Original Research Article

Morphogenotyping of *Cyclosa bifida* (Arachnida: Araneidae) from Odisha, India

Abstract

Morphogenotyping involves analyzing an individual's morphology and DNA sequences to identify structural and genetic differences. Among the arthropod group, the spider is the most diversified, primarily female, and solely predatory. The surveys were carried out in the morning, from 5:00 AM to 8:00 AM, and in evening from 5:00 PM to 6:00 PM, well before darkness. With the help of identification keys and based on morphological features, all collected spiders were identified and made taxonomical positions. Then DNA was isolated from the single female captured *C. bifida* species. Quality was evaluated on 2% Agarose Gel; a single band of high-molecular weight DNA has been observed. Isolated DNA was amplified with *cytochrome oxidase subunit I (COI) gene* Specific Primer (LCO 1490 and Chelicerate Reverse 2). The consensus sequence was generated for each sample using BioEdit version 6.0.7 and were searched over the GenBank database usingBasic Local Alignment Search Tool (BLAST) against the spider genomes previously present in GenBank. The complete sequences were deposited in GenBank with accession no. PQ478048.1.This morphogenotyping investigation will demonstrate the need for additional research on several unidentified or perplexed spiders. Thus, research suggests that the *bifida* species found in West Bengal and Odisha are more genetically similar and closer to one another than those found in Kerala. These discrepancies would result from two distinct evolutionary pathways.

Key words: Spider, *Cyclosa bifida*, Morphogenotyping, Odisha, insect pests

1. INTRODUCTION

Morphogenotyping involves analyzing an individual's morphology and DNA sequences to identify structural and genetic differences. This approach integrates the study of physical traits with genetic profiling. The groundbreaking research on spider morphogenotyping is represented in the current discussion. Spiders are categorised under the class Arachnida and are members of the order Araneae. They are a very successful evolutionary group within the Arthropoda phylum (Miller & Sac, 2011). According to Raiz Tabasum et al. (2018), they are among the most prevalent groups of predatory creatures in the animal kingdom. One characteristic that distinguishes spiders from other arachnids is the presence of a pedicel. The cephalothorax, which houses the male reproductive organ, two pedipalps, eight leg appendages, and sensory organs, makes up the spider's body. The female reproductive system and spinnerets, which are specialised for producing silk, are found near the back of the abdomen (Sebastian & Peter, 2009). Spiders are fascinating and incredibly varied invertebrates that are especially common in tropical and subtropical areas. As natural predators, they are essential in controlling insect pests (Saha et al., 2016). Spiders are an important group, being the second-largest order within the Arachnids. One of the most ubiquitous and cosmopolitan families is Araneidae, also known as orb-weavers. Common habitats for orb-weavers include grasslands, gardens, agricultural areas, and woodlands. They weave spiral, wheel-shaped webs. These spiders lack a stridulating organ, have eight spiny or hairy legs, and eight identically formed eyes grouped in two rows. They are highly specialised predators that are usually huge, colourful, and have three claws. Orb-weavers are polyphagous carnivores that eat a wide variety of prey, including eggs, larvae, and other life stages. They are useful partners for farmers because of their important involvement in pest management (Marc et al., 1999). Spiders are extremely sensitive to even slight alterations in the biological, chemical, and physical aspects of their environments. They are useful bio-indicators of environmental changes and have a significant impact on natural and agricultural ecosystems (Jansen, 1997). Because they are easily recognised and respond differently to natural and man-made disturbances, they serve as biological indicators that aid in evaluating the health of ecosystems (Marc et al., 1999; Pearce & Venier, 2006). Among the arthropod group, this order is the most diversified, primarily female, and solely predatory. The ecosystems that spiders live in depend on them (Bennett, 2001). The conservation status of 99.5% of the spider species has not yet been evaluated by the IUCN globally (Seppälä et al 2018). Globally, they account for 52,510 species in 4,406 genera belonging to 135 families as of 1st December 2014 (World Spider Catalog, 2024).

With many species still undiscovered in the area and throughout India, spider research in Odisha is still mostly unexplored. Because of a lack of taxonomists, zoologists, or experts in arachnology, there is very little data on spiders in different states of India (Meher et al., 2024). Spiders are one of the least studied arthropods, while being essential for controlling invertebrate populations in ecosystems (Russell-Smith, 1999). Although there is a wealth of information about birds and mammals in Odisha, little is known about spider species, despite their critical ecological importance. The members of the *Cyclosa* genus are renowned for creating tiny, round orb webs embellished with linear decorations composed of trash and prey remnants, known as trash line orb-weavers. These embellishments are thought to draw in prey while aiding in the spider's concealment, described by Anton Menge in 1866. The members of the genus *Cyclosa* are found all over the world (Tanasevitch, 2020). They prefers dark, damp woodland with a good shrub cover and is found on shrubs and trees, usually evergreen, in damp or shaded conditions. The word "*Cylosa*" comes from the Greek and means "to move in a circle," alluding to their habit of spinning webs. Certain *Cyclosa* species, notably *C. ginnaga* and *C. argenteoalba*, face upwards when they wait for prey, in contrast to the majority of orb-weavers, which usually face downwards (Nakata & Zschokke, 2010). There is no sufficient availability of taxonomical study and identification key of spiders; it creates more confusion for taxonomist to identify only on the basis of morphological character. Moreover this group's sexual dimorphism, polymorphism, and absence of identification keys for juveniles make morphological identification difficult and time-consuming (Coddington & Levi, 1991; Magalhaes et. al., 2017). Thus molecular identification is prime necessary for accurate identification of spider. DNA barcoding has been widely used in the last decade in biodiversity research for accurate species identification (Hebert et. al., 2003; Tyagi et. al., 2019) as well as identifications also occurred on the basis of sequences of *cytochrome oxidase subunit I* (COI) gene. During our survey, very few *Cyclosa bifida* were captured from the paddy field. The morphology of *Cyclosa bifida* (Doleschall, 1859) created more confusion with the morphology of other species of the genus *Cyclosa*. So here authors took first attempt to address morphogenotyping study on *C. bifida* collected from Kalahandi district, Odisha. The *C. bifida* species found in the Kalahandi district of Odisha is the first report from Kalahandi. This kind of research is very significant from a taxonomical and zoological perspective. This morphogenotyping investigation will demonstrate the need for additional research on several unidentified or perplexed spiders.

