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Labeling of Spider with DNA Barcode

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Abstract

The COI gene in mitochondria is used to identify unique sequence for DNA barcode. Spiders are found to be habitat specific and their morphological characters misguide researchers due to the molting stagesin juveniles. DNA barcodes are Molecular signatures which are used to identify spider species.Using this technique we were able toassign DNA barcodes tosixdifferent families of spider species in Beed district of Maharashtra state. Since these barcodes are specific to each species, they can help in identifying and differentiating closely related species and sub-species.

Keywords: Barcode, DNA, Label, Spider, Beed, India

Introduction

Morphological and anatomical features and environmental habitat characters are tools of classical taxonomy. DNA Barcode, a unique mitochondrial COI gene sequence, for taxonomic identification and study of divergences within and between species of plants, ferns, mosses, algae and fungi, protists and prokaryotes is an advanced and effective taxonomic tool [1-7]. This method is applicable to all organisms, unicellular or multicellular. Researchers have used it to identify various genus and species. Till today more than 45,884 spider species and subspecies belonging to 109 families have been recorded worldwide in the form of World Spider Catalog 2015 [3, 8-12]. Databanks like Gene Bank and BOLD, are reference providers of unique "barcodes". Spider species identification is difficult and complicated due to metamorphosis of juveniles and sexual dimorphism of species [10,13-14].

In India, Department of Biotechnology (DBT), National Centre for Cell Science (NCCS, Pune), Indian Council for Agricultural Research (ICAR) India, at national level; University of Delhi, and 'PHCDBBDS at BAMU (Aurangabad-MS) at the university level and few colleges are now recognized as leading centers for barcoding of species of a variety of microbes, fungi, plants, and animals. Sebastian and Peter, and Keshwani et. al. [15] have contributed in recording more than 2500 species by matching morphological and anatomical characters of spiders. Considering the significant application of DNA Barcoding as molecular taxonomic tool and the potential of COI as a rapid and accurate identification technique for spider diversity, the present work has been undertaken to record spiders by DNA barcode from Beed district of Maharashtra State.

Methodology

A survey was conducted in various seasons to establish sampling stations in Beed district of Maharashtra State. Spider samples were collected and species identification was done using databases or reference books.

The barcode data generated was statistically analyzed by employing Randomized Block Design (RBD), ANOVA software and students 't' test. For molecular biology experiments, some advanced softwares like Genesen, Genetool, Arliqueen, etc. were utilized. Nearly 302samples were processed for photography and DNA extraction of either entire spider or part of it, like tissue or leg clip (Promega Corporation, Madison, WI). DNA concentration of 100ng/µl and <1000ng/µl concentrations were directly processed for Cytochrome c Oxydase I PCR amplification. Primer such as LepF, LepR with LCO, HCO and LCO, HCO (both with M13 phage tail) as well as cocktail concentrations of LCO, HCO (both with M13 phage tail) with Lep F, Lep R and Lep F, Lep R individually were used (Table No. 1and 2). The PCR thermal regime included the following steps: 94°C for 3 minute; 5 replicates of 94°C for a minute, 45°C for 40 seconds, and 72°C for one minute; 35 cycles for a minute at 94°C, 40 seconds at 51°C, 72°C for a minute; and concluding with seven minutes at 72°C. Primers used for PCR amplification, cycle sequencing on Gen Amp PCR System (ABI Biosystems, Grand Island, NY) and for each specimen and data were availed through BOLD Systems and blast analogarithm of NCBI to obtain Taxon ID tree.

Results and Discussions

Spiders were collected from sampling stations by surveying Beed district region of Maharashtra State. Data was collected in each geographically and demographically important region. Out of 302 specimens, System showed 6 families, 11 genera and 03 species.

