects from India. (a) Number of publications found in *Science direct* with the key word 'Insect DNA barcodes India'. (b) Number of sequences by order. (c) Representation of families, genera and species under different orders. (d) Specimen sources from which DNA barcodes were generated from India. (e) Comparison of sequences submitted to BOLD among mega-diverse countries

India, occupying about 2% of the earth's landmass, is among the top ten mega-diverse nations of the world in terms of insect diversity, harboring about 7.10% of the world's insect fauna [20]. India currently holds an estimated 63,760 described insect species, including 658 families representing 27 orders, out of which 21,166 species are endemic [20]. Of these, eight orders, viz. Coleoptera, Lepidoptera, Orthoptera, Diptera, Hemiptera, Odonata, Hymenoptera and Thysanoptera, constitute the bulk, representing 94% of the insect fauna [21]. The total number of species described from India has been steadily increasing but at a very slow pace [22]. To explore and document the insect diversity in such a vast landmass with diversified ecological zones, simple, rapid, and accurate methods such as DNA barcoding is required. The Barcode of Life Data System (BOLD) is an international repository for barcode records, storing specimen data, images, sequences and their trace files. BOLD comprises of 142,398 insect species barcodes, out of which only 2758 barcodes are for Indian specimens until 2015 according to Jalali et al. [23]. In the present review, we analyzed the DNA barcode data of insects from India obtained from BOLD (www.boldsystems.org) to understand

the present status and to discuss what needs to be done in the future.

Current status of insect DNA barcoding in India

With the wide use of Sanger sequencing and next-generation sequencing (NGS) technologies, the cost of obtaining each sequence read of a target region has come down drastically. The advantage of low-cost sequencing technologies encouraged a positive growth in the use of DNA barcoding in scientific studies from India, as illustrated by its use in different studies (Fig. 1a). BOLD comprises 852,657 insect species barcodes from 12 mega-diverse countries. The highest number of barcodes is reported from Costa Rica, which accounts for 77%, followed by South Africa, China and Mexico, each accounting for around 5%, whereas India contributes only 1.53% (Fig. 1c). India stands in seventh position among given megadiverse countries, with 13,152 sequences (including 10,570 COI-5P, and other appropriate barcode markers advocated for by the Consortium for the Barcode of

Table 1 DNA barcoding of insects from India to the rank of species

Orders	Number of DNA barcodes retrieved from BOLD				Total Sequences with BINs	Sequences without BINs	BIN count	Number of Families present in India [#]	Number of species described from India [#]	Number of species bar-coded from India (%)
	Sequences	Families	Genera	Species#						
Hemiptera	3402 (25.87)	56 (21.21)	322 (19.65)	391 (16.78)	1992	1410	476	92	6479	6.03
Lepidoptera	3430 (26.08)	38 (14.39)	414 (25.26)	687 (29.48)	2689	741	621	84	15,000	4.58
Diptera	2116 (16.09)	36 (13.64)	141 (8.60)	332 (14.25)	1430	686	343	87	6337	5.24
Coleoptera	1665 (12.66)	41 (15.53)	307 (18.73)	373 (16.01)	1102	563	435	114	17,455	2.14
Thysanoptera	862 (6.55)	4 (1.52)	62 (3.78)	89 (3.82)	780	82	123	7	686	12.97
Hymenoptera	842 (6.40)	37 (14.02)	202 (12.32)	209 (8.97)	646	196	311	57	12,605	1.66
Odonata	301 (2.29)	11 (4.17)	65 (3.97)	117 (5.02)	222	79	100	19	463	25.27
Blattodea	234 (1.78)	5 (1.89)	25 (1.53)	31 (1.33)	158	76	41	12	186	16.67
Ephemeroptera	154 (1.17)	13 (4.92)	41 (2.50)	44 (1.89)	142	12	57	12	124	35.48
Orthoptera	82 (0.62)	8 (3.03)	40 (2.44)	41 (1.76)	61	21	40	21	1033	3.97
Neuroptera	26 (0.20)	3 (1.14)	4 (0.24)	5 (0.21)	23	3	8	12	312	1.60
Siphonaptera	19 (0.14)	1 (0.38)	1 (0.06)	3 (0.13)	7	12	2	8	46	6.52
Embioptera	5 (0.04)	1 (0.38)	2 (0.12)	2 (0.09)	2	3	1	2	31	6.45
Mantodea	3 (0.02)	1 (0.38)	3 (0.18)	2 (0.09)	2	1	2	11	174	1.15
Psocoptera	3 (0.02)	1 (0.38)	2 (0.12)	0 (0.00)	3	0	3	16	105	0.00
Trichoptera	3 (0.02)	3 (1.14)	3 (0.18)	2 (0.09)	3	0	3	27	1046	0.19
Zygentoma	2 (0.02)	2 (0.76)	2 (0.12)	1 (0.04)	2	0	2	3	28	3.57
Dermaptera	1 (0.01)	1 (0.38)	1 (0.06)	1 (0.04)	1	0	1	7	298	0.34
Strepsiptera	1 (0.01)	1 (0.38)	1 (0.06)	0 (0.00)	1	0	1	4	21	0.00
Phasmatodea	1 (0.01)	1 (0.38)	1 (0.06)	0 (0.00)	0	1	0	-	-	0.00
Total	13,152	264	1639	2330	9266	3886	2570	595	62,429	3.73

