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DNA Barcoding of Two Economically Important edible insect species, Asian giant hornet, *Vespa magnifica* and a Lepidopteran, *Xyleutes* sp from Manipur for Solving species Ambiguity

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ABSTRACT: DNA barcoding is a standardized and widely used method to distinguish categories and proper identification of insect species. The two edible insects belonging to Hymenopteran and Lepidopteran order were collected as a delicious food item by different ethnic communities of Northeast India, especially in Manipur. Molecular identification was carried out by using COI gene. Phylogenetic analysis using BLAST sequences revealed that the species were *Vespa magnifica* and *Xyleutes* sp. COI gene sequences was submitted to NCBI and the Accession number were ON514039 (*Vespa magnifica*) and ON533749 (*Xyleutes* sp). The species conformity was carried out based on mitochondrial COI gene sequences, molecular evolutionary divergence and phylogenic status of insect species. The present study the great scope of DNA barcoding technique using COI gene sequences was used for identification and documenting of edible insects in the region (Manipur).

Keywords: DNA barcode, COI gene sequence, Edible insect, Phylogenetic analysis.

INTRODUCTION

Eating insect has been a part of human existence since time immemorial. Entomophagy is described as the practice of consumption of insects by human being. Over 2000 species of edible insects have been consumed by more than 300 ethnic groups from 113 countries (MacEvilly, 2000). Various countries like South Africa, Angola, China, Zaire, India, Thailand, Mexico, New Zealand, Korea, Japan, Australia and USA used insect as food (Shantibala *et al*., 2012). Moreover, the United Nations has recommended entomophagy as a potential solution to the shortage of world food supplies (Van Huis, 2013). They have played an important part in the history of human nutrition in large proportions of the population in many underdeveloped and developing countries such as Africa, Asia and Latin America (Bodenheimer, 1951).

In Manipur around 70 different edible insect species were consumed (Shantibala *et al*., 2021). Among these, Asian giant hornet and giant Lepidopteran insect larvae were economically important species for the various communities of the State. For Asian giant hornetall the stages, larvae, pupa and adult were consumed and for the giant Lepidopteran, only larval stages are eaten. These were consumed either by fried, roasted, or cooked along with other ingredients according to consumer's choice and even eaten as raw. However, correct identification of the species is a big challenge, therefore to solve the species ambiguity, DNA barcoding with a genetic marker of genomic DNA was done for proper identification of these two edible insect species. Correct identification of the species is prime essential for proper documentation and scientific validation for record as well as future reference. Therefore, the study was designed based on COI gene sequence analysis using DNA barcode techniques in documenting the two edible insect species found in Manipur to assemble these edible insects in NCBI Library of DNA barcodes. A tree-based phylogeny approach was studied for both the edible insects.

MATERIALS AND METHODS

Asian giant hornet and the larva of giant lepidopteran insects (Fig. 1) were collected from Senapati District (25.3203° N, 94.1514° E) of Manipur and preserved in 99.80% diluent of molecular grade.DNA was isolated from samples and the quality were evaluated on 1.0 % agarose gel, a single band of high-molecular weight DNA has been observed. Fragment of COI gene was amplified by LCO and HCO primers. A single discrete PCR amplicon band of 700 bp was observed when resolved on agarose gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with LCO and HCO primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of COI gene was generated from

forward and reverse sequence data using aligner software. The COI gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on maximum identity score first fourteen (14) sequences were selected and aligned using multiple alignment software program Clustal W. The phylogenetic tree was drawn based on the distance neighbor-joining method using MEGA software. FASTA format of consensus sequences of respective specimens were submitted in Gene Bank with the respective Voucher Specimen number (SSS.104 & SSS.101A) to get the Accession number from NCBI (https://www.ncbi.nlm.nih.gov/).

Fig. 1. Photographs of (a) Women selling Asian giant hornet, *Vespa magnifica* at local markets of Senapati District, Manipur(b) Adult of *V. magnifica* (c) Larvae of *V. magnifica* and(d) larva of giant Lepidopteran insect, *Xyleutes* sp.

RESULTS

The partial coding sequence of cytochrome coxidase subunit I (COI) gene of the Asian giant hornet and the larva of giant lepidopteran insects were amplified and consensus sequence of 680bp and 597bp respectively was generated using aligner software. COI gene sequences were submitted to NCBI and the Accession number were ON514039 (*Vespa magnifica*) and ON533749 (*Xyleutes* sp). Nucleotide sequences showed high similarity with the specimens *V.magnifica* and *Xyleutes* sp and the phylogenetic analysis also revealed they belong to the sameclan (Fig. 2). The entomophagy practices of these edible insects were recorded and documented through structured questioner with the local community.