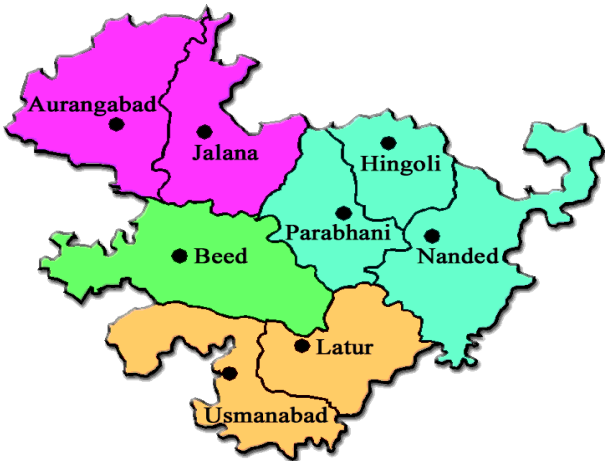


Figure 1: showing the Beed region of spider collection

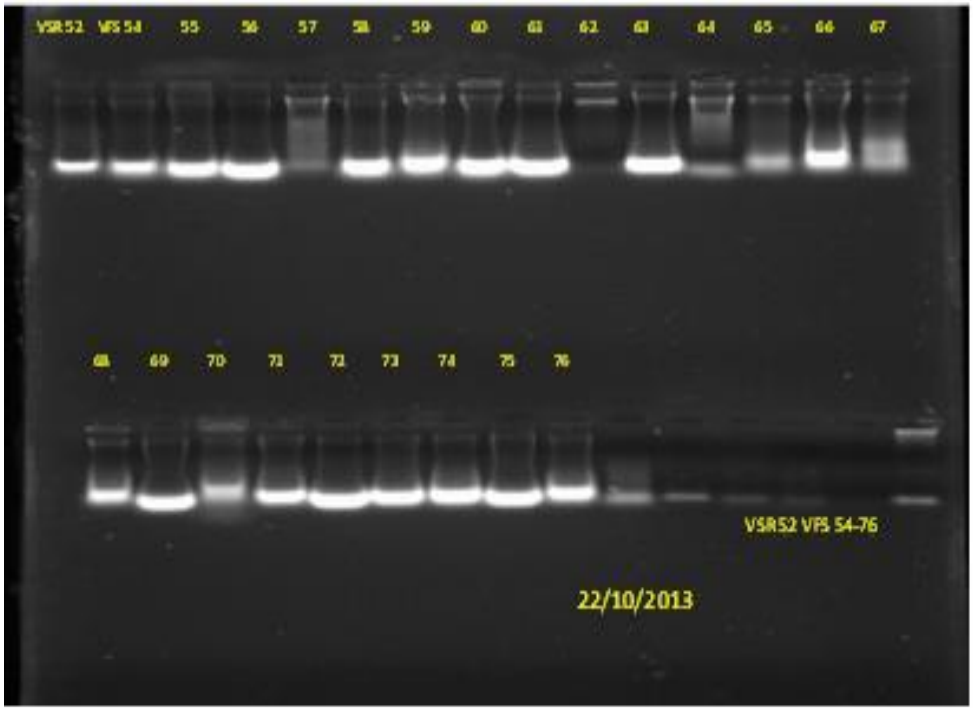
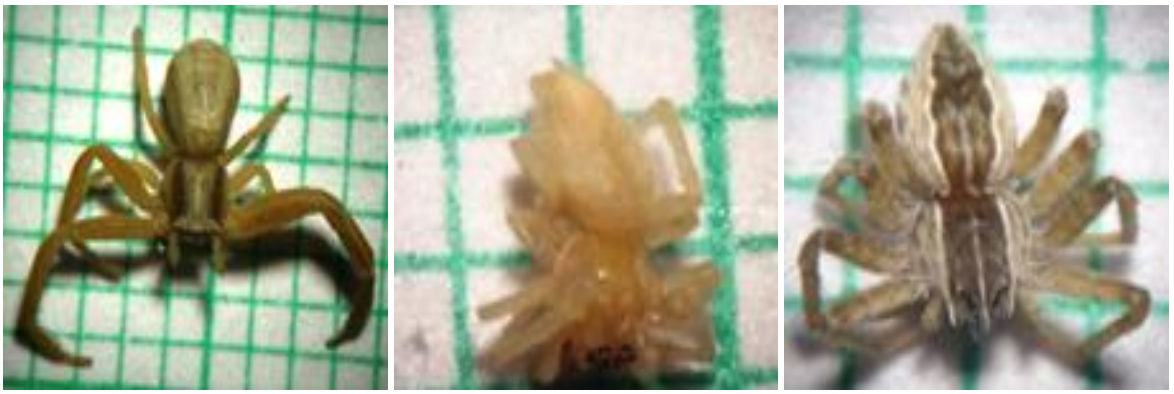


Figure 2: Showing PCR products of samples



Runciniasps .Cheiracanthiumsps. Lycosasps.

