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Morphological Profiling and DNA Barcoding of Agroecosystem Spiders from the Paddy Field of Namakkal District, Tamil Nadu

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Abstract

Our study primarily aimed to investigate the diversity of spiders and assess the comparative effectiveness of DNA barcoding and morphological evaluation for the identification of spiders. The main objective of our study was to explore the diversity of spiders and compare the effectiveness of DNA barcoding and morphological evaluation for species identification. The study was conducted to document the spider diversity in the paddy fields of Namakkal District, Tamil Nadu. There were found to be ten different families and a total of 22 different spider species. The arrangement of eyes was found to be a key factor in identifying spider species, and the eye pattern of the spiders was observed and noted for certain species to validate the morphological identification. However, due to the morphological ambiguity of the two species, molecular characterization was done to confirm their identity. The COI gene was sequenced for Olios (440 bp) and Hogna (590 bp), and the selected spider species were identified as *Olios suavis* and *Hogna insularum*, respectively. Based on our findings, it can be inferred that DNA barcoding is a more reliable method, particularly in cases where morphological characteristics are ambiguous, such as with immature spiders.

Keywords: agroecosystem; paddy field; spider diversity; eye pattern; DNA barcoding.

Introduction

Spiders are a diverse group of invertebrates that make up a significant portion of the ecosystem's fauna (1). The World Spider Catalogue contains taxonomic information on approximately 46,000 spider species across 114 families (2). Several studies have described the diversity and abundance of spider species in various agroecosystems. Spiders are generally found in areas with suitable temperature and moisture levels that fall within their physiological tolerances, making them important for land preservation strategies. They are well-represented among the predators found in paddy fields, and spider surveys have been conducted in rice-growing regions across Asia (2, 3). However, identifying spider species can be challenging due to difficulties in morphological characterization (4). The need for adults, sexual dimorphisms, and discrepancies in genital morphology frequently make it difficult (5). In recent years, there has been a significant surge in the advancement and utilization of molecular tools for investigating microbial diversity and identifying isolates (6). To address these challenges, DNA barcoding

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has emerged as a reliable and cost-effective tool for identifying and delimiting new species across various animal groups (7).

The Barcode of Life Data System (BOLD) is an informatics platform designed to streamline the utilization of DNA barcoding for the identification of specimens and the exploration of new species (8). It gathers specimen metadata and sequences, provides tools for the analysis of data and publication, and enables species discrimination through the allocation of each COI sequence cluster to a Barcode Index Number (BIN) (9). DNA barcoding has become widely accepted for its applications, in recognizing cryptic species, discovering new species, revising taxonomy and conducting faunal assessments (10 - 13).

BOLD currently contains an extensive database with 6.8 million records encompassing 587,000 BINs, including 117,000 spider records assigned to more than 10,000 BINs (accessed 13 April 2019). Although the extent of prior research on spiders has varied, only two studies from Canada and Germany have sought to develop an extensive DNA barcode library for a national spider fauna (14, 15). The necessity for similar endeavours in other regions, particularly South Asia, is evident, considering the limited availability of barcode records for spider species in India (73 species) and 41 species in Pakistan (8, 16, 17). Therefore, this study aimed to estimate the efficacy of DNA barcoding for identifying spider species from the Nanjaiedayar paddy field in Namakkal District, India, for the first time. The study also assessed the efficacy of DNA barcoding by downloading conspecific sequences from the Gen Bank database.

Materials and Methods

Sample site and sample collection

Live spider samples were collected from the Nanjaiedayar paddy field in Namakkal District, Tamil Nadu, India during the periods of 2019-2021. These sampling sites consisted of monoculture paddy fields surrounded by strips or bunds covered with grass. Samples were collected based on using sweep netting through visual search (18). Sampling was done for three seasons monsoon, winter and summer in the paddy field.

Sample storage

Insects were gathered in plastic jars (4×6 inches) in 75% ethanol. Collected specimens were brought to the laboratory in the Department of Zoology, Kandaswami Kandar's College, Paramathi Velur, Namakkal District of Tamil Nadu. Spiders were moved to clean glass vials (20 ml) for morphological study after being cleaned with alcohol and using forceps. The vials were filled with Odd Man's solution which consisted of 70% ethanol, 15% glacial acetic acid and 15% glycerol.

Morphological identification

Morphological characterization of spiders was examined using a dissection microscope and spider species were classified based on available keys (19). The characterized spider specimens were recorded using a dissecting microscope and a digital camera to assist in documentation.

