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Journal of Advanced Scientific Research

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ISSN
0976-9595
Research Article

USE OF COI GENE FOR MOLECULAR IDENTIFICATION AND PHYLOGENETIC STUDY OF *ACETES INDICUS* FROM MUMBAI COAST, MAHARASHTRA, INDIA

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ABSTRACT

Crustaceans are one of the most studied metazoan group of marine invertebrates because of their immense morphological diversity. Members of family Sergestidae from Infraorder Penaeidea are small sized shrimps and considered under non-penaeid category. It is difficult to identify them by classical methods and need taxonomical expertise for morphological identification. The purpose of this study is to provide alternative for morphological identification using molecular approach. DNA Barcoding molecular technique supports the classical taxonomy by producing *Cytochrome c Oxidase Subunit I* (COI) gene barcode. DNA barcoding of *A. indicus* was carried out and its genetic population was studied. This technique was further used to study molecular phylogenetic evolution of family Sergestidae. The study shows that *Acetes indicus* found in Indian waters fishing area 51 and fishing area 71 belong to the same genetic population with respect to COI gene. *A. indicus* is closely related to *A. serrulatus* as compared to other family members of Sergestidae.

Keywords: *Acetesindicus*, Sergestidae, DNA barcoding, COI gene, phylogenetic analysis.

1. INTRODUCTION

Shrimps are economically important due to high export value. Penaeid shrimps, because of their large size are mostly exported whereas small shrimps or non-penaeid shrimps are utilized by locals. In Maharashtra, non-penaeid shrimps are found in abundance and have immense value in market as they are consumed fresh as well as in dried form [1, 2]. Non-penaeid shrimps promote economy as well as play significant role in marine food chain and help maintain ecological balance. Dol net fishery is carried out throughout Maharashtra and non-penaeid shrimps comprise significant catch of it. In Maharashtra, non-penaeid shrimps include *Nematopalaemon tenuipes, Exhippolysmata ensirorstris* and *Acetes* species which contributes 10.6% of total marine fish production and represent as one of the important fishery resources of the state [3].

Earlier studies on *Acetes* spp were done covering vast geographical area and identification keys were prepared using morphological characters [4]. *Acetes* was reported in high percentage in the zooplankton off Versova [5]. The larval abundance and biology of *Acetes indicus* in Bombay waters have been studied [6, 7]. The non-penaeid prawns and stock evaluation of *Acetes indicus* along the Maharashtra coast concluded that *Acetes indicus* contribute 74.4% of the *Acetes* landing and 51.2% of total non-penaeid prawn catch [8]. The genetic diversity, morphometric characterization and intras-pecific and interspecific variation of genera *Acetes* were reported from the west coast of Peninsular Malaysia [9, 10].

The species of *Acetes* are small shrimps about 1 to 4 cm in length. The body is translucent or semi-translucid, with black eyes and several pairs of red pigment spots on the basis and endopods of uropods, based on which the species are identified. Because of small size and fragile nature, it becomes difficult to identify them using classical taxonomy and hence the need for molecular technique is strongly felt. Recently, molecular methods are predicted to offer new and more accurate method for species identification and producing phylogenetic relation among species. Molecular biomarkers are robust tools to pick out the species' genetic building and the evolutionary records of populations and have been found beneficial for correlation among taxonomic ranks and molecular divergence [11]. Recently, use of

molecular marker like mitochondrial DNA in marine resources management has given acceptable results [12]. Mitochondrial markers like cytochrome oxidase subunit I gene (COI), Mt 16S ribosomal RNA (16S rRNA) gene and randomly amplified polymorphic DNA (RAPD) were used for molecular identification and phylogenetic examination of Penaeidae species [13-19].

The present study envisages first genetic record of *Acetes indicus* from India. Interspecific and intraspecific phylogenetic relationship between members of family Sergestidae was also studied with respect to mitochondrial COI gene. This research encourages use of molecular technique DNA barcoding in phylogenetic investigation; ecological and population genetic studies; and develops our understanding of evolutionary biology and other related disciplines.