Theridionsps .Eustalasps.

Figure 3: showing some of the images of spiders of Beed district

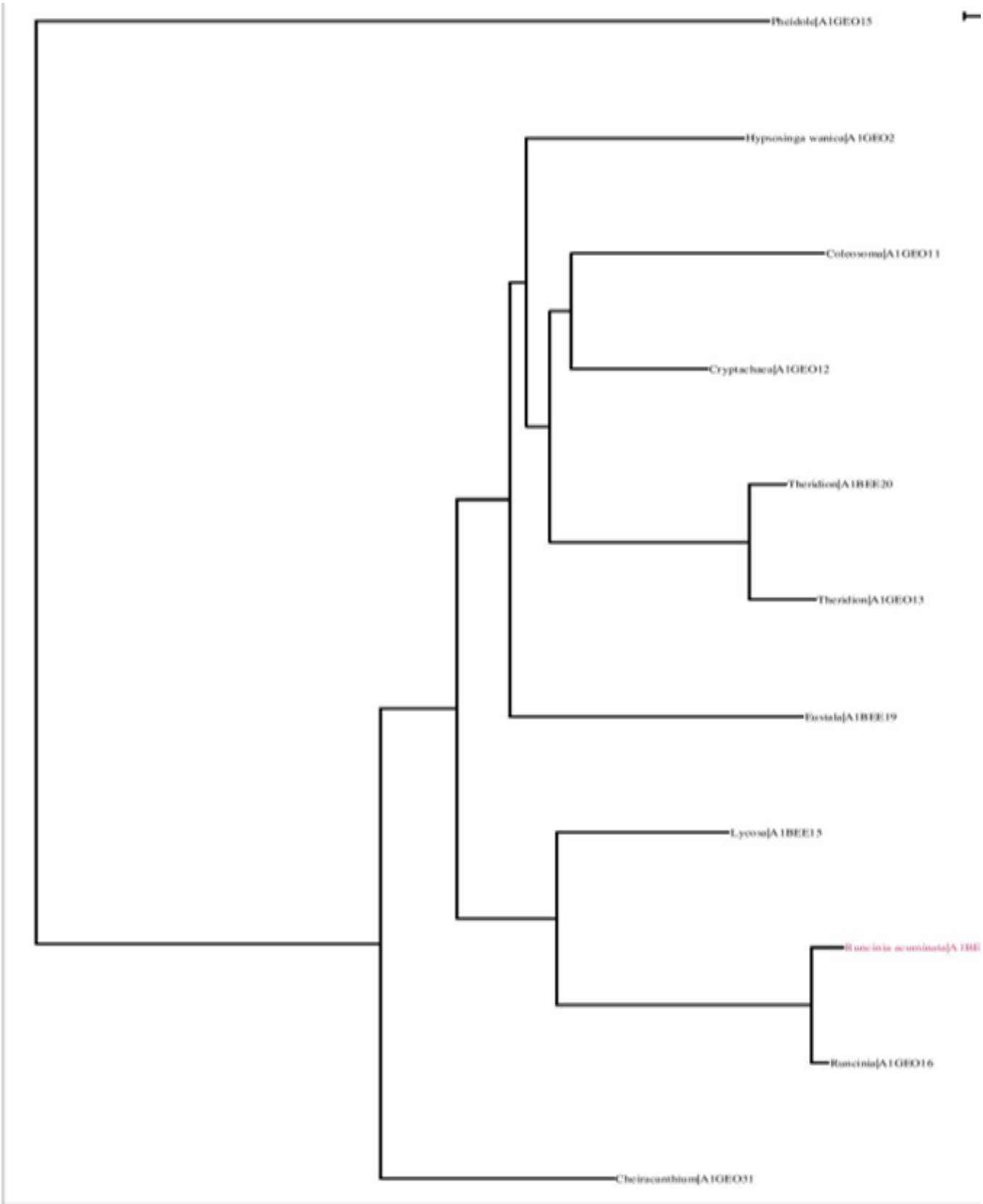


Figure 4: Showing Family tree data of the spider species of Beed district

Table 1: showing Sequence of primer used for PCR

S. No	Primer Code	Sequences
1	$LepF_1$	5′-ATTCAACCAATCATAAGATATTGG-3′
2	$LepF_2$	5′-TGATTTTTTGGACATCCAGAAGTTTA-3′
3	LCO_{1490}	5′GGTCAACAAATCATCATAAAGATATTGG-3′
4	COI-HCO_{2198}	5′TAAACTTCAGGGTGACCAAAAAATCA-3′
5	LCO1490_ti	5′TGTAAAACGACGGCCAGTGGTCAACAAATCATAAAGATATTGG 3′
6	HCO_{2198}_ti	5′CAGGAAACAGCTATGACTAAACTTCAGGGTGACCAAAAAATCA3′
7	DgLCO-1490	5′-GGTCAACAAATCATAAAGAYATYGG-3′

Table 2: showing region wise distribution of the species

Sr. No	Sample ID	BOLD Process ID	Family	Taxon name of specimen	No of species found
1	A1BEE9|448[0n]	| ISCS1362-14|	Thomisidae	*Runcinia acuminate*	01
2	A1BEE15|558[0n]	|ISCS1359-14	Lycosidae	*Lycosa*	01
3	A1BEE19|552[0n]	|ISCS1360-14|	Tetragnathidae	*Eustala*	02
4	A1BEE20|550(ON	ISCS1361-14|		*Theridion*|	03
5	A1GEO2|528[0n]	ISCS1366-14|	Araneidae	*Hypsosingawanica*	02
6	A1GEO11|482[0n]	| ISCS1363-14|	Theridiide	*Coleosoma*	02
7	A1GEO12|600[0n	| ISCS1368-14|]		*Cryptachaea*	02
8	A1GEO13|479[0n]	|ISCS1364-14|		*Theridion*	02
9	A1GEO15|578	ISCS1380-15		*Pheidole*	01
10	A1GEO16|449	ISCS1365-14		*Runcinia acuminate*	02
11	A1GEO31|573[0n]	|ISCS1367-14|	Eutichuridae	*Cheiracanthium*	01

Diversity and distributions:

COI sequences >420 bp were recovered from the specimens analyzed (302). Among these records, 14% were fully compliant with the "barcode standard" as they possessed a sequence >500 bp with less than 1% Ns, and involved a record that was based on bidirectional sequence analysis. Sequencing success improved during the study. The joint morphological and DNA barcode analysis revealed spiders representing 6 families: Lycosidae, Tetragnathidae, Arnidae, Theridiidae and Eutichuridae, and 11 genera.

Discussion