Molecular characterization of selected spider species

The two spiders were professionally identified before it was sequenced for the barcode region of the mitochondrial COX1 gene. The specimens were initially stored in plastic bottles before being maintained in 100% ethanol vials. For DNA isolation purposes, one or more legs were removed from these specimens and stored in vials containing absolute ethanol at a temperature of -20º C until further use. Identification of spider species was carried out by examining distinguishing features like female epigyne (genitalia), male pedipalp (genitalia),

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cheliceral teeth etc. and other relevant features using a stereo zoom microscope (Lawerence and Mayo) and keys available in (20, 21).

DNA extraction, amplification and sequencing

DNA extraction, PCR, and Sanger sequencing were performed at the United State Government National Library of Medicine using standard protocols. Using sterile forceps, a single leg was removed from each specimen and transferred into a well in a 96-well microplate pre-filled with 30 µl of 95% EtOH. Subsequently, DNA extraction was carried out by subjecting the samples to overnight tissue lysis at 56°C overnight followed by a column-based protocol (22). PCR enables the targeted and swift amplification of specific DNA or RNA sequences (23). The COI-50 barcode area was amplified using the primers C_LepFolF and C_ LepFolR in PCR. This primer cocktail includes an equal volume of LepF1. /LCO1490 (24) and LepR1 (25) /HCO2198 (24), respectively. The targeted COI region was amplified using 2 µL of DNA template in a 12.5 µL reaction mixture containing standard PCR components, following the PCR protocol: 94°C (1 min), 5 cycles of 94°C (40 s), 45°C (40 s), 72°C (1 min); 35 cycles of 94°C (40 s), 51°C (40 s), 72°C (1 min) and final extension of 72°C (5 min). The amplicons were subjected to analysis using a 2% agarose E-gel 96 system (In-vitrogen Inc.) and sequencing was performed bidirectionally using the Big Dye Terminator Cycle Sequencing Kit (v3.1) on an Applied Bio-systems 3730XL DNA Analyzer. Using Codon Code Aligner (Codon Code Corporation, USA), sequences were put together, aligned, and modified. MEGA 5 was used to check the sequences to make sure there were no stop codons.

Data analysis

Using Chromas Pro version 1.34, the sequences gathered were modified, and fasta formatted files were produced (Technelysium P Ltd., Tewantin, Queensland, Australia). The obtained sequences were subjected to comparison with the NCBI database using the BLAST tool and with the BOLD database using the species identification tool. A similarity cut-off of 97% was used for species identification (25). To assess the efficacy of DNA barcoding for species identification, we have additionally retrieved a few conspecific and congeneric sequences supplied by others from various geographical locations. The downloaded sequences, along with the sequences generated in this study, were subjected to neighbour joining (NJ) clustering analysis using MEGA 6 software (26). The *Mygalomorph, Tarantula* and *Heterophrictus* sp. (Sample code, ADB118) was used as an out-group. In MEGA 6, the Kimura 2 parameter was used to estimate both the intra- and interspecific nucleotide divergence (26). To confirm the existence of a DNA barcoding gap, a dot plot illustrating the nucleotide divergence within each species compared to the nucleotide divergence to its closest neighbour was generated.

Results and Discussion

Spider diversity from the paddy field was documented from 2019 to 2021 for three different seasons and results were already published (27). During the study period of the present study, 22 spider species were recorded including *Argiope catenulata, Argiope anasuja, Argiope epicta, Araneus ventricosus, Araneus diadematus, Neoscona crucifera, Clubiona terrestris, Sergiolus montanus, Hersilia caudata, Hogna aspersa, Hogna insularum* (Fig. 1a), *Peucetia viridans, Oxyopes macilentus, Telamonia dimidiata, Telamonia elegans, Heteropoda venatoria, Olios suavis* (Fig. 1b), *Olios millet, Tetragnatha elongata, Tetragnatha guatemalensis, Tetragnatha laborious* and *Thomisus lobosus* belonging to 10 families (Table 1).

