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***Research Article** (Open Access)*

TAXONOMY OF *CYRTOPHORA UNICOLOR* DOLESCHALL, 1857 (ARANEAE, ARANEIDAE) – AN INTEGRATED APPROACH

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ABSTRACT
Spider taxonomy has over the years been recognized as a demanding field of study due to the complex morphological and anatomical features of spiders and genitalic polymorphism (Jocque, 2002). The spider genus *Cyrtophora* Simon, 1864 belongs to family Araneidae Clerck, 1757 and subfamily Cyrtophorinae Simon, 1895. *Cyrtophora unicolor,* known as the "Red tent spider" appears reddish brown in color and builds large tent web, characteristic of subfamily Cyrtophorinae. After Pocock (1900) reported the species from Sikkim, subsequent report came more than a century later, in 2014, from the Western Ghats. This study describes the species and gives its *Cytochrome oxidase 1* (*Cox1*) sequence. Morphological taxonomy gives a vivid description of the appearance of the species. The cephalothorax, abdomen and genitalia are explained and illustrated. Males are rarely found, due to extreme sexual dimorphism; they are much smaller, and seldom found in the webs. The web architecture and ecology of the species are slightly different from its congeners. The species builds webs stationed high above the ground, among trees. Complex morphological characters often constrain spider identification and molecular markers aid in overcoming these impediments. Though RNA sequences and histone protein markers have been occasionally used for molecular characterization of spiders, DNA – based identification methods have proven to offer the most promising approach to overcome taxonomic barriers. The mitochondrial marker *Cox1*, widely used for species identification, is sequenced for the species. The combination of traditional taxonomy and molecular data is a good model to adopt. Spider studies in India will greatly benefit from such a model as documentation of spider diversity of the country is far from complete. An integrated approach in taxonomy is the need of the hour.

***Keywords**: Spider, Taxonomy, Cyrtophora, Cytochrome oxidase 1, Integrated approach*

INTRODUCTION
Taxonomy, the science behind discovering, naming, describing, and classifying organisms based on shared characteristics, has been central to biology for centuries. The intricate morphological and anatomical differences among related organisms make the role of a taxonomist essential. It is estimated that over 15,000 taxonomists are needed to accurately identify 10 to 15 million species based on morphology (Hebert *et al.,* 2003). This highlights the importance of taxonomy in biology. Spider taxonomy, in particular, has long been recognized as challenging due to the complex morphological and anatomical features of spiders and genitalic polymorphism (Jocque, 2002). Documenting spider biodiversity in India remains incomplete, necessitating further taxonomic expertise and investigation. This study aims to contribute to that effort.
An alternative to morphological characters is molecular markers, many of which have been well characterized and are abundant in animal tissues. Complex morphological characters often constrain spider identification and molecular markers aid in overcoming these impediments. Cytochrome oxidase subunit1 (*Cox1)* has been established as a universal barcode for all animal species and is already an identifier for numerous insect taxa. The utility of this marker in identifying spider species is also well founded (Tanikawa *et al*., 2010; Franzini *et al.,* 2013). The *cox1* region is useful for unveiling

phylogenetic relationships among populations and it is also used as the primary DNA barcode throughout the world. This study employs *cox1* marker in molecular characterization of *Cyrtophora* species under consideration.

MATERIALS AND METHODS

2.1. Morphological Taxonomy

Spiders were collected by holding a jar open beneath them and tapping the spider into it with the lid; the specimens were then transferred to collection bottles containing 75% ethyl alcohol. Field photographs were taken using a digital camera. The collected specimens were identified up to species level soon after collection following available literature. Stereomicroscope Leica M205C with advanced automontage software is used for detailed description and documentation. Epigyne and internal genitalia were examined on clearing with 10% Potassium hydroxide (KOH).