* ZSI, 2012; Values in parentheses represent per cent share in total; Abbreviations: BINs = Barcode Index Numbers, BOLD = Barcode of Life Data Systems; #Number of sequences with proper species identification has been considered

Life for animals), representing 2330 species collected from different geographical locations across India (Fig. 1e). In the present review, barcode data were downloaded from BOLD (including NCBI mined sequences) up to April, 2022. The data is available on BOLD under the dataset DS-ININD20 (<https://doi.org/10.5883/DS-ININD20>). For details on data acquisition and processing please refer to Supplementary file 1.

The species belongs to 20 different orders, which are (in decreasing number of samples) Lepidoptera, Hemiptera, Diptera, Coleoptera, Thysanoptera, Hymenoptera, Odonata, Blattodea, Ephemeroptera, Orthoptera, Neuroptera, Siphonaptera, Embioptera, Mantodea, Psocoptera, Trichoptera, Zygentoma, Dermaptera, Strepsiptera and Phasmatodea (Fig. 1b; Table 1). Among these orders, the largest number of sequences was generated for Lepidoptera (26.08%), followed by Hemiptera (25.87%), Diptera (16.09%), Coleoptera (12.66%), Thysanoptera (6.55%), Hymenoptera (6.40%), Odonata (2.29%), Blattodea (1.78%), Ephemeroptera (1.17%) and the rest of the eleven orders comprise < 1%.

The highest species coverage was achieved for, Lepidoptera with 687 species (29.48%), followed by Hemiptera with 391 spp. (16.78%), Coleoptera 373 spp. (16.01%), Diptera 332 spp. (14.25%), Hymenoptera 209 spp. (8.97%), Odonata 117 spp. (5.02%), Thysanoptera 89 spp. (3.82%), and Ephemeroptera with 44 spp. (2.22%), while the remaining 12 orders together comprise < 3% (Table 1).

In India, ~62,429 species of insects belonging to 595 families were described (21), but only 2330 species from 264 families possess DNA barcodes (Table 1). Among the total number of species described from India, only 3.73% of insects have been barcoded. In Lepidoptera, 15,000 described species from 84 families are reported from India. Among these, 4.58% of the species belonging to 38 families feature DNA barcodes. Major families represented among the barcode material are Erebiidae (146 species), Nymphalidae (80 spp.), Sphingidae (76 spp.), Geometridae (64 spp.), Noctuidae (63 spp.), and Saturniidae (54 spp.) (Fig. 2a). Hemiptera comprise 6479 species from 92 families, where around 6.03% of the species from 56 families are