2. MATERIAL AND METHODS

2.1. Sample collection and identification

Fresh samples of *Acetes indicus* were collected (2017-2018) from three major fishing centres of Mumbai, viz. New Ferry Wharf (Bhaucha Dhakka) located- 18° 57′ 22.97″ N, 72° 50′ 57.34″ E from southeast Mumbai; Sassoon Dock, located 18° 54′ 41.81″ N, 72° 49′ 34.11″ E, the terminal point of the Mumbai suburban; Versova, located - 19° 8' 6.3384" N, 72° 48' 48.3228" E from northwest part of Mumbai (fig. 1).

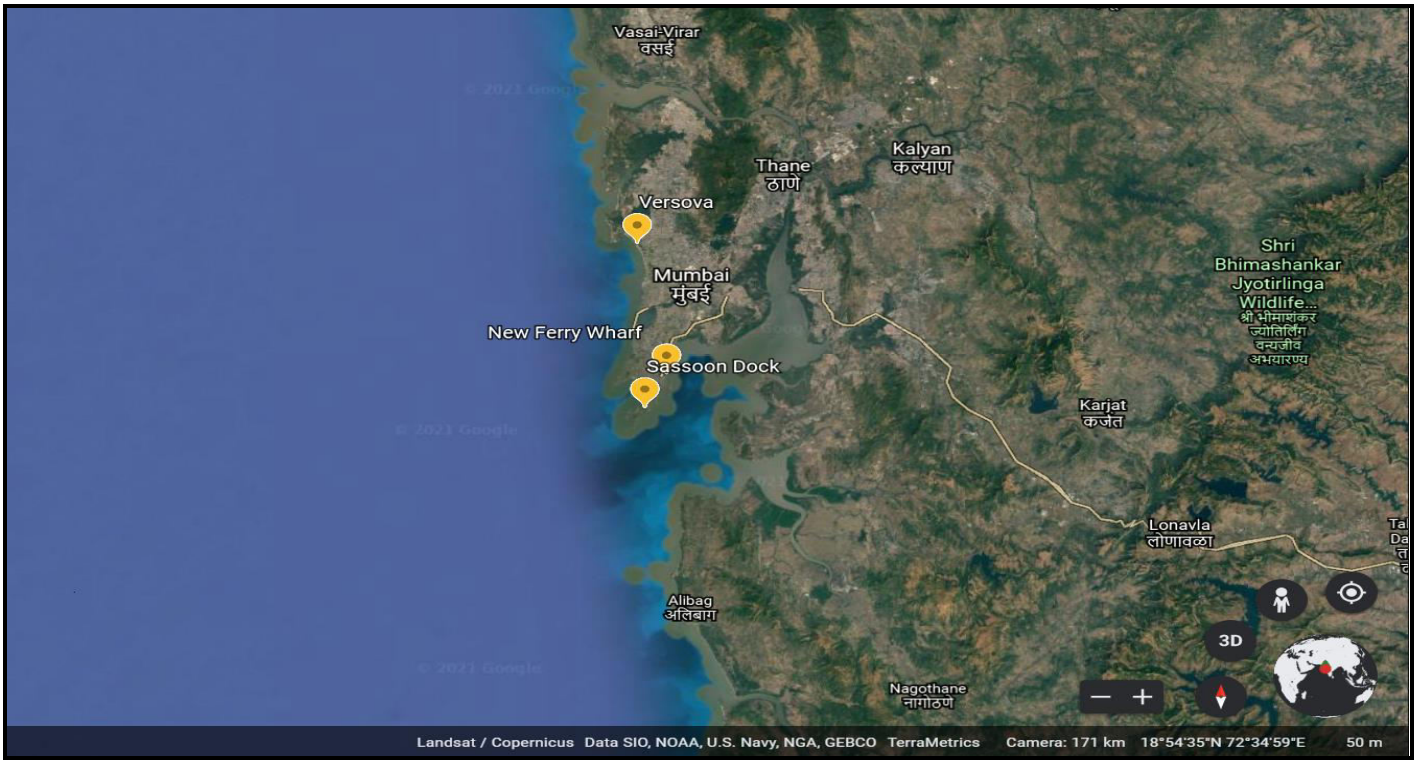


Fig. 1: Google earth screenshot represents three major collection sites New ferry wharf, Sassoon Dock and Versova of Mumbai region, west coast of Maharashtra

The Food and Agricultural Organisation (FAO) had divided world marine area into 19 major fishing zones and assigned as fishing areas with numbers. The Indian Ocean involves three fishing areas- 51, 57 and 58 called as Western Indian Ocean, Eastern Indian Ocean and Indian Ocean, Antarctic respectively [20]. Western coast of Maharashtra fishing ground fall into Fishing Area 51.

