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IDENTIFICATION AND DOCUMENTATION OF EDIBLE INSECTS OF *VESPA AFFINIS* IN NAGALAND BY USING DNA BARCODE TECHNIQUES

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ABSTRACT: DNA bar-coding is an ordered strategy that utilizes a little genetic marker in insect DNA to distinguish animal categories, as well as unidentified species. The present investigation manages the utilization of COI gene sequences in the distinguishing proof and documentation of edible insects in Nagaland. More than 2000 types of edible insects are recorded in more than 300 ethnic groups all around the world. It is evaluated that more than 200 types of species of edible insects and other invertebrates are consumed in North East India. In the preliminary study, *Vespa affinis* was selected and morphologically identified with the help of local tribals in Nagaland. *Vespa affinis* was identified by using phylogenetic tree analysis, and COI gene sequences were submitted in NCBI to received the accession number (MN820554), BOLD (ACH6486), and COI gene sequence were submitted in BOLD to generated DNA barcode for edible insects of *Vespa affinis*.

INTRODUCTION: *Vespa affnis* Linnaeus (English: Lesser Banded Hornet, Sinhales: Debara and Tamil: Kulavikalai) is a hymenopteran insect of the family Vespidae, native to Sri Lanka and the Asia Pacific region [1, 2]. The larvae and pupae of Vespa sp. (Hymenoptera: Vespidae) is consumed by various tribes and communities in North-East India [3, 4]. The nutritional value of this species has been reported as 50.13 g of protein, 13.29 g of carbohydrate, and 25.33 g of fats per 100 g species [5]. Senthil Kumar *et al.,* (2008) reported that this insect species have been used traditionally for the treatment of arthritis. So far, no mechanistic study has been carried out to investigate the beneficial property of this insect species against oxidative stress.

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An enormous number of ethnic individuals are additionally occupants right now in India, and they likewise have tremendous customary information on viable use of consumable insects and other edible invertebrates, which were obtained through experience and normally passed on by oral conventions as a monitored mystery of specific families [6]. It is feared that the methods of identification of edible insects and practices of entomophagy may be limited to a certain population, where it cannot be recorded locally and disappeared with that particular group of people. Hence, it needs to be modified to bioinformatics documentation.

Although, in traditional taxonomy, morphological characters are taken as key aspects for the identification of insect species. The classical identifications become a little ambiguous in differentiation between closely related species, but molecular-based taxonomy helps in deciphering the differences between concomitant species. Therefore, to help resolve the issues, we analyzed the complete mitochondrial gene sequence of the

edible insect Vespa *affinis*. DNA barcoding refers to the technique of sequencing a short fragment of the mitochondrial cytochrome c-oxidase subunit I (COI) gene. The "DNA barcode," from a taxonomically unknown specimen and performing comparisons with the reference library of barcodes of known species origin to establish a species-level identification [7]. In the present work, we examine the COI, mitochondrial gene for bar-coding approach to assemble edible insect of *Vespa affinis* in a global library of DNA barcodes at Bold systems. A tree-based approach was used to study phylogeny.

MATERIALS AND METHOD: The insect was collected from a local market in Dimapur, Nagaland and preserved in 100 % ethanol. DNA was extracted from body tissues using CTAB or Kit-based methods. The genes were amplified using PCR. Each PCR reaction for testing the amplification efficiency and development of multiplex PCR assays for DNA barcode primers contained 1 µl DNA template (25 ng), 2 µl 10 × reaction buffer, 0.5 µl $MgCl_2$ (50 pM), 1µl dNTPs mix (10 mM), 1µl forward primer (10 pM), 1 µl reverse primer (10 pM), 0.5 µl Taq polymerase (5 U/pi) and the final volume 25 µl will be adjusted with molecular grade water. Primers are standard available for COI gene amplification. COIF- GGTCAACAAATCATAAAGATATTGG - Tm 51 ºC COIR- TAAACTTCAGGGTGAC CAAAAA A TCA - Tm 53 ºC

Phylogenetic Tree Analysis: An analysis for similar sequences using BLASTN was performed, and sequence alignment was performed using the Clustal Omega program. A Phylogenetic tree was drawn based on the distance neighbor-joining method in Geneious v.9.0.2 8. FAST format of the COI gene sequence was submitted in Gene Bank to get accession number from NCBI (https:// www. ncbi.nlm.nih.gov/), and Barcode sequences of the sampled specimen are available online in the Database of BOLD. (http://www.boldsystems.org).