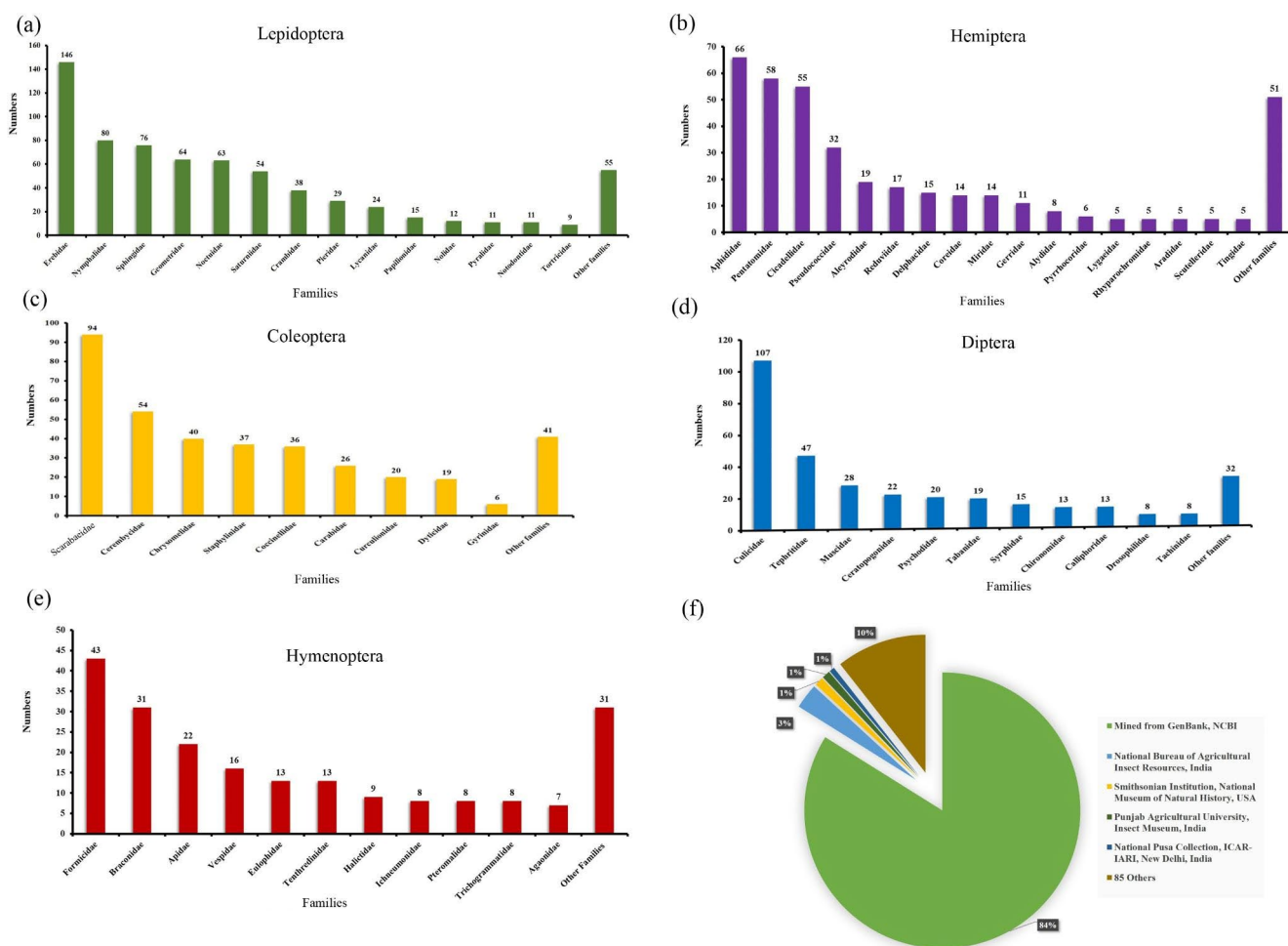


Fig. 2 DNA barcodes of mega-orders represented by families. (a) Lepidoptera. (b) Hemiptera. (c) Coleoptera. (d) Diptera. (e) Hymenoptera. (f) DNA barcodes contributed by different institutes from India