Material Examined: 5 ♀♀, 2 ♂♂, Pathiramanal island, Alappuzha district, Kerala, S. India (9°58′12" N; 76°16'48" E); 2 ♀♀, Thattekad Bird Sanctuary, Ernakulam district, Kerala, S. India (10º07'48" N, 76º40'48" E; 280m a.s.l.)

2.2. DNA Barcoding

2.2.1. DNA extraction: DNA isolation was done with Qiagen DNeasy® kit according to manufacturer's protocol for Purification of DNA from Animal Tissue.

Procedure

a. Tissue (spider leg) is cut into small pieces and placed in a 1.5 ml microcentrifuge tube.

b. 20 µl proteinase K is added. Contents of the microcentrifuge tube are mixed thoroughly by vortexing. Tube is incubated at 56°C until the tissue is completely lysed. Vortexing is done occasionally during incubation to disperse the sample, or placed in a thermomixer, shaking water bath, or on a rocking platform.

c. After vortexing again for 15s, 200 µl Buffer AL is added to the sample, and mixed thoroughly by vortexing. Then 200 µl ethanol (96–100%) is added, and mixed again thoroughly by vortexing.

d. The mixture from step 3 (including any precipitate) is pipette into the DNeasy Mini spin column placed in a 2 ml collection tube, and centrifuged at 6000 x g (8000 rpm) for 1 min. Flow-through and collection tube are discarded.

e. DNeasy Mini spin column is placed in a new 2 ml collection tube, 500 µl Buffer AW1 is added, and centrifuged for 1 min at 6000 x g (8000 rpm). Flow-through and collection tube are discarded.

f. DNeasy Mini spin column is placed in a new 2 ml collection tube, 500 µl Buffer AW2 is added, and centrifuged for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Flow-through and collection tube are discarded.

g. The DNeasy Mini spin column is placed in a clean 1.5 ml or 2 ml microcentrifuge tube, and 200 µl Buffer AE is pipetted directly onto the DNeasy membrane. Incubation is carried out at room temperature for 1 min, and then centrifuged for 1 min at 6000 x g (8000 rpm) to elute.

h. Elution as described in step g is repeated for maximum DNA yield.

2.2.2. PCR amplification and sequencing: DNA extract of each species was subjected to PCR amplification of a 657bp region near the 5' terminus of *cox1* gene as per standard protocol (Hebert *et al*., 2003). Primers used were: forward primer (LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer (HCO 2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR reactions were carried out in 96-well plates, 50 µL reaction volume containing 5 µL GeNeiTM Taq buffer, 1 µL GeNeiTM Taq DNA polymerase (1 U/µL), 2 µL DNA (50 ng/µL), 1 µL GeNeiTM 10mM dNTP mix, 2.5 µL (20 pmol/µL) forward primer, 2.5 µL (20 pmol/µL) reverse primer and 36 µL sterile water. Thermo cycling comprises an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. PCR was performed using a C1000™ Thermal Cycler. The amplified products were analyzed on a 1.5% agarose gel electrophoresis as

suggested by Sambrook and Russell (2001). The amplified products were sent to Applied Biosystems, Bangalore for sequencing. Each specimen was bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI BLAST. All sequences were uploaded on GenBank.

RESULTS AND DISCUSSION

3.1. Taxonomic Description

Female: *C. unicolor* is peculiar in its web architecture. It finds retreat in curled dry leaves at the centre of an extended dome shaped web at a height of 5 to 15m from the ground (Fig 1A).
Cephalothorax: Orange-brown; fovea a rounded pit; carapace with large tubercles laterally; few black setae, sparse white setae in anterior half (Fig 1B). Eyes: Both rows recurved; lateral and median eyes distinctly elevated from carapace; row of AME wider than row of PME; ME quadrangle wider than long. Sternum: wider than long, orange-brown with black pigmentation; covered with black macrosetae (Fig 1C). Labium: twice as wide as long, orange-brown, front end rounded and with white rim. Chelicerae: orange, apically darker, 4 promarginal and 3 retromarginal teeth. Leg formula 1243; femora orange-brown with blurry dark annulations and very dark brown apically, tibiae brown with light annulations highlighted by white setae; brown metatarsi and tarsi.