2. MATERIALS AND METHODS

2.1 STUDY AREA

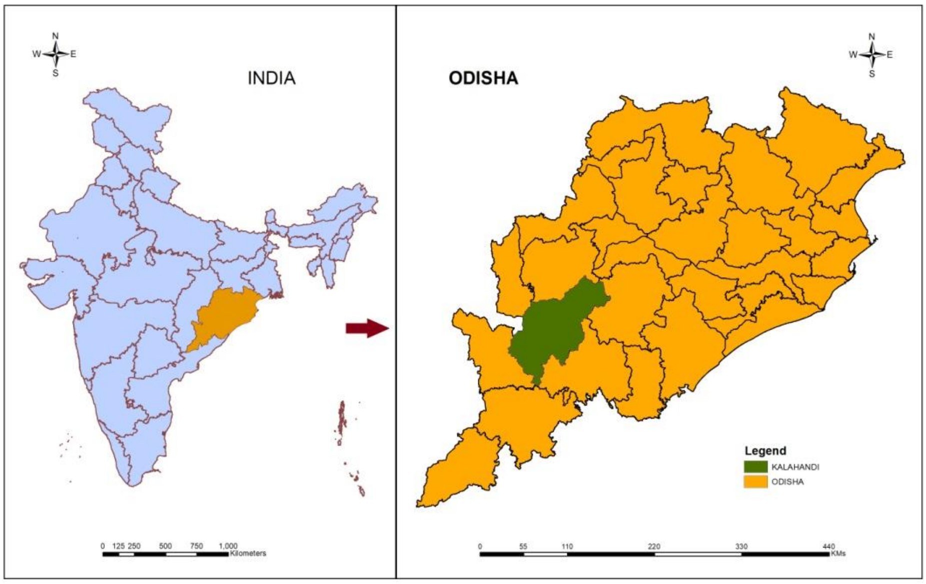


Figure 1 Map of Kalahandi district,-Odisha, India

The study site was Kalahandi District Southern Odisha with Latitudinal extent 19°55′09″North to 83°10′27″East **(Fig.1)**. The collection of data was made from different sites of Kalahandi district, Odisha, eastern India. Kalahandi is a district of Odisha in India, which is situated between 19.3N and 21.5N latitudes and 82.20E and 83.47E longitudes, has an area of 8,364.89 square kilometer. The topography of Kalahandi consists of plain land, hills and mountains. The climate of this district is quite extreme remaining mostly dry except during monsoon. Kalahandi, Odisha experiences an average of 1378.2 mm of rainfall annually, most of which falls during the monsoon season from June to September. May is the hottest month, and December is the coldest, with a mean daily low temperature of about 11°C.

2.2 SPIDER COLLECTION AND IDENTIFICATION


(a) (b)

Figure 2(a) Collection Site (Paddy field) and, (b) *C. bifida* on a leaf

Only paddy fields were used for the survey, which was conducted throughout one cropping season from June to October 2024. Each study site had an approximate area of approximately three acres, and the sampling covered every block in the Kalahandi district **(Fig. 2)**. Prior to the investigation, farmers' permission was obtained to collect on their property. Kharif and Rabi are the two crops that are harvested in this district each year. Kharif crops are typically harvested in October or November, at the end of the monsoon season, after being planted from June to September.

The surveys were carried out in the morning, from 5:00 AM to 8:00 AM, and in evening from 5:00 PM to 6:00 PM, well before darkness. These paddy fields were often damp due to water deposition; they were dry when they were harvested. Spider collecting was conducted in the Bargarda district of Odisha using the approach described by Priyadarshini and Mahapatra (2023). Ten quadrats were sampled within each one-acre farming plot using the quadrat method, for a total of thirty quadrats per study site. Every quadrat was 5 x 5 meters in size, and sampling was done consistently throughout the plot. Along transects, the quadrats were chosen to include the primary paddy crop, adjacent fringe vegetation, and bunds—elevated margins within paddy fields. Each quadrat was placed at least ten feet apart to prevent overlap.

During each stage of vegetative growth, each site was visited twice a day for three days. The information gathered from these visits was then merged for analysis. Experts and pertinent literature helped classify the collected specimens to the species level after they were identified using morphological descriptions and images from B.K. Tikader's Handbook of Spiders (1987) (Howell & Jenkins, 2004; Siliwal, 2005). A Stereo Zoom Binocular

Microscope was used to analyse the samples that were obtained. Some specimens were kept in 70% alcohol and deposited in the animal house of department of zoology, Maa Manikeshwari University for in-depth research and future use, while others were returned to their original environments.

