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Phylogenetic analysis of cox i gene in identification of spiders

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Abstract
Morphological diversity refers to diversity of species at the genetic or molecular level. In order to study the diversity at the genetic level the taxonomic method DNA barcoding is used. The most commonly used barcode region for animals and some protists is found in mitochondrial DNA (Mt-DNA) *i.e.* a portion of the cytochrome oxidase I (Mt-Cox I) gene. The cox gene has a frequency of faster mutation rate and are highly conserved among the species hence Mt-Cox I sequence was used for the practical method of species identification. In the present study, the most dominant female spiders collected were *Argiope aemula, Nesticodes rufipes, Oxyopes lineatype, Leucauge decorata, Nephila kuchli, and Nephila philipis*. These spiders were preserved in 70% ethanol and DNA was extracted. The amplification of the gene and PCR analysis was done by treating forward and reverse primers. The Cox I gene was sequenced for BLAST sequence similarity search. The phylogenetic analysis revealed the relationship between molecular and morphological taxonomy. The six species with different families have raised from a common ancestor. At each branch point lies the most recent common ancestor of all the groups descended from that branch point. The four descendents *N. rufipes, N. kuchli, N. philipis and O. lineatype* raised from one common ancestor, but O. *lineatype* emerged as an out group species from the others. *Argiope aemula* and *Laucauge decorata* raised from the other common ancestor, indicating the homology sharing.

Keywords: Biodiversity, spider species Mt-Cox I, BLAST sequence, morphological taxonomy, phylogenetic analysis

Introduction
Spiders belonging to the order Araneae of class Arachnida are the most abundant and potential generalist predator of insect pests [6]. Most of them are terrestrial and few are aquatic also. The spiders are different from other insects in, presence of pedipalpi and the head is not differentiated in to different parts (seen in others). The legs of spiders have coxa, trochanter, a patella and a metatarsus. Spiders also have spinnerets and differences in their eyes. The spiders are the biological agents which capture and eats on many other insects like ants, bugs, mites etc. and thus helps in crop protection. As for as biodiversity of spiders are concerned there is a significant record of the wide variety of species in world, India and also in Karnataka. In the present life science world the word biodiversity is taking a various meanings. Basically it refers to varieties of life forms present on the earth. It is often defined as the totality of genes, species and ecosystem of a region. Biodiversity is not distributed evenly on earth and is richest in the tropics. Some of the traditional types of biological diversity methods used are taxonomic diversity, ecological diversity, morphological diversity and functional diversity. Morphological diversity refers to the diversity at the genetic or molecular level. In order to study the diversity at the genetic level the taxonomic method DNA barcoding is used. It uses a designated portion of a specific gene or genes to identify an organism to species [4]. The most commonly used barcode region for animals and some protists is found in mitochondrial DNA (Mt-DNA) i.e. a portion of the cytochrome oxidase I (Mt-COX I) gene. The DNA barcoding represents a promising approach to resolve the taxonomic impediment of difficulties in species identification [7]. The Mt-COXI gene sequence is more suitable for the DNA barcoding because of its faster mutation rate and the sequences are highly conserved among the species.
The present study aims at, recording the most dominant species from the study area. The female spiders were selected to study the morphological diversity, as they exhibit the sexual size dimorphism. The MT-Cox sequence from different families of spiders were used as the molecular source in order to draw the phylogenetic relationship among the selected species.

Materials and Methods

Specimen Collection

Spiders were collected from the surroundings of Turahalli forest 8km off from the Banashankari temple of South Bangalore. The total area of the forest is 590 acres, with 888 mt elevation. The coordinates are 12, 8816831^0 N, 77.5249823^0 E. The forest is well protected by the Karnataka forest department. The flora and fauna of the area include figs (*Ficus tinctoria*) neralemara (*Syzygium cumini*). The most common herb is the *Byttneria herbacea*. Animals spotted were Hares Jackals, Lizards, Mongoose, etc. The forests bird population includes great horned Owls, Mynas, Bablers, and more. The variety of spider diversity was found in this region hence the area was selected. The collection was done by a visual encounter method and hand collection method. Female spiders were collected, and preserved in 70% ethanol for further usage.

DNA extraction and PCR analysis

100mg of spider tissue was weighed and frozen in dry ice and the thugh was added with 200 µl of cTAB homogenize 0.5ml cTAB was vortexed vigorously and incubated at 60°C for 1h.To the lysate, 0.5 ml of phenol - chloroform, Iso-amyl alcohol was added and mixed for 2-3 minutes. It was centrifuged at 10000 rpm for 15 min at 4ºC. The upper aqueous layer was taken in a new tube, to which double the volume of cold 100% ethanol was added and 3M sodium acetate was added and was incubated for1h minutes at 4^0C, Centrifuged at 10000 rpm for 15 min. The supernatant, was removed and DNA pellet was washed in 70% ethanol and centrifuged at 5000 rpm for 10 minutes. Again the supernatant was removed, the DNA pellet was air dried and was finally dissolved in TE buffer.