Approximately 42,055 of spider fauna including 3821 genera and 110 families have been reported globally [16]. 1520 spider species of 377 genera and 60 families have been reported in India [17]. According to Gazetteer of India, Maharashtra State, records show a total of 90 species of spiders belonging to 14 families [18-19]. Some researchers of Indian spiders are Majumdar and Tikader [20], Reddy and Patel [21], Sadana and Goel [22], Biswas *et al*. [23], Hippargi *et al.* [24] ; Patel and Vyas [25], Patel [26,27], Biswas and Biswas [28], Keswani *et al*. [29].

Reports on Spider from different regions of Maharashtra listed 32 families which represents 53.33 % of spider families. Among these 18 families have been newly reported by Hippargi *et al*. [24], Kashmeera and Sudhirkumar [30].

In the present study, we have recorded 11genera from Beed district belonging to 6 families. Considering the available data on spider species reported from Maharashtra it is suggested that more efforts must be

taken to produce barcoding data for identifying the spiders in the region. This indicates that the spider diversity from Maharashtra needs further long-term detailed studies using some additional methodologies. Among 60 DNA barcode species of spiders, 43 new species of spiders were identified by Warudkar [31]. Zoological Survey of India reported 664 barcode species of spiders from Araneae to the database reference library of spiders from India [32]. Till today, 21% - 28% species have been identified by DNA Barcode. NCBI and BOLD Data revealed that Indian spiders are very poorly represented in both the databases. More efforts should be made to build rich and comprehensive database for spiders from India [33]. The authors emphasized on the necessity to determine the ranges of intra- and inter-specific variation among different taxonomic groups and the need fora platform for a uniform and practical method of species identification.

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- Principal, Deogiri College, Aurangabad.

Conflicts of interest: The authors stated that no conflicts of interest.

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