Abdomen: Reddish brown in appearance, triangular to sub triangular, longer than wide, with a pair of prominent pointed humeral humps (Fig 1D); numerous small orange-brown sclerotized plates; prominent sigilla. Epigynum: sinuous anterior rim (Fig 1F), median sclerotized part pentagonal; large, round spermathecae (Fig. 1G).

Measurements (in mm): TL 16.75, CL 7.13, CW 6.25, Clypeus height 0.33. Sternum (length/width) 3.15/3.28. Labium (length/width) 0.90/1.65. AL 10.38, AW 11.75.
Eye diameter: AME 0.27, ALE 0.19, PME 0.23, PLE 0.22.
Inter-ocular distance: AME – AME 0.405, AME – ALE 0.562, AME – PME 0.682, PME –PME 0.393, PME – PLE 0.583.
Variation (range, mean±SD): TL 12.30 ±17.5, 14.55 ± 2.14; CL 5.45 ± 7.70, 6.25 ± 0.98; CW 4.97 ± 7.13, 5.70 ± 0.86; n=5.

Table 1. Leg and pedipalp measurements in *C. unicolor*

Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
I	7.58	3.13	5.33	5.75	2.15	23.94
II	7.18	3.03	4.93	5.38	2.13	22.65
III	4.50	2.00	2.50	2.75	1.79	13.54
IV	6.63	2.88	3.89	4.78	1.88	20.06
Pedipalp	2.38	1.23	1.52	-	2.63	7.76

Male: Male was unknown until Framenau (2018) discovered it from Christmas islands in Australia.

Cephalothorax: Cephalic region protruding over clypeus; fovea longitudinal; sparse black setae. Eyes: Both rows of eyes recurved; MOQ wider than long. Sternum: wider than long, orange-brown; covered with few black macrosetae. Labium: twice as wide as long, dark brown, front end rounded and with white rim. Chelicerae: orange-brown with black pigmentation; three very small retromarginal teeth and four promarginal teeth. Leg formula 1243; legs orange-brown with dark annulations.

Abdomen**:** Oval in dorsal view, posterior end elevated into a distinct tip; posterior tip very dark olive-gray; covered with a few silver setae, setal sockets dark brown; ventrum milky-gray with two large lighter patches; spinnerets orange-brown with dark pigmentation. Palp: median apophysis appears as a mesally directed hook; embolus sickle-shaped with broad base; terminal apophysis with distinct sclerotized ridges (Fig. 1H).

Distribution: *Cyrtophora unicolor* is known from Southern Taiwan (Chen & Tso, 2004), China (Song *et al*. 1999) and Japan (Miyashita, 2002) in the north, Papua New Guinea (Framenau, 2008) and the Philippines (Barrion & Litsinger, 1995) as its eastern border, Christmas Island (Framenau, 2008) and Indonesia (Hasselt, 1882; Pocock, 1897; Chrysanthus, 1959) to the South, and Thailand (Karsch, 1878), Sri Lanka (Pocock, 1900) and Myanmar (Thorell, 1895; Pocock, 1900) and India (Pocock, 1900; Elizabeth *et al*., 2014; Malamel, 2018) in the East.

India: Sikkim (Pocock, 1900), Kerala (Elizabeth *et al*., 2014; Malamel, 2018).

