MOLECULAR TAXONOMY AND PHYLOGENY OF ORB WEAVING SPIDERS (ARANEAE: ARANEIDAE) IN KERALA

Thesis submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

Under the Faculty of Science

UNIVERSITY OF CALICUT

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December 2025

DECLARATION

I, **SHILPA K. R.**, hereby declare that the work presented in the thesis titled, "**MOLECULAR TAXONOMY AND PHYLOGENY OF ORB WEAVING SPIDERS (ARANEAE: ARANEIDAE) IN KERALA**" submitted to the University of Calicut in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Zoology is a bonafide record of the research work carried out by me under the supervision of **Dr. Sr. ANIS K. V.**, Assistant Professor (Retd.), Department of Zoology, St. Joseph's College (Autonomous), Irinjalakuda and **Dr. SUDHIKUMAR A. V.**, Associate Professor, Department of Zoology, Christ College (Autonomous), Irinjalakuda, affiliated to the University of Calicut. No part of the thesis has formed the basis for the award of any degree, diploma, or similar titles of any university. The contents of the thesis have undergone a plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index was found to be within the permissible limit. I also declare that this thesis is free from AI-generated content.

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Shilpa K. R.

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ABBREVIATIONS

+G	Gamma distribution
+I	Proportion of Invariable sites
AER	Anterior Eye Row
AGE	Agarose Gel Electrophoresis
AIC	Akaike Information Criterion
ALE	Anterior Lateral Eye
AME	Anterior Median Eye
BI	Bayesian Inference
BIC	Bayesian Information Criterion
BOLD	Barcode of Life database
COI	Cytochrome Oxidase subunit I
ESS	Effective Sample Size
GTR	General Time Reversible
H3	Histone 3
HKY	Hasegawa-Kishino-Yano model
HPD	Highest posterior density
K2P	Kimura 2-Parameter
LE	Lateral Eyes
PME	Posterior Median Eyes
lnL	Maximum Likelihood value
MA	Median Apophysis
MCMC	Markov Chain Monte Carlo
ME	Median Eyes
ML	Maximum Likelihood
MP	Maximum Parsimony
MSA	Multiple Sequence Alignment
Mya	Million years ago
NCBI	National Center for Biotechnology Information
NJ	Neighbour Joining
PCR	Polymerase Chain Reaction
PER	Posterior Eye Row
PLE	Posterior Lateral Eye
PME	Posterior Median Eye
TIM2	Transitional model 2
TIM2ef	Transitional model 2 (equal frequencies)
TIM3	Transitional model 3
TN93	Tamura-Nei 1993 model
TPM2	Transitional Pseudo-model 2
TrN	Tamura and Nei model

Nothing in biology makes sense except in the light of evolution

- Theodosius Dobzhansky

സംഗ്രഹം

വട്ടവല ചിലന്തികൾ എന്ന പേരിൽ വ്യാപകമായി അറിയപ്പെടുന്ന അരാനിഡേ വിഭാഗം ലോകത്തിലെ മൂന്നാമത്തെയും, ഇന്ത്യയിലെ രണ്ടാമത്തെയും വലിയ ചിലന്തി കുടുംബത്തെ പ്രതിനിധീകരിക്കുന്നു. വൈവിധ്യമാർന്ന ആവാസ വ്യവസ്ഥകളിൽ വസിക്കുന്ന ഇത്തരം ചിലന്തികൾ, അന്റാർട്ടിക്ക ഒഴികെയുള്ള ലോകത്തിലെ മറ്റെല്ലാ ഭൂപ്രദേശങ്ങളിലും സാധാരണയായി കാണപ്പെടാറുണ്ട്. ആൺ, പെൺ ചിലന്തികളുടെ വലുപ്പത്തിലുള്ള വ്യത്യാസം, ഒരേ വർഗ്ഗത്തിൽപ്പെടുന്ന ചിലന്തികളുടെ നിറത്തിലും, ഉദരത്തിലുമുള്ള വ്യത്യാസങ്ങൾ, ക്രിപ്റ്റിക് സ്പീഷീസുകൾ എന്നിവയെല്ലാം അരാനിഡേയുടെ വർഗ്ഗീകരണത്തെ സാരമായി സ്വാധീനിക്കുന്നുണ്ട്. ആയതിനാൽ ഈ മേഖലയിൽ ശാസ്ത്രീയമായ പഠനങ്ങളും, പുനരവലോകനങ്ങളും അനിവാര്യമാണ്. കേരളത്തിലുള്ള ഈ ചിലന്തി കുടുംബത്തിൻറെ വർഗ്ഗീകരണത്തിലും ജനിതക വിവരങ്ങളിലും പരിണാമ പഠനത്തിലും ഗണ്യമായ സംഭാവന നൽകുക എന്ന ലക്ഷ്യത്തോടെയാണ് ഈ പഠനം പ്രതിഫലിപ്പിക്കുന്നത്. ചിലന്തികളുടെ വിശദമായ രൂപശാസ്ത്ര പഠനവും COI, H3 ജീനുകളുടെ ബാർകോഡിംഗും സംയോജിപ്പിച്ചുകൊണ്ട് 23 ജീനസുകളിൽപ്പെട്ട 59 സ്പീഷീസുകളെ കേരളത്തിൽ നിന്നും രേഖപ്പെടുത്തുവാനായി സാധിച്ചു. ലെവിയരാനിയസ് വിരിഡിവെൻട്രിസ്, കോറിസോപെസോയിഡ്സ് ഓറിയന്റാലിസ് തുടങ്ങിയ രണ്ടിനം ചിലന്തികളെ ആദ്യമായി ഇന്ത്യയിൽ നിന്ന് റിപ്പോർട്ട് ചെയ്തു. വികസിപ്പിച്ചെടുത്ത 109 ബാർകോഡുകളിൽ, 38 ബാർകോഡുകൾ ജീൻ ബാങ്കിലേക്ക് പുതിയ കൂട്ടിച്ചേർക്കലുകളായിരുന്നു. രൂപഘടനയിൽ വെല്ലുവിളികൾ ഉയർത്തുന്ന ചില സ്പീഷീസുകളുടെ സ്വത്വവും, നെഫില പിലിപസിൽ കളർ മോർഫിന്റെ സാന്നിദ്ധ്യവും സ്ഥിരീകരിച്ചു. മൂന്ന് ജോഡി ക്രിപ്റ്റിക് സ്പീഷീസുകളെ തിരിച്ചറിഞ്ഞു. നെയ്ബർ ജോയിനിങ്, മാക്സിമം പാർസിമോണി, മാക്സിമം ലൈക്ലിഹുഡ്, ബയേഷ്യൻ ഇൻഫെറെൻസ് എന്നിവയുടെ അടിസ്ഥാനത്തിൽ അരാനിഡേയുടെ പരിണാമ ചരിത്രവും, ബന്ധവും പുനർ നിർണ്ണയിച്ചു. നെഫിലിനുകളുടെയും, സിജിലിനിയകളുടെയും സ്ഥാനവും, മോണോഫൈലിയും സ്ഥിരീകരിച്ചു. അക്കുസിലാസ്, അരക്നുറ, ആർജിയോപിനേ, ബിജോഅരാനീയസ്, അരാനിയെല്ല, പ്ലെബ്സ് എന്നിവയുടെ മോണോഫൈലെറ്റിക് ഉത്ഭവം വെളിപ്പെടുത്തി. അരാനീയസ്, സൈക്ലോസ എന്നിവയുടെ പോളിഫൈലിയും സ്ഥിരീകരിക്കുവാൻ സാധിച്ചു. അരാനിഡ ചിലന്തികളുടെ പരിണാമ വ്യതിയാന സമയം കണ്ടെത്താനുള്ള ആദ്യ ശ്രമമായി ഈ പഠനത്തെ രേഖപ്പെടുത്താം. സ്പീഷീസുകളുടെ സ്വത്വം നിർണ്ണയിക്കുന്നതിൽ COI ബാർകോഡിംഗിന്റെ വിശ്വാസ്യത അടിവരയിട്ടുറപ്പിക്കാം. ഒന്നിലധികം ജനിതക മാർക്കറുകൾ സംയോചിപ്പിച്ച് പരിണാമ ബന്ധങ്ങൾ പരിഹരിക്കാൻ സാധിക്കുമെന്ന് ഈ പഠനം തെളിയിക്കുന്നു.

പ്രധാന പദങ്ങൾ: ബാർകോഡിംഗ്, ഡൈവേർജൻസ് സമയം, വട്ടവല ചിലന്തികൾ, സിസ്റ്റമാറ്റിക്സ്, കേരളം

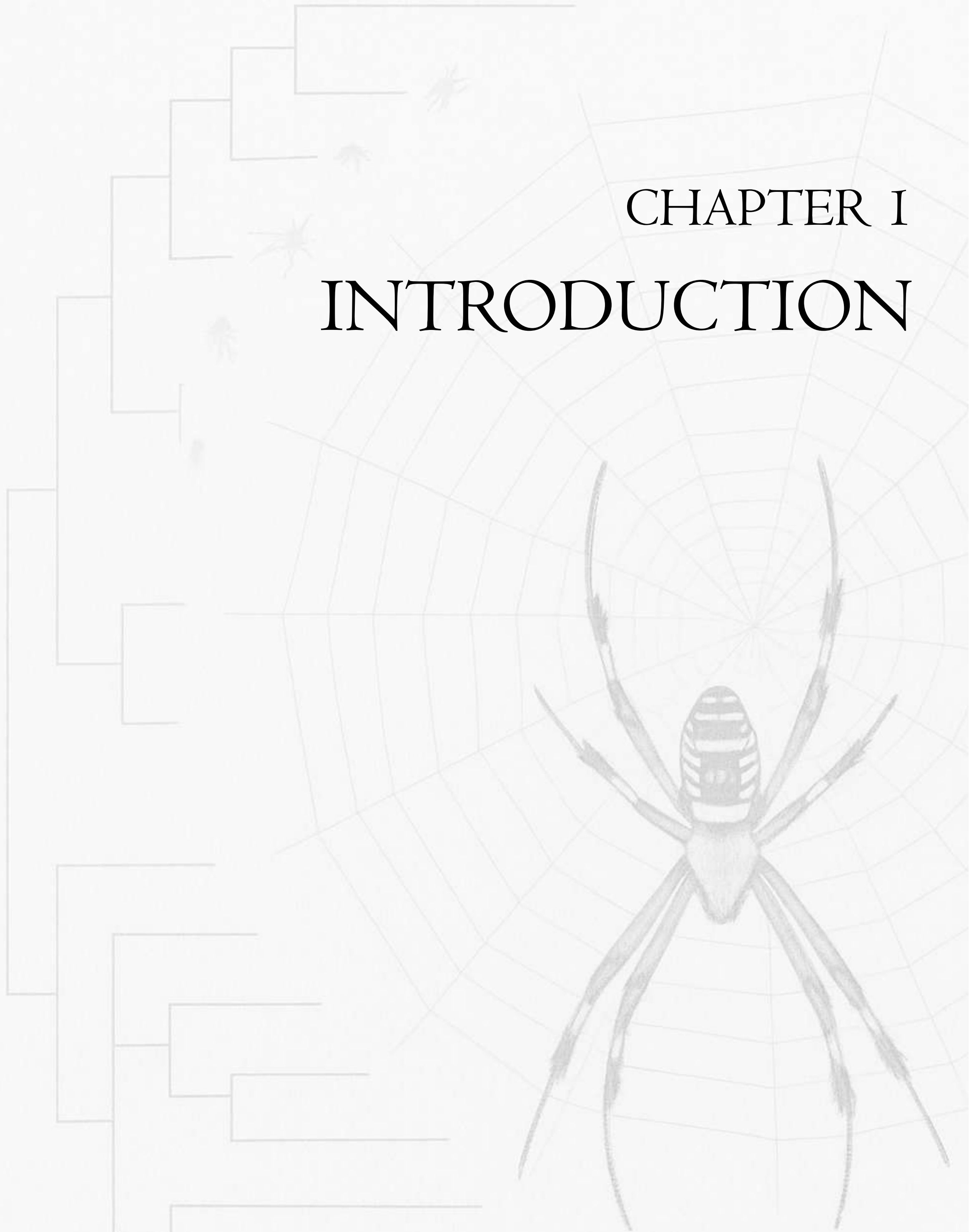
ABSTRACT

The family Araneidae, commonly known as orb-weaving spiders, represents the third-largest spider family in the world and the second-largest in India. These spiders inhabit a wide range of ecosystems and are distributed across all landmasses except Antarctica. Ongoing revisions are necessitated by challenges such as extreme sexual size dimorphism, colour polymorphism, and abdominal variability among conspecifics, as well as the existence of cryptic species. This study presents a focused investigation on the araneids of Kerala, aiming to make a substantial contribution to the taxonomy, molecular data, and phylogenetic understanding of the group. Detailed morphological examination and barcoding of COI and H3 genes have resulted in an updated checklist of araneids in Kerala, including 59 species belonging to 23 genera. *Leviaraneus viridiventris* and *Chorizopesoides orientalis* were reported from India for the first time. A total of 109 barcodes were generated, among these, 38 were new additions to GenBank. Species identity of certain morphologically challenging species and the existence of colour morphs in *Nephila pilipes* were confirmed. Three pairs of cryptic species were identified. Phylogenetic trees were reconstructed using different methods such as Neighbour Joining, Maximum Parsimony, Maximum Likelihood and Bayesian Inference. The placement and monophyly of nephilines and zygiellines were confirmed. The monophyletic origin of *Acusilas*, *Arachnura*, Argiopinae, *Bijoaraneus*, *Araneilla*, and *Plebs* was revealed. The genera *Araneus* and *Cyclosa* exhibited polyphyly. The study is the first comprehensive, successful attempt to date the divergence of araneids. The present study underscores the effectiveness of COI barcoding in species-level identification and demonstrates the value of incorporating multiple genetic markers to resolve phylogenetic relationships.

Keywords: Barcoding, Divergence time, Orb web spiders, Systematics, Kerala

CHAPTER I

INTRODUCTION



"Biodiversity, the planet's most valuable resource, is on loan to us from our children"

- ***Edward O. Wilson***

Life is the most fascinating facet of our planet, taking different forms, from microscopic prokaryotes to giant whales and intelligent humans. These life forms have developed exceptional chemical, physical, and structural characteristics that play a crucial role in the functioning of Earth's ecosystems (Patrick, 1997). The totality of life on Earth can be referred to as biodiversity. Since the term 'biodiversity' was first introduced at the National Forum on Biodiversity held in Washington, D.C., in 1986, numerous scientists have proposed various definitions of the concept, tailored to different contexts. Among them, the most widely accepted was proposed during the UN Conference on Environment and Development in 1992. "Biodiversity is the variability among living organisms from all sources, including inter alia, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species, and of ecosystems" (Convention on Biological Diversity, 1995, Article 2). Though each definition relies on a different perspective, all underline three fundamental biodiversity components - composition, structure, and function (Groves et al., 2002).

A major question that preoccupied environmentalists and naturalists during the 1990s was the significance of biodiversity. Biodiversity holds importance across economic, scientific, ecological, cultural, and recreational dimensions (Mortan & Hill, 2014). Estimating the planet's total magnitude of biological diversity is an immensely challenging task. The number could range from millions to trillions. Each day, new species emerge while others face extinction. Scrupulous characterisation at each level of organisation is key to accurate biodiversity analysis, as every natural community presents distinct features in both species composition and abundance (Lovejoy, 1997). Managing this valuable diversity would be impossible without a thorough understanding of life forms at all levels. Scientists and biodiversity enthusiasts are diligently working to characterise, validate, and quantify the full spectrum of life.

1.1. Spiders: A megadiverse arachnid lineage

Land-dwelling arthropods represent the most prevailing and successful group of animals, accounting for 97% of global biodiversity (Lovejoy, 1997). One among them is spiders, which belong to the order Araneae of the class Arachnida under the subphylum Chelicerata. Araneae holds the status of a mega-diverse order of Arachnida, alongside the order Acari, and ranks as the 7th largest animal order (Sebastian & Peter, 2009) with 52536 accepted species under 4406 genera (World Spider Catalog [WSC], 2025). Spiders reside in a remarkable range of environments, spanning from sweltering parched deserts to dense rainforests and swampy mangroves, as well as from intricate hypogean layers to windswept alpine zones. The presence of an exoskeleton, a segmented body, and jointed appendages are plesiomorphic traits shared by these predatory organisms and other arthropods. The abdominal silk glands and spinnerets, the silk-producing organ, along with the modified pedipalps in males, contribute to the distinctiveness compared to other Arachnida orders, such as Pseudoscorpions, Opiliones, Amblypygi, Scorpions, Uropygi, Palpigradi, Ricinulei, Schizomida and Solifugae (Platnick, 2020). Spider fossils can be traced back to the Devonian period (Penney & Selden, 2011; Selden & Penney, 2010).

The pronounced morphological features underpin their evolutionary success across the planet. Prosoma and opisthosoma, two prominent body parts of a spider, are linked by a tubelike structure called the pedicel. The prosoma, also called as cephalothorax possess the taxonomically significant features such as eyes, fovea, chelicerae, and jointed appendages. The number of eyes and its arrangement, along with characteristic structures like spikes, lumps, and surreal extensions, have key taxonomic roles. Chelicerae, the structure helping in predation, are located on the ventral side of the prosoma. They have teeth and a sharp fang, which has an opening of the venom gland and used to inject the venom. The ventrum of the cephalothorax also features the mouthparts and sternum. Additionally, the cephalothorax holds one pair of palps and four pairs of seven-segmented walking legs. In male spiders, the palps are modified to function as intromittent organs, which will be species specific.

The opisthosoma, also called as abdomen, exhibits a wide-range of variations in shape and colouration. Ventral side of the abdomen hold epigastric furrow, openings of the book lungs, and the spinnerets. Female spiders have a specialised structure called the epigyne, external to the opening of the epigastric furrow, which complements the pedipalps of conspecific males. Spiders also have an alimentary canal with a specialized sucking stomach, an open circulatory system with the respiratory pigment hemocyanin, book lungs, and tracheae for respiration, and Malpighian tubules for the excretion. Their sensory structures include the eyes, tarsal organs, tactile hairs, trichobothria, slit organs, and proprioceptors.

Being a carnivore in the food chain, spiders mostly prey upon other arthropods, especially insects. Larger mygalomorphs can consume small birds and rodents using different predatory strategies. Spiders exhibit sexual dimorphism, primarily in colour and size. Female spiders are adapted to fulfil multiple roles, such as producing eggs and safeguarding the next generation, whereas males are primarily evolved to perform sperm transfer, essential for fertilization. This drives male spiders to develop competitive traits, relying on their skill and courage to find and secure a mate.

Upon maturity, male spiders must find and court a female by engaging in different courtship behaviours. Courtship behaviour across Araneae is diverse, ranging from web-tapping and vibrating to intricate courtship dances. Once mating is accomplished, the female spider lays eggs and encloses them in an egg sac. Protection strategies vary among species; some construct inconspicuous egg sacs that blend with their surroundings for camouflage, while others carry the egg sac with them. Once hatched, spiderlings grow to adulthood through a process called moulting. After a few initial moults, spiderlings disperse themselves through a method known as ballooning. During their juvenile and subadult stages, they focus on catching prey and evading predators, ultimately maturing into reproductive adults. To evade predators, they have developed different survival strategies.

Spiders may appear minor and irrelevant; nevertheless, they hold considerable ecological, economic, and cultural importance. Being the primary predators of insects, they have a fundamental role in preserving the ecological balance within both natural and agricultural ecosystems. Additionally, spiders pony up the food web dynamics, serving as prey for various other organisms. Two unique aspects of spiders, silk and venom, are particularly significant to humanity. Researchers continue to identify exceptional spider venom compounds that have promising medical applications. Similarly, the remarkable properties of spider silk inspire advancements in material science. Apart from this, spiders serve as model organisms in evolutionary, behavioural, and ecological research. Furthermore, as bioindicators, they provide valuable insights into environmental changes and ecosystem degradation.

1.2. Araneidae: Nature's web weavers

Not all spiders build a similar type of web. Web structure fluctuates across different groups of spiders, which helps in field identification at the family and genus levels to some extent. Orb web is the one that is observed commonly. The major group that weaves orb webs are the members of the family Araneidae, often referred to as orb-weaving spiders. Fossil records from the Lower Cretaceous pointed out likenesses between ancient and modern araneids (Penney & Ortuño, 2006). Araneids exhibit an extensive disparity in size, colour, shape, and behaviour, which promotes their distribution across continents. This large, globally dispersed group is generally found in all ecosystems with vegetation. To date, 3154 araneid species under 198 genera have been documented worldwide (WSC, 2025), making them the third largest spider family. Among them, 188 species belonging to 34 genera have been reported from India, including the endemic ones (Caleb & Sankaran, 2025).

Araneids employ a sit-and-wait predatory strategy, using their webs as home territories. When the prey becomes entangled, they quickly approach and subdue it through a spin-wrap-attack method (Sebastian & Peter, 2009). Although araneids generally construct orb webs and rest in a head-down position at the hub, web features such as shape, number of radials and spirals, hub design, and decorations vary among genera. A notable exception within this group is the genus *Cyrtophora* Simon, 1864, which builds tent webs instead of orb webs. Despite these variations, araneids share several defining features.

All araneids are three-clawed and ecribellate, having an entelegyne genitalia. They typically have eight eyes arranged in two rows, with the lateral eyes close together and widely separated from the medians, often situated on tubercles. Their chelicerae are strong and non-divergent. The epigyne is completely or partially sclerotised with a scape that varies in shape and length among genera, while the pedipalps feature a prominent median apophysis and haematodocha. The abdomen varies greatly in shape, colour, and texture, ranging from lightly chitinized, as in *Singa* C. L. Koch, 1836, to heavily, as in *Gasteracantha* Sundevall, 1833 often provided with humps and tubercles. Though not all araneids exhibit certain features that make the group more exciting. One among them is the construction of web decorations, known as stabilimentum. Extreme sexual size dimorphism and sexual cannibalism are particularly common among orb-weaving spiders (Elgar & Nash, 1988; Robinson & Robinson, 1980). The taxonomic hierarchy of araneids is as follows.

Kingdom – **Animalia** Linnaeus, 1758
Phylum – **Arthropoda** von Siebold, 1848
Subphylum – **Chelicerata** Heymons, 1901
Class – **Arachnida** Lamarck, 1801
Order – **Araneae** Clerck, 1757
Suborder – **Opisthothelae** Pocock, 1892
Infraorder – **Araneomorphae** Koch, 1850
Superfamily – **Araneoidea** Latreille, 1806
Family – **Araneidae** Clerck, 1757

Family Araneidae have been classified into different subfamilies: Araneinae, Argiopinae, Cyrtarachninae, Cyrtophorinae, Gasteracanthinae, Maastophorinae, Nephilinae, and Zygiellinae (Whyte & Anderson, 2017). Ongoing debates surround the classification and placement of certain groups within the family.

1.3. Taxonomy: Organizing life's complexity

Researchers continue to work on arranging the planet's astounding diversity to comprehend and manage it better. The theoretical study of classification, including its principles, procedures, and rules, is called taxonomy (Simpson, 1961). Although the practice of grouping organisms dates back to Aristotle, it gained widespread acceptance following the publication of *Systema Naturae* by Linnaeus in 1735. The Linnean system of classification, which relies on the morpho-anatomical features of organisms for their identification and categorisation, is now referred to as classical, traditional, or orthodox taxonomy (Martinez-Goss & Arguelles, 2020). The system works on the morphological resemblances and appearances of organisms. Despite being over two centuries old, the system continues to be widely accepted within the scientific community due to its hierarchical system of classification and the binomial system of nomenclature (Uno et al., 2001).

Linnaeus focused on common morphological features among organisms rather than their evolutionary relationships. Over time, the classification system evolved to incorporate the phylogenetic aspects, a field now known as systematics. As a core component of systematics, taxonomy remains popular for its simplicity, accessibility, and cost-effectiveness. Earlier, taxonomists figured out the identity of an organism by employing keys, comparing the images from the literature, and studying type specimens.

Taxonomical approaches to Araneae date back to the 18th century with Linnaeus, but the publication of Clerck's *Svenska Spindlar* in 1757 is considered the foundational work in arachnological classification (Platnick & Raven, 2013). The history of spider classification is enriched by the inputs of pioneering researchers such as Eugene Simon and Charles Athanase Walckenaer, among others. Initial studies on Indian spiders were done during the British colonial period by arachnologists like Octavius Pickard-Cambridge, Tamerlan Thorell, and Eugene Simon. Post-independence, Indian arachnologist B. K. Tikader emerged as a key figure, providing significant insights into the Indian spider fauna. It was further advanced by successors, who played a pivotal role in elevating Indian arachnology. While taxonomic practices developed rather slowly (Platnick & Raven, 2013) during the 20th century, advancements in microscopy and imaging techniques have revolutionised spider taxonomy. Araneidae, one of the families described by Clerck (1757), has marked its presence in numerous arachnological studies.

The classical taxonomic approach boosted arachnology but also presents several limitations. Spiders display complex morphological features, predominantly in their genitalia, which require specialised expertise to construe accurately. Environmental factors can influence morphological traits, resulting in phenotypic plasticity that muddles up species identification. Many early descriptions of spiders were terse and lacked detailed illustrations (Li, 2020), leading to taxonomic ambiguities. Furthermore, the loss of type specimens hinders the validation of species descriptions. The prevalence of cryptic species leads to underestimations of biodiversity. Extreme sexual dimorphism, a trait found among araneids, further complicates taxonomic efforts. Genera like *Araneus* Clerck, 1757 and *Neoscona* Simon, 1864 have been used as catch-all categories, leading to taxonomic inflation. These limitations contribute to frequent misidentifications, synonymies, and unnecessary splitting or lumping of taxa. Without accurate taxonomic understanding, assessing species distributions becomes difficult, which impedes comparative studies and conservation efforts.

1.4. Molecular approach: Taxonomic revolution

Taxonomy, whose ultimate goal is to characterise and arrange the vast diversity of life in an orderly manner (Trüper & Krämer, 1981), has grown substantially by incorporating various methodologies. Among these, the molecular approach has emerged as the most recent advancement, utilising tools such as DNA barcoding and genetic analysis. Unlike the classical approach, which relies on morphological features, the molecular taxonomy leverages integral biomolecules like DNA, RNA, and proteins to classify organisms. The genomes of all living organisms have short gene sequences that are conserved among conspecifics but show significant interspecific differences. DNA barcoding exploits these conserved markers to confirm the identity of organisms.

Although the concept of DNA barcoding dates back to the 1980s, it gained worldwide acceptance by the zoological taxonomists following the efforts of Herbert, Stoeckle and their colleagues (Hebert et al., 2003a, b; Stoeckle, 2003). DNA barcoding has proven to be a timely revolution in the history of taxonomy. Its efficacy extends beyond species identification to applications in phylogenetics, population genetics, species boundary delineation, genetic variability assessment, conservation biology, biodiversity studies, and community ecology (DeSalle & Goldstein, 2019; Fišer Pečnikar & Buzan, 2014; Purty & Chatterjee, 2016; Stoeckle, 2003). The prominent advantage of DNA barcoding is its consistency across all life stages of an organism, as the genome remains unchanged throughout its lifecycle. Moreover, cryptic species, often overlooked in traditional taxonomy, have been effectively resolved using molecular approaches. Incorporating molecular tools into spider taxonomy has settled issues related to phenotypic plasticity and sexually dimorphic forms.

However, despite its growing importance, molecular taxonomy cannot operate independently. DNA barcoding faces limitations in distinguishing recently diverged species and those arising from hybridisation events. Additionally, the absences of a universal barcode valid to all domains of life mean that specific groups of organisms necessitate distinct molecular markers. The robustness of DNA barcoding relies heavily on the establishment of reference sequences from taxonomically validated specimens. Therefore, a unifying approach that combines molecular methods with traditional taxonomy is essential for improving the accuracy of species identification and bridging gaps in our understanding of biodiversity (Miller, 2007).

1.5. Phylogeny: The story of ancestors to descendants

Theodosius Dobzhansky once stated, “Nothing in biology makes sense except in the light of evolution” (Dobzhansky, 1973). This context gained prominence as the field of phylogeny advanced. The Tree of Life conceptualises that all living domains originated from a single common ancestor. Through the action of evolutionary forces, as defined by Darwin (1859) in “*On the Origin of Species*”, these ancestors diversified to form the biodiversity we observe today. The likenesses between organisms reflect their evolutionary relatedness, and the field of biology that investigates these relationships is known as phylogeny.

Phylogeny seeks to estimate rates of speciation and extinction while exploring how and why these rates have varied across evolutionary time, geographical regions, and taxonomic groups. Understanding these dynamics is important for unravelling the ecological and evolutionary processes that create biological diversity (Morlon, 2014). Phylogenetic analysis delves into the evolutionary history of organisms, representing it in the form of a tree. In such trees, the root represents the ancestor, branches symbolise descendants, and each branching point signifies a speciation or divergence event. Darwin (1837) studied the morphological characteristics of a red coral and a marine organism and sketched the first evolutionary tree in his *Notebook B: Transmutation of Species*. Since then, substantial efforts across the globe have been dedicated to constructing a comprehensive Tree of Life.

The progression of phylogenetic research has offered profound insights into taxonomy, eventually leading to the emergence of systematics, a discipline that integrates taxonomy and phylogeny. In traditional taxonomy, phylogeny is implicit, whereas in systematics, it is explicit. Systematics encompasses various schools, including phenetics, which is based on the overall similarity or dissimilarity of characters, cladistics, focusing on synapomorphies and evolutionary systematics, which considers both ancestral and derived traits. Phylogenetic approaches have accelerated fields such as comparative physiology (Garland & Bennett, 2005), ecology (Wanntrop et al., 1990), diversification studies (Morlon, 2014), and extinction analysis (Purvis, 2008). Phylogeny has numerous applications, including studying character evolution, reconstructing ancestral genes, estimating biogeographic relationships, understanding the origin and epidemiology of human diseases, and prioritising species conservation (Hillis, 1997).

Initially, phylogenetic studies relied on morphological characteristics. Over time, statistical approaches enhanced the accuracy and reliability of results. The advent of DNA barcoding further revolutionised phylogenetic analysis. Along with the evolution of computer science, innovative methods to determine evolutionary relationships have been introduced (Horiike, 2016). Phylogenetic analysis involves three key components: selecting a dataset, establishing criteria to assess how well the data fit the candidate trees, and using pertinent methods to gauge and validate the results (Hillis, 1997). While morphological datasets often pose challenges due to the complexity of defining, delimiting, and ordering character states, molecular data has proven more robust and trustworthy. The databases such as the National Centre for Biotechnology Information (NCBI), DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL), and Barcode of Life Data (BOLD) systems has increased the accessibility of molecular datasets. However, the results of phylogenetic analysis highly depend on the datasets and methods. Using inappropriate methods and models can lead to false conclusions, highlighting the importance of methodological rigour in phylogenetic research.

1.6. Divergence time: Milestones of evolutionary change

The estimation of divergence time has evolved along with phylogenetic analysis. Divergence time is the point in the evolutionary history at which two lineages diverged from their most recent common ancestor, indicating the age of speciation or other evolutionary events. The degree of difference between two species is proportional to the time passed since their evolutionary separation (Zuckerkandl & Pauling, 1965). By combining morphological data, molecular data, fossil records and geological information, researchers can build time-calibrated phylogenies using computational algorithms.

Divergence time evaluation plays a significant role in understanding the age of evolutionary events, reconstructing biogeographic histories, linking evolutionary changes with environmental or geological events. The dating of evolutionary divergences using variations was first presented in 1965 (Zuckerkandl & Pauling, 1965). Since then, various studies gave the divergence time of different organisms, thereby progressing the Tree of Life. This progress has incredibly improved diverse fields, ranging from epidemiology to community ecology. Technological and computational developments have refined divergence time assessment. Software such as BEAST, MrBayes, and RAxML employs advanced algorithms, including Bayesian inference and maximum likelihood, to construct and calibrate phylogenetic trees. Additionally, relaxed molecular clock models have enabled researchers to accommodate rate heterogeneity, addressing one of the key challenges in molecular evolution.

Both morphological and molecular datasets can be considered in divergence time analysis. In morphological approaches, traits are coded as discrete characters, assuming a relatively steady rate of character evolution. However, the utility of morphological data is limited due to non-clock-like evolutionary patterns, subjective interpretation, and sparse fossil evidence. On the other hand, molecular datasets are preferred for their reckonable rates of change, higher resolution, and objective nature, making them generally more reliable for divergence time estimation.

1.7. Significance of the study

This study focuses on the molecular taxonomy and phylogeny of Araneidae in Kerala, India. Although members of the family are cosmopolitan and frequently recorded in spider diversity studies, their taxonomic status remains chaotic. Even though it is the third-largest spider family, traditional taxonomic approaches are limited by certain challenges, including sexual dimorphism, cryptic species, inadequate illustrations, ambiguous diagnostic features, and lack of timely revisions. Hence, error prone genus-level groupings and incorrect placement of species are common in this family. Advances in molecular tools, such as DNA barcoding and phylogenetic analysis are helping the researchers to resolve these taxonomic ambiguities.

This study utilizes these tools to make an accurate and stable classification of Araneidae in Kerala. Kerala, one of the biodiversity rich states, offers a unique opportunity to enrich the understanding of spider diversity, and the findings of this study can help in future investigations. The major outcome of this research is the development of an updated checklist of araneids in Kerala, which contributes to the database of Indian spiders. Moreover, the incorporation of phylogenetic and divergence time analyses has enriched the understanding of evolutionary history of araneids in Kerala.

Also, the phylogenetic findings of this study can serve as a foundation for formulating hypotheses about the evolutionary and biogeographical patterns of the family. Thus, this study provides a foundation for the molecular taxonomy and phylogeny of araneids and highlights the need for more detailed investigations into this ecologically and scientifically significant spider family.

1.8. Objectives of the study

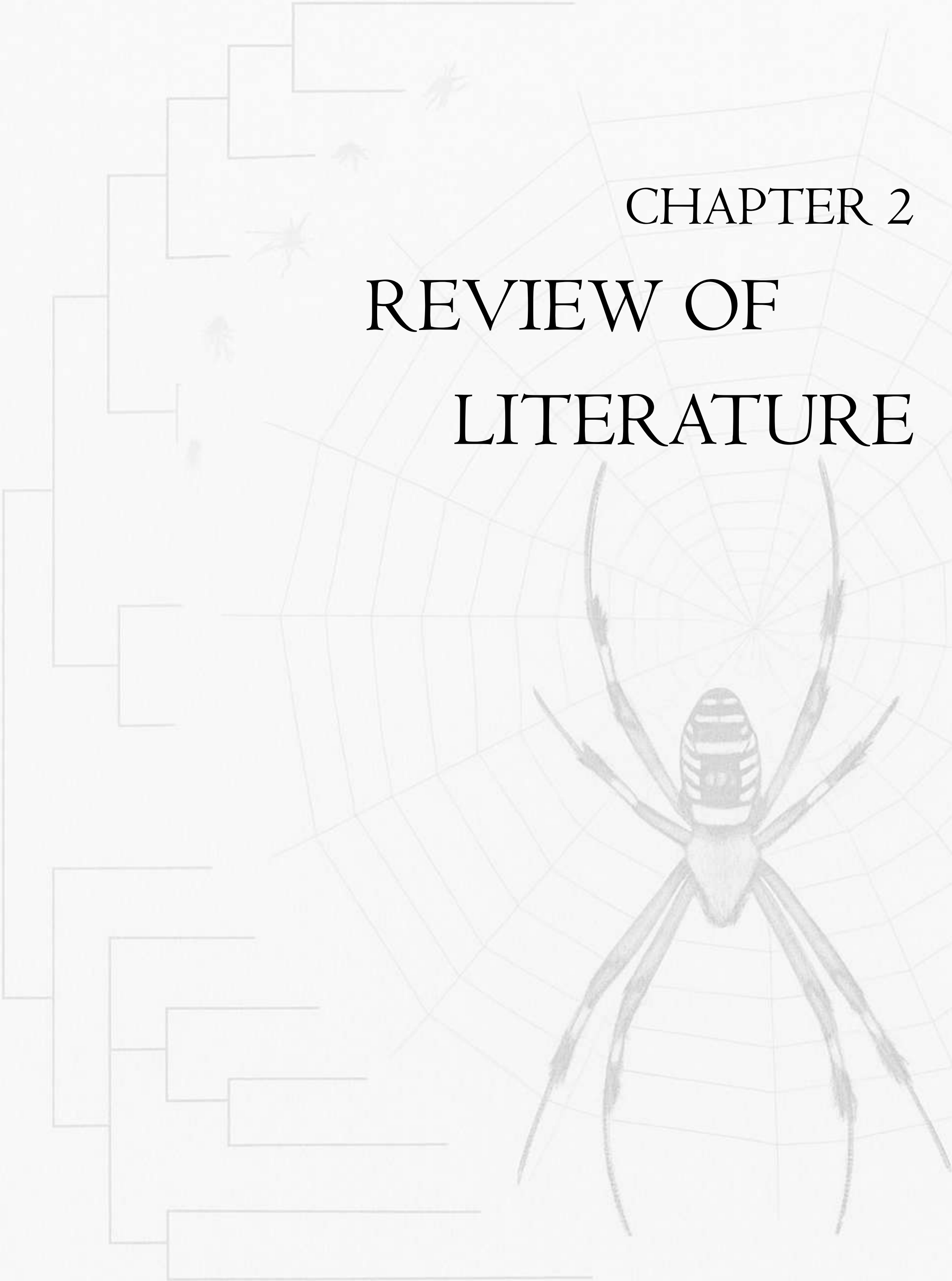
1. Prepare a checklist of araneids found in Kerala.
2. Barcoding of Araneidae of Kerala using molecular markers.
3. Study the interrelationships between collected and identified members of Araneidae in Kerala.
4. Study the relationship of the araneids of Kerala with other araneids across the Oriental and Palearctic regions.
5. Study the divergence time of the family Araneidae.

1.9. Limitations of the study

Kerala, one of the Indian states rich in biodiversity and home to diverse ecosystems, spans an area of 38,863 sq. km. (Kerala Soil Survey, 2024). Conducting periodic visits to all ecosystems within a limited timeframe of three years posed significant challenges. Therefore, a random selection of sampling was undertaken to represent all districts and ecosystem types in Kerala. Identification of certain specimens was restricted to the genus level due to the lack of type specimen information, inadequate diagnostic features, and the absence of comprehensive keys. During DNA barcoding, the validated reference sequences were limited in the existing databases. The rarity of some species, such as *Arachnura angura* Tikader, 1970, *Paraplectana gravelyi* (Tikader, 1961), and *Cyrtophora citricola* (Forsskål, 1775), further limited the study. Molecular analyses were also limited by the less availability of specimens and unsuccessful amplification.

CHAPTER 2

REVIEW OF LITERATURE



“***If I have seen further, it is by standing on the shoulders of giants***”

\- ***Isaac Newton***

The present study was undertaken to advance the taxonomic understanding of the family Araneidae in Kerala, India. Conducting a broad literature review is an essential part of the research process, as it allows researchers to understand extant knowledge and identify gaps in the field, and improve the methodologies effectively.

Spiders are one among the dominant predators in terrestrial ecosystems and they have been dispersed and survived across different habitats utilising their morphological and behavioural adaptations. The taxonomic studies of spiders have a history of approximately 260 years. Arachnologists have used the advancements in methodologies, integrating new microscopy and molecular techniques, and greatly improved the accuracy and scope of spider taxonomy.

Araneids are always documented in spider diversity studies across different ecosystems. Hence the need for a thorough evaluation of their taxonomic status is high. Identifying gaps in the literature has helped in designing the current study, aiming to resolve taxonomic ambiguities and enrich the understanding of evolutionary relationships among araneids. In Kerala, being one among the biodiversity rich state, such an effort necessary as it will make significant contributions in defining the evolutionary trajectories of araneids. This section begins with a review to provide the current taxonomic status of araneids, followed by a search on how DNA barcoding has contributed in araneid classification. Afterward, an extensive analysis of existing phylogenetic research, encompassing both morphological and molecular approaches, is presented.

2.1. Global and regional diversity of araneids

The spider family Araneidae, commonly known as orb-weavers, represents one of the most diverse and widely distributed groups of spiders. Their ability to build intricate, wheel-shaped orb webs exemplifies remarkable evolutionary adaptations. Morphologically, araneids exhibit a wide range of body sizes and colours, often characterised by distinctive abdominal patterns. Their web-building behaviour has not only inspired advancements in biomimetic research (Naderinejad et al., 2023; Regassa et al., 2021) but has also contributed to space research (Zschokke et al., 2021).

Araneidae is the first described spider family (Clerck, 1757). He proposed the genus *Araneus* and characterised 70 species within it. According to the current araneid classification, 17 of these species, now distributed across six genera, are valid (WSC, 2025). A significant contribution was made by Eugene Simon (1864 – 1903), who described 42 genera and 212 species under the family Araneidae. Being the third largest family, with 3131 species and 191 genera globally (WSC, 2025), araneids are found in almost all ecosystems with vegetation. The diversity of araneids was reported during several explorations across the world. A notable attempt was made by Herbert W. Levi (1964 – 2013) in the Neotropics. Other attempts were from Southeast Asia (Barrion & Litsinger, 1995), China (Yin et al., 1997, 2012), Canada (Dondale et al., 2003), Portugal's Serra da Estrela Natural Park (Sousa, 2006), Socotra Archipelago (Grasshoff & van Harten, 2007), Japan (Tanikawa, 2007, 2009), Turkey (Türkeş & Mergen, 2008), Southern Brazil (Rodrigues & Mendonça Jr., 2011), Korea (Kim & Lee, 2012), France (Lecigne, 2021), South Africa (Dippenaar–Schoeman et al., 2022a, 2022b), Spain (Morano, 2023), and Europe (Ibrahimi et al., 2024).

A comprehensive study exclusively focused on Indian araneids was done by Tikader (1982), including 92 species from 30 genera. Spider explorations done in West Bengal (Saha & Raychaudhuri, 2004; Roy et al., 2017), Madhya Pradesh (Gajbe, 2005), Kedarnath Wildlife

Sanctuary, Uttarakhand (Quasin & Uniyal, 2010), Indo-Gangetic plains (Priyadarshini et al., 2015), Eastern Ghats (Palem et al., 2017), and Nilgiris (Dharmaraj et al., 2018) had further enriched the Indian araneid diversity along with the characterisation of new species such as *Zilla globosa* Saha & Raychaudhuri, 2004, *Larinia mandlaensis* Gajbe, 2005, *Araneus bastarensis* Gajbe, 2005, and *Cyrtophora bituberculata* Roy, Saha & Raychaudhuri, 2017. The book *Spiders of India* (Sebastian & Peter, 2009) provided a detailed characterisation of Indian spiders, documenting 34 species from 17 genera. In India, 188 species from 34 genera have been reported to date (Caleb & Sankaran, 2025).

Kerala, the state of India, is renowned for its rich biodiversity. Its ecosystems range from windy highland elevations to saline coastal regions. Most of Kerala forms part of the Western Ghats, one of the world's biodiversity hotspots. Although several studies have been conducted to explore the araneofauna in different ecosystems of Kerala, a study exclusively for araneids has not been reported yet. Studies to explore the spider diversity in Parambikulam Wildlife Sanctuary (Patel, 2003), irrigated rice ecosystems across varying elevations (Sebastian et al., 2005a), Mangalavanam (Sebastian et al., 2005b), Kuttanad rice agroecosystem (Sudhikumar et al., 2005a), Mannavan Shola forest (Sudhikumar et al., 2005b), Kavvayi river basin (Jose et al., 2018), Wayanad (Rajeevan et al., 2019; Shabnam et al., 2021; Sudhin, 2022), sacred grooves of Northern Kerala (Sumesh & Sudhikumar, 2020), Montane cloud forests (Paul, 2021), Poovar mangrove patches (Vishnudas et al., 2021), Muriyad Kol Wetlands (Nafin, 2022), Shendurney Wildlife Sanctuary (Sudhin & Sen, 2023), and Karuvatta, a coastal island in the Vembanad Lake (George et al., 2024) has enriched the understanding of araneid diversity in Kerala. Notably, in all araneofaunal diversity studies in Kerala, the family Araneidae consistently ranks first or second as the most diverse spider family. A comprehensive pictorial guide on Kerala's spiders, including 44 araneids belonging to 21 genera, was presented by Sudhikumar (2021), which enhanced field identification of spiders.

Sebastian & Peter (2009) divided the family Araneidae into five subfamilies, including Araneinae, Argiopinae, Cyrtarachninae, Cyrtophorinae and Gasteracanthinae. Subsequent revisions updated as Araneinae, Argiopinae, Cyrtarachninae, Cyrtophorinae, Gasteracanthinae, Maastophorinae, Nephilinae, and Zygiellinae (Whyte & Anderson, 2017). However, with the continuous integration of molecular methodologies, the sub-familial arrangement is subject to ongoing refinement. Efforts to restructure the family based on molecular insights are reviewed and discussed later in this section. Following, the taxonomic status of common genera found in India is reviewed.

2.2. Taxonomic status: A review of Indian araneid genera

As microscopic techniques advanced, previously described species were revised to enhance taxonomic clarity. Given the vast number of reported species, compiling a comprehensive global revision remains a formidable challenge. Consequently, taxonomic studies have increasingly focused on araneids from specific geographical regions. Herbert W. Levi made significant contributions to the taxonomy of araneids through extensive revisional studies and developed a key for American araneids, including 65 genera (Levi, 2002). Similarly, Dippenaar-Schoeman & Jocque´ (1997) published a key for African araneids while Tikader (1982) presented a key for Indian araneids. Here is a review of the taxonomic studies of common araneid genera in India.

Based on two species from Africa, Simon (1895) described the genus *Acusilas*. Schmidt & Scharff (2008) later revised the genus, adding two new species and redescribing existing ones. Their work included Scanning Electron Microscopic (SEM) images and microscopic photographs of the chelicerae, spinnerets, and genitalia. Currently, this genus has 10 species (WSC, 2025). Among these, *Acusilas coccineus* Simon, 1895 is the only one reported from India (Roy et al., 2017).

Early taxonomists placed members of *Anepsion* in the genus *Paraplectana*. Later, they have been updated to a genus (Strand, 1929). A significant revision of the genus was done by

Chrysanthus (1961, 1969), including 12 species. Subsequent studies identified new species across different regions, bringing the total number of *Anepsion* species to sixteen (WSC, 2025). In 2011, Jose described *Cyrtarachne keralensis,* which was later synonymised with *Anepsion maritatum* (O. Pickard-Cambridge, 1877) by Malamel et al. (2015), the only species reported from India.

The genus *Arachnura* was first characterised by Vinson (1863) with the identification of a species from the Reunion Islands in the Indian Ocean. To date, taxonomists have described additional species, reporting 12 species in total (WSC, 2025). Among these, *Arachnura melanura* Simon, 1867, was reported in India (Sen & Sureshan, 2020). *Arachnura angura* Tikader, 1970 was also reported from India and is endemic to the country. Although members of this genus have been reported from various parts of the world, no thorough revisions have been reported.

The genus *Araneus* was first characterised by Clerck (1757) with the identification of 70 species, many of which were later synonymised or reassigned to other genera and families. Despite this, it is the largest genus in the family, with 551 species (WSC, 2025). Levi (1991) conducted a study including all Neotropical and Mexican species, which is considered a milestone, redescribing 144 species with detailed genitalia illustrations and a comprehensive species key for males and females. In India, *Araneus hirsutulus* was the first reported species (Stoliczka, 1869), with 22 species recorded to date (Caleb & Sankaran, 2025). Although Levi (1991) noted that *Araneus* species cannot be readily distinguished by body shape, colour, pattern, leg length, or eye ratio, many species have still been reported based on these features. The genus has often been used as a dumping ground (Scharff et al., 2020) for species that do not fit into other groups, making it chaotic. Taxonomic efforts of the 21st century are focusing on resolving this confusion through synonymization (Sherwood, 2021), genus transfers (Bosmans & Hervé, 2021; Framenau, 2019) and the establishment of new genera (Framenau et al., 2022; Mi et al., 2024b; Tanikawa et al., 2021).

The colourful and intriguing genus *Argiope* was first reported by Audouin (1826) with three species and currently includes 89 known species (WSC, 2025). *Argiope* is among the well-studied genera in the family, thanks to significant revisions by Levi (1983) and Jäger (2012). Levi's (1983) comprehensive study of Western Pacific species in the subfamily Argiopinae included *Argiope*, *Gea*, and *Neogea*, categorising *Argiope* into seven species groups. Jäger (2012) explored the ground-breaking concept of using broken emboli in the species identification of *Argiope*. By examining 316 broken emboli from 834 females, he demonstrated their utility in matching conspecifics and identifying cryptic species. This led to the removal of some synonymies and the synonymization of others, covering 41 species from 63 countries. Of the nine species reported in India (Caleb & Sankaran, 2025), eight were included in the study done by Tikader (1982).

Another genus within the subfamily Argiopinae is *Gea*, which was first characterised by Koch (1843). This small genus includes 13 species worldwide (WSC, 2025), seven of which were re-characterised by Levi (1983) alongside *Argiope*. A recent study by Mi et al. (2024a) offered extensive morphological descriptions accompanied by detailed illustrations. Three species represent *Gea* in Indian spider fauna (Caleb & Sankaran, 2025).

The genus *Bijoaraneus* is the most recently established among those discussed here, resulting from the integration of molecular techniques in taxonomy. During the revision of certain *Araneus* species, Tanikawa et al. (2021) characterised this genus, transferring three species from *Araneus* and two from *Epeira*, thereby classifying a total of five species under *Bijoaraneus* (WSC, 2025). *Bijoaraneus mitificus* (Simon, 1886), which is widely distributed across the Oriental and Indo-Malayan regions, is the only species recorded from India (Caleb & Sankaran, 2025).

Menge (1866) established the genus *Cyclosa*, whose members are notable for constructing stabilimenta in their webs. *Cyclosa* has become a diverse group within the family, comprising 180 species worldwide (WSC, 2025). A thorough revision was conducted by Levi

(1999), who examined 51 Neotropical and Mexican species, describing 38 as new. Levi identified two hidden palpal diagnostic features, the conductor tooth and the median apophysis. In India, the genus is represented by 18 species (Caleb & Sankaran, 2025), with Tikader (1982) reporting ten. Subsequent revisions by Keswani (2013) and Dixit & Ade (2017) provided habitus photographs and microscopic images. However, these lacked proper labelling and detailed diagnostic features for the species considered.

The genus *Cyrtophora* is represented globally by 49 species (WSC, 2025). In 1864, Simon first described this genus with the species *Cyrtophora citricola*, which remains valid to date. In India, the genus comprises eight species (Caleb & Sankaran, 2025), four of which (*Cyrtophora bituberculata* Roy et al., 2017; *Cyrtophora bidenta* Tikader, 1970; *Cyrtophora jabalpurensis* Gajbe & Gajbe, 1999; *Cyrtophora ksudra* Sherriffs, 1928) have only been reported from their respective type localities and have not been recorded elsewhere in India or globally.

Archer (1951) established the genus *Eriovixia* by transferring four species from *Araneus*. It is currently represented by 33 species worldwide (WSC, 2025), including nine recently described species (Mi & Li, 2021). Han & Zhu (2010) revisited this Southeast Asian genus, providing insights into its biogeography. In India, eight species have been identified (Caleb & Sankaran, 2025), two of which, *Eriovixia gryffindori* (Ahmed et al., 2016) and *Eriovixia kachugaonensis* (Basumatary et al., 2019), are endemic to India.

Spiny-backed orb weavers, members of the subfamily Gasteracanthinae, are very fascinating araneids due to their uniquely modified abdomens. Among them, the genus *Gasteracantha*, described by Sundevall (1833), comprises 86 species (WSC, 2025), frequently recorded in tropical diversity studies but lacking a comprehensive revision. Ten species were reported from India (Caleb & Sankaran, 2025). Another genus in this subfamily is *Thelacantha*, in which only two species have been reported (WSC, 2025). Of these, *Thelacantha brevispina* (Doleschall, 1857) is widely distributed, including India, while *Thelacantha cuspidata* has not been recorded since Dahl (1914). Originally placed in *Gasteracantha*, *Thelacantha* was reclassified by Benoit (1964) and Emerit (1974). Attempt to review the taxonomic and biogeographic status of the subfamily, resulted in a detailed morphological characterisation, genitalia illustrations, and photographs (Macharoenboon et al., 2021). Additionally, they also documented intraspecific colour polymorphism in *Thelacantha brevispina*, identifying four distinct morphs.

The subfamily Nephilinae, comprising *Herennia*, *Nephila*, and *Nephilengys*, remains a subject of debate regarding its placement in Araneidae. *Herennia*, characterised by Thorell (1877), includes 11 species globally (WSC, 2025), with *Herennia multipuncta* (Doleschall, 1859) as the sole representative in India (Caleb & Sankaran, 2025). Known as coin spiders, this genus was revised by Kuntner (2005), who identified *H. multipuncta* as the only widespread, synanthropic, and invasive species, while the other ten are restricted to Australia and its islands. *Nephila* was originally described based on *Nephila maculata* (Leach, 1815), later synonymised with the widespread *Nephila pilipes* (Fabricius, 1793). Four species were reported in India (Tikader, 1982), all recently synonymised under *N. pilipes* (Sankaran et al., 2020), revealing the colour polymorphism of *N. pilipes* in the Indian population. The genus *Nephilengys*, described by Koch (1872), consists of only two species (WSC, 2025), commonly known as hermit spiders. Kuntner (2007) provided a comprehensive monograph on pantropical hermit spiders, detailing their morphology with SEM images of the eye region, chelicerae and genitalia.

The genus *Larinia*, described by Simon (1874), is globally represented by 62 species (WSC, 2025). Later, Levi (1975) and Harrod et al. (1991) tried to revise the genus, but the attempts were taxon-limited. In India, Tikader (1982) reported two species with genitalic illustrations, while the remaining seven Indian *Larinia* (Caleb & Sankaran, 2025) have been recorded exclusively from their type localities. Another genus extensively studied by Levi is *Parawixia*. Pickard-Cambridge (1904) transferred eight species from *Epiera* to establish *Parawixia* along with a newly described species. Among 29 currently valid species (WSC, 2025), 28 were included in Levi's (1992) revision. Only one species, *Parawixia dehaani* (Doleschall, 1859), has been reported from India (Tikader, 1982).

Plebs, described by Joseph & Framenau (2012), is another recently diagnosed genus. In their study, they transferred 15 species from *Araneus* and *Epiera* and described seven new species, bringing the total to 22 globally recognised species (WSC, 2025). Also, this study provided habitus images, SEM images of spinnerets, and detailed genitalia photographs. In India, the genus is represented by two endemic species (Joseph & Framenau, 2012).

The genus *Poltys*, described by Koch (1843) with *Poltys illepidus*, remains an intriguing yet elusive group due to its exceptional camouflage. Despite their rarity in the field, 42 species have been reported globally (WSC, 2025), though 21 have not been documented beyond their original descriptions. Smith (2006) revised the Australian *Poltys*, identifying 19 species, and provided a species key with SEM images of genitalia and the cephalic region. Five *Poltys* species have been recorded from India (Caleb & Sankaran, 2025), and three are known only from their type localities. Although a comprehensive revision is needed, their scarcity poses a challenge.

Another small genus, *Chorizopes*, includes 29 species (WSC, 2025). First described by Pickard-Cambridge (1871) with a single species, its type species was later redescribed by Levi (1964). No further taxonomic revisions have been conducted. In India, 11 species are documented (Caleb & Sankaran, 2025), but many are known only from their type localities with limited diagnostic features, highlighting the need for a thorough morphological reassessment.

The genus *Neoscona* is widely distributed across diverse ecosystems, making its members among the most commonly encountered araneids. With 124 reported species (WSC, 2025), many were transferred from *Araneus* or *Epiera*. Simon (1864) recognised the need to reclassify certain *Epiera* species, leading to the establishment of *Neoscona*. American *Neoscona* was studied by Berman & Levi (1971), identifying 17 species, of which 15 remain valid. They highlighted epigynal structure, abdominal colour patterns in females, palpal features, and tibial macrosetae in males as key diagnostic traits. In India, 29 *Neoscona* species have been documented (Caleb & Sankaran, 2025), with Tikader (1982) describing 17 species. Contrary to Berman & Levi (1971), Tikader observed abdominal colour and pattern polymorphism in female *Neoscona mukerjei* Tikader, 1980. While reviewing the Indian species, it was found that 13 are known only from their type locality, and 12 were described solely on female morphology, weakening their taxonomic robustness.

The taxonomic status of several genera within the family Araneidae remains unresolved, with varying degrees of revision across different groups. Genera such as *Acusilas, Argiope, Gea, Thelacantha, Nephila, Herennia, Nephilengys,* and *Plebs* have been well-characterised, possessing a strong morphological foundation and molecular approaches serve as supplementary evidence. Conversely, genera such as *Arachnura, Araneus*, *Cyrtophora,* and *Chorizopes* require more comprehensive taxonomic assessments to establish a clearer framework. Meanwhile, several other genera, including *Anepsion, Cyclosa, Eriovixia, Gasteracantha, Larinia, Parawixia, Poltys,* and *Neoscona*, exhibit taxonomic ambiguities that could be effectively resolved through molecular investigations. Applying integrative taxonomic approaches to resolve these gaps will enhance species delineation.

2.3. The emergence of molecular taxonomy in animal systematics

By the late 20th century, the taxonomic community faced significant challenges including a lack of consensus on the morphological characters to be universally adopted, a growing taxonomy deficit (May, 1988), and a decline in government and funding agency support for taxonomic research (Convention on Biological Diversity [CBD], 1995). In response, the International Barcode of Life (iBOL) project was proposed to establish a global repository of DNA sequences, leading to the launch of the Barcode of Life Database (BOLD) in 2005.

The potential of DNA barcoding, particularly using mitochondrial gene Cytochrome Oxidase subunit 1 (COI) sequences in taxonomy, was first explored by Hebert et al. (2003a). Their study assessed the accuracy of DNA barcoding at higher taxonomic levels and species levels using 200 closely related lepidopteran species, ultimately yielding successful results. COI sequences were proposed as the foundation for a universal bio-identification system for animals (Hebert et al., 2003a), demonstrating efficacy in distinguishing closely related species across all animal phyla, except Cnidaria (Hebert et al., 2003b).

Despite its potential, DNA barcoding in species identification faced criticism. Taxonomists feared it might overshadow the Linnaean system and shift taxonomy solely to DNA-based methods (Ebach & Holdrege, 2005). Concerns also arose that it could compete for funding (Blaxter et al., 2004; DeSalle, 2006). The need for classical taxonomy training and biodiversity inventories was highlighted (Prendini, 2005). A major limitation was its reliance on fresh or cryopreserved specimens, whereas most described species are museum-preserved (Hillis, 1987). However, Fang et al. (2002) demonstrated successful DNA extraction from formalin-fixed museum specimens. However, DNA barcoding complements rather than replaces traditional taxonomy, aiding species assignment when morphological traits are unclear (Schindel & Miller, 2005).

Subsequent studies validated the efficacy of DNA barcoding across various taxa, including birds (Hebert et al., 2004), springtails (Hogg & Hebert, 2004), gastropods (Remigio & Hebert, 2003), fish (Ward et al., 2005), primates (Lorenz et al., 2005), and spiders (Barrett & Hebert, 2005), reinforcing its applicability. Further research demonstrated that an integrative taxonomic approach, incorporating both morphological and molecular data, has set new benchmarks for data quality, ensuring that morphological information remains essential in resolving the taxonomic crisis (Hubert & Hanner, 2015; Patwardhan et al., 2014; Tautz et al., 2003).

2.4. Molecular taxonomy in Araneae: A global perspective

The utilization of DNA barcoding in spider taxonomy was initiated by Barrett & Hebert (2005), who successfully demonstrated the efficiency of mitochondrial COI gene as a molecular marker in the identification of spider species. They suggested molecular approach as a solution to overcome the limitations of morphological taxonomy, such as sexual dimorphism, dependence on adult specimens, and descriptions based on single-sex. Their study, including 168 species from various genera, showed that the mean sequence divergence between congeneric species was significantly higher than intraspecific divergence, with a 2% threshold achieving 100% accuracy in identification.

The efficacy of the COI marker was tested across different spider groups. Greenstone et al. (2005) examined its potential in identifying immature spider stages focussing on the families Anyphaenidae, Lycosidae, Linyphiidae, and Miturgidae in Texas, USA and confirmed a high success rate. Similarly, Petersen et al. (2007) sequenced the COI marker from exuviae of Central American tarantulas (*Brachypelma*), demonstrating that DNA sequencing from shed exoskeletons could enable species identification without harming populations, hence resolving the conservation concerns. Similarly, several studies used the DNA barcoding principles to resolve the ambiguities in different spider groups. The global acceptance of DNA barcoding for spider identification led to the establishment of DNA barcode reference libraries across various regions (Ashfaq et al., 2019; Astrin et al., 2016; Blagoev et al., 2013; Miller et al., 2013).

While these studies focussed on the potential of COI markers, the existence of a barcode gap and differences in genetic divergence between spider species across morphological and geographical scales were examined by Čandek & Kuntner (2015). By analysing 1,203 barcodes, they confirmed that DNA barcoding remains a reliable tool for species identification across large geographical scales. They also confirmed the presence of barcode gaps in Tetragnathidae and Lycosidae and a significant overlap in Araneidae. Accurate genus and family-level assignments using DNA barcoding are feasible, though identification accuracy may be influenced by sequence quality and database completeness (Coddington et al., 2016).

The potential of other markers was also tested. Astrin et al. (2006) suggested another mitochondrial gene, the 16S rRNA gene, as a potential marker other than COI in pholcids. On the other hand, Wang et al. (2018) found lower species identification success rate for 16S rRNA in other families. Cao et al. (2016) focussed on the nuclear region, internal transcribed spacer 2 (ITS2), suggesting a multi-locus DNA barcoding approach yields more accurate results than relying only on mitochondrial COI. Moreover, the capability of an integrative approach that combined morphological, geographical, and molecular data to resolve taxonomic ambiguities was well-studied by Oh et al. (2022), Tahir et al. (2019), and Xu et al. (2015).

In India, Gaikwad et al. (2017) made the first attempt to use COI sequencing in spider identification. Their study contributed 43 new sequences to the existing database. Unlike Barrett & Hebert (2005), they found a species discrimination threshold of 3%, rather than 2%. Tyagi et al. (2019) later contributed 85 novel barcodes of 22 morphospecies. Their species delimitation threshold ranged between 2.6% and 3.7%. Additionally, their study suggested cryptic diversity in certain species. The findings highlight that DNA barcoding is a powerful tool for specimen identification and species discovery in Indian spiders.

2.5. Taxonomy of Araneidae: A molecular view

The family Araneidae ranks fourth among spider families in terms of available barcode sequences (Barcode of Life Database [BOLD], 2025), with 20,068 barcodes, representing 734 species across 74 countries. As one of the most diverse spider families, Araneidae includes numerous ignored genera that require a thorough characterisation. and diagnosis. DNA barcoding has enabled a better picture of certain taxa, improving their classification.

