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RESEARCH ARTICLE

PRELIMINARY STUDY ON IDENTIFICATION OF SPIDERS USING MITOCHONDRIAL DNA

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ABSTRACT

Taxonomy provides us with basic understanding about the components of biodiversity which is necessary for effective decision making about conservation and sustainable use. Taxonomy is a vital component of biodiversity management, as the first step towards protecting and benefiting from biodiversity is sampling, identifying and studying biological specimens. DNA barcoding offers taxonomists the opportunity to greatly expand and eventually complete a global inventory of life's diversity. The utility of DNA barcoding in identifying spider species was revealed in this study. The study established that the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core of a global bioidentification system for animals. The study also demonstrated that COI identification system will provide a reliable, cost-effective and accessible solution to the current problem of species identification. A comparative study of CO1 sequence of nine families of spiders and their phylogenetic analysis was performed and it showed their relationship with evolution of web building behaviour. Thus this study revealed the relationship between molecular and morphological taxonomy.

INTRODUCTION

Biodiversity is the variety of all life forms - the different plants, animals, fungi and micro-organisms, the genes they contain and the ecosystems of which they form a part. Biodiversity is essential to sustaining the living networks and systems that provide us all with health, food, wealth, fuel and the vital services of our lives depend on. Sampling, identifying, and studying biological specimens are among the first steps toward protecting and benefiting from biodiversity. DNA 'barcoding' offers rapid and low cost ways to monitor and manage biodiversity.

Spiders form one of the largest groups of invertebrate animals, nearing 40,000 known species (Platnick, 2013). They provide important model systems for studies of sociality, mating systems, and sexual dimorphism. The complete spider fauna is known for only a very few, small areas of globe, thanks to the dedicated efforts over many years of enthusiastic arachnologists. However, our knowledge of the diversity of spiders is imperfect, deficient or negligible. More information would become available if arachnologists were able to easily identify the spiders they encounter. DNA-based identification systems like DNA barcoding represent a promising approach to resolve the taxonomic impediment of difficulties in species identification. The technique identifies known species and records new ones by sequencing a specific, short area of mitochondrial DNA called cytochrome c oxidase subunit I (COI). It is supposed to provide a uniform, practical method of species identification. Comparative study of CO1 sequence of different families of spiders and their phylogenetic analysis will reveal the relationship between molecular and morphological taxonomy.

MATERIALS AND METHODS

Specimen collection: Spiderswere collected from premises of Christ College campus. Collection was conducted by hand picking method. Female spiders alone were collected and placed directly in absolute alcohol for preservation. Cytochrome *c* oxidase 1 (CO1) sequences from nine species (*Argiope anasuja*, *Clubiona drassodes*, *Oxyopes birmanicus*, *Pardosa birmanica*, *Stegodyphus sarasinorum*, *Tetragnatha mandibulata*, *Chilobrachys hardwicki*, *Theridion manjithar* and *Thiania bhamoensis*) of spiders were examined in this study. The CO1 sequences were obtained through the sequence analysis of specimens. The mitochondrial gene regions encoding cytochrome oxidase subunit 1 (COI) was sequenced from samples of ninespecies. COI was selected as they have shown to be successful for phylogenetic reconstruction of spiders at species level (Vink *et al*., 2002).

DNA extraction and analysis: The first step in the phylogenetic analysis was extracting DNA from the spiders,

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which can be used in subsequent PCR analysis. DNA was extracted from the leg of the preserved spiders. Muscle tissue in arthropod legs is good source of mitochondrial DNA (mtDNA) (Prendini, 2005). Extraction of genomic DNA from the samples provided was accomplished by standard protocol of chloroform: isoamyl alcohol method.

Next step was the amplification of the CO1 gene region of the mt DNA. This was done using the PCR machine. In this step primers are used which selectively amplifies the CO1 gene. So the amplified products contain only the CO1 gene. Forward primer used was C1-J-1751 and reverse primer was C1-N-2191 (Bond, 2004).

It takes three more steps to obtain the sequences from the purified PCR product: a sequencing PCR step, a precipitation and adding formamide step, and finally the actual sequencing step with an automated sequencer.

Phylogenetic analysis was conducted using CO1 sequences of nine species of spiders. Phylogenetic trees provide an indirect record of the speciation events that have led to the present-day species. They show how species or populations are related by common ancestry. Together with information on ecological and morphological features of the species, we can gain information about the causes, processes and patterns of speciation. In order to carry out a phylogenetic analysis of spiders, DNA sequences were obtained. This invoked three major steps: DNA extraction, amplification of the genes and sequencing. After obtaining the CO1 gene sequences, phylogenetic analyses were performed to reveal the evolutionary relationship of the species. Sequences were aligned in BioEdit, using ClustalW Multiple Alignment (Tamura *et al*., 2007).

Neighbour-joining analysis, implemented in MEGA version 4 (Tamura *et al*., 2007) was employed to examine relationships among taxa. The neighbour joining method has been shown to be computationally efficient and has a record of recovering trees that are at least as good as those generated by alternate methods (Nei& Kumar, 2000). To root the trees, an out group was added to the data. In this study, *Chilobrachys hardwicki*, a mygalomorph spider, which too distantly related to the other taxa was used as out group.