Evolutionary divergence and phylogenetic analysis. The percentage of evolutionary divergence of the specimen at COI gene sequence level with its most closely related species accessible from NCBI GenBank database and corresponding phylogenetic tree were constructed with Neighbor-joining method using MEGA software (Table 1, 2 and Fig. 2). The evolutionary history was inferred by using the Maximum Likelihood method. The COI gene sequence was BLAST and based on maximum identity score first fourteen sequences along with consensus sequence of selected edible insects *V. magnifica* and Lepidopteran insect separately and aligned using multiple alignment software program Clustal W. The two samples showed 99.85% similarity with sequences of *V. magnifica* isolate (MZ191806.1) and 97.49% and 97.41% similarity with sequences of *Lepidoptera* sp. (GU662870.1) and *Xyleutes strix* (AB983482.1) respectively in GenBank.

DISCUSSION

DNA barcoding was used for the rapid automatic identification of species. In the identification of animal groups, the segment of the COI gene, ITS1 and ITS2 genes has been selected as the main molecular marker (Dai *et al*., 2012; Hebert *et al.*, 2003). Studies have shown that COI sequences have relatively low success rates for species identification in some taxon groups, such as some Dipteran insects (Meier *et al*., 2006), it is necessary to test the ability of COI gene to identify Vespa species (Wang *et al*., 2022). A checklist of the species in the subfamily Vespinae is presented by Carpenter and Kojima (1997). Okuyama, *et al.* (2017) supported species identification using a complete COI gene sequence in the tropical hornet of *Vespa affinis*. From India out of the 15,000 described species of Lepidoptera belonging to 84 families, 4.58% of the species under 38 families were feature from DNA barcodes. Therefore, DNA barcoding has proved to be an efficient tool for accelerating species discovery of small moths both in tropical (Lees *et al.,* 2014; Lopez-Vaamonde *et al*., 2019) and temperate areas (Mutanen *et al*., 2013; Huemer *et al*., 2020). DNA barcoding was shown as a powerful tool to discriminate species and reveal cases of cryptic diversity (Kim *et al*., 2020). Around the world many DNA barcoding projects are successful because of collaboration among molecular labs and taxonomic experts across the globe (Lopez-Vaamonde *et al*., 2021).

Table 1: Sequences producing significant alignments with closely related species accessible from NCBI GenBank.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Vespa magnifica isolate KYY-23	1251	1251	100%	0.0	99.85%	MZ191806.1
Vespa magnifica isolate KYY-22	1240	1240	100%	0.0	99.56%	MZ191805.1
Vespa magnifica isolate KYY-21	1240	1240	100%	0.0	99.56%	MZ191804.1
Vespa magnifica isolate KYY-16	1240	1240	100%	0.0	99.56%	MZ191799.1
Vespa magnifica	1240	1240	100%	0.0	99.56%	MT137097.2
Vespa magnifica isolate KYY-19	1232	1232	98%	0.0	99.85%	MZ191802.1
Vespa mandarinia voucher VEUN20	1197	1197	96%	0.0	99.54%	MN477949.1
Vespa magnifica isolate KYY-13	1194	1194	95%	0.0	99.85%	MZ191796.1
Vespa magnifica isolate KYY-17	1192	1192	95%	0.0	99.85%	MZ191800.1
Vespa mandarinia isolate KMP142	1186	1186	96%	0.0	99.24%	KT257114.1
Vespa magnifica isolate KYY-20	1173	1173	93%	0.0	99.84%	MZ191803.1
Vespa magnifica isolate KYY-24	1182	1182	94%	0.0	99.84%	MZ191807.1
Vespa magnifica isolate M - COX	1240	1240	100%	0.0	99.56%	OP253678.1
Vespa magnifica	1240	1240	100%	0.0	99.56%	NC 064062.1