For example, Franzini et al. (2013) conducted an integrative study to examine *Cyrtophora* species in South Africa. By sequencing the COI gene and the nuclear Histone 3 (H3) gene, along with genitalic examination, they identified the presence of multiple *Cyrtophora* species. Similarly, Gregorič et al. (2015) provided new taxonomic insights into bark spiders, *Caerostris*, in Madagascar. They amplified and analysed COI and nuclear gene, 28S rDNA, from 50 specimens to evaluate the species boundaries and led to the description of six new species.

The usefulness of combining multiple lines of evidence in species delimitation was further demonstrated by Spasojevic et al. (2016). Combining COI barcoding and morphometric analysis, they successfully distinguished six *Araniella* species, emphasizing the potential of DNA barcoding to enhance species classification, even within small, morphologically similar genera. In 2018, Tan employed a multigene approach to resolve the ambiguities in *Argiope hoiseni,* whose females exhibit significant similarities in genital structure with other species. Elizabeth (2019) utilised molecular data to identify *Cyrtophora* species from the Western Ghats, and five species were documented.

The utilisation of molecular approaches in taxonomic studies has played a key role in both synonymization and the re-evaluation of previously synonymized species. Smith (2006) conducted a revision of Australian *Poltys* species, combining DNA barcoding and behavioural traits, and led to the synonymization of several species and the determination of cryptic taxa. The study also resulted in the description of five new species. Similarly, Tanikawa et al. (2006) used the barcoding principles to clarify the identity of a Japanese spider previously identified as *Pasilobus bufoninus*. Through partial sequencing of the COI gene, they confirmed that the Japanese population was conspecific with *Pasilobus hupingensis* from China.

Levi (1983) synonymised *Argiope chloresis* and *Argiope chloreides* based on morphological examination however, Tan et al. (2019b) revisited using the molecular approach and removed the synonymy. Advanced microscopy of genitalic structures further reinforced their separation, leading to the removal of their synonymy. In the study done by Yuan & Zhang (2020), genetic distance analysis supported the synonymy of *Nephila laurinae* and *Trichonephila* *antipodiana*. However, the lack of type specimens prevented a comprehensive morphological characterisation, leaving the taxonomic status of these species unresolved.

Major gaps in araneid taxonomy were profound due to polymorphism, sexual dimorphism, and extreme male dwarfism. Tanikawa et al. (2010) successfully distinguished three *Cyrtophora moluccensis* (Doleschall, 1857) colour morphs (green, red, and yellow) as three distinct species through a combined approach. Their analyses revealed that there is considerable genetic distances among these morphs, and phylogenetic reconstruction using the neighbour-joining method placed them in separate clades. Magalhaes et al. (2017) tried to assess the phylogenetic relationships between males and their supposed females of *Micrathena*. Their findings successfully associated males and females. This study highlights the importance of incorporating multiple data sources to resolve taxonomic ambiguities in sexually dimorphic spider groups.

In some cases, the integrative approach has led to the establishment of new genera. One such effort was done by Framenau et al. (2022), who described *Socca*, a new Australo-Pacific genus reporting 12 species while revising the genus *Backobourkia*. Similarly, Tanikawa et al. (2021) described two new genera, *Aoaraneus* and *Bijoaraneus*. More recently, Tanikawa & Petchard (2023) described *Leviaraneus* while reviewing specific species groups within *Araneus*.

An integrative approach, with DNA barcoding and traditional morphological examination, is a powerful tool in resolving taxonomic ambiguities. However, molecular studies have not been uniformly applied across all araneid groups or geographical regions. In India, for instance, comprehensive molecular investigations on Araneidae remain scarce, leaving substantial gaps in our understanding of the group's diversity.

2.6. Molecular phylogenetics of spiders: A heuristic approach

A well-resolved phylogeny serves as a foundational framework for organising and interpreting patterns of biodiversity, facilitating predictive classification (Coddington, 2005). Traditionally, phylogenetic relationships were inferred based on shared morphological traits. However, morphological analyses alone often posed challenges due to convergent evolution, phenotypic plasticity, and cryptic diversity. The advent of molecular phylogenetics revolutionised the field and greatly enhanced the resolution of phylogenetic trees.

According to the WSC (2025), the earliest documented phylogenetic study on spiders was conducted by Purcell (1910), who investigated the evolution of the tracheal system in Araneae through a detailed examination of tracheal tubes and book lung anatomy. Subsequently, studies have been conducted emphasising certain characters to elucidate the evolutionary relationships, based on the morphological traits. Some spider groups focused on were Hersiliidae (Baehr & Baehr, 1987), Deinopoidea (Coddington, 1990; Griswold et al., 1998), Nephilins and Tetragnathidae (Hormiga et al., 1995), Araneiodea (Griswold et al., 1998), Gnaphosidae (Platnick, 1990), and Mesothelae (Haupt, 2003).

Although molecular phylogenetics had been explored in the animal kingdom earlier (Field et al., 1988), its application in elucidating evolutionary relationships among spider groups gained momentum with the integration of DNA barcoding into spider taxonomy. Bolzern et al. (2013) combined morphological and molecular data to clarify the phylogenetic placement of the *Tegenaria-Malthonica* complex within the family Agelenidae. Hazzi & Hormiga (2023) investigated the phylogeny of tropical wandering spiders (Ctenidae), with a particular emphasis on eye conformation. The study was constrained by limited taxon sampling, which was later addressed by Hazzi et al. (2025) during the extensive phylogenetic analysis incorporating global museum collections. Labarque et al. (2018) examined the evolutionary relationships of Drymusidae in Africa, while Booysen & Haddad (2021) focused on the Afrotropical Gnaphosidae.

The segmented spiders of the family Liphistiidae were studied by Sivayyapram et al. (2024), who provided a clearer phylogenetic framework alongside insights into their biogeography. The molecular phylogeny of wolf spiders, Lycosidae, was investigated by

Piacentini & Ramírez (2019), whereas Huber et al. (2018) critically reevaluated the morphological phylogeny of Pholcidae, presenting a well-supported evolutionary framework. Salticidae, the largest spider family, was extensively studied by Maddison et al. (2014, 2017). In the family Tetragnathidae, molecular phylogenetic studies have primarily focused on the genera *Meta* (Kallal & Hormiga, 2018a) and *Leucauge* (Ballesteros & Hormiga, 2021).

Although phylogenetic studies at the family and genus levels have significantly enriched arachnology across various regions, a comprehensive phylogenetic framework for Araneae as a whole remains limited. One of the earliest large-scale attempts was made by Coddington & Levi (1991), who proposed a cladistic structure of Araneae, identifying three major monophyletic groups, Mesothelae, Mygalomorphae, and Araneomorphae. Later in 2005, Coddington revisited the existing phylogenetic literature and fossil evidence to reaffirm the monophyly of Araneae. He highlighted the evolutionary complexity of Deinopoidea and Araneoidea, suggesting instances of convergent evolution. He confirmed that monophyletic groups are defined by synapomorphies, whereas misclassifications result in paraphyletic or polyphyletic groupings.

Building on these findings, Wheeler et al. (2017) done a phylogenetic analysis of araneofauna using large-scale transcriptomic data. Their study combined genetic data from six molecular markers from 932 species representing 115 spider families. In addition to supporting the monophyly of Mesothelae, Mygalomorphae, and Araneomorphae, the study provided strong support for the Synspermiata clade and identified Trogloraptoridae and Caponiidae as sister groups to Dysderoidea. Kulkarni et al. (2023) made significant contributions to spider systematics and phylogenetics by combining data from 125 families. With the help of six molecular markers, the existence of major clades, as suggested by Coddington & Levi (1991), was confirmed. Additionally, the authors emphasised the important role of data quality in reconstructing evolutionary relationships.

2.7. Phylogenetic insights into Araneidae

Early studies depended on morphological synapomorphies, but conflicts in the taxonomic classification of orb weavers led to confusions in the phylogenetic relationships of araneids. Given the vast diversity within this group and the limited availability of specimens across different regions, most studies were geographically restricted.

Initial research focused on resolving the phylogenetic relationships within Araneoidea with the primary objective of identifying reliable synapomorphies. Levi (1978) hypothesised that orb webs may have evolved multiple times due to their energy-conserving advantages. Additionally, he cautioned that similarities between the webs of Uloboridae and Araneidae could complicate interpretations of their evolutionary trajectories. Consequently, he proposed that male palpal morphology, rather than web or claw characteristics, would serve as a more reliable phylogenetic indicator for araneids. This was supported by Heimer & Nentwig (1983), and they determined that traits associated with the eye region, leg spinnation, respiratory organs, and cheliceral morphology had limited utility for phylogenetic inference, whereas epigynal and web characteristics were comparatively more informative. They also provided strong evidence for the monophyly of Araneoidea.

Levi & Coddington (1983) identified the rotation of the bulb within the cymbium, the presence of a median and terminal apophysis, and the shortening of the abdomen as the three key synapomorphic traits present in all araneids. They emphasised that synapomorphies are essential for grouping taxa in phylogenetic reconstructions. If a trait is absent in a particular lineage, it may have been secondarily lost or never present in the first place. Coddington (1990) reaffirmed the monophyly of Araneoidea, building upon the synapomorphic traits identified by Levi & Coddington (1983). Ramos et al. (2005) provided further insights into araneoid evolution by supporting the positive genitalic divergence model, demonstrating a significant correlation between sexual genitalic dimorphism and sexual size dimorphism. Dimitrov et al. (2017)

reinforced the previous findings through a molecular analysis. Their study, the first to include representatives from all araneoid families, analysed 363 terminal taxa using sequence data from six molecular markers. Despite supporting the previous findings, the monophyly of Orbicularia was rejected, underscoring the complexity of orb web evolution.

Scharff & Coddington (1997) conducted the first dedicated phylogenetic study of Araneidae, analysing 82 morphological and behavioural characters to establish a foundational framework. Two major clades were identified within the family, Araneinae and Argiopidae, the latter encompassing the subfamilies Gasteracanthinae, Caerostreinae, Micratheninae, Xylethreinae, Encyosaccinae, Eurycorminae, Arciinae, Cyrtarachninae, Argiopinae, Cyrtophorinae, and Arachnurinae. While Gasteracanthinae and Micratheninae were found to be non-monophyletic, the monophyly of *Cyrtarachne* was confirmed, along with the polyphyletic nature of Cycloseae, Micratheneae, and Anepsieae. The monophyly of Araneidae as a whole was supported by four synapomorphic traits, including specific male palpal and ocular features. They also explored evolutionary patterns of certain traits, finding that stabilimenta evolved independently at least nine times and were lost once, while sexual dimorphism arose three times but reverted five times in certain genera. This study remains a foundational framework of araneid phylogeny.

Building upon these findings, Kallal et al. (2018) used transcriptomic data to improve the phylogenetic framework of Araneidae. With the help of phylogenetic methods and orthology assessments, they confirmed the monophyly of Araneidae. The results supported the earliest divergence of the subfamily Zygiellinae and Nephilinae. The study also highlighted that lower gene counts can lead to the misrepresentation of evolutionary relationships though the study was limited by taxon sampling.

Later significant contributions were made by Scharff et al. (2020), marking the first attempt to explain the araneid phylogeny with a balanced representation of taxa from both the Northern and Southern hemispheres. This attempt used five markers and included major araneoid lineages as outgroups. Members from Zygiellinae, Nephilinae, and other araneids were included in the ingroup. The results confirmed the sister-group relationship between Zygiellinae with other araneid clades. Within the ARA clade, ten informal groups, such as Caerostrines, Micrathenines, Eriophorines, Backobourkiines, Gasteracanthines, Argiopines, Cyrtarachnines, Mastophorines, Nuctenines, and Zealaraneines, were identified. The study also confirmed the polyphyly of *Araneus*, *Argiope*, *Eriophora*, *Larinia*, and *Parawixia*. They stated that the classical orb web is a plesiomorphic trait, and stabilimenta evolved independently at least five times. Female gigantism was identified as the primary factor for sexual dimorphism.

Beyond family-level analyses, several studies have focused on specific genera within Araneidae. Kuntner (2002) conducted a morphological survey of the genus *Perilla*, analysing 82 morphological and behavioural traits, confirming the placement of *Perilla* within Araneinae. In contrast, Framenau et al. (2010b) integrated morphological and molecular data to establish the Australasian genus *Backobourkia*, identifying the basal flange of the median apophysis as a synapomorphic trait. Phylogenetic analysis placed *Backobourkia* within the coxal hook clade of Araneinae and supported its monophyly, along with a close relationship to *Eriophora*. Framenau et al. (2010a) conducted a phylogenetic analysis to determine the placement of *Demadiana* within the subfamily Arkyinae.

Magalhaes & Santos (2012) revealed that *Chaetacis* is nested within *Micrathena* and should be considered a subgroup rather than a distinct genus. *Micrathena* males and females were successfully matched using morphological, genetic, and geographical data (Magalhaes et al., 2017). Gregorič et al. (2015) conducted the first species-level evolutionary analysis of *Caerostris* through a molecular approach and confirmed the monophyly of African *Caerostris* species. Geographic genetic variations in *C. sexcuspidata* and *C. sumatrana* suggested intraspecific variation across regions.

The study on Australian *Zygiellinae* (Kallal & Hormiga, 2018b) supported the early findings, with the subfamily as the earliest diverging lineage in Araneidae. The formation of leaf retreats in web hubs evolved at least three times. The study on Indomalayan *Chorizopes* examined palpal homologies and araneid palpal sclerite evolution (Kallal & Hormiga, 2019). The correlation between orb web loss and changes in palpal morphology led to complex relationships among *Artonis*, *Chorizopesoides*, and *Porcataraneus*. However, weak phylogenetic support prevented the definitive placement of *Chorizopes* within Araneidae.

Tan et al. (2019a) examined the evolutionary relationships of Peninsular Malaysian *Actinacantha*, *Gasteracantha*, *Micracantha*, and *Thelacantha* using five markers, supporting the monophyly of *Gasteracanthinae*, contradicting Scharff & Coddington (1997), but suggested the paraphyly of *Gasteracantha*, despite limited taxon sampling. Cabra-García & Hormiga (2020) conducted a total evidence study on *Wagneriana*, integrating morphological, behavioural, and molecular data. Two species, *Wagneriana carimagua* and *W. uropygialis*, were nested within *Parawixia* and *Alpaida*, respectively, while the remaining *Wagneriana* species formed three main clades, with relationships heavily influenced by methodological choices. The study also established a new genus, *Popperaneus*, and revived *Paraverrucosa*.

Yu et al. (2022) studied *Cyphalonotus* and *Poltys* using five markers, rejecting previous hypotheses and recovering a well-supported *Cyphalonotus* + *Poltys* clade. They confirmed the independent evolution of extreme sexual dimorphism in *Poltys* as a multiple convergent evolutionary outcome. Hormiga et al. (2023) examined *Paraplectanoides* using six molecular markers, confirming its placement within Araneidae and identifying a sister-group relationship with *Nephilinae*. Their findings contradicted Kuntner et al. (2023), who proposed a separate family for *Paraplectanoides*, raising concerns about the stability and monophyly of Araneidae. The study also opposed splitting Araneidae into multiple families.

The taxonomic placement of *Nephilinae* within Araneidae remains argued and studies are still ongoing to confirm whether it should retain subfamily status or be recognised as a separate family. Hong-Chun et al. (2004) analysed *Nephila* from China and India using the data of three markers, concluding that *Nephila* is more closely related to Araneidae and should be included within it. On the other hand, Kuntner et al. (2008) examined *Clitaetra, Herennia, Nephila,* and *Nephilengys* using a character matrix including morphological and behavioural traits and placed Nephilids at the base of the Araneoidea clade, supporting family status. The study also proposed that the evolution of male and female body size has resulted in extreme sexual size dimorphism, but did not explained the evolutionary pressures and mechanisms driving that changes.

Kuntner et al. (2013) used morphological and behavioural data with molecular sequences from eight genes. The analysis strongly supported the monophyly of *Herennia* and *Clitaetra* but found *Nephilengys* and *Nephila* to be diphyletic, challenging previous assumptions. The study confirmed the monophyly of *Nephilidae*, *Zygiellidae*, and *Araneidae*, rejecting the tetragnathid hypothesis. Yong et al. (2021) used a mitogenome approach to understand the relationship between *Nephila* and *Trichonephila*. Despite limited taxon sampling, their findings, based on 13 protein-coding and 2 rRNA genes, supported the inclusion of both genera in *Araneidae*.

Numerous studies have explored the phylogeny of Araneidae, with many focusing on specific groups. While these studies provide valuable insights, they often contribute to increasing uncertainty rather than resolving existing ambiguities. Depending on the methodologies used, the findings keep on varying, ultimately leading to conflicting conclusions. Kuntner et al. (2023) reviewed the increasing information in family-level classifications, suggesting a monophyletic structure for taxonomic validity. The authors supported a "splitting" approach to maintain distinct families for further studies.

As discussed, phylogenetic relationships can be understood by using morphological, behavioural, and genetic data, but an integrative approach provides more robust and reliable conclusions. Phylogenetic studies based on morphological character matrices use Maximum Parsimony (MP) method while integrative approaches depend on Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Using different analytical methods and extensive taxon sampling are significant for phylogenetic reconstructions. Irrespective of the ongoing debates on the phylogenetic relationships within the family, the monophyly of the family Araneidae is well supported. However, there are gaps in the understanding of araneid phylogeny, as most studies focus on specific groups or are geographically limited. Several genera, including *Araneus*, *Neoscona*, and *Argiope*, require thorough phylogenetic analysis to explain their evolutionary relationships.

2.8. Dated phylogeny of spiders: A review

Time-calibrated phylogenies, constructed using morphological or molecular data in combination with fossil records and geological evidence, play a vital role in understanding evolutionary events, speciation patterns, and biogeographic histories over time. Hines (2008) estimated the divergence time of bumble bees (*Bombus*) and concluded their origin in the Old World, approximately 25–40 million years ago (Ma), during the transition from the Eocene to the Oligocene. Similarly, Boudinot et al. (2016) estimated the divergence time and origin of the spider ant genus *Leptomyrmex*. Their findings supported a Neotropical origin, followed by a dispersal event to Australia around 29 Ma during the Oligocene. Some studies to date the phylogeny of certain spider groups have been reported.

The study by Jeyaprakash & Hoy (2009) focused on chelicerates, including spiders, scorpions, mites, and ticks. By analysing the molecular data, they confirmed the monophyly of chelicerates and suggested that the diversification of these groups had occurred in the late Palaeozoic era. Their analysis estimated the divergence of the spider-scorpion clade at approximately 397 ± 23 Ma. Bidegaray-Batista & Arnedo (2011) explored the ground-dwelling spiders of the genus *Parachtes* (family Dysderidae), endemic to the Western Mediterranean. They suggested that the divergence of this group occurred during the Oligocene, congruent with the time of the separation of region’s main islands.

Starrett et al. (2013) reconstructed a Haemocyanin gene tree to explore the phylogeny and diversification of Mygalomorphs. They successfully recovered deep phylogenetic nodes and multiple mygalomorph clades. The findings suggested that the diversification of Mygalomorphs happened before that of the extant Araneomorphs. A notable study by Wood et al. (2013) focused on archaeid spiders, a group with fossil evidence in the Northern hemisphere but extant species restricted to the Southern hemisphere. Using both morphological and molecular datasets, the authors suggested that the diversification of archaeid spiders occurred with the breakup of Pangaea into Laurasia and Gondwana. This study is an example, rare yet well-supported, of biogeographic patterns shaped by ancient continental drift.

Euophryines (family Salticidae) were studied by Zhang & Maddison (2013) by examining nucleotide data from four markers, confirming its monophyly. Phylogenetic reconstruction resulted in the separate clades of Old and New World Euophryines, suggesting that most diversification within the group occurred during the Cenozoic era following the geographic separation of these regions. The study also identified New Guinea and the Caribbean islands as key hotspots of diversity for this salticid lineage.

Ceccarelli et al. (2016) studied the coastal spider genus *Amaurobioides* (family Anyphaenidae) to compare the patterns of Gondwanan vicariance with long-distance dispersal. Using the data from four molecular markers, it was concluded that ancestral species have undergone many long-distance dispersal events, from the mid-Miocene to the Pleistocene. The study emphasised rafting as the primary dispersal mechanism over ballooning. Li et al. (2024) focused on Psilodercids (family Psilodercidae) in Southeast Asia. Divergence time estimates placed the origin of Psilodercidae at approximately 129.85 Mya, with significant diversification events occurring in the late Cretaceous. The study identified tropical Asia as a key biodiversity hotspot for this group and suggested *in situ* speciation as the major driver of diversification.

Kuntner et al. (2013) have also made some conclusions on the origin and diversification of Nephilids. Their findings confirmed that *Nephila jurassica* does not belong to Nephilidae, rejecting the Jurassic period as the origin. Instead, *Paleonephila*, dated to approximately 40 Ma, was identified as the oldest known fossil of this group. The study suggested an African or Asian origin of nephilids. To understand the geographical history of the South American tropics, Bartoleti et al. (2018) examined the diversification of *Nephila clavipes*. The study highlights the usage of widely distributed species in studying the evolutionary patterns of complex regions. Geological and climatic events had resulted in the formation of four separate lineages within the species. Their findings supported a Pleistocene connection between the Amazonian and Atlantic rainforests through the central Cerrado region. Turk et al. (2020) reconstructed the ancestral states to understand the biogeographic history of golden orb-weaving spiders (Nephilidae). By analysing geographical origins and intercontinental colonisation patterns, the study tested two hypotheses, an 'out of Africa' origin (Afrotropic) and an 'out of West Burma' origin (Indomalaya and/or Australasia). The results supported the second hypothesis.

The developments in molecular techniques, computational tools, and statistical models have promoted divergence time analyses. While there are several phylogenetic studies of Araneae, divergence time analyses remain limited, leaving gaps in our understanding of their evolutionary history. These kinds of efforts have the potential to resolve the conflicts in biogeographical hypotheses and provide a better picture of spider evolution.

2.9. Fossil evidence of araneids

Fossil evidence plays a vital role in providing direct temporal reference points, helping the researchers to estimate lineage divergence and calibrate molecular clocks (Magalhaes et al., 2020). By preserving the morphological characteristics of extinct species, fossils give valuable insights into character transformations within lineages (Anderson, 2012). The paleoaraneological fauna is also rich. While spider fossils date back to the Palaeozoic era, they have been recovered from the Mesozoic and Cenozoic eras as well, with the highest diversity observed in the Mesozoic (Selden & Penney, 2010).

Because of the soft-body, most spider fossils are preserved in amber. The Eocene Baltic amber represents the key source of fossilised spiders, followed by Oligocene-Miocene Chiapas amber from Mexico and Miocene amber from the Dominican Republic (Selden & Penney, 2010).

The earliest study of spider fossils was done by Kundmann (1737), who described a true spider encased in amber. Later, Koch & Berendt (1854), Giebel (1856), and Petrunkevitch (1942, 1958) contributed significantly. The discovery of Mesozoic spider fossils popularised in the mid-20th century, with reports emerging from Cretaceous amber deposits in Canada (McAlpine & Martin, 1969), France (Schlüter, 1978), Spain (Alonso et al., 2000), and Myanmar (Rasnitsyn & Ross, 2000).

A total of 1,427 valid fossil spider species have been documented to date (Dunlop et al., 2023). Fossil spiders have been documented across various groups, including Anapidae (Wunderlich, 2004d), Mimetidae (Harms & Dunlop, 2009), Mygalomorphs (Wunderlich, 2004a), Oonopidae (Marusik & Wunderlich, 2008; Penney, 2000), Pholcidae (Huber & Wunderlich, 2006), Psilodercidae (Magalhaes et al., 2021), Scytodoidae and Sicariidae (Magalhaes et al., 2022), Tetragnathidae, Zygiellidae (Wunderlich, 2004b), Theridiidae (Wunderlich, 2008), Zodariidae (Wunderlich, 2004f), and Zoropsidae (Wunderlich, 2004e). The fossil discoveries have significantly contributed to the dated phylogenies. Magalhaes et al. (2020) conducted a thorough evaluation of 67 unique fossils for calibration purposes. The authors emphasised the importance of the validation of fossils in calibrations, particularly through the careful identification of synapomorphies. The study also provided a list of 41 key fossils and a revised set of 23 calibrations.

The fossil record of Araneidae has inconsistencies, with several early reports lacking proper descriptions (Barthel & Hetzer, 1982; Giebel, 1856; Presl, 1822). The family is believed to have originated in the Cretaceous (Dunlop et al., 2023). The first formal description of an araneid fossil was done by Heyden (1859), who identified *Gea kuantzi*. Eskov (1984) proposed the Jurassic family Juraraneidae as an early orb-weaver lineage; however, this classification was later questioned by Selden (1989). Further studies have significantly expanded knowledge of paleoaraneid diversity. Wunderlich (2004c) introduced a new subfamily, Miraraneinae, and several genera, including *Palaeonephila* (*P. brevis, P. curvata, P. dilitans, P. fibula, P. longipes*), *Eonephila* (*E. bitterfeldensis, E. excellens, E. longembolus*), and *Luxurionephila* (*L. spinifera*) in Nephilinae. A breakthrough in araneid fossil research was made by Penney & Ortuño (2006), who described the oldest orb-weaving spider fossil by describing the genus *Mesozygiella*, with a species *M. dunlopi*. This specimen was recovered from Lower Cretaceous amber from Álava, Spain and became the strongest evidence for the presence of Araneidae in this period.

Before this study, several fossils supposedly belonging to Araneidae had been reported, including specimens from Canadian amber (McAlpine & Martin, 1969), Siberian amber (Eskov & Wunderlich, 1995), and Lebanese amber (Wunderlich, 2004g). But, these specimens were juveniles, and their placement within Araneidae remained uncertain due to the lack of morphological characterisation. Later, *Molinaranea mitnickii* from middle Miocene Dominican amber (Saupe et al., 2010), and *Eochorizopes* (Wunderlich, 2011) and *Minutunguis* (Wunderlich, 2012) from Baltic amber were reported. Recently, Poinar Jr. (2015) described a new araneid genus, *Pulchellaranea*, from Cenozoic Dominican amber, further enriching the known fossil diversity of araneids.

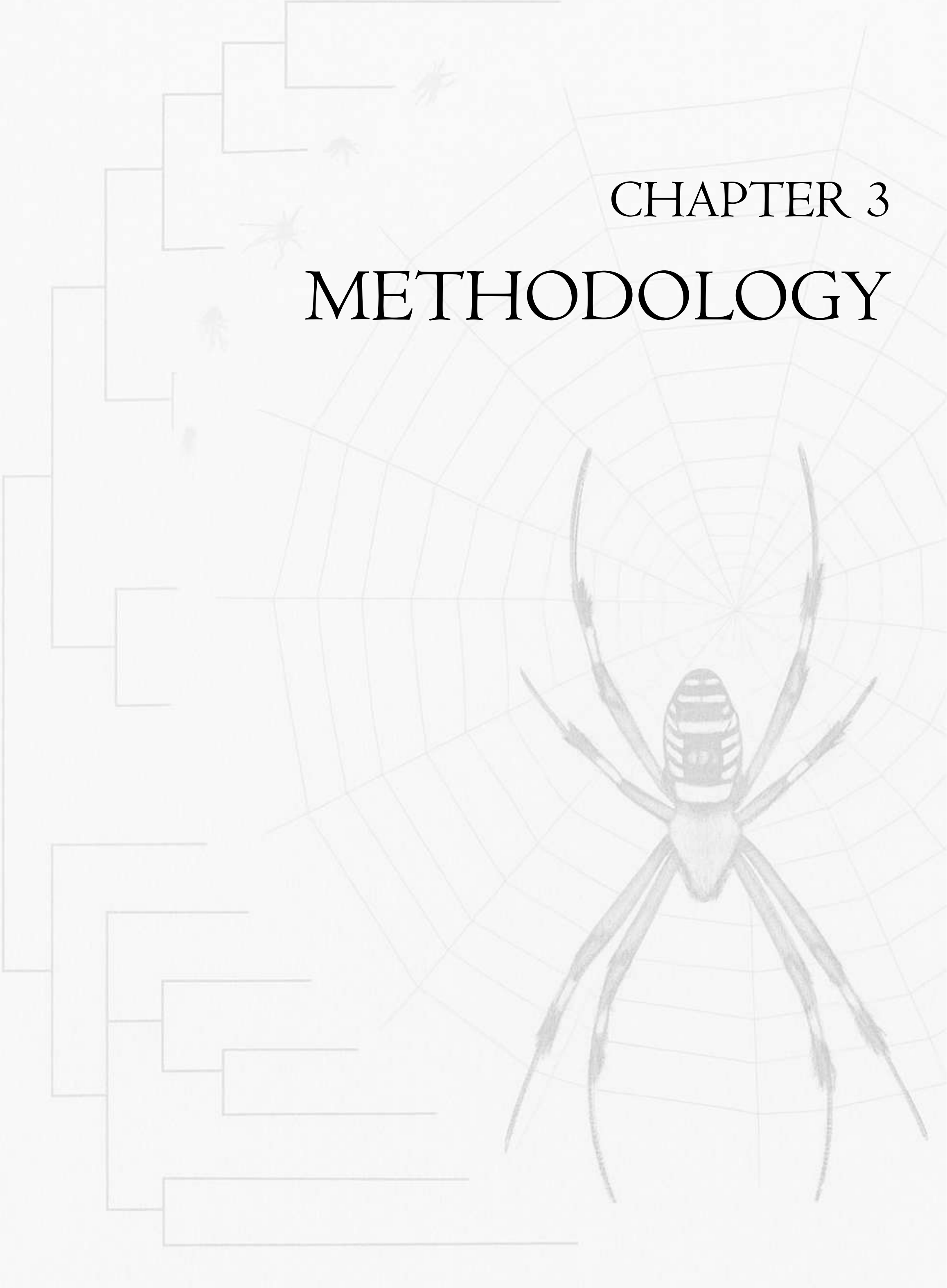
A review of the available literature highlights the ecological and research significance of spiders, particularly araneids. Developments in microscopy, such as SEM imaging and stereo zoom microscopy, have improved taxonomic resolution, leading to synonymization and the establishment of new genera. Molecular tools, especially DNA barcoding, have strengthened araneid taxonomy, with COI as the primary marker and additional genes (16S rRNA, 12S rDNA, 18S rDNA, 28S rDNA, H3, and ITS2) expanding the scope of molecular identification. In phylogenetic research, a total evidence approach, integrating morphological, behavioural, and molecular datasets, has proven invaluable for resolving evolutionary relationships. Divergence time analysis and fossil calibrations have further refined our understanding of araneid evolution and biogeographic patterns.

However, significant gaps remain. The evolutionary basis of extreme sexual dimorphism, polymorphism, and single-sex descriptions in araneids continues to pose challenges. Taxonomic ambiguities persist in genera such as *Araneus*, *Neoscona*, and *Cyclosa*, where modern imaging techniques have yet to be applied. The molecular data availability is slanted toward certain groups, and others underrepresented. While Scharff et al. (2020) provided a better understanding, it is geographically limited, and required a broader sampling. Even though there are fossil evidences, no successful attempt has been made to construct a dated phylogeny of Araneidae.

This study considers several of these gaps by providing a checklist of araneids from Kerala, that have been prepared by combining morphological and molecular data. Thus, contributing to the broader understanding of araneid phylogenetics. Additionally, it represents the first successful attempt to date araneid phylogeny over an evolutionary time scale. The study highlights the importance of continuous efforts using morphology, molecular data, and fossil evidence in refining araneid systematics and evolutionary research.

CHAPTER 3

METHODOLOGY



“***Look deep into nature, and then you will understand everything better***”

- ***Albert Einstein***

Methodology plays a crucial role in all types of research, whether quantitative or qualitative. A well-structured method provides a clear and logical framework for the study, justifies the research design, ensures data reliability, and facilitates efficient planning and organisation. It encompasses a systematic description of the sequential steps and procedures undertaken during the research process.

This section discusses the various methods, tools and techniques used to achieve the research objectives of the present study. It is organised into three major headings—morphological analysis, molecular analysis, and molecular data analysis—each further divided into relevant subsections.

3.1. Morphological analysis

This section gives a detailed account of the fieldwork and strategies used for the traditional morphological examination.

3.1.1. Study area: The present study was conducted in Kerala, a biodiversity-rich state in southern India. Kerala is bordered by the Arabian Sea to the west and the Western Ghats, a recognised biodiversity hotspot, to the east, spanning over an area of 38,863 sq. km. (Kerala Soil Survey, 2024). The state is home to eighteen wildlife sanctuaries, two tiger reserves, five national parks, one community reserve and a myristic swamp (Kerala Forest Department, 2025). The state encompasses different habitat types, including tropical evergreen rainforests, montane forests, riparian forests, semi-evergreen forests, deciduous forests, plantations such as teak, cashew, and rubber, and unique ecosystems such as mangroves, wetlands, grasslands, myristic swamps and shola forests (Forest Survey of India, 2019; Kerala Forest Department, 2025).

The interplay of climatic variations, diverse geography and multiple habitat types contributes to the exceptional biodiversity of the state. However, climate change, driven by anthropogenic activities, has led to shifting and overlapping seasonal patterns. Administratively, Kerala is divided into fourteen districts, which can be broadly grouped as northern Kerala, including the districts Kasaragod, Kannur, Kozhikode, Malappuram, and Wayanad; central Kerala, including Palakkad, Thrissur, Ernakulam, Idukki, and Kottayam; and southern Kerala including Alappuzha, Pathanamthitta, Kollam, and Thiruvananthapuram.

3.1.2. Sampling strategy: Specimens were collected from 54 sites across the state (Figure 3.1), ensuring that at least three sites per district were covered. Sampling sites were selected randomly and aimed to represent diverse habitats of the state, including tropical rainforests, tropical evergreens, deciduous forests, protected areas, mangroves, wetlands, and various plantations (Figure 3.2). The sampling sites included the protected areas, government and private properties. Considering the heterogeneity of these habitats, a flexible and random site-based sampling approach was adopted. An active search method wherein a systematic trajectory was fixed, with adjustments of the path based on vegetation density and terrain. This approach was adopted due to the high variability among sites while ensuring broad taxonomic representation across Kerala’s diverse habitats. The details of sampling sites are given in Table 3.1.

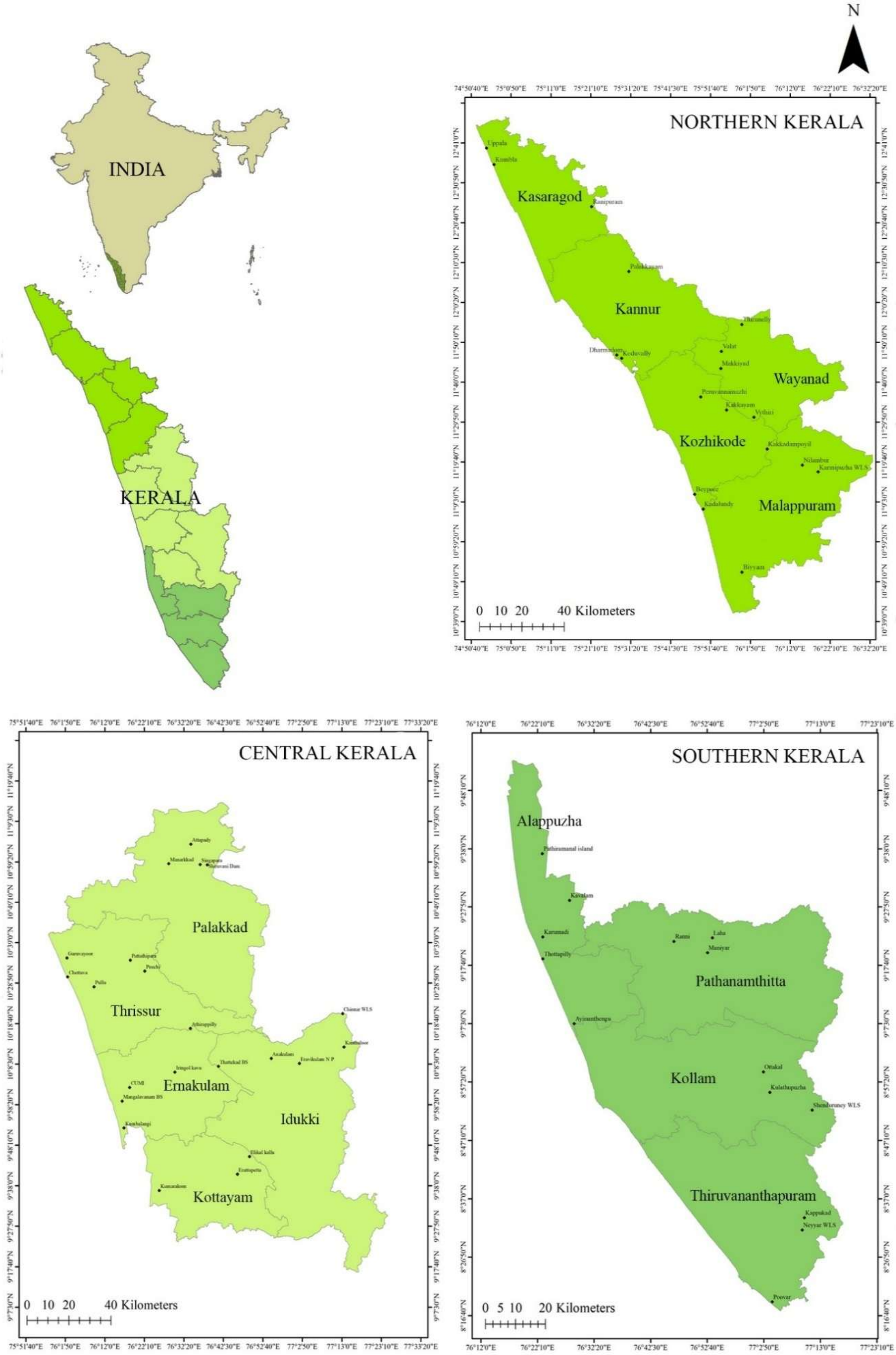


Figure 3.1. Map showing the geographical locations of 54 sampling sites.

Table 3.1. Details of the sampling sites, including the geographical coordinates, habitat type and collection time

Sl. No.	District	Sampling site	Coordinates	Habitat	Collection time (month, year)
1	Kasaragod	Kumbla	12°35'29.99"N, 74°56'32.22"E	Mangroves	September, 2022
2		Ranipuram	12°24'45.00"N, 75°21'24.00"E	Tropical evergreen shola forest, grassland	September, 2022
3		Uppala	12°39'40.97"N, 74°54'34.53"E	Mangroves	September, 2022
4	Kannur	Dharmadom	11°46'57.25"N, 75°27'48.11"E	Mangroves	March, 2022
5		Koduvally	11°46'05.37"N, 75°29'01.80"E	Mangroves	March, 2022
6		Palakkayam	12°08'13.67"N, 75°30'54.23"E	Grassland	March, 2022
7	Wayanad	Makkiyad	11°43'30.50"N, 75°54'18.00"E	Semi-evergreen forest, grassland	September, 2022
8		Thirunelly	11°54'43.08"N, 75°59'40.30"E	Tropical evergreen forest	September, 2022
9		Valat	11°47'52.13"N, 75°54'27.01"E	Plantation (banana, coffee)	April, 2022
10		Vythiri	11°31'04.27"N, 76°02'44.96"E	Tropical evergreen forest	July, 2023
11	Kozhikode	Beypore	11°11'26.92"N, 75°47'38.62"E	Mangroves	November, 2022
12		Kadalundy	11°07'40.59"N, 75°49'48.43"E	Mangroves	November, 2022
13		Kakkayam	11°32'55.68"N, 75°55'45.85"E	Tropical evergreen forest, moist deciduous forest	December, 2021
14		Peruvannamuzhi	11°36'16.74"N, 75°49'10.22"E	Tropical evergreen forest, riparian forest	December, 2021
15	Malappuram	Biyyam	10°51'35.08"N, 75°59'43.22"E	Mangroves, coastal area	August, 2022
16		Chungathura, Nilambur	11°18'52.68"N, 76°15'05.26"E	Plantation (teak)	December, 2023
17		Kakkadampoyil	11°22'58.51"N, 76°06'07.00"E	Grassland	December, 2023

18		Karmipuzha WLS	11°17'9.18"N, 76°19'4.88"E	Semi-evergreen forest	December, 2023
19	Palakkad	Attapady	11°03'47.83"N, 76°34'09.45"E	Tropical evergreen forest	July, 2022
20		Mannarkkad	10°58'53.69"N, 76°28'28.69"E	Plantations (cashew, banana)	January, 2022
21		Shiruvani Dam	10°58'35.85"N, 76°38'23.89"E	Tropical evergreen forest, riparian forest	January, 2024
22		Singapara	10°58'41.07"N, 76°36'34.05"E	Tropical evergreen forest, riparian forest	January, 2024
23	Thrissur	Athirappilly	10°17'27.17"N, 76°34'02.69"E	Tropical evergreen forest	August, 2021 & August, 2023
24		Chettuva	10°30'24.38"N, 76°02'25.57"E	Mangroves, coastal area	May, 2023
25		Guruvayoor	10°35'9.20"N, 76° 2'14.70"E	Mangroves	August, 2023
26		Pattathipara	10°34'35.85"N, 76°18'34.59"E	Plantation (rubber)	August, 2023
27		Peechi	10°31'51.48"N, 76°22'17.68"E	Tropical evergreen forest, man-made garden	December, 2024
28		Pullu	10°27'55.40"N, 76°09'13.92"E	Kole wetland	August, 2021 & February, 2022
29	Ernakulam	Iringol kavu	10°06'30.31"N, 76°30'01.95"E	Lowland evergreen forest	April, 2024
30		Kumbalangi	9°52'29.52"N, 76°16'57.42"E	Mangroves	October, 2024
31		Mangalavanam BS	9°59'14.70"N, 76°16'28.41"E	Mangroves	July, 2023
32		Thattekad BS	10°07'56.70"N, 76°41'15.81"E	Tropical evergreen forest, moist deciduous forest	February, 2023
33		CUMI, Carborundum Universal Limited	10°02'40.29"N, 76°18'25.43"E	Plantation (teak), scrubland	October, 2022
34	Idukki	Chinnar WLS	10°21'11.65"N, 77°13'13.47"E	Dry deciduous forest, riparian forest	February, 2024

35		Eravikulam N P	10°08'41.14"N, 77°02'05.34"E	Montane forest, grassland	October, 2021
36		Kanthaloor	10°12'48.44"N, 77°13'33.18"E	Plantation (tea, coffee)	February, 2024
37		Anakulam	10° 9'56.71"N, 76°54'50.81"E	Riparian forest	April, 2024
38	Kottayam	Erattupetta	9°40'52.95"N, 76°46'08.15"E	Plantations (rubber)	August, 2022
39		Illikal kallu	09°45'19.74"N, 76°49'13.26"E	Grassland	August, 2022
40		Kumarakom	9°36'48.30"N, 76°26'02.77"E	Mangrove, paddy field	November, 2024
41	Alappuzha	Karumadi	9°22'35.33"N, 76°23'07.81"E	Paddy field	March, 2023
42		Kavalam	9°29'01.89"N, 76°27'55.51"E	Paddy field	March, 2023
43		Pathiramanal island	9°37'10.29"N, 76°23'01.26"E	Mangroves, semi-evergreen	October, 2021 & March, 2023
44		Thottapilly	9°18'46.58"N, 76°23'07.21"E	Mangroves	March, 2023
45	Pathanamthitta	Laha	9°22'25.80"N, 76°53'35.20"E	Grassland	May, 2024
46		Maniyar	9°19'51.09"N, 76°52'42.73"E	Tropical evergreen	May, 2024
47		Ranni	9°21'49.33"N, 76°46'41.94"E	Plantation (rubber)	May, 2024
48	Kollam	Ayiramthengu	9°07'30.22"N, 76°28'45.28"E	Mangroves	May, 2023
49		Kulathupuzha, Thenamala	8°55'32.18"N, 77°03'55. 15"E	Riparian forest	May, 2023
50		Ottakal, Thenmala	8°59'06.3"N, 77°02'45.3"E	Tropical evergreen forest	May, 2023
51		Shenduruney WLS	8°52'27.15"N, 77°11'30.62"E	Tropical evergreen and semi-evergreen forest	May, 2023
52	Thiruvananthapuram	Kappukad	8°33'42.1"N, 77°10'06.8"E	Tropical evergreen, deciduous forest	December, 2022
53		Neyyar Wildlife Sanctuary	8°31'34.2"N, 77°09'44.5"E	Tropical evergreen	December, 2022
54		Poovar	8°19'03.15"N, 77°04'21.94"E	Mangroves	December, 2022

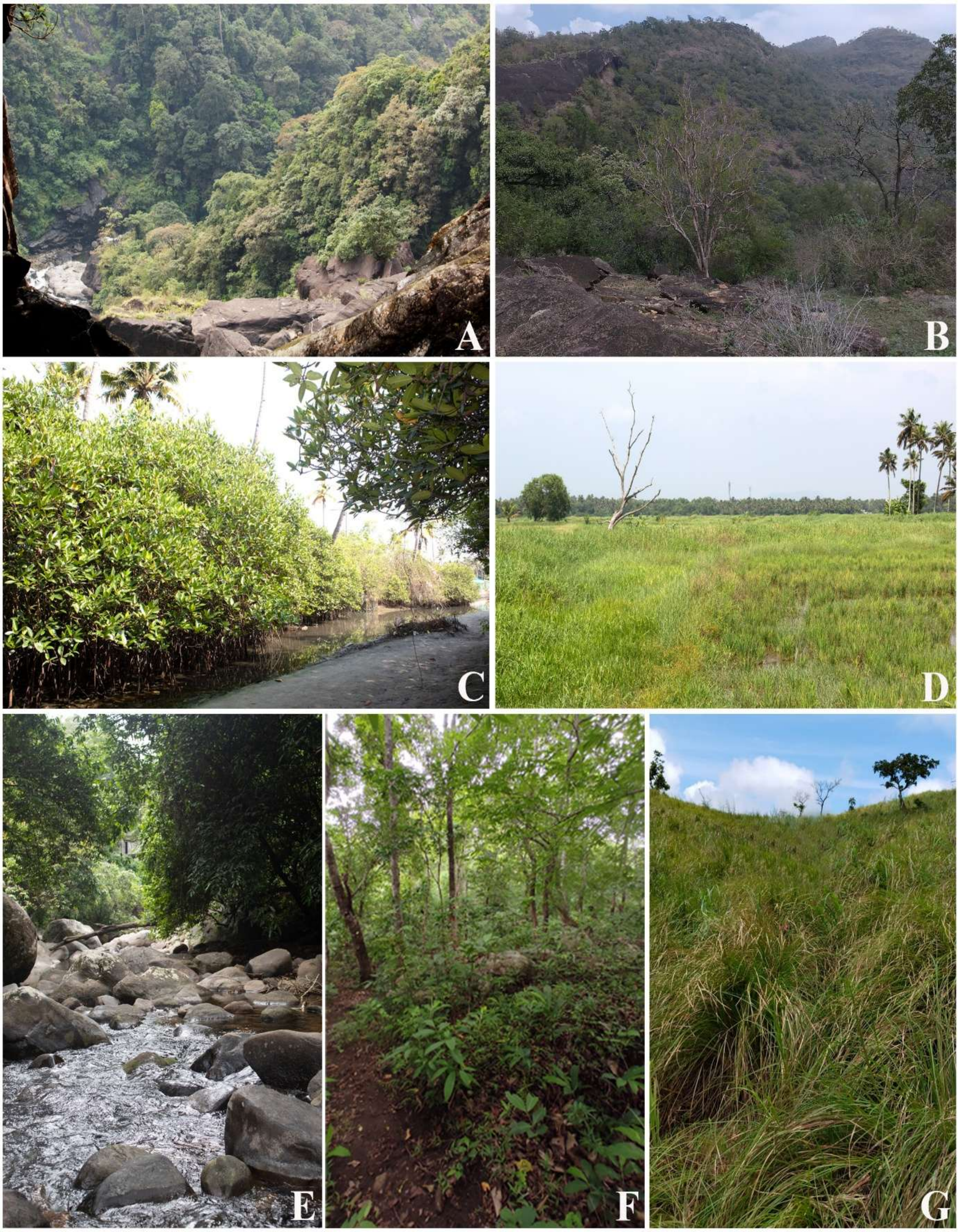


Figure 3.2.a. Various habitats from which the collection of specimens is done: (A) Tropical evergreen forest, (B) Dry deciduous forest, (C) Mangrove, (D) Kole wetland, (E) Riparian forest, (F) Semi-evergreen forest, (G) Grassland

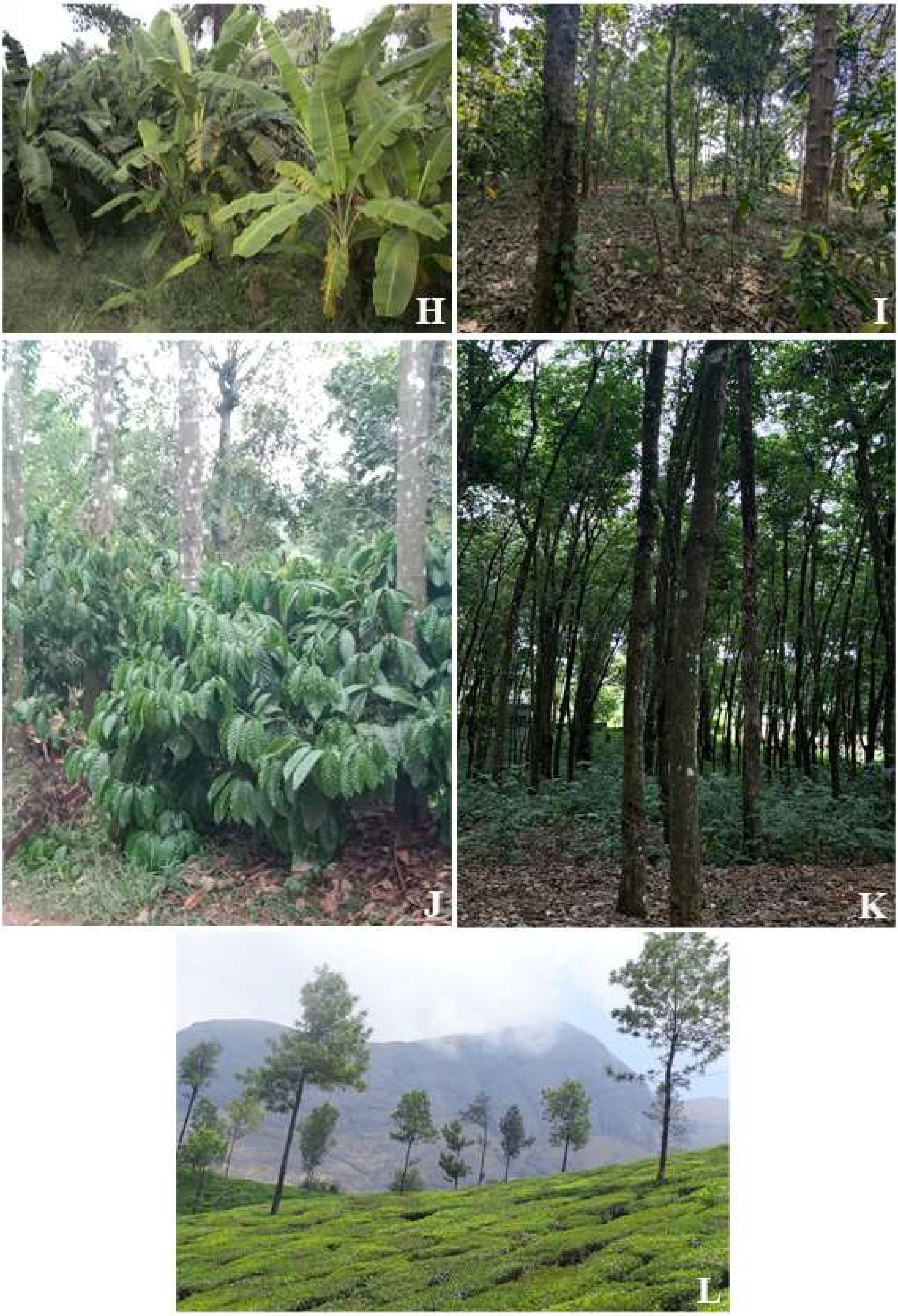


Figure 3.2.b. Various habitats from which the collection of specimens is done: (H) Banana plantation, (I) Teak plantation, (J) Coffee plantation, (K) Rubber plantation, (L) Tea plantation

3.1.3. Sampling period: The sampling was done over 42 months (July 2021 – December 2024). Each site was visited once, except a few required additional visits for further collection or analysis (Table 3.1). Specimen collection was carried out twice a day, during morning (6.00 a.m. to 12.00 p.m.) and evening (5.00 p.m. to 8.00 p.m.). Sampling was conducted actively throughout the year, except during June and July, due to the monsoon.

3.1.4. Specimen collection methods: Compared to other active spider families, araneids are sedentary and only move actively upon encountering an attack. They build orb webs and either occupy the hub or rest in a retreat associated with the web. Hence, capturing araneids includes spotting the orb webs and actively searching the premises of the web. Depending only on a particular collection method will not be effective as variations in the sampling sites exist, hence, a combination of the following methods was used.

a. **Visual searching and hand collection:** The most appropriate method to collect araneids that exist within 1-3 meters from the ground is to search visually and collect them by hand (Coddington et al., 1991). A soft paintbrush or blunt-ended forceps were used to gently tap the specimen into the collecting bottles (Sebastian & Peter, 2009). This method was used in all kinds of habitats (Figure 3.3 A).

b. **Beating and shaking:** This method can be employed in habitats with complex bushes, shrubs, and small trees. It involves beating the branches with a strong twig or vigorous shaking. This led to the dislodging of the spiders into a white plastic or cotton sheet placed below the shaken plant, followed by a thorough inspection for any fallen araneids, which will be captured with collection vials (Figure 3.3 B).

c. **Using the sweep net:** Using a sweep net is ideal for collecting the araneids inhabiting the grasslands and agroecosystems. In this simple procedure, a sweep net of respective length, made of heavy fabric, is swept back and forth through the vegetation. This results in the trapping of spiders (Figure 3.3 C).

d. **Litter collection:** Certain araneids lay their egg in leaf litter or rotting twigs and barks near the ground (Kuntner et al., 2009). Hence, considering litters during the collection of araneids is essential. Ground litter samples were collected and spread on a white plastic or cotton sheet, followed by sorting the litter along with a thorough search for the spider and capturing it before its escape. It is a time-consuming process and is applicable in any habitat that yields a good amount of litter (Figure 3.3 D).

Figure 3.3. Different collection methods used for sampling: (A) Visual searching on vegetation, (B) Beating and shaking vegetation onto a collecting sheet, (C) Sweeping through grasses with a sweep net, (D) Litter collection and sorting.

3.1.5. Specimen documentation: Certain details were noted on site, including the locality, habitat features, web features, and other notable observations. Later, these details were elaborated and entered into the files for proper documentation. The photographs of certain live araneids were taken in the field, and some others were brought to the lab alive. Equipment used to capture live images includes Canon EOS 1300D (W), Canon EF-S 55-250mm Zoom lens, Canon EF-S 18-55mm Zoom lens, and Canon EF 100mm f/2.8L IS USM Macro lens. The images were further processed in Adobe Photoshop CS 2020.

3.1.6. Specimen preservation and storage: After capturing, the specimens were transferred into labelled plastic vials, containing either 70% or 100% ethanol, in which they were stored. Usually, 5 - 6 specimens of a species were collected. 50% of the specimens were transferred to 70% ethanol for morphological examination, and the rest to 100% ethanol for DNA isolation. However, for rare species, the entire specimens will be stored in 100% ethanol. After being brought to the lab, the specimens for morphological examination were provisionally identified, catalogued and stored in a closed rack for a detailed examination. These storage bottles were regularly checked for damage, and refilling of the ethanol was done to prevent drying up. Specimens for the DNA extraction were also provisionally identified, catalogued, and stored at -20°C until the molecular process began.

3.1.7. Morphological examination and identification: Detailed morphological examination of each specimen was done to reveal its taxonomic identity. The major equipment used for this was a Leica M205C stereomicroscope (Figure 3.4). Microscopic photographs of the morphological features and genitalia were captured using a Leica DMC4500 digital camera attached to Leica M205C stereomicroscope along with the software package Leica Application Suite (LAS) Windows version 4.3.0. All measurements were recorded in millimetres (mm).

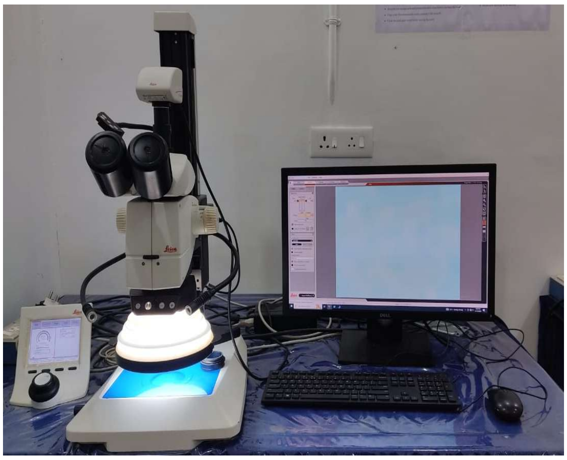


Figure 3.4. Leica M205C stereomicroscope attached with Leica DMC4500 digital camera.

Genitalia were dissected out and cleared in 10% potassium hydroxide (KOH) solution. As the epigyne of araneids are heavily sclerotised, heating the KOH solution was a required step, in the case of certain specimens. Species confirmation was done using various keys (Tikader, 1982; Barrion & Litsinger, 1995; Yin et al., 1997; Levi, 2002; Tanikawa, 2009). Most of all, the literatures were accessed from the WSC (2025). The species identity of certain specimens has not been revealed due to various reasons, such as damaged specimens and genitalia, lack of proper literature, etc.

All the examined specimens were deposited in the Centre for Animal Taxonomy and Ecology (CATE) reference collections, Department of Zoology, Christ College (Autonomous), Irinjalakuda, Kerala, India.

3.1.8. Figures, illustrations and maps: All figures, illustrations and maps included in this thesis were originally developed as part of the present study. Microscopic images and photographs were captured as mentioned in sections 3.1.7. and 3.1.5., respectively. All images were processed in Adobe Photoshop 2020 (Adobe Inc., 2020). The illustrations were made using the online platform draw.io version 26.2.14. (JGraph Ltd., 2023). All maps presented were generated using ArcGIS version 10.8 (Esri, 2020).

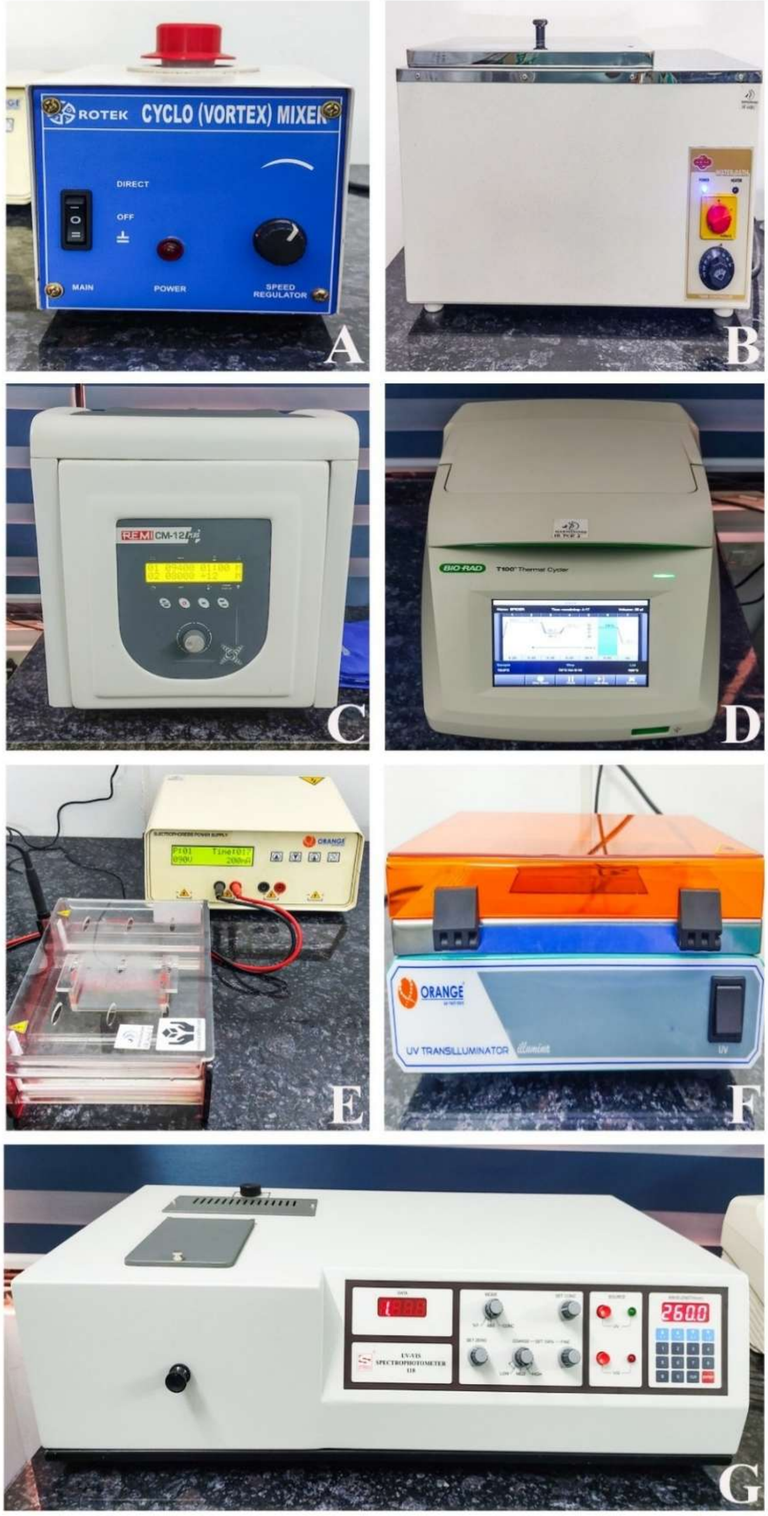
3.2. Molecular analysis

This section describes the molecular laboratory protocols followed to generate genetic data from the collected specimens. Steps from genomic DNA extraction and quality assessment to the amplification and sequencing of selected genetic markers are detailed below.

3.2.1. Genomic DNA extraction: Genomic DNA from specimens stored at -20°C was isolated using the DNeasy Blood and Tissue Kit (Qiagen), following the spin column purification protocol. To minimise contamination, legs from one side of the spider’s body, including the coxal segments, were used for DNA isolation; however, for smaller specimens, the entire body was utilised. The tissue was lysed completely, and the released DNA was allowed to bound to spin columns, which were subsequently washed and eluted. All molecular procedures were conducted using the laboratory facilities at Marigenome, Ernakulam, Kerala. The detailed steps followed for DNA extraction are described below.