barcoded, in which Aphididae (66 spp.), Pentatomidae (58 spp.) and Cicadellidae (55 spp.) covered the majority, while the remaining families contributed 212 species (Fig. 2b). Furthermore, in Coleoptera, 17,455 species were described under 114 families, where only 2.14% species belonging to 41 families exhibit DNA barcodes, among which Scarabaeidae (94 spp.), Cerambycidae (54 spp.), Chrysomelidae (40 spp.) and Staphylinidae (37 spp.) are the most covered families (Fig. 2c). In the case of Diptera, 2116 barcodes have been generated from 382 species covering 36 families. This indicates only 5.24% of dipteran species are barcoded among 87 families with 6337 species described from India. The majority of species covered are from Culicidae (107 spp.), Tephritidae (47 spp.) and 178 species from the other 36 families (Fig. 2d). In the mega diverse order Hymenoptera, 12,605 species have been described under 57 families, where only 209 species belonging to 37 families are barcoded, constituting 1.66% of the known Indian Hymenoptera species diversity, with Formicidae (43 spp.), Bracnoidae (31 spp.), Apidae (22 spp.) and 113 species belonging

to other 34 families (Fig. 2e). However, there are few orders like Ephemeroptera (124 spp.), Odonata (463 spp.), Thysanoptera (686 spp.) and Blattodea (186 spp.) represented by only a smaller number of described species from India. But, 44.35% of described ephemeropterans, 16.63% of odonatanans, 14.58% of thysanopterans and 14.52% of Blattodea show more per cent share in number of species barcoded in respective orders (Table 1). Based on BOLD public data for specimens with or without sequences of any kind, for percentage of species assigned to various taxa out of total species, the Indian dataset has a higher percentage of Hemiptera (16.78% vs. 5.67%), Thysanoptera (3.83% vs. 1.79%) and Odonata (5.02% vs. 1.12%) when comparing the total species counts of the same Order list.

The Barcode Index Number (BIN) system is used to assign groups of similar COI-5P sequences a single OTU (operational taxonomic unit) through sequence clustering methods and it is suggested as a proxy in place of a species level identification. Sequences which possess quality criteria of more than 300 bp in the Folmer barcode