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Fig. 1a *Hogna insularum*

Fig.1b *Olios suavis*

Table 1: Spider diversity in the selected paddy field

Order	Family	Species
Araneus	Araneidae	*Argiope catenulata*
		Argiope anasuja
		Argiope pepicta
		Araneus ventricosus
		Araneus diadematus
		Neoscona crucifera
	Clubionidae	*Clubiona terrestris*
	Gnaphosidae	*Sergiolus montanus*
	Hersiliidae	*Hersilia caudate*
	Lycosidae	*Hogna aspersa*
		Hogna insularum [NI]
	Oxyopidae	*Peucetia viridians*
		Oxyopes macilentus
	Salticidae	*Telamonia dimidiate*
		Telamonia elegans
	Sparassidae	*Heteropoda venatoria*
		Olios millet
		Olios suavis [NI]
	Tetragnathidae	*Tetragnatha elongate*
		Tetragnatha guatemalensis
		Tetragnatha laboriosa
	Thomisidae	*Thomisus lobosus*

Eye arrangement is the key factor for the identification of spider species, to validate the morphological identification of the selected spider, the eye pattern of the spiders was observed and the detailed eye pattern of certain spider species was noted and elaborated in Fig. 2. The spider studied included the grass cross spider, orb-weaver, two striped jumpers, giant crab spider, green lynx spider, pumpkin spider, barn spider, green crab spider, sac spider, elongate stilt spider, silver long-jawed orb weaver, Guatemalan long-jawed spider, wolf spider, two-tailed spider, lean lynx spider and rice bug. A few key characters of the spider family based on eye patterns were discussed here. Family Araneidae is also known as orb weavers with distinctive araneid eyes. Sac spider belongs to the family Clubionidae, family Gnaphosidae is also called ground spider and this spider often has an oval-shaped eye pattern. Lycosidae was also named wolf spiders with uniform round eyes. Lynx spiders come under the family Oxyopidae, Family Salticidae is also called jumping spider, Sparassidae is known as Giant crab spider, Long-Jawed orb weavers belongs to the family Tetragnathidae and Crab spider comes under Thomisidae.

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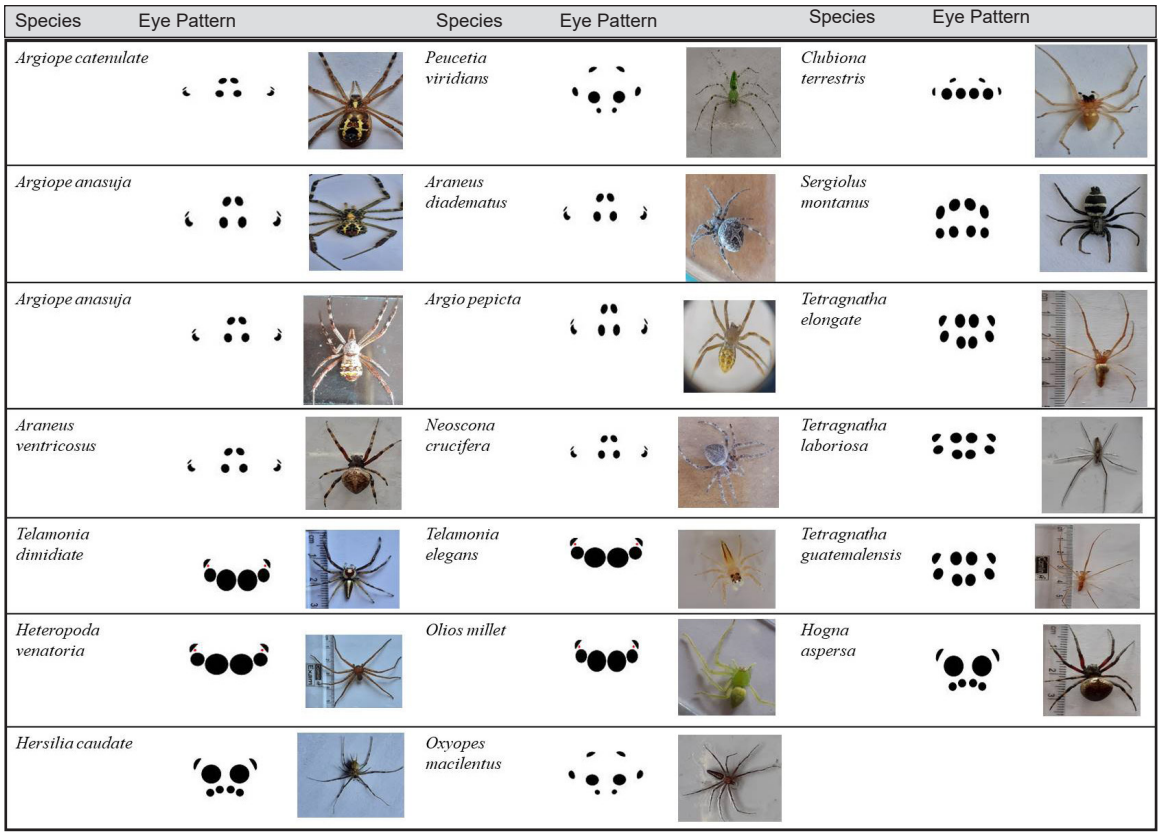