3.2. DNA Barcoding

PCR products were easily produced and aligned as no insertions, deletions or stop codons were observed and 1st frame of DNA sequences were chosen from ORF finder for submission. The visualized PCR product contained only discrete single bands, indicating that sequences obtained were mitochondrial DNA and not nuclear pseudogenes. Sequence of *C. unicolor* was submitted on NCBI GenBank with accession number KJ361518.1.
The sequence is

>***Cyrtophora unicolor***
ACGTTATATTTAGTATTTGGAGCTTGAGCCTCTATAGTGGGGACAGCTATAAGAGTTTTAATC
CGTGTAGAATTAGGACAACCTGGAAGGTTTATTGGGGATGACCAACTTTACAATGTAATTGT
CACTGCCCATGCTTTTGTTATAATTTTTTTTATAGTTATGCCTATTTTAATTGGGGGATTCGGT
AATTGGCTGGTCCCTCTAATGTTAGGTGCCCCAGATATGGCATTTCCTCGAATAAATAATTTA
AGTTTTTGGTTATTACCTCCTTCCTTATTTCTTTTGGTCATTTCCTCTATAGTCGAGATGGGGG
TAGGGGCAGGTTGAACTATTTACCCTCCTTTGGCGAGACTAGAAGGCCATTCTGGGAGGTCC
GTGGATTTTGCCATTTTTTCTCTTCATTTAGCTGGGGCTTCGTCTATTATAGGTGCTATCAATT
TTATTTCTACCATTATTAATATGCGGTCTTACGGGATAGGTATAGAAAAAGTTCCCCTCTTTG
TGTGGTCCGTATTAATTACCGCAGTTCTTTTACTTTTATCTTTACCCGTTCTAGCAGGAGCTAT
CACTATATTATTAACGGATCGAAATTTTAATACCTCTTTTTTTGACCCTTCGGGAGGGGGTGA
TCCTATTTTATTTCAACATTTATTT

In *C. unicolor* humeral humps and red sclerotized plates on dorsal abdomen are the distinguishing features. Sigilla are prominent. Sinuous rim of epigyne is another distinct feature. Spermathecae are large and round (Framenau, 2008). *Cyrtophora unicolor*, like *C. moluccensis* and *C. bidenta*, prefers serene habitats. Web is situated at a height of 5m – 15m. It finds retreat in dry, curled leaf at the hub of the web, which is a unique feature of the species. *Cyrtophora unicolor* was reported for the first time from the Western Ghats. This was also the first confirmed report for the species from India in more than 100 years. Pocock documented the species from northeastern India as early as 1900, but the species was not cited by Tikader (1982) in his monograph on Indian spiders. Siliwal *et a*l. (2005), Mathew *et al*. (2008) and Keswani *et al*. (2012) too have not cited this species. The current study confirmed Pocock's report. This is only a range extension since this species is confirmed for Sri Lanka and a few other neighbouring countries viz. Myanmar, Thailand, Taiwan and Philippines (Song *et al*., 1999; Tso & Chen, 2004). Considering the presence of the species in Sri Lanka, the report from the Western Ghats is not surprising.

CONCLUSION

Cyrtophora unicolor is different from other congeners in morphology and web architecture. The species is characterized by a pair of shoulder humps and numerous orange-brown sclerotized plates on the abdomen. Epigyne has a sinous rim and distinct sclerotized epigyne that has a pentagonal central part. The species builds webs high above the ground – between 5 to 15m. The spider takes retreat in a dry curled leaf at the hub. DNA tools can significantly complement taxonomic studies. This is an effort towards assembling the spiders of India into a universal library of DNA barcodes. The combination of traditional taxonomy and molecular data is a good model to adopt.