2.3 MORPHOLOGICAL STUDY

In order to evaluate the shape, size, colour, and other characteristics of different body sections, morphological analysis is an essential stage in zoological research. Photographing specimens in detail is also crucial **(Figs. 3 & 4)**. We first verified the captured spider's taxonomic position based on important features before establishing the systematic classification.

Identification Key:

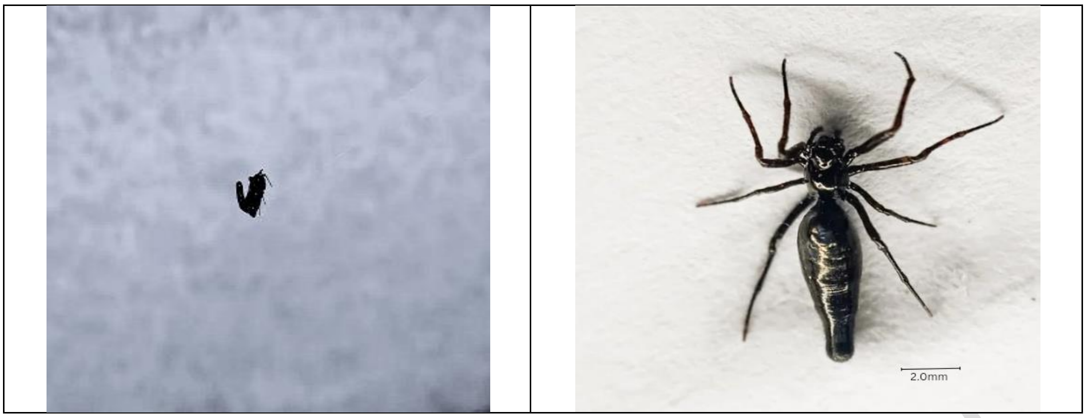
(Handbook of Spider by B. K. Tikader 1987)

1. Carapace flat with distinct thoracic furrow; abdomen anteriorly very high and provided with at least one pair of shoulder humps .... *Cyrtophora*

Carapace not flat, thoracic furrow may or may not distinct; abdomen anteriorly not high and shoulder humps mayor may not present 2

2. Posterior median eyes very close, nearly touching; carapace provided with a V-shaped junction between cephalic and thoracic region .... *Cyclosa*

The spider was classified using the following literature (Tikader, 1987; Howell & Jenkins, 2004; Siliwal, 2005): Phylum: Arthropoda, Subphylum: Chelicerata, Class: Arachnida, Order: Araneae, Family: Araneidae, Genus: *Cyclosa*, Species: *bifida*. Morphometric measurements were taken after identification because they are essential for differentiating between spiders and insects.



(a) (b)

Figure 3 (a) *C. bifida* hanging ventrally in its net and, (b) Dorsal view of *C. bifida*

(a) (b)

Figure 4 (a) Dorsal view of *C. bifida* and, (b) Egg pouch of *C. bifida*

2.4 MOLECULAR WORK

DNA was isolated from the single female captured *C. bifida* species. Quality was evaluated on 2% Agarose Gel; a single band of high-molecular weight DNA has been observed. Isolated DNA was amplified with *cytochrome oxidase subunit I (COI) gene* Specific Primer (LCO 1490 and Chelicerate Reverse 2) using Veriti® 96 well Thermal Cycler. A single discrete PCR amplicon band of ~700 bp was observed **(Fig. 5)**. The PCR amplicon was bead purified and further subjected to Sanger Sequencing.