Fig. 2: Eye pattern of spider from selected paddy field

Due to the morphological ambiguity of the two species (*Olios* spp. and *Hogna* spp.), they were selected for molecular characterization for additional confirmation of the species. To confirm these species, molecular characterization was done by sequencing the COI gene (Table 2) of *Olios* spp. (440 bp) and *Hogna* spp. (590 bp). The presence of two species and two genera was revealed by DNA barcoding. The obtained sequences were also compared to sequences that are currently available at Gen Bank. The selected spider was identified as *Olios suavis* (ON897744.1) and *Hogna insularum* (ON897743.1) based on molecular characterization.

Table 2: Selected spider species for barcoding

Species	N	DN	TN	PI
[NI]*Olios suavis*	1	9	2748340	USW04573.1
[NI]*Hogna insularum*	1	11	2893257	USW04572.1

N- Number of individuals, DN- Distance to nearest Neighbour species, TN- Taxon Number, PI- Protein ID, [NI]- New species of the family to India

Discussion

DNA sequencing is a laboratory technique employed to determine the precise sequence of nucleotides, also known as bases within a DNA molecule [28]. To create a barcode reference library for spiders, agroecosystem spiders from Namakkal District were molecularly

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identified using morphological methods. In comparison to a molecular method, the morphological evaluation had a percentage accuracy of approximately 80%. 88% accuracy in the morphological explanation of the species in a study on spiders that inhabit crops was found [29]. Less accuracy in the morphological evaluation may be caused by the absence of identification keys and the deprivation of young spiders' distinguishing characteristics.

Identification of spiders based on morphology presents challenges, particularly for young spiders. These stages lack reliable diagnostic features like genitalia, which spider taxonomists rely on the most [30]. In similar scenarios, DNA barcoding emerges as an equally significant and dependable method [25]. DNA barcoding proves beneficial, particularly in cases where morphological identification is inconclusive [31]. In the present study also morphological identification of spiders collected from a paddy field was done and the eye pattern of the spider was also observed. Discrimination between juveniles and adult spiders from Prince of Wales Island [32], Alaska using morphology and DNA barcoding [16] was done successfully.

Blagoev and Dondale [33] used a combination of conventional and molecular methods to identify one new species of the Lycosidae family. Similarly, the present study also attempted to identify spider species based on morphological and molecular characterization. In certain circumstances, misidentification of species may also occur and this could be due to a mismatch of the specimens available at GenBank. Using DNA barcoding, Tahir et al. [17] successfully identified five different species of spiders. COI and cytochrome b are better for determining species boundaries than 16s rRNA genes [34]. Thus, this is the reason for selecting COI gene sequencing in the present investigation. Additionally, Nicolas et al. [35] discovered that the 16sRNA gene is 2.5% less useful for identifying species than mitochondrial genes. Many other taxonomic groupings, including butterflies, aphids, salamanders, and araneids, likewise lack a noticeable barcoding gap [31].

Many authors have criticised DNA barcoding as a method [36, 23]. Despite some criticism, DNA barcoding is experiencing more popularity and in the future it will become a standard identification protocol for diverse organisms. This is due to its ease, speed and reliability for various animal groups including spiders. NJ clustering analysis is frequently employed in addition to the genetic divergence measure to evaluate the efficiency of DNA barcoding in quickly differentiating animal species [37]. According to Coddington et al. [38], DNA barcoding makes it possible to accurately assign unidentified spider taxa to a higher taxonomic rank.

The results of the current study imply that the COI gene carries sufficient data to identify spiders down to the species level. For the *Olios suavis* and *Hogna Insularum* spiders of the Namakkal district, our study has also produced a complete DNA barcode library. From the current research, it can be inferred that when DNA barcoding is used in conjunction with morphological studies, their accuracy and reliability are boosted.

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