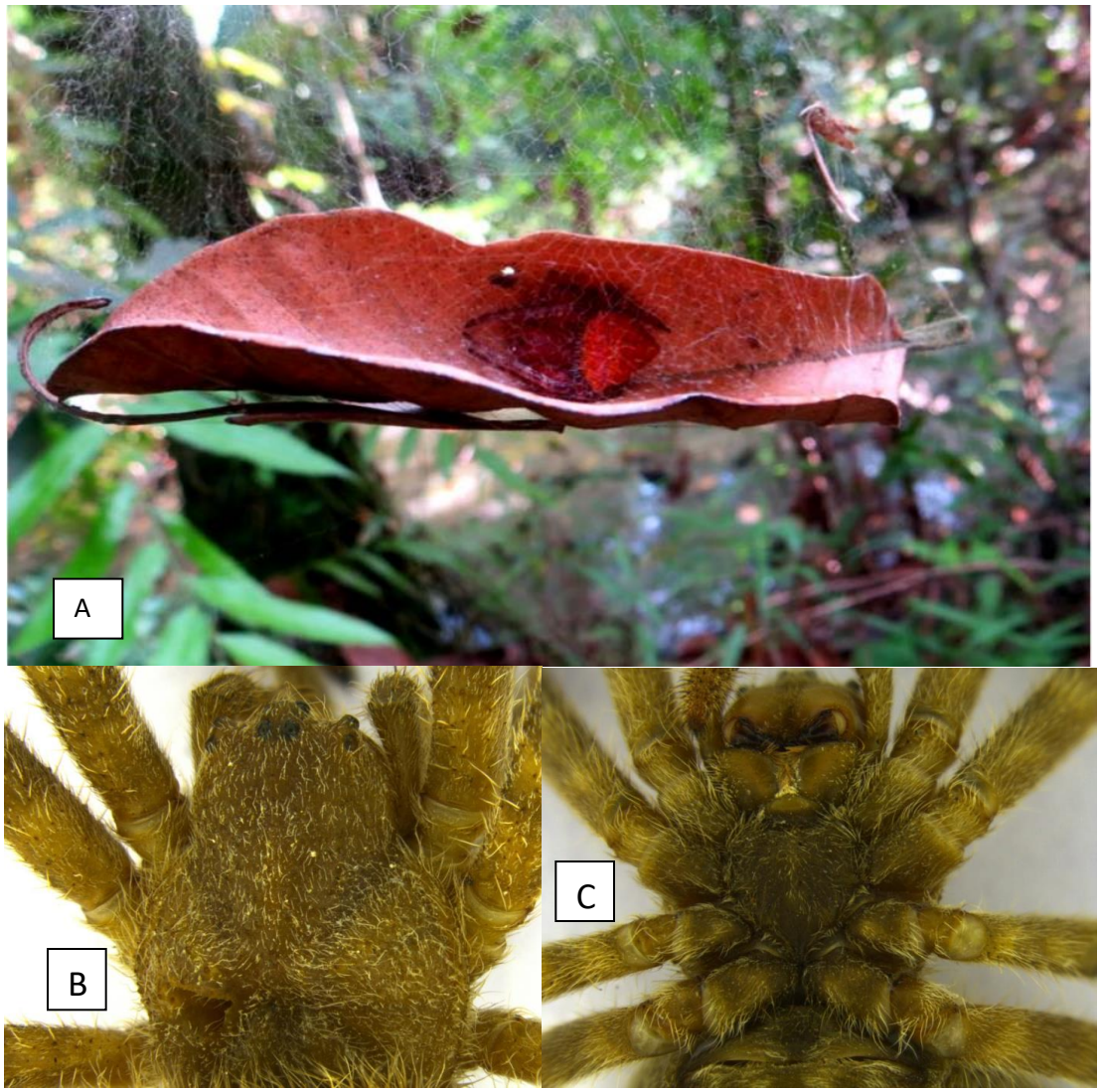
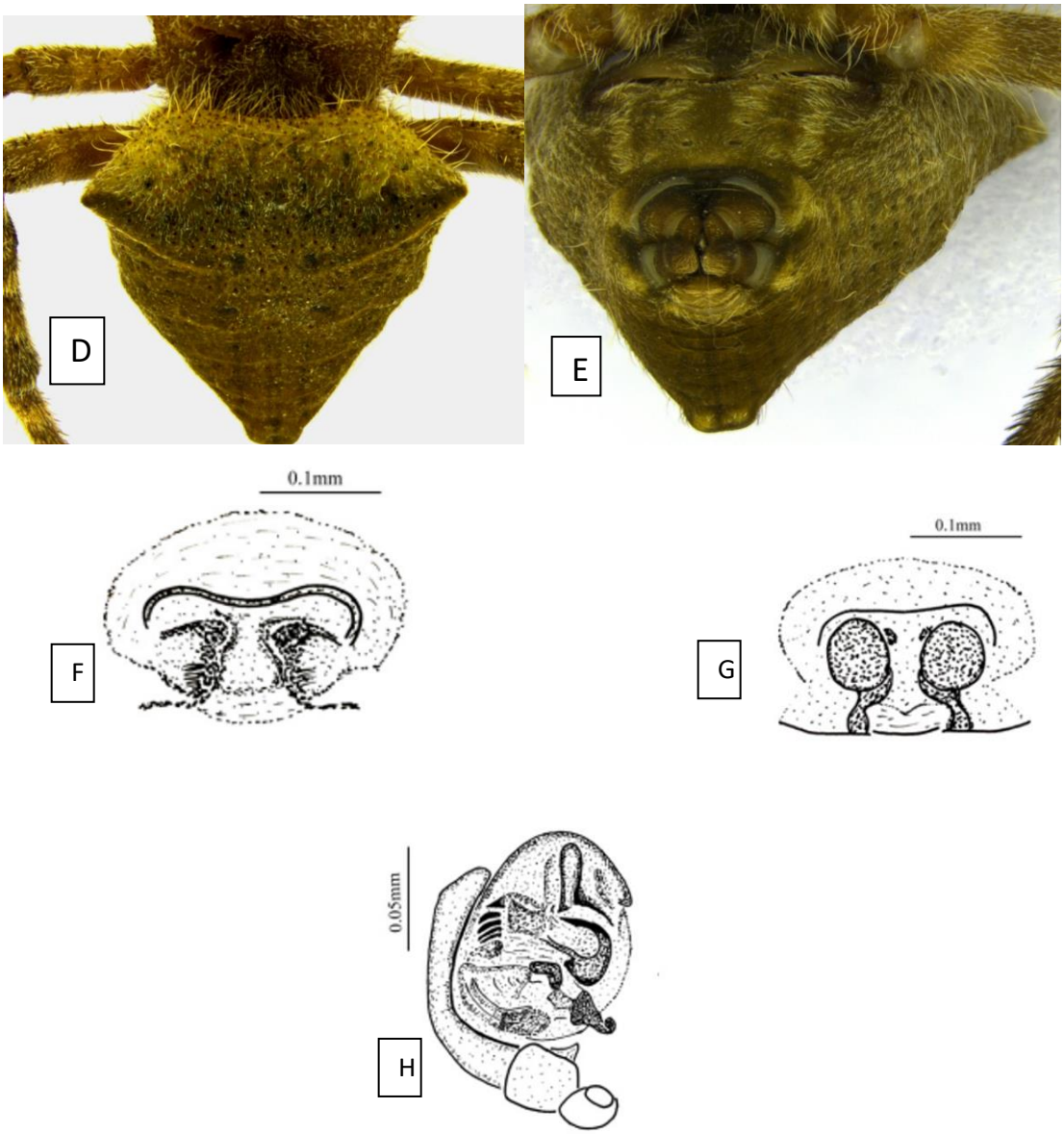


Figure 1: Morphological features of *Cyrtophora unicolor* *A – Spider in its curled leaf retreat at the centre of the web, B - Cephalothorax, C – Sternum*



Contd…* Figure 1: Morphological features of *Cyrtophora unicolor *D – Abdomen (dorsal), E – Abdomen (ventral), F – epigyne (external view), G – vulva, H – pedipalp (retrolateral view)*

Abbreviations
TL – Total Length
CL – Cephalothorax length
CW – Cephalothorax Width
AL – Abdomen Length
AW – Abdomen Width
AME – Anterior Median Eyes
ALE – Anterior Lateral Eyes
PME – Posterior Median Eyes
PLE – Posterior Lateral Eyes

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