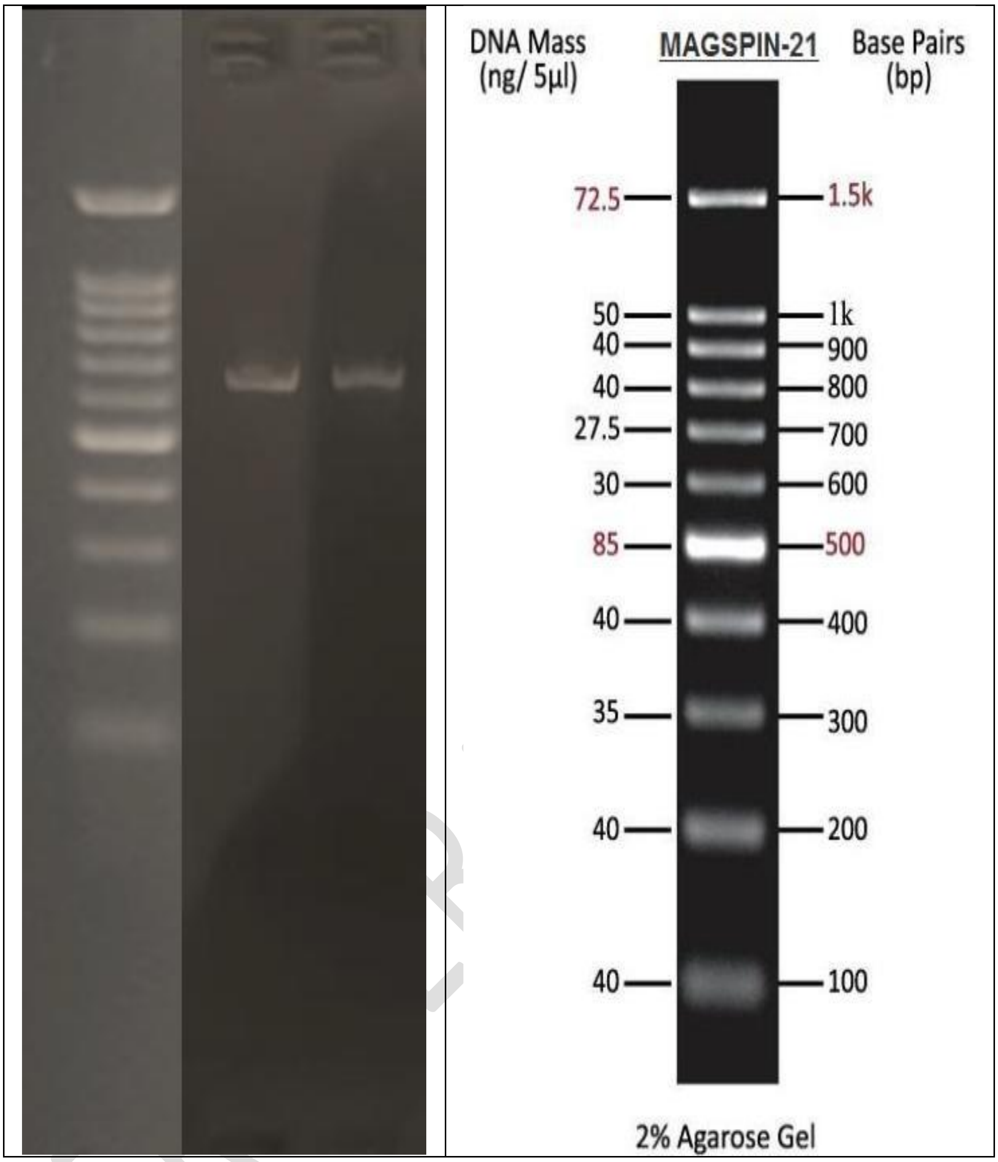


Figure 5 2% Agarose gel showing single 700 bp of *cytochrome oxidase subunit I (COI) gene Amplicon*.

2.4.1 DNA quantification The concentration of each DNA sample was measured by Nanodrop (Biotech instruments, USA). DNAwas stored at−80^{0} C for further use. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. A ratio of ~1.8 to 2.0 is generally accepted as “pure” for DNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

2.4.2 PCR Amplification PCR was performed in a total volume of 25 µl containing 10 pmol each of forward and reverse primers, 2.5 mM of MgCl2, 200 µM each of the four deoxyribonucleotide triphosphates (dNTPs), 0.5 U of Taq DNA polymerase, 1x concentration of PCR buffer (Invitrogen, Life Technologies, Brazil) and 50 to 100 ng of isolated bacterial genomic DNA. The template was denatured by heating at pre-denaturation of 95 °C for 5 min. This was followed by 39 cycles of denaturation 30 sec at 95 °C, 45 sec annealing and 1 min elongation at 72°C, with a final extension of 7 min at 72°C. The amplicons were resolved in 1.5% agarose gel using 0.5x tris-acetate-EDTA (TAE) buffer. Bi-directional DNA sequencing reaction of PCR amplicon was carried out with LCO 1490 & Chelicerate Reverse 2 primers using BDT v3.1 Cycle sequencing kit on ABI 3500Dx Genetic Analyzer

Primers Used:

LCO 1490- GGTCAACAAATCATAAAGATATTGG

Chelicerate Reverse 2- GGATGGCCAAAAAATCAAAATAAATG

Sequencing Instrument Used: For sequencing of isolated DNAABI 3730XL instrument was used in the lab of Heredity Biosciences, Bhubaneswar.

2.4.3 Bioinformatics study

The consensus sequence was generated for each sample using BioEdit version 6.0.7 and were searched over the GenBank database usingBasic Local Alignment Search Tool (BLAST) against the spider genomes previously present in GenBank (NCBI WEB SITE) **(Fig. 6)**. The complete sequences were deposited in GenBank with accession no. PQ478048.1. The mtCOI sequence of *C. bifida* (our isolated strain KSP02) were compared with the whole world samples of gene COI of *Cyclosa* species found from other countries by Maximum likelihood method of the world **(Fig. 7)**; further, it also compared with the COI gene sequence of Indian *C. bifida* **(Fig. 8)** using Multiple Sequence Alignment (MSA) based on the sequences available in NCBI GenBank.

2.4.4 Molecular Phylogenetic analysis

The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. This analysis involved 16 nucleotide sequences. The Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pair wise deletion option). Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). The horizontal lines (branches) in the tree denoted the evolutionary distance between the connected nodes, representing genetic divergence **(Figs. 7 & 8)**.