RESULT: The retrieved barcode sequences were also judged with previous sequences in Gene bank, and the specimen (Sample1-COI1F.ab) that showed 99 % similarity with other specimens in the dataset were considered that particular species available in the Gene bank data.COI gene sequence was submitted to NCBI Gene bank and get accession numbers (MN820554), BOLD number (ACH6486), and sample sequence submitted in BOLD to a generated barcode with DOI number: dx.doi.org/10.5883/BOLD:ACH6486. **Fig. 1, Fig. 2,** and **Fig. 3**). Multiple sequence alignment of **Table 1** BLAST analysis showed that the observed sequence of *Vespa affinis* 99 % homology with the sequence of Gene Bank accession number of MH036512.1. The pairwise alignment view of *Vespa affinis* nucleotide similarity is 99 % of 635/636 the no gap between the voucher and samples (0/636).

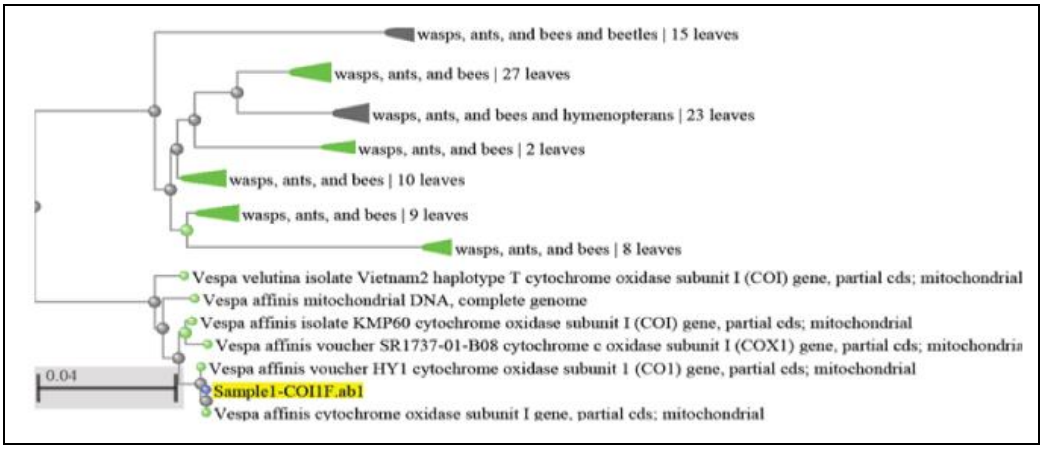
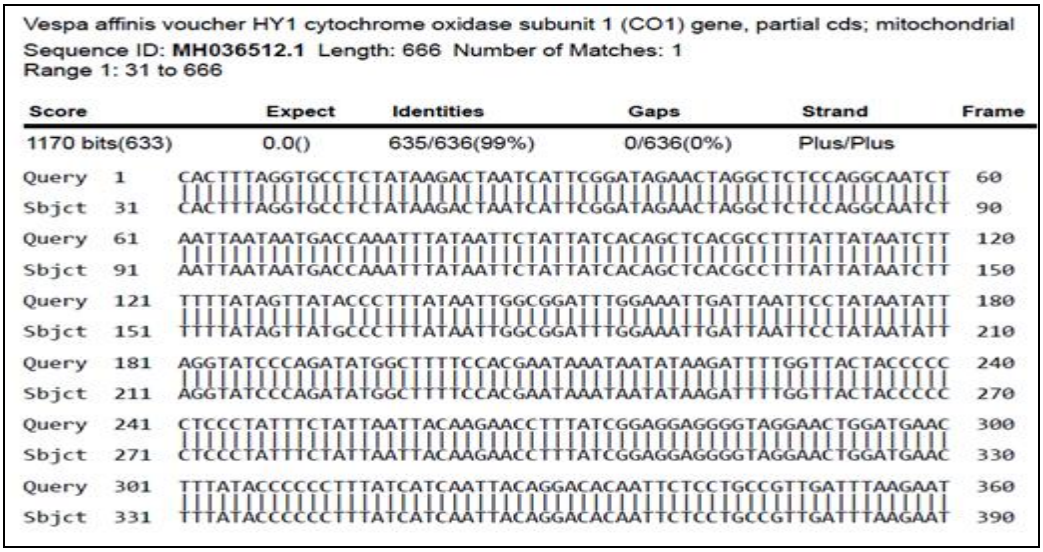