The specimens of *Acetes indicus* were morphologically identified using field identification key [21] and authenticated by CMFRI, Mumbai.

2.2. DNA Extraction, Amplification and Sequencing

Acetes samples were brought to the laboratory in ice box. Genomic DNA from the fresh muscle tissues were extracted using modified CTAB method [22, 23]. The purity of extracted DNA was confirmed by Agarose Gel Electrophoresis (AGE) technique. The mitochondrial COI gene was amplified by polymerase chain reaction (PCR) technique using forward primer LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and

reverse primer HCO2198 (5′-TAAACTTCAGGGTGA CCAAAAAATCA-3′) [24]. Polymerase Chain Reaction was carried out in the GeneAmp 9700 Applied Biosystem thermal cycler by mixing 2.5 µl of 10X buffer, 2 µl of 10 mMdNTP, 1µl of Taq Polymerase, 10 p. mol of each primer and 100 ng concentration of DNA, making the total volume 25 µL. Optimized cyclic parameters were 5 min at 96°C initial denaturation; 35 cycles of 30 sec at 95°C denaturation, 30 sec at 50°C annealing, 30 sec at 72°C extension and final extension for 10 min at 72°C. DNA Sequencing was performed by Sanger's Sequencing Method and outsourced from Eurofins, Bangalore, India.

2.3. Sequence Analysis

The raw sequence data of COI gene of *A. indicus* were analysed and modified by various bioinformatics tools and software's. Chromas Version 2.6.4 software [25] was used to trim the raw forward and reverse sequences. Alignment was done by Multiple Alignment online software [26] and merged by online tool Emboss Merger. The algorithm Basic Local Alignment Search Tool (BLAST) and BLASTx search were applied to compare nucleotide and protein sequences from GenBank. The partial mitochondrial COI gene sequences of *A. indicus* were deposited in International database NCBI BankIt/GenBank. These sequences were allotted with Accession numbers and were published in NCBI.

2.4. Genetic Population and Phylogenetic Analyses

A. indicus was the only species database available from Fishing Area 51. Therefore, genetic data of *A. indicus* from F.A.71 was retrieved from GenBank and it was compared with F.A.51 to study the intraspecific relationship. Multiple alignment of protein sequences of COI gene was carried out using Mul Alintool. The interspecific relationship in family Sergestidae was studied by retrieving genetic data from GenBank. MEGA version 7.0.26 software [27] was used to construct phylogenetic relationships among shrimp species from family Sergestidae.

3. RESULTS AND DISCUSSION

3.1. Generation of DNA barcode

Agarose gel (0.8%) containing Ethidium Bromide was prepared to run pure gDNA with 1kb DNA Ladder. The gel displays obtained pure gDNA without RNA contamination (fig. 2). Amplified COI gene by PCR technique was displayed on 1.5% agarose gel with 100bp Ladder and was visible at 700 bp (fig .3). DNA sequences were successfully submitted to NCBI and published in GenBank with Accession No. MK331952, MK770780 and MK784109. DNA barcodes of *A. indicus* COI gene from various sites were generated by DNA Barcode Generator online tool (table 1).

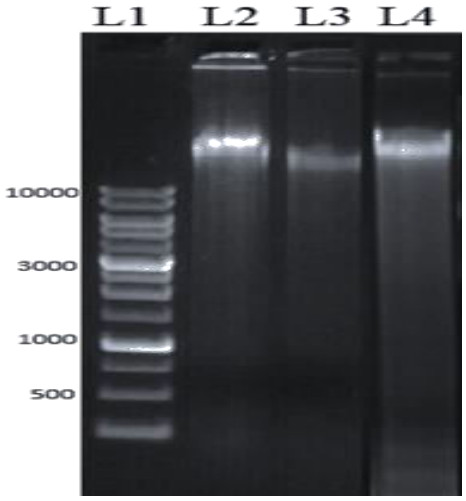


Fig. 2: Gel image represents puregenomic DNA.L1 signifies 1 kb DNA Ladder. L2 toL4 stand for species *Acetes indicus*from New Ferry Wharf, Sassoon Dock and Versova respectively