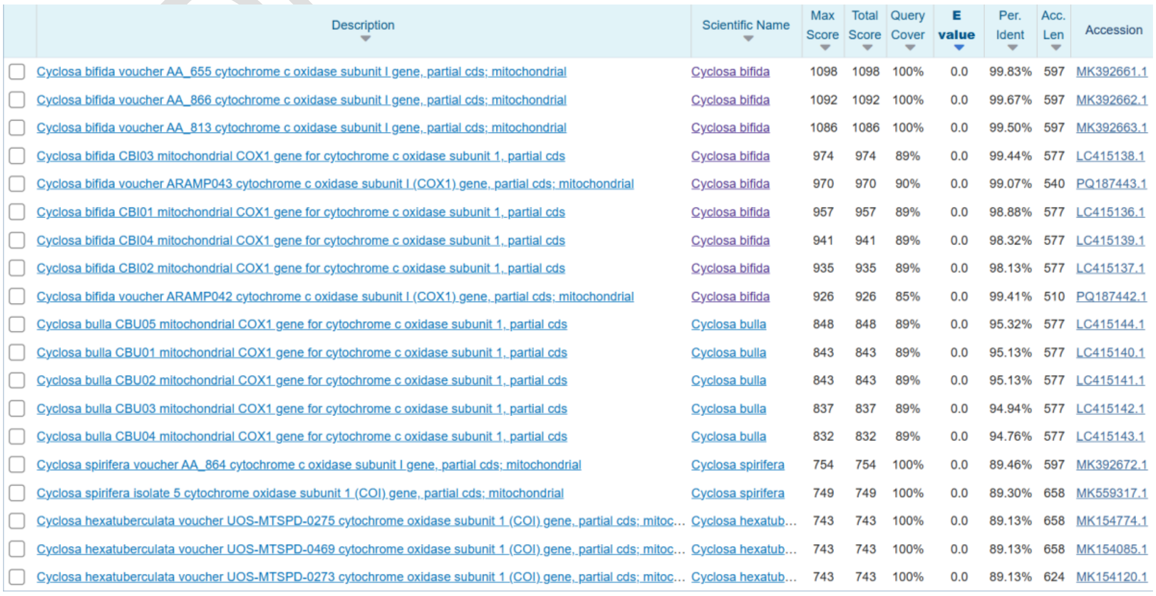
3. RESULT

During the collection process, 187 spiders were gathered from various Kalahandi district paddy fields. The individuals were categorised into 26 genera, 93 species, and 9 families based on morphotaxonomy. During our study, a single female spider was caught, although a sizable number of others were gathered. Thus, for the purpose of morphogenotyping, or detailed morphological and genetical research based on the sequencing of the mitochondrial gene (mt) Cytochrome Oxidase subunit I (COI), we concentrated on single species. The phylum Arthropoda, sub-phylum Chelicerata, class Arachnida, order Araneae, family Araneidae, genus *Cyclosa*, and species *bifida* were all proven to be members of the gathered species based on their morphological study. This species was photographed **(Figs. 2 (b), 3 & 4)**. The most recognisable characteristic of *C. bifida* is its unique abdomen structure, which is sometimes described as having a bifid (split or forked) appearance. This is where the species' name originates. For a more thorough analysis of this species, measurements were also taken of every leg portion of *C. bifida* **(Table 1)**. They are used for orb-building and catching prey, and like most orb-weavers, they have long, thin legs.

Table 1 Length of the leg segments of *C. bifida* (in mm; Female)

Parts of leg	Ist Leg (in mm)	IInd Leg (in mm)	IIIrd Leg (in mm)	IVth Leg (in mm)
Coxa	0.4	0.3	0.4	0.4
Trochantor	0.2	0.2	0.1	0.2
Femur	1.3	1.8	0.7	1.8
Patella	o.3	0.4	0.1	0.4
Tibia	1.8	1.4	0.5	1.4
Tarsus and Claw	1.2	1.7	1.1	1.7
Total	5.2 mm	5.8 mm	2.9 mm	5.9 mm

Following receipt of the BLAST report from the NCBI site, there was strong confirmation that the specimen, using morphological keys, was indeed *C. bifida*. According to phylogenetic analysis and nucleotide homology, the entire BLAST result, which is displayed in Figure 6, indicated a high degree of similarity with the top. The *C. bifida* strain with accession number MK392661.1 and our *C. bifida* strain (KSP02) shared 99.83% of similarities. It was also identical to strains of *C. bifida* with accession numbers MK392662.1, MK392663.1, LC415138.1, and PQ187443.1, with 99.67%, 99.50%, 99.44%, and 99.07% respectively. Although the Kalahandi species of *C. bifida* was genetically similar to the *C. bifida* strain, it was equally genetically distinct from other species of the genus *Cyclosa* worldwide **(Fig. 7)**. However, it was more genetically similar to the West Bengal (Kolkata) strain of *C. bifida*, which has accession number MK392662.1 **(Fig. 8)**.