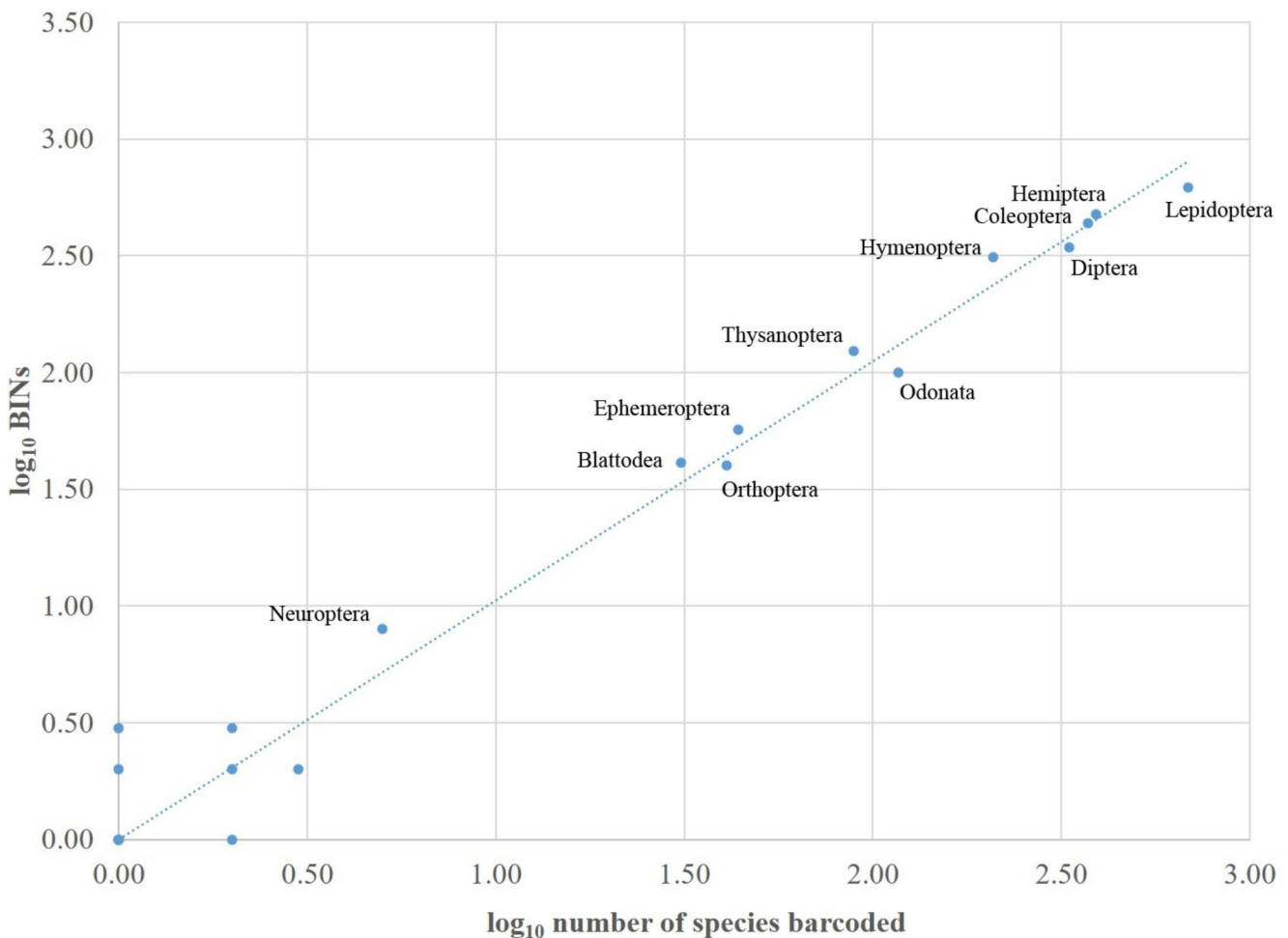


Fig. 3 BIN count versus number of known species with barcodes for 19 orders of Indian insects. Points above the line represent orders with more BINs than number of species

region, and less than 1% uncertain base calls (Ns) are assigned BIN. Amongst, 10,570 COI-5P sequences from India 9266 sequences have been assigned BINs and 1304 sequences have not. The remaining 2583 specimens either have sequences in other markers, or lack them altogether. There are 2570 BINs in the present dataset. BINs counts and known species ratios for 19 orders revealed that 11 orders possess far more BINs than known barcoded species (Fig. 3). Orders Lepidoptera, Odonata, Orthoptera, Siphonaptera and Embioptera possess far less BINs than known barcoded species. The orders Strepsiptera and Psocoptera shows 3 and 1 BIN, respectively, but the specimens are only identified at the genus level. Similarly, in Embioptera two species have been assigned to a single BIN. The probable reason for more sequence clusters (here more BINs) than identified species (known species) is identifying the presence of species by current taxonomic methods. Similarly, less sequence clusters than identified species may be due to insufficient sampling programmes or when BIN sharing by different species is common [50]. Among total barcodes, the

majority (11,035) of sequences were submitted to GenBank NCBI directly by different authors (Fig. 1f). However, more than 80 different institutes have contributed to DNA barcoding of Indian species. Alarming, the data shows that, among the 13,152 sequences, only 645 sequences are barcode compliant, only 1004 possess images and 4769 have GPS coordinates. This shows there is a wide gap in collecting and uploading metadata along with sequences. There is a huge opportunity to work on the barcoding of Indian insects as evident by the large percentage of known insect species yet to be barcoded (Fig. 4).