- The legs from one side of the specimen were dissected out and placed on a sterile glass slide, allowing the evaporation of ethanol. 25 mg of the tissue was weighed and transferred into a sterile pre-cooled mortar. To enhance the lysis, the tissue was ground thoroughly along with 180 µl Buffer ATL. The whole contents were pipetted out into a sterile 2.0 ml microcentrifuge tube.
- Added 20 µl Proteinase K, followed by vortexing (Figure 3.5 A) for 10-15 seconds. The microcentrifuge tube was incubated at 56°C for complete lysis of the tissue (around 2.5 to 3 hours) in a water bath (Figure 3.5 B). During the incubation period, the tube was taken out and vortexed (Figure 3.5 A) occasionally.
- After incubation, 200 µl Buffer AL was added to the mixture, followed by a thorough vortexing (Figure 3.5 A) for 10-15 seconds. Added 200 µl ethanol (96–100%) and vortexed (Figure 3.5 A) again.
- The entire mixture was carefully pipetted into the DNeasy Mini spin column, placed in a 2 ml collection tube, and subjected to centrifugation (Figure 3.5 C) at 8000 rpm for 1 minute.
- Discarded the flowthrough, and the spin column was placed in a new 2 ml collection tube. 500 µl Buffer AW1 was added to the spin column and again subjected to centrifugation (Figure 3.5 C) at 8000 rpm for 1 minute.
- Discarded the flowthrough, and the spin column was placed in a new collection tube. 500 µl Buffer AW2 was added to the spin column. It was then kept for centrifugation (Figure 3.5 C) at 14,000 rpm for 3 minutes. Discarded the flowthrough, and the spin column was placed in a sterile 1.5 ml microcentrifuge tube for elution.

- Carefully added 100 µl Buffer AE to the centre of the spin column membrane and incubated at room temperature for 1 minute. It was then centrifuged (Figure 3.5 C) at 8000 rpm for 1 minute. The step was repeated for maximum DNA yield.



A - Vortex
Cyclo (Vortex) Mixer
ROTEK Instruments

B - Water bath
KEMI Lab Equipments

C - Cooling micro centrifuge
CM-12 Plus
REMI Lab World

D - Thermalcycler
96 well T100
Bio-Rad Laboratories

E - Electrophoresis unit
Mini horizonatal unit
(Standard model)
Orange, Oprl Bio Sciences

F - Transilluminator
UV - BLB Research
Orange, Oprl Bio Sciences

G - Spectrophotometer
UV-VIS 118
SYSTRONICS India

Figure 3.5. Various instruments used during DNA extraction and amplification: A-G (Instrument, Model, Manufacturer).

3.2.2. DNA quantification: Assessing the quality and quantity of the extracted DNA is a crucial step, and UV spectrophotometry was used for this purpose. For this, 100 µl of the eluted DNA sample was transferred to a cuvette containing 1900 µl of distilled water. Similarly, a blank was prepared using 100 µl Buffer AE and 1900 µl of distilled water. The absorbance of the sample was measured at 260 nm and 280 nm using a UV spectrophotometer (Figure 3.5 G). The concentration of DNA was calculated using the formula:

$$\text{Concentration} = \text{Absorbance at 260 nm} \times 50 \times \text{Dilution factor}$$

The ratio of absorbance at 260 nm to 280 nm was calculated to determine the DNA quality, with values between 1.7 and 2.0 considered indicative of good quality. Samples exhibiting ratios outside this range were discarded, and DNA isolation was repeated.

3.2.3. Selection of genetic markers: In the present study, fragments of two loci, mitochondrial cytochrome oxidase subunit I (COI) and nuclear histone 3 (H3), were used for species confirmation and phylogenetic analysis. COI is widely accepted as the primary marker for the identification of animals (Hebert et al., 2003a) and has proved its potential in spider species identitification, including cryptic groups (Barrett & Hebert, 2005; Coddington et al., 2016, Spasojevic et al., 2016; Wang et al., 2018; Tan et al., 2019b).

The H3 gene has been used in DNA barcoding since the proposal of the H3 barcode hypothesis (Hake & Allis, 2006), and it has been applied in spider taxonomy and phylogenetics (Dimitrov et al., 2017; Kallal & Hormiga, 2018b; Tan, 2018; Scharff et al., 2020). The availability of reference sequences in public databases such as GenBank and BOLD further supported the selection of these markers.

Even though the initial plan was to include additional markers, such as 16S rRNA (mitochondrial), 28S rDNA (nuclear), and 18S rDNA (nuclear), the unavailability of reference sequences and certain practical constraints ultimately led to the selection of COI and H3.

3.2.4. PCR amplification of target markers: The selected markers were amplified from the extracted DNA sample using the principles of Polymerase Chain Reaction (PCR) (Figure 3.5 D). Each PCR reaction was performed in a total volume of 25 µl, including 12.5 µl Takara Emerald Green 2X PCR master mix, 1 µl forward primer, 1 µl reverse primer, 4 µl template DNA and 6.5 µl distilled water. For COI amplification, the forward primer LCO1490 was paired with different reverse primers, HCO 2198, chelicerate-R1, and chelicerate-R2 (Table 3.2). For H3 amplification, the forward primer, H3aF, and reverse primer, H3aR, were used (Table 3.2).

Table 3.2. Primer sequences used for COI and H3 amplification

Marker	Primer	Sequence	Reference
COI	LCO1490	5’-GGTCAACAAATCATAAAGATATTGG-3’	Folmer et al. (1994)
	HCO2198	5’-TAAACTTCAGGGTGACCAAAAAATCA-3’	Folmer et al. (1994)
	chelicerate-R1	5’-CCTCCTCCTGAAGGGTCAAAAAATGA-3’	Barret & Hebert (2005)
	chelicerate-R2	5’-GGATGGCCAAAAAATCAAAATAAATG-3’	Barret & Hebert (2005)
H3	H3aF	5’-ATGGCTCGTACCAAGCAGACVGC-3’	Colgan et al. (1998)
	H3aR	5’-ATATCCTTRGGCATRATRGTGAC-3’	Colgan et al. (1998)

A standardised thermal profile was utilised for the amplification of both COI and H3 (Table 3.3), however, for the samples that failed to amplify, a touch-up step was introduced to enhance amplification efficiency (Cheng & Kunter, 2014). Primer sequences and thermal profiles for each sample are detailed below (Tables 3.4 & 3.5).

Table 3.3. Thermal regimes used for the amplification process

PCR pI	Initial denaturation at 94°C for 5 min, followed by 35 cycles of 45 sec denaturation at 94°C, 45 sec annealing with a gradient from 45°C to 50°C and 45 sec extension at 72 °C with a final extension of 5 min at 72 °C and cooling to 4°C
PCR pII (Cheng & Kuntner, 2014)	Initial denaturation at 94°C for 2 min followed by 20 cycles of touch-up amplification (50 sec denaturation at 94°C, 1 min annealing at 46–52°C, 50 sec extension at 72°C), followed by 15 cycles of 50 sec denaturation at 94°C, 1 min annealing at 52°C, 50 sec extension at 72°C, and a 7 min final extension at 72°C
PCR pIII	Initial denaturation at 94°C for 5 min, followed by 35 cycles of 45 sec denaturation at 94°C, 45 sec annealing with a gradient from 47°C to 52°C and 45 sec extension at 72 °C with a final extension of 5 min at 72 °C and cooling to 4°C

Table 3.4. Primer pairs and temperature profiles for generating COI sequences

Sl. No.	Voucher specimen No.	Forward primer	Reverse Primer	PCR profile
1	ARAMP007	LCO1490	chelicerate-R2	PCR pII
2	ARAMP008	LCO1490	chelicerate-R2	PCR pII
3	ARAMP012	LCO1490	chelicerate-R2	PCR pII
4	ARAMP015	LCO1490	HCO2198	PCR pII
5	ARAMP016	LCO1490	HCO2198	PCR pII
6	ARAMP017	LCO1490	HCO2198	PCR pI
7	ARAMP018	LCO1490	HCO2198	PCR pI
8	ARAMP019	LCO1490	chelicerate-R2	PCR pII
9	ARAMP020	LCO1490	HCO2198	PCR pII
10	ARAMP021	LCO1490	HCO2198	PCR pI
11	ARAMP022	LCO1490	HCO2198	PCR pII
12	ARAMP023	LCO1490	HCO2198	PCR pII
13	ARAMP024	LCO1490	HCO2198	PCR pII
14	ARAMP025	LCO1490	chelicerate-R2	PCR pII
15	ARAMP026	LCO1490	HCO2198	PCR pII
16	ARAMP027	LCO1490	HCO2198	PCR pI
17	ARAMP028	LCO1490	HCO2198	PCR pI
18	ARAMP029	LCO1490	HCO2198	PCR pII
19	ARAMP030	LCO1490	chelicerate-R1	PCR pII

20	ARAMP031	LCO1490	HCO2198	PCR pII
21	ARAMP032	LCO1490	chelicerate-R2	PCR pII
22	ARAMP033	LCO1490	HCO2198	PCR pI
23	ARAMP034	LCO1490	HCO2198	PCR pII
24	ARAMP035	LCO1490	HCO2198	PCR pII
25	ARAMP036	LCO1490	HCO2198	PCR pII
26	ARAMP037	LCO1490	HCO2198	PCR pII
27	ARAMP038	LCO1490	HCO2198	PCR pII
28	ARAMP039	LCO1490	HCO2198	PCR pII
29	ARAMP041	LCO1490	HCO2198	PCR pII
30	ARAMP042	LCO1490	HCO2198	PCR pII
31	ARAMP043	LCO1490	HCO2198	PCR pII
32	ARAMP044	LCO1490	chelicerate-R2	PCR pII
33	ARAMP045	LCO1490	HCO2198	PCR pII
34	ARAMP046	LCO1490	HCO2198	PCR pII
35	ARAMP047	LCO1490	HCO2198	PCR pII
36	ARAMP048	LCO1490	HCO2198	PCR pII
37	ARAMP049	LCO1490	HCO2198	PCR pII
38	ARAMP050	LCO1490	HCO2198	PCR pII
39	ARAMP051	LCO1490	HCO2198	PCR pII
40	ARAMP052	LCO1490	HCO2198	PCR pII
41	ARAMP053	LCO1490	chelicerate-R2	PCR pII
42	ARAMP054	LCO1490	HCO2198	PCR pII
43	ARAMP055	LCO1490	HCO2198	PCR pII
44	ARAMP056	LCO1490	HCO2198	PCR pII
45	ARAMP057	LCO1490	HCO2198	PCR pII
46	ARAMP058	LCO1490	chelicerate-R2	PCR pII
47	ARAMP060	LCO1490	chelicerate-R2	PCR pII
48	ARAMP061	LCO1490	chelicerate-R2	PCR pII
49	ARAMP063	LCO1490	chelicerate-R2	PCR pII
50	ARAMP065	LCO1490	chelicerate-R2	PCR pII
51	ARAMP066	LCO1490	chelicerate-R2	PCR pII
52	ARAMP067	LCO1490	HCO2198	PCR pII
53	ARAMP068	LCO1490	HCO2198	PCR pII
54	ARAMP069	LCO1490	HCO2198	PCR pII

55	ARAMP070	LCO1490	HCO2198	PCR pII
56	ARAMP071	LCO1490	chelicerate-R1	PCR pII
57	ARAMP073	LCO1490	chelicerate-R1	PCR pII
58	ARAMP074	LCO1490	chelicerate-R1	PCR pII
59	ARAMP075	LCO1490	chelicerate-R1	PCR pII
60	ARAMP076	LCO1490	chelicerate-R1	PCR pII
61	ARAMP077	LCO1490	HCO2198	PCR pII
62	ARAMP078	LCO1490	HCO2198	PCR pII
63	ARAMP079	LCO1490	HCO2198	PCR pII
64	ARAMP080	LCO1490	chelicerate-R2	PCR pII
65	ARAMP082	LCO1490	chelicerate-R2	PCR pII
66	ARAMP083	LCO1490	chelicerate-R2	PCR pII
67	ARAMP084	LCO1490	HCO2198	PCR pII
68	ARAMP085	LCO1490	chelicerate-R1	PCR pII
69	ARAMP086	LCO1490	chelicerate-R1	PCR pII
70	ARAMP087	LCO1490	chelicerate-R2	PCR pII
71	ARAMP088	LCO1490	chelicerate-R2	PCR pII
72	ARAMP089	LCO1490	chelicerate-R2	PCR pII
73	ARAMP090	LCO1490	chelicerate-R2	PCR pII
74	ARAMP091	LCO1490	HCO2198	PCR pII
75	ARAMP092	LCO1490	chelicerate-R2	PCR pII
76	ARAMP093	LCO1490	chelicerate-R1	PCR pII
77	ARAMP095	LCO1490	chelicerate-R2	PCR pII
78	ARAMP096	LCO1490	chelicerate-R2	PCR pII

Table 3.5. Primer pairs and temperature profiles for generating H3 sequences

Sl. No.	**Voucher specimen No.**	**Forward primer**	**Reverse Primer**	**PCR profile**
1	ARAMP007	H3aF	H3aR	PCR pIII
2	ARAMP008	H3aF	H3aR	PCR pIII
3	ARAMP012	H3aF	H3aR	PCR pIII
4	ARAMP015	H3aF	H3aR	PCR pIII
5	ARAMP016	H3aF	H3aR	PCR pIII
6	ARAMP017	H3aF	H3aR	PCR pIII
7	ARAMP018	H3aF	H3aR	PCR pIII
8	ARAMP019	H3aF	H3aR	PCR pIII

9	ARAMP020	H3aF	H3aR	PCR pIII
10	ARAMP021	H3aF	H3aR	PCR pIII
11	ARAMP022	H3aF	H3aR	PCR pIII
12	ARAMP023	H3aF	H3aR	PCR pIII
13	ARAMP024	H3aF	H3aR	PCR pIII
14	ARAMP025	H3aF	H3aR	PCR pIII
15	ARAMP026	H3aF	H3aR	PCR pIII
16	ARAMP027	H3aF	H3aR	PCR pIII
17	ARAMP028	H3aF	H3aR	PCR pIII
18	ARAMP032	H3aF	H3aR	PCR pIII
19	ARAMP034	H3aF	H3aR	PCR pIII
20	ARAMP043	H3aF	H3aR	PCR pIII
21	ARAMP049	H3aF	H3aR	PCR pIII
22	ARAMP052	H3aF	H3aR	PCR pIII
23	ARAMP054	H3aF	H3aR	PCR pIII
24	ARAMP060	H3aF	H3aR	PCR pIII
25	ARAMP061	H3aF	H3aR	PCR pIII
26	ARAMP067	H3aF	H3aR	PCR pIII
27	ARAMP070	H3aF	H3aR	PCR pIII
28	ARAMP078	H3aF	H3aR	PCR pIII
29	ARAMP082	H3aF	H3aR	PCR pIII
30	ARAMP084	H3aF	H3aR	PCR pIII
31	ARAMP096	H3aF	H3aR	PCR pIII
32	ARAMP097	H3aF	H3aR	PCR pIII
33	ARAMP098	H3aF	H3aR	PCR pIII
34	ARAMP099	H3aF	H3aR	PCR pIII

3.2.5. Amplicon quality assessment: The PCR products were subjected to Agarose Gel Electrophoresis (AGE) (Figure 3.5 E). Electrophoresis has been an integral technique in molecular biology for separating molecules based on their charge and size, providing critical information about the integrity and quality. In the present study, 1.2% agarose gel was used. Following electrophoresis, the gels were visualised using a transilluminator (Figure 3.5 F).

Samples exhibiting intense bands upon staining with SYBR Green were selected for subsequent sequencing.

3.2.6. DNA Sequencing: Purification of the PCR product and partial sequencing of the markers were done at GeneSpec, Kakkanad, Ernakulam, Kerala, which was an outsourced service. Partial sequencing of the markers was done based on Sanger Sequencing.

3.2.7. Sequence processing and preliminary analysis: The Sanger sequencing results were reviewed before proceeding further. Each sequence was carefully examined for contamination, and any noise was manually corrected using BioEdit (Hall, 1999). Primary screening of these developed sequences was performed using BLAST (Altschul et al., 1990) by NCBI. Query coverage and percentage identity were used to confirm species identification. Since both genes are protein-coding, the sequences were translated to check for stop codons using the online tool provided by Expasy (Gasteiger et al., 2003). Finally, these validated sequences were submitted to the GenBank database at NCBI.

3.3. Molecular data analysis

This section outlines the analytical approaches towards the molecular dataset. The methods include genetic distance computation, phylogenetic tree construction using various algorithms, and divergence time estimation. The software and programs used for the analysis are also described.

3.3.1. Genetic divergence analysis: The genetic divergence of the sequences of morphologically challenging specimens was assessed by calculating the pairwise genetic distance (Nei, 1979). Pairwise genetic distance quantifies genetic divergence by measuring the differences in base pairs between sequences. COI sequences from the concerned specimens were selected, and their homologous sequences were retrieved from GenBank, followed by multiple sequence alignment in ClustalX (Thompson et al., 1997). Gaps were trimmed using MEGA11 (Tamura et al., 2021). After confirming the quality with Gblocks (Castresana, 2000), the best-fit nucleotide substitution model for the alignment was determined and the calculation of pairwise genetic distance in MEGA11 (Tamura et al., 2021). The number of base substitutions per site between sequences was calculated using the K2P model (Kimura, 1980). 1st, 2nd, and 3rd codon positions were included, and all ambiguous positions were removed using the pairwise deletion option.

3.3.2. Phylogenetic reconstruction: Phylogenetic analysis of the araneids in Kerala was done based on the molecular data obtained from the partial sequencing of two genes, COI and H3, through this study. To understand the phylogenetic relationships of araneids in Oriental and Palearctic regions, sequences were retrieved from GenBank (Table S1). For the phylogenetic analysis, two species (*Leucauge decorata* and *Leucauge fastigata*) belonging to the family Tetragnathidae were used as the outgroups, as they show a sister relationship with the family Araneidae. Both gene trees and trees based on concatenated sequences were reconstructed. Steps for phylogenetic reconstruction are illustrated in Figure 3.6. To analyse the phylogenetic relationships of araneids, four different methods were used:

1. Neighbour Joining (NJ) (Saitou & Nei, 1987): A stepwise clustering method that uses a distance matrix.

   This method sequentially finds and connects the possible operational taxonomic unit (OTU) pairs connected by a common node, and finally joins those pairs with the shortest internal branch, thus providing the shortest possible tree. Ease, speed and computational efficiency made this method popular over other distance-based phylogenetic methods. However, its accuracy may be compromised in complex evolutionary scenarios, and the assumption of equal evolutionary rates can affect its reliability. NJ analysis was conducted using MEGA11 (Tamura et al., 2021) in the study.
2. Maximum Parsimony (MP) (Fitch, 1971): An exhaustive search method based on character states.

   During the exhaustive search, the algorithm starts by considering an initial tree based on the input data, followed by inferring the character changes explaining all nodes. Similarly, all possible topologies are analysed, and the topology with the minimum number of character changes will be the optimal tree. This method can be applied to both morphological and molecular data and does not assume a substitution model. However, this method is less reliable for highly variable sequences due to long-branch attraction, and it does not provide any statistical support for nodes. In the present study, MP analysis was done in MEGA11 (Tamura et al., 2021).

3. Maximum Likelihood (ML) (Felsenstein, 1981): An exhaustive search method using character states.

   The probability of each character in the ancestral nodes will be calculated by the algorithm and provide a likelihood score for the tree topology. After calculating the likelihood of all possible tree topologies, the algorithm chose the most likely tree as the best one. Unlike the MP method, this method requires a specific substitution model and provides statistical support through bootstrapping. Even though the method is time consuming and computationally intensive, the analysis can handle large data with different rates of evolution. In the present study, ML analysis was done using RAxML (Stamatakis, 2014).

4. Bayesian Inference (BI) (Huelsenbeck & Ronquist, 2001): An exhaustive search method using character states.

   Based on the input data, the probability of a tree to be correct is calculated in this method. This method uses Bayes' theorem, where the posterior probability of a tree topology is determined from the prior probability and likelihood. The analysis uses the Markov Chain Monte Carlo (MCMC) algorithm, and the results are statistically supported by posterior probability values. This method can handle more complex substitution models, and yield accurate and consistent trees. However, it is computationally intensive, and the results are dependent on the priors and models. MrBayes (Ronquist et al., 2012) was used to perform this analysis.

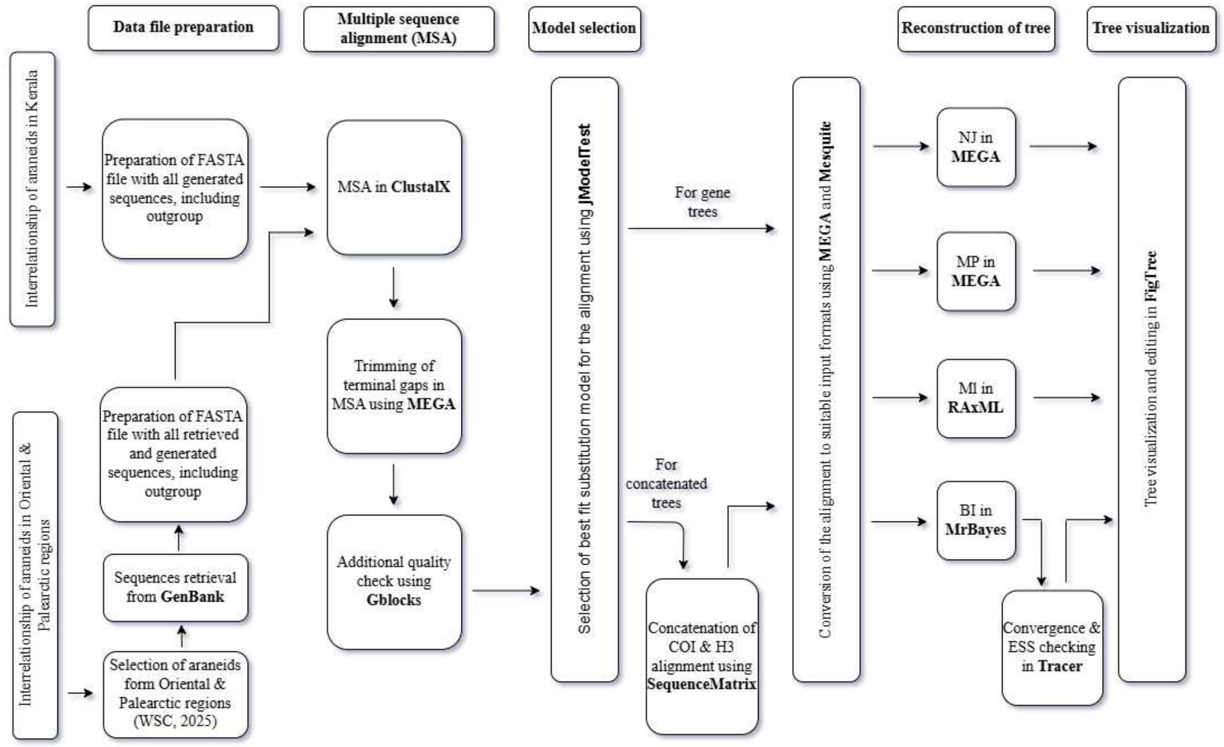


Figure 3.6. Illustration of the step-by-step procedure for the phylogenetic analysis.

3.3.3. Divergence time estimation: The time-calibrated phylogenetic tree of araneids was reconstructed using BEAST (Bouckaert et al., 2019). For the estimation of divergence time, a partitioned dataset was used, which includes the sequences developed in the current study and those retrieved from GenBank. The analysis used a Bayesian MCMC approach. The XML file was generated using the BEAUti utility within the software package. Details of the models and priors used are given along with the results. Two calibration points were applied (Table 3.6). Following the analysis, convergence was assessed in Tracer (Rambaut et al., 2018), and the final tree was visualised using FigTree (Rambaut, 2012).

Table 3.6. Molecular clock calibration points for araneids

Si. No	Calibration point (C)	Period	Placement in the tree
1	*Mesozygiella dunlopi* (C1)	115 – 121 Mya	Assigned to the root of the tree as it is considered the stem araneoid (Magalhaes et al., 2020)
2	*Palaeonephila dilitans* (C2)	43 – 47.8 Mya	Assigned to entire nephilines as it is considered the stem Nephilinae (Magalhaes et al., 2020)

3.3.4. Software and computational tools:

Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990): BLAST is an online program developed by NCBI to perform similarity searches on sequence data. It helps to find the highest-scoring optimal local alignments with the query sequence. BLAST, specifically BLASTn, was utilised for the preliminary identification of sequences, thereby confirming taxonomic identity and identifying homologous sequences for downstream analysis.

BEAST version 2.7.7 (Bouckaert et al., 2019): Bayesian Evolutionary Analysis Sampling Trees (BEAST) is a software package developed for Bayesian phylogenetic analysis. This software is particularly significant for evolutionary time estimates. The software allows the user to incorporate fossil calibrations, select the clock model, and change prior distributions. Different applications in this software package, such as Tree Annotator, BEAUti, DensiTree, etc., make the user experience user-friendly. In the present study, divergence time estimations were performed using BEAST.

BioEdit version 7.7.1 (Hall, 1999): This freely available program serves as the biological sequence alignment editor. It is the primary application for viewing, editing and aligning the molecular data. In the present study, it was used to analyse the raw sequence data and enhance its quality by checking for contamination and editing the noise.

ClustalX version 1.81 (Thompson et al., 1997): This is the graphical interface of the widely used alignment tool, ClustalW. The software can take nucleotide and amino acid sequences for multiple sequence alignment using a progressive approach. Downweighing the sequences and adjusting the weight matrix according to closeness between the sequences enables the software to produce more accurate alignments. Multiple sequence alignment for the analysis was done by ClustalX.

ExPASy Translate Tool (Gasteiger et al., 2003): This is an online tool used to translate nucleotide sequences into amino acid sequences. The tool was developed by the Swiss Institute of Bioinformatics (SIB). The tool checks the functional coding regions without frameshifts or stop codons by translating the sequence. In the present study, this tool was used to check the stop codons and validate the sequences.

FigTree version 1.4.4 (Rambaut, 2012): This program helps to visualise the phylogenetic trees created from different phylogenetic software. The user-friendly graphical interface of this program allows the user to modify different the tree components such as rooting positions, node labels, tip labels etc. All trees reconstructed in this study were visualised and edited using FigTree.

Gblocks version 0.91b (Castresana, 2000): It is a program to improve the quality of the multiple sequence alignment by removing poorly aligned positions and highly divergent regions of the sequences. Since it has a high processing speed, it is very suitable for large datasets. The program divides the entire multiple sequence alignment into several blocks and carefully checks for gaps and highly varying regions. This program was used to remove the positions that were not aligned perfectly, thereby ensuring only conserved regions were used for the phylogenetic analysis.

GenBank (Benson et al., 2013): GenBank is a publicly available database of nucleotide sequences and provide data such as mRNA with coding regions, genomic DNA segments, and ribosomal RNA genes, with their associated protein translations. The database is maintained by NCBI and serves as the central repository for genetic sequences submitted by researchers worldwide, enhancing data sharing, comparison and retrieval. GenBank was employed to access the homologous sequences and to deposit the newly generated sequences in this study.

jModelTest version 2.1.10 (Posada, 2008): The major function of this software is to find the best-fit nucleotide substitution model for a given alignment, which is an important prerequisite for phylogenetic analysis. Computing the likelihood of different evolutionary models gives the best fit through a statistical criterion such as the Akaike Information Criterion (AIC) and

Bayesian Information Criterion (BIC). Evolutionary model selection for ML analysis, BI, and divergence time estimation was done using this program.

MEGA version 11.0.13 (Tamura et al., 2021): Molecular Evolutionary Genetics Analysis is a comprehensive software package developed for performing molecular evolutionary analyses and constructing phylogenetic trees. This was the primary software employed as it was used for the multiple sequence alignment checking, model selection for preliminary trees, calculation of pairwise genetic distance and construction of phylogenetic trees using NJ and MP methods.

Mesquite version 3.80 (Maddison & Maddison, 2023): Mesquite is a modular software developed to support phylogenetic analysis, population genetics and non-phylogenetic multivariate analysis. This software helps the biologist to visualise, format, organise and analyse the character matrices. This software was utilised to generate different formats of the input files compatible with different phylogenetic analyses.

MrBayes version 3.2.7a (Ronquist et al., 2012): This widely accepted software is used for Bayesian phylogenetic analysis. MrBayes uses the MCMC approach to compute the posterior probabilities. It can handle large datasets and allows the user to apply different, even complex, evolutionary models that best fit the data, including partitioned data. In the present study, MrBayes was employed to infer phylogenetic relationships using the BI approach.

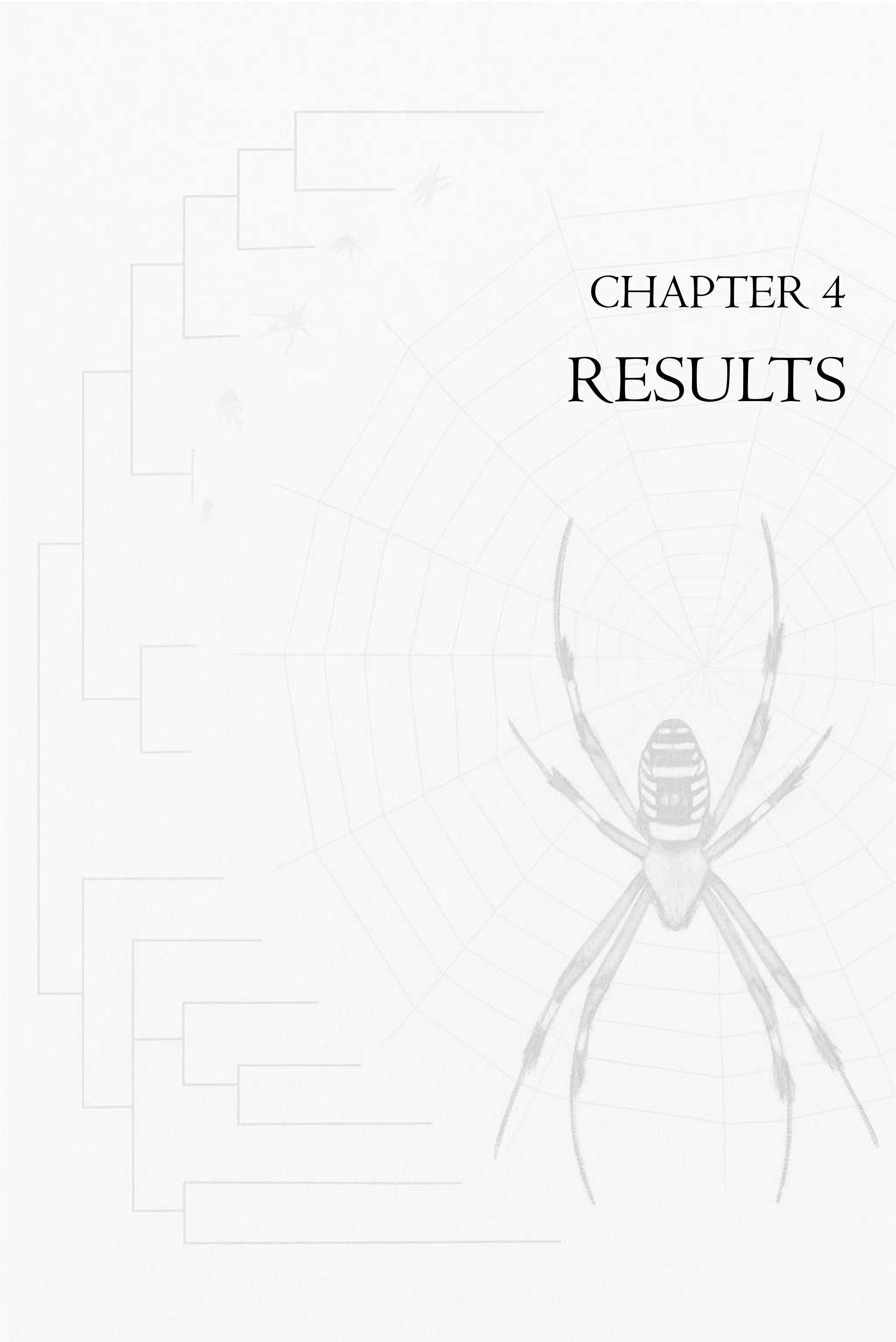
RAxML version 1.5 b1 (Stamatakis, 2014): Randomized Axelerated Maximum Likelihood (RAxML) is a high-performance program used to construct phylogenetic trees through the ML approach. With an effective algorithm and parallel processing, the program analyse large datasets. Due to its capability in building accurate phylogenetic trees, this software was selected to conduct the ML analysis.

SequenceMatrix version 1.6.7 (Vaidya et al., 2011): This program is very useful while using the concatenated dataset. The program combines multiple sequence alignments of different genes and generate a single alignment. The program can generate output files compatible with major phylogenetic software like BEAST, MrBayes, and RAxML. This program was utilised to concatenate the alignments and to partition the data.

Tracer version 1.7.2 (Rambaut et al., 2018): This graphical tool is used along with the Bayesian phylogenetic analysis, which enables the visualisation of the convergence of MCMC runs. This tool accept input files generated from different software, such as MrBayes, BEAST, RevBayes, etc. In the present study, Tracer helped to validate the reliability of Bayesian phylogenetic analyses by assessing the convergence and effective sample size.

CHAPTER 4

RESULTS



“***What we observe is not nature itself, but nature exposed to our method of questioning***”

- *Werner Heisenberg*

The results section of a thesis is a crucial part of the whole research process. By systematically presenting the findings, this section provides a foundation for further interpretation and analysis, thereby enhancing the validity of the study. This section presents the key findings of the current study.

The section begins with the morphological characterisation of different genera in the family Araneidae, followed by a checklist of araneids found in Kerala. Subsequent sections include the key findings from barcoding, phylogenetic analysis and divergence time analysis.

4.1. Checklist of family Araneidae in Kerala

From the field study spanning 42 months (July 2021 – December 2024), in fourteen districts of Kerala, a total of 735 specimens were collected. After thorough morphological examinations, 59 species from 23 genera (Table 4.1.1) belonging to the family Araneidae were identified. The species-level identity of 80.1% of the collected specimens was successfully determined (Figure 4.1.1). Genus-level identification was achieved for 18% of specimens, while the remaining specimens could only be identified to the family level. This limitation was primarily due to the subadult condition of the specimen or damage to the specimen.

Eleven species were found to be very common in Kerala through this study, while seven species were rarely seen (Table 4.1.2). This categorization is based only on field observations, with species frequently encountered across sampling sites considered common, and those recorded only once classified as rare. A larger number of species were recorded from the genus *Neoscona,* followed by *Cyclosa*. Of the total genera identified, 16 are represented by single species (Figure 4.1.2).

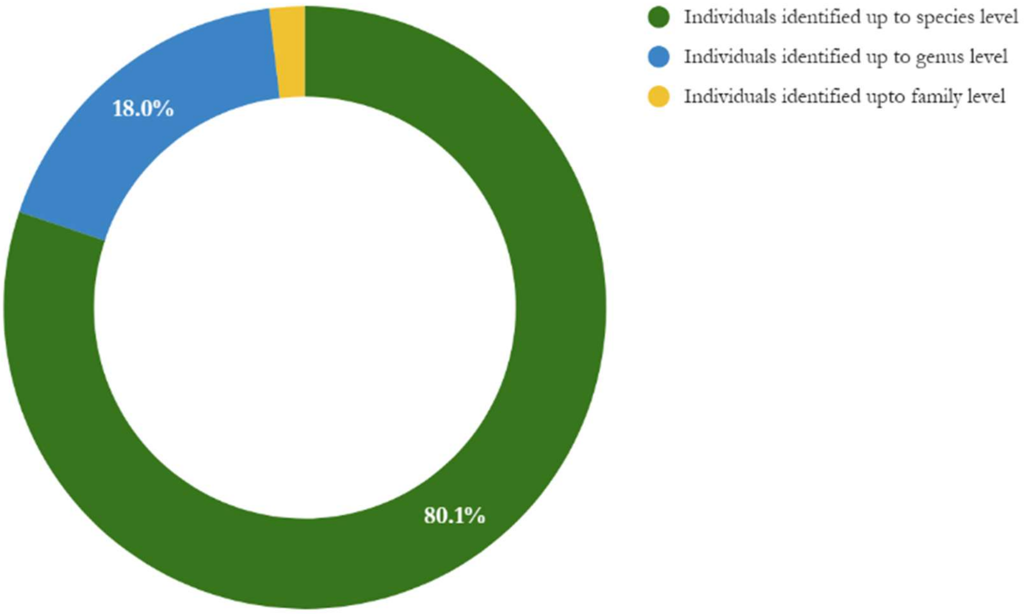


Figure 4.1.1. Pie chart showing the proportion of araneid specimens identified to different taxonomic levels in the current study (total number = 735).

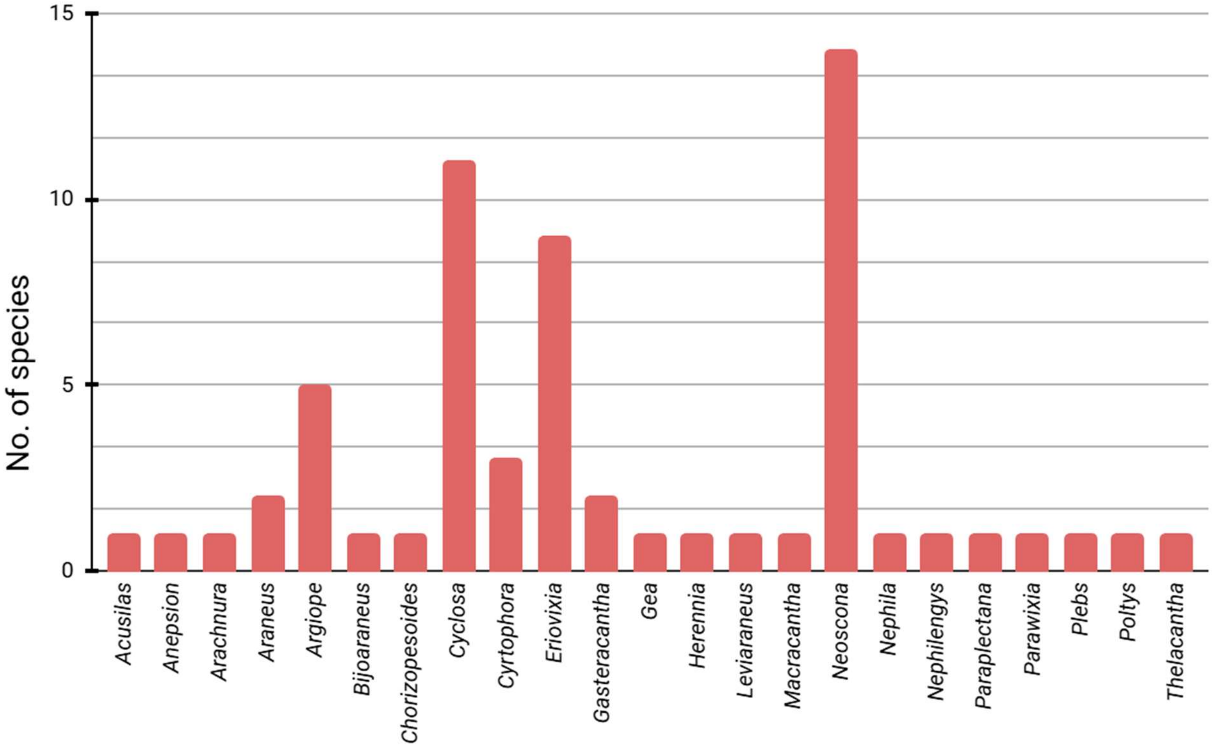


Figure 4.1.2. Bar graph representing the number of species identified from each genus.

Table 4.1.1. Checklist of araneids in Kerala

Sl. No.	Genus	Sl. No.	Species
1	*Acusilas*	1	*Acusilas coccineus* Simon, 1895
2	*Anepsion*	2	*Anepsion maritatum* (O. Pickard-Cambridge, 1877)
3	*Arachnura*	3	*Arachnura melanura* Simon, 1867
4	*Araneus*	4	*Araneus tubabdominus* Zhu & Zhang, 1993
		5	*Araneus* sp. 1
5	*Argiope*	6	*Argiope aemula* (Walckenaer, 1841)
		7	*Argiope anasuja* Thorell, 1887
		8	*Argiope catenulata* (Doleschall, 1859)
		9	*Argiope pulchella* Thorell, 1881
		10	*Argiope versicolor* (Doleschall, 1859)
6	*Bijoaraneus*	11	*Bijoaraneus mitificus* (Simon, 1886)
7	*Chorizopesoides*	12	*Chorizopesoides orientalis* (Simon, 1909)
8	*Cyclosa*	13	*Cyclosa bifida* (Doleschall, 1859)
		14	*Cyclosa confraga* (Thorell, 1893)
		15	*Cyclosa hexatuberculata* Tikader, 1982
		16	*Cyclosa mulmeinensis* (Thorell, 1887)
		17	*Cyclosa purnai* Keswani, 2013
		18	*Cyclosa quinqueguttata* (Thorell, 1881)
		19	*Cyclosa spirifera* Simon, 1889
		20	*Cyclosa* sp. 1
		21	*Cyclosa* sp. 2
		22	*Cyclosa* sp. 3
		23	*Cyclosa* sp. 4
9	*Cyrtophora*	24	*Cyrtophora cicatrosa* (Stoliczka, 1869)
		25	*Cyrtophora moluccensis* (Doleschall, 1857)
		26	*Cyrtophora unicolor* (Doleschall, 1857)
10	*Eriovixia*	27	*Eriovixia excelsa* (Simon, 1889)
		28	*Eriovixia gryffindori* Ahmed, Khalap & Sumukha, 2016
		29	*Eriovixia laglaizei* (Simon, 1877)
		30	*Eriovixia poonaensis* (Tikader & Bal, 1981)

		31	*Eriovixia sakiedaorum* Tanikawa, 1999
		32	*Eriovixia* sp. 1
		33	*Eriovixia* sp. 2
		34	*Eriovixia* sp. 3
11	*Gasteracantha*	35	*Gasteracantha dalyi* Pocock, 1900
		36	*Gasteracantha geminata* (Fabricius, 1798)
12	*Gea*	37	*Gea spinipes* C. L. Koch, 1843
13	*Herennia*	38	*Herennia multipuncta* (Doleschall, 1859)
14	*Leviaraneus*	39	*Leviaraneus viridiventris* (Yaginuma, 1969)
15	*Macracantha*	40	*Macracantha hasselti* (C. L. Koch, 1837)
16	*Neoscona*	41	*Neoscona bengalensis* Tikader & Bal, 1981
		42	*Neoscona elliptica* Tikader & Bal, 1981
		43	*Neoscona nautica* (L. Koch, 1875)
		44	*Neoscona panchganiensis* Tikader & Bal, 1981
		45	*Neoscona punctigera* (Doleschall, 1857)
		46	*Neoscona theisi* (Walckenaer, 1841)
		47	*Neoscona usbonga* Barrion & Litsinger, 1995
		48	*Neoscona vigilans* (Blackwall, 1865)
		49	*Neoscona yptinika* Barrion & Litsinger, 1995
		50	*Neoscona* sp. 1
		51	*Neoscona* sp. 2
		52	*Neoscona* sp. 3
17	*Nephila*	53	*Nephila pilipes* (Fabricius, 1793)
18	*Nephilengys*	54	*Nephilengys malabarensis* (Walckenaer, 1841)
19	*Paraplectana*	55	*Paraplectana* sp.
20	*Parawixia*	56	*Parawixia dehaani* (Doleschall, 1859)
21	*Plebs*	57	*Plebs mitratus* (Simon, 1895)
22	*Poltys*	58	*Poltys columnaris* Thorell, 1890
23	*Thelacantha*	59	*Thelacantha brevispina* (Doleschall, 1857)

Table 4.1.2. Commonly and rarely found araneids in Kerala

Commonly found araneids	Rarely found araneids
1. *Anepsion maritatum* (O. Pickard-Cambridge, 1877)	1. *Araneus tubabdominus* Zhu & Zhang, 1993
2. *Argiope pulchella* Thorell, 1881	2. *Argiope versicolor* (Doleschall, 1859)
3. *Cyclosa confraga* (Thorell, 1893)	3. *Chorizopesoides orientalis* (Simon, 1909)
4. *Cyclosa hexatuberculata* Tikader, 1982	4. *Eriovixia gryffindori* Ahmed, Khalap & Sumukha, 2016
5. *Cyclosa quinqueguttata* (Thorell, 1881)	5. *Neoscona usbonga* Barrion & Litsinger, 1995
6. *Cyrtophora cicatrosa* (Stoliczka, 1869)	6. *Neoscona yptinika* Barrion & Litsinger, 1995
7. *Eriovixia laglaizei* (Simon, 1877)	7. *Paraplectana* sp.
8. *Eriovixia poonaensis* (Tikader & Bal, 1981)	
9. *Gasteracantha geminata* (Fabricius, 1798)	
10. *Neoscona nautica* (L. Koch, 1875)	
11. *Neoscona vigilans* (Blackwall, 1865)	

4.1.1. Identification key to the araneid genera found in Kerala

1. Tarsus and metatarsus together longer than tibia and patella, straited cheliceral boss in both sexes ………………………………………………………………………................… **2**

Tarsus and metatarsus together not longer than tibia and patella, non-straited cheliceral boss ………………………………………………………………………………... **4**

2. Elongated abdomen, carapace humps present, epigyne without a conspicuous septum, long tapering embolic conductor, large orb webs between trees ……………………...… ***Nephila***

Abdomen otherwise, carapace humps absent, epigyne with distinguishing septum, large and sigmoidal embolic conductor, orb webs against substratum …………………...…….. **3**

3. Females with oval abdomen, four conspicuous brightly coloured spots on the ventrum, strong carapace spines, males with smooth embolus. Large orb webs built against the substratum in a single plane with silken tubular retreat above the orb…………………………………………………………………….…….. ***Nephilengys***

Females with lobed abdomen and, warty carapace, males having an embolus with a conspicuous distal hook. Orb webs build against substrate in the plane of the substrate with the hub touching the substrate …..……………………………………...…….. ***Herennia***

4. Cephalic region highly elevated and abruptly slopes the thoracic region, spinnerets surrounded by black, elevated, sclerotised rings, hard abdomen with dorsal spines ……………………………………………………………………………………... **5**

Cephalic region otherwise, spinnerets not surrounded by sclerotised rings, abdomen not hard without dorsal spines ……………………………………………………...……. **7**

5. Ventral tubercle absent ……………………………………..………………... ***Macracantha***

Ventral tubercle present …………………………………………………..…………. **6**

6. Three pairs of pointed, short or long dorsal spines, brightly coloured abdomen ………………………………………..…………………………...… ***Gasteracantha***

Three pairs of blunt-ended, very short dorsal spines on tubercles, a conspicuous pair of mid-dorsal white circular patches …………………………….……..……... ***Thelacantha***

7. PE row procurved, arrangement of long, strong legs in X-shape during resting in the web, epigyne lacks scape but has a prominent septum ……………….…………………... **8**

PE row straight or recurved, arrangement of legs otherwise during resting time, epigyne with scape ………………………………………………………….…………...… **9**

8. PMEs are closer to each other than with PLEs, heavily sclerotised epigynum, non-bifurcated MA ……………………………………………………..….......…. ***Argiope***

PMEs are placed at an equal distance as with PLEs, weakly sclerotised epigynum, bifurcated MA ……………………………………………………………...…... ***Gea***

9. Abdomen wider than longer, conspicuous black patches over uniformly, brightly coloured, smooth abdominal dorsum ……………………………………………….…..... **10**

Abdomen longer than wide, without conspicuous black patches ……………….…… **11**

10. Highly elevated cephalic region, convex abdominal dorsum, prominent, wrinkled, narrowing scape ……………………………………………………….………..... ***Anepsion***

Slightly elevated cephalic region, highly convex abdominal dorsum, less conspicuous, very short scape-like depression on the anterior epigynal margin ………….….. ***Paraplectana***

11. Strongly convex carapace, three pairs of lateral abdominal tubercles and three vertically arranged caudal tubercles ……………………………………..….….… ***Chorizopesoides***

Carapace may or may not be convex, abdominal and caudal tubercles may or may not be present ………………………..……………………………………………….………. **12**

12. Pear-shaped carapace, anterior-most edge of the cephalic region protruded out, forming a prominent tubercle in which the MEs are located, LEs are pushed far from the ocular quad ……………………………………………………………..……………..……... ***Poltys***

Carapace otherwise, no protrusions from the cephalic region, LEs are not pushed far from the ocular quad ……………………………………………………..…….…. **13**

13. Abdominal dorsum with a pair of conspicuous shoulder tubercles, sometimes pointed and hard ……………………………………………………………………….….….... **14**

Abdominal dorsum without conspicuous shoulder tubercles ……………..….….…….. **16**

14. Bifurcated anterior abdominal margin forming a pair of pointed shoulder tubercles, elongated posterior end with prominent three caudal tubercles .... ***Arachnura***

Anterior abdominal margin not bifurcated, lacks an elongated posterior end and caudal tubercles …………………………………………………………..……..…….. **15**

15. Large, triangular abdomen, presence of a pair of hard, conspicuous lateral tubercles near the LEs, heavily sclerotised epigynum with short beak-like narrowing scape ………………………………………………………...……….…...… ***Parawixia***

Slightly elongated, subtriangular abdomen, lacks cephalic tubercles, epigynum moderately sclerotised with smooth long scape with a U-shaped ending ………………………… ***Plebs***

16. Cephalic and thoracic regions are separated clearly ……………………….…….…… **17**

Cephalic and thoracic regions are not separated clearly ……………………………….……. **19**

17. Less prominent separation of cephalic and thoracic region, flat carapace, abdomen with at least one pair of dorsal tubercles, characteristic darker area between epigyne and spinnerets bordered by bright white narrow bands or spots with white spots in the area, builds tent web ………………………………………………………………...…...…… ***Cyrtophora***

Prominent U-shaped junction between cephalic and thoracic region, cephalic and thoracic region raised equally, with or without abdominal tubercles, abdomen otherwise ……………………………………………………………………..……..………. **18**

18. PMEs very close, either elongated or globular abdomen with at least one pair of tubercles, either dorsal or caudal or both, moderately sclerotised epigynum with short wrinkled scape, orb webs are decorated with stabilimentum …………………………...………..… ***Cyclosa***

PMEs are not very close, quadrilateral abdomen with sequentially arranged black patches on the dorsum, lacks abdominal tubercles, weakly sclerotised oblong epigynum without scape, builds a retreat at the centre of the orb webs .... ***Acusilas***

19. Subtriangular abdomen, triangular tongue-like epigyne with short, completely fused scape ………………………………………………………………………….…. ***Eriovixia***

Subtriangular, elliptical or ovoid abdomen with either smooth or wrinkled, long or short, basally fused scape ………………………………………………………...……..…… **20**

20. Females having U-shaped or heart-shaped epigynum with short scape, males lack a ventral hook on coxa I ……………………………………………………………...……. **21**

Females having an epigyne with a long, broad or narrow, wrinkled or smooth scape, males having a ventral hook on coxa I …………………………………………………….…… **22**

21. Dorsum of the oval abdomen having a black kidney-shaped patch with other black coloured lines and patches, U-shaped epigynum with very short and inflexible scape and short copulatory duct, males have strong ventral spines on femur II ……………………………………………………………..….......………. ***Bijoaraneus***

Bright green coloured sub-triangular abdomen with conspicuous brownish red lateral markings, heart-shaped epigynum with short tongue-like scape and twisted copulatory ducts, males having dorsal groove on femur II ……………..………..………. ***Leviaraneus***

22. Females with transverse thoracic groove, epigyne with distinct moderate to long, wrinkled scape lacking lateral lobes, males have complex palpal structures with narrow cymbium ………………………………………………………………………………... ***Araneus***

Females with longitudinal thoracic groove, epigyne with distinct moderate to long smooth scape with one or two pairs of lateral lobes, males having complex palpal structures with broad cymbium ………………………………………………………..…….…..… ***Neoscona***

4.1.2. Taxonomic account of araneids documented from Kerala

Family Araneidae Clerck, 1757 (Orb-weavers)

The world's third-largest spider family, Araneidae, includes small to large-sized members. Typically, araneids weave nearly vertical orb webs with sticky spirals. However, certain members weave the modified orb webs. Webs being the home territory, araneids usually occupy the hub in a head-down position; however, some might take shelter in the retreats associated with the orb webs. Members of this cosmopolitan family possess eight eyes, arranged in two rows, usually recurved. The lateral eyes are spaced apart from the median eyes and usually situated on prominent to less prominent tubercles.

The family is highly diverse as the members show variable carapace and abdominal features. Members of the family possess strong, non-divergent chelicerae with a lateral condyle and two rows of strong teeth. These three-clawed spiders have six spinnerets. Female genitalia are heavily or partially sclerotised with a distinguishable scape or septum. Male palpi are even complex with additional sclerites and structures like haematodocha. Male araneids are usually smaller than females, and some even show extreme sexual size dimorphism.

Genus I. ***Acusilas* Simon, 1895**

Type species: *Acusilas coccineus* Simon, 1895

Diagnostic features: Species of this genus can be distinguished from other araneids by the following features. Instead of one, males have two lateral condyles on the chelicerae. The fused conductor and the slender triangular MA have a distal hook. Presence of brace-like stipes and radix with spiral embolus. Females have a prominent U-shaped cephalic groove that demarcates the narrow head and thoracic region. ALE is in the proximity of AME, and the posterior eye row is strongly recurved. Epigyne is weakly sclerotised with an anterior bulge and characteristic transverse rim and septum.

This genus has a wide distribution across Asia, with a representative species from Africa. So far, ten species have been reported under this genus (WSC, 2025). In the present study, one species belonging to *Acusilas* was identified.

1. ***Acusilas coccineus* Simon, 1895 (Scarlet *Acusilas*)** (Figure 4.1.3 A-G)

   Acusilas coccineus Simon, 1895: 785 (♀); Yaginuma, 1960: 59, Fig. 58 (♀); Namkung, 1964: 37, Fig. 23 (♀); Hu, 1984: 88, Fig. 78.1-2 (♀); Schmidt & Scharff, 2008: 18, Figs. 7A-B, 10A-D, 11A-B, 12A, 13A, 18A-B, 19A-E, 20A-B, 21A-B (♀♂); Sankaran & Sebastian, 2018: 391, Figs. 1A-E, 2A-G (♀♂).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Medium-sized spider with darker cephalothorax and appendages. Males are usually smaller than females. Females build characteristic regular vertical orb webs having a retreat, a rolled dried leaf facing the ground, in a sector of the web that lacks spirals. Usually found in evergreen and semi-evergreen forests.

   Distribution: Asia

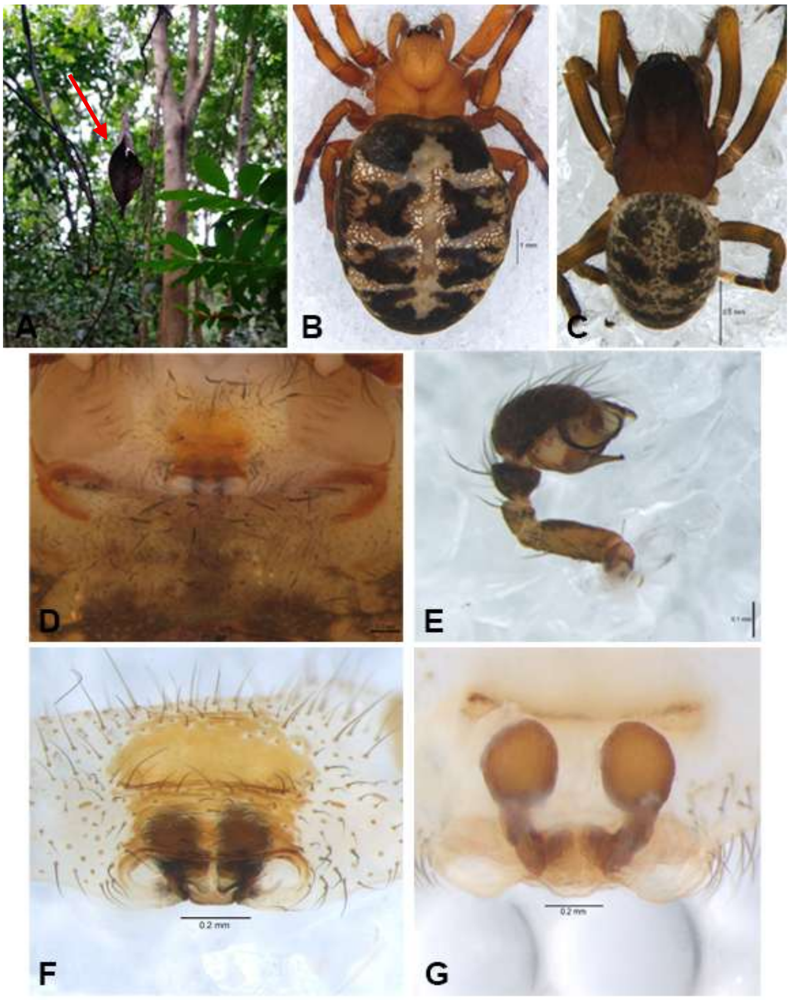


Figure 4.1.3. *Acusilas coccineus* Simon, 1895: (A) Retreat of adult female, (B) Female dorsal view, (C) Male dorsal view, (D) Epigyne in situ, (E) Palp retrolateral view, (F) Epigyne ventral view, (G) Epigyne dorsal view. Scales: B 1mm; C 0.5 mm; D-G 0.2 mm.

Genus II. ***Anepsion*** **Strand, 1929**

Type species: *Anepsion rhomboides* (L. Koch, 1867)

Diagnostic features: Both males and females of this genus have a raised cephalic region with dorsal bulging, while the thoracic region is nearly flat and overlaid by the abdomen. MEs are very close and located at the centre of the broad cephalic region, forming a narrow strip of clypeus, while the LEs are pushed away to the antero-lateral margin of the carapace. Males have short and slender embolus, whereas females have an epigyne with a short to long, highly wrinkled, distinct scape.

This genus has a wide distribution across Asia and Australia. Sixteen species belonging to this genus have been identified (WSC, 2025). The genus is represented by one species in this study.

1. ***Anepsion maritatum* (O. Pickard-Cambridge, 1877) (Button spider)** (Figure 4.1.4 A-G)

Anepsion maritatum Thorell, 1877: 356 (♀); O. Pickard-Cambridge, 1877: 32, Fig. 7 (♀♂); Simon, 1905: 63; Yaginuma & Wen, 1983: 196, Fig. 4 A-B (♀); Malamel et al., 2015: 478, Figs. 1 A-O, 2 A-I (♀♂); Tanikawa & Yamasaki, 2019: 11, Fig. 1 A-F (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: They are small-sized spiders that build medium to large orb webs and rest in the hub. The carapace has a bright yellow to orange colour, while the round, shiny abdomen is white with distinct black markings. The species has been spotted in habitats with high humidity, including tropical evergreen forests, rubber plantations, mangroves and coastal areas.

Distribution: India, Sri Lanka, Thailand, Japan, China, Indonesia.

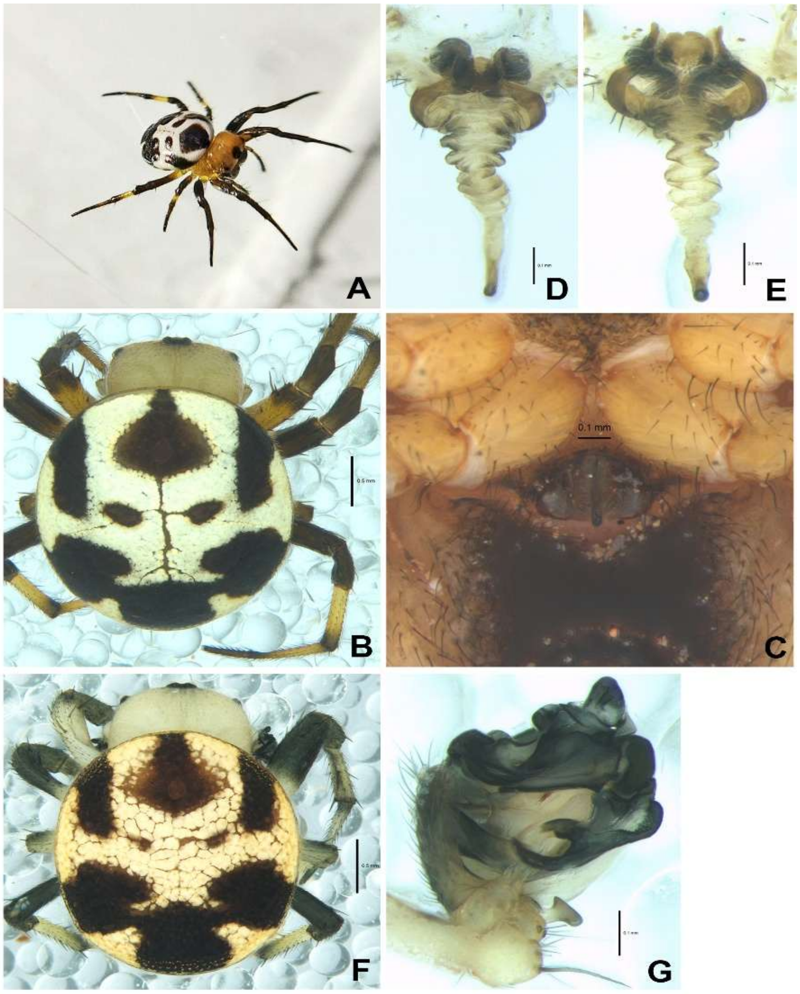


Figure 4.1.4. *Anepsion maritatum* (O. Pickard-Cambridge, 1877): (A) Female habitus, (B) Female dorsal view, (C) Epigyne in situ, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: B & F 0.5 mm; C-E, F 0.1 mm.

Genus III. ***Arachnura* Vinson, 1863**

Type species: *Arachnura scorpionoides* Vinson, 1863

Diagnostic features: Female members of this fascinating genus can be distinguished by their elongated abdomen resembling a scorpion's tail. This movable posterior end of the abdomen extends beyond the spinnerets. The anterior end of the abdomen forms a V-shaped depression following gradual narrowing and ends with tapering posterior tubercles. AME are on a prominent tubercle projecting anteriorly. Partially sclerotised epigyne with short, broad scape.

To date, twelve species have been identified under this genus, which is distributed across Africa, Asia and Australia (WSC, 2025). In the present study, one species belonging to *Arachnura* was identified.