	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☐	Cyclosa bifida voucher AA_655 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Cyclosa bifida	1098	1098	100%	0.0	99.83%	597	MK392661.1
☐	Cyclosa bifida voucher AA_866 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Cyclosa bifida	1092	1092	100%	0.0	99.67%	597	MK392662.1
☐	Cyclosa bifida voucher AA_813 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Cyclosa bifida	1086	1086	100%	0.0	99.50%	597	MK392663.1
☐	Cyclosa bifida CBI03 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bifida	974	974	89%	0.0	99.44%	577	LC415138.1
☐	Cyclosa bifida voucher ARAMP043 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Cyclosa bifida	970	970	90%	0.0	99.07%	540	PQ187443.1
☐	Cyclosa bifida CBI01 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bifida	957	957	89%	0.0	98.88%	577	LC415136.1
☐	Cyclosa bifida CBI04 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bifida	941	941	89%	0.0	98.32%	577	LC415139.1
☐	Cyclosa bifida CBI02 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bifida	935	935	89%	0.0	98.13%	577	LC415137.1
☐	Cyclosa bifida voucher ARAMP042 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	Cyclosa bifida	926	926	85%	0.0	99.41%	510	PQ187442.1
☐	Cyclosa bulla CBU05 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bulla	848	848	89%	0.0	95.32%	577	LC415144.1
☐	Cyclosa bulla CBU01 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bulla	843	843	89%	0.0	95.13%	577	LC415140.1
☐	Cyclosa bulla CBU02 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bulla	843	843	89%	0.0	95.13%	577	LC415141.1
☐	Cyclosa bulla CBU03 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bulla	837	837	89%	0.0	94.94%	577	LC415142.1
☐	Cyclosa bulla CBU04 mitochondrial COX1 gene for cytochrome c oxidase subunit 1, partial cds	Cyclosa bulla	832	832	89%	0.0	94.76%	577	LC415143.1
☐	Cyclosa spirifera voucher AA_864 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	Cyclosa spirifera	754	754	100%	0.0	89.46%	597	MK392672.1
☐	Cyclosa spirifera isolate 5 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	Cyclosa spirifera	749	749	100%	0.0	89.30%	658	MK559317.1
☐	Cyclosa hexatuberculata voucher UOS-MTSPD-0275 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cyclosa hexatub...	743	743	100%	0.0	89.13%	658	MK154774.1
☐	Cyclosa hexatuberculata voucher UOS-MTSPD-0469 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cyclosa hexatub...	743	743	100%	0.0	89.13%	658	MK154085.1
☐	Cyclosa hexatuberculata voucher UOS-MTSPD-0273 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitoc...	Cyclosa hexatub...	743	743	100%	0.0	89.13%	624	MK154120.1

Fig.6. Related strain sequences obtained from NCBI BLAST producing significant alignments

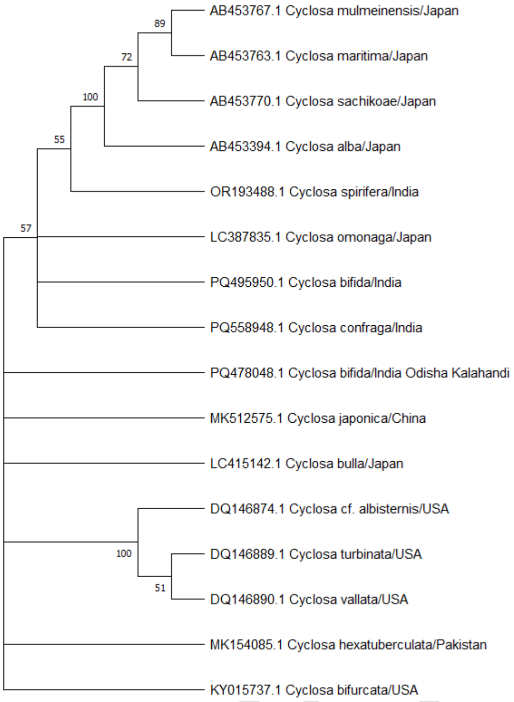


Fig.7.Molecular phylogenetic analysis of *C. bifida* by maximum likelihood method of the world

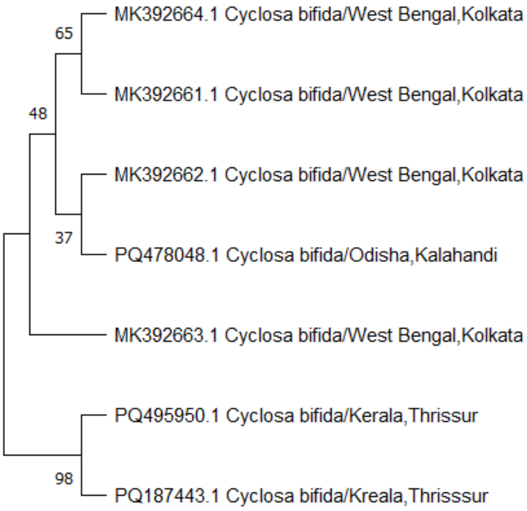


Fig.8.Molecular phylogenetic analysis of *C. bifida* by maximum likelihood method of India

4. DISCUSSION

The minimal number of *C. bifida* collected in paddy fields in Kalahandi district. However, Priyadarshini and Mahapatra's survey in 2023 in the Bargarh district's paddy fields did not report any collection of *C. bifida*, although they did capture other species of the genus. However, other researchers' findings indicated that *C. bifida* was present in different locations (Shirbhate & Shirbhate, 2017; Sunil et al., 2018; Asima & Prasad, 2022). Although *C. bifida* was shown to be less prevalent in paddy fields, it may be found in green vegetables. Since males of this species have not ventured outdoors, our survey's male collection was zero.

Certain traits are displayed by the species *C. bifida*: The abdomen appears to be separated into two halves due to a thin constriction or a distinguishing marking **(Fig. 3(b))**. *C. bifida* frequently exhibits a brown to dark greyish colouration. The entire body is often one colour **(3 (b) & 4 (a))**, with no other discernible colour seen. Its colouration pattern aids in its environmental integration. Cyclosa spiders are also known for their camouflage behaviour; the spider often adds debris or silk structures to the web as a form of camouflage. They sit in the middle or near the web's hub and blend into the environment in the shaded places that are generally undisturbed and unspotted. So in the laboratory, female *C. bifida* made and designed her egg pouch in a spider case roof as shown in Figure 4(b).