Probable obstacles for the shortfall in Indian insect DNA barcoding

Major factors that might have hindered DNA barcoding of insects in India are discussed below:

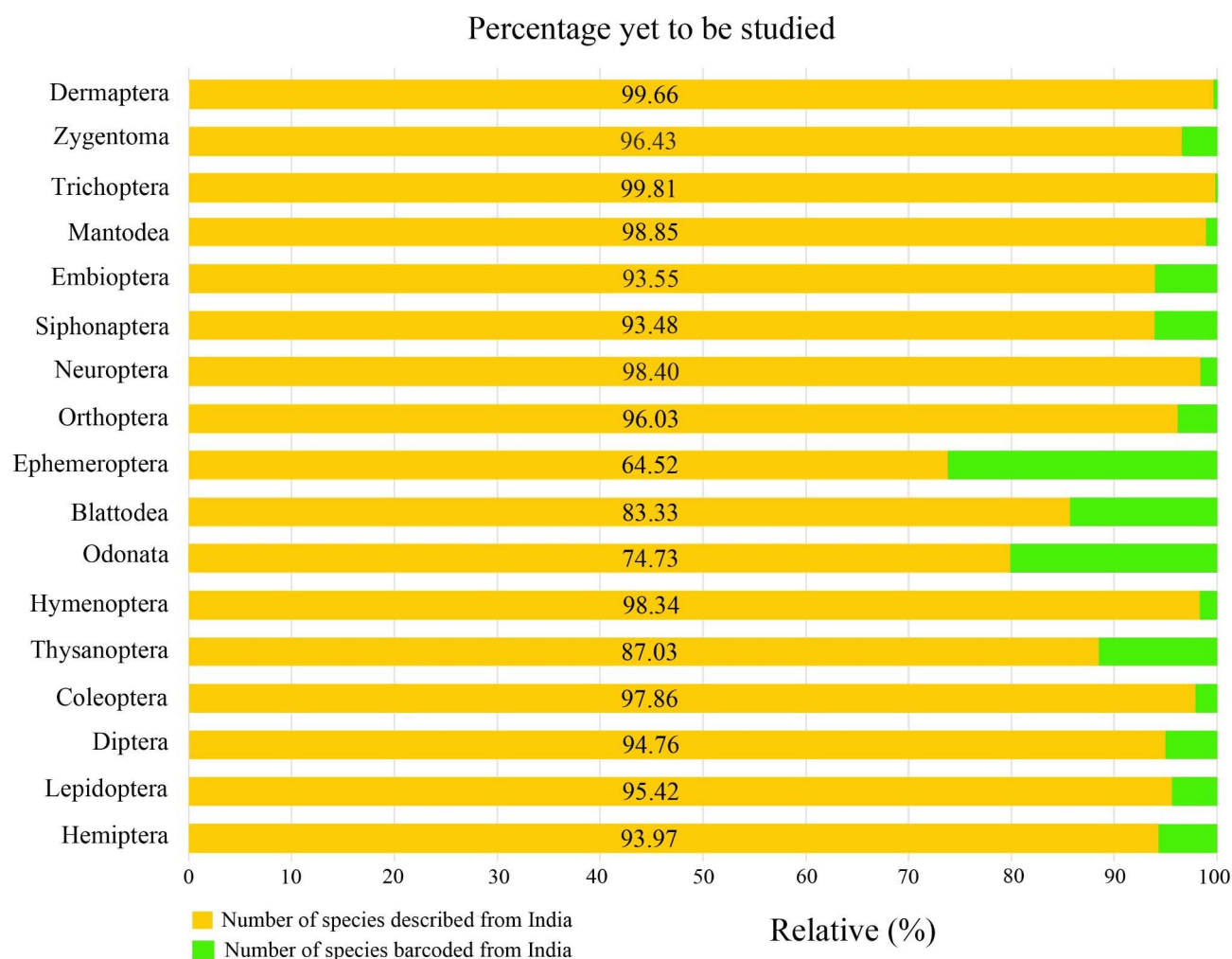


Fig. 4 Relative percentage of species yet to be barcoded from India

Dominance of conventional/traditional taxonomic practices

Roonwal [24] estimated Indian insect diversity to be approximately 100,000 species, and estimates of a recent global biodiversity assessment put the number at 10 to 15 times higher [25]. Studies on Indian insect diversity started from the Linnaeus period/pre-independence by foreign workers, although pioneers like Ramakrishna Ayyar, Narayanan, Pruthi, Mani and a few others also made such efforts through traditional taxonomy [25]. Alfred et al. [26], listed about 155 Indian traditional insect taxonomists who immensely contributed to the exploration of insect species across different orders. The Zoological Survey of India (ZSI) reported 341 insect faunal experts who have contributed to Indian insect diversity in its Indian Fauna Expert Data Base [27]. Even after two decades, DNA barcoding has not made a major inroad in India except in some economically important groups of insects [23]. Although the number of publications found in “Science Direct” using the key words ‘insect DNA barcodes India’ has shown an increasing trend during the last decade, the pace has been sluggish (Fig. 1a).

Lack of expertise and collaborations

Despite being a mega-diverse country, presently there are only a few institutes like ZSI, Forest Research Institute (FRI), Indian Agricultural Research Institute (IARI), National Bureau of Agricultural Insect Resources (NBAIR), and certain central/state Universities, organizations and non-governmental organizations are working in the field of insect taxonomy. Among these institutions, approximately less than 200 people are presently working on insect taxonomy and a very few are focused towards molecular/integrative taxonomy. Most of the experts are working in the orders Hemiptera, Lepidoptera, Diptera and Coleoptera, which constitutes 80% of sequences barcoded (Table 1). This representation clearly shows the taxonomic impediment in other groups.