1. ***Arachnura melanura* Simon, 1867 (Scorpion tailed spider)** (Figure 4.1.5 A-E)

   Arachnura melanura Simon, 1867: 17 (♀); Tanikawa, 1991: 12, Fig. 1-6 (♀♂); Yin et al., 1997: 122, Fig. 46 a-d (♀); Castanheira et al., 2019: 164, Fig. 9 A-E (♀); Sen & Sureshan, 2020: 286, Fig. 1-5 (♀).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Male spiders of this species are much smaller than the females and are rare to spot. Usually, females construct orb webs in low vegetation and rest in the web, mimicking a wilted leaf. Upon disturbance, they raise their tail over their body, a behaviour resembling the scorpion. Since they show a high level of mimicry, it is hard to spot them in their natural habitat. They have been spotted in evergreen and moist deciduous forests.

   Distribution: India, Japan, Southeast Asia, Australia.

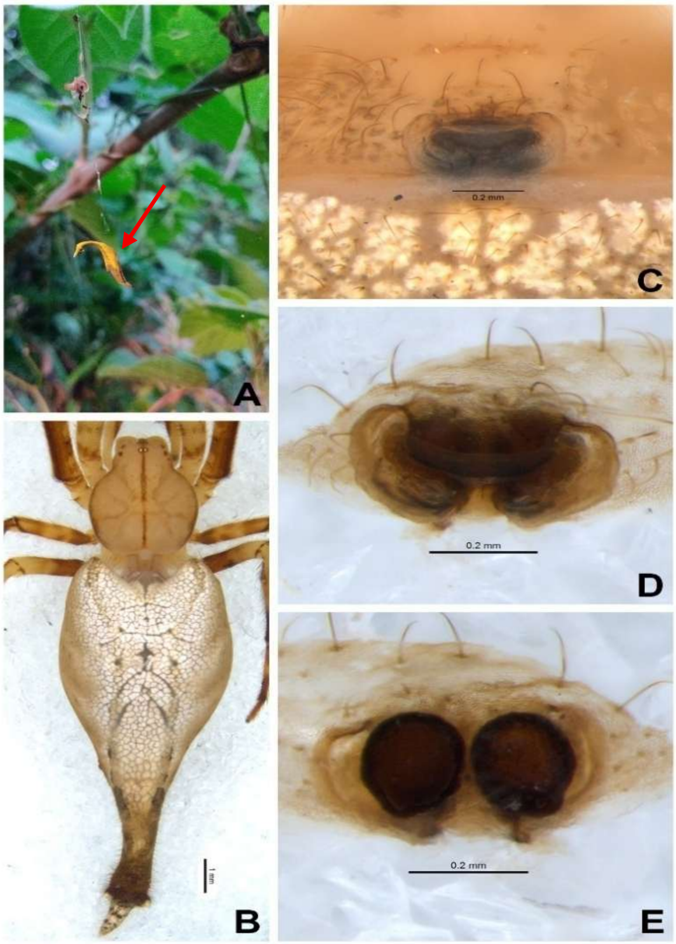


Figure 4.1.5. *Arachnura melanura* Simon, 1867: (A) Female habitus, (B) Female dorsal view, (C) Epigyne in situ, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: B 1 mm; C-D 0.2 mm.

Genus IV. ***Araneus*** **Clerck, 1757**

Type species: *Araneus angulatus* Clerck, 1757

Diagnostic features: This is one among the specious genus in the order Araneae. Members of this genus show high variations in size, shape, colour, and even in genital structures. However, they share certain features in common. The thoracic groove of females will be transverse, while that of males will be longitudinal. LEs are very close and always situated on distinct tubercles. Among species, females can be distinguished by the plates in the epigynum, which have a distinct scape, long or short, wrinkled, and attached to the base. Males have a hook on coxa I and heavy spinnation in the appendages, particularly in the tibial segment. The shape of the embolus determines the male species' identity. Palpal patella possesses two long curved setae. Palpal structures are complex by the presence of subterminal and terminal apophysis, distal hematodocha. Almost all spiders usually do not rest in the hub during daytime rather hide in a retreat made by curling the leaves secured with silk.

This genus has a wide distribution across the globe with 550 representatives (WSC, 2025). Even though two species were recognised from this genus, only one could be identified to the species level.

1. ***Araneus tubabdominus* Zhu & Zhang, 1993** (Figure 4.1.6 A-G)

   Araneus tubabdominus Zhu & Zhang, 1993: 36, Fig. 1-7 (♀♂); Sen et al., 2021: 19864, Fig. 1-5 (♀)

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Male and female spiders have similar shapes and colour; however, the male spiders are a bit smaller than the females. They have been spotted in paddy fields but not in Kole wetlands. With specialised silk, both males and females build long tunnel-like retreats using the dried inflorescence or curling the long leaf blade. Retreats are also used to protect their egg sacs. These spiders are in pale brown, which helps them in camouflaging with the dried leaves and inflorescences.

   Distribution: India and China

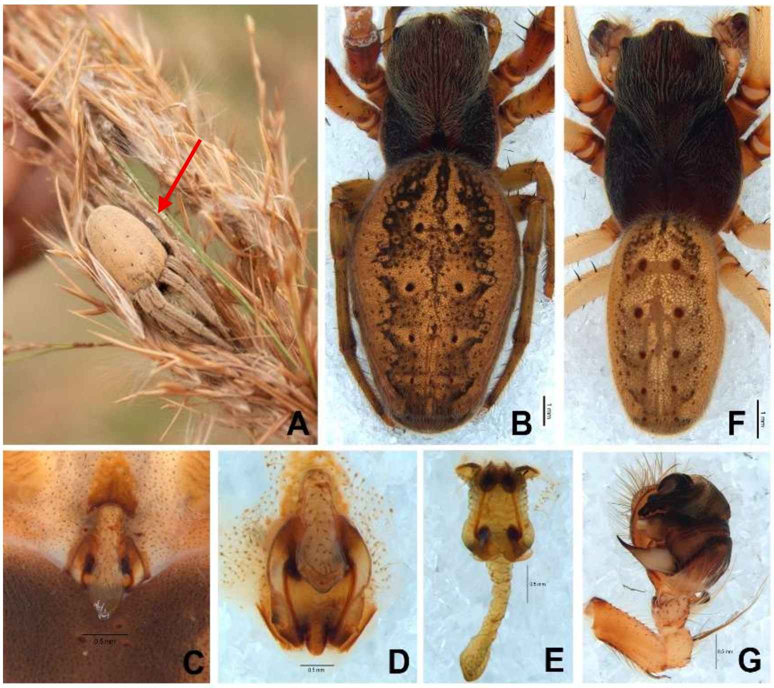


Figure 4.1.6. *Araneus tubabdominus* Zhu & Zhang, 1993: (A) Female habitus, (B) Female dorsal view, (C) Epigyne in situ, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp ventral view. Scales: B, F 1mm; C-E, G 0.5 mm.

Genus V. ***Argiope*** **Audouin, 1826**

Type species: *Argiope lobata* (Pallas, 1772)

Diagnostic features: Species belonging to this genus can be easily identified from their natural habitat due to the bright colours and presence of stabilimentum made with specialised silk that resembles a signature. This group is one among those that exhibit high sexual size dimorphism. They can be distinguished from other araneids by their procurved posterior eye row and X-shaped arrangement of strong and long legs during rest in the web. PMEs are very close to each other, which separates them from others in the subfamily Argiopinae. Females have a heavily sclerotised epigyne with an anterior bulge that lacks a scape, but always have a distinguishing septum that divides the atrium into two. Females are separated by the shape and arrangement of the posterior plate formed by this septum. Males have a complex palp with a small median apophysis, a large conductor and embolus and lack a terminal apophysis.

Argiope has a wide distribution across the landmasses with 89 recognised species (WSC, 2025). In the present study, five species belonging to *Argiope* were identified.

1. ***Argiope aemula*** **(Walckenaer, 1841) (Oval cross spider)** (Figure 4.1.7 A-G)

   Argiope aemula Walckenaer, 1841: 118 (♀); Thorell, 1877: 364 (♂); Tikader, 1970: 29, Fig. 17b (♀); Yin, 1978: 4, Fig. 9A-C (♀); Levi, 1983: 273, Figs. 9-10, 29-35(♀♂); Yaginuma, 1986: 114, Fig. 59.6 (♂); Jäger, 2012: 281, Fig. 3-7 (♂).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Males are much smaller than females. Female spiders, having yellow and black striped abdomen, construct large orb webs with X-shaped stabilimentum in bushy lower vegetation and rest in the hub in a head-down position for most of the day. Red to brown brightly coloured males are rare to find alone, but during the mating season, they can be found in the webs of female spiders. Even though they were found mostly in grasslands, they have been spotted in evergreens and mangroves also.

Distribution: South and Southeast Asia.

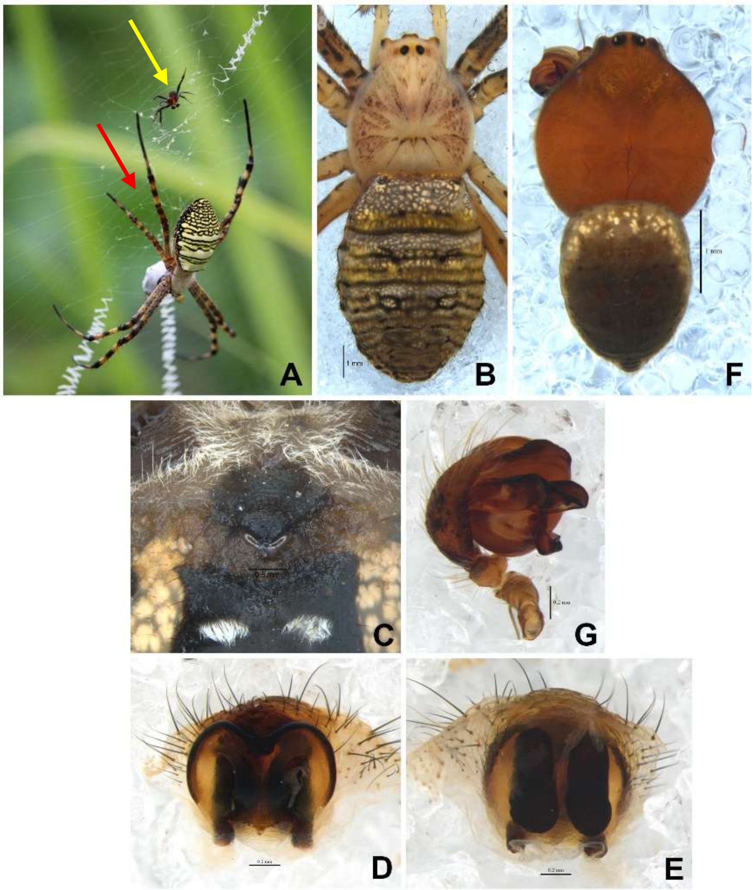


Figure 4.1.7. *Argiope aemula* (Walckenaer, 1841): (A) Female and male habitus, (B) Female dorsal view, (C) Epigyne in situ, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: B, F 1mm; C-E, G 0.2 mm.

2. ***Argiope anasuja* Thorell, 1887 (St. Andrews cross spider)** (Figure 4.1.8 A-D)

Argiope anasuja Thorell, 1887: 162 (♀); Gravely, 1921: 412, Fig. 3a (♀); Levi, 1983: 293, Fig. 167-172 (♀♂); Jäger, 2012: 299, Fig. 84-87 (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Similar to the previous one, males of these species are also very small compared to the female spiders and differ in shape and colour. Males are usually found in the web of female spiders during the mating season. The female spider has a pentagonal abdomen with transverse silvery white and brown to black stripes. This species shows resemblance to *Argiope pulchella* in appearance, but a detailed examination of female genitalia helps to distinguish them. Even though this species has been reported from forest habitats with complex vegetation, in the present study, it was spotted only in grasslands with low and less complex vegetation.

Distribution: Seychelles, Iran, South Asia, Australia.

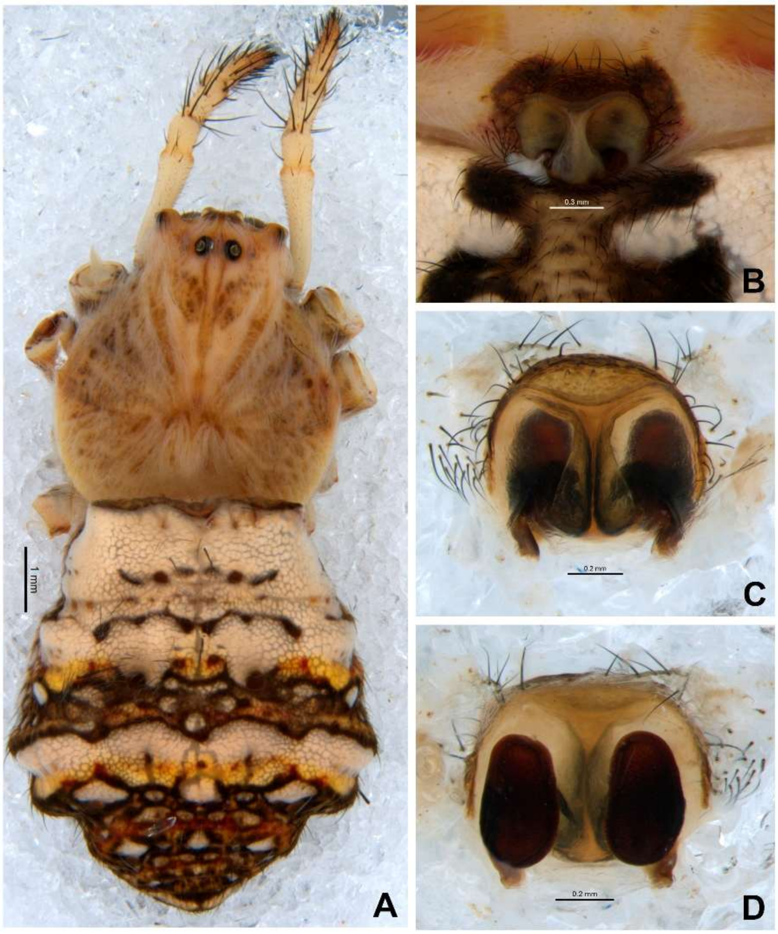


Figure 4.1.8. *Argiope anasuja* Thorell, 1887: (A) Female dorsal view, (B) Epigyne in situ, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: A 1mm; B-D 0.2 mm.

3. ***Argiope catenulata* (Doleschall, 1859) (Grass cross spider)** (Figure 4.1.9 A-E)

Argiope catenulate Doleschall, 1859: 30, pl. 9, Fig.1 (♀); Thorell, 1859: 299 (♀); Yin, 1978: 4, Fig. 10 A-C (♀); Tikader & Biswas, 1981: 35, Fig. 56-58 (♀); Levi, 1983: 274, Fig. 36-41 (♀♂); Jäger, 2012: 282, Fig. 8-9 (♀♂); Brown & Henderson, 2019: 98, Fig. 2-5 (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Unlike the previous ones, males and females of this species look similar, although slight variations in size exist. As the common name indicates, these species are exclusively found in habitats having grass vegetation, including grasslands and paddy fields. They build small to medium-sized orb webs and rest at the hub. Their oval abdomen is decorated with transverse yellow, silvery white, and brown patterns. In the present study, this species has also been spotted in grasslands, Kole wetlands, and paddy fields.

Distribution: South Asia, Southeast Asia, Northern Australia.

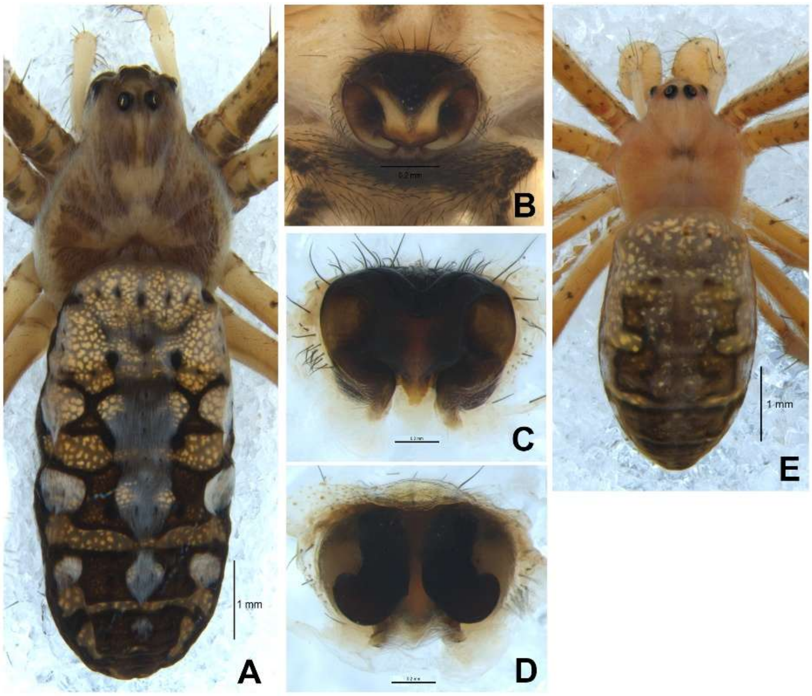


Figure 4.1.9. *Argiope catenulata* (Doleschall, 1859): (A) Female dorsal view, (B) Epigyne in situ, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male subadult dorsal view. Scales: A, E 1mm; B-D 0.2 mm.

4. ***Argiope pulchella* Thorell, 1881 (Garden cross spider)** (Figure 4.1.10 A-G)

Argiope pulchella Thorell, 1881: 74 (♀); Thorell, 1887: 158 (♂); Gravely, 1921: 412, Fig. 3b (♀); Tikader, 1970: 27, Fig. 17a (♀); Levi, 1983: 304, Fig. 238-250 (♀♂); Yin et al., 1989: 64, Fig. 4 A-F (♀♂); Jäger, 2012: 304, Fig. 115-118 (♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: This species looks similar to *Argiope anasuja,* as the female spiders also have a pentagonal abdomen with transverse yellow and brown bands. Extreme sexual size dimorphism is also exhibited in this species. Female spiders build large, perfect orb webs and rest at the hub in a head-down position. When disturbed, they fall to the ground, mimicking as dead and later return to the web. Males often build webs in proximity to females' webs. Webs of both males and females have X-shaped stabilimentum. The webs are built at a height of one meter from the ground up to high in the trees. The male spiders often break their embolus during mating and use it as a mating plug in the females' genitalia. This species can be found in almost all habitats, from dry deciduous to waterlogged Kole wetlands. They are often considered as synanthropic since they are also found in human-inhabited areas. In the present study, it was identified in all the habitats.

Distribution: India, Pakistan, China, and Indonesia.

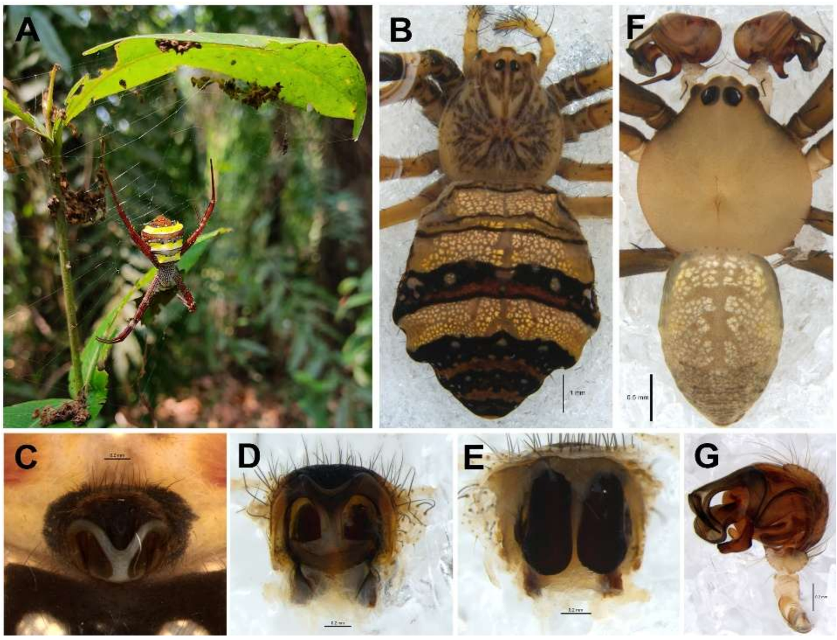


Figure 4.1.10. *Argiope pulchella* Thorell, 1881: (A) Female habitus, (B) Female dorsal view, (C) Epigyne in situ, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: B 1mm; C-E, G 0.2 mm; F 0.5mm.

5. ***Argiope versicolor* (Doleschall, 1859)** (Figure 4.1.11 A-F)

Argiope versicolor Doleschall, 1859: 31, pl. 9, Fig. 10 (♀); L. Koch, 1871: 35 (♀); Thorell, 1890: 95 (♂); Levi, 1983: 305, Figs. 3-4, 251-262 (♀♂); Yin et al., 1989: 65, Fig. 6 A-F (♀♂); Tyagi et al., 2019: Fig. S2.2, S3.26 (♀).

(Complete list of references in WSC, 2025)

Habitat and natural history: This species resembles *Argiope pulchella* in appearance, behaviour, and web-building patterns, as both of them belong to the same species group, but it is not found in all habitats as the former.

Remarks: Clear demarcation of this species from *Argiope* pulchella needed to be studied further.

Distribution: China, South and Southeast Asia.

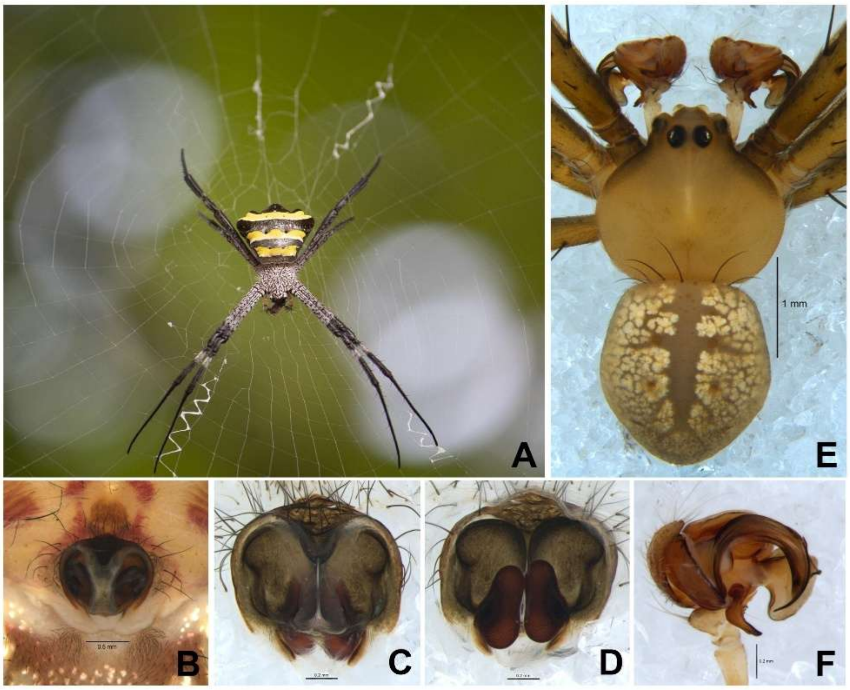


Figure 4.1.11. *Argiope versicolor* (Doleschall, 1859): (A) Female habitus, (B) Epigyne in situ, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male dorsal view, (F) Palp retrolateral view. Scales: B 0.5 mm; C-D, F 0.2 mm; E 1 mm.

Genus VI. ***Bijoaraneus*** **Tanikawa, Yamasaki & Petcharad, 2021**

Type species: *Bijoaraneus mitificus* (Simon, 1886)

Diagnostic features: Members of this genus were previously placed under the genus *Araneus*. Females can be distinguished by the short, inflexible scape. Males either lack or have an inconspicuous terminal apophysis and lack a subterminal apophysis. Also, the males are characterised by the presence of strong ventral spines on femur II and the lack of a coxal tubercle on coxa I. This genus has a wide distribution across Asia and a sparse distribution over Australia, with an overall of five recognised species (WSC, 2025). In the present study, one species belonging to this genus was identified.

1. ***Bijoaraneus mitificus*** **(Simon, 1886) (Kidney garden spider)** (Figure 4.1.12 A-E)

   Bijoaraneus mitificus Simon, 1886: 150 (♀); Workman, 1896: 39, pl. 39 (♀♂); Tikader, 1963: 43, Fig. 4a-c (♀♂); Levi, 1974: 271 (♀); Hu, 1984: 95, Fig. 89.1-2 (♀); Barrion & Litsinger, 1995: 638, Fig. 405a-h (♀); Tanikawa et al., 2021: 98, Figs. 2C, 5A-I (♀♂).

   (Complete list of references in WSC, 2025)

Habitat and natural history: Both males and females of this species have similar size and colouration. Both have a nearly globular abdomen with a particular kidney-shaped black patch and markings over pale white dorsum, and the ventrum is green. They are found in secondary forests and gardens. The spider usually hides on a retreat made of leaves and specialised silk during the daytime and constructs webs not beyond three meters high.

Distribution: China, South and Southeast Asia.

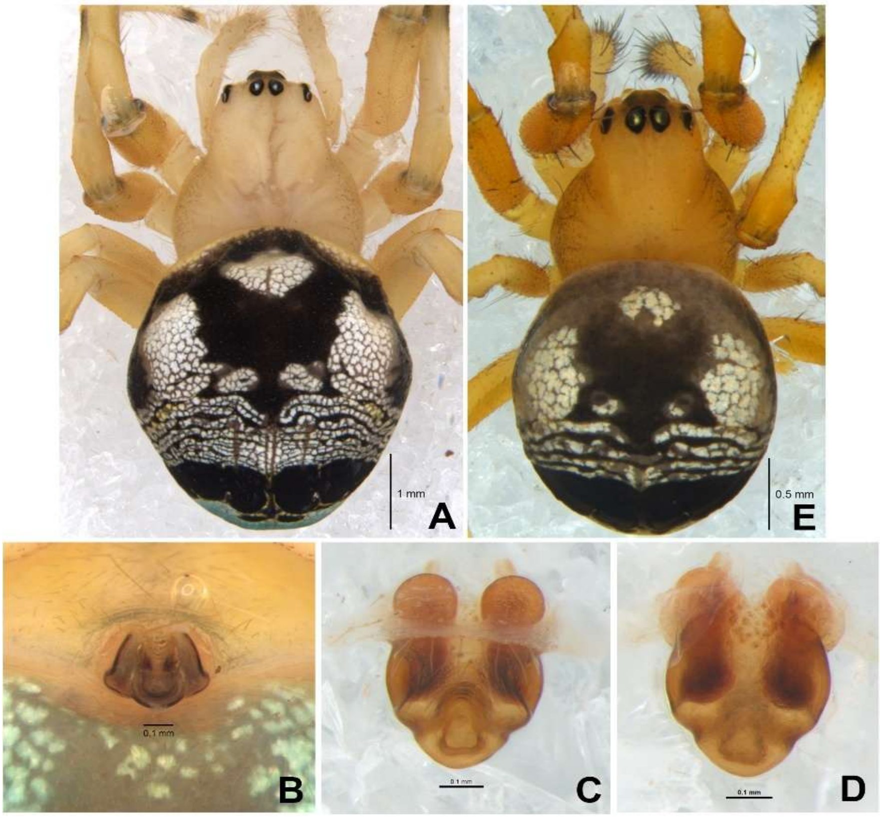


Figure 4.1.12. *Bijoaraneus mitificus* (Simon, 1886): (A) Female dorsal view, (B) Epigyne in situ, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male dorsal view. Scales: A 1 mm; B-D 0.1 mm; E 0.5 mm.

Genus VII. ***Chorizopesoides*** **Mi & Wang, 2018**

Type species: *Chorizopesoides wulingensis* (Yin, Wang & Xie, 1994)

Diagnostic features: This genus is very similar to another araneid genus, *Chorizopes,* as both have an elevated thoracic region. However, the members of *Chorizopesoides* possess three pairs of lateral and three pairs of vertically arranged caudal tubercles on the abdomen. Serrated teeth and their arrangement also distinguish this genus from *Chorizopes*. Absence of terminal apophysis, digitiform paracymbium and short scape further supports the identification of the members. This genus has been described recently with four species (WSC, 2025) having a distribution across China and Vietnam. In the present study, one species belonging to *Acusilas* was identified, but it was a subadult specimen.

1. ***Chorizopesoides orientalis* (Simon, 1909)** (Figure 4.1.13 A-B)

   Chorizopesoides orientalis Simon, 1909: 119 (♀); Levi, 1964: 214, Figs. 21-26 (♀); Mi & Wang, 2018: 83, Figs. 4A-D, 5A-C, 6A-E (♀♂).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: These spiders are very small, with a total length of not more than 5mm. Both male and female spiders have similar colouration. These rare species build orb webs in lower vegetation and in the current study it was spotted from the coastal area.

   Remarks: First report from India.

   Distribution: India, China and Vietnam.

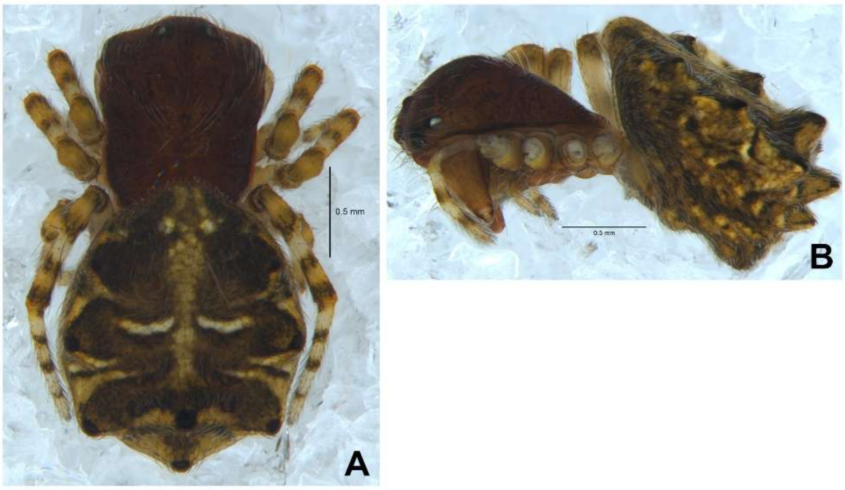


Figure 4.1.13. *Chorizopesoides orientalis* (Simon, 1909): (A) Female subadult dorsal view, (B) Female subadult lateral view. Scales: A-B 0.5 mm

Genus VIII. ***Cyclosa* Menge, 1866**

Type species: *Cyclosa conica* (Pallas, 1772)

Diagnostic features: This genus includes small-sized spiders that build small to medium-sized, perfect orb webs. The members of this genus can be identified in their natural habitat as they construct an evident stabilimentum along the vertical axis of the web with specialised silk, dried leaves, debris, and remains of the prey. Egg sacs are also attached to this stabilimentum. The spider will rest within the stabilimentum by camouflaging themselves. Species belonging to this genus are characterised by a distinct U-shaped groove that visibly demarcates the cephalic and thoracic regions. PMEs are closely situated, almost seem like touching each other. Males have only one long patellar seta. Abdomen of females often possess tubercles and humps whose epigyne is partially sclerotised with a weak, mostly wrinkled scape.

This group has a worldwide distribution with 180 recognised species (WSC, 2025). In the present study, eleven species of *Cyclosa* were captured and revealed the species identity, except for four.

1. ***Cyclosa bifida* (Doleschall, 1859) (Black *Cyclosa* spider)** (Figure 4.1.14 A-F)

   Cyclosa bifida Doleschall, 1859: 38, pl. 2, Fig. 8 (♀); Simon, 1895: 779, Fig. 852; Tikader, 1966: 152, Fig. 31-34 (♀); Barrion & Litsinger, 1995: 608, Fig. 381a-g (♀); Petcharad et al., 2014: 27, Fig. 1-5 (♀♂).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: These small black coloured spiders have an elongated abdomen. They build small to medium-sized orb webs decorated with vertical stabilimentum made with the prey debris. The posterior end of the female's abdomen extends beyond the spinnerets and appears like a tail. Male spiders are smaller than the females, with characteristic four white spots arranged in an X-shape on the dorsal side of the abdomen. This species has been spotted in evergreen and semi-evergreen forests, and they build their webs in not more than 3 meters height from the ground.

   Distribution: South and Southeast Asia.

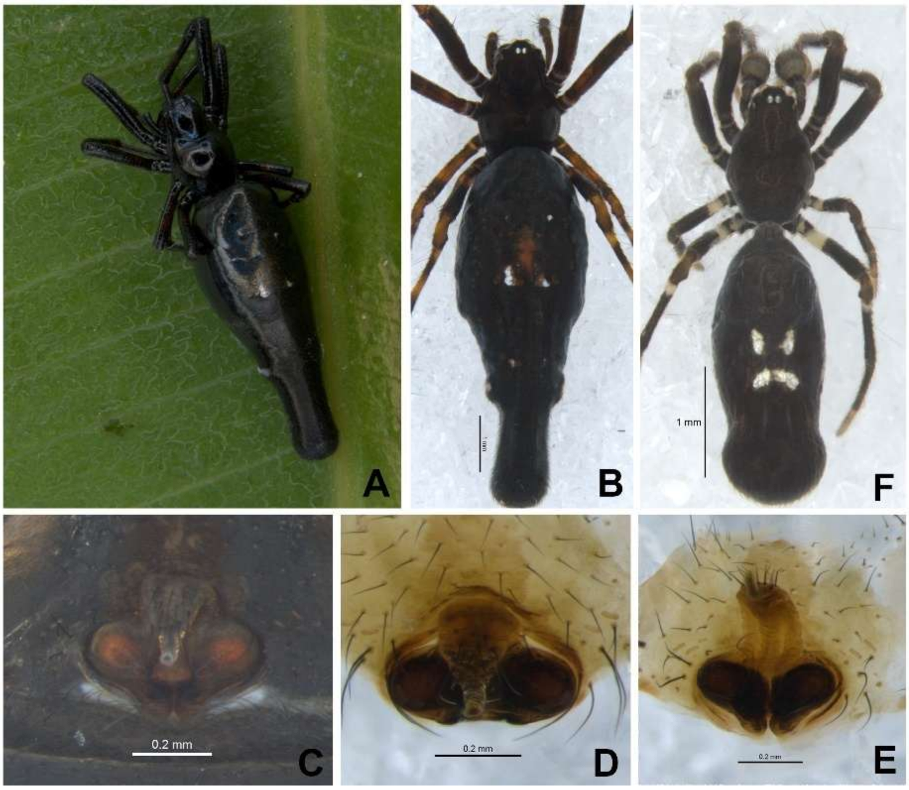


Figure 4.1.14. *Cyclosa bifida* (Doleschall, 1859): (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male subadult dorsal view. Scales: B, F 1 mm; C-E 0.2 mm.

2. ***Cyclosa confraga* (Thorell, 1893) (Trash line *Cyclosa* spider)** (Figure 4.1.15 A-D)

Cyclosa confraga Thorell, 1893: 239 (♀); Simon, 1909: 104; Tikader, 1982: 193, Fig. 372-376 (♀); Keswani, 2013: 64, Fig. 2A-F (♀).

(Complete list of references in WSC, 2025)

Habitat and natural history: Only the females of this species have been reported yet. Their dark to pale brown colour camouflages well in the vertical stabilimentum. Female spiders usually construct small orb webs and rest in the middle of the stabilimentum. Upon disturbances, they move away by creating a gap in the stabilimentum. They also have an elongated abdomen with a small, pointed caudal process. In the current study, they were spotted in all the sites having mangrove habitats and rarely in evergreens.

Distribution: South and Southeast Asia

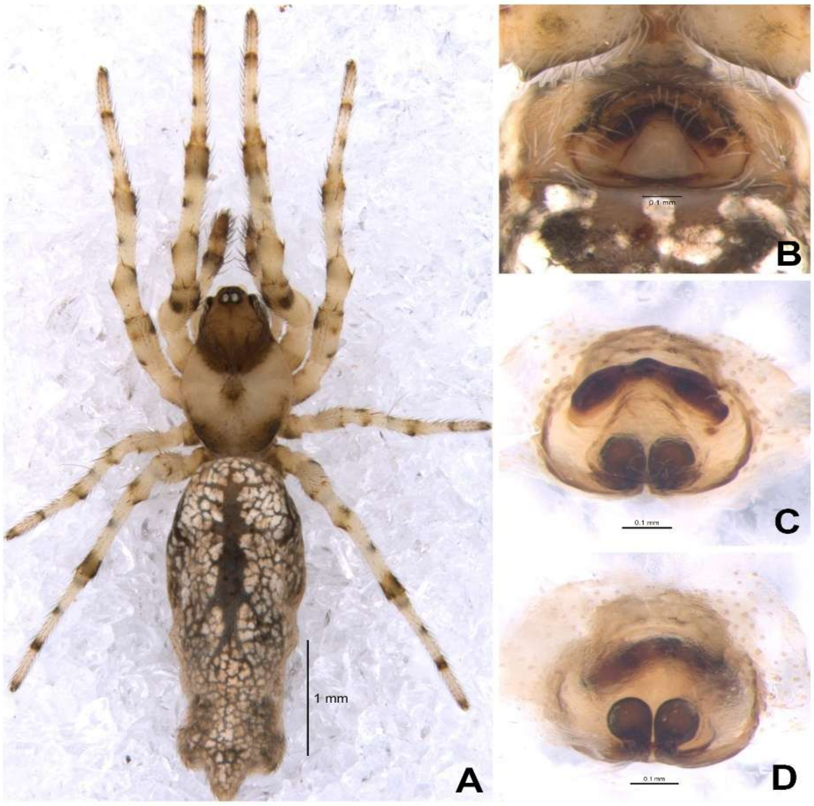


Figure 4.1.15. *Cyclosa confraga* (Thorell, 1893): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: A 1 mm; B-D 0.2 mm.

3. ***Cyclosa hexatuberculata* Tikader, 1982 (Brwon *Cyclosa* spider)** (Figure 4.1.16 A-G)

Cyclosa hexatuberculata Tikader, 1982: 197, Fig. 382-387 (♀♂); Dixit & Ade, 2017: 948, Fig. 3 A-I, 7 A-I (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: This is a common *Cyclosa* species found in almost all habitats. Female spiders have an elliptical abdomen with a characteristic number of tubercles, while the males are smaller than the females with an oval abdomen lacking the tubercles. However, both of them possess characteristic silvery white patches on the brown abdomen. They also produce stabilimentum and occupy the centre as other members of the genus do. They have been identified from all the habitats, including the gardens.

Distribution: India, Pakistan.

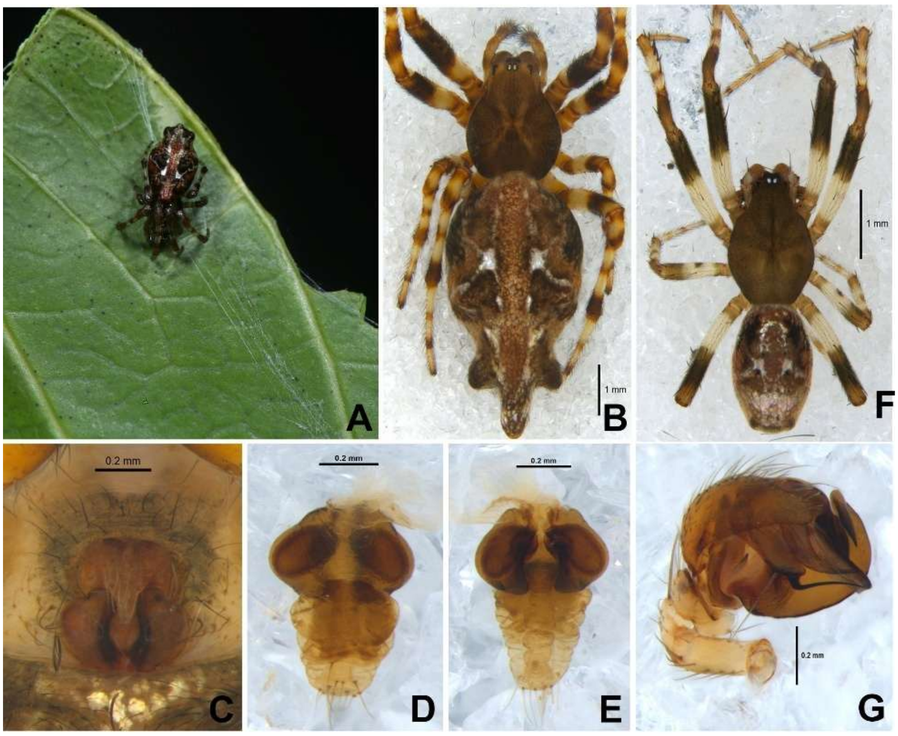


Figure 4.1.16. *Cyclosa hexatuberculata* Tikader, 1982: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: B, F 1 mm; C-E, G 0.2 mm.

4. ***Cyclosa mulmeinensis* (Thorell, 1887)** (Figure 4.1.17 A-D)

Cyclosa mulmeinensis Thorell, 1887: 221 (♀); Simon, 1909: 104; Yaginuma, 1963: 21, Fig. 1 (♀); Tikader, 1982: 187, Fig. 356-360 (♀); Barrion & Litsinger, 1995: Figs. 370a-g, 371a-e (♀); Yin et al., 1997: 253, Fig. 164a-j (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: This species is one of the *Cyclosa* species that have a globular abdomen. These dark brown coloured spiders have a pair of characteristic shoulder tubercles. Unlike other species, they did not form a proper vertical stabilimentum; instead built a discontinuous one. It is not a common species found in all habitats. In the present study, individuals of this species have been spotted in the mangrove ecosystem and high-altitude plains.

Distribution: Asia.

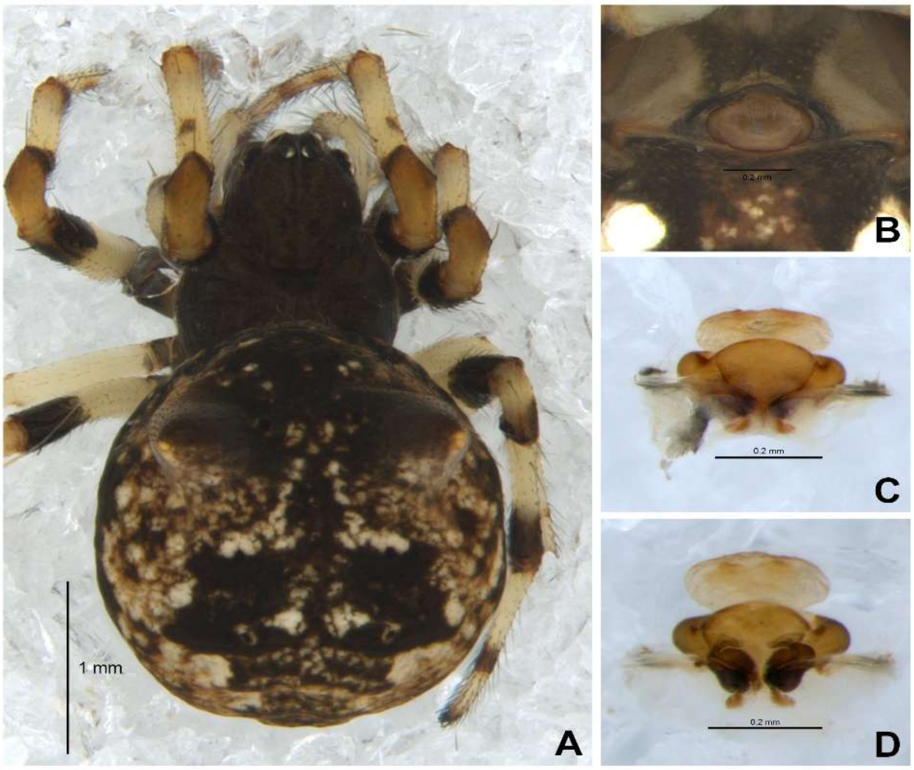


Figure 4.1.17. *Cyclosa mulmeinensis* (Thorell, 1887): (A) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: A 1 mm; B-D 0.2 mm.

5. ***Cyclosa purnai* Keswani, 2013** (Figure 4.1.18 A-E)

Cyclosa purnai Keswani, 2013: 76, Fig. 8 A-H (♀); Dixit & Ade, 2017: 951, Fig. 6 A-I (♀).

Habitat and natural history: This species has structural similarities with *Cyclosa hexatuberculata*. Only female spiders of this species have been reported yet, and they have a characteristic silvery brown longitudinal band on the dark brown coloured dorsum of the abdomen, extending from the anterior to the posterior end. Like others, they also build vertically oriented stabilimentum and house themselves in the centre. Only a single individual of this species has been collected in the study, which was from the tropical evergreens of Thenmala, Kollam.

Distribution: India

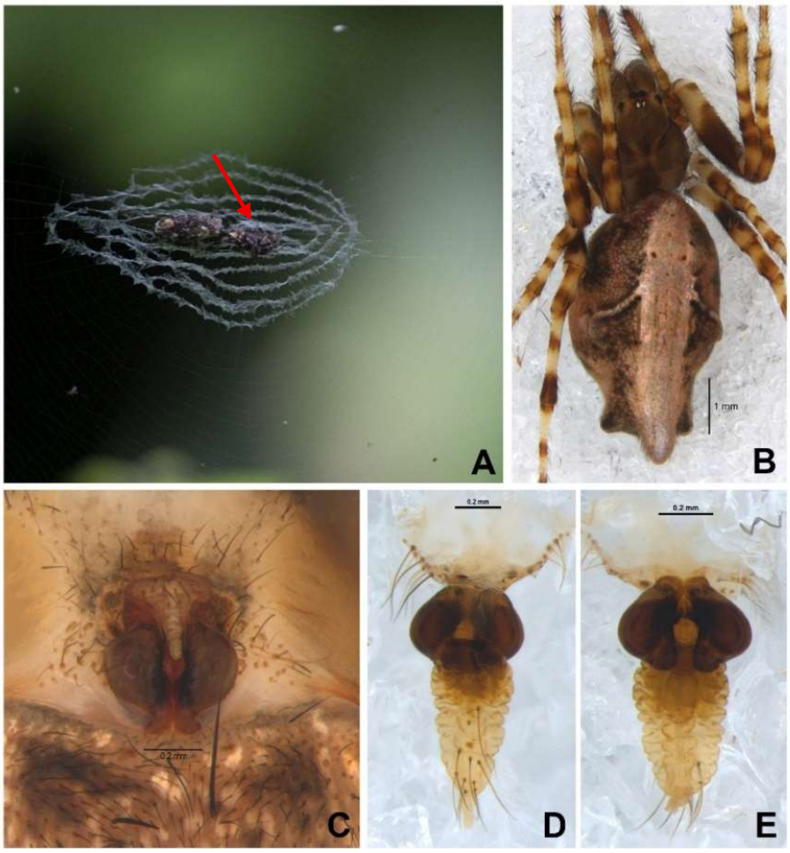


Figure 4.1.18. *Cyclosa purnai* Keswani, 2013: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: B 1 mm; C-E 0.2 mm.

6. ***Cyclosa quinqueguttata* (Thorell, 1881) (Spherical *Cyclosa* spider)** (Figure 4.1.19 A-F)

Cyclosa quinqueguttata Thorell, 1881: 113 (♂); Simon, 1889: 338; Tikader, 1982: 189, Fig. 361-366 (♀); Tanikawa, 1993: 61, Fig. 190-197 (♀♂); Yin et al., 1997: 254, Fig. 165a-f (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Males and females of this species look different in colour, pattern, and size. Typically, the females are small, not beyond 6 mm, and males are smaller than that. Females have an elliptical abdomen with a pair of characteristic mid-dorsal tubercles. Males are darker in colour with a pair of characteristic mid-dorsal white spots. They also decorate their web and occupy the centre to camouflage themselves. This species was spotted in different habitats, including evergreens, deciduous forests, mangroves, and paddy fields.

Distribution: China, South and Southeast Asia

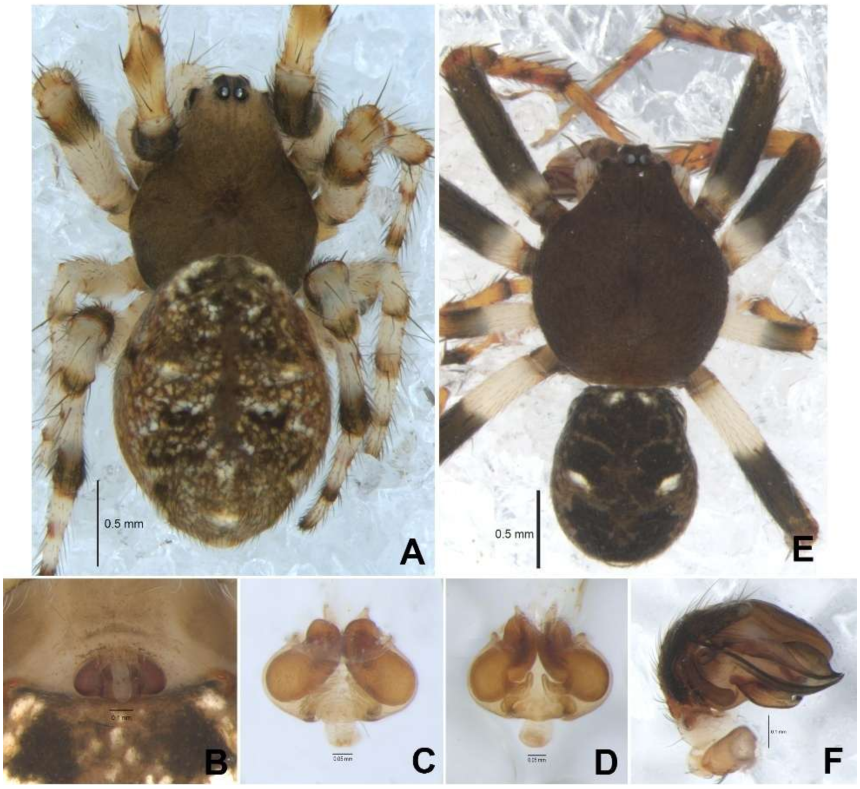


Figure 4.1.19. *Cyclosa quinqueguttata* (Thorell, 1881): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male dorsal view, (F) Palp retrolateral view. Scales: A, E 0.5 mm; B, F 0.1 mm; C-D 0.05 mm.

7. ***Cyclosa spirifera* Simon, 1889** (Figure 4.1.20 A-E)

Cyclosa spirifera Simon, 1889: 337 (♀♂); Tikader, 1982: 195, Fig. 377-381 (♀); Tyagi et al., 2019: Figs. S2.7, S3.1-2 (♀).

(Complete list of references in WSC, 2025)

Habitat and natural history: These spiders look similar to *Cyclosa hexatuberculata*. They also possess a characteristic number of abdominal tubercles, which can be considered an identification trait. These spiders build stabilimentum differently from others. They usually decorate their web with white coloured concentric rings around the hub with specialised silk. Sometimes, the hub possesses additional decorations made with the prey remains. Unlike *Cyclosa hexatuberculata,* they are not seen in all habitats. During the present study, they have only been spotted in evergreen and semi-evergreen forests with complex vegetation.

Distribution: India, Pakistan.

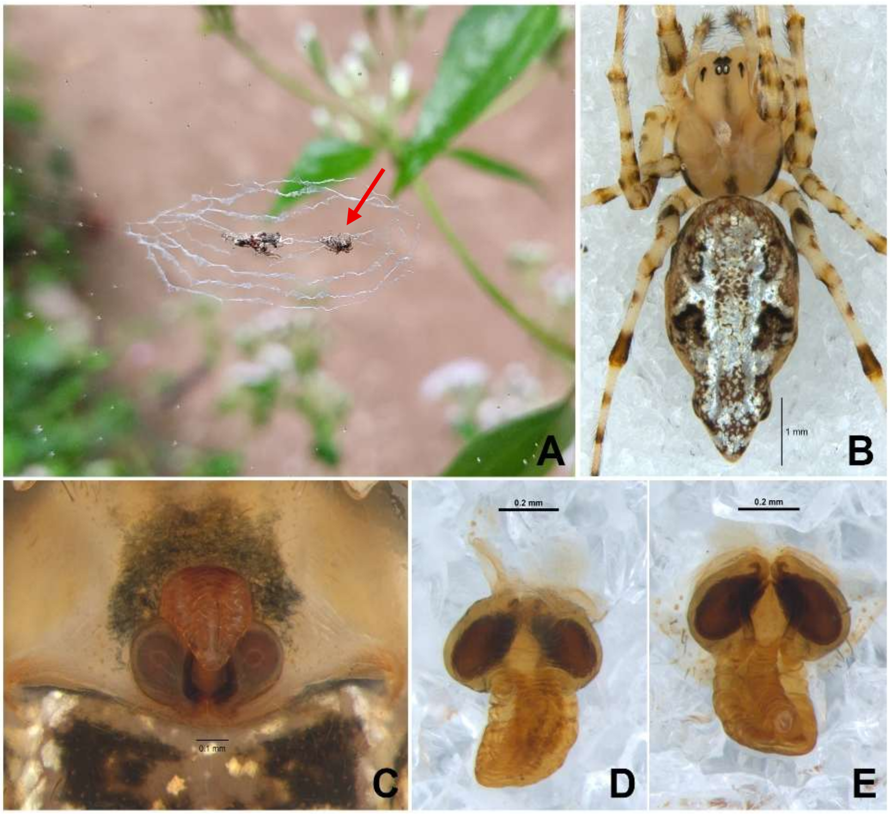


Figure 4.1.20. *Cyclosa spirifera* Simon, 1889: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: B 1 mm; C-E 0.2 mm.

Genus IX. ***Cyrtophora* Simon, 1864**

Type species: *Cyrtophora citricola* (Forsskål, 1775)

Diagnostic features: *Cyrtophora* is an exceptional group in the family Araneidae regarding the web. Unlike other araneids, members of this genus build specialised dome-shaped horizontal webs with viscid threads. A layer of fine mesh-like threads is woven below the dome. The spider occupies the centre of this structure, which can be considered the hub. A few egg sacs can be observed in the web of adult female spiders as a string of beads. Specialised web construction can be considered the diagnostic feature of this genus. Additionally, most of the members possess paired tubercles on the anteriorly high abdomen. Members of this genus have been radiated in all realms with 47 recognised species (WSC, 2025). In the present study, three species belonging to *Cyrtophora* were identified.

1. ***Cyrtophora cicatrosa* (Stoliczka, 1869) (Common tent web spider)** (Figure 4.1.21 A-E)

Cyrtophora cicatrosa Stoliczka, 1869: 242, pl. 20, Fig. 5 (♀); Simon, 1895: 775; Tikader & Biswas, 1981: 32, Fig. 45-46 (♀); Yin et al., 1997: 279, Fig. 188a-f (♀); Tyagi et al., 2019: Figs. S2.8, S3.23-24 (♀).

(Complete list of references in WSC, 2025)

Habitat and natural history: These spiders are abundant and common in all habitats, and they can be considered synanthropic since they can be seen near human constructions. Females are small, not beyond 1 cm, with two pairs of prominent black abdominal tubercles. The dorsal and ventral sides of the abdomen have a characteristic pattern formed with pale brown, white and black markings. As described in the diagnostic features of *Cyrtophora*, this species constructs dome-shaped webs and rests in the centre. Every adult female lays their eggs in their web itself in a line upwards from the centre of the dome.

Distribution: Australia, South and Southeast Asia.

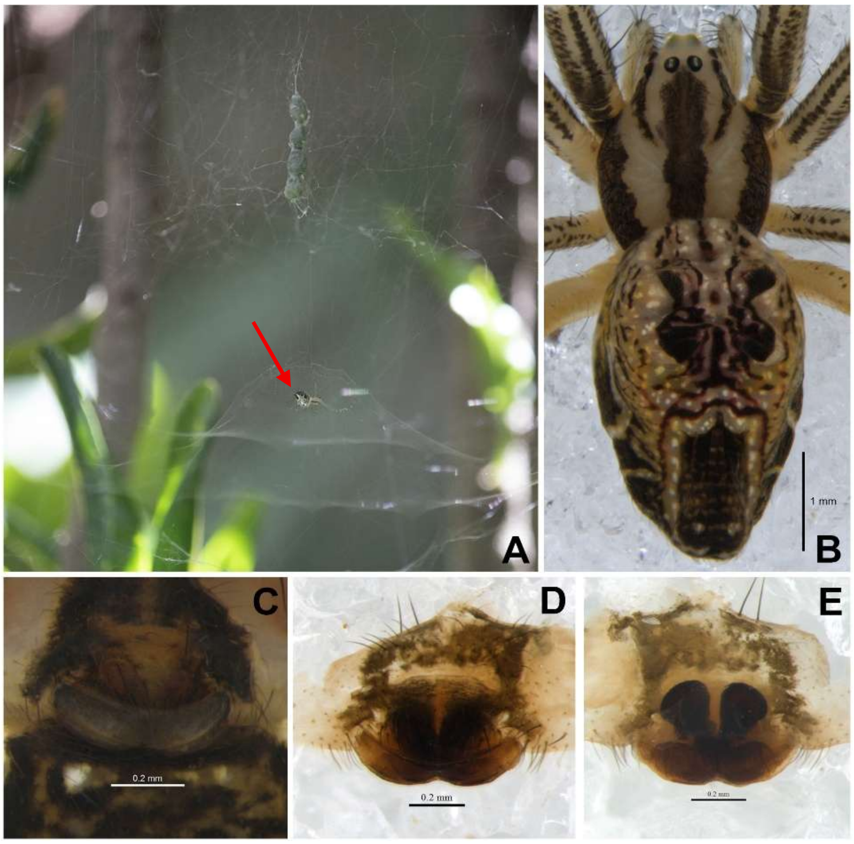


Figure 4.1.21. *Cyrtophora cicatrosa* (Stoliczka, 1869): (A) Female habitus with egg sacs, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: B 1 mm; C-E 0.2 mm.

2. ***Cyrtophora moluccensis* (Doleschall, 1857) (Dome tent web spider)** (Figure 4.1.22 A-H)

Cyrtophora moluccensis Doleschall, 1857: 418 (♀); Simon, 1895: 770, 775, Fig. 846 (♀♂); Yaginuma, 1958: 14, Fig. 2F-H (♀♂); Nentwig et al., 2019: 35.

(Complete list of references in WSC, 2025)

Habitat and natural history: This is another species that exhibits a high level of sexual size dimorphism. An adult female builds large tent webs along with many additional supporting threads to the nearby vegetation. Occasionally, these spiders can be seen in a group by building communal webs that cover a large area. Males are very small and only during the mating time they are only seen near the female's web. They require humid habitats with water bodies nearby. In the present study, they have only been spotted in the mangrove ecosystems.

Distribution: Australia, America, and Asia.

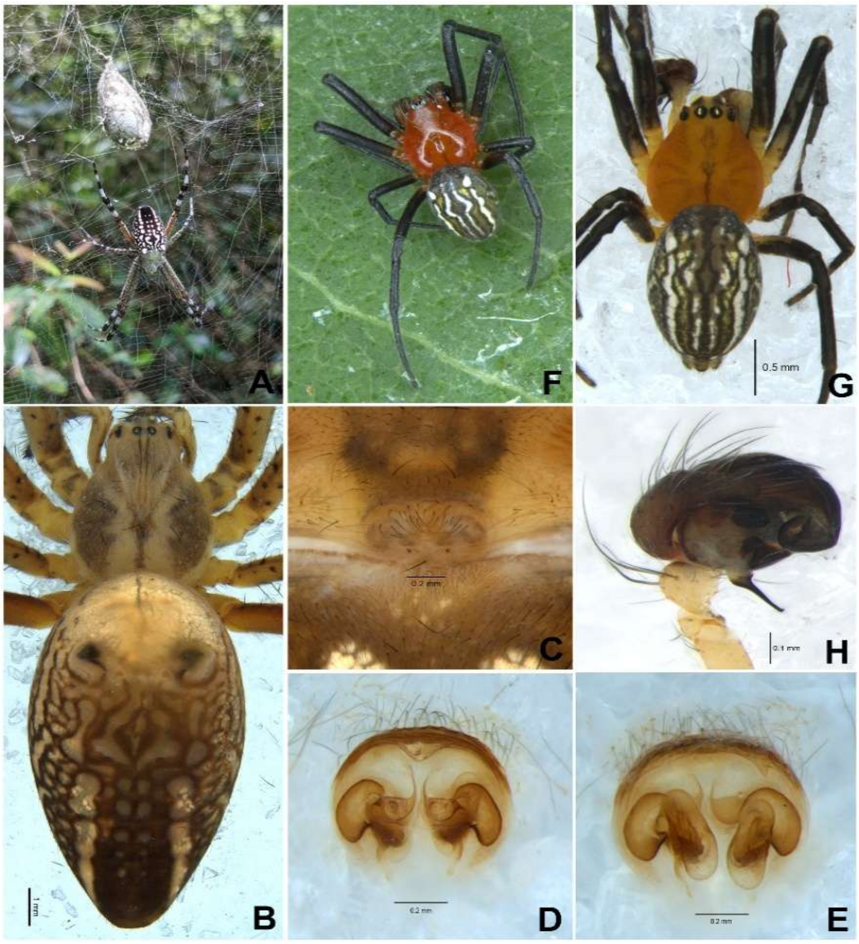


Figure 4.1.22. *Cyrtophora moluccensis* (Doleschall, 1857): (A) Female habitus with egg sac, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male habitus, (G) Male dorsal view, (H) Palp retrolateral view. Scales: B 1 mm; C-E 0.2 mm; G 0.5 mm; H 0.1 mm.

3. ***Cyrtophora unicolor* (Doleschall, 1857) (Brown tent web spider)** (Figure 4.1.23 A-G)

Cyrtophora unicolor Doleschall, 1857: 419 (♀); Simon, 1895: 775; Yaginuma, 1968: 36, Fig. 8-9 (♀). Barrion & Litsinger, 1995: 587, Fig. 366a-i (♀); Yin et al., 1997: 287, Fig. 196a-c (♀); Malamel, 2018: 112, Figs. 2A-B, 3A-I (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Like others, females of this species construct messy, large tent webs, but unlike others, they rest in retreats made by rolling a dried leaf, which will be placed at the centre of the web. Their webs are usually untidy as they have the prey's remains, twigs, leaves and egg sacs scattered in the web. Females are brown with prominent shoulder humps. Males are smaller than females and are dark coloured. They have been spotted in different habitats, including monotypic plantations and mangroves, but are abundant in evergreen and semi-evergreen forests.

Distribution: Asia and Australia.