The abdomen is significantly longer and broader than the cephalothorax, despite the body being small and compact. The carapace has a smooth, delicate surface and is oval to inverted conical in shape. The abdomen is roughly 5.5 mm in size. As is evident from the slightly elevated abdominal end portion, the abdomen ends with a pointed projection. The carapace has a U-shaped intersection of the abdomen and cephalothorax. In lateral view, the epigynal scape with the narrow tip pointed out is apparent with bilobes. Because they are somewhat bigger than the anterior and lateral eyes, the posterior and anterior median eyes are easily seen. They have long, thin legs **(Fig. 9)**.

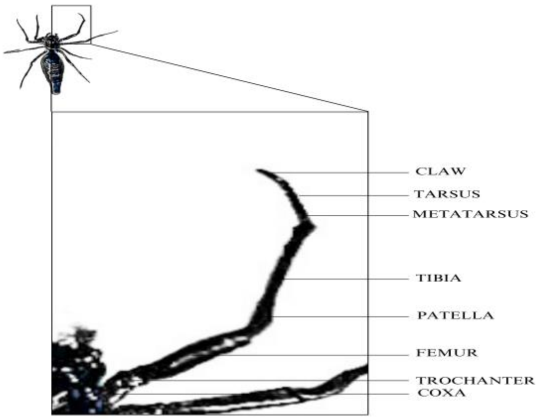


Fig. 9 Different parts of *C. bifida*

The genus *Cyclosa* is characterised by a spherical, pointed abdomen that extends behind spinnerets into the tail. An oblique groove typically separates the anterior narrow cephalic portion of the cephalothorax. The ocular quadrangle, which is made up of two rows of eyes, forms a trapezium and is narrower behind than in front. Although each leg is the same length, the first pair is marginally longer **(Figs. 3(b) & 9)**. According to Petcharad et. al. (2014), these physical characteristics might differ; the carapase may be black or pale brown, and the female's abdomen may be extended but not always slender. We discovered a single, glossy, dark-coloured abdomen. Pale brown legs with dark brown stripes are possible (Petcharad et al., 2014). These characteristics are found in our specimen, but Dixit's description and image of our specimen do not match those of Dixit et al. (2017), which contradict both our results and the findings of Petcharad et. al. (2014).

Table 2 Distance matrix of Indian COI gene sequences of *C. bifida*.

Spider species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
PQ478048.1_C._bifida/Odisha, Kalahandi							
PQ495950.1_C._bifida/Kerala,Thrissur	0.7211009						
PQ187443.1_C._bifida/ Kerala,Thrissur	0.7037037	0.6296296					
MK392664.1_C._bifida/West Bengal, Kolkata	0.6566164	0.6587156	0.6592593				
MK392663.1_C._bifida/West Bengal, Kolkata	0.6582915	0.6568807	0.6574074	0.0033501			
MK392662.1_C._bifida/West Bengal, Kolkata	0.6549414	0.6605505	0.6611111	0.0016750	0.0050251		
MK392661.1_C._bifida/West Bengal, Kolkata	0.6566164	0.6587156	0.6592593	0.0000000	0.0033501	0.0016750	

According to Figure 6, the isolated strain of *C. bifida* from Kalahandi, Odisha, differed equally from the other *Cyclosa* species. However, this species belonged to the primary clade, but the Indian species *C. bifida*, which has accession number PQ495950.1, belonged to a different sub-clade. Because they belonged to the same clade, our isolated strain KSP02 of *C.*

bifida from Kalahandi was more closely linked to the strain from Kolkata (West Bengal) with accession number MK392662.1. However, because they belonged to the same lineage, the other two strains of *C. bifida* that were isolated from the same location and had accession numbers MK392661.1 and MK392664.1 were quite similar **(Fig.8)**. Three of the four *C. bifida* strains that were isolated from Kolkata belonged to the same clade, whereas the fourth strain, accession number MK39266.31, was in a different clade. Eight *C. bifida* strains from India, including our strain KSP02, were found in the molecular phylogenetic tree using the Maximum Likelihood approach. Two further strains of *C. bifida* from Kerala, with accession numbers PQ495950.1 and PQ187443.1 are also found in a different lineage. Thus, research suggests that the *bifida* species found in West Bengal and Odisha are more genetically similar and closer to one another than those found in Kerala. These discrepancies would result from two distinct evolutionary pathways.

5. CONCLUSION

Morphogenotyping involves analyzing an individual's morphology and DNA sequences to identify structural and genetic differences, is the prime importance of study of animals to confirmation of systematic position of animals. Two strains of *C. bifida* from Kerala, with accession numbers PQ495950.1 and PQ187443.1, are also found in a different lineage from other strain of *C. bifida* of India. Thus, research suggests that the *bifida* species found in West Bengal and Odisha are more genetically similar and closer to one another than those found in Kerala. These discrepancies would result from two distinct evolutionary pathways.

1.

2.

3.

8. REFERENCES

1. Asima, A., & Prasad, G. (2022). Araneid spiders of Shendurney wildlife sanctuary in southern Western Ghats, India. Entomon, 47(3), 337-340.
2. Bennett, R. G. (2001). Spiders (Araneae) and araneology in British Columbia. *Journal of the Entomological Society of British Columbia*, *98*, 83-90.
3. Coddington, J. A., & Levi, H. W. (1991). Systematics and evolution of spiders (Araneae). *Annual review of ecology and systematics*, 565-592.
4. Dixit, G. S., & Ade, P. P. (2017). Revision of spiders from the genus Cyclosa (Araneae: Araneidae) with description of three new species and the first record of male of C. conica and C. purnai from India. International Journal of Researches in Biosciences, Agriculture and Technology, 5(2), 945-953.
5. Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. evolution, 39(4), 783-791.
6. Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313-321.
7. Howell, W. M., & Jenkins, R. L. (2004). Spiders of the eastern United States: a photographic guide. Pearson Education.
8. Jansen, A. (1997). Terrestrial invertebrate community structure as an indicator of the success of a tropical rainforest restoration project. *Restoration Ecology*, *5*(2), 115-124.
9. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
10. LEVI, H.W., LEVI, L.R., ZIM, H.S. and STREKALOVSKY, N., 2002. Spiders and Their Kin. New York: Golden Books Publishing Company, pp. 1-220.
11. Magalhaes, I. L., Martins, P. H., Nogueira, A. A., & Santos, A. J. (2017). Finding hot singles: matching males to females in dimorphic spiders (Araneidae:

Micrathena) using phylogenetic placement and DNA barcoding. *Invertebrate Systematics*, *31*(1), 8-36.

12. MARC, P.A., CANARD, A. and YSNEL, F., 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agriculture, Ecosystems & Environment, vol. 74, no. 1-3, pp. 229-273. http://dx.doi.org/10.1016/S0167-8809(99)00038-9.
13. Meher, D., Gahir, N., Majhi, H., Bag, G., Majhi, D., Choudhury, S. R., & Panigrahi, S. K. (2024). Research Article Spider Diversity of Maa Manikeshwari University Campus, Bhawanipatna, Odisha. *Research Journal of Pharmacy and Life Sciences: Volume*, *5*(1), 72-84.
14. MILLER, J.A. and SAC, P.D.(2011). Landscape biodiversity of tropical forest Spider communities in Vietnam (Arachnida: araneae). Treubia, vol. 38, pp. 53-70.
15. Nakata, K., & Zschokke, S. (2010). Upside-down spiders build upside-down orb webs: web asymmetry, spider orientation and running speed in Cyclosa. Proceedings of the Royal Society B: Biological Sciences, 277(1696), 3019-3025.
16. Pearce, J. L., & Venier, L. A. (2006). The use of ground beetles (Coleoptera: Carabidae) and spiders (Araneae) as bioindicators of sustainable forest management: a review. *Ecological indicators*, *6*(4), 780-793.
17. Petcharad, B., Bumrungsri, S., Douangboubpha, B., & Tanikawa, A. (2014). The first records of Cyclosa bifida (Araneae: Araneidae) from Thailand and Laos with redescription of the male and female. Acta Arachnologica, 63(1), 27-30.
18. Priyadarshini, D., & Mahapatra, P. K. (2023). Effect of abiotic factors and seasonal influence on spider diversity in rice agroecosystem of Bargarh District, Odisha, India. Nova Geodesia, 3(4), 146-146.
19. Raiz Tabasum, N., Nagaraj, B., Shantakumari, S., Sreenivasa, V., & Sai Sandeep, Y. (2018). Assessment of spider diversity and composition along the Tungabardhra irrigation channel at Ballari, Karnataka. *International Journal on Biological Sciences*, *9*(1), 36-44.
20. Russell-Smith, A. (1999). The spiders of Mkomazi Game reserve. Mkomazi: The Ecology, Biodiversity And Conservation Of A Tanzanian Savanna.(M. Coe et al., eds). Royal Geographical Society, London.
21. Saha, S., Roy, T. K., & Raychaudhuri, D. (2016). Survey on spider faunal diversity of Darjeeling tea plantations. *Munis Entomology & Zoology*, *11*(2), 622-635.

22. Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution, 4(4), 406-425.
23. SEBASTIAN, P.A. and PETER, K.V., 2009. Spiders of India. Hyderabad: Universities Press, pp. 1-15
24. Seppälä, S., Henriques, S., Draney, M. L., Foord, S., Gibbons, A. T., Gomez, L. A., & Cardoso, P. (2018). Species conservation profiles of a random sample of world spiders I: Agelenidae to Filistatidae. *Biodiversity data journal*, (6).
25. Shirbhate, M. V., &Shirbhate, A. M. (2017). Diversity and distribution of spider fauna (family-Araneidae) in and around Katepurna Sanctuary, Akola, India.
26. Siliwal, M., Molur, S., & Biswas, B. K. (2005). Indian spiders (Arachnida: Araneae): updated checklist 2005. ZOOS'PRINT JOURNAL, 20(10), 1999-2049.
27. Sunil, J. K., Sudhikumar, A., Davis, S. A. M. S. O. N., & Sebastian, P. (2008). Preliminary studies on the diversity of spider fauna (Araneae: Arachnida) in Parambikulam Wildlife Sanctuary in Western Ghats, Kerala, India. Journal of the Bombay Natural History Society, 105(3), 264-273.
28. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.
29. Tanasevitch, A. V. (2020). On linyphiid spiders collected by Antoine Senglet on Corsica, with the description of a new species (Araneae, Linyphiidae). Revue suisse de Zoologie, 126(1), 43-51.
30. Tikader, B. K. (1987). Hand book of Indian spiders. Ed. Director Zool. Surv. India, Calcutta: 251 pp.
31. Tyagi, K., Kumar, V., Kundu, S., Pakrashi, A., Prasad, P., Caleb, J. T., & Chandra, K. (2019). Identification of Indian spiders through DNA barcoding: cryptic species and species complex. *Scientific Reports*, *9*(1), 14033.
32. World Spider Catalog (2024). World Spider Catalog. Version 25.5. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on {date of access}. doi: 10.24436/2