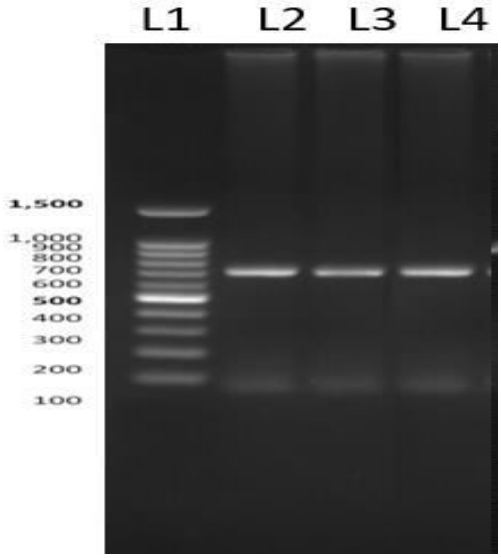
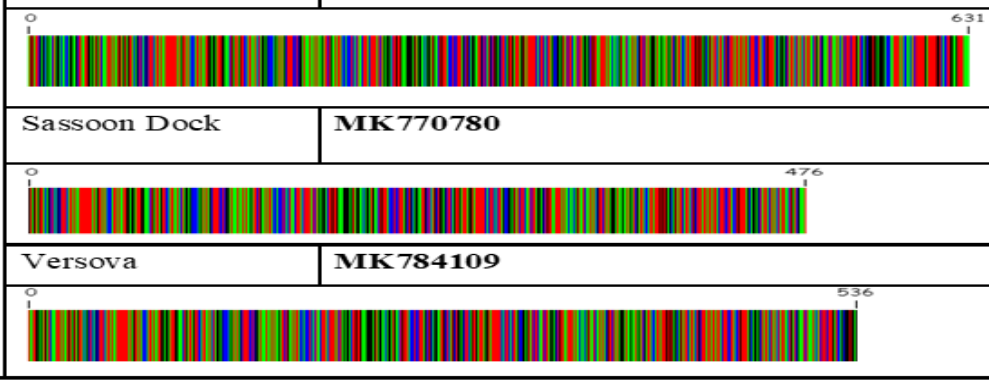


Fig. 3: Gel image represents amplified COI gene by PCR technique. L1 signifies 100bp DNA Ladder. L2 to L4 stand for species *Acetes indicus* from New Ferry Wharf, Sassoon Dock and Versova respectively

Table 1: The table represents barcode generated of *Acetes indicus* with respect to COI gene from New Ferry Wharf, Sassoon Dock and Versova respectively

Acetes indicus	New Ferry Wharf	**MK331952**
	Sassoon Dock	**MK770780**
	Versova	**MK784109**



3.2. Intraspecific and Interspecific Relationships

Intraspecific relation was studied between *A. indicus* collected from New Ferry Wharf, Sassoon Dock and Versovasites. Multiple alignment tool by comparing protein sequences derived from DNA sequences reveals the relation between geographically isolated species. The zoogeographical comparison was carried out between *A. indicus* found in F.A.51 and F.A.71 (Accession No. HQ630492). Protein sequences alignment study was carried out between three generated sequences from F.A.51 and from F.A.71 (fig. 4). The studied species were found to be from the same population and poor genetic diversity seen with respect to Mt COI gene in water bodies as there is not yet isolation of water mass on the globe.

The genetic population structure of species is influenced by many aspects such as evolutionary history of species, oceanographic or geographic patterns of the distribution area and life history of species. Due to passive dispersal during larval stages and absence of obvious physical barriers such as topological barriers, results in a pattern consistent with gene flow. Many of the marine crustacean species show no or comparative little genetic differentiation across a wide range of distribution [28-31]. To establish powerful and effective conservation and management policy for the species, it is required to have understanding of genetic structure of the population. The study of gene flow suggested by the genetic diversity determines the units of species conservation and resource management. Multiple population can be assumed as single unit when gene flow among species is not constrained whereas local population can be treated as an independent unit when gene flow is restricted [32-36].