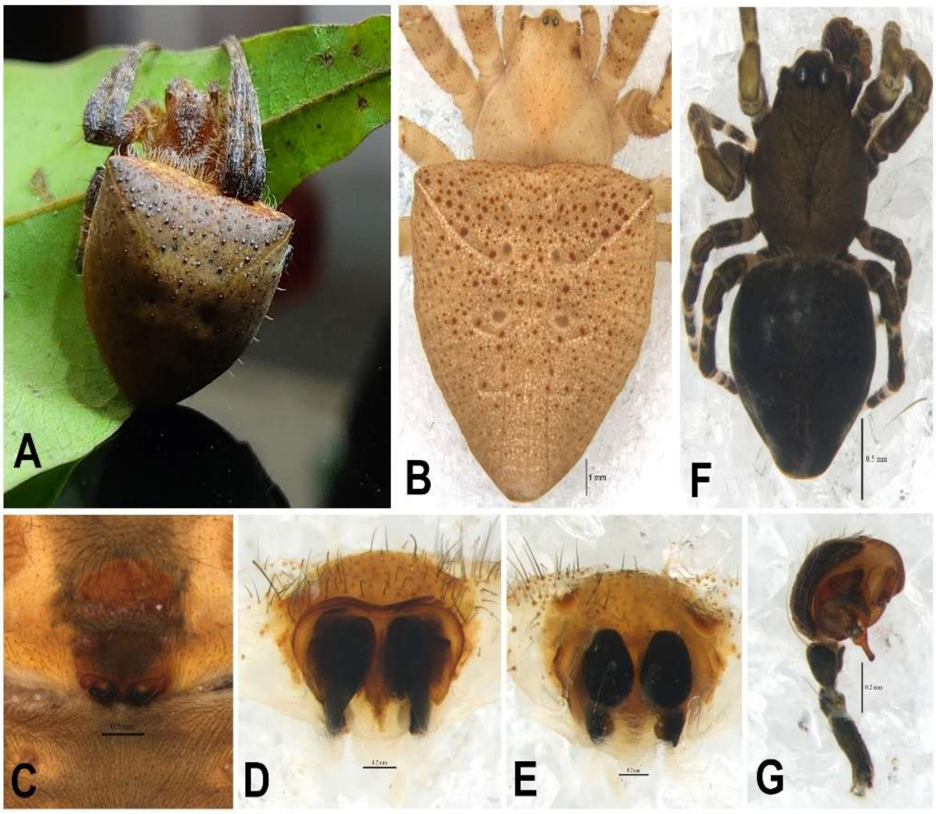


Figure 4.1.23. *Cyrtophora unicolor* (Doleschall, 1857): (A) Female habitus (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: B 1 mm; C-E, G 0.2 mm; F 0.5 mm.

Genus X. ***Eriovixia* Archer, 1951**

Type species: *Eriovixia rhinura* (Pocock, 1900)

Diagnostic features: This genus is commonly called bird-dropping spiders, including small to medium-sized spiders. Female spiders have a sub-triangular abdomen tapering posteriorly sometimes with caudal humps and extensions. They have a tongue-like, nearly triangular epigynum with a blunt-ended ended short scape completely fused with the atrium. Males are smaller and more spinous than females. They don’t have macrosetae in the patella segment of the pedipalp. Males can be distinguished from other araneids by the size of the median apophysis and the presence of additional spurs. Members of this genus are distributed across Africa and the southern and southeastern countries of Asia. To date, 33 species have been identified from this genus (WSC, 2025). In the present study, eight species have been collected. Among them, species identity was revealed for five.

1. ***Eriovixia excelsa* (Simon, 1889) (Dark bird dropping spider)** (Figure 4.1.24 A-D)

   Eriovixia excelsa Simon, 1889: 337 (♀); Tikader & Bal, 1981: 25, Fig. 50-54 (♀♂); Grasshoff, 1986: 118 (♀♂); Tso & Tanikawa, 2000: 129, Fig. 17-22 (♀♂); Mi et al., 2010: 41, Fig. 1-8 (♀♂); Han & Zhu, 2010: 2616, Figs. 1C, 6A-C (♀).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Females of this species have a folium made of pale brown to white patches on the black to dark brown dorsum of the abdomen. They also have a characteristic black spot at the posterior end of the abdomen. Usually, they build medium orb webs and rest at the hub during nighttime. In the daylight, they will hide under leaves. In the present study this species has been spotted in mangrove ecosystem and rubber plantations.

   Distribution: South and Southeast Asia, introduced to Africa.

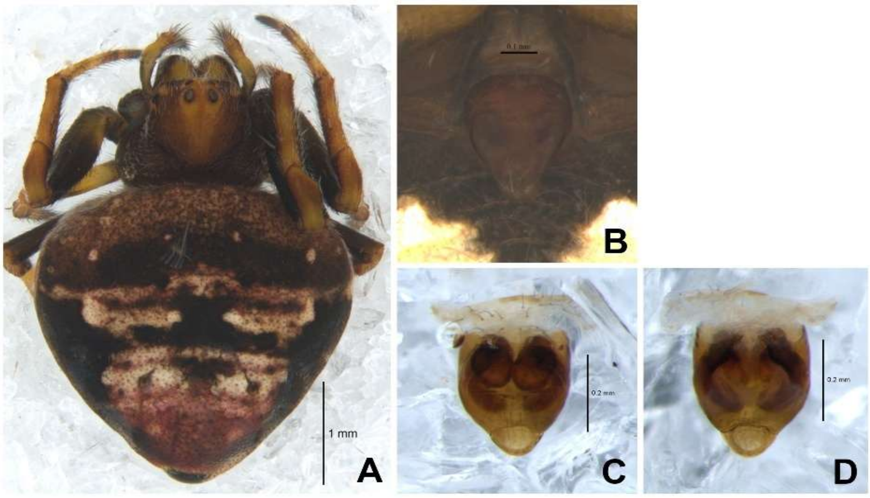


Figure 4.1.24. *Eriovixia excelsa* (Simon, 1889): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: A 1 mm; B-D 0.2 mm.

2. ***Eriovixia gryffindori* Ahmed, Khalap & Sumukha, 2016** (Figure 4.1.25 A-D)

Eriovixia gryffindori Ahmed, Khalap & Sumukha, 2016: 25, Fig. 1-6 (♀).

Habitat and natural history: This species has been reported only from India, and the males are unknown. The sub-triangular abdomen of the female has a tapering, raised, and downwardly bent posterior extension. The dorsum of the abdomen possesses pale green folium. They are medium-sized spiders which are nocturnally active and hide themselves in tree bark and dried leaves by camouflaging themselves. The species was rarely spotted in the present study, and it was from the riparian ecosystem.

Distribution: India

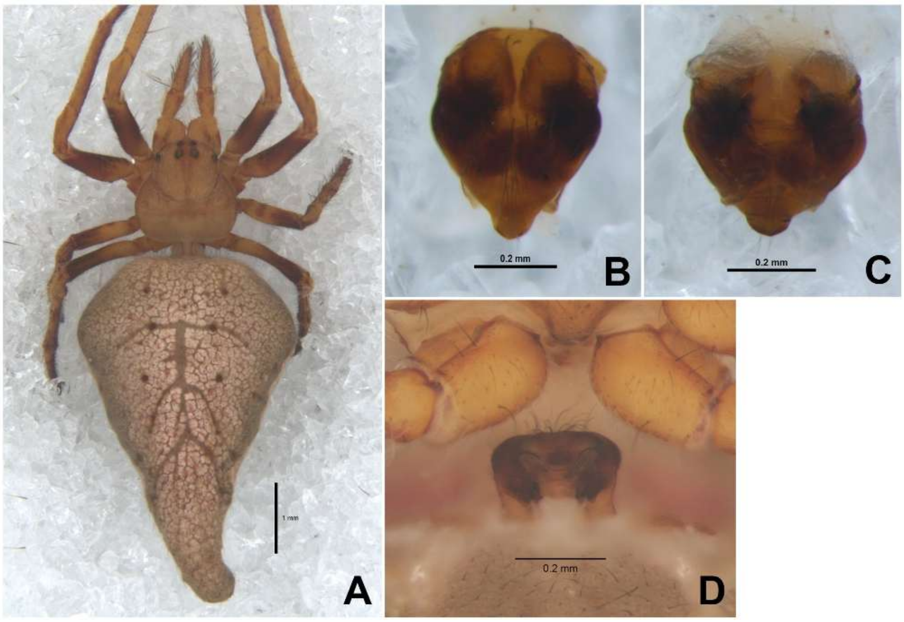


Figure 4.1.25. *Eriovixia gryffindori* Ahmed, Khalap & Sumukha, 2016: (A) Female dorsal view, (B) Epigyne ventral view, (C) Epigyne dorsal view, (D) Epigyne insitu. Scales: A 1 mm; B-D 0.2 mm.

3. *Eriovixia laglaizei* (Simon, 1877) (Common bird-dropping spider) (Figure 4.1.26 A-F)

Eriovixia laglaizei Simon, 1877: 77 (♀); Simon, 1895: 820, Fig. 871-872 (♀♂); Tikader & Bal, 1981: 27, Fig. 55-58 (♀♂); Grasshoff, 1986: 118; Yin et al., 1997: 296, Fig. 206a-e (♀♂); Han & Zhu, 2010: 2623, Fig. 3A-B, 10A-I (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: This is a common species that can be found in gardens and all types of ecosystems. Like others, they also build webs during dusk and in daytime they hide themselves under the leaves. Both males and females are pale grey in colour and possess a subtriangular abdomen, however, males are smaller than the females. Sometimes the posterior end of the abdomen of females extends and looks like a short tail. In the present study, they have been collected from almost all the ecosystems having complex vegetation, including evergreens, deciduous, and mangroves.

Distribution: South and Southeast Asia.

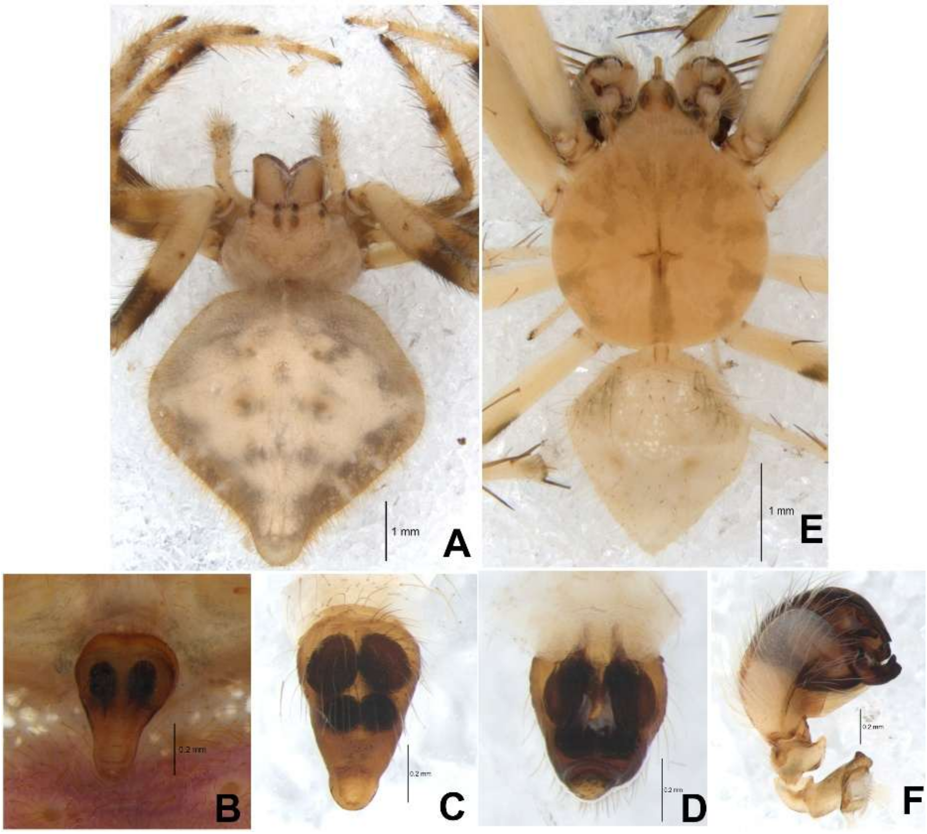


Figure 4.1.26. *Eriovixia laglaizei* (Simon, 1877): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male dorsal view, (F) Palp retrolateral view. Scales: A, E 1 mm; B-D, F 0.2 mm.

4. *Eriovixia poonaensis* **(Tikader & Bal, 1981)** (Figure 4.1.27 A-F)

Eriovixia poonaensis Tikader & Bal, 1981: 29, Figs. 59-62 (♀); Grasshoff, 1986: 118 (♀); Yin et al., 1990: 115, Figs. 280-282 (♀); Mi & Wang, 2016: 731, Fig. 7-12 (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: This species has similarities with *Eriovixia laglaizei* in appearance, abdominal patterns and genital structures. However, they are not as common as the former. Females possess a branched longitudinal grey band on their pale white abdominal dorsum. They have also been spotted in ecosystems having complex vegetation. Unlike the former, individuals of this species have been spotted in their webs.

Distribution: India, China, Thailand, Malaysia.

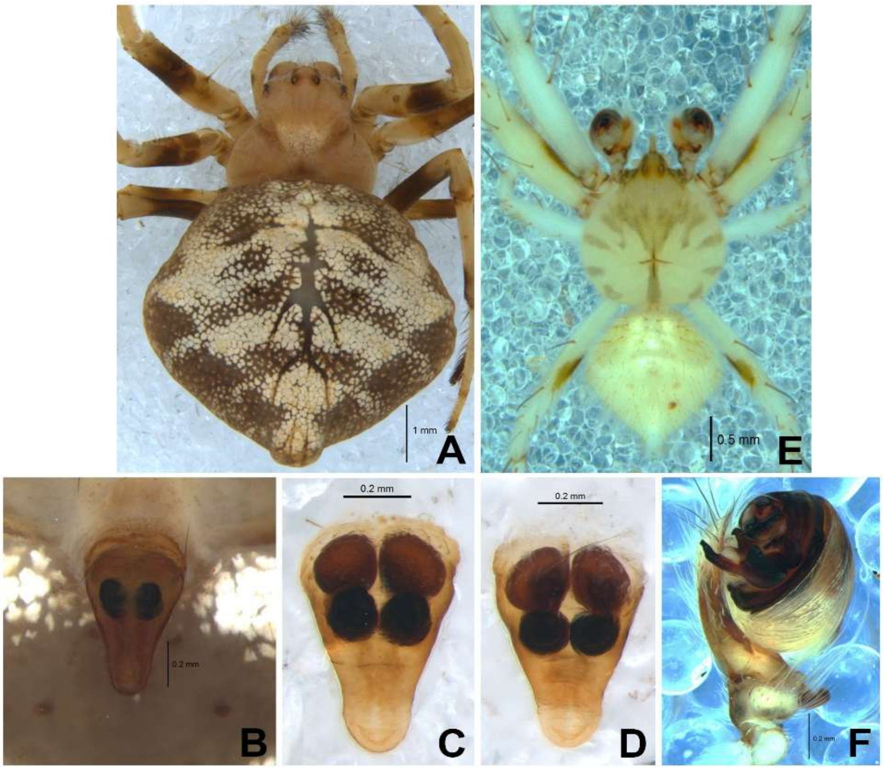


Figure 4.1.27. *Eriovixia poonaensis* (Tikader & Bal, 1981): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male dorsal view, (F) Palp retrolateral view. Scales: A 1 mm; B-D, F 0.2 mm; E 0.5 mm.

5. *Eriovixia sakiedaorum* **Tanikawa, 1999 (Black button spider)** (Figure 4.1.28 A-E)

Eriovixia sakiedaorum Tanikawa, 1999: 45, Fig. 11-17 (♀♂); Han & Zhu, 2010: 2629, Figs. 4B-C, 13A-E (♀♂); Asalatha & Prasadan, 2019: 169, Fig. 2a-b (♀).

(Complete list of references in WSC, 2025)

Habitat and natural history: Males and females of this small species are black in colour with a globular abdomen. Females possess two lateral white markings. Like other members of *Eriovixia*, this species is active during the night and builds orb webs. They have only been spotted in evergreens and semi-evergreen forests.

Distribution: India, China, Taiwan, Japan.

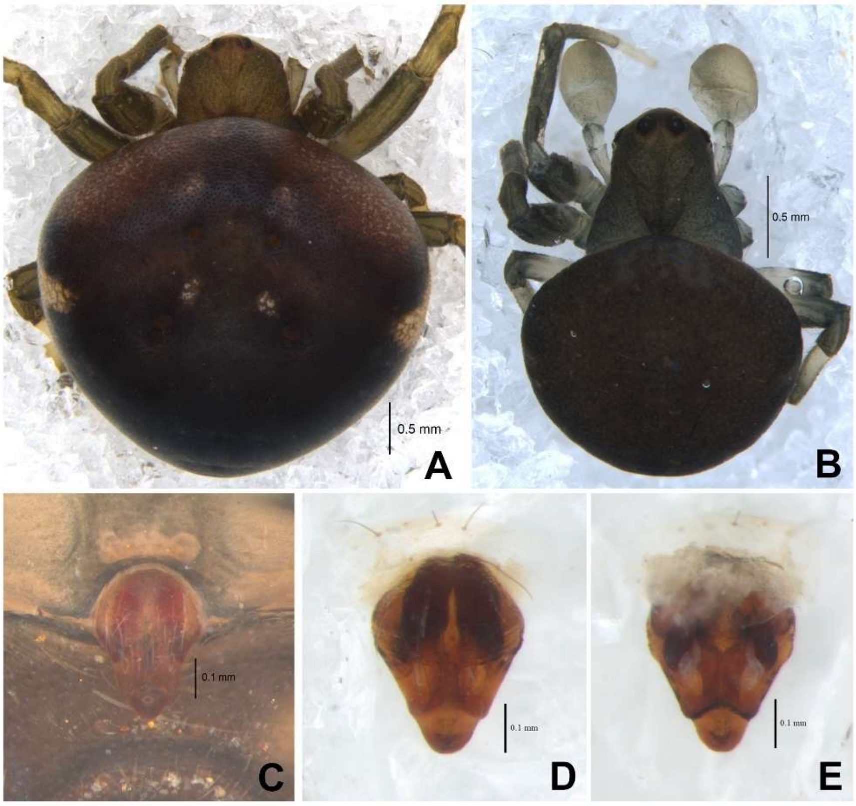


Figure 4.1.28. *Eriovixia sakiedaorum* Tanikawa, 1999: (A) Female dorsal view, (B) Male subadult dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: A-B 0.5 mm; C-E 0.1 mm.

Genus XI. ***Gasteracantha*** **Sundevall, 1833**

Type species: *Gasteracantha cancriformis* (Linnaeus, 1758)

Diagnostic features: This genus includes brightly coloured medium to large female spiders. However, the males are much smaller than the females and have black to dark brown colour. They have a hard body with three pairs of abdominal spines. The central bulge between the epigastric furrow and spinnerets and the placement of spinnerets on a cone surrounded by heavily sclerotised annulations are the distinguishing features of this genus. They have a broad, elevated cephalic region. Females have a sclerotised epigyne with a basally fused, short, tongue-like scape. Male pedipalps lack patellar macrosetae and have a median apophysis spur and long, tapering embolus.

Members of *Gasteracantha* have been radiated worldwide, with 85 recognised species (WSC, 2025). The study identified two species.

1. ***Gasteracantha dalyi* Pocock, 1900 (Long-horned spiny spider)** (Figure 4.1.29 A-F)

   Gasteracantha dalyi Pocock, 1900: 232 (♀); Tikader, 1982: 67, Fig. 135-138 (♀).

   Habitat and natural history: Females are bright yellow to orange coloured with a pair of long, arched abdominal median spines. Males are still unknown. They construct perfect vertical orb webs and the females rest at the hub. Usually, they prefer high humidity and shady habitats. In the current study this species has been recorded only from the tropical evergreen forests.

   Remarks: Reporting male for the first time, but could not characterise due to the subadult condition of the specimen.

   Distribution: Pakistan, India

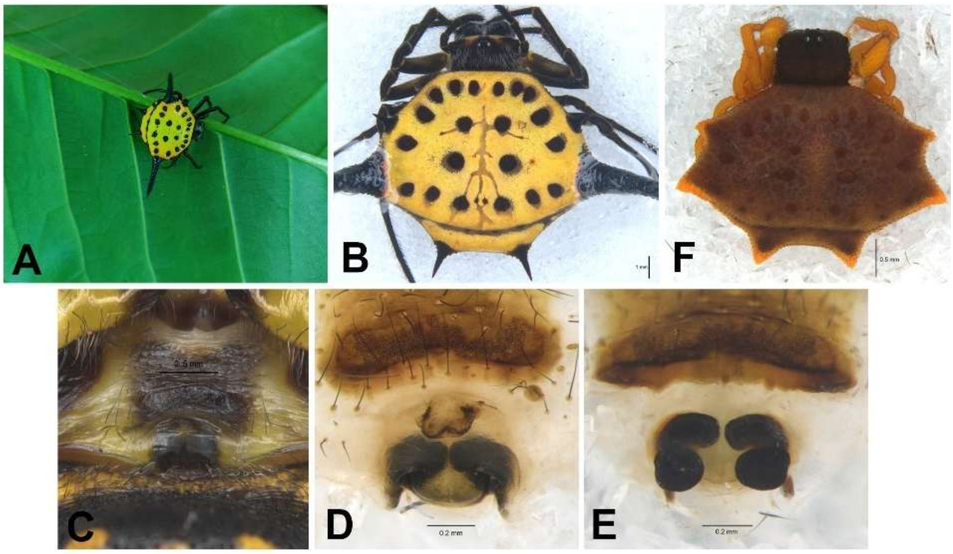


Figure 4.1.29. *Gasteracantha dalyi* Pocock, 1900: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male subadult dorsal view. Scales: A 1 mm; B, F 0.5 mm; D-E 0.2 mm.

2. ***Gasteracantha geminata* (Fabricius, 1798) (Oriental spiny spider)** (Figure 4.1.30 A-G)

Gasteracantha geminata Fabricius, 1798: 292 (♀); C. L. Koch, 1837: 16, Fig. 260 (♀); Simon, 1895: 836, Figs. 883-887 (♀♂); Tikader, 1982: 53, Figs. 107-110 (♀); Sankaran et al., 2015: 147, Figs. 1A-F, 2A-F (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: This is a common species that can be found in almost all habitats. Females have a horizontally elongated abdomen with fused anterior and median pairs of spines. They possess three characteristic white transverse bands on the black to dark brown dorsum of the abdomen. Tinges of yellow and red are also visible at the base of the spines. Males are much smaller and dull in colour. Both males and females construct orb webs and rest at the hub during the day. Sometimes, juvenile females build small, fluffy ball-like structures made with specialised silk and arranged serially in the radials, which can be considered as stabilimentum. In the present study, this species has been recorded from all the habitats except grasslands, Kole wetlands and paddy fields.

Distribution: India, Sri Lanka.

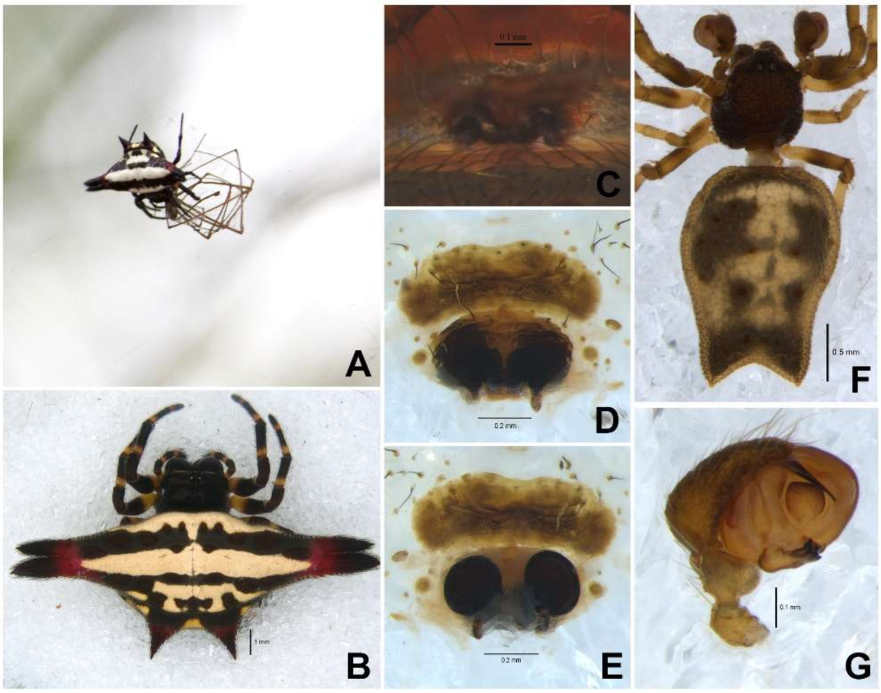


Figure 4.1.30. *Gasteracantha geminata* (Fabricius, 1798): (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: A 1 mm; F 0.5 mm; D-E 0.2 mm; C, G 0.1mm.

Genus XII. ***Gea* C. L. Koch, 1843**

Type species: *Gea spinipes* C. L. Koch, 1843

Diagnostic features: Members of this genus are medium-sized sized that highly resemble *Argiope* species in the general appearance and resting position in the orb web. However, they can be distinguished by having equally spaced PEs. Also, the cephalic and the thoracic regions are raised equally. Females have a weakly sclerotised epigyne that lacks a scape but has a prominent median septum dividing the atrium, forming two equal depressions. The anterior lip of the septum and the posterior septal plate play an important role in characterising the species. Males are smaller than females, but do not exhibit high sexual size dimorphism as in *Argiope*. Males have only one patellar seta in the pedipalp. Median apophysis is bifurcated and possesses a long curved filiform embolus.

This genus has a worldwide distribution with 13 identified species (WSC, 2025). In the present study, one species belonging to *Gea* was identified.

1. ***Gea spinipes* C. L. Koch, 1843 (Orange-headed orb web spider)** (Figure 4.1.31 A-D)

   Gea spinipes C. L. Koch, 1843: 101, Fig. 823 (♀); Levi, 1983: 326, Fig. 362-375 (♀♂); Yin et al., 1989: 67, Fig. 7A-C (♀); Chakrabarti, 2009: 128, Fig. 1-7 (♀); Mi et al., 2024a: 79, Figs. 3A-L, 4A-E, 7E-H (♀♂).
   (Complete list of references in WSC, 2025)

   Habitat and natural history: Females of this species have a subtriangular abdomen with white patches on a greyish brown abdomen. They are rarely found during day daytime and build large vertical webs at night. This species builds their webs in high humid and shady regions of evergreen and semievergreen forests. In the present study they have been spotted only from two regions.

   Distribution: China, South and Southeast Asia.

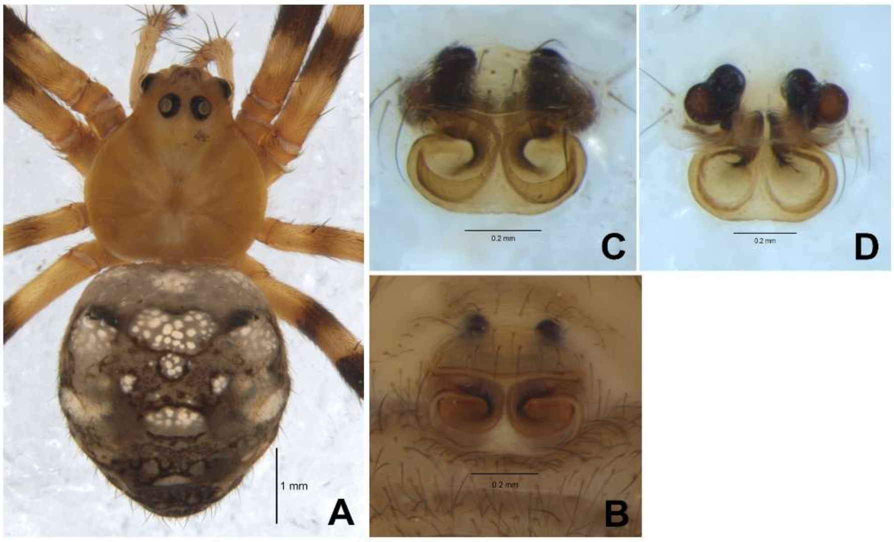


Figure 4.1.31. *Gea spinipes* C. L. Koch, 1843): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: A 1 mm; B-C 0.2 mm.

Genus XIII. ***Herennia*** **Thorell, 1877**

Type species: *Herennia multipuncta* (Doleschall, 1859)

Diagnostic features: Members of this genus are unique in building orb webs. The spiders built small to medium orb webs in tree trunks, rocks and walls in the plane of the substrate, and the hub will be touching the substrate. Medium pale brown to grey coloured females, having long legs, rest at this hub, camouflaging themselves so that it is difficult to spot them. They show extreme sexual dimorphism in terms of size and appearance. Females, having a lobed abdomen, possess a sclerotised epigynum with two distinct chambers formed by the septum. Males can be distinguished by having an apical tegular apophysis, an embolus hook and a large sigmoid-shaped conductor. Both males and females have straited cheliceral boss. Members of this genus are distributed across the tropical belt with 11 identified species (WSC, 2025). The only representative from India was also spotted during the study.

1. ***Herennia multipuncta*** **(Doleschall, 1859) (Bark hugging spider)** (Figure 4.1.32 A-H)

   Herennia multipuncta Doleschall, 1859: 32, pl. 1, Fig. 5 (♀); Thorell, 1877: 371; Simon, 1894: 759, Fig. 828, 835 (♀♂); Tikader, 1982: 106, Fig. 202-204 (♀); Kuntner, 2005: 397, Figs. 1A-G, 2A-D, 3A-C, 4A-E, 5A-F, 6A-F, 7A-F, 8A-F, 9A-F, 10A-F, 11A-F, 12A-F, 13A-F, 14A-C, 15 (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Females of this species are medium-sized spiders, while the males are very small. Females, typically flat, have a pale brown abdomen with a dark carapace and build small orb webs on tree trunks and rest at the hub, camouflaging themselves. Bright red coloured to brown males can be seen in the proximity of this web during the breeding time. In the present study, they have been spotted in all evergreen, semi-evergreen and deciduous forests.

Distribution: India, China, Vietnam, Indonesia.

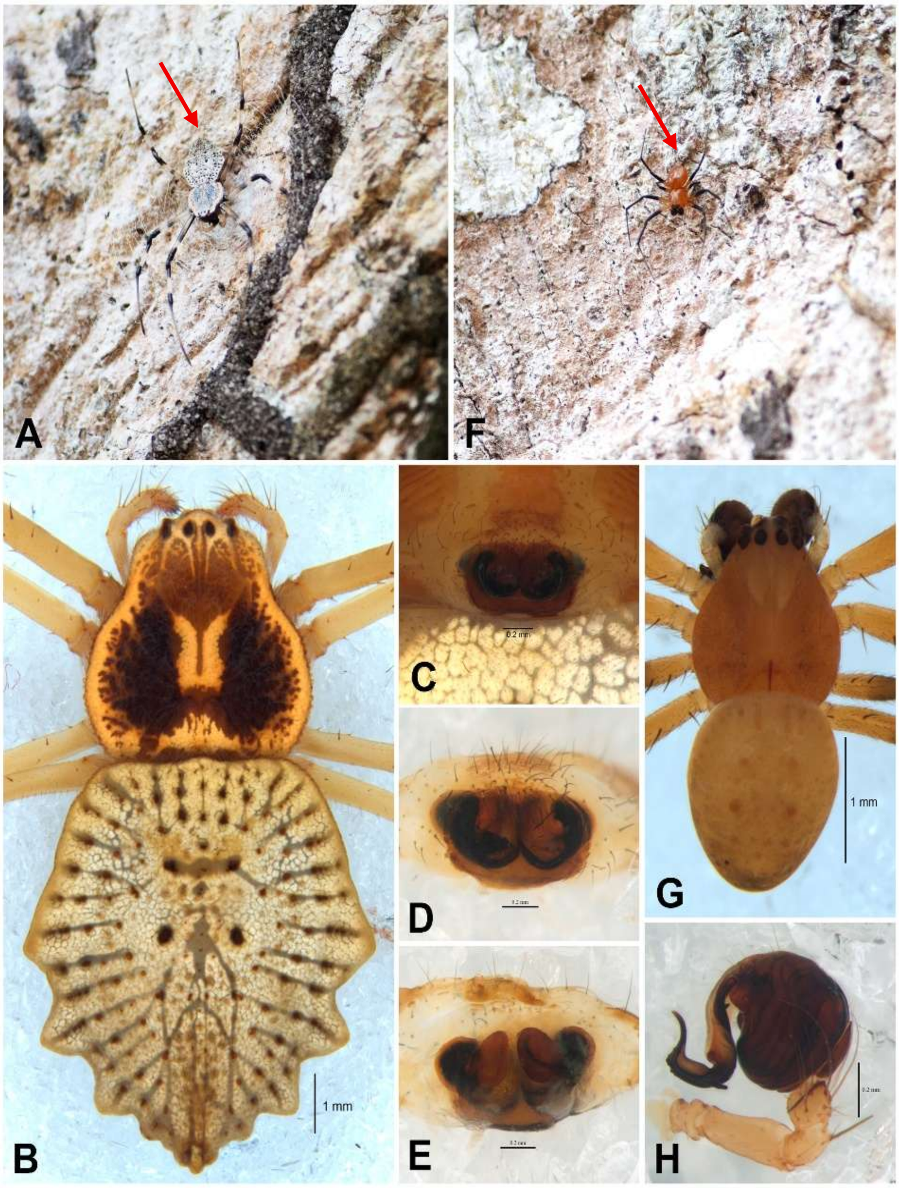


Figure 4.1.32. *Herennia multipuncta* (Doleschall, 1859): (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male habitus, (G) Male dorsal view, (H) Palp ventral view. Scales: B, G 1 mm; C-E, H 0.2 mm.

Genus XIV. ***Leviaraneus*** **Tanikawa & Petcharad, 2023**

Type species: *Leviaraneus viridiventris* (Yaginuma, 1969)

Diagnostic features: *Leviaraneus* is one among the species group that has been recently upgraded to genus status from *Araneus*. Both males and females have a similar appearance. Males have only one patellar macrosetae in their pedipalp and lack a ventral hook on coxa I and a dorsal groove on femur II. Their embolus is S-shaped, long and filiform. Females have a partially sclerotised, heart-shaped epigyne with a short, tongue-like, U-shaped scape. All the members of this genus are bright green in colour.

Members of this genus are mainly distributed in Southeast Asian countries. To date, three species have been recognised under this genus (WSC, 2025), and among them, only one has been reported in the present study.

1. ***Leviaraneus viridiventris* (Yaginuma, 1969)** (Figure 4.1.33 A-F)

   Leviaraneus viridiventris Ohno & Yaginuma, 1969: 21, Fig. 3a-f (♀♂); Yin et al., 1990: 22, Fig. 51-57 (♀♂); Shilpa et al., 2023: 133, Figs. 1, 2A-E, 3A-E, 4A-C (♀); Tanikawa & Petcharad, 2023: 121, Fig. 2A-H (♀♂).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Both males and females of this spider have an inverted triangle abdomen with blunt posterior end. They have a bright green abdominal dorsum with dark brown comma-shaped lateral patches from anterior to the posterior end. This species has been spotted in the evergreen forest and garden during the present study. Both the male and female specimens were found in the retreat near the medium orb web.

   Remarks: First report from India (Shilpa et al., 2023).

   Distribution: India, China, Taiwan, Japan.

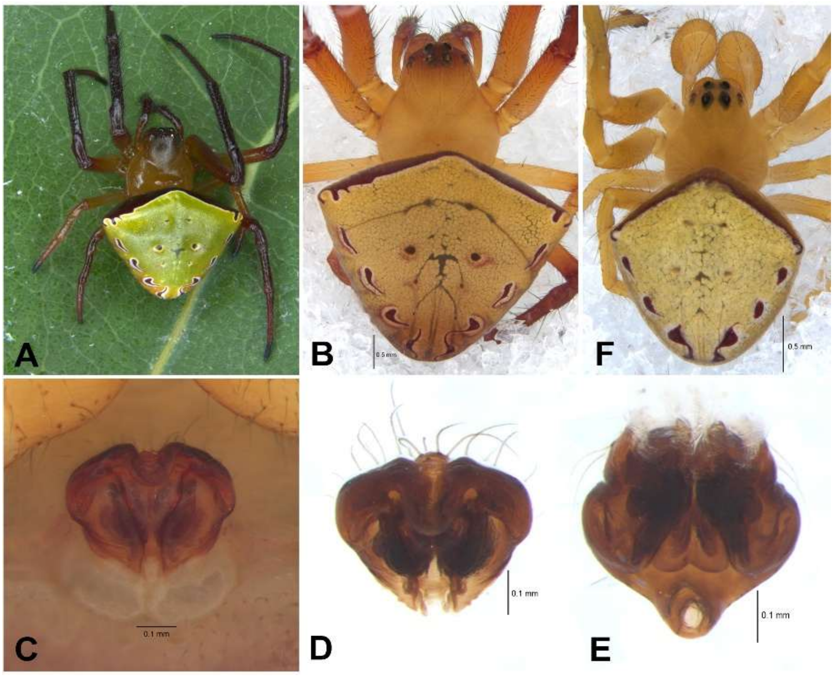


Figure 4.1.33. *Leviaraneus viridiventris* (Yaginuma, 1969): (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view. Scales: B, F 0.5 mm; C-E 0.1 mm.

Genus XV. ***Macracantha*** **Simon, 1864**

Type species: *Macracantha arcuata* (Fabricius, 1793)

Diagnostic features: Members of this genus were initially placed under *Gasteracantha* as they possess three pairs of abdominal spines, but later Emerit (1974) characterised a new genus, *Macracantha*, and transferred them. Carapace has a raised mid-region. Octagonal abdomen with sigilla on both dorsal and ventral sides. They also have a well-developed median spine longer than the other two pairs. Unlike *Gasteracantha*, members of this genus are concentrated in South and Southeast Asia. To date, only two species have been recorded from this genus. In the present study, one species was identified.

1. ***Macracantha hasselti*** **(C. L. Koch, 1837) (Hasselt's spiny spider)** (Figure 4.1.34 A-C)

   Macracantha hasselti C. L. Koch, 1837: 29, Fig. 267 (♀); Emerit, 1974: 29; Tikader & Biswas, 1981: 33, Fig. 47-49 (♀); Yin et al., 1997: 96, Fig. 25a-f (♀♂); Macharoenboon et al., 2021: 45, Figs. 9C-D, 12D-I.

   (Complete list of references in WSC, 2025)

Habitat and natural history: Females of this species are bright yellow in colour with conspicuous black sigilla and three pairs of abdominal spines. Median spines are the largest and most pointed of the others. They build vertical orb webs in between branches of trees or shrubs and rest at the hub. Usually, they prefer shady regions. During the current study, this species was collected from a semi-evergreen forest.

Distribution: India, Pakistan, China, Southeast Asia.

Figure 4.1.34. *Macracantha hasselti* (C. L. Koch, 1837): (A) Female subadult habitus, (B) Female subadult dorsal view, (C) Female subadult ventral view. Scales: A-B 1 mm.

Genus XVI. ***Neoscona*** **Simon, 1864**

Type species: *Neoscona arabesca* (Walckenaer, 1841)

Diagnostic features: Members of this genus are the most common araneids in India. They can be spotted in all the ecosystems, from waterlogged swamps to arid deserts. Usually, the members are brown in colour with characteristic abdominal patterns. Some of the species exhibit abdominal colour polymorphism. Most of the members are nocturnally active, and during the day, they hide in the shade of leaves or retreats associated with the orb webs. These medium to large spiders share similarities with the species belonging to *Araneus* regarding their behavioural patterns. Females have a longitudinal thoracic groove that distinguishes them from *Araneus*. Males have two patellar macrosetae on the pedipalp and have a broad cymbium. Epigynum is sclerotised and has a tongue-like, prominent, smooth scape basally fused, with one or two pairs of lateral lobes. Members of this genus are distributed globally, with 124 recognised species.

This common group has been spotted in all the ecosystems under study and has been identified with eleven species.

1. ***Neoscona bengalensis* Tikader & Bal, 1981 (Spotted orb weaver)** (Figure 4.1.35 A-E)

   Neoscona bengalensis Tikader & Bal, 1981: 15, Fig. 22-25 (♀); Mukhtar, 2012: 1714 (♂). (Complete list of references in WSC, 2025)

   Habitat and natural history: Only females of this medium to large-sized spider have been reported. They have a brown coloured, sub-triangular abdomen with numerous hairs. Epigynum is partially sclerotised with a long, tongue-like scape that bends downwards at the base. They use curled leaves as retreats for hiding during the daytime and return to the web at dawn. They have been spotted in the evergreen and semi-evergreen forests during the study.

   Distribution: India, Pakistan, and Bangladesh.

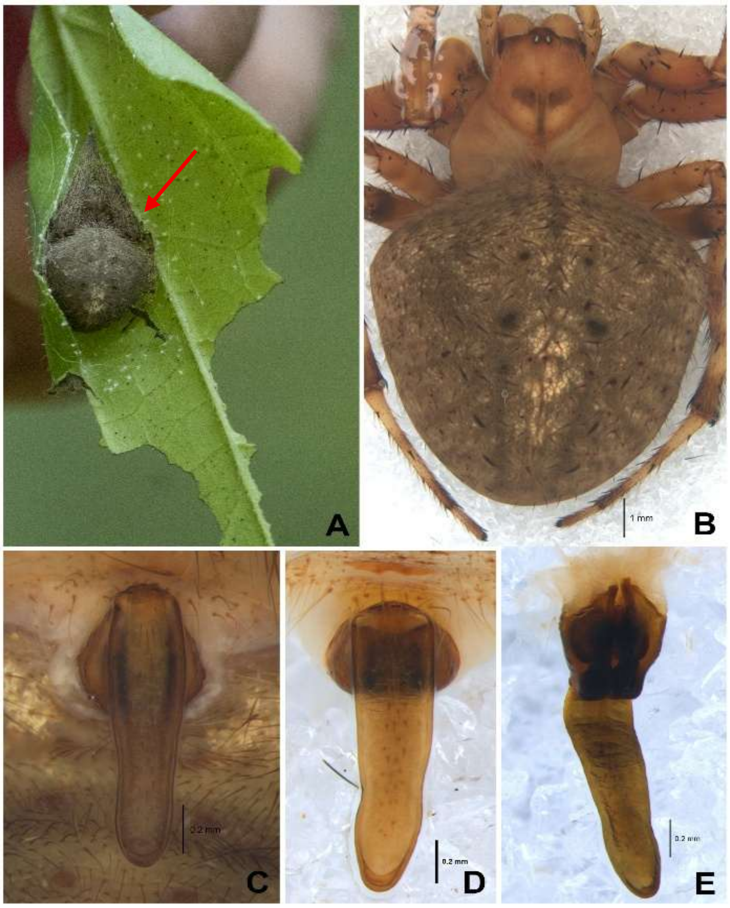


Figure 4.1.35. *Neoscona bengalensis* Tikader & Bal, 1981: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: A 1 mm; C-E 0.1 mm.

2. ***Neoscona elliptica* Tikader & Bal, 1981**(Figure 4.1.36 A-G)

Neoscona elliptica Tikader & Bal, 1981: 24, Fig. 45-49 (♀♂); Yin & Wang, 1982: 260, Fig. A-L (♀♂); Grasshoff, 1986: 118.

(Complete list of references in WSC, 2025)

Habitat and natural history: Both males and females of this species have a similar appearance, however, males are smaller than females. They have a pale brown to yellowish elliptical abdomen on which a characteristic trident-like, dark brown or black coloured anterior marking can be observed. They are abundantly found in lowland grasslands. Like others, they build their webs as dawn starts, and for the whole daytime they will be hidden inside the curled grass leaf blades, closed with the silk. During the study, they have been spotted in paddy fields, Kole wetlands and grasslands.

Distribution: India, Bangladesh, China, Thailand, Laos.

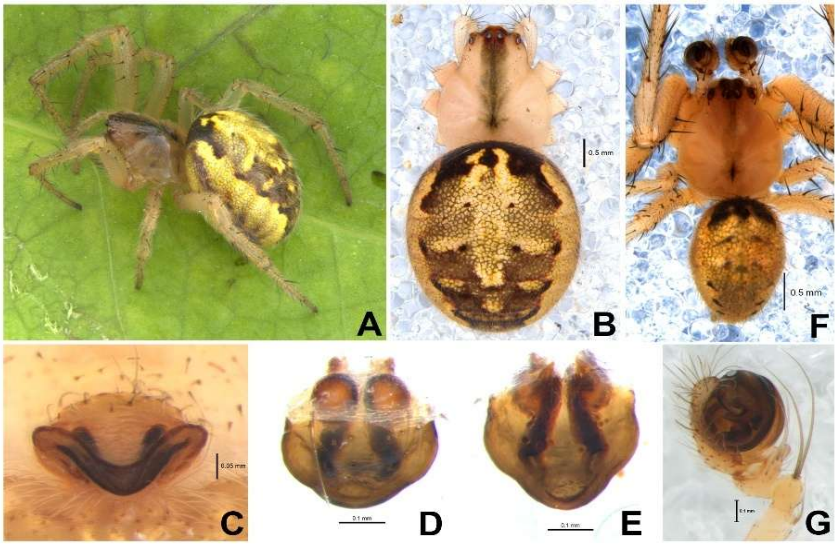


Figure 4.1.36. *Neoscona elliptica* Tikader & Bal, 1981: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: A, F 0.5 mm; C 0.05 mm; D-E, G 0.1 mm.

3. ***Neoscona nautica* (L. Koch, 1875) (Grey sphere spider)** (Figure 4.1.37 A-E)

Neoscona nautica L. Koch, 1875: 17, pl. 2, Fig. 2 (♀♂); Petrunkevitch, 1930: 320, Fig. 197-199 (♀♂); Yaginuma, 1955: 17, pl. 1, Figs. 5, 15 (♀♂); Berman & Levi, 1971: 498, Figs. 13, 111-120, 132 (♀♂); Tikader & Bal, 1981: 12, Fig. 14-17 (♀); Mi et al., 2024b: 306 (♀).

(Complete list of references in WSC, 2025)

Habitat and natural history: This is another species of *Neoscona* that can be commonly found in almost all habitats. These dark brown coloured spiders have a rounded, sub-triangular abdomen with characteristic black folium-like markings. Like others, they will be in their webs only during the nighttime. During the study, they have been spotted in all habitats except grasslands, paddy fields, and Kole wetlands.

Distribution: Asia, America (introduced), and Africa (introduced).

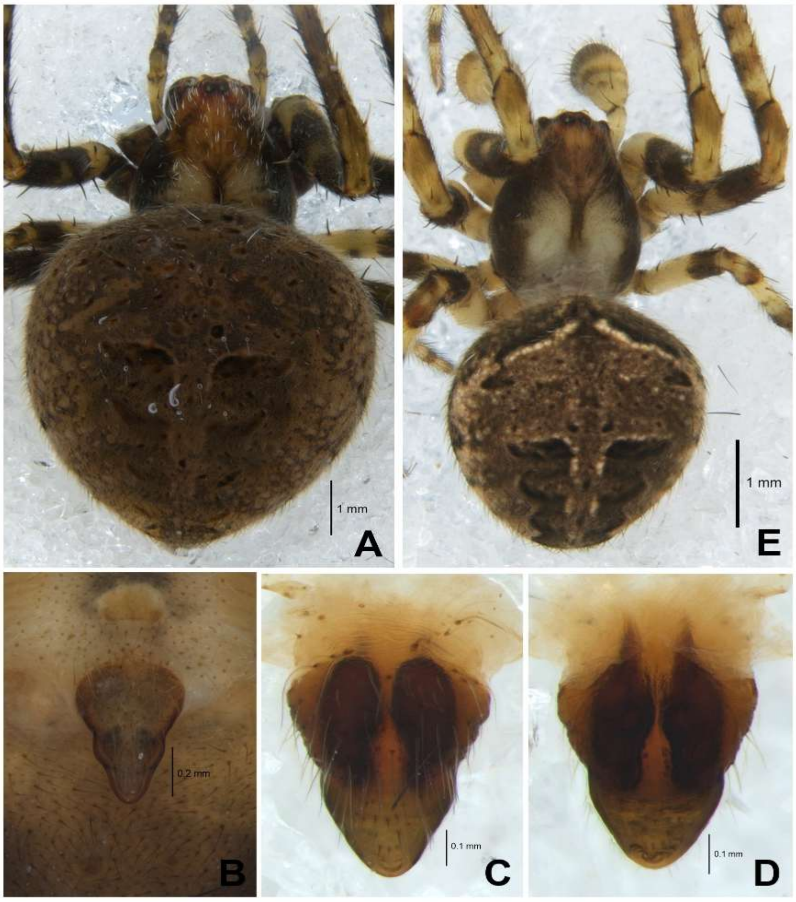


Figure 4.1.37. *Neoscona nautica* (L. Koch, 1875): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male subadult dorsal view. Scales: A, E 1 mm; B 0.2 mm; C-D 0.1 mm.

4. ***Neoscona panchganiensis* Tikader & Bal, 1981** (Figure 4.1.38 A-D)

Neoscona panchganiensis Tikader & Bal, 1981: 48, Fig. 103-106 (♀); Tikader, 1982: 227, Fig. 440-443 (♀).

Habitat and natural history: This species has only been reported from India. Details regarding the natural history and web-building behaviour of this species are not detailed. In the present study, only one specimen was spotted in a evergreen forest, which was hiding inside a curled dried leaf.

Remarks: This species was originally placed under the genus *Araneus*, which was recommended for a genus transfer to *Neoscona* in this study.

Distribution: India.

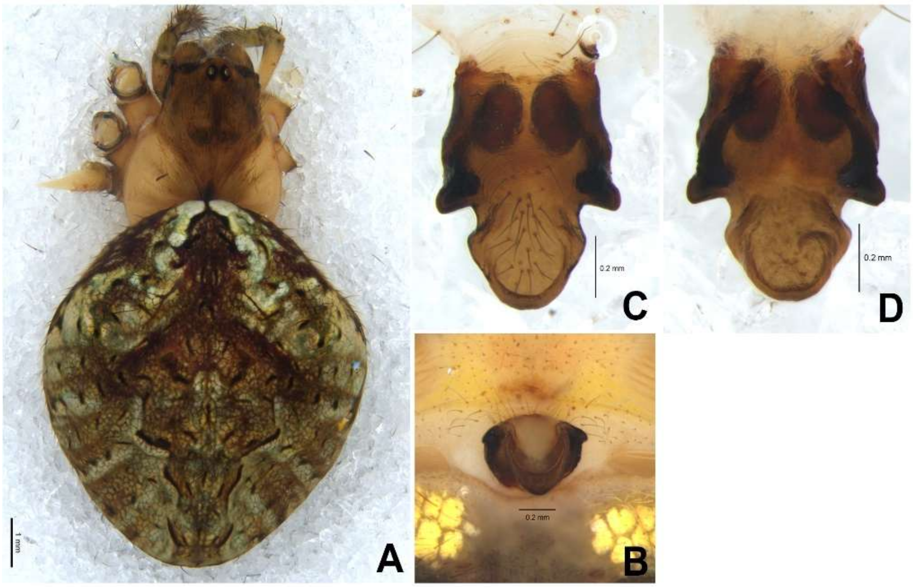


Figure 4.1.38. *Neoscona panchganiensis* Tikader & Bal, 1981: (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: A 1 mm; B-D 0.2 mm.

5. ***Neoscona punctigera* (Doleschall, 1857)** (Figure 4.1.39 A-G)

Neoscona punctigera Doleschall, 1857: 420 (♀); Simon, 1895: 828 (♀); Bösenberg & Strand, 1906: 230, pl. 11, Fig. 206 (♀♂); Tikader & Bal, 1981: 20, Fig. 36-39 (♀); Yin et al., 1997: 360, Fig. 256a-h; Saaristo, 2010: 41, Fig. 4.47-50 (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Males are smaller than the brown-coloured females. Females have a subtriangular abdomen and usually hide under the leaf shades during the day. They construct perfect large orb webs and sit at the hub during nighttime. Even though they suit all habitats, they are not as common as *Neoscona* nautica. During the current study, they have been collected from the evergreen forests, mangroves and grassland.

Distribution: Asia and Australia.

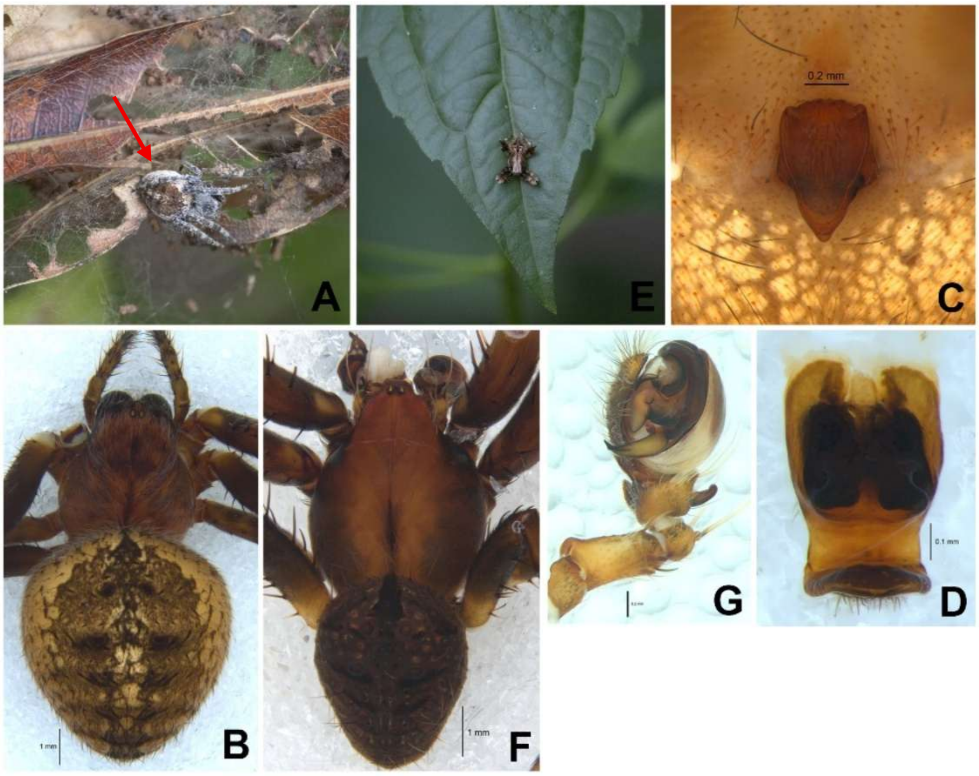


Figure 4.1.39. *Neoscona punctigera* (Doleschall, 1857): (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne dorsal view, (E) Male habitus, (F) Male dorsal view, (G) Palp retrolateral view. Scales: B, F 1 mm; C, G 0.2 mm; D 0.1 mm.

6. ***Neoscona theisi* (Walckenaer, 1841) (Common orb spider)** (Figure 4.1.40 A-C)

Neoscona theisi Walckenaer, 1841: 53, pl. 18, Fig. 4 (♀); F. O. Pickard-Cambridge, 1904: 470, pl. 44. Fig. 9-10 (♀♂); Yaginuma, 1955: 17, pl. I, Fig. 3 (♀♂); Patel, 1975: 158, Fig. 1a-g (♀♂); Grasshoff, 1986: 69, Figs. 90-100 (♀♂); Zamani et al., 2020: 36, Figs. 11A-B, 14A, D, 17A-C (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Like others this widely distributed species build their web during the night time and hide inside a curled leaf during the daylight. Females have an oval to subtriangular abdomen with a characteristic white cross shaped patch on the dark brown dorsum. In the present study they have been only spotted once from the grassland ecosystem.

Distribution: Asia, Australia, Pacific islands (introduced).

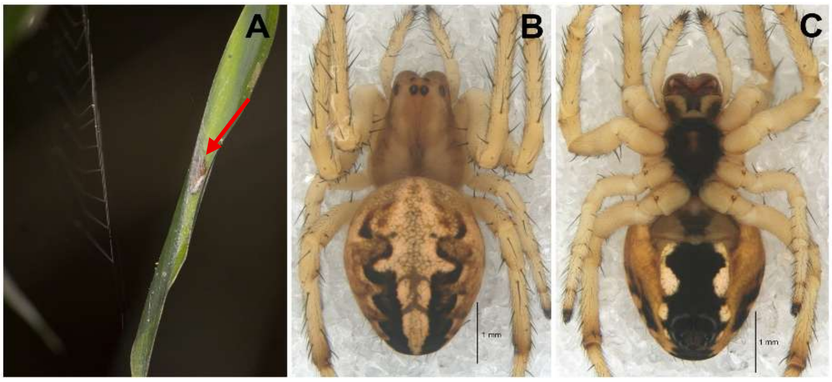


Figure 4.1.40. *Neoscona theisi* (Walckenaer, 1841): (A) Female subadult habitus, (B) Female subadult dorsal view, (C) Female subadult ventral side. Scales: B, F 1 mm; C, G 0.2 mm; D 0.1 mm.

7. ***Neoscona usbonga* Barrion & Litsinger, 1995** (Figure 4.1.41 A-D)

Nesoscona usbonga Barrion & Litsinger, 1995: 620, Figs. 391a-j, 392a-g (♀♂); Asalatha & Prasadan, 2020: 98, Fig. I1-e (♂).

Habitat and natural history: Epigynum of the female have similarities with the *Neoscona puntigera*, but can be distinguished based on having a prominent lateral tubercle at the base of the scape and a U-shaped, round-ended scape. During the study, a female specimen of this species was identified from the garden, which was captured from the retreat made by a curled leaf.

Distribution: India, Philippines.

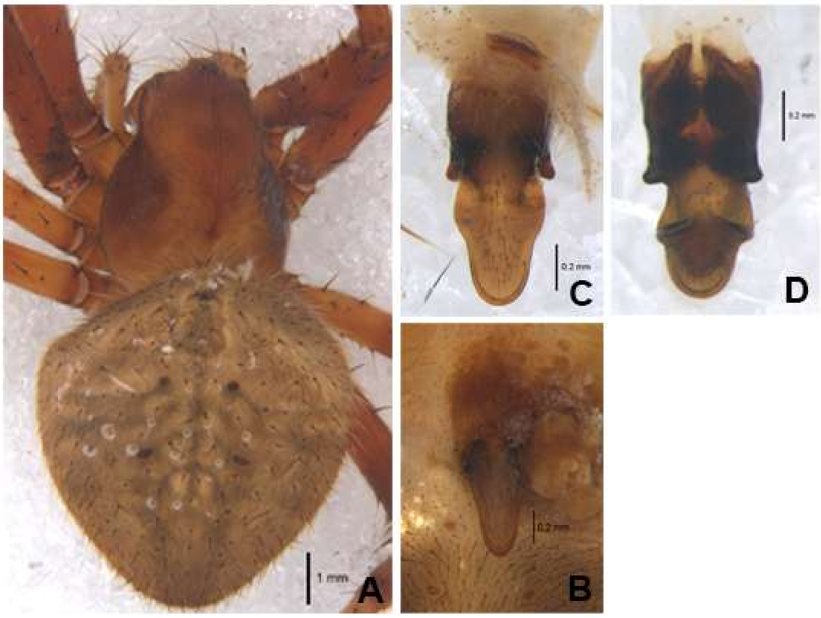


Figure 4.1.41. *Neoscona usbonga* Barrion & Litsinger, 1995: (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral, (D) Epigyne dorsal. Scales: A 1 mm; B-D 0.2 mm.

8. ***Neoscona vigilans* (Blackwall, 1865) (Brown legged spider)** (Figure 4.1.42 A-G)

Neoscona vigilans Blackwall, 1865: 342 (♀); Yaginuma, 1955: 19, pl. I, Fig. 7 (♀); Tikader & Bal, 1981: 18, Figs. 31-35 (♀♂); Grasshoff, 1986: 95, Figs. 141-145 (♀♂); Zamani et al., 2025: 3, Figs. 3A-E, 4A-B (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: These spiders can be seen commonly in gardens and other ecosystems having less complex vegetation. Both males and females have a reddish-brown abdomen with some having dark brown folium on the dorsum. Females can be distinguished by having a characteristic epigynal scape, having a median constriction and a pair of prominent median lateral lobes. Even though they are nocturnally active, they have been spotted in their webs during the daytime in the present study.

Distribution: Africa, Asia, New Guinea.

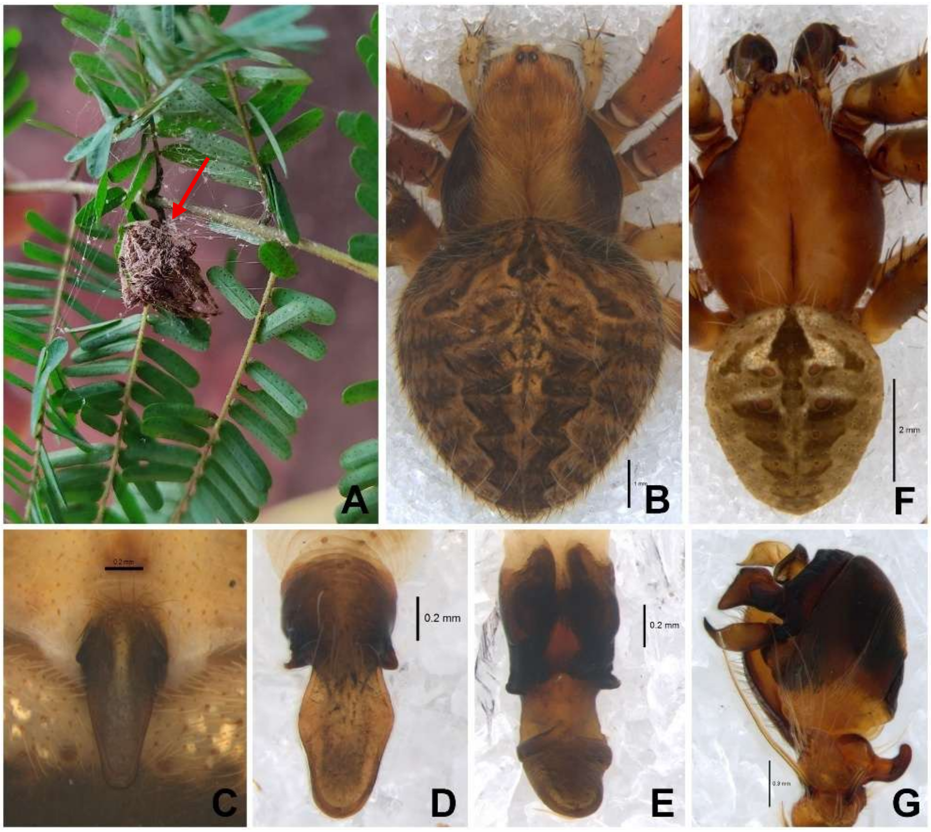


Figure 4.1.42. *Neoscona vigilans* (Blackwall, 1865): (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral, (E) Epigyne dorsal, (F) Male dorsal, (G) Palp ventral view. Scales: B 1 mm; C-E, G 0.2 mm; F 2mm.

9. *Neoscona yptinika* Barrion & Litsinger, 1995 (Figure 4.1.43 A-B)

Neoscona yptinika Barrion & Litsinger, 1995: 620, Fig. 390a-l (♂); Sen et al., 2011: 370, Figs. 8-14, 16 (♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Only males of this species have been reported yet and no enough literatures available stating their web building behaviour and natural habitats. During the present study a single specimen of this species was spotted in semi-evergreen forest. The spider was hiding under the leaf of a Jack in the bush plant.

Distribution: India, Philippines.