RESULT

NJ treehas drawn using *Chilobrachys hardwicki* as out group (Figure1). A detailed examination of the NJ tree revealed evidence for the clustering of taxonomically allied species. Two distinct clades were obtained; one clade of non-web weavers and other with web weavers. *T. bhamoensis, O. birmanicus, P. birmanica, C. drassodes* and *S. sarasinorum* were found in one clade. Among these, *T. bhamoensis, O. birmanicus, P. birmanica* and *C. drassodes* were non weavers. *S. sarasinorum* makes sheet web. *T. bhamoensis* and *O. birmanicus* are monophyletic.*T. manjithar, T. mandibulata* and *A. anasuja* were found in other clade. Among these *T. manjithar* makes cob web. *T. mandibulata* and *A. anasuja* are orb web weavers. They are found to be monophyletic.

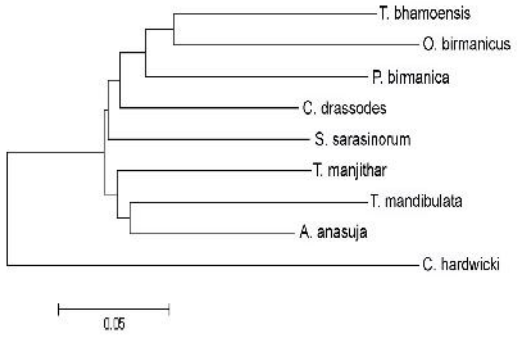

Figure 1. Neighbour Joining Tree showing evolutionary relationship among spiders

Studies of Coddington (1991) revealed that evolution of web building has been from irregular to more regular webs. During the course of evolution amount of silk used in web reduced as an attempt to economize amount of protein used. Tree also suggests evolutionary loss of web weaving behaviour. *S. sarasinorum* of family Eresidae is the only cribellate spider among these spiders studied. The loss of the cribellum, a structure that produces fibers contributing stickiness to prey snares and which is invariably associated with a set of accessory structures, has been studied in orb web-weavers and shown to have been lost once during the evolutionary history of the group, but never regained. Cribellum is also absent in non weavers**.**

Stegodyphus sarasinorum is a social spider. Social spiders live in colonies with high levels of inbreeding and high relatedness among colony members (Johannesen *et al*., 2007). This mating system, combined with high turnover rates of colonies (Croucher *et al*., 2004) and both dispersal and colony founding by mated females, prevents gene flow and provides conditions that may purge genetic variation and lower individual fitness quickly. The inbred nature of social spiders led to speculate that social species are unstable in evolutionary time and constitute evolutionary dead ends, which could explain the rarity of social spiders and presence of cribellum in them. Two features might limit the ability to diversify and speciate, namely extreme inbreeding and obligate group living, both of which narrow the species' ecological niche.

DISCUSSION

DNA barcoding: DNA barcodingis a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome. DNA barcode sequencesare very short relative to the entire genome and they can be obtained reasonably quickly and cheaply. DNA barcoding can serve a dual purpose as a new tool in the taxonomists toolbox supplementing knowledge as well as being an innovative device for non-experts who need to make a quick identification.

The main goal of DNA barcoding is to facilitate rapid identification of potentially unidentified taxa in global biodiversity assessment and conservation. DNA barcoding has also focused on the development of a global barcoding

database as a species identification tool for large taxonomic assemblages of animals, representing a quick and easy method for non-specialists to identify disparate specimens. The identification process through DNA barcoding is relatively straight-forward, and depends upon the quantifiable matching of a genetic marker region called DNA barcode. Significance of DNA barcode region is its relatively fast mutation rate, which results in significant variation between species. DNA barcode should be present in most of the taxa of interest and sequencable without species - specific PCR primers, short enough to be easily sequenced with current technology and provide a large variation between species yet a relatively small amount of variation within a species.

Phylogenetic analysis: Phylogenetics is the science of estimating and analyzing evolutionary relationships. Molecular biology often helps in determining genetic relationships between different organisms. Nucleic acids (DNA and RNA) and proteins are 'information molecules' in that they retain a record of an organism's evolutionary history. The approach is to compare nucleic acid or protein sequences from different organisms using computer programs and estimate the evolutionary relationships based on the degree of homology between the sequences. Nucleic acids and proteins are linear molecules made of smaller units called nucleotides and amino acids, respectively. The nucleotide or amino acid differences within a gene reflect the evolutionary distance between two organisms. In other words, closely related organisms will exhibit fewer sequence differences than distantly related organisms.

One advantage of the molecular approach in determining phylogenetic relationships over the more classical approaches, such as those based on morphology or life cycle traits, is that the differences are readily quantifiable. Sequences from different organisms can be compared and the number of differences can be established. These data are often expressed in the form of 'trees' in which the positions and lengths of the 'branches' depict the relatedness between organisms.

So this study demonstrated that DNA barcoding using standard segment of mitochondrial gene - cytochrome *c* oxidase I (CO1) is effective in discriminating spider species. It provides a uniform, practical method of spider identification. This study also demonstrated an easy and rapid method of identifying spiders instead of conventional difficult methods. Comparative study of CO1 sequence of nine families of spiders and their phylogenetic analysis showed their relationship with evolution of web building behaviour. Thus this study revealed the relationship between molecular and morphological taxonomy.

A DNA-based approach to species identification will not lead to the displacement of taxonomists. Tautz *et al*., (2002) make the important distinction that, unlike genes in GenBank, species are not facts but hypotheses. The scientific name does double duty as both shorthand for this hypothesis and as the handle used to collate information on the species. The validity of DNA-based species identification systems depends on establishing reference sequences from taxonomically confirmed specimens, a process requiring the cooperation of a diverse group of scientists and institutions. Moreover, further analysis of the type demonstrated here is essential to determine the ranges of intra- and inter-specific variation among different taxonomic groups. Once completed, these studies will provide the platform for a uniform, practical method of species identification, a result with broad scientific implications.

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