Table 2: Sequences producing significant alignments with closely related species accessible from NCBI GenBank.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Lepidoptera sp. BOLD:AAF2341	1020	1020	100%	0.0	97.49%	GU662870.1
Xyleutes strix MZB.Lepi 144	987	987	96%	0.0	97.41%	AB983482.1
Autosticha modicella JCS-06-0175	843	843	100%	0.0	92.13%	KF523749.1
Autosticha sichuanica NKU-TZL0031	837	837	100%	0.0	91.96%	MZ234712.1
Phthonerodesscotarcha CCDB-15745-C06	826	826	100%	0.0	91.65%	KF398518.1
Phthonerodesscotarcha 11ANIC-08556	822	822	99%	0.0	91.62%	KF399173.1
Cosmopterigidae sp. BIOUG19653-H04	819	819	99%	0.0	91.44%	MH417307.1
Noctuidae gen. noctBioLep01 sp.	815	815	100%	0.0	91.30%	JQ573436.1
Lepidoptera sp. BOLD:AAD8186	815	815	100%	0.0	91.30%	HM409736.1
Lepidoptera sp. BOLD:AAA0891	815	815	99%	0.0	91.30%	HM402005.1
Punctulata palliptera isolate NKU – T2L0004	809	809	99%	0.0	91.39%	MZ234708.1
Paysandisia archon JBA – 06-0011	806	806	100%	0.0	91.11%	KF491991.1
Lichenaula sp. ANIC263	806	806	100%	0.0	91.09%	KF397267.1
Lepidoptera sp. BOLD:AAH8242	806	806	100%	0.0	91.11%	HM906916.1

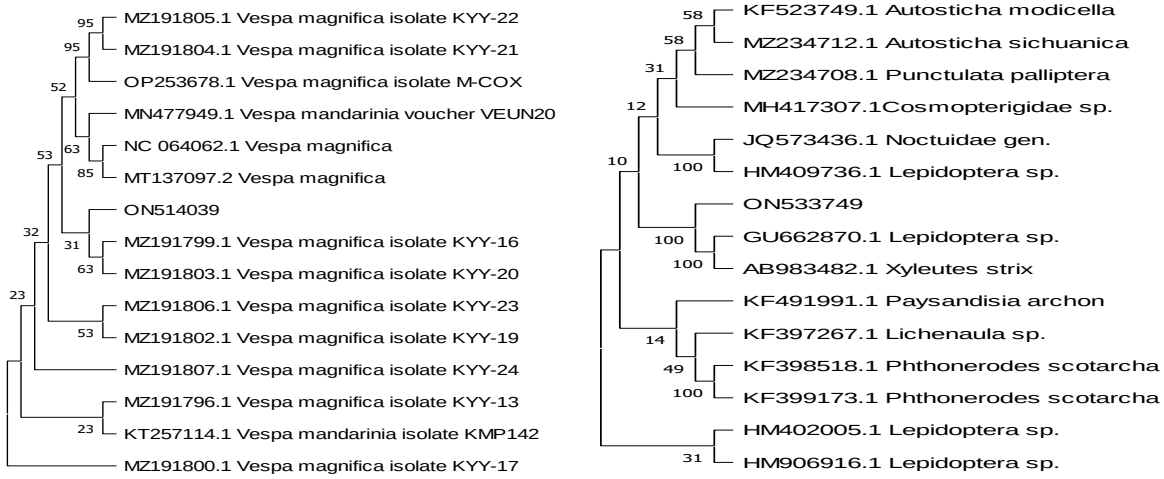

Fig. 2. Phylogenetic analysis of *Vespa magnifica* and *Xyleutes* sp Lepidopteran insect inferred by Neighbor - joining method.

CONCLUSIONS

The wild species of selected edible insects are eaten fondly by different ethnic people of the State; serving of this insect curry or in fried form along with other ingredients as per the consumers' choice. They are expensive selling at the price of Rs. 2000 – 5000 per hive or 7-8 insect larvae because of their demands in local markets as well as wild in nature. Insects are characterized by rich species diversity and large populations so as nutritive resources; they can be widely exploited and have great development potential. DNA barcode technique has a great scope for the identification and documentation of edible insects, animals, etc. The selected samples will be sent for complete mitochondrial genome which will be helpful for the studies of population genetic structure, conservation and genetic programmes and evolution of species and the published mitogenomes will be a promising marker to study and understand phylogenetic relationship. The comprehensive data generated from present study would be useful in further implication for

edible insects for development of diagnostic guide at molecular level.

In India, there are a very few focused national and international projects which include such collaborations. Financial support/funding is one of the most important problems in the field of taxonomic research. Collaborations between national institutions focusing on molecular aspects, and joining forces with international platforms like the Centre for Biodiversity Genomics, will improve the DNA barcoding status of Indian insects. Indian scientists should focus on specimen-based group-specific DNA barcode libraries with national-level campaigns.

FUTURE SCOPE

Establishment of mass breeding insectaries with modern artifact such as raising them in an artificial diet or through biotechnological intervention could provide hope for golden aspects for income generation. Scientific validation and updating of traditional wisdom in bioprospecting have assumed greater significance. There is a need for more and more analysis of insect biodiversity for the development of virgin resources and their industrialization, particularly in North East India.:

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Conflict of Interest. None.

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