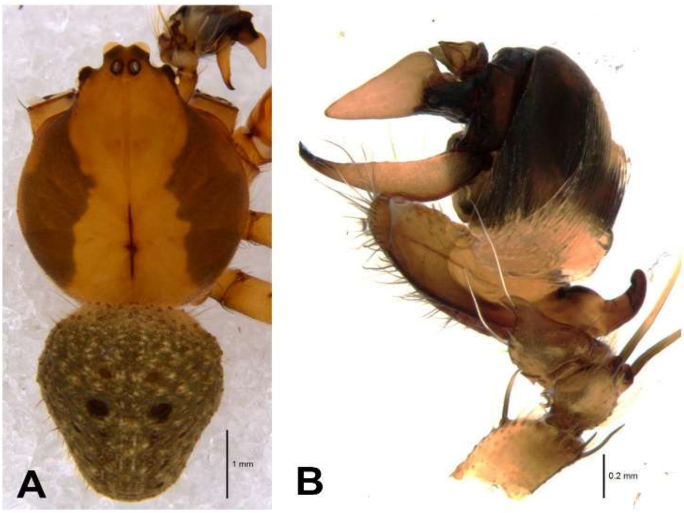


Figure 4.1.43. *Neoscona yptinika* Barrion & Litsinger, 1995: (A) Male dorsal view, (B) Palp retrolateral view. Scales: A 1 mm; B 0.2 mm.

Genus XVII. ***Nephila*** **Leach, 1815**

Type species: *Nephila pilipes* (Fabricius, 1793)

Diagnostic features: This interesting group of spiders are one of the largest spider groups in the world. The females of these spiders can grow up to several millimetres and construct very large orb webs in woody trees, in which even a small bird can be trapped. Species belonging to this genus show extreme sexual dimorphism. Males can be spotted in the web of adult females. Female members of this species can be distinguished by their characteristic abdomen, which is elongated. The legs of the females are very long which measuring a palm size when it is fully extended. Epigynum of females, which is less complex than other araneids with a spherical spermatheca and short copulatory duct, are partially sclerotised and devoid of a prominent scape. Males also have an elongated abdomen but are significantly smaller than females, with a long tapering embolus and devoid of additional palpal sclerites.

This widely distributed genus has eight identified species (WSC, 2025). Only one species of this genus has been reported from India which was also spotted in the present study.

1. ***Nephila pilipes* (Fabricius, 1793) (Giant wood spider)** (Figure 4.1.44 A-G)

   Nephila pilipes Fabricius, 1793: 424; Leach, 1815: 134, Fig. 110 (♀); Tikader, 1962: 566 (♀); Barrion et al., 1988: 248, Figs. 1, 2a-e (♀); Harvey et al., 2007: 422, Figs. 1-2, 4-5, 19-31 (♀♂); Sankaran et al., 2020: 592, Figs. 1A-D, 2A-C, E-F, 3A-H (♀♂).

   (Complete list of references in WSC, 2025)

   Habitat and natural history: This is the most widely distributed species in the genus. Females are large with an elongated abdomen and construct large vertical orb webs mostly in woody trees. They will rest at the hub throughout the day. They also show different colour morphs, such as the one with two longitudinal bright yellow stripes and yellow spots in black coloured abdomen with black legs and a completely black-coloured abdomen with red-coloured legs. Males are extremely small than females and have a bright red colour. They can be spotted in the web of the females along with some other kleptoparasites. Sometimes, food caches can also be seen in the web of females. Usually, females come to the forest floor to lay eggs in the egg sacs constructed in the shallow depressions of the ground. This species was spotted in all the evergreen and semi-evergreen forests during the present study.

   Distribution: South and Southeast Asia, China, Australia.

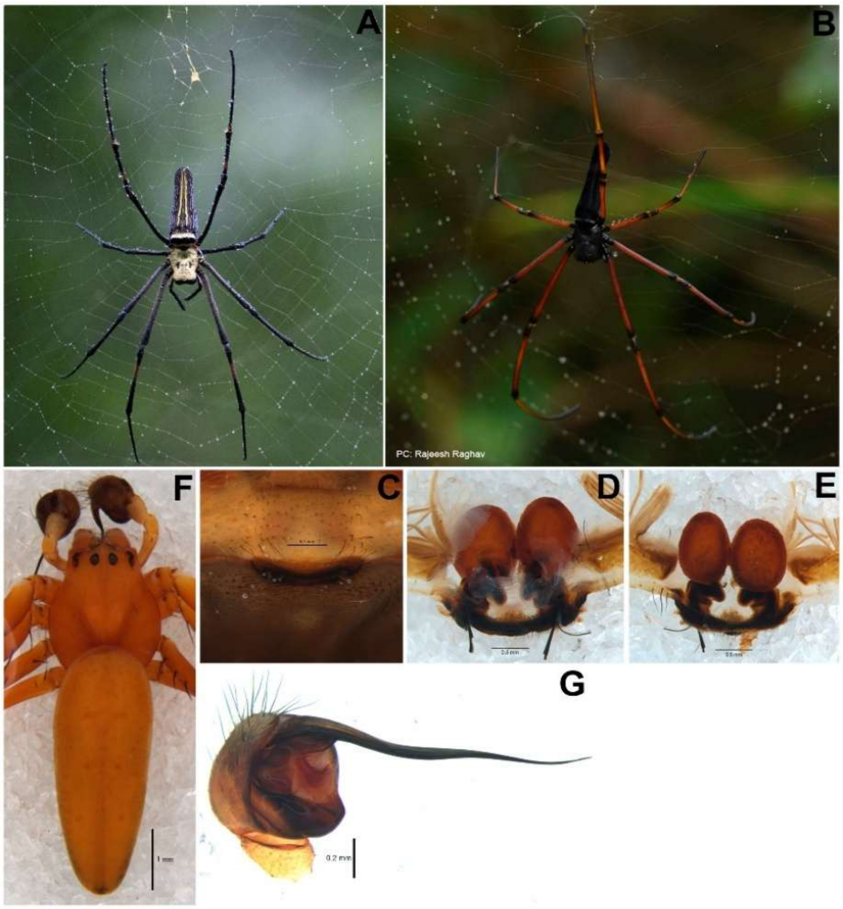


Figure 4.1.44. *Nephila pilipes* (Fabricius, 1793): (A) Female habitus, (B) Female black morph habitus, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (B) Palp retrolateral view. Scales: C-E 0.5 mm; F 1 mm; G 0.2 mm.

Genus XVIII. ***Nephilengys* L. Koch, 1872**

Type species: *Nephilengys malabarensis* (Walckenaer, 1841)

Diagnostic features: This is another genus that shows extreme sexual dimorphism. Females of this group are larger with a brown abdominal dorsum, while the ventrum has bright yellow or orange characteristic markings. They also possess strong spines on the carapace near the eye region. Epigynum is heavily sclerotised and lacks a scape. Males are much smaller than females in bright red colour. They have a long, smooth embolus with distal enlargement and a conductor that is extremely sigmoid and rigid. Both males and females have a striated boss in their chelicerae. Females build large orb webs against trees, rocks or building roofs with a characteristic tubular retreat. This genus is distributed across the landmasses of Asia and Australia with two recognised species (WSC, 2025). In the present study, one species is recorded.

1. ***Nephilengys malabarensis*** **(Walckenaer, 1841) (Asias Hermit spider)** (Figure 4.1.45 A-F)

Nephilengys malabarensis Walckenaer, 1841: 103 (♀); Thorell, 1878: 123 (♂); Tikader, 1977: 181, Fig. 12A-C (♀); Kuntner, 2007: 119, Figs. 22A-C, 23A-E, 24A-B (♀♂).

(Complete list of references in WSC, 2025)

Habitat and natural history: Females of this species construct large vertical orb webs against the large tree trunks. They also built the characteristic tubular retreat made of specialised silk, from the hub to the substrate, so that they could escape to the retreat upon encountering a threat. The hub in the adult female's web will be significantly shifted towards the upper part. They spend the daytime either by occupying the hub or hiding in the retreat. Adult male spiders, which are significantly smaller, can be seen in the webs of female spiders. They were spotted in evergreen and semi-evergreen forests during the study.

Distribution: Asia.

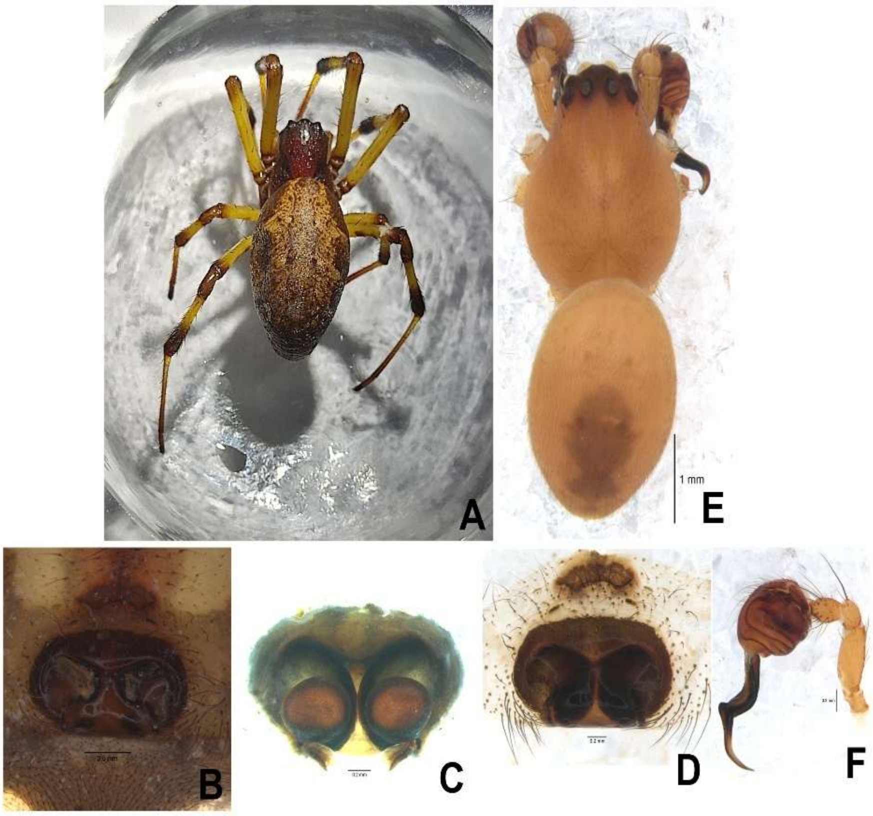


Figure 4.1.45. *Nephilengys malabarensis* (Walckenaer, 1841): (A) Female dorsal, (B) Epigyne insitu, (C) Epigyne dorsal view, (D) Epigyne ventral view, (E) Female black morph habitus, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Male dorsal view, (F) Palp ventral view. Scales: B 0.5 mm; C-D, F 0.2 mm; E 1mm.

Genus XIX. ***Paraplectana*** **Brito Capello, 1867**

Type species: *Paraplectana thorntoni* (Blackwall, 1865)

Diagnostic features: This genus can be considered a rare araneid, as most of the species have not been reported after their first discovery. Also, males of any species have not been reported except for one. All the females of this species are brightly coloured from red to pink in combination with distinct black patches, which mimic the ladybird beetles. The number of black patches is even considered as a taxonomic trait. They build webs only during the nighttime and sit on the top of the leaves during the daytime. They are usually found on low vegetation. Females possess a partially sclerotised epigynum with a prominent median septum and lack the scape.

This genus has representatives from Asia and Africa with 13 identified species (WSC, 2025). In the present study, only a single specimen of this genus was recorded but its species identity is not yet revealed.

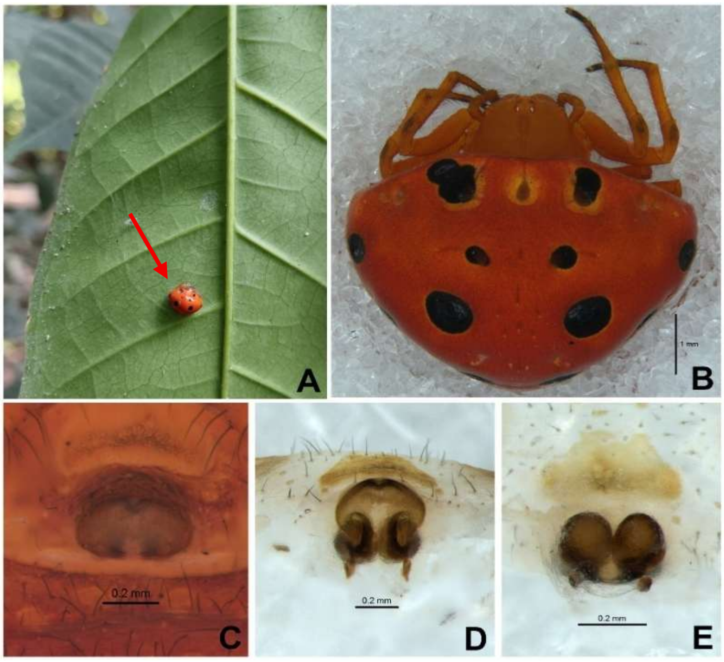


Figure 4.1.46. *Paraplectana* sp.: (A) Female habitus, (B) Female dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view. Scales: B 1 mm; C-D, F 0.2 mm; E 1mm.

Genus XX. ***Parawixia* F. O. Pickard-Cambridge, 1904**

Type species: *Parawixia destricta* (O. Pickard-Cambridge, 1889)

Diagnostic features: Females of this group are moderately large and have a thick body with a triangular abdomen with vertices. They are usually in black to dark brown colour with two characteristic tubercles on the anterior edge and on either side of the cephalic region. Females have a partially sclerotised epigynum with an oval atrium and a short, pointed, tail-like scape that is completely fused at the basal region. Males are distinguished by the shape of the paramedian apophysis, and they possess only one patellar macrosetae.

Members of this genus are distributed in all biological realms with 29 identified species (WSC, 2025). One representative of this genus was recorded in the study.

1. ***Parawixia dehaani* (Doleschall, 1859) (Abandoned orb web spider)** (Figure 4.1.47 A-D)

   Parawixia dehaani Doleschall, 1859: 33, pl. 2, Fig. 7 (♀); Pocock, 1900: 225, Fig. 72 (♀); Tikader, 1982: 212, Figs. 414-418 (♀♂); Yin et al., 1997: 133, Fig. 48a-i (♀♂); Nentwig et al., 2019: 34.

   (Complete list of references in WSC, 2025)

   Habitat and natural history: Females of this species are large and have a characteristic dark brown coloured inverted triangle marking on the abdominal dorsum. Also, they possess a pair of pointed shoulder tubercles. They build large orb webs at a height of not more than three meters and rest either at the bridge line of the web or a retreat associated with the web, rarely at the hub. Upon disturbance, they fall down and mimic as dead by pulling their legs towards their bodies and later return to the web. They have been spotted in all evergreen and semi-evergreen forests.

   Distribution: New Guinea, South and Southeast Asia.

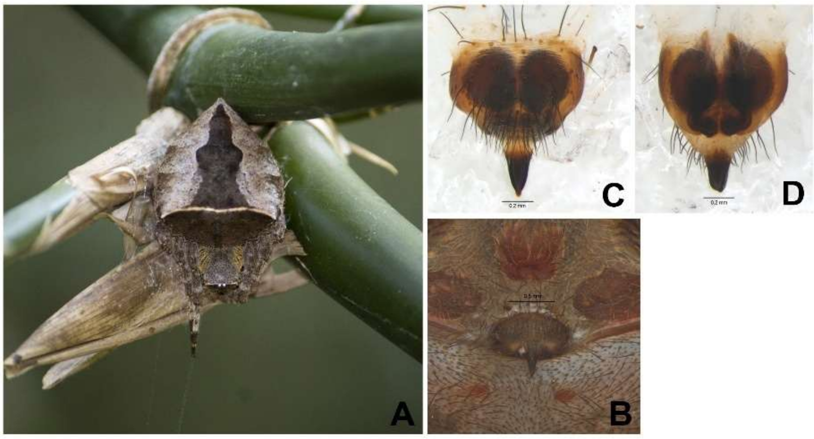


Figure 4.1.47. *Parawixia dehaani* (Doleschall, 1859): (A) Female habitus, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: B 0.5 mm; C-D 0.2 mm.

Genus XXI. ***Plebs*** **Joseph & Framenau, 2012**

Type species: ***Plebs eburnus*** (Keyserling, 1886)

Diagnostic features: Members of this genus include medium-sized, colourful spiders with a characteristic folium-like pattern on the abdominal dorsum. They have a pair of anterior tubercles on the abdomen. Males are also similar to females in appearance, but smaller than them. Females have an oblong-shaped epigynum with a long and smooth scape. Males possess a paramedian apophysis and an elongated transverse median apophysis. Both males and females have a light-coloured inverted U-shaped marking and a pair of anterior light spots on either side of the spinnerets.

Representatives of this genus are scattered across Asia and Australia, with 22 identified species (WSC, 2025). One species belonging to this genus was recorded in the study.

1. ***Plebs mitratus*** **(Simon, 1895) (Green orb weaver)** (Figure 4.1.48 A-E)

   Plebs mitratus Simon, 1895: 805 (♀); Joseph & Framenau, 2012: 333, Fig. 34A-F (♀); Paul et al., 2016: 103, Figs. 1A-B, 2A-L (♀♂).

   Habitat and natural history: Females of this species are larger than males and have a dark brown to reddish folium with pale lines and patches on the abdominal dorsum. The lateral sides of the abdomen are green in colour, with the characteristic ventral markings of the genus. They build medium orb webs with vertical stabilimentum made with specialised silk.

These diurnal spiders are found only in forests of higher altitudes. This species has been only spotted from the evergreens at higher altitudes.

Distribution: India.

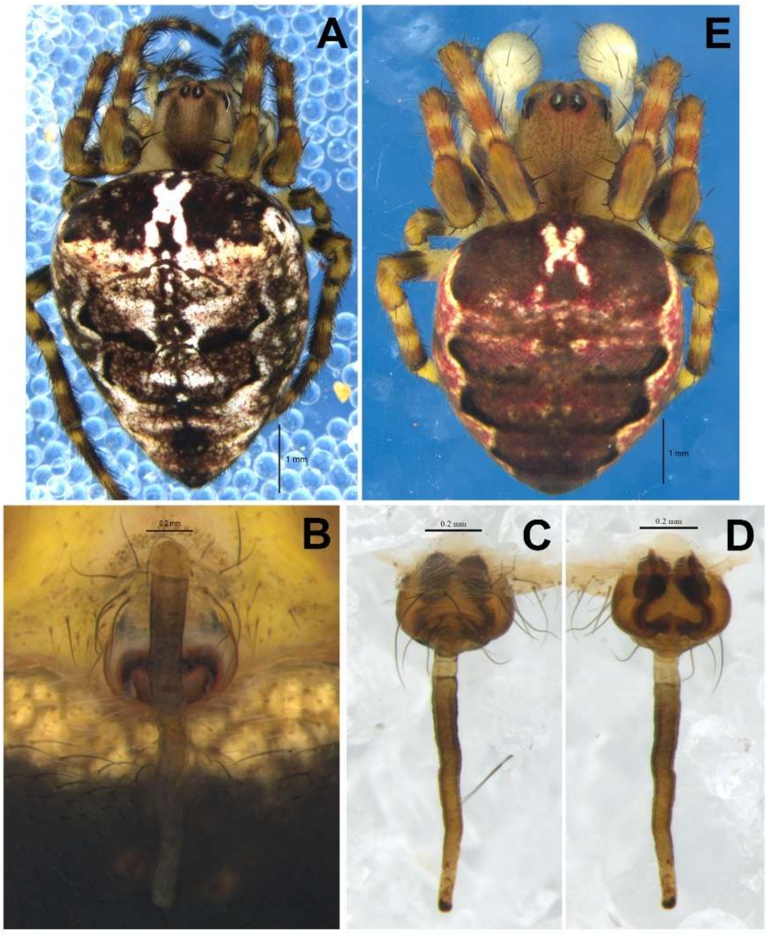


Figure 4.1.48. *Plebs mitratus* (Simon, 1895): (A) Female dorsal view, (B) Epigyne insitu, (C) Epigyne ventral view, (D) Epigyne dorsal view, (E) Male subadult dorsal view. Scales: A, E 1 mm; B-D 0.2 mm.

Genus XXII. ***Poltys*** **C. L. Koch, 1843**

Type species: *Poltys illepidus* C. L. Koch, 1843

Diagnostic features: This is an interesting araneid genus in regard to its appearance. Females are medium-sized spiders, while the males are extremely small. The members have a pear-shaped carapace and a doubly domed appearance in the lateral view. The genus can be distinguished by its characteristic eye pattern. Median eyes are situated on prominent tubercles, while the lateral eyes are pushed far from them. The epigyne is bulbous, having a thin scape. Males lack endite teeth and coxal hooks and have less complex palpal structure than their sister groups.

To date, 42 species have been recorded from this genus with a wide distribution across Africa, Asia, and Australia.

1. ***Poltys columnaris*** **Thorell, 1890 (Tree stump spider)** (Figure 4.1.49 A-D)

   Poltys columnaris Thorell, 1890: 87 (♀); Smith, 2006: 88, Figs. 223-225 (♀); Tanikawa, 2007: 91, Figs. 289-294, 754-756 (♀♂); Kulkarni & Smith, 2013: 4524, Fig. 1-3 (♀).

   Habitat and natural history: Females of this species have an interesting abdominal shape which has elevated anteriorly. They are active during nighttime by building orb webs. During nighttime, they can be spotted on the web, while in daytime, they will be somewhere in the proximity of the web. Since they have a favourable colour and shape to mimic the twigs, they will remain in the nearby branches, and it is difficult to find them. They can be found in forests and gardens. In the present study, one specimen of this species was collected from the garden during the night.

   Distribution: India, Sri Lanka, Indonesia, Japan.

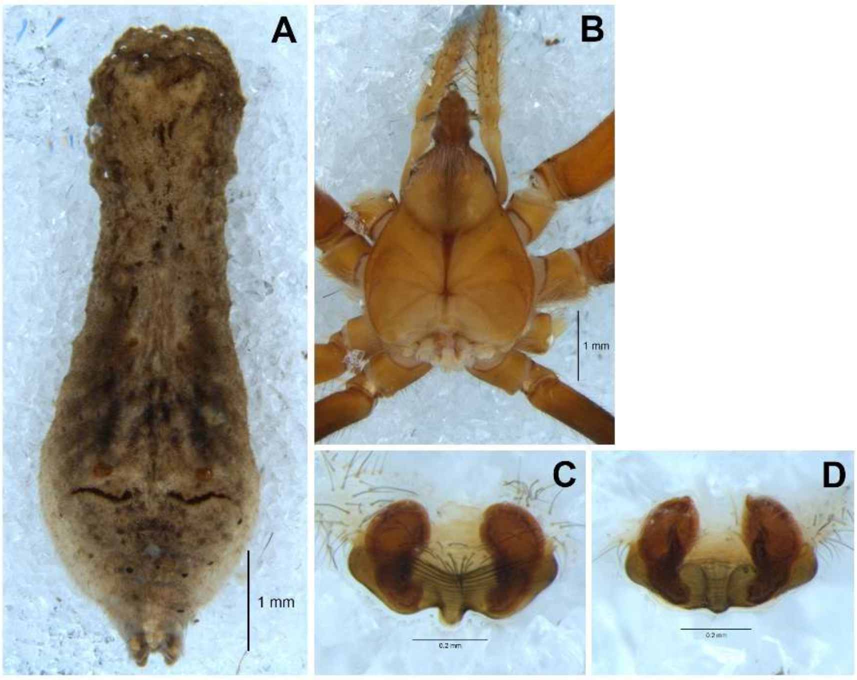


Figure 4.1.49. *Poltys columnaris* Thorell, 1890: (A) Female abdomen dorsal view, (B) Female cephalothorax dorsal view, (C) Epigyne ventral view, (D) Epigyne dorsal view. Scales: A-B 1 mm; C-D 0.2 mm.

Genus XXIII. ***Thelacantha*** **van Hasselt, 1882**

Type species: *Thelacantha brevispina* (Doleschall, 1857)

Diagnostic features: This genus was placed as a subgenus of *Gasteracantha,* and later it was elevated to a separate genus. They share similarities with the former in many aspects. Like in the former group, three pairs of abdominal spines are present, but the spines are in the form of tubercles with a slight protuberance at the tip. Females are medium-sized spiders having a heavily sclerotised oblong epigynum with a less distinct short scape. Even though males have a similar shape, they are very much smaller than females without colourful patches on the abdominal dorsum, as in females.

This genus has only two identified species with wide distribution across the continents (WSC, 2025).

1. ***Thelacantha brevispina*** **(Doleschall, 1857) (Double spotted spiny spider)** (Figure 4.1.50 A-G)

   Thelacantha brevispina Doleschall, 1857: 423 (♀); Thorell, 1878: 17, 294; Tikader, 1970: 38, Fig. 23d (♀); Emerit, 1974: 57, Figs. 17, 18A-B, 19A-C, 26A (♀♂); Tan et al., 2019a: 47, Figs. 1H, 12A-E (♀); Macharoenboon et al., 2021: 47, Figs. 10A-E, 12J-L (♀).

   Habitat and natural history: This is the only species of this genus that has a wide distribution. Despite the similarities with *Gasteracantha*, this species has a dark body with colourful patches, especially a pair of mid-dorsal white patches. However, certain colour morphs of this species have been reported. Males are smaller than females with pale to dark brown bodies. They build perfect medium to large orb webs and sit at the hub. Sometimes, males can also be seen hanging in the female's web. They can be found in all types of ecosystems. In the present study, they have been spotted in all the habitats considered except the paddy field, Kole wetlands and grasslands.

   Distribution: Africa, Asia, and Australia.

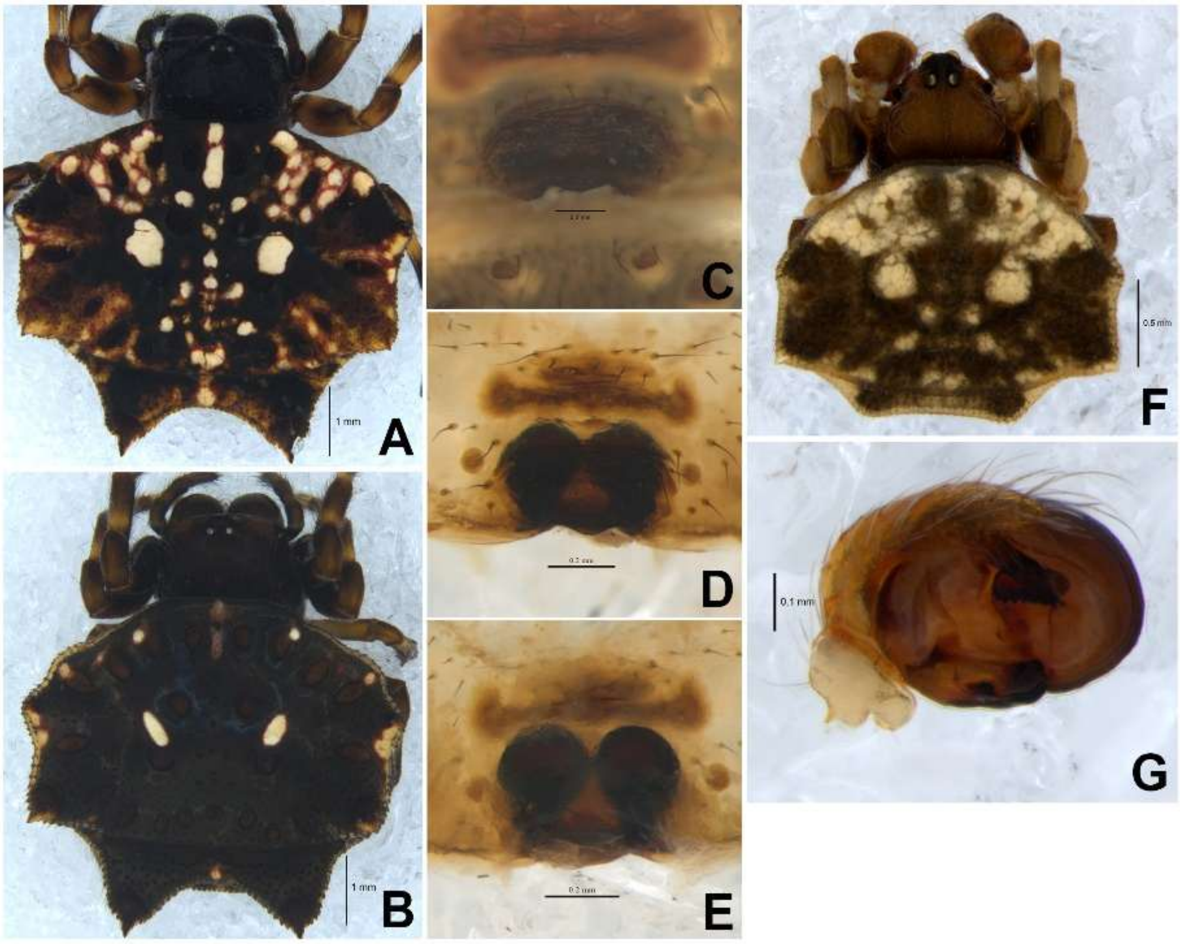


Figure 4.1.50. *Thelacantha brevispina* (Doleschall, 1857): (A) Female dorsal view, (B) Female colour morph dorsal view, (C) Epigyne insitu, (D) Epigyne ventral view, (E) Epigyne dorsal view, (F) Male dorsal view, (G) Palp retrolateral view. Scales: A-B 1 mm; C-E 0.2 mm; F 0.5 mm; G 0.1 mm.

4.2. Barcoding of araneids in Kerala

For barcoding the araneids in Kerala, 84 specimens were chosen for DNA extraction. A 100% success rate was achieved during DNA isolation, following the steps mentioned in section 3.2.1. DNA samples with an optical density (OD) ratio at 260/280 nm between 1.7 and 2.0 were selected (Table S1 & S2).

Among the 84 successful DNA samples, all of them were subjected to COI gene amplification, and 36 were subjected to H3 gene amplification using the primer pairs and thermal profiles mentioned in Tables 3.4 and 3.5, with 92.865% (78 samples) and 94.4% (34 samples) success rates, respectively. All the successful PCR amplicons produced bands when visualised after gel electrophoresis (Figure 4.2.1 & 4.2.2).

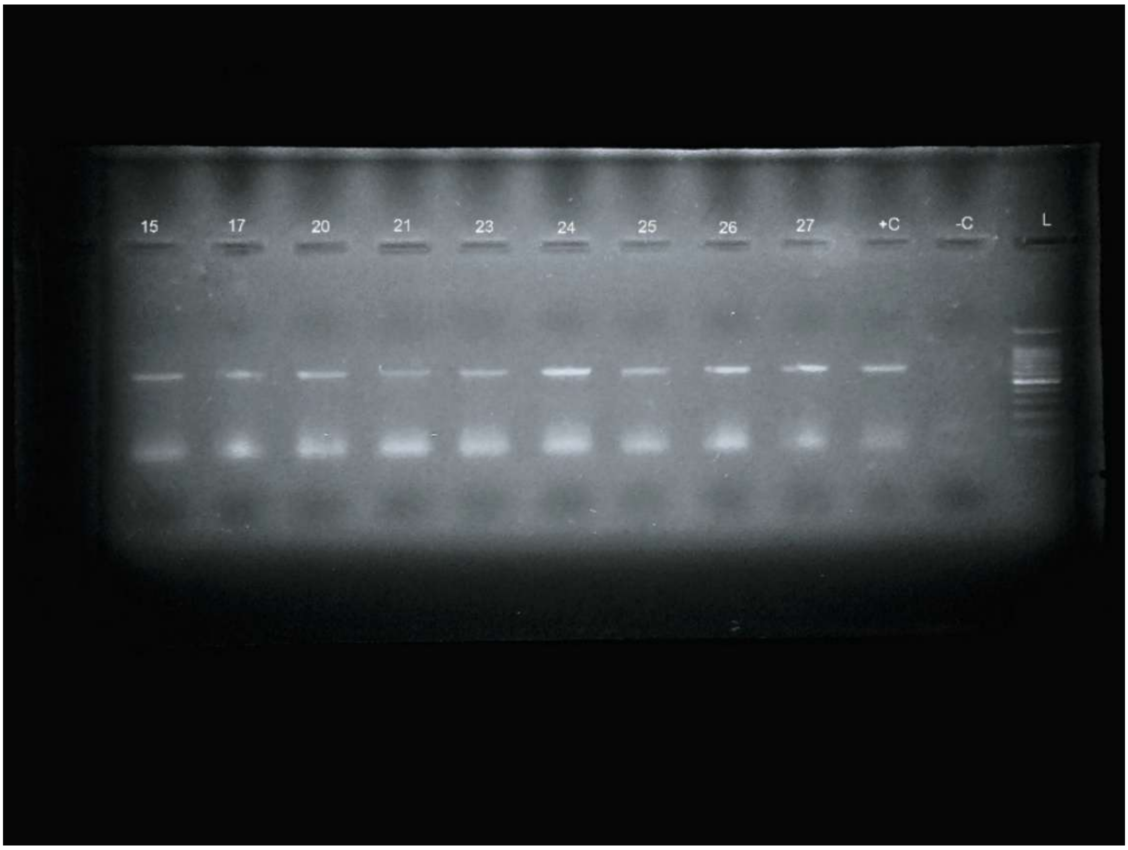


Figure 4.2.1. Gel visualised under UV transilluminator: COI amplicons of ARAMP015, ARAMP017, ARAMP020, ARAMP021, ARAMP023, ARAMP024, ARAMP025, ARAMP026, ARAMP027, (L) Ladder, (-C) Negative control, (+C) Positive control.

Figure 4.2.2. Gel visualised under UV transilluminator: H3 amplicons of ARAMP015, ARAMP016, ARAMP020, ARAMP022, ARAMP023, ARAMP024, ARAMP025, ARAMP026, ARAMP027, ARAMP028, ARAMP032, ARAMP034, ARAMP043, (L) Ladder, (-C) Negative control, (+C) Positive control.

Partial sequences of COI and H3 were successfully sequenced, except for one for each gene. Thus, a total of 110 sequences were developed in the present study. All the developed sequences were checked for contamination and stop codons, resulting in sequence lengths ranging from 422 to 578 bp, with an average of 558 bp for the COI gene and 254 to 312 bp, with an average of 297 bp for the H3 gene. All the COI and H3 sequences obtained in this study were subjected to a BLAST search in the NCBI nucleotide database to validate species-level identifications. The results of the BLAST search are summarised in Table S1 and S2.

The sequences were submitted to GenBank, and accession numbers were obtained for 109 records (Table S1 & S2). A total of 53 araneid species were successfully barcoded using the COI gene and 33 species using the H3 gene. Of the 109 barcodes submitted to GenBank, 28 COI barcodes and 10 H3 barcodes represent the first entries in the database, thereby contributing to the global reference library of the barcodes.

4.2.1. Genetic distance of *Arachnura melanura* specimens

Original alignment = 558 positions

Gblocks alignment = 526 positions (94%)

Pairwise genetic distance analysis revealed that the genetic distance between the voucher samples is zero (Table 4.2.3), which shows that all three voucher samples belong to the same species. The genetic distance between the voucher samples and the *Arachnura melanura* reference sequence is zero. Hence, it can be concluded that these three specimens belong to the species *Arachnura melanura*.

Table 4.2.1. Genetic distance inferred from COI sequences of *Arachnura melanura*. *Sequences developed for the present study

	1	2	3	4	5	6	7	8
A. feredayi (MT724704.1)								
A. higginsi (MK420059.1)	0.0828							
A. melanura (ARAMP029) *	0.1870	0.1749						
A. melanura (ARAMP031) *	0.1870	0.1749	0.0000					
A. melanura (ARAMP032) *	0.1870	0.1749	0.0000	0.0000				
A. melanura (KJ957945.1)	0.1870	0.1749	0.0000	0.0000	0.0000			
A. logio (KJ957944.1)	0.1974	0.1826	0.1610	0.1610	0.1610	0.1610		
A. scorpionoides (KJ957946.1)	0.1872	0.1845	0.1150	0.1150	0.1150	0.1150	0.1518	

4.2.2. Genetic distance of *Cyclosa bifida* specimens

Original alignment = 545 positions

Gblocks alignment = 540 positions (99%)

The analysis shows that the genetic distance between the three voucher specimens is less than 3%, indicating they are conspecific. Also, the average genetic distance between the voucher specimens and the reference *Cyclosa bifida* sequences is 0.006. Hence, it can be concluded that the voucher specimens, ARAMP041, ARAMP042, and ARAMP043, belong to the species *Cyclosa bifida*.

Table 4.2.2. Genetic distance inferred from COI sequences of *Cyclosa bifida*. *Sequences developed for the present study

	1	2	3	4	5	6	7	8
C. bifida (ARAMP042) *								
C. bifida (ARAMP043) *	0.0039							
C. bifida (ARAMP041) *	0.0020	0.0056						
C. bifida (MK392662.1)	0.0079	0.0112	0.0056					
C. bifida (PQ478048.1)	0.0059	0.0093	0.0037	0.0019				
C. bifida (MK392663.1)	0.0059	0.0093	0.0037	0.0056	0.0037			
C. spirifera (MK392677.1)	0.1287	0.1343	0.1275	0.1298	0.1276	0.1231		
C. mulmeinensis (PQ558947.1)	0.1475	0.1547	0.1474	0.1545	0.1522	0.1474	0.1597	

4.2.3. Genetic distance of *Eriovixia poonaensis* specimens

Original alignment = 483 positions

Gblocks alignment = 476 positions (98%)

Genetic distance analysis of ARAMP065 and ARAMP067 shows that their distance is less than 3%, which indicates the chance of conspecificity. The average genetic distance of the specimens with *Eriovixia poonaensis* sequences is less than 3%, while it is 5.5% with the *Eriovixia laglaizei* sequence, which indicates that the specimens belong to *Eriovixia poonaensis*.

Table 4.2.3. Genetic distance inferred from COI sequences of *Eriovixia poonaensis*. *Sequences developed for the present study

	1	2	3	4	5	6	7
E. poonaensis (KT383762.1)							
E. poonaensis (KT383689.1)	0.0021						
E. poonaensis (ARAMP065)*	0.0228	0.0257					
E. poonaensis (ARAMP067)*	0.0042	0.0063	0.0286				
E. poonaensis (MK392775.1)	0.0460	0.0437	0.0619	0.0506			
E. laglaizei (MK420106.1)	0.0437	0.0414	0.0619	0.0483	0.0021		
E. poonaensis (MK392776.1)	0.0437	0.0414	0.0619	0.0483	0.0063	0.0042	

4.2.4. Genetic distance of *Neoscona punctigera* specimens

Original alignment = 545 positions

Gblocks alignment = 545 positions (100%)

ARAMP087 and ARAMP088 show a genetic distance of 0.0037, concluding that they are conspecific. The average genetic distance between the voucher specimens and the *Neoscona punctigera* sequences is 3%, which can be considered the specimens as *Neoscona punctigera*.

Table 4.2.4. Genetic distance inferred from COI sequences of *Neoscona punctigera*. *Sequences developed for the present study

	1	2	3	4
N. punctigera (ARAMP087)*				
N. punctigera (ARAMP088)*	0.0037			
N. punctigera (MF467584.1)	0.0074	0.0111		
N. punctigera (JN817151.1)	0.0577	0.0617	0.0597	

4.2.5. Genetic distance of genus *Neoscona bengalensis* specimens

Original alignment = 560 positions

Gblocks alignment = 542 positions (96%)

The genetic distance analysis to reveal the identity of the *Neoscona* voucher specimens resulted in the conspecificity of ARAMP084 and ARAMP085 with a genetic distance of 0.2%. ARAMP083 showed an average distance of 0.003 from the former specimens, indicating conspecificity. These three voucher specimens have a genetic distance of >3% with *Neoscona vigilans* and *Neoscona nautica* reference sequences. This indicates that the voucher specimens neither belong to *Neoscona vigilans* nor *Neoscona nautica*.

Table 4.2.5. Genetic distance inferred from COI sequences of *Neoscona bengalensis*. *Sequences developed for the present study

	1	2	3	4	5
N. bengalensis (ARAMP084)*					
N. bengalensis (ARAMP085)*	0.0020				
N. bengalensis (ARAMP083)*	0.0040	0.0018			
N. vigilans (MK154773.1)	0.0892	0.0923	0.0923		
N. nautica (OQ520327.1)	0.0984	0.0944	0.0923	0.1006	

4.2.6. Genetic distance of *Gea* specimens

Original alignment = 491 positions

Gblocks alignment = 428 positions (87%)

Voucher specimens, ARAMP075 and ARAMP076, show a genetic distance of 0.0047, indicating their conspecificity. At the same time, the average genetic distance between the voucher specimens and the *Gea spinipes* sequences is 0.043, which clearly says that the voucher specimens do not belong to the species *Gea spinipes*.

Table 4.2.6. Genetic distance inferred from COI sequences of the genus *Gea*. *Sequences developed for the present study

	1	2	3	4
G. spinipes (KJ957965.1)				
G. spinipes (OQ871572.1)	0.0609			
Gea sp. (ARAMP075)*	0.0642	0.0226		
Gea sp. (ARAMP076)*	0.0613	0.0253	0.0047	

4.2.7. Genetic distance of *Gasteracantha dalyi* specimens

Original alignment = 557 positions

Gblocks alignment = 557 positions (100%)

Genetic distance analysis of *Gasteracantha* voucher specimens, ARAMP070 and ARAMP073, revealed a 0.19% genetic difference between the specimens. This shows that both are conspecific. An average distance of 4.3% with homologous sequences indicates that the specimens are not congeneric.

Table 4.2.7. Genetic distance inferred from COI sequences of *Gasteracantha dalyi*. *Sequences developed for the present study

	1	2	3	4
G. dalyi (ARAMP070)*				
G. dalyi (ARAMP073)*	0.0019			
M. hasselti (MT584903.1)	0.0447	0.0412		
M. hasselti (MK392767.1)	0.0466	0.0433	0.0091	

4.2.8. Genetic distance of *Neoscona vigilans* specimens

Original alignment = 536 positions

Gblocks alignment = 536 positions (100%)

The genetic distance between the specimens ARAMP092 and ARAMP082 is zero, thus, both belong to the same species. The average genetic distance between the voucher specimens and *Neoscona vigilans* sequences is 0.003, indicating that the specimens belong to the species *Neoscona vigilans*.

Table 4.2.8. Genetic distance inferred from COI sequences of *Neoscona vigilans*. *Sequences developed for the present study

	1	2	3	4	5
N. vigilans (ARAMP092)*					
N. vigilans (MK154395.1)	0.0000				
N. vigilans (ARAMP082)*	0.0000	0.0000			
N. vigilans (MK559319.1)	0.0037	0.0037	0.0021		
N. vigilans (MK154431.1)	0.0075	0.0075	0.0063	0.0037	

4.2.9. Genetic distance of genus *Thelacantha brevispina* specimens

Original alignment = 551 positions

Gblocks alignment = 551 positions (100%)

ARAMP020 and ARAMP074 can be concluded as conspecific according to the results of genetic distance analysis. They are genetically distant by only 0.2%. The average distance between the voucher specimens and *Thelacantha brevispina* sequences is 0.002, indicating that the voucher specimens belong to the genus *Thelacantha brevispina*.

Table 4.2.9. Genetic distance inferred from COI sequences of *Thelacantha brevispina*. *Sequences developed for the present study

	1	2	3	4
T. brevispina (ARAMP020)*				
T. brevispina (MT584920.1)	0.0000			
T. brevispina (MT584921.1)	0.0019	0.0018		
T. brevispina (ARAMP074)*	0.0019	0.0037	0.0055	

4.2.10. Genetic distance of *Nephila pilipes* specimens

Original alignment = 569 positions

Gblocks alignment = 569 positions (100%)

The voucher specimens of the genus *Nephila*, ARAMP077 and ARAMP017, are genetically distant by 1.7%. It can be considered conspecific as it is less than the threshold value. The average distance between the voucher specimens and the *Nephila pilipes* sequences is 0.008, indicating that the specimens belong to the species *Nephila pilipes*.

Table 4.2.10. Genetic distance inferred from COI sequences of *Nephila pilipes*. *Sequences developed for the present study

	1	2	3	4	5	6	7
N. pilipes (ARAMP077)*							
N. pilipes (DQ779231.1)	0.0054						
N. pilipes (DQ779253.1)	0.0073	0.0018					
N. maculata (AY052591.1)	0.0165	0.0107	0.0089				
N. pilipes (DQ779242.1)	0.0184	0.0125	0.0107	0.0018			
N. pilipes (ARAMP017)*	0.0171	0.0110	0.0092	0.0000	0.0018		
N. kuhlii (MN341002.1)	0.0184	0.0125	0.0107	0.0018	0.0035	0.0018	

4.2.11. Genetic distance of *Argiope pulchella* and *Argiope versicolor* specimens

Original alignment = 551 positions

Gblocks alignment = 533 positions (96%)

The analysis revealed that ARAMP034 has 0.38% and 0.57% genetic difference with ARAMP035 and ARAMP038, and ARAMP035 shows only 0.19% difference with ARAMP038. The difference between ARAMP036 and ARAMP037 is 0.95%. Whereas the ARAMP036 has 2.89%, 3.29%, 3.49% difference with ARAMP034, ARAMP035, and ARAMP038, respectively, and ARAMP037 has a distance of 2.69%, 3.09%, and 3.29% with ARAMP034, ARAMP035, ARAMP038. This shows that the former three belong to the same species, and the latter two are conspecific. Also, the average genetic distance between the two groups is 0.031. Hence, it can be concluded that ARAMP034, ARAMP035, and ARAMP038 are genetically different from ARAMP036 and ARAMP037, suggesting two different species. The average genetic distance between the former group with the reference *Argiope pulchella* sequences is 0.0004, while with reference *Argiope versicolor* it is 0.05, indicating the former group belongs to *Argiope pulchella*. Similarly, the average distance between the latter group with reference *Argiope pulchella* sequences is 0.031, and with reference *Argiope versicolor* sequences is 0.02, indicating they belong to *Argiope versicolor*.

Table 4.2.11. Genetic distance inferred from COI sequences of *Argiope pulchella* and *Argiope versicolor*. *Sequences developed for the present study

	1	2	3	4	5	6	7	8	9	10	11
A. versicolor (ARAMP036)*											
A. versicolor (ARAMP037)*	0.009										
A. versicolor (KJ957991.1)	0.027	0.017									
A. versicolor (PQ721811.1)	0.006	0.012	0.028								
A. pulchella (ARAMP034)*	0.028	0.026	0.017	0.037							
A. pulchella (MK153906.1)	0.028	0.026	0.017	0.037	0.000						
A. pulchella (ARAMP035)*	0.032	0.030	0.017	0.039	0.003	0.003					
A. pulchella (ARAMP038)*	0.034	0.032	0.019	0.041	0.005	0.005	0.001				
A. versicolor (MK392649.1)	0.030	0.028	0.015	0.037	0.001	0.001	0.001	0.003			
A. pulchella (MK153865.1)	0.032	0.030	0.017	0.039	0.003	0.003	0.003	0.005	0.001		
A. versicolor (MK392647.1)	0.027	0.025	0.015	0.028	0.013	0.013	0.013	0.015	0.011	0.013	

4.2.12. Genetic distance of *Cyclosa hexatuberculata* and *Cyclosa spirifera* specimens

Original alignment = 536 positions

Gblocks alignment = 536 positions (100%)

Genetic distance analysis of *Cyclosa* voucher specimens resulted in two conspecific groups. The first one includes the specimens ARAMP045, ARAMP046, and ARAMP047 with zero genetic difference. The average genetic distance of the second group, that is ARAMP048, ARAMP050, ARAMP053, ARAMP054, and ARAMP055, is 0.002, indicating their conspecificity. The first group shows a 2.2% average distance between the *Cyclosa hexatuberculata* and a 3.3% average distance with *Cyclosa spirifera* reference sequences. Thus, the first group belong to the species *Cyclosa hexatuberculata*. The second group has an average distance of 0.023 with *Cyclosa spirifera* and 0.032 with *Cyclosa hexatuberculata* reference sequences, indicating that the specimens belong to the species *Cyclosa spirifera*. The average genetic distance between *Cyclosa hexatuberculata* group and *Cyclosa spirifera* group is 0.0524, which is greater than 3%, indicating two groups are not conspecific.

Table 4.2.12. Genetic distance inferred from COI sequences of *Cyclosa hexatuberculata* and *Cyclosa spirifera*. *Sequences developed for the present study

	1	2	3	4	5	6	7	8	9	10	11	12	13
C. spirifera (ARAMP050) *													
C. spirifera (ARAMP055) *	0.0000												
C. spirifera (ARAMP048) *	0.0021	0.0021											
C. spirifera (ARAMP054) *	0.0000	0.0000	0.0000										
C. spirifera (MK559316.1)	0.0000	0.0000	0.0021	0.0000									
C. spirifera (ARAMP053) *	0.0042	0.0041	0.0064	0.0043	0.0037								
C. hexatuberculata (MK154774.1)	0.0042	0.0041	0.0064	0.0043	0.0037	0.0075							
C. hexatuberculata (ARAMP045) *	0.0523	0.0516	0.0556	0.0540	0.0506	0.0505	0.0462						
C. hexatuberculata (ARAMP047) *	0.0523	0.0516	0.0553	0.0538	0.0486	0.0486	0.0446	0.0000					
C. spirifera (MK392672.1)	0.0546	0.0539	0.0577	0.0562	0.0507	0.0506	0.0466	0.0020	0.0019				
C. hexatuberculata (KT383709.1)	0.0522	0.0515	0.0557	0.0541	0.0486	0.0486	0.0444	0.0000	0.0000	0.0019			
C. spirifera (MK392670.1)	0.0522	0.0515	0.0552	0.0537	0.0486	0.0485	0.0445	0.0061	0.0056	0.0075	0.0059		
C. hexatuberculata (ARAMP046) *	0.0523	0.0516	0.0553	0.0538	0.0486	0.0486	0.0446	0.0000	0.0000	0.0019	0.0000	0.0056	

4.3. Phylogenetic relationships of araneids in Kerala

Gene trees of COI and H3 were constructed separately using four different methods of phylogenetic analysis. Additionally, the phylogenetic trees of the concatenated dataset were also reconstructed using the four methods. Thus, a total of twelve trees were reconstructed in the present study to understand the interrelationships of araneids in Kerala.

4.3.1. COI gene tree reconstruction

A total of 76 COI barcodes were generated in the present study. After the genetic distance analysis, 55 sequences were selected for the phylogenetic analysis based on the COI sequences. This represents 52 araneid species recorded from this study. The original MSA alignment included 573 bp positions, which were trimmed to 541 bp (94%) Gblocks alignment. Mitochondrial COI partial sequences of *Leucauge decorata* (MK057512) and *Leucauge fastigata* (MK392881) were selected as the outgroup species.

Model selection for the maximum likelihood analysis and Bayesian inference was done using jModelTest. The analysis supported GTR+G+I as the best fit nucleotide substitution model with the lowest BIC score as 18039.76, the lowest AIC value as 17515.96, and the Maximum Likelihood value (lnL) as 8635.98.

The neighbour-joining gene tree was constructed in MEGA11 using 1000 bootstrap replicates. As MEGA11 does not support the GTR model for NJ analysis, the tree was generated using the TN93 substitution model with a gamma distribution (+G = 0.95). Pairwise deletion was selected to treat the missing data in the MSA. All three codon positions were included in the analysis (Figure 4.3.1).

The most parsimonious tree was also constructed in MEGA11. The number of bootstrap replications and the selection of codon position were the same as the NJ analysis. The Tree Bisection Reconnection (TBR) method was used to search the parsimonious tree (Figure 4.3.2).

The maximum likelihood gene tree was built in RAxML with 1000 bootstrap replicates. Maximum likelihood and a rapid bootstrap approach were chosen for the analysis, which was based on the nuclear substitution model, GTR+G+I. The analysis resulted in the final ML optimization likelihood score of 88661.743 (Figure 4.3.3).

Bayesian Inference analysis was done in MrBayes with 2 million MCMC generations. In the analysis based on the GTR+G+I substitution model, every 500th tree was saved. Of the total trees sampled (6002), 5942 trees were 99% credible. The arithmetic mean of the marginal likelihoods for the two runs was 8667.75. Effective Sample Size (ESS) values of all model parameters were >200 (Figure 4.3.4).

In all four trees, the members of the subfamily Nephilinae (highlighted in red) consistently formed a monophyletic clade, positioned as a sister group to the remaining araneid taxa. This relationship received strong support in the Bayesian Inference (BI) tree, with a posterior probability of $p = 1$. Similarly, the monophyly of the subfamily Argiopinae (blue) was evident across all trees ($p=0.89$). The monophyly of *Neoscona* (pink) appeared monophyletic only in the NJ tree, while in all others, it was represented as paraphyletic. Likewise, two species of the *Araneus* (orange) were a monophyletic group in NJ and MP trees, while in ML and BI trees, they showed paraphyletic relationships. Even though several species of *Cyclosa* (green) clustered together in a monophyletic clade across all trees, the genus as a whole exhibited a polyphyletic pattern. Other genera, including *Eriovixia*, *Cyrtophora*, and members of the subfamily Gasteracanthinae, were scattered across the trees, displaying either polyphyletic or paraphyletic relationships.

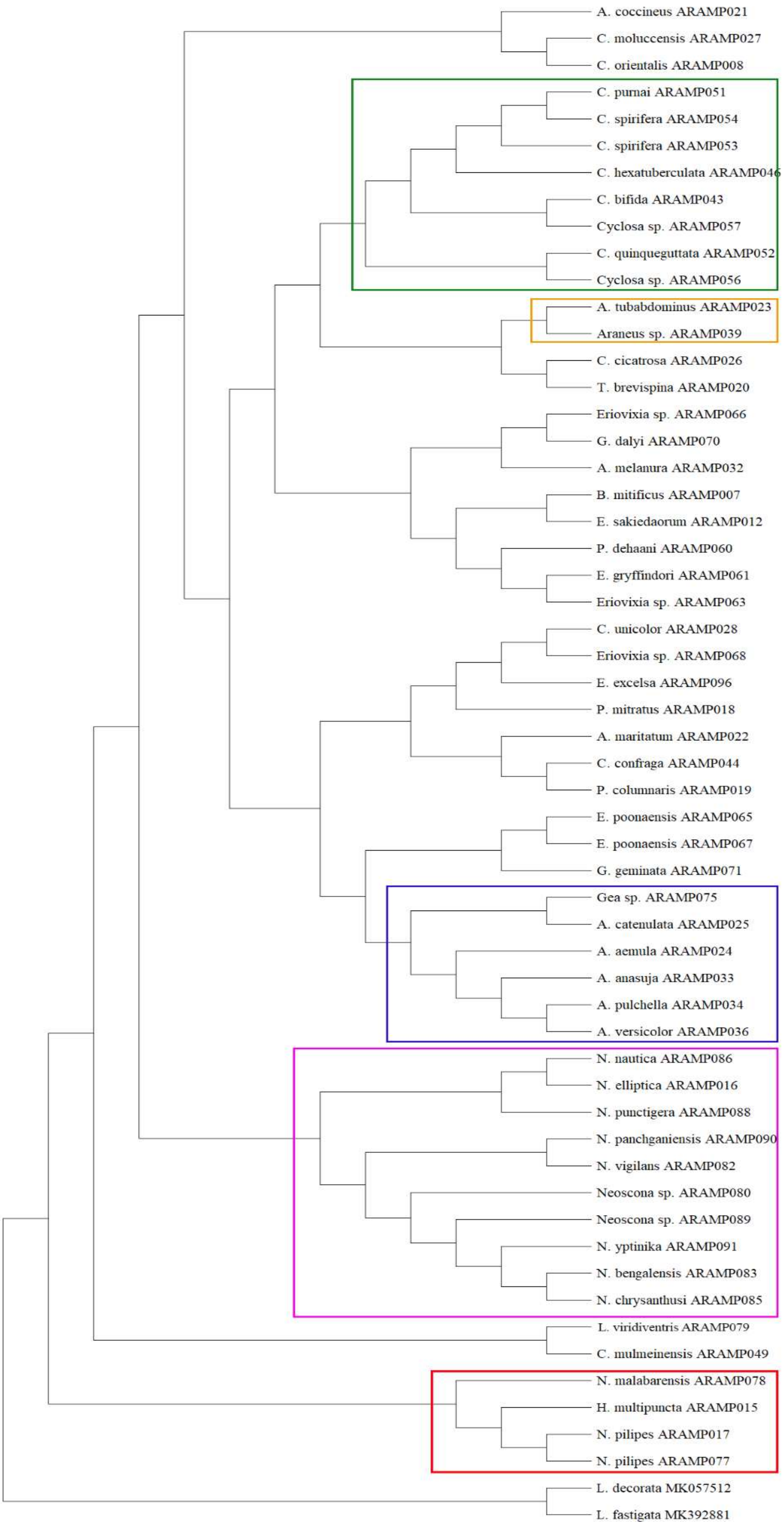


Figure 4.3.1. Neighbour joining tree based on the COI sequence data of araneids in Kerala.

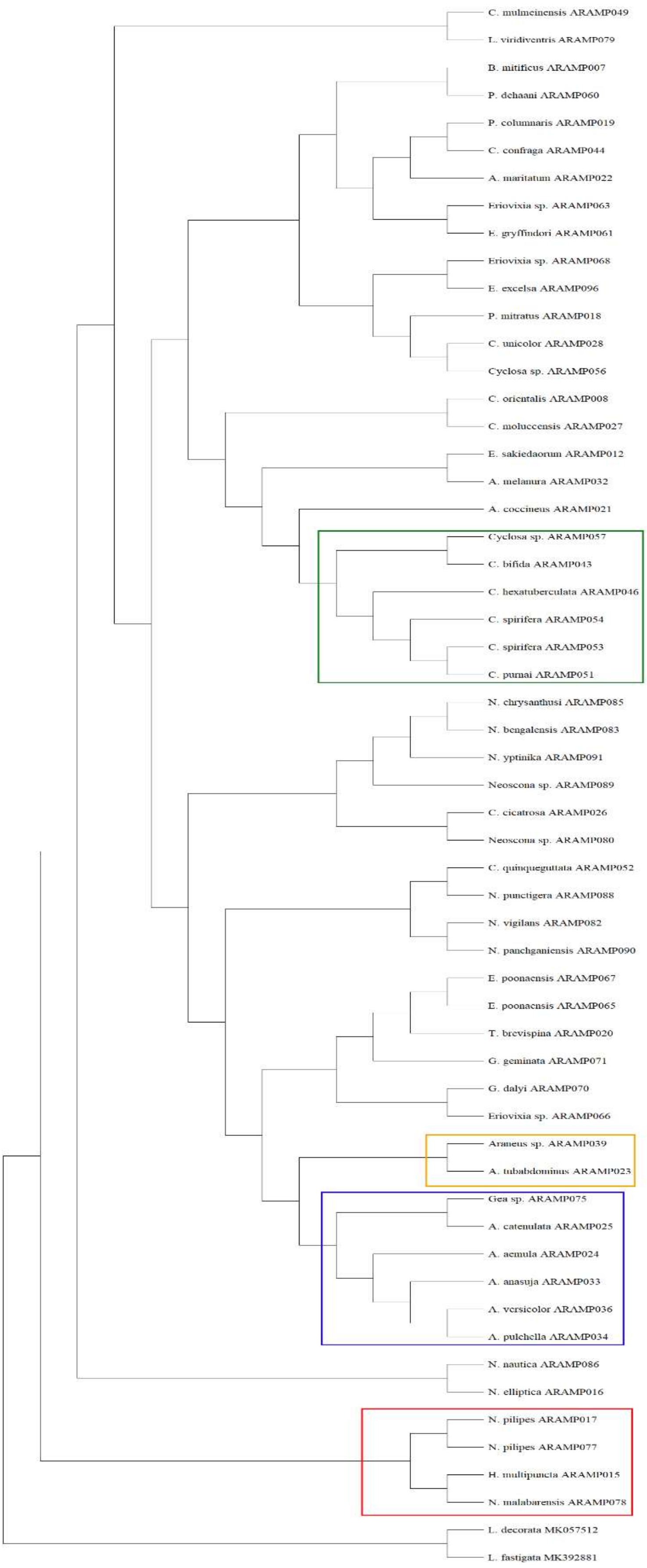


Figure 4.3.2. Most parsimonious tree based on the COI sequence data of araneids in Kerala.

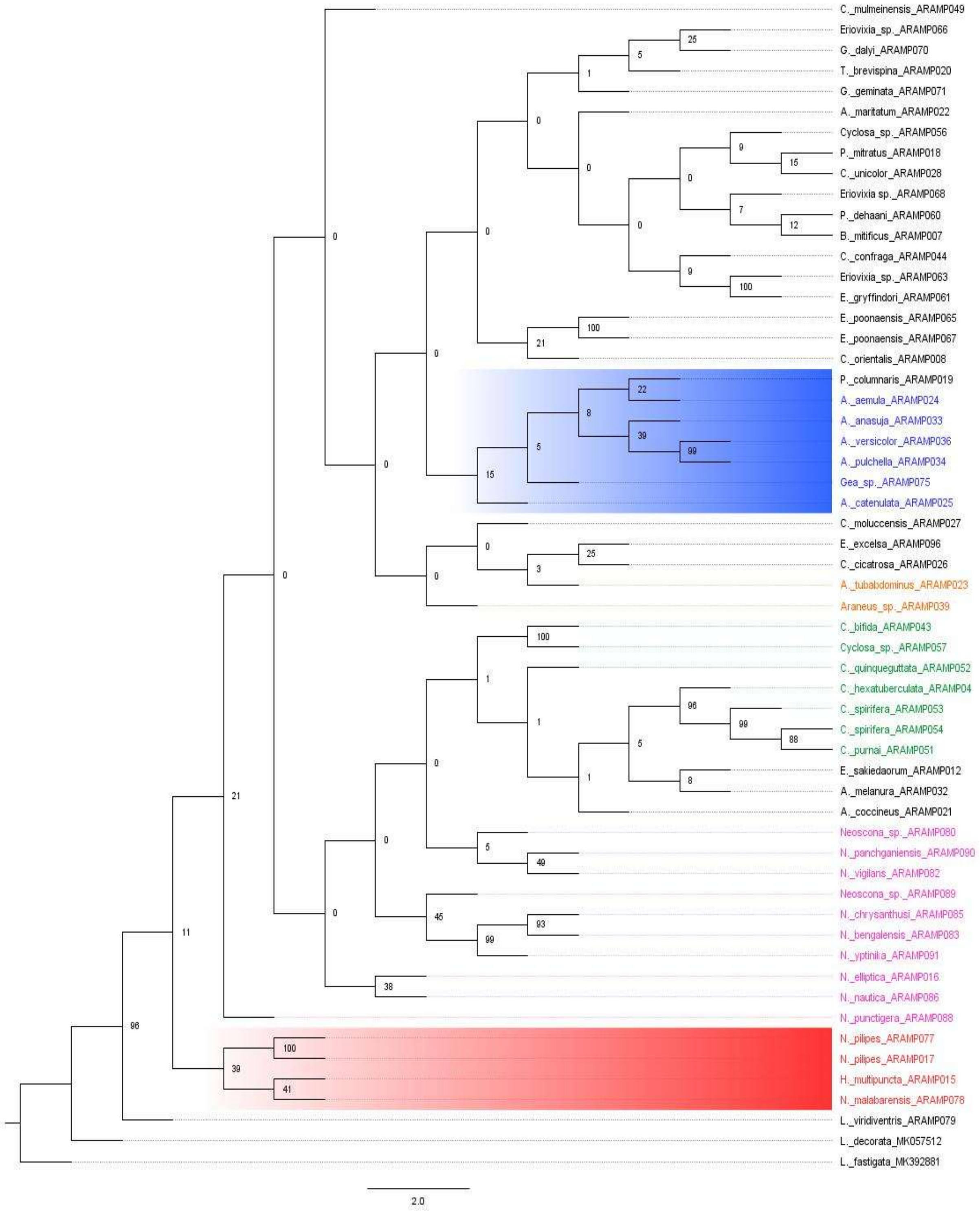


Figure 4.3.3. Maximum likelihood tree based on the COI sequence data of araneids in Kerala.