Taxonomic labs in India are also constrained with regards to equipment and adequate facilities coupled with protocol hindrances. The DNA extraction procedure differs with the group of insects, which needs to be standardized or refined. Furthermore, around the world many DNA barcoding projects are successful because of collaboration among molecular labs and taxonomic experts across the globe. In India, there are a very few focused national and international projects which include such collaborations. This may be because of conservativeness of traditional taxonomists towards molecular taxonomy, lack of expertise in particular groups of insects with less economic importance, complex

biodiversity laws and rules etc., which are influencing the slow pace of barcode generation.

Lack of continuous funding

Large DNA barcode projects need long term financial support to generate well curated specimen-based reference libraries. Financial support/funding is one of the most important problems in the field of taxonomic research. Pires & Marinoni [49], quoted US\$ 5.00 for each sequence, which is not a large amount in developed countries, but in developing country like India it makes a vast difference without any Barcoding ‘campaigns’ like, Fish-BOL [17], or the Sponge Barcoding Project database (available at <http://www.spongebarcoding.org/>)[15].

Incorrect sequences and lack of meta data in public databases

One possible reason for major gap in present study may stem from most barcode data originated from GenBank. It is evident from our analyses that many Indian workers are submitting DNA barcodes directly to GenBank. In practice, all barcode sequences contained in either database should have been derived from a vouchered specimen, which was initially identified by a taxonomic expert. However, there are many sequences without a proper species identification. There are many stages in DNA barcoding which result in incorrect sequences, it may be due to taxonomic misidentification, poor DNA isolation method, endoparasites in insects, and PCR based errors [51].

GenBank is a much larger database and act as a sequence repository, performing basic sequence and taxonomic quality checks of the submitted sequences. However, BOLD is more of an integrated web service which also stores images, collection metadata and sequence chromatograms and allows users to identify likely misidentifications and contaminants and report these to the platform. The BIN system on BOLD also provides an automated means to validate submitted sequences to see if taxonomic assignment matches the COI OTU they are assigned. Although, BOLD periodically mines and curate data from GenBank, the errors which occur during data generation and submission can't be avoided without focused curatorial efforts from the scientific community.