The PCR mixture (final volume of 20 µL) contained 2 µL of DNA, 10 µL of Red Taq Master Mix 2x (Amplicon) and 1µM of each complementary primer specific forward and reverse. The samples were denatured at 94ºC for 5 minutes, and amplified using 40 cycles of 94ºC for 30 seconds, 44ºC for 30 seconds, and 72ºC for 1 minute for followed by a final elongation at 72ºC for 10 minutes. The optimal numbers of cycles have been selected for amplification of the gene.

Table 1: Primers used for Cox gene

Gene	Primer pair	Sequence details	Tm	Product size (bp)
Cox	Lco1490FP	GGTCAACAAATCATAAAGATATTGG	51.3	710
	hco2198RP	TAAACTTCAGGGTGACCAAAAAATCA	53.2	

Purification of PCR products

Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore).

Sequencing

The PCR product was sequenced using the LCO F primers. Sequencing reactions were performed using a ABI PRISM® Big Dye TM Terminator Cycle Sequencing Kits with Ampli Taq® DNA polymerase (FS enzyme) (Applied Biosystems). The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were re suspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Bio systems).

Phylogenetic analysis:

Based on BLAST analysis the Mt-Cox sequence, of these species has been matched, compared and phylogenetic tree was constructed.

Results and Discussion

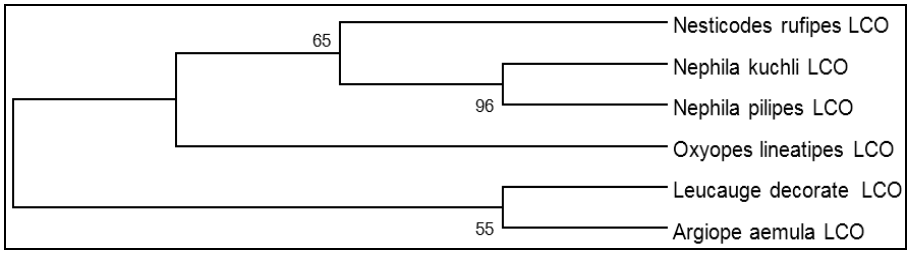

Fig 1: The Maximum Likelihood method of evolutionary relationship among spiders

In the present study the most dominant female spiders collected from the study area were, *Argiope aemula, Nesticodes rufipes, Oxyopes lineatypes, Laucauge decorata, Nephila kuchli, and Nephila philipis*. The Mt- COX I gene was sequenced and was used for the practical method of species identification. The statistical inference, maximum likelihood method revealed the relationship between molecular and morphological taxonomy. This method also involved in finding the evolutionary relationship which yields the highest probability of evolving the observed data [3].

The phylogenetic analysis, was done based on maximum likelihood method. The maximum likelihood method revealed that, the six species with different families have raised from a common ancestor. The branch point or internal nodes represent the most recent common ancestor. At each branch point lies the most recent common ancestor of all the groups descended from that branch point. The two species *Laucauge decorata* and *Argiope aemula* raised from one common branch point. *Laucauge decorata* belongs to tetragnathidae family is commonly called as long jawed orb weaver or decorative silver orb spider. The body shape and leg shape has silver black and yellow marking. Studies have revealed that evolution of web building has been from irregular to more regular webs [1]. The web is slanted rather than vertical. The *Laucauge* spider rests in the middle of the web with its underside facing upwards.

Argiope aemula belongs to Aranidae family exhibits sexual size dimorphism, where females are greatly larger than males. It shows female gigantism or male dwarfism. Sexual Size Dimorphism, (SSD) and morphometric analysis of *Argiope anasuja* has revealed that the females are four times larger than males in their total body length [5]. The web pattern of

these species are in Zig-Zag form resembling the letter, hence they are commonly called as signature spiders. The other four descendants raised from one common ancestor, but *O. lineatype* belongs to Oxyopidae family is commonly called as jumping spider, golden lynx spiders, emerged as an out group species from the others. The *O. lineatype* was different from the other species, in not constructing the web and are called as ambush predators. The setae are present on the legs to trap the predators. This could be the reason for *O. lineatype* to arise as an out group.

The branch point represented the most recent common ancestor for orb weavers *Nephila kuchli, Nephila philips, and Nesticodes rufipes. N. rufipes* emerged out as an out group which belongs to family Theridiidae and is commonly called as red house spider. It has dark brown globular abdomen, the front half of a spider and legs are red brown in color. *Nesticodes* builds a small tangled web in dark corners of home or under the rims of garden pots.

Extreme sexual size dimorphism species of orb-weaving spiders with large females and small males is seen in *Nephila* [2]. *Nephila kuchli* and *Nephila Philips* belongs to family Nephilidae also has the characteristic feature of exhibiting sexual size dimorphism. Morphologically the legs of *Nephila* species has alternating dark and light banding pattern. The web pattern is highly complex, builds non sticky, fine meshed barrier webs.

Conclusion

The current study demonstrated that phylogeny draws the relationship among individuals or groups of organisms. The biomolecules DNA, RNA and proteins will serve as the sources for the construction of phylogenetic tree. The mitochondrial gene cytochrome oxidase enzyme has a frequency of faster mutation rate and are highly conserved among the species hence can serve as a molecular source for constructing the molecular phylogeny. The bioinformatics software, BLAST programme serves as the search engine for finding the homology between the DNA or proteins from the different organism. Maximum likelihood method marks as an extensive use for the analysis of construction of phylogenetic tree among distantly related sequences.

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