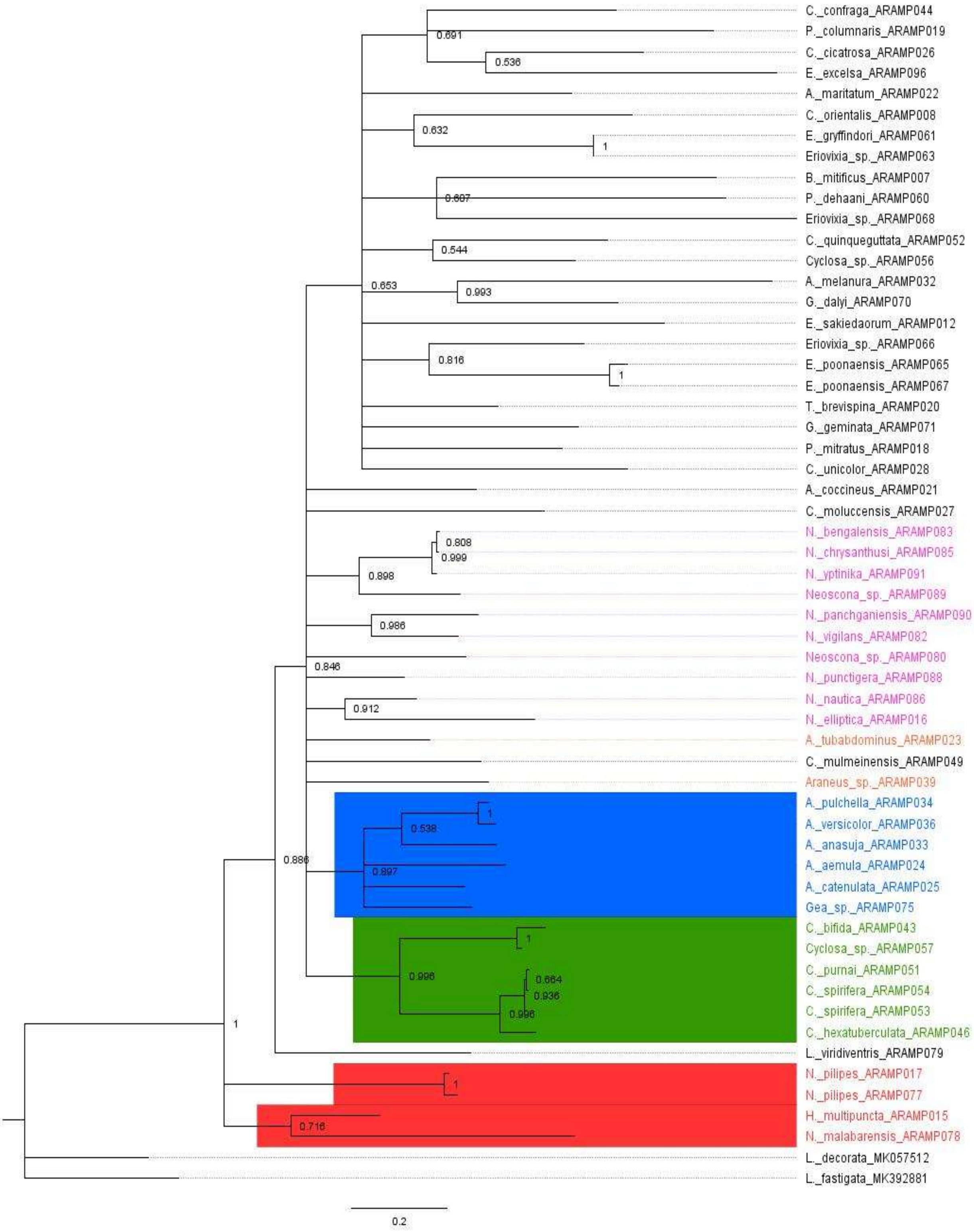


Figure 4.3.4. Bayesian Inference tree based on the COI sequence data of araneids in Kerala.

4.3.2. H3 gene tree reconstruction

In the present study, 33 H3 barcodes were generated. All 33 sequences, representing 33 araneid species, were selected for the phylogenetic analysis. The original MSA alignment included 303 bp positions, which were trimmed to 288 bp (95%) Gblocks alignment. Nuclear H3 partial sequences of *Leucauge decorata* (MK071606) and *Leucauge fastigata* (MK071605) were selected as the outgroup species.

Model selection for the maximum likelihood analysis and Bayesian inference was done using jModelTest. The analysis supported TPM2+I+G as the best fit nucleotide substitution model with the lowest BIC score and AIC value. However, since RAxML and MrBayes do not support this model, the model with the next lowest values was used to construct the trees. It was GTR+G+I with 4414.61 BIC score and 4154.65 AIC value, and 2003.44 Maximum Likelihood value (lnL).

The neighbour-joining gene tree was constructed using 1000 bootstrap replicates. The tree was generated using the K2P substitution model with a gamma distribution (+G = 0.97). Pairwise deletion was selected to treat the missing data in the MSA. All three codon positions were included in the analysis (Figure 4.3.5).

The MP tree was also constructed using 1000 bootstrap replications. During the tree construction using the Tree Bisection Reconnection (TBR) algorithm, all three codon positions were included (Figure 4.3.6).

The maximum likelihood H3 gene tree was built with the same parameter settings as the COI gene tree in RAxML with 1000 bootstrap replicates. The analysis resulted in the best tree with a likelihood score of 1997.687 (Figure 4.3.7).

The MCMC generations for the Bayesian Inference tree were 2 million. In the analysis based on the GTR+G+I substitution model, sample frequency was set to 100, and of the 29883 tree samples, 29583 trees were included in the 99% credible set. The arithmetic mean of the marginal likelihoods for the two runs was 2029.58. Effective Sample Size (ESS) values of all model parameters were >200 (Figure 4.3.8).

In all four trees, the members of the subfamily Nephilinae (red) and Argiopinae (blue) maintained the monophyletic relation, as in the COI gene trees. All the members of *Neoscona* (pink) were grouped in all the trees, along with a paraphyletic relation with *Poltys columnaris*. Unlike COI gene trees, the species of *Eriovixia* (violet) clustered together in all four trees, showing a paraphyletic relation with *Gasteracantha dalyi*. Similarly, *Cyrtophora* species (yellow) were grouped along with a paraphyletic relation with *Acusilas coccineus*. Additionally, all the members of the subfamily Gasteracanthinae, except *Gasteracantha dalyi*, clustered together. Clustering of *Cyclosa* species was not observed; rather, a paraphyletic nature was visible.

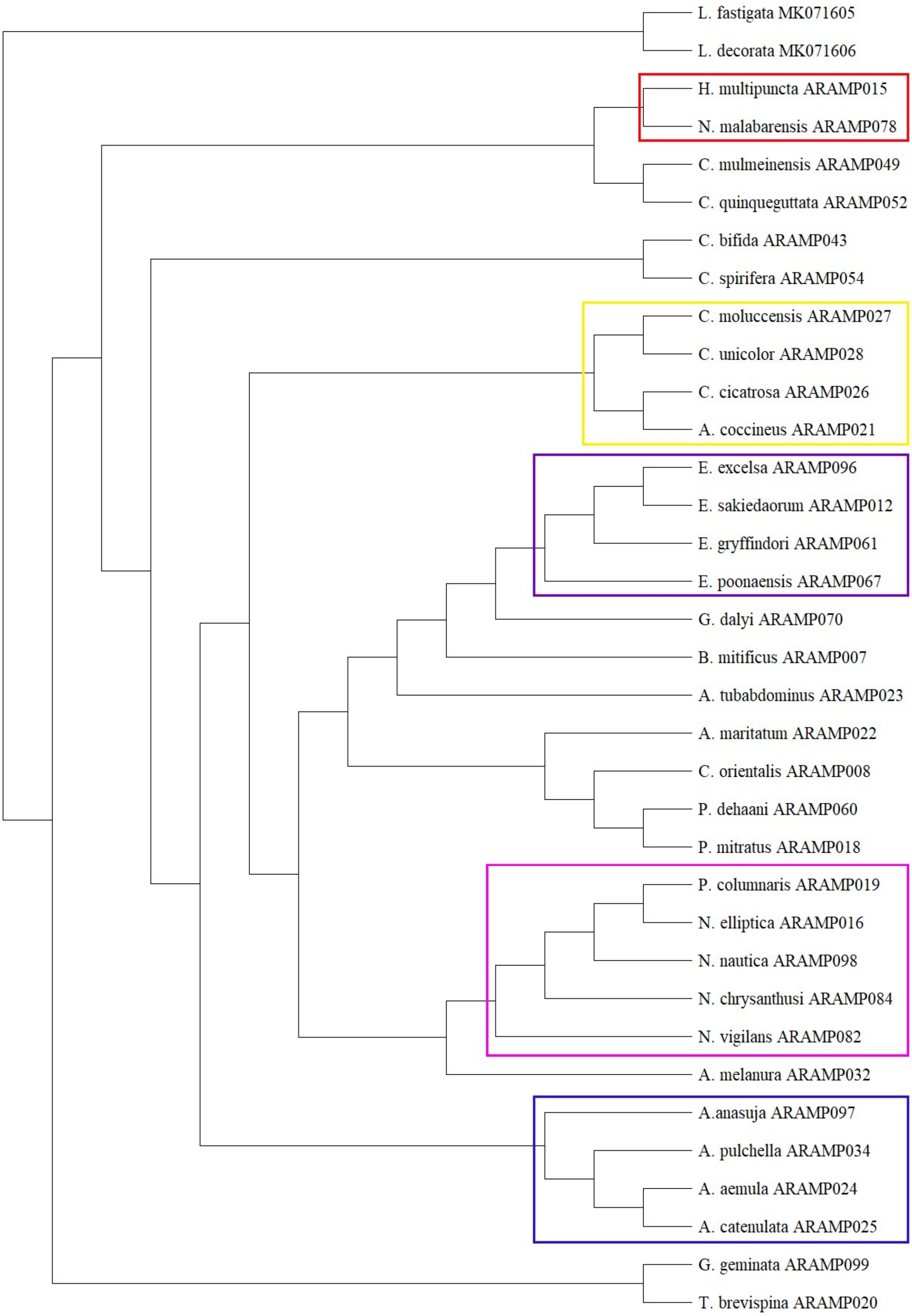


Figure 4.3.5. Neighbour joining tree based on the H3 sequence data of araneids in Kerala.

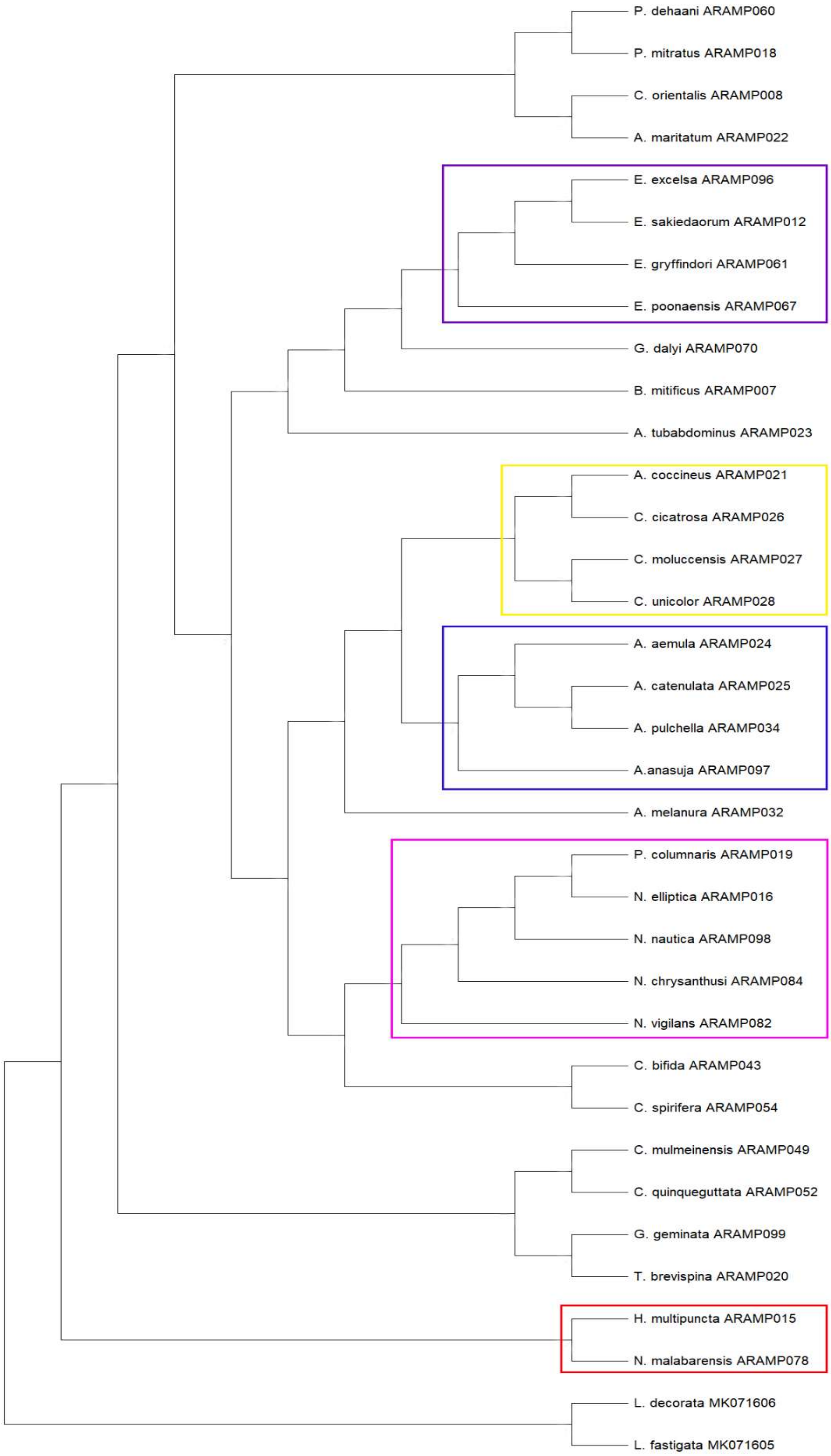


Figure 4.3.6. Maximum parsimony tree based on the H3 sequence data of araneids in Kerala.

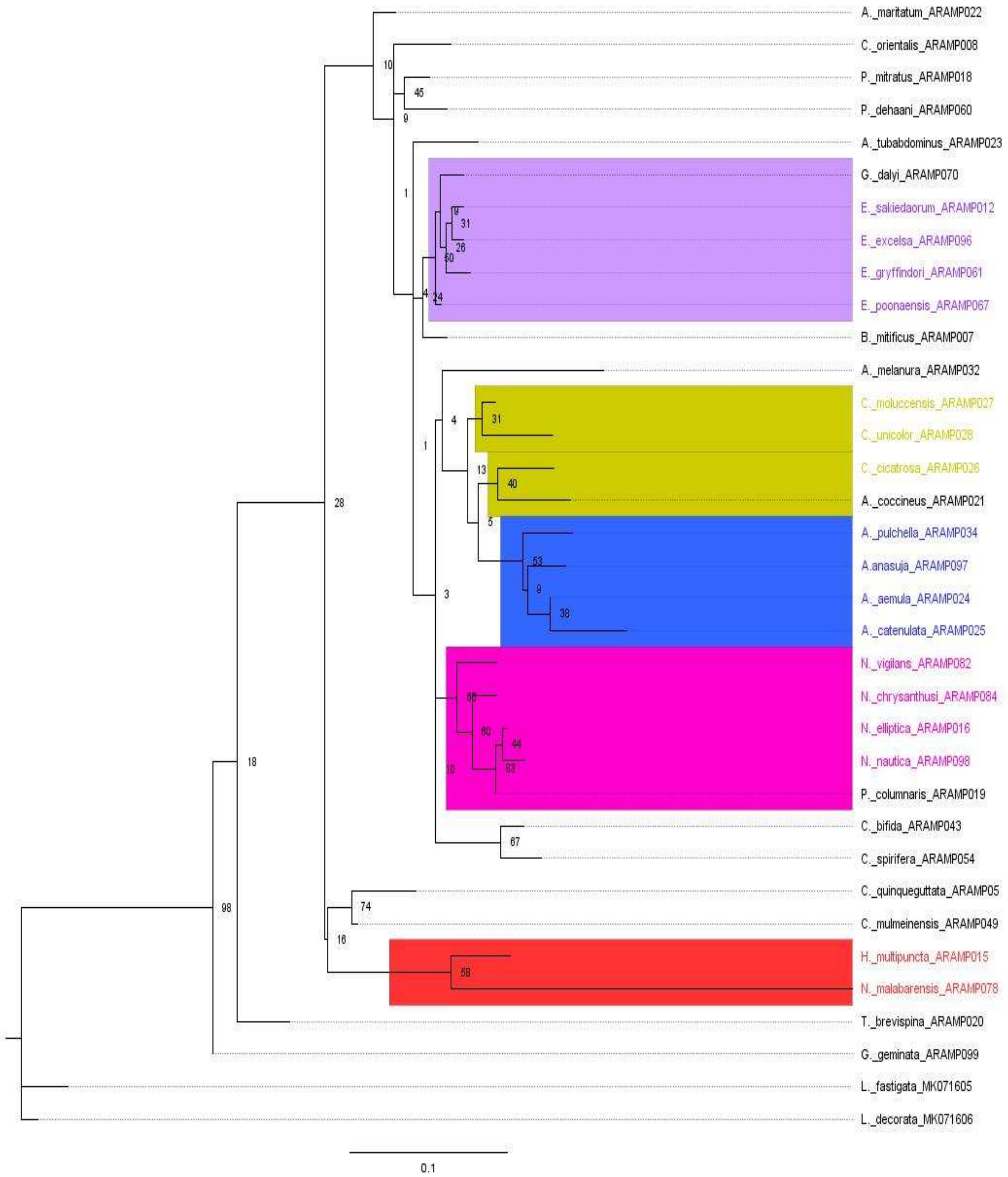


Figure 4.3.7. Maximum likelihood tree based on the H3 sequence data of araneids in Kerala.

Figure 4.3.8. Bayesian Inference tree based on the H3 sequence data of araneids in Kerala.

4.3.3. Concatenated (COI + H3) tree reconstruction

In the present study, both COI and H3 barcodes were generated for 33 species. All 33 species were included in the concatenated dataset for the phylogenetic analysis. Sequences of the outgroup species were the same as those of the COI and H3 gene trees. The MSA alignment of COI sequences had 664 bp positions, and H3 sequences had 367 bp positions, upon concatenation, they gave 1031 bp length sequences.

Model selection was done using jModelTest for both gene alignments separately. TrN+I+G was the best model recommended by jModelTest. However, since this model is not supported by either RAxML or MrBayes, HKY+I+G were selected as it had the next lowest values (BIC score = 14191.11, AIC value = 13858.24, Maximum Likelihood value (lnL) = 6855.12). Similarly, for H3, TPM2+I+G was the best model, and for the same reason, K80+I+G was selected (BIC score = 4498.27, AIC value = 4775.55, Maximum Likelihood value (lnL) = 2178.13).

With 1000 bootstrap replicates, the NJ tree was constructed based on the TN93 model with a 0.73 gamma parameter. Pairwise deletion was selected to treat the missing data in the MSA (Figure 4.3.9). The most parsimonious tree search was also done for 1000 bootstrap replications. The Tree Bisection Reconnection (TBR) algorithm was used for the tree construction (Figure 4.3.10).

The maximum likelihood tree for the concatenated dataset was done using the Maximum likelihood and rapid bootstrap option with 1000 bootstrap replicates. Although models were calculated for each partition, GTR+G+I was the model used to build the ML tree, as RAxML only supports GTR variants. Optimization likelihood for the analysis was 9426.60 (Figure 4.3.11).

During the Bayesian Inference analysis in MrBayes, above mentioned two models were set for each partition with 2 million MCMC generations. 2972 trees were included in the 99% credible set. The arithmetic mean of the marginal likelihoods for the two runs was 9275.20. Effective Sample Size (ESS) values of all model parameters were >200 (Figure 4.3.12).

As the gene trees, the monophyly of subfamily Nephilinae (red), subfamily Argiopinae (blue) and genus *Neoscona* (Pink) was observed in the trees based on the concatenated dataset. BI and ML trees were more resolved than the gene trees. In ML and BI trees, all the *Eriovixia* species were grouped with a moderate support (p = 0.74, bootstrap = 30), which showed a paraphyletic relation to other species. In the NJ and ML trees, *Araneus* showed a sister relation with the *Neoscona* clade, but with very low support. Members of the subfamily Gasteracanthinae clustered together in all four trees except *Gasteracantha dalyi*, which was paraphyletic to the *Eriovixia* group in all four trees. *Cyclosa* showed a polyphyletic distribution across the trees, with two groups, *C. bifida* and *C. spirifera* in one and *C. quiquegutteta* and *C. mulmiensis* in another. Polyphyly of the genus *Cyrtophora* was also observed in all four trees.

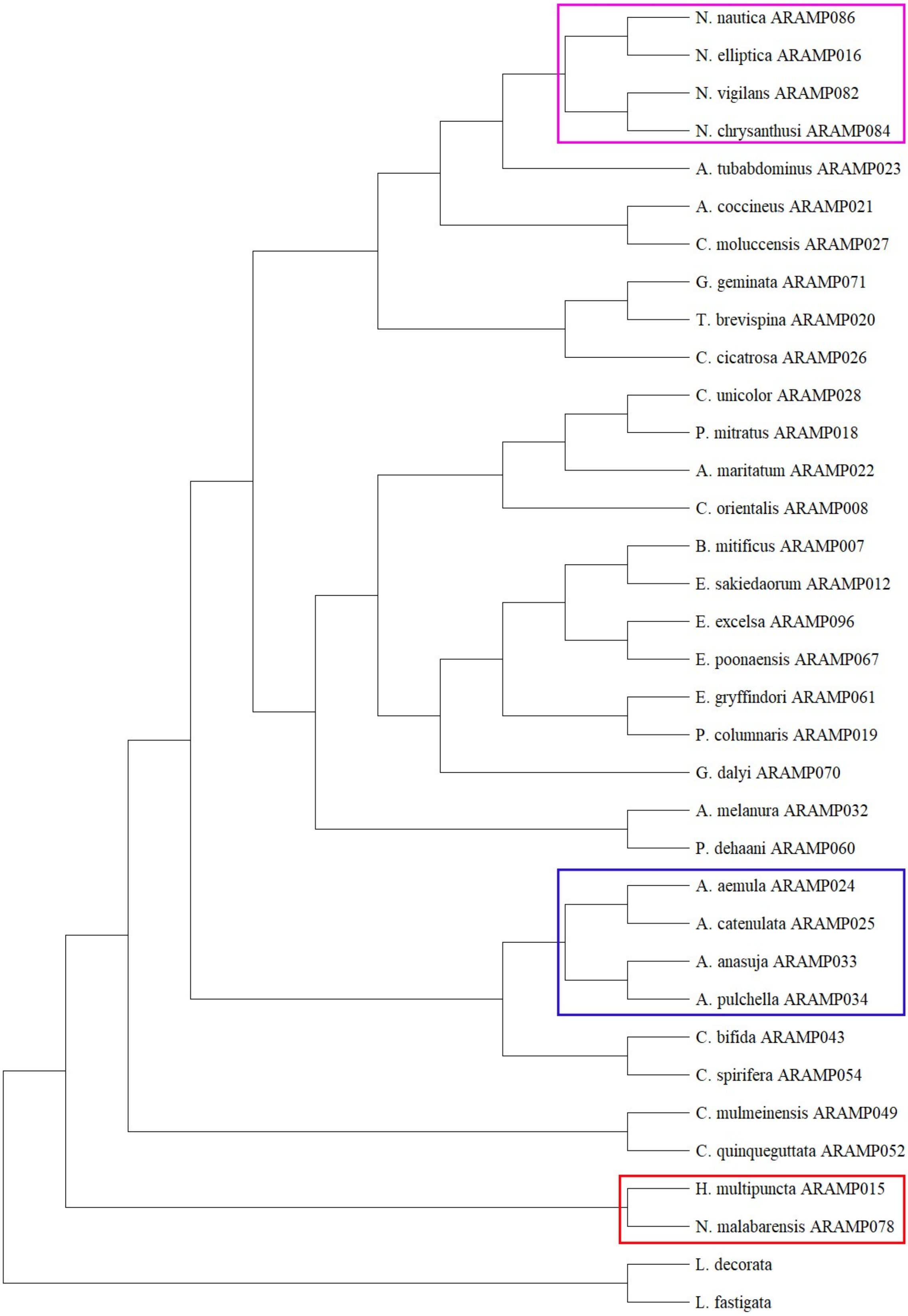


Figure 4.3.9. Neighbour joining tree based on the concatenated dataset of araneids in Kerala.

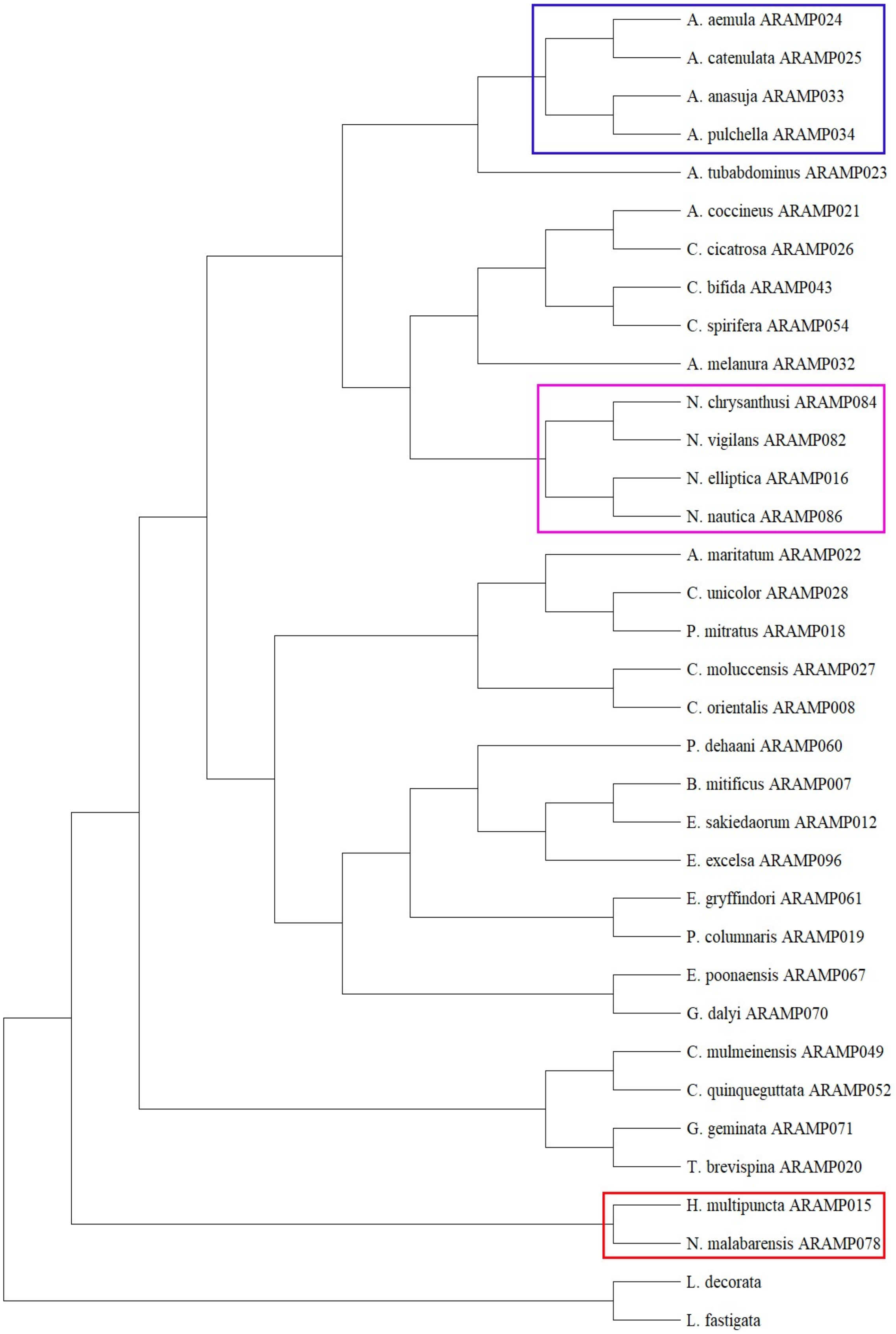


Figure 4.3.10. Parsimonious tree based on the concatenated dataset of araneids in Kerala.

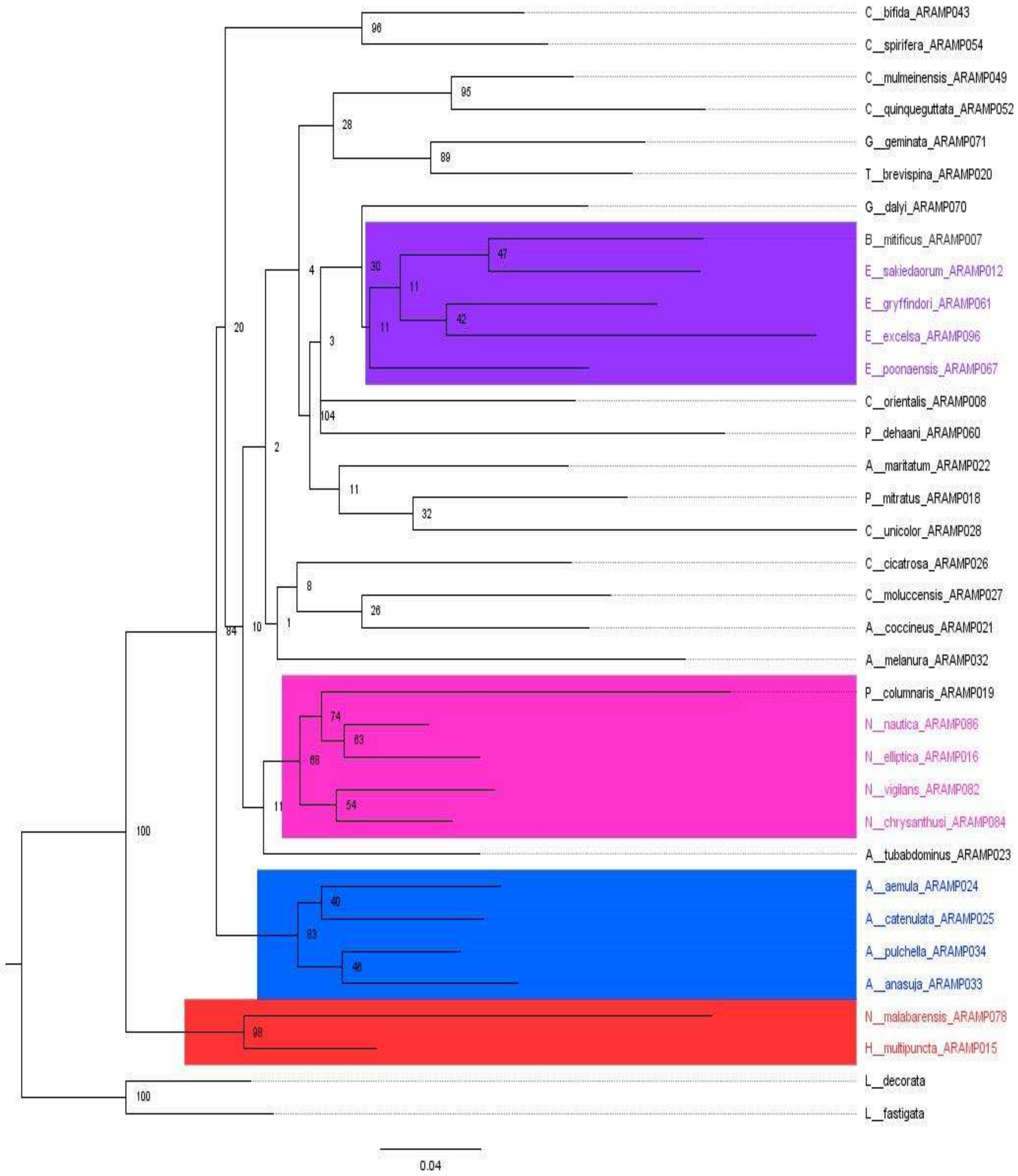


Figure 4.3.11. Maximum Likelihood tree based on the concatenated dataset of araneids in Kerala.

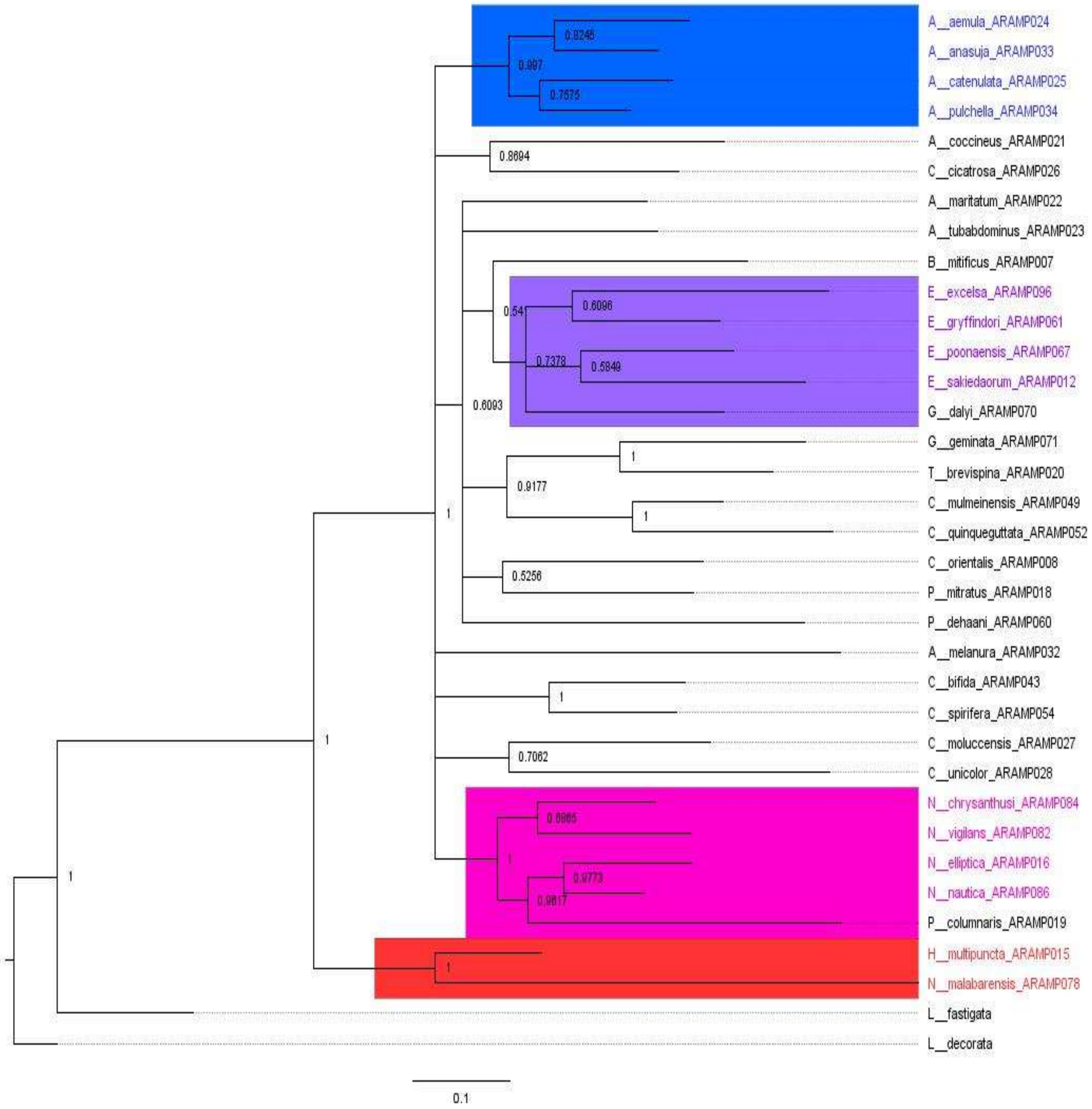


Figure 4.3.12. Bayesian Inference tree based on the concatenated dataset of araneids in Kerala.

4.4. Phylogenetic relationships between araneids in Kerala, Oriental, and Palearctic regions

The present study investigated the phylogenetic interrelationships between araneids from Kerala and those from the Oriental and Palaearctic regions. As in the previous analysis, gene trees were constructed separately for the mitochondrial COI gene, the nuclear H3 gene, and the concatenated dataset using four different phylogenetic methods. Consequently, a total of twelve phylogenetic trees were generated to explore and compare the evolutionary relationships among araneid taxa across these biogeographic regions.

4.4.1. COI gene tree reconstruction

For the COI gene tree reconstruction, 55 COI sequences, developed in the present study, were combined with COI sequences of araneid species reported from Oriental and Palaearctic regions, resulting in a dataset consisting of 253 sequences. The additional sequences were retrieved from GenBank, with careful selection to ensure that only published and taxonomically validated sequences were added (Table S1 in appendix).

The original MAS alignment had 906 bp, which was trimmed to 535 bp (59%) during Gblocks alignment. Outgroup species were the same as the previous analysis (*Leucauge decorata* (MK057512) and *Leucauge fastigata* (MK392881)). jModelTest suggested TIM2+I+G as the best substitution model for the dataset with the lowest AIC and BIC values. However, this was not selected for further analysis as both RAxML and MrBayes do not support this mode. Hence, the model with the next lowest values, GTR+I+G (BIC = 66411.09, AIC = 64192.87, lnL = 31578.43).

Details of each phylogenetic method are summarised in Table 4.4.1. Trees obtained from NJ (Figure 4.4.1), MP (Figure 4.4.2), ML (Figure 4.4.3) and BI (Figure 4.4.4) methods are represented below.

Table 4.4.1. Comparative summary of parameters and settings used in the reconstruction of the COI gene tree for araneids from Kerala, Oriental and Palaearctic regions. NJ Neighbour Joining, MP Maximum Parsimony, ML Maximum Likelihood, BI Bayesian Inference

Method	No. of bootstrap replicates /generations	Model used	Additional details
NJ	1000	TN93 (+G = 0.41)	Missing data treatment - Pairwise deletion
			All three codon positions were included
MP	1000		Tree search algorithm - Tree Bisection Reconnection (TBR)
			All three codon positions were included
ML	1000	GTR+G+I	ML + rapid bootstrap approach
			ML optimization likelihood score = 31844.282
BI	10 million	GTR+G+I	Sample frequency = 1000
			99% credible trees = 14852
			Arithmetic mean of two runs = 31888.67
			Parametric ESS > 200

Monophyly of the genera *Cyclosa*, *Neoscona* and subfamilies Nephiliane and Argiopinae were observed in none of the trees; rather, they formed multiple monophyletic clusters, rendering either a paraphyletic or polyphyletic relationship. However, in all the trees, genera such as *Bijoaraneus*, *Hipsosinga*, *Plebs*, *Eriovixia*, and *Araniella* formed monophyletic clades. In all trees subfamily Nephiliane formed two clusters, including all the *Nephila pilipes* in one clade and all the other nephilines in another clade. Subfamily Argiopinae formed a large monophyletic clade, including all its species except a few showed paraphyly. Paraphyly of the subfamily Gasteracanthinae were observed in all trees.

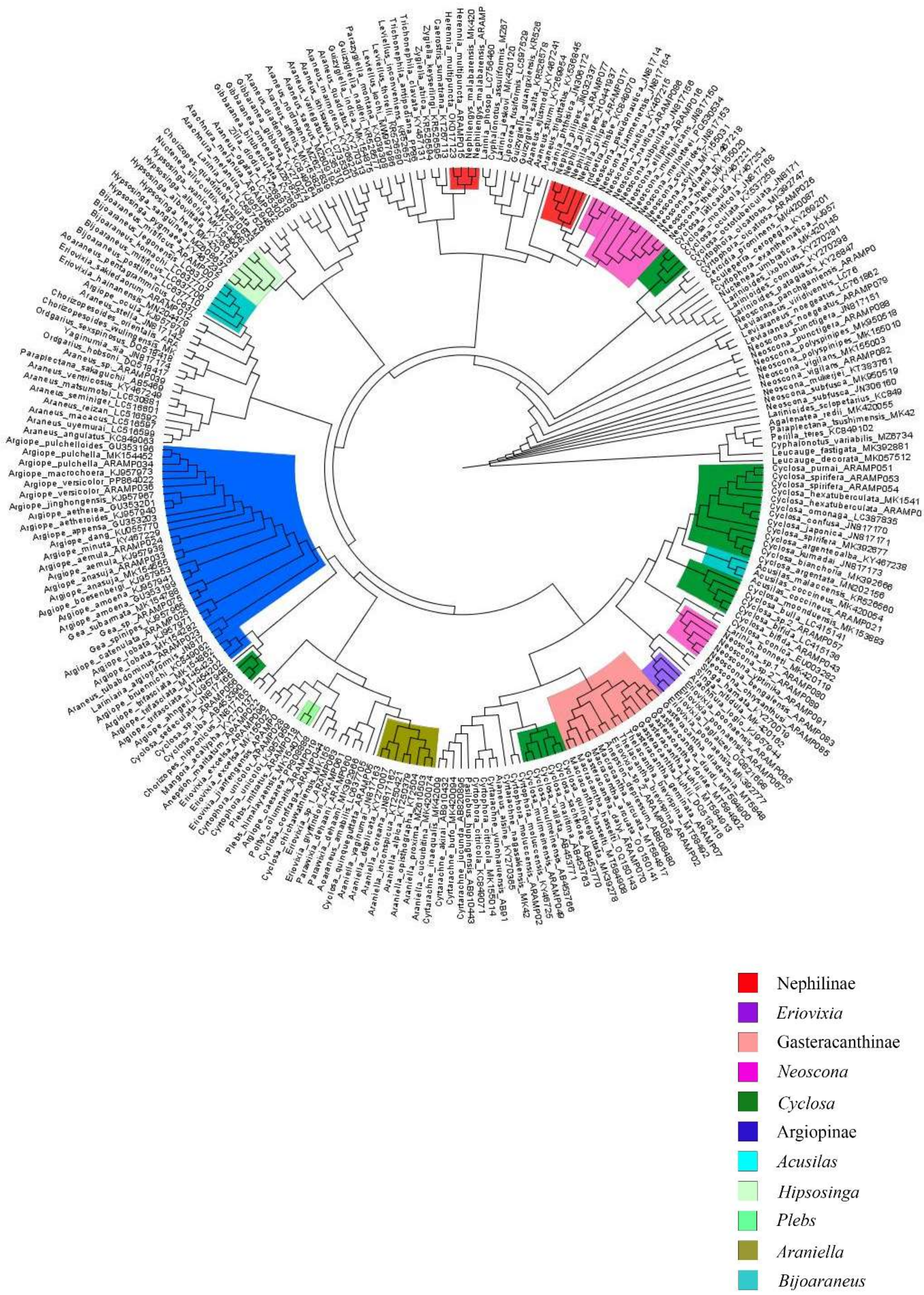


Figure 4.4.1. Neighbour joining COI gene tree for araneids from Kerala, Oriental and Palaearctic regions.

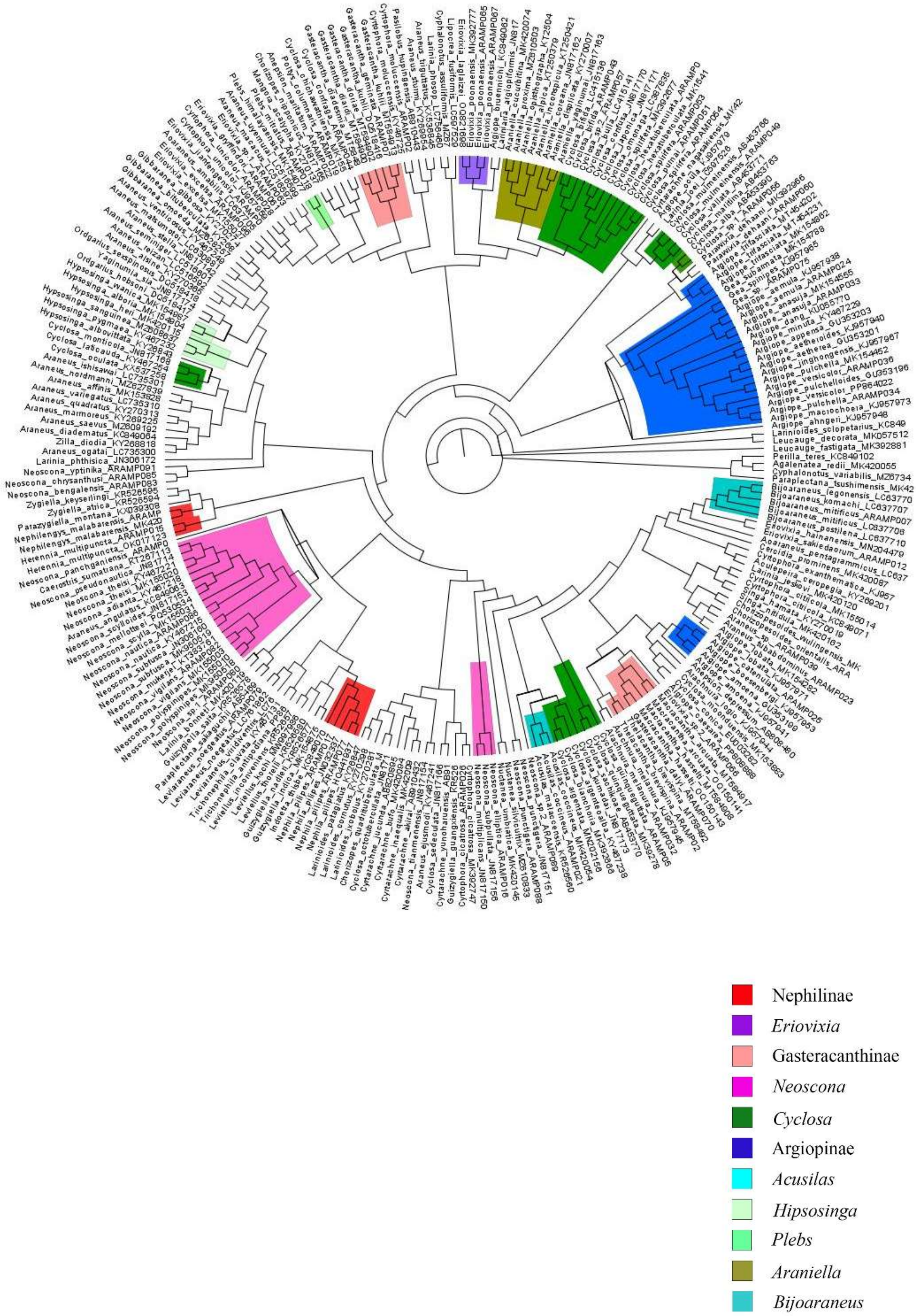


Figure 4.4.2. Most parsimonious COI gene tree for araneids from Kerala, Oriental and Palaearctic regions.

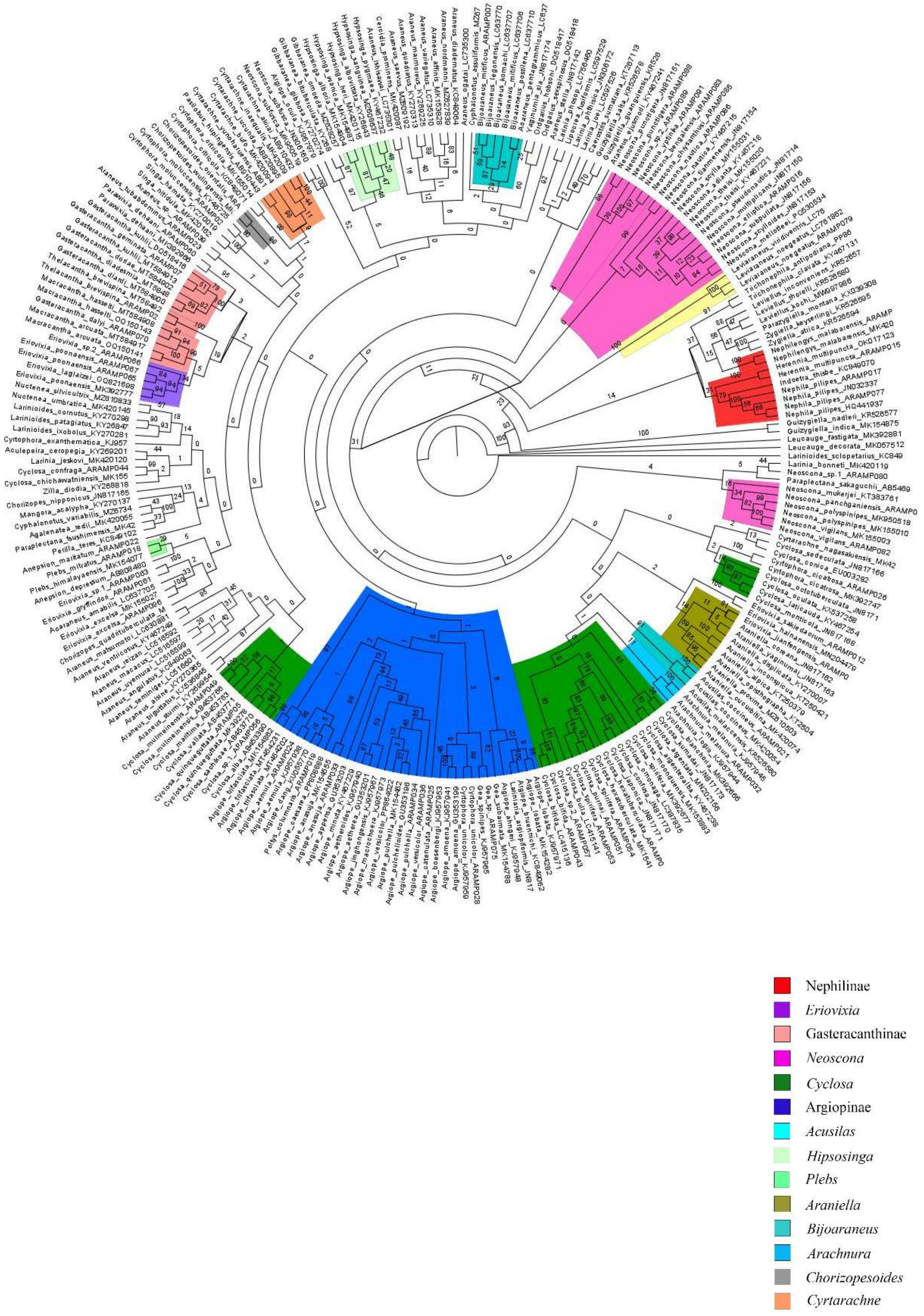


Figure 4.4.3. Maximum likelihood COI gene tree for araneids from Kerala, Oriental and Palaearctic regions.

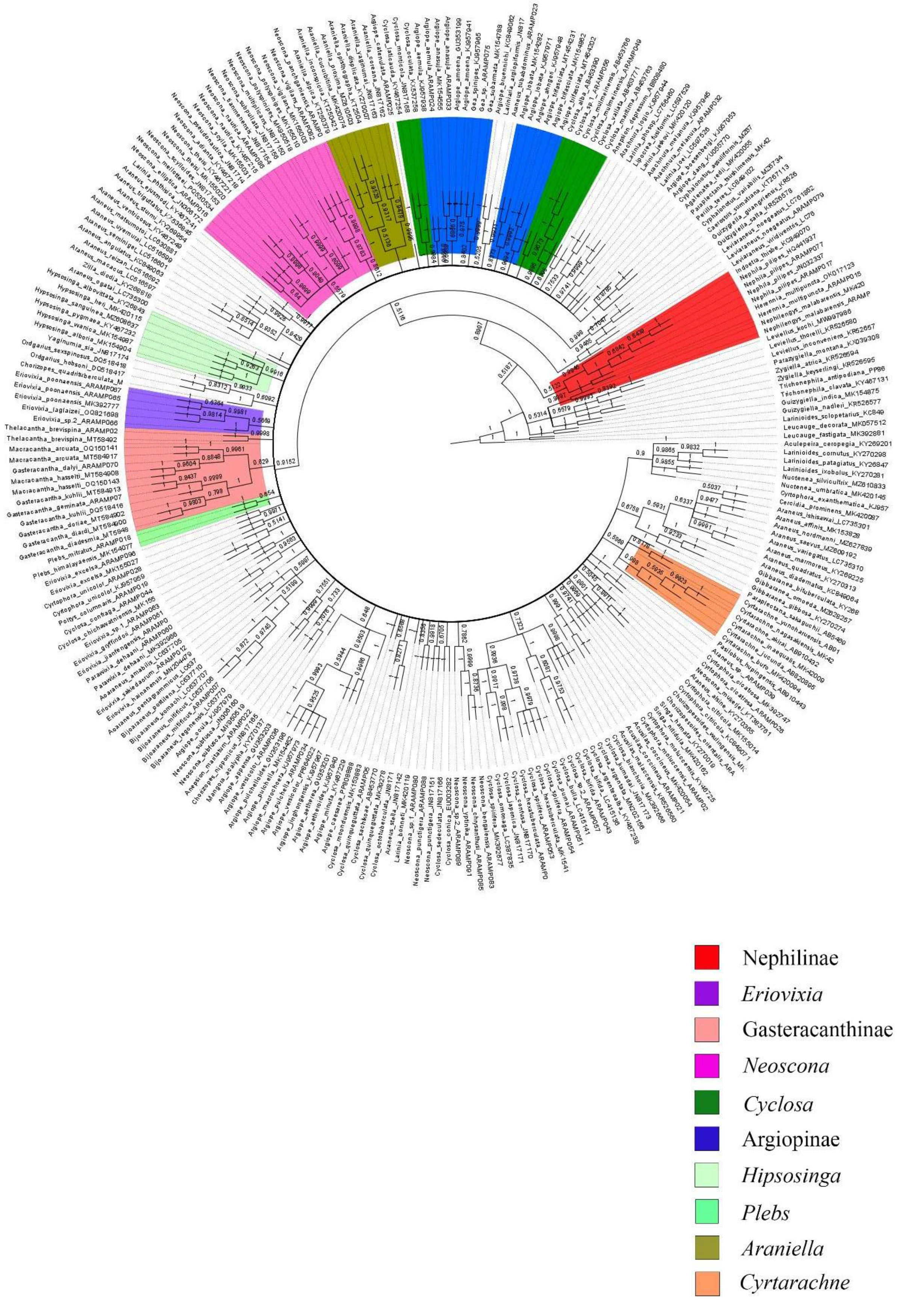


Figure 4.4.4. Bayesian Inference COI gene tree for araneids from Kerala, Oriental and Palaearctic regions.

4.4.2. H3 gene tree reconstruction

For the H3 gene tree reconstruction, 33 H3 sequences, developed in the present study, were combined with H3 sequences of araneid species reported from Oriental and Palaearctic regions, resulting in a dataset consisting of 94 sequences. Published and taxonomically valid sequences were retrieved from GenBank (Table S1 in appendix).

The original MAS alignment had 340 bp, which was trimmed to 320 bp (94%) Gblocks alignment. Outgroup species were *Leucauge decorata* (MK071606) and *Leucauge fastigata* (MK071605). jModelTest suggested GTR+ G+I as the best substitution model for the dataset (BIC = 9731.54, AIC = 8977.87, lnL = 4288.93). Details of each phylogenetic method are summarised in Table 4.4.2. Trees obtained from NJ (Figure 4.4.5), MP (Figure 4.4.6), ML (Figure 4.4.7) and BI (Figure 4.4.8) methods are represented below.

Table 4.4.2. Comparative summary of parameters and settings used in the reconstruction of the H3 gene tree for araneids from Kerala, Oriental and Palaearctic regions. NJ Neighbour Joining, MP Maximum Parsimony, ML Maximum Likelihood, BI Bayesian Inference

Method	No. of bootstrap replicates /generations	Model used	Additional details
NJ	1000	K2P (+G = 0.93)	Missing data treatment - Pairwise deletion
			All three codon positions were included
MP	1000		Tree search algorithm - Tree Bisection Reconnection (TBR)
			All three codon positions were included
ML	1000	GTR+G+I	ML + rapid bootstrap approach
			ML optimization likelihood score = 4286.33
BI	5 million	GTR+G+I	Sample frequency = 1000
			99% credible trees = 7427
			Arithmetic mean of marginal likelihoods = 4375.10
			Parametric ESS > 200

All the H3 gene trees were more resolved than the COI gene trees. Monophyly of the Subfamily Nephilinae and its basal placement in the araneid clade were maintained across all trees. Similarly, monophyly of genera such as *Cyrtophora*, *Arachnura*, *Eriovixia*, *Acusials* and *Bijoaraneus* and the subfamily Argiopinae were also congruent in all four trees. Only in the BI tree, *Cyrtophora* and *Acusilas* remained paraphyletic.

Members of the genus *Cyclosa* are scattered across the trees, suggesting a polyphyletic nature of the genus. *The Poltys* species was always embedded in the *Neoscona* clade, exhibiting a paraphyletic nature. Similarly, all members of the subfamily Gasteracanthinae remained in their monophyletic clade, except *Gasteracantha dalyi*, which was changing its position across the trees.

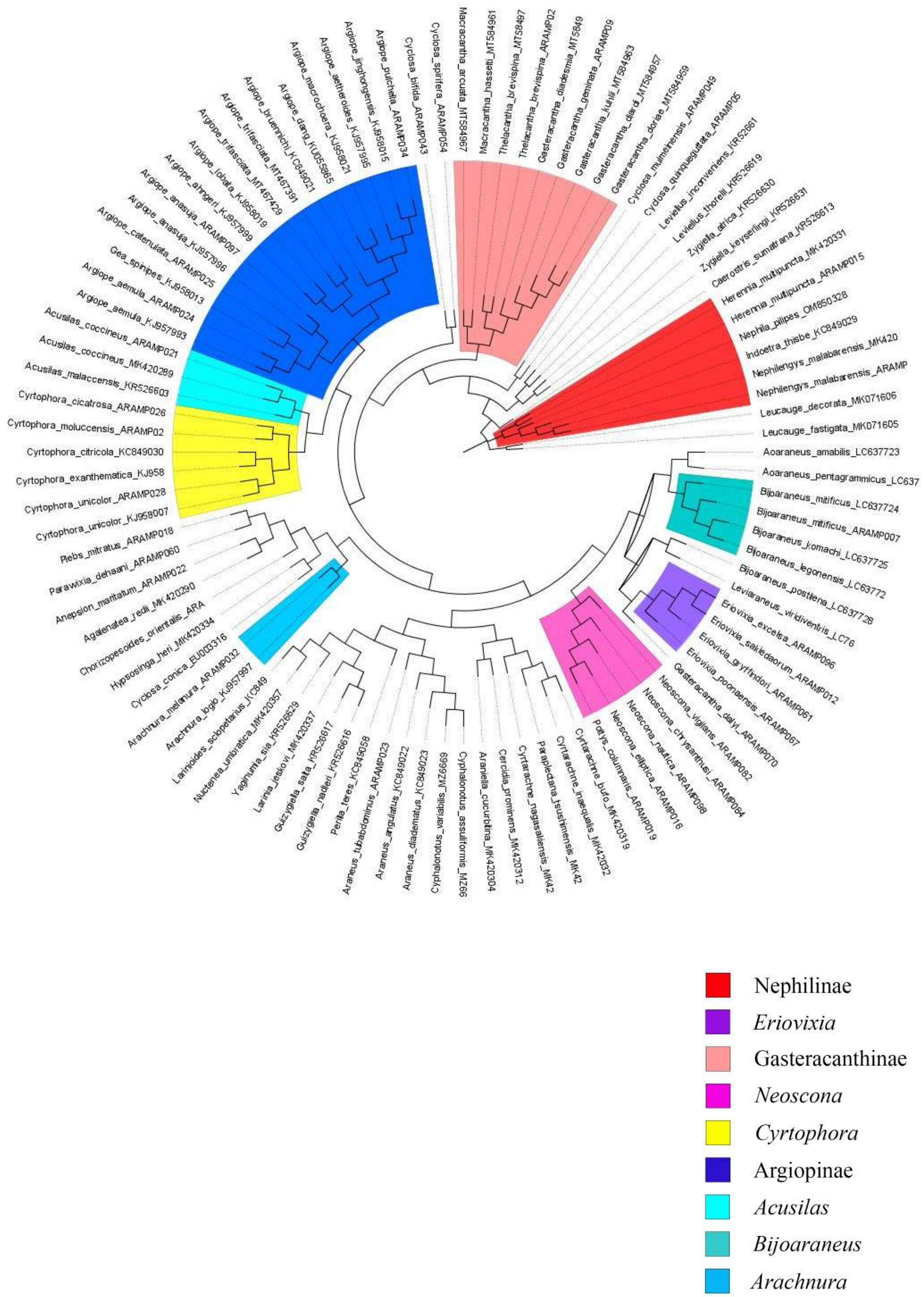


Figure 4.4.5. Neighbour Joining H3 gene tree for araneids from Kerala, Oriental and Palaearctic regions.