Future prospects for DNA barcoding research in India

Need for collaborations

Although DNA barcoding alone may not be a sufficient tool for species description, it can complement the identification and description of insect specimens with morphology, natural history and other data. It will accelerate the pace of species discovery by allowing taxonomists to rapidly sort specimens and by highlighting divergent taxa that may represent new species [28]. Traditional taxonomists need to collaborate with molecular biologists to find new insights and to utilize this tool in a synergistic way. Collaborations between national institutions focusing on molecular aspects, joining forces with international platforms like the Centre for Biodiversity Genomics, will improve the DNA barcoding status of Indian insects.

Specimen-based DNA barcoding libraries for specific groups

Many countries have proved that highly focused group-specific barcode libraries will help to generate DNA barcode data in an accelerated way [29, 30]. Only a few prior studies have employed DNA sequences for species identification of specific insect groups from India, such as the subfamily Plusiinae [31]; spiders [32]; thrips [33]; and mosquitoes [34]. Indian scientists should focus on specimen-based group-specific DNA barcode libraries with national level campaigns.

Integration of new species descriptions with DNA barcodes

In recent times many Indian taxonomists are integrating DNA barcodes with isolated species descriptions [35–37]. These include reviews, revisions of small, specific groups of insects at the generic level [38, 39] and studies resolving cryptic species problems [6]. Taxonomists should add DNA barcode data to new species descriptions and taxonomic revisions. It will also provide insights into intraspecific variation, understanding the phylogenetic relationship amongst the species, besides enriching DNA barcode reference libraries.

Barcoding of museum collections

Museum specimens are valuable resources for generating information regarding the population dynamics, population genetics, geographic ranges (spatial and altitudinal), phenological and ecological changes, and evolutionary

changes (both genetic and morphological) of a given taxon [40, 41]. Recent studies worldwide show the usefulness of mini-barcodes (short fragment of barcode region) generated from museum specimens through sequencing technologies like pyrosequencing, Illumina sequencing etc. [42, 43]. The available technologies and networking among all the Indian museums together on a single platform will open the doors for the mini-barcoding era.

Utilization of next-generation sequencing (NGS) technologies

DNA barcoding is a standardised and widely used method. The standard DNA barcoding protocol relies majorly on Sanger sequencing, but next-generation sequencing (NGS) technologies can improve and supplement the standard DNA barcoding [44–47]. NGS can advantageously replace Sanger sequencing in certain DNA barcoding studies where a larger number of specimens needs to be analysed. It can be used to sequence different PCR products simultaneously, including co-amplified products [48]. By adopting these emerging scientific tools, one can generate more data for both fresh as well as museum insect specimens.

Conclusions

The ever-growing populations of biodiversity rich countries such as India are facing enormous challenges in balancing economic development, food security and protecting biodiversity. In a post-COVID-19 world, the recovery from this unprecedented impact on economies may inhibit the balancing act between economic development and conservation of biodiversity. Systematics, which is the backbone of biodiversity research, is already facing tremendous challenges. Traditional taxonomy played an important role in the identification of more than 1.4 million species of insects world-wide for the last two centuries. However, the pace at which we have achieved this is not sufficient to document the entire biota before it goes extinct. Hence, novel technologies have gained momentum in documenting biodiversity rapidly and economically, led by DNA barcoding. India being one of the mega-diverse countries looks forward to contributing in a sizeable way to achieve the United Nations Sustainable Development Goals (SDGs) and targets. However, the present review brings to light the alarming status of DNA barcoding in India, where only 3.97% of described species of insects were captured. We fear that in the era of genomics, the delay in developing DNA barcode reference libraries for insects will leave us behind in documenting the rich biodiversity of India.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s11033-022-07628-2>.

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Authors' contributions P.R.S. conceived the study, T.A.E. provided data and N.L.N. and S.S. sorted and analysed the data, P.R.S. and N.N.R. wrote the manuscript. K.S., T.A.E. and N.M.M. contributed critically to subsequent manuscript improvement.

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Data Availability The dataset has been released publicly; researchers can access the dataset at using DOI: <https://doi.org/10.5883/DS-ININD20>.

Declarations

Conflict of interest Authors declare no conflicts of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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