Cladograms are tree like diagrams which represents genetic evolutionary history of organisms to study molecular phylogenetics. It expresses pedigrees of organisms supporting molecular data *i.e.,* DNA or protein sequences [37]. The interspecific relationship in family Sergestidae was studied and phylogenetic tree was constructed using MEGA version 7.0.26 software [38] (fig. 5) with the help of Pairwise Distance matrix of *A. indicus* and species sequences from family Sergestidae outside F.A.51 (table 2). Poisson correction method was used to construct pairwise distance matrix and cladogram was built using Neighbor-joining (NJ) method [39]. The tree was constructed by bootstrap method [40] with 500 replications. The constructed tree illustrates that *A. indicus* found in F. Area 51 and 71 belong to common genetic population. *A. serrulatus* and *A. chinensis* are the most recent common ancestors of *A. indicus*. *A. americanus* and *A. japonicus* are sister taxa which have common ancestor *A. chinensis* whereas *A. sibogae* is the out group. Poisson correction method was used to construct pairwise distribution by amino acid substitution per site between sequences. The three sequences of *A. indicus* shows close intraspecific relation with each other deliberated from different sites, hence can say they belong to same genetic population. *A. indicus* from fishing area 51 and Malaysia fishing area 71 shares similar protein sequences with divergence of 0.008, indicating that they belong to same population. There is no genetic geographical isolation found in *Acetes indicus* species. *Acetes serrulatus* from Malaysia and *Acetes chinensis* from China found to be the most recent ancestor with the divergence of 0.421-0.445. The optimal tree with the sum of branch length 1.730 is shown with 128 positions in the final dataset. The nucleotide frequencies are 28.17% (A), 36.45% (T/U),

18.24% (C), and 17.14% (G) with overall mean distance of 0.494. The transition/transversion rate ratios for purines (k1) is 165.367 and for pyrimidines (k2) is 1.526. The overall transition/transversion bias is R = 32.629, where R = [A*G*k1+T*C*k2]/ [(A+G)*(T+C)]. There were total of 477 positions in the final dataset and all positions containing gaps and missing data were eliminated.

Morphological character base phylogeny stated that *A. indicus* is closely related to *A. chinensis* followed by *A. japonicus* and *A. natalensis* [41]. The COI gene phylogenetic relationship of *A. indicus* make monophyletic group with *A. serrulatus* along the West Coast of Peninsular Malaysia [9]. The present molecular study also states that, monophyletic group *A. indicus* make paraphyly clade with *A. serrulatus* and *A. chinensis*.

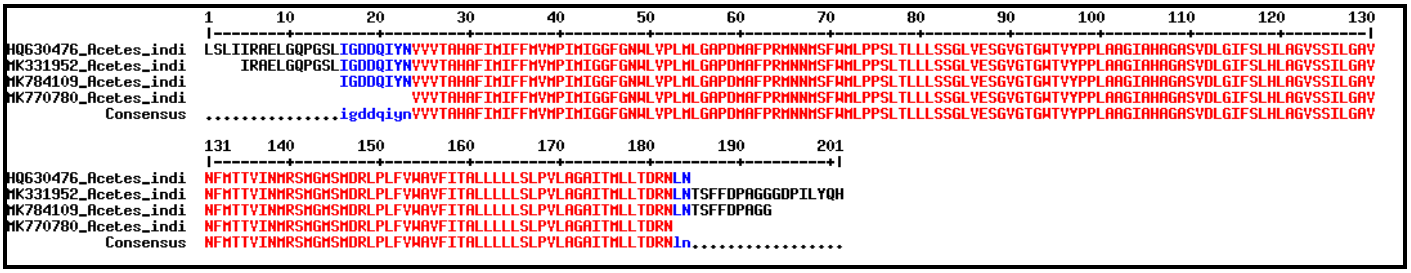


Fig. 4: The protein sequences alignment result of *Acetesindicus* from three different sites of Mumbai related with Malaysia fishing area 71.