FIG. 1: PHYLOGENETIC ANALYSIS FOR EDIBLE INSECT OF VESPA AFFINIS

TABLE 1: BLAST ANALYSIS OF SEQUENCE FOR EDIBLE INSECT OF *VESPA AFFINIS*

S. no.	Specimen Name	Total Score	Query Cover	E value	Per ident	Accession
1	*Vespa affinis*	1178	99 %	0.0	99.84	MH036512.1
2	*Vespa affinis*	1133	100	0.0	98.5 %	KJ147242.1
3	*Vespa affinis*	1099	100%	0.0	97.66	NC039134.1


Vespa affinis voucher HY1 cytochrome oxidase subunit 1 (CO1) gene, partial cds; mitochondrial
Sequence ID: **MH036512.1** Length: 666 Number of Matches: 1
Range 1: 31 to 666

Score	Expect	Identities	Gaps	Strand	Frame
1170 bits(633)	0.0()	635/636(99%)	0/636(0%)	Plus/Plus	

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Query  1    CACTTTAGGTGCCTCTATAAGACTAATCATTCGGATAGAACTAGGCTCTCCAGGCAATCT  60
Sbjct  31   CACTTTAGGTGCCTCTATAAGACTAATCATTCGGATAGAACTAGGCTCTCCAGGCAATCT  90
Query  61   AATTAATAATGACCAAATTTATAATTCTATTATCACAGCTCACGCCTTTATTATAATCTT  120
Sbjct  91   AATTAATAATGACCAAATTTATAATTCTATTATCACAGCTCACGCCTTTATTATAATCTT  150
Query  121  TTTTATAGTTATACCCTTTATAATTGGCGGATTTGGAAATTGATTAATTCCTATAATATT  180
Sbjct  151  TTTTATAGTTATGCCCTTTATAATTGGCGGATTTGGAAATTGATTAATTCCTATAATATT  210
Query  181  AGGTATCCCAGATATGGCTTTTCCACGAATAAATAATATAAGATTTTGGTTACTACCCCC  240
Sbjct  211  AGGTATCCCAGATATGGCTTTTCCACGAATAAATAATATAAGATTTTGGTTACTACCCCC  270
Query  241  CTCCCTATTTCTATTAATTACAAGAACCTTTATCGGAGGAGGGGTAGGAACTGGATGAAC  300
Sbjct  271  CTCCCTATTTCTATTAATTACAAGAACCTTTATCGGAGGAGGGGTAGGAACTGGATGAAC  330
Query  301  TTTATACCCCCCTTTATCATCAATTACAGGACACAATTCTCCTGCCGTTGATTTAAGAAT  360
Sbjct  331  TTTATACCCCCCTTTATCATCAATTACAGGACACAATTCTCCTGCCGTTGATTTAAGAAT  390
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FIG. 2: PAIRWISE ALIGNMENT VIEW OF VESPA AFFINIS

FIG. 3: DNA BARCODE ANALYSIS OF *VESPA AFFINIS* IN BOLD

DISCUSSION: During the survey, *Vespa affinis* was collected from the forest and take out brood to use as food sources it was recorded by various researchers in Northeast of India [4]. Hence, the edible insects of V. affinis were identified by using DNA barcode methods that result well supported with Hisashi Okuyama *et al.,* (2017). Our result shows 99% similarity with voucher specimens in NCBI library. Our edible samples of *Vespa affinis* showed similarity with the voucher specimen of MH036512.19 and KJ147242.1 [10] and NC039134.1 Okuyama, *et al., (*2017) supported the result where they have done a complete COI gene sequence of tropical hornet of *Vespa affinis* [11]. In addition, Prachurjya Dutta *et al.,* who have first reported and demonstrated that there is a potential for anti-oxidant in edible insect, Vespa affinis in Northeast India [12].

CONCLUSION: DNA barcoding has a broad scope of application in the confirmation of animals or fishes or insect species, along with the support of traditional morph metrical analysis. The wide scope of DNA barcoding shows the high level of exactness which has made DNA standardized identification examination in scientific classification evolutionary. It helps in the studies of phylogeographic investigation, handled nourishments distinguishing proof and species complex recognizable proof. Thus, the DNA barcode techniques are also used for the identification and documentation of edible animals such as edible insects, snails and frogs for further uses.

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