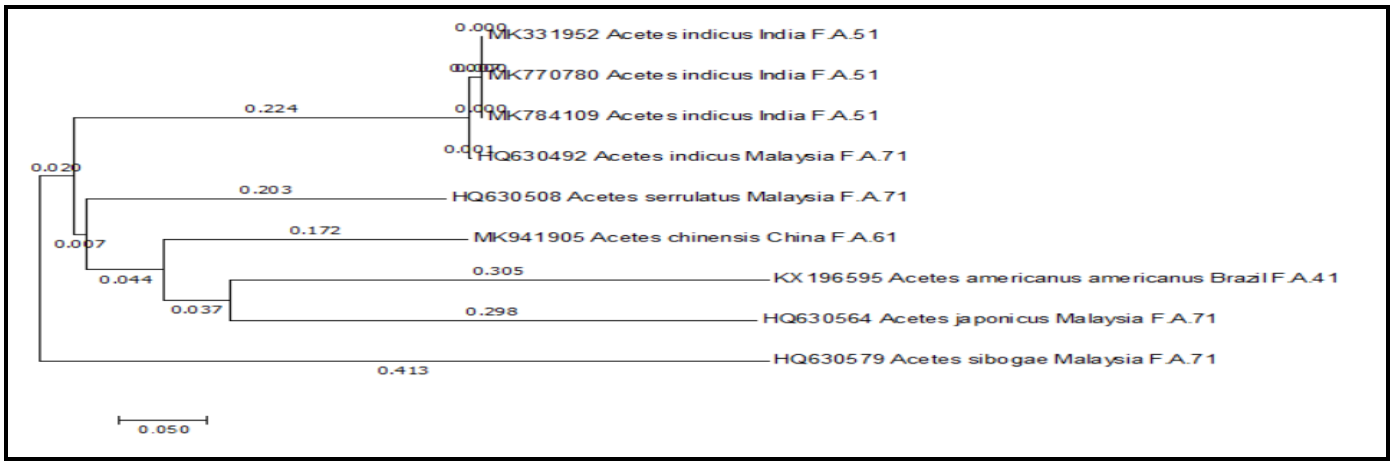


Fig. 5: Phylogenetic tree representing relationship between species from family Sergestidae with respect to COI protein gene sequence using NJ method.

Table 2: Pairwise Distance Matrix of species from family Sergestidae found Outside Fishing Area 51 associated to COI gene protein sequences

		1	2	3	4	5	6	7	8	9	10
1	MK331952 Acetes indicus India F.A.51										
2	MK770780 Acetes indicus India F.A.51	0.000									
3	MK784109 Acetes indicus India F.A.51	0.000	0.000								
4	HQ630492 Acetes indicus Malaysia F.A.71	0.008	0.008	0.008							
5	MK941905 Acetes chinensis China F.A.61	0.433	0.433	0.433	0.421						
6	HQ630508 Acetes serrulatus Malaysia F.A.71	0.445	0.445	0.445	0.433	0.421					
7	KX196595 Acetes americanus americanus Brazil F.A.41	0.633	0.633	0.633	0.618	0.575	0.604				
8	KF977240 Acetes japonicus China F.A.61	0.470	0.470	0.470	0.458	0.409	0.470	0.589			
9	HQ630564 Acetes japonicus Malaysia F.A.71	0.647	0.647	0.647	0.633	0.445	0.562	0.604	0.217		
10	HQ630579 Acetes sibogae Malaysia F.A.71	0.662	0.662	0.662	0.662	0.741	0.633	0.725	0.741	0.792	

4. CONCLUSION

Acetesindicus is an important constituent of non-penaeid fishery of Maharashtra. There are very less genetic studies carried out on these small fragile delicate but ecologically important non-penaeid shrimp. Samples were collected from three major landing centers of Mumbai. Pure genomic DNA was extracted from standardized CTAB method and COI gene was amplified by PCR technique. Sequences were published in NCBI with Accession number MK331952, MK770780 and MK784109 and barcodes were generated. The Accession number MK331952 is the first genetic record in GenBank, of *A. indicus* found in F.A.51. The intraspecific relation of *A. indicus* found in different areas form a common genetic population. Interspecific relation of *A. indicus* states that it is closely related to *A. serrulatus* with 0.443 distance followed by *A. chinensis* with 0.433 pair wise distance.

The taxonomy is considered on the basis of morphological characters but morphological data are insufficient to conclude phylogenetic relationship. This issue is overcome by molecular technique DNA barcoding and its application. Generating DNA barcodes and its submission contributes towards building the gene library and thus serving the scientific community. DNA barcoding helps in detecting food adulteration and hence beneficial for the society. Population studies are generally concerning morphological characters but now a days it is supported by genetical records.

5. ACKNOWLEDGMENT

This publication was supported by UGC MINOR PROJECT F 47-973/14 (General/621 WRO) XII PLAN.

Conflict of interest

The authors declare no conflict of interest.

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