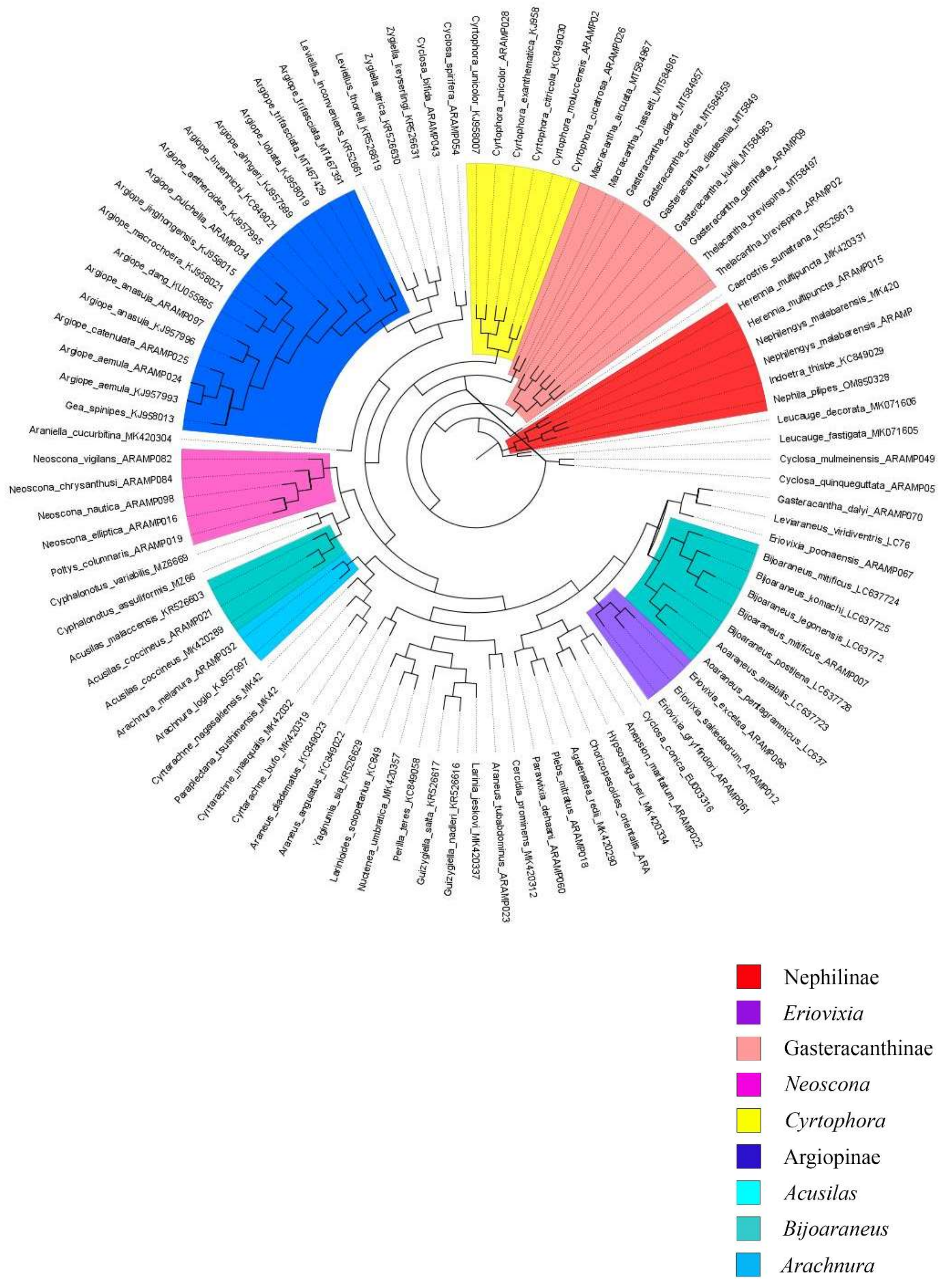


Figure 4.4.6. Maximum Parsimony H3 gene tree for araneids from Kerala, Oriental and Palaearctic regions.

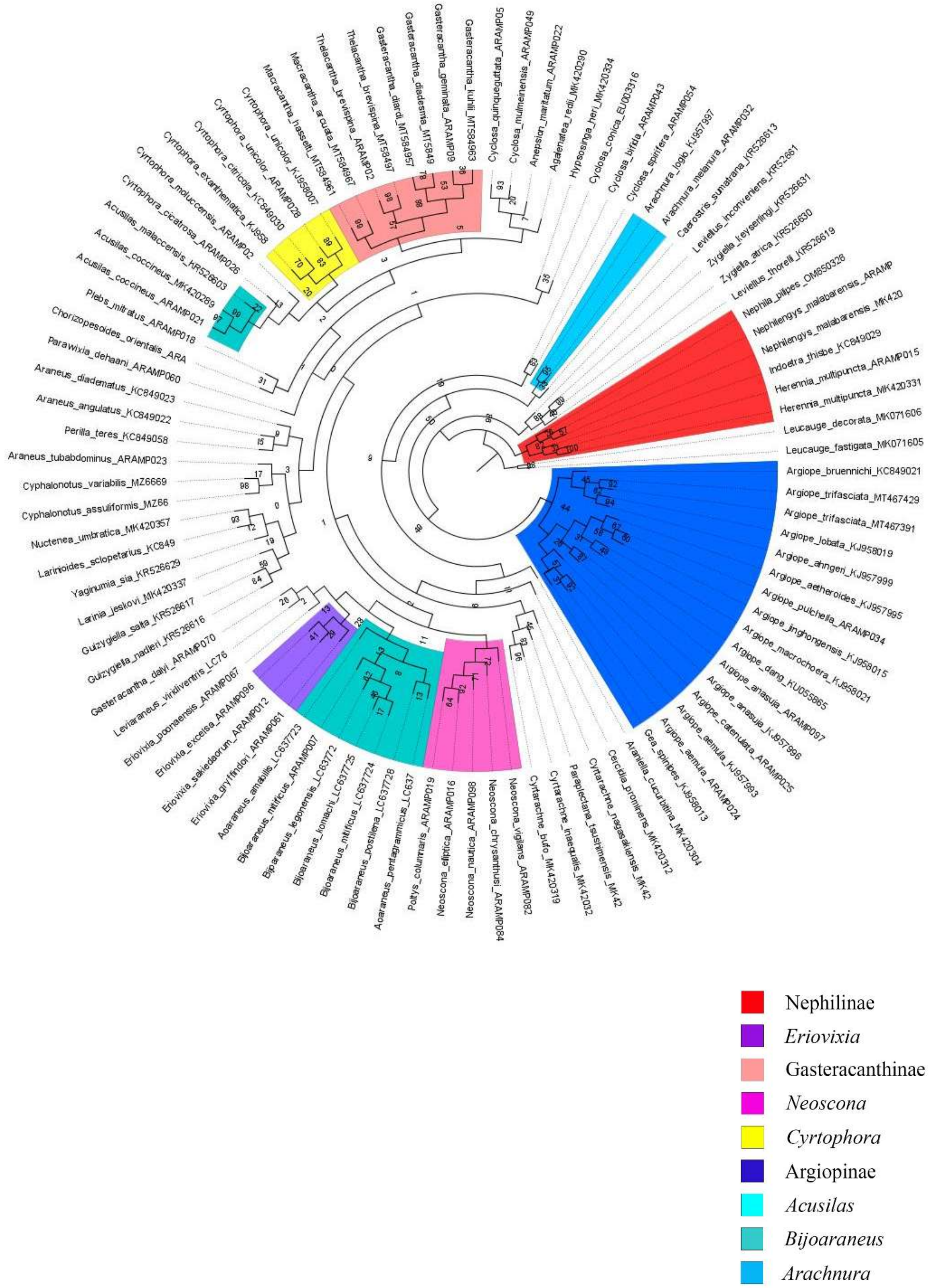


Figure 4.4.7. Maximum Likelihood H3 gene tree for araneids from Kerala, Oriental and Palaearctic regions.

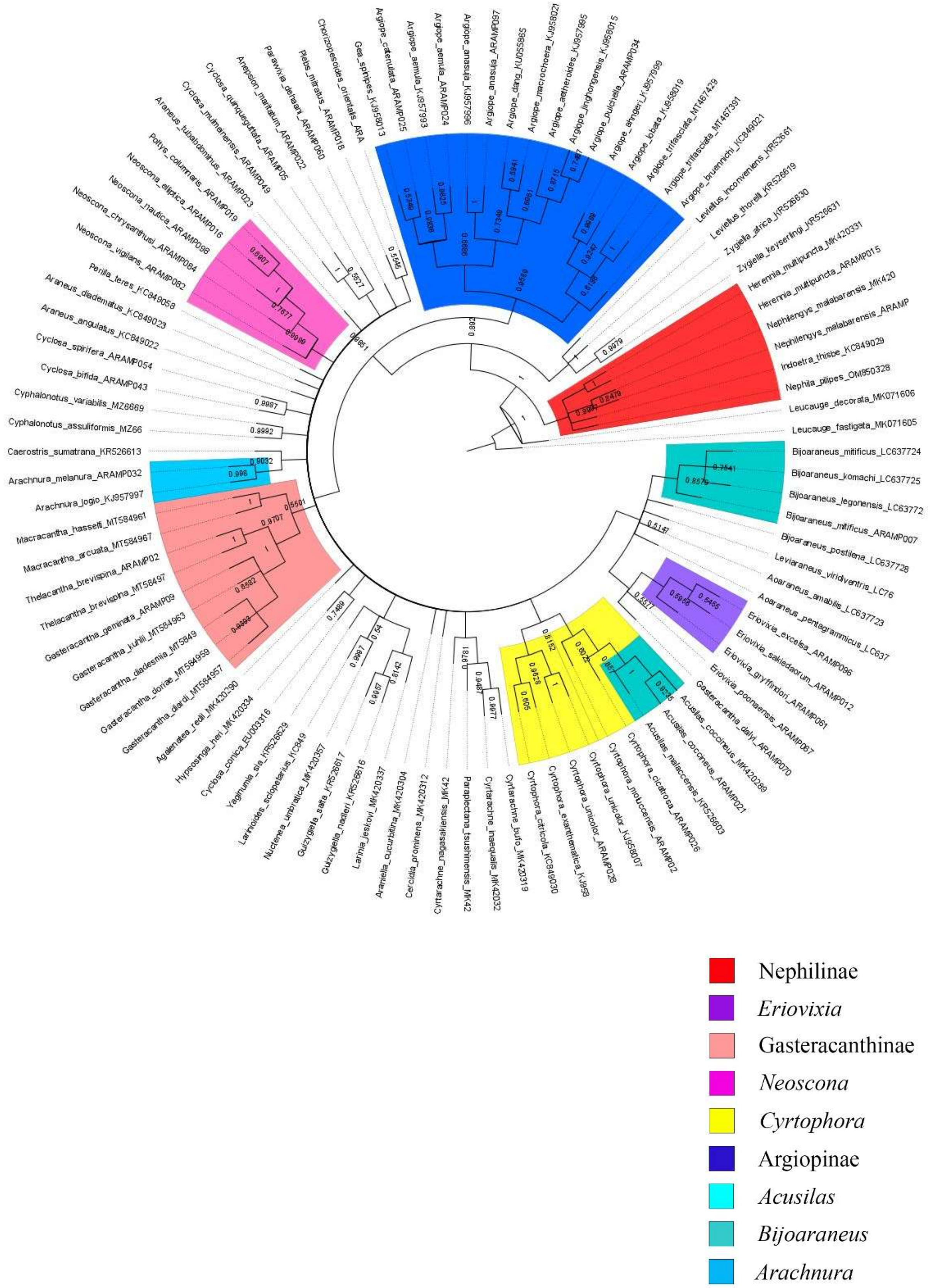


Figure 4.4.8. Bayesian Inference H3 gene tree for araneids from Kerala, Oriental and Palaearctic regions.

4.4.3. Concatenated tree reconstruction

For the concatenated (COI+H3) gene tree reconstruction, COI and H3 sequences from 33 species, developed in the present study, were combined with the COI and H3 sequences of araneid species reported from Oriental and Palaearctic regions, resulting in 94 terminals in the trees. All sequences were retrieved from GenBank (Table S1 in appendix).

The MSA alignment of COI sequences had 1278 bp positions, and H3 sequences had 369 bp positions; upon concatenation had a total of 1647 bp positions. Model selection was done using jModelTest for both gene alignments separately. GTR+G+I was the best model recommended for the COI sequence alignment (BIC = 45406.86, AIC value = 44386.55, lnL = 21995.27). Similarly, for H3, TIM3+I+G was the best model with the lowest scores (BIC score = 10066.91, AIC = 9300.40, lnL = 4454.20). Since MrBayes does not support TIM3+I+G directly, and it is the restricted form of GTR+G+I with equal base frequencies, GTR+I+G was used instead.

Details of each phylogenetic method are summarised in Table 4.4.3. Trees obtained from NJ (Figure 4.4.9), MP (Figure 4.4.10), ML (Figure 4.4.11) and BI (Figure 4.4.12) methods are represented below.

Table 4.4.3. Comparative summary of parameters and settings used in the reconstruction of the concatenated tree for araneids from Kerala, Oriental and Palaearctic regions. NJ Neighbour Joining, MP Maximum Parsimony, ML Maximum Likelihood, BI Bayesian Inference

Method	No. of bootstrap replicates /generations	Model used	Additional details
NJ	1000	TN93 (+G = 0.41)	Missing data treatment - Pairwise deletion
MP	1000		Tree search algorithm - Tree Bisection Reconnection (TBR)
ML	1000	GTR+G+I	ML + rapid bootstrap approach
			ML optimization likelihood score = 22490.85
BI	5 million	GTR+G+I	Sample frequency = 1000
			99% credible trees = 2972
			Arithmetic mean of marginal likelihoods = 27141.15
			Parametric ESS > 200

The concatenated dataset produced more resolved trees than the gene trees. The monophyly of subfamily Nephilinae and Argiopinae was repeated in all trees. In NJ and ML trees, subfamily Gastercanthinae formed a monophyletic clade, while in ML and BI trees, it has been split into two separate clades, rendering a paraphyletic relationship. Genera such as *Bijoaraneus*, *Archnura*, *Acusilas*, and *Cyrtophora* were observed as monophyletic clades across the trees. In ML and NJ trees, *Cyrtophora* clade showed a sister relationship with the Argiopinae clade. In all trees, *Neoscona*, clustered in a single clade, but it cannot be called monophyletic, as the *Poltys* species were embedded in the clade, showing a paraphyly.

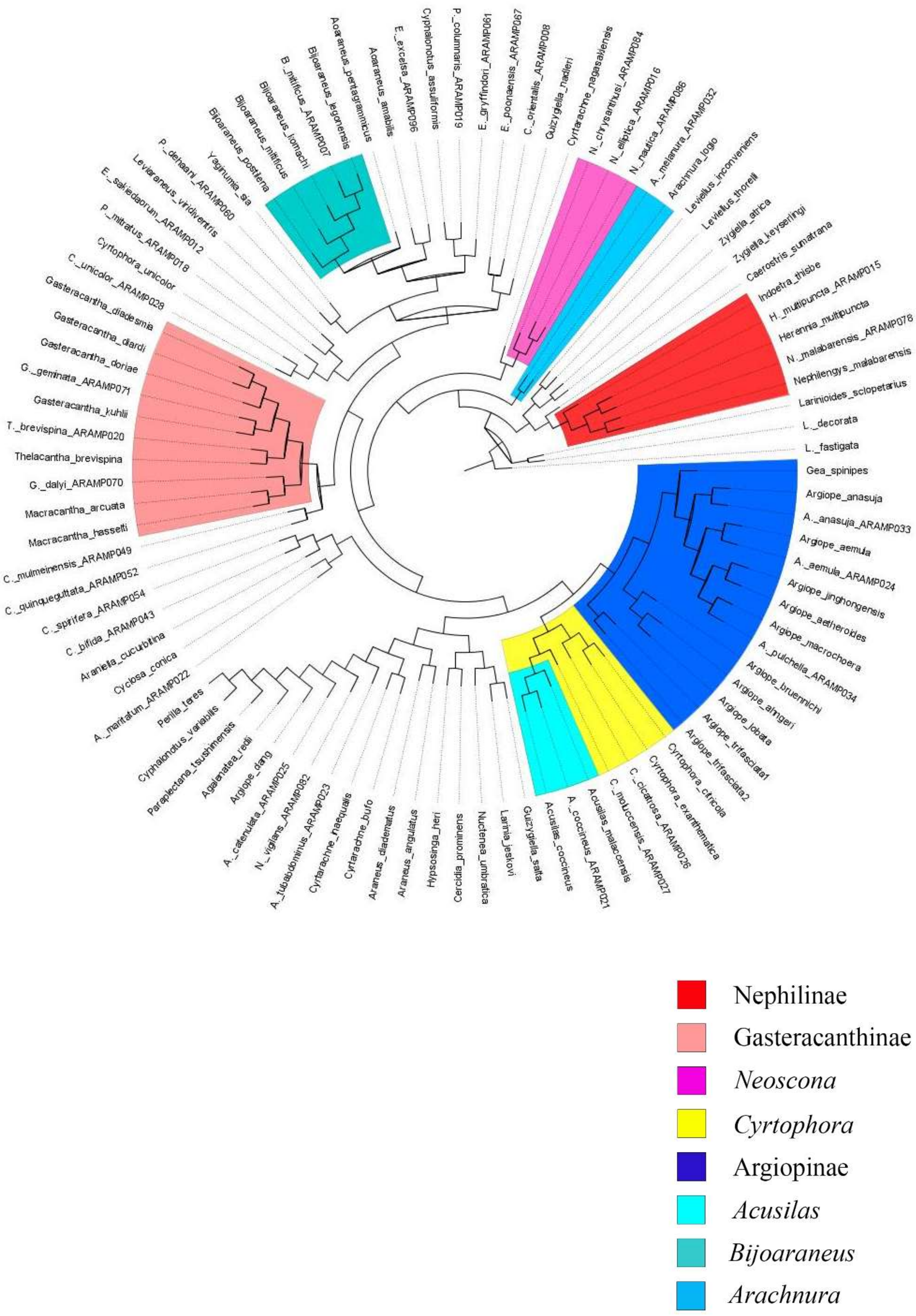


Figure 4.4.9. Neighbour Joining concatenated tree for araneids from Kerala, Oriental and Palaearctic regions.

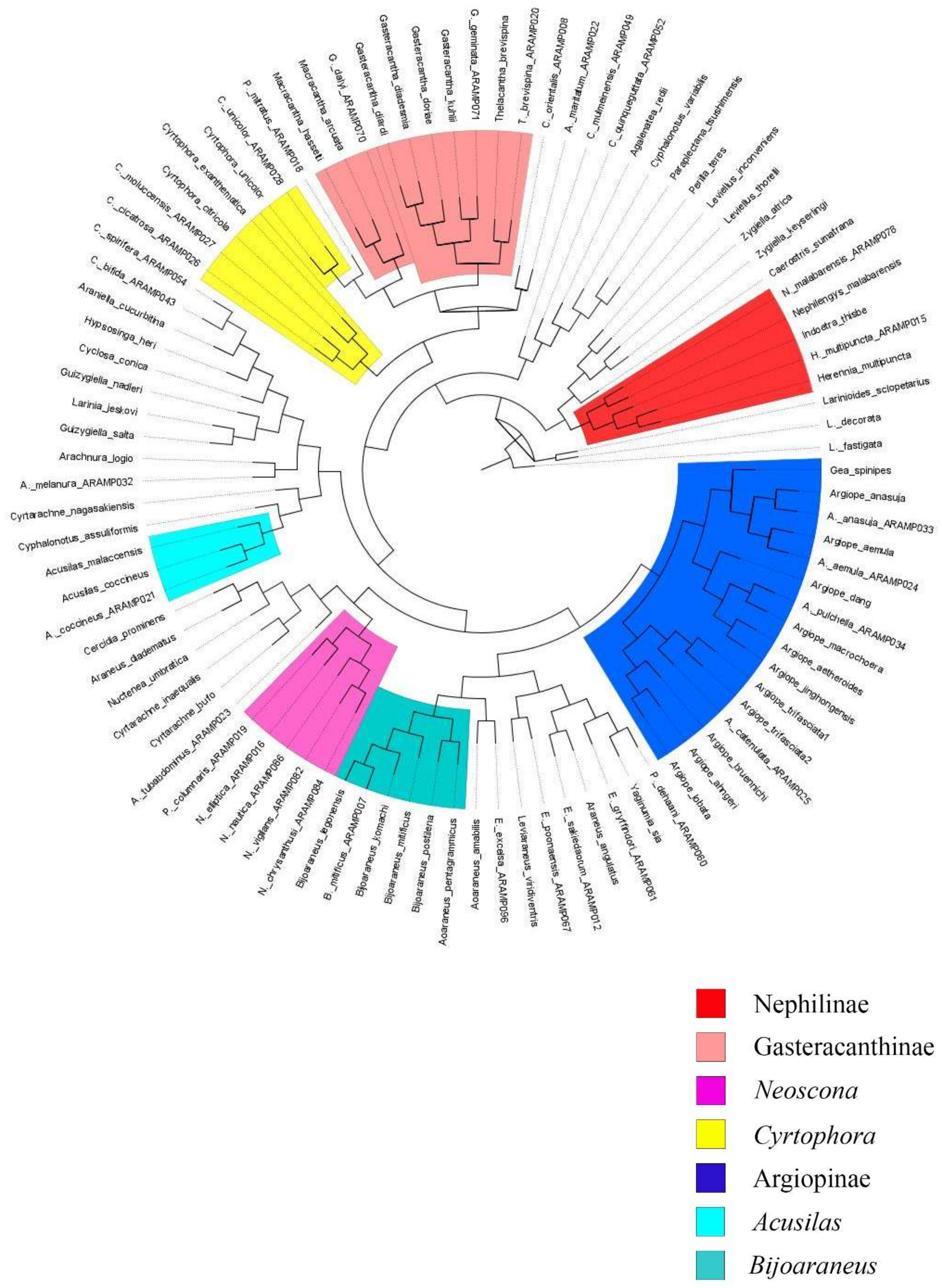


Figure 4.4.10. Maximum Parsimony concatenated tree for araneids from Kerala, Oriental and Palaearctic regions.

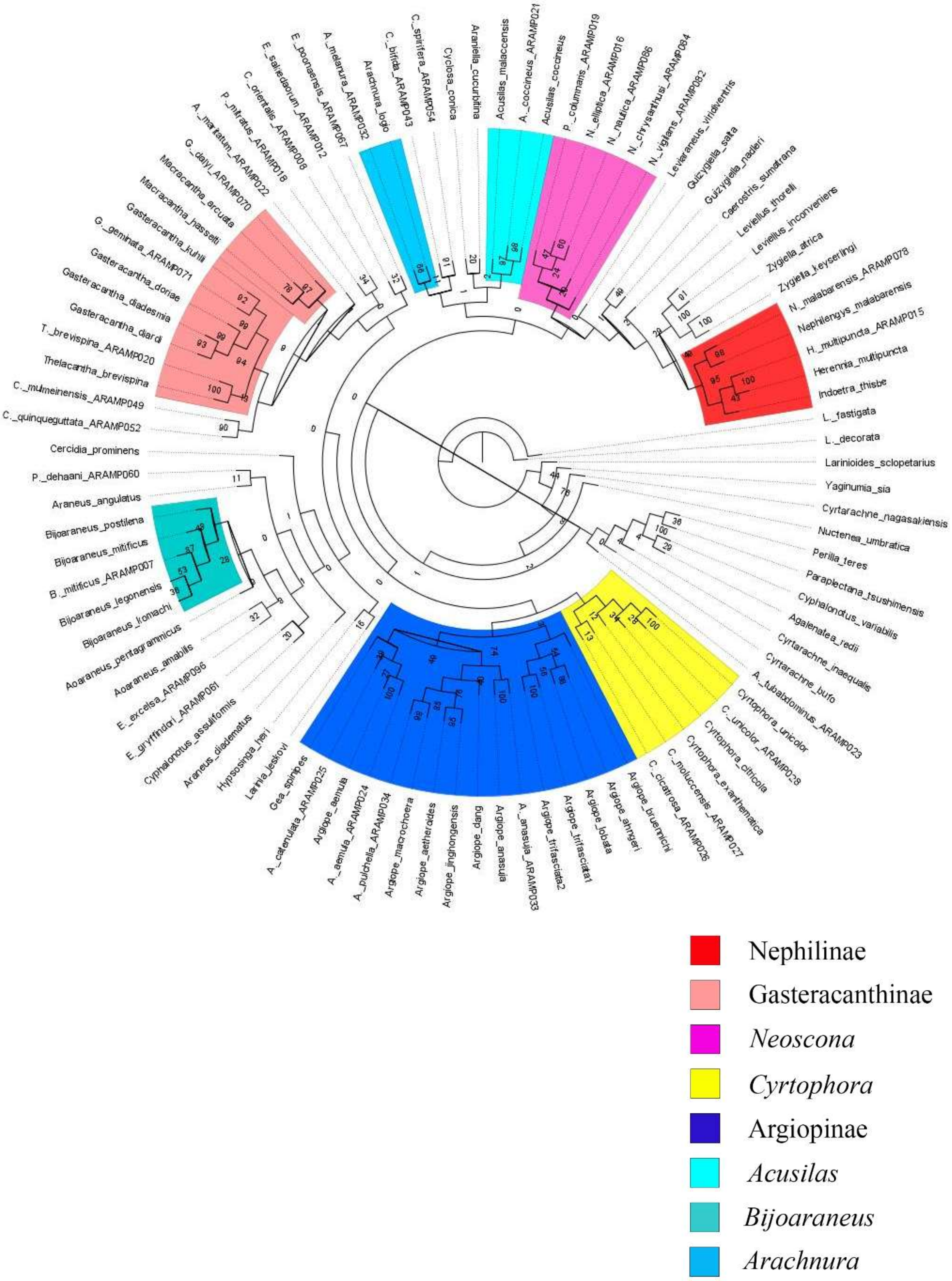


Figure 4.4.11. Maximum Likelihood concatenated tree for araneids from Kerala, Oriental and Palaearctic regions.

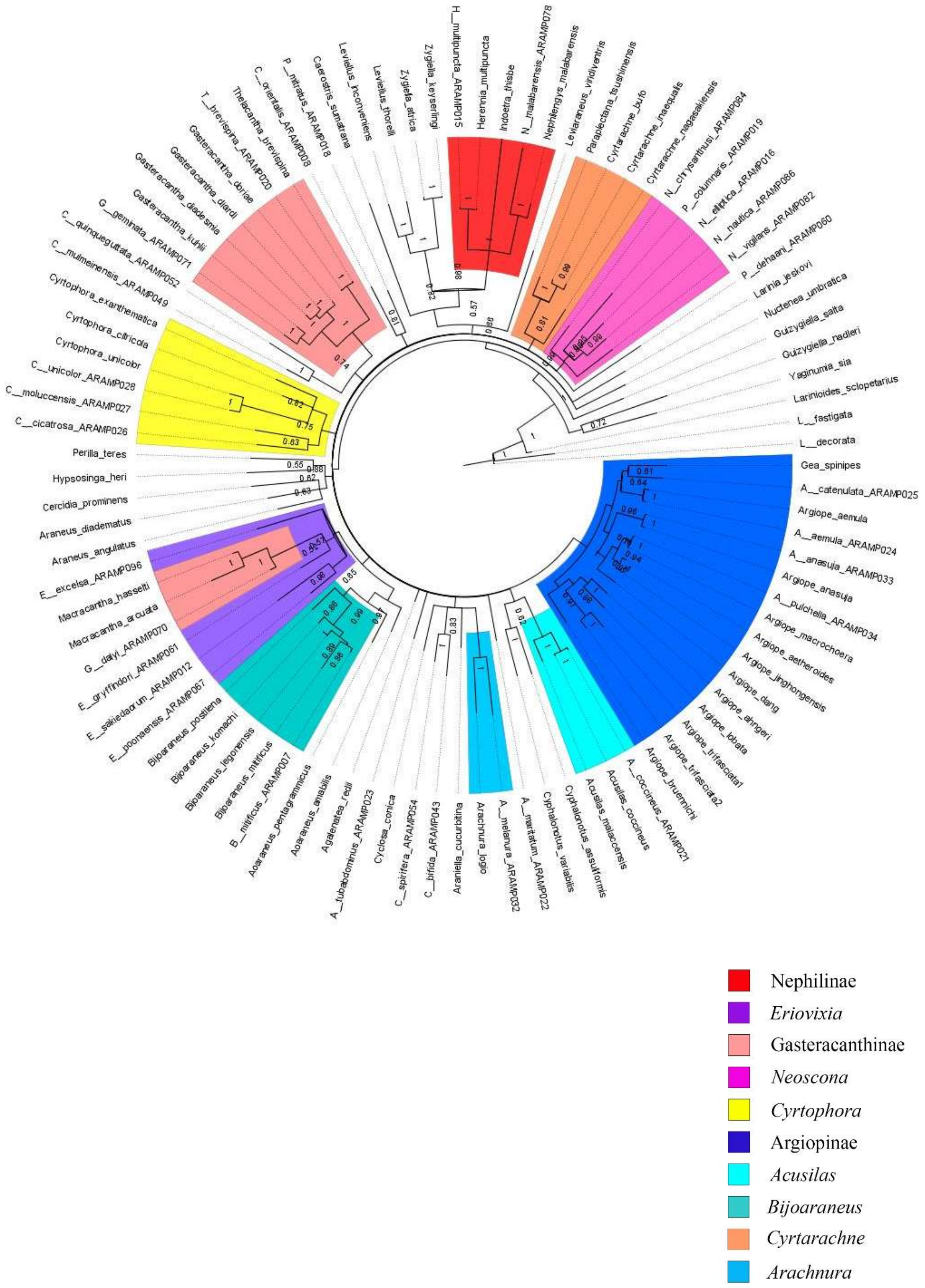


Figure 4.4.12. Bayesian Inference concatenated tree for araneids from Kerala, Oriental and Palaearctic regions.

4.5. Divergence of araneids

The divergence time of araneids was estimated using BEAST v2.7.7. For this, a partitioned dataset including mitochondrial COI and nuclear H3 gene sequences from 83 extant araneid species across the Oriental and Palearctic regions was prepared (Table S1). The outgroup species were the same species used during the phylogenetic analysis. No fossil taxa were used as a tip in this analysis.

The MSA of COI sequences resulted in 788 bp positions and the MSA of the H3 gene had 340 bp positions, thus a total of 1128 bp positions upon concatenation. Model selection was done using jModelTest for both gene alignments separately. TIM2+G was the best model for COI alignment with BIC score = 33751.36, AIC value = 32934.20, and lnL = 16292.1. While for H3 alignment, TIM2ef+I+G was the best scored with BIC = 9250.51, AIC = 8588.10, and lnL = 4121.05.

The site and clock model for both the partitions were unlinked, while the tree was linked. Since the model suggested by the jModelTest for both partitions are the restricted versions of GTR, the substitution model for both partitions were opted as GTR, but the input relative substitution rates were as per the jModelTest result (Table 4.5.1). The clock model used for both partitions was the relaxed clock log normal with varying mean clock rate. The linked trees were implemented using the Birth-Death tree prior. The clade comprising all the araneids was constrained to be monophyletic based on the phylogenetic analysis done during this study. Two points were used to calibrate the tree (Table 3.6). The analysis was done for 50 million MCMC generations sampled every 1000 trees. The convergence of the runs was evaluated using Tracer v1.7.2, and the ESS for all the parameters were above 200. Tree Annotator v10.5.0 was used to burn-in 10% of the trees and to compile the trees.

The analysis resulted in an ultrametric tree (Figure 4.5.1) whose monophyletic clades were congruent with the Bayesian Inference tree that resulted from the phylogenetic analyses

(Figure of concatenated BI tree in O4). The monophyletic clades, such as the subfamily Nephilinae, Argiopinae, Gasteracanthinae and the genera like *Arachnura*, *Neoscona*, *Cyrtarachne*, *Eriovixia*, *Bijoaraneus*, *Cyrtophora*, and *Acusilas*, were retrieved in the divergence time tree with supporting posterior values. The stem age of the family was estimated at 115.39 mya (95% HPD: 113.4 – 117.3). The crown ages of each subfamily and genus are summarised in Table 4.5.2.

Table 4.5.1. Relative substitution rates between nucleotide pairs under the best substitution models for COI and H3 MSA as per jModelTest

	TIM2+G	**TIM2ef+I+G**
R(a) [AC]	0.4079	2.3460
R(b) [AG]	8.9585	6.6172
R(c) [AT]	0.4079	2.3460
R(d) [CG]	1.0000	1.0000
R(e) [CT]	6.9778	8.4524
R(f) [GT]	1.0000	1.0000

Table 4.5.2. Mean crown age estimates and 95% highest posterior density (HPD) intervals for major monophyletic clades inferred from the time-calibrated Bayesian phylogeny.

Subfamily	**Estimated crown age (mya)**	**95% HPD (mya)**
Nephilinae (Pale green)	18.50	16.56 – 20.39
Argiopinae (Pale yellow)	39.78	18.09 – 64.67
Gasteracanthinae (Green)	32.61	18.11 – 48.1
Genera	**Estimated crown age (mya)**	**95% HPD (mya)**
Arachnura (Pale pink)	40.79	5.07 – 35.12
Neoscona (Pale violet)	28.59	10.32 – 49.59
Cyrtarachne (Pale blue)	25.45	6.86 – 47.67
Eriovixia (Blue)	22.4	5.92 – 28.46
Bijoaraneus (Pale red)	11.92	3.86 – 21.87
Cyrtophora (Pale orange)	35.36	16.91 – 56.63
Acusilas (Cyan)	20.18	4.85 – 39.68

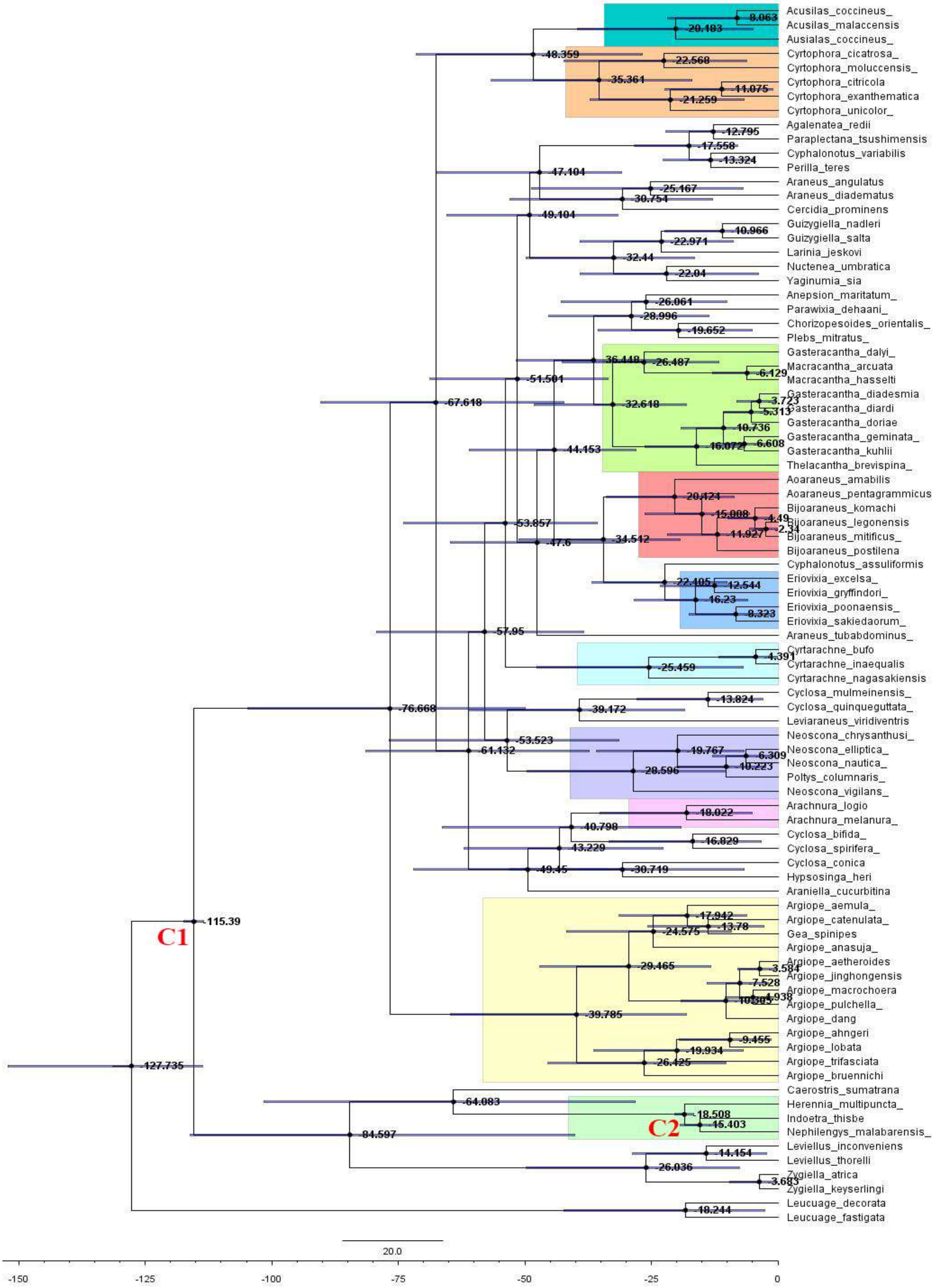


Figure 4.5.1. BEAST inferred time-calibrated tree showing divergence times of Araneidae lineages. Node bars represent 95% HPD intervals; node labels show mean ages in Mya. Highlighted clades correspond to well-supported monophyletic groups. C1 and C2 represent the two calibration points.

CHAPTER 5

DISCUSSION

"***The more I study science, the more I believe in the complexity of nature and the need for humility***"

\- ***Robert Ballard***

This section can be considered as the most important part of a thesis, as the researchers validate the results obtained through the research process. Here, the researcher will compare the results with the previous understandings to substantiate the findings or counter the previous arguments. Thus, a good discussion proves the hypothesis by including the findings and possible practical implications, highlighting the significance of the research.

This section has been organised into five subsections, starting with validating the results of the identification of araneids in Kerala using morphological characteristics. Following, the results of barcoding are validated, emphasising the importance of mitochondrial COI in spider species identification. Phylogenetic relationships between araneids have been discussed later, along with the diversification of araneids in light of evolutionary time.

5.1. Araneids in Kerala

Family Araneidae is the third-largest spider family across the geographical landmasses (WSC, 2025). They are cosmopolitan and can be found in all ecosystems except the cold and harsh environment of Antarctica. These orb-weaving spiders are very common in a biodiversity-rich state like Kerala, India and have been reported from all the major ecosystems.

In 2008, Sunil et al. surveyed Parambikulam Wildlife Sanctuary, documenting 27 araneid species. Sebastian et al. (2005a) studied spiders in irrigated rice ecosystems across varying elevations and reported 25 araneid species spanning 11 genera, while Sudhikumar et al. (2005a) reported that araneids comprised 26.40% of the total species documented from Kuttanad rice agroecosystem. 12 species under 8 araneid genera were reported from Mangalavanam, an ecosensitive mangrove ecosystem (Sebastian et al. in 2005b). Likewise, Sudhikumar et al. (2005b) had documented 17 araneid species belonging to 9 genera from Mannavan Shola forest. Jose et al. (2018) studied the spider diversity in Kavvayi River Basin and resulted in the documentation of 24 species from Araneidae. Rajeevan et al. (2019) investigated riparian forests, reserve forests and coffee plantations, reporting 22 araneids from 9 genera. In 2020, Sumesh & Sudhikumar explored the sacred grooves of Northern Kerala, identifying 51 species under 19 araneid genera. Shabnam et al. (2021) examined spider diversity in coffee, tea, and rubber plantations in Wayanad, listing 23 species from 13 genera. Cashew plantations were also surveyed, revealing 17 araneid species (Smitha & Sudhikumar, 2020). Montane cloud forests were explored by Paul (2021), reporting 29 araneids, while Nafin (2022) focused on Muriyad Kol Wetlands, documenting 34 araneids. Shendurney Wildlife Sanctuary was explored by Sudhin & Sen (2023), documenting 35 species across 17 genera. Araneids in Karuvatta, a coastal island in the Vembanad Lake, were studied by George et al. (2024) and reported 17 species from 11 genera and Vishnu et al. (2021) explored the Poovar mangrove patch and documented 14 species from 10 araneid genera.

Likewise, different araneids have been reported from different parts of Kerala. However, a comprehensive study exclusively on the araneid fauna of the entire state is currently lacking. Hence the primary objective of this study was to prepare a checklist of araneids in Kerala. A total of 60 species representing 23 araneid genera were reported through this study. From India, 188 araneid species under 34 genera have been reported so far (Caleb & Sankaran, 2025). Hence, through this study, it is revealed that 32% of the Indian araneid species are present in Kerala, representing 68% of Indian araneid genera.

The present study provides the first report of *Leviaraneus viridiventris* (Yaginuma, 1969) from India (Shilpa et al., 2023). Similarly, *Chorizopesoides orientalis* (Simon, 1909) was also reported for the first time from India. However, the specimen was subadult, and the morphological characterisation of the genitalia was not possible. The presence of cryptic species in the family Araneidae has been identified in the study. Their morphology needs to be characterised thoroughly, along with the molecular barcodes. In the case of eleven species, only generic identity was revealed. To confirm their species identity, more specimens and a detailed morphological approach supported by molecular evidence are needed.

During the study, certain concerns and challenges in the classical taxonomic approach towards the family Araneidae were identified. A revolution in the study of Indian araneid fauna can be observed during the late 20th and early 21st century (WSC, 2025), and they are supported by very little literature, that too, with improper diagnosis and poor illustrations (Reddy & Patel, 1992; Gajbe, 2004; Biswas & Biswas, 2007). The family should be approached carefully and in detail while shedding taxonomic light, as they exhibit a high level of variation in morphology and behaviour. They are one among the groups that have adopted complex genitalia during the evolutionary course of time (Scharff & Coddington, 1997), and hence, while describing a species, both males and females should give equal consideration. However, more than 50% of Indian araneids are characterised based on a single sex (WSC, 2025), mostly female. Here lies the other concern.

Abdominal polymorphisms and the existence of colour morphs in females have been reported in several araneid genera such as *Neoscona* (Tikader, 1982), *Nephila* (Sankaran et al., 2020), *Thelacantha* (Macharoenboon et al., 2021), etc. Hence, only with a detailed characterisation of the complex palp and epigyne, the identity of a species can be validated. But, in certain cases, new species have been described only based on abdominal colour variations, patterns, number of abdominal patches and tubercles (Keswani, 2013; Patel, 1988; Patel & Reddy, 1993; Gajbe, 2004). Lack of a proper record of the type specimens also makes the taxonomic attempts difficult. These concerns should be addressed as a high priority, as it is making the group taxonomically threatened and vulnerable to being misled.

5.2. Barcoding of araneids in Kerala

Barcoding was a crucial step in the present study. Through barcoding mitochondrial COI and nuclear H3 gene fragments, the morphological identification of the araneids in Kerala was validated. A total of 109 barcodes, including 76 COI and 33 H3, were developed in the present study. Of these, 28 COI and 10 H3 barcodes were the first recorded sequences of the representative species, thus contributing to the global reference library of the molecular world. H3 barcodes were mainly utilised during the phylogenetic analysis, whereas COI barcodes played a vital role in confirming the species identity of confusing and subadult specimens and in validating the identity of morphologically identified araneids.

Genetic distance of some morphologically challenging species such as *Arachnura melanura*, *Cyclosa bifida*, *Cyclosa hexatuberculata*, *Cyclosa spirifera*, *Eriovixia poonaensis*, *Neoscona punctigera*, *Neoscona bengalensis* and *Gea* species were done. The analysis revealed the conspecificity of these species respectively. During the morphological examination of the above-mentioned species, there were discrepancies in the morphological characters including the genitalia. The specimens of each species showed variations in the abdominal colours and patterns. However, the genetic divergence analysis using the COI barcodes revealed their conspecificity by having a genetic distance less than 3%. Tikader (1982) had reported abdominal polymorphism in *Neoscona mukerjei*. From this analysis, it is clear that abdominal polymorphism is existing in other araneid species also, especially the ones belong to the genera *Cyclosa* and *Neoscona*.

The identity of the specimens belonging to the genus *Gea* was not revealed through the genetic divergence analysis using COI barcodes. Though the specimens were found to be conspecific, the genetic distance between the *Gea spinipes* reference sequences showed discrepancies. This could only be resolved through a thorough comparative morphological examination of the specimens with the specimens belonging to the reference sequence.

A visible and striking example of the existence of colour morphs in spiders is *Nephila pilipes*. Indian members of *Nephila pilipes* exist in two colour morphs, the common yellow-black morph and the completely black morph. COI barcoding was done for both colour morphs. The pairwise genetic distance analysis revealed their conspecificity (distance = 0.017), and the results highly support the findings of Sankaran et al. (2020), who reported the colour morphs of *Nephila pilipes* through a detailed morphological examination.

In this family, where extreme sexual size dimorphism predominantly exists, matching females and males caught separately from their natural habitat is very difficult. Even so, the females and males of a species do not show significant differences in their barcodes. In the present study, females and males of three species, *Gasteracantha dalyi*, *Neoscona vigilans* and *Thelacantha brevispina*, were matched using COI barcoding. Females of the three species exhibited a pairwise genetic distance of less than 3% with male specimens, respectively, indicating the conspecificity. Magalhaes (2017) had already proved the potential of COI barcodes in associating the male and female spiders. This analysis can also be considered as evidence in matching the males and females using COI barcodes. The males of *Gasteracantha dalyi* has not been reported yet. Hence, we are reporting it for the first time, however, since the specimen was in subadult condition, the morphological characterisation is impossible.

The existence of cryptic species in the araneofauna has been studied by Gregorič et al. (2025), Oh et al. (2022), and Tyagi et al. (2019). Through this study, we propose three pairs of cryptic species. They are *Argiope pulchella* Thorell, 1881 and *Argiope versicolor* (Doleschall, 1859), *Cyclosa hexatuberculata* Tikader, 1982 and *Cyclosa spirifera*, Simon, 1889, *Eriovixia laglaizei* (Simon, 1877) and *Eriovixia poonaensis* (Tikader & Bal, 1981). All these are morphologically indistinguishable from each other. The genetic divergence analysis using COI barcodes revealed that *A. pulchella* group and *A. versicolor* group are not conspecific. Similarly, *C. hexatuberculata* and *C. spirifera* group showed considerable genetic distance. As the amplification of *E. laglaizei* specimens was unsuccessful, the molecular support for that pair cannot be provided through this study. We are proposing these three pairs of species to be cryptic, however, it should be confirmed only through a detailed examination of on their morphological features, along with multigene barcodes from multiple specimens inhabiting different regions.

The specimen belonging to *Chorizopesoides orientalis* (voucher no ARAMP008) was initially identified as a member of the genus *Chorizopes,* but the species identification was not done since it was subadult. With the help of the COI barcode, it was confirmed that the specimen belongs to the genus *Chorizopesoides* and showed 93.04 percent identity with *Chorizopesoides wulingensis*. Thus, this study supports Barrett & Hebert (2005) that COI barcodes can be efficiently used in species identification of spiders, especially in a family like Araneidae, whose members show a high degree of variations in morphology, genitalia, and behaviour, even the conspecifics.

However, there were challenges in using COI barcodes for species identification. A proper reference sequence that is morphologically validated is required for species identification using molecular barcodes. Although GenBank holds a large number of reference sequences for araneids, the ones that are supported by published data are fewer. Also, they accept the sequences without morphological validation, which leads to heaping of misleading sequences, even so, their terms and conditions for sequence submission are being modified. Hence, careful retrievals of the reference sequences are needed for further analysis. Similarly, an appropriate threshold value is required for the species identification using DNA barcodes. In the case of araneofauna, a delimitation threshold value between 2% to 3.6% is used according to the group under consideration (Barrett & Hebert, 2005; Candek & Kuntner, 2015; Gaikwad et al., 2017). Later, Tyagi et al. (2019) extended this range to 2.6% to 3.7%. Hence, careful selection of the delimitation threshold is required according to the group. In this study, a threshold value of 3% was used for species identification, as a barcode gap exists in the family Araneidae (Candek & Kuntner, 2015).

5.3. Interrelationship of araneids in Kerala

The phylogenetic relationships of araneids in Kerala were revealed through this study. The analysis was based on the partial sequences of the mitochondrial COI gene and the nuclear H3 gene. Phylogenetic trees for the gene COI, H3 and concatenated data were reconstructed. Phylogenetic analysis was conducted using four different methods: Neighbour Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). Thus, a total of 12 trees were constructed in this analysis.

The COI gene tree was reconstructed based on 55 sequences representing 52 araneid species. Of these, 13 species were the only representatives of their respective genus. Hence, to understand the phylogenetic nature of that particular genus was not possible, however, their relationship with other genera in the family can be retrieved. In all four trees, the outgroup species were placed outside the ingroup clade, representing the monophyly of the family Araneidae. Subfamilies such as Nephilinae, Argiopinae, and Gasteracanthiane were retrieved in the trees. The monophyly of Nephiliane and its placement as the basal lineage of the family Araneidae was congruent in all trees. Similarly, congruence can be observed in the subfamily Argiopinae, which appeared as monophyletic. Members of the subfamily Gasteracanthinae were scattered among trees, tending to be paraphyletic.

The phylogenetic nature of the genus *Neoscona* showed discrepancies across the trees. In NJ tree, all them members gathered to be monophyletic, while in other trees they were scattered and exhibiting a paraphyletic nature. Similar condition was exhibited with the genus *Cyclosa*. They appeared monophyletic only in the NJ tree, and rendered a polyphyly in other trees. Even so, *Cyclosa confraga* and *Cyclosa mulmiensis* were always placed with other taxa, especially sharing a paraphyly with *Poltys columnaris* and *Leviaraneus viridiventris*. Since only two representatives from the genus *Araneus* were able to be included in this analysis. Based on those two, it is not efficient to conclude on the phylogenetic nature of this largest genus. However, their occurrence patterns across the trees can be mentioned. Although in both NJ and MP trees, two species formed a monophyletic clade, their paraphyly in ML and BI trees should be highlighted. A polyphyletic nature of the genera *Cyrtophora* and *Eriovixia* has been observed in all trees.

The H3 gene tree reconstruction was based on 33 sequences representing 33 araneid species. Among them, 12 species were the only representative of their genus. As in COI gene trees, the outgroup was properly placed outside, separating the araneid clade. Even though they are only represented by two species, *Herennia multipuncta* and *Nephilengys malabarensis*, the subfamily Nephilinae maintained its monophyly across the trees. Gasteracanthinae rendered polyphyly by forming a monophyletic clade including *Gasteracantha geminata* and *Thelacantha brevispina* and a scattered lineage of *Gasteracantha dalyi*. The genus *Argiope* also maintained its monophyly in all trees. Unlike COI gene trees, members of the genus *Cyrtophora* clustered in a monophyletic clade. *Cyrtophora* clade exhibited a sister relation to the Argiopinae clade along with *Acusilas coccineus*, which was congruent in H3 gene trees. Similarly, the paraphyly of *Cyclosa* and the clustering of the *Eriovixia* were congruent in all trees. Another congruence observed was the appearance of *Poltys columnaris* within the monophyletic clade of *Neoscona*. Despite this observation, a sister relationship between these two genera cannot be confirmed due to the limited representation of *Poltys*. As the genus *Araneus* was also represented by only a single species, their phylogenetic nature cannot be confirmed through the H3 gene trees obtained through this analysis.

The concatenated phylogenetic tree was based on the partitioned dataset including COI and H3 sequences from 33 araneid species. 12 terminals were the only representation of their respective genera. The tree was rooted on the outgroup species, which was placed outside the ingroup clade. With the representation of *Herennia multipuncta* and *Nephilengys malabarensis*, the subfamily Nephilinae appeared to be monophyletic in all four trees. The genus *Argiope* also maintained its monophyly across the trees. As in the H3 gene trees, the subfamily

Gasteracanthinae seems to be polyphyletic, with a monophyletic clade including *Gasteracantha geminata* and *Thelacantha brevispina* and a scattering lineage of *Gasteracantha dalyi*.

The polyphyletic nature of the genus *Cyclosa* was congruent across the trees. Even though *Eriovixia* rendered a paraphyletic nature in NJ and MP trees, they were clustered together in a clade in ML and BI trees. However, they cannot be called a monophyletic clade as *Bijoaraneus mitificus* and *Gasteracantha dalyi* were paraphyletic to the clade, yet with moderate support. *Neoscona* species were clustered together, exhibiting a monophyletic nature. However, in ML and BI trees, *Poltys* showed a similar relation, as in H3 gene trees, with moderate to high support. Polyphyly of *Cyrtophora* is congruent in all four trees. *Araneus*, with a single species representation, was scattered across the trees, either rendering a sister relationship with *Neoscona* or *Argiope*. Hence, its phylogenetic nature cannot be confirmed.

Most of the monophyletic clades were retrieved in all three categories of trees. The placement of two outgroup species was congruent in the COI gene tree, H3 gene tree and concatenated tree, which highlighted the monophyly of the family Araneidae as suggested by Scharff & Coddington (1997) and Scharff et al. (2020). Although the overall topology of these trees looks different, certain phylogenetic relationships maintained the congruence throughout. The phylogenetic nature of the subfamily Nephilidae and its basal placement within the ingroup clade highly support the literature (Hong-Chun et al., 2004; Kuntner et al., 2013; Kallal et al., 2018).

Similarly, the subfamily Argiopinae repeated the monophyly in gene trees and concatenated trees, as suggested by Levi (1983) and Scharff & Coddington (1997). However, through the multigene approach, Scharff et al. (2020) claimed the polyphyly of *Argiope,* which was not observed in any of the reconstructed trees. In none of the trees, the subfamily Gasteracanthinae expressed monophyly, which is congruent with the results of Scharff & Coddington (1997). Tan et al. (2019a) had suggested the monophyly of the subfamily. This discrepancy may be resolved by including more members of the subfamily.

The analysis also suggests the polyphyly of the genus *Araneus* (Scharff & Coddington, 1997; Scharff et al., 2020), but a confirmation is limited due to the low number of taxa. The paraphyly of the genus *Eriovixia* was confirmed as this relationship was repeated in all trees. Scharff et al. (2020) got *Neoscona* as a monophyletic clade with strong bootstrap and posterior probability support, however, it was taxon-limited. In the present analysis, it was able to include more members of the genus. Only in the COI NJ gene tree, *Neoscona* expressed monophyly. In the rest, they clustered together in a clade, paraphyletic to *Poltys columnaris*. Even so, this paraphyly cannot be confirmed as the genus *Poltys* was taxon-limited. Including more members of *Poltys* might help to resolve this relationship better.

As the literature suggested (Scharff & Coddington, 1997; Scharff et al., 2020), the polyphyly of *Cyclosa* is confirmed. The genus *Cyrtophora* showed a paraphyletic relation in concatenated trees, polyphyly in COI gene trees and monophyly in H3 gene trees. They also expressed a sister group relation with the Argiopines, suggested by Scharff & Coddington (1997) and Scharff et al. (2020). This discrepancy in the phylogenetic relationship of *Cyrtophora* can be resolved by including more gene barcodes.

It is evident that H3 gene trees were resolved better than the COI gene trees. This might be because the slowly evolving H3 gene has more information on the divergence of araneid lineages than the fast-evolving COI gene. However, compared as a whole, the concatenated trees drew the resolved phylogenetic relationships of araneids. Similarly, ML and BI trees are more efficient in concluding the phylogeny of araneids in Kerala than the NJ and MP trees. It is suggested that including more taxa in single-species genera might resolve the trees better and tackle the discrepancies existing in the phylogeny of araneids in Kerala.

5.4. Interrelationships of araneids in Kerala with Oriental and Palearctic araneids

The phylogenetic relationships of araneids in Kerala with the Oriental and Palaearctic araneids were reconstructed in this analysis. The reconstruction was based on the mitochondrial COI gene and the nuclear H3 gene sequences. Gene trees of COI and H3 were analysed separately, followed by the analysis of concatenated trees. Neighbour Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) were the methods used to construct the trees. Thus, a total of 12 trees were constructed in this analysis.

The COI gene trees were reconstructed utilising 253 COI gene sequences across the Oriental and Palaearctic regions, including the sequences developed in the present study. Of these, 18 terminals were single-species representatives of their respective genera. Thus, understandings about their phylogenetic relationships are limited in this study and can be resolved better by including more taxa. In all, COI gene trees acquired through four different phylogenetic methods, the outgroup species were placed outside the ingroup clade, representing the monophyly of the family Araneidae. Genera that maintained monophyly with high support across all the trees are *Acusilas*, *Araneilla*, *Bijoaraneus*, *Hypsosinga*, *Leviaraneus*, and *Plebs*. The polyphyly of *Cyclosa*, *Eriovixia*, and *Neoscona* was congruent in all trees. The genus *Aoaraneus*, rendered a polyphyly, but *Aoaraneus pentragramicus* always showed a paraphyletic relation with the *Bjioaraneus* clade.

The subfamily Nephilinae was represented as two clades in all trees, one including *Nephila* and *Indoetra* species and the other with *Herennia* and *Nephilengys* species, rendering a paraphyletic relationship between the clades. The subfamily Zygiellinae showed a polyphyly across the trees. The phylogenetic status of the subfamily Argiopinae was polyphyletic in the MP and BI trees, while all the species grouped in a monophyletic clade except *Argiope ocula* and *Argiope caesarea* in the NJ and ML trees. Similarly, the subfamily Gasteracanthinae also showed discrepancies in their phylogenetic status across the trees. They showed monophyly in the NJ tree and polyphyly in MP trees, whereas a paraphyletic relationship was observed in both ML and BI trees. The genus *Cyrtarachne* also provided confusion regarding its phylogenetic placement. In all trees, *Cyphalonotus variabilis*, *Perilla teres*, *Paraplectana tsushimensis*, and *Agalenatea redii* grouped in a clade which was placed as the basal clade of the ingroup in the NJ and MP trees, while within the group in the ML and BI trees.

H3 gene trees were also reconstructed, based on 94 H3 sequences, including the outgroup species. The placement of outgroups and the monophyly of the family Araneidae were congruent across the H3 gene trees. The subfamily Nephilidae was monophyletic and was placed as the basal clade among the ingroups. This placement and relationship appeared in all trees. Similarly, the monophyly of the subfamily Argiopinae was also maintained across the trees. In all trees, except the MP tree, the subfamily Gasteracanthinae formed a monophyletic clade without including *Gasteracantha dalyi*, which always gave a paraphyletic relationship with the *Eriovixia* clade. In the MP tree, the placement of *Gasteracantha dalyi* was congruent with other trees, however, the monophyletic clade including other members of the subfamily was split into two paraphyletic clades, the *Gasteracantha* + *Thelacantha* clade and *Macracantha* clade. Members of the subfamily Zygiellinae formed a clade along with *Leviellus* and were placed outside the ingroup araneids as Nephilinae.

Monophyly of *Acusilas, Arachnura*, *Bijoaraneus*, and *Cyrtophora* was repeated across the trees. In the NJ tree, *Argiope*, *Cyrtophora*, and *Acusilas* clades shared a common ancestor. But this relation was not observed in the MP tree. Whereas in the ML and BI tree, *Cyrtophora* and *Acusilas* shared a paraphyletic relationship. Likewise, there was congruence in the paraphyletic relationship between *Bijoaraneus*, *Aoaraneus*, and *Leviaraneus*. The genera *Cyrtarachne* and *Paraplectana* shared paraphyly across the trees. Members of the genus *Eriovixia* clustered together and exhibited a paraphyly with *Gasteracantha dalyi*. Similarly, members of the genus *Neoscona* clustered in a clade exhibiting a paraphyletic relationship with *Poltys*. Polyphyly of *Cyclosa* was repeated in H3 gene trees as in COI gene trees.

It was found that 94 terminals from the COI and H3 gene trees had both COI and H3 sequence data, and hence they were partitioned to reconstruct the concatenated phylogenetic trees of araneids in the Oriental and Palaearctic regions. As in the gene trees, the outgroups were properly placed and exhibited the monophyly of the family Araneidae. A congruence existed in the monophyly of the subfamily Nephilinae, and its basal placement within the ingroup clade was achieved in all trees except the BI tree. Similarly, the monophyly of the subfamily Zygiellinae were repeated, and its basal placement was congruent in all trees except the BI tree. The phylogenetic relationship of the subfamily Gasteracanthinae was not congruent across the trees. In the NJ tree, they formed a monophyletic clade, while in the MP and ML trees, they exhibited a paraphyletic relationship. However, in the BI tree, they showed a polyphyly with two distinct monophyletic clades, the *Gasteracantha* + *Thelacantha* clade and the *Macracantha* clade. The subfamily Argiopinae exhibited a monophyletic clade across the trees, except in the NJ tree, in which *Argiope dang* and *Argiope catenulata* were separated from the monophyletic clade, resulting in a polyphyly.

In congruence with the gene trees, *Acusilas*, *Arachnura*, and *Bijoaraneus* exhibited monophyletic relationships, while *Cyclosa* showed high polyphyly, and *Eriovixia* appeared as paraphyletic. Similarly, a paraphyletic relationship was exhibited by *Bijoaraneus* and *Aoaraneus*. The genus *Cyrtophora* exhibited a paraphyly, and conflicts were observed in its relationships with *Acusilas* and Argiopinae. Only in the ML tree, these three clades shared a common ancestor, while in the BI tree, Argiopinae and *Acusilas* shared the ancestor, and in the NJ tree, *Cyrtophora* and *Acusilas* diverged from a common ancestor. As in the H3 gene trees, *Neoscona* appeared to be monophyletic with an additional paraphyletic relationship with *Poltys*. However, this pattern was absent in the NJ tree. The *Cyrtarachne* + *Paraplectana* clade was observed only in the BI tree.

The monophyly of the family Araneidae was revealed in this analysis, which is congruent with the results of Scharff & Coddington (1997) and Scharff et al. (2020). The subfamily Nephilinae formed a monophyletic clade and was positioned as the basal clade in both the H3 gene tree and the concatenated tree, while the subfamily was distributed into two clades having a paraphyletic relationship. However, it is evident that the subfamily is positioned as the basal clade within the araneid ingroup, strongly supporting the placement of Nephilidae as a subfamily of Araneidae (Scharff & Coddington, 1997; Hong-Chun et al., 2004; Kuntner et al., 2013; Kallal et al., 2018).

The discrepancies regarding the phylogenetic status of the subfamily Gasteracanthinae were observed in all trees, which supports the polyphyly of the subfamily as suggested by Scharff & Coddington (1997). Even so, all the NJ trees revealed a monophyly for the subfamily, which supports Tan et al. (2019a). This could be resolved through a multigene approach. Monophyly of the subfamily Argiopinae was congruent throughout the H3 gene trees and concatenated trees, while the genus *Argiope* showed a polyphyletic nature. This observation was also made by Scharff et al. (2020). The monophyly of the genus *Cyrtophora* observed in the analysis was supported by literature (Scharff & Coddington, 1997). A sister relationship between the Argiopinae and *Cyrtophora* was suggested by Scharff & Coddington (1997) and Kallal et al. (2018), which was observed in H3 gene trees and concatenated trees. The placement of the subfamily Zygiellinae and Nephilinae as the basal clade is congruent with the findings of Kallal & Hormiga (2018a, b), who suggested Zygiellinae and Nephilinae as the earliest diverged lineages of the family Araneidae.

The monophyly of the genera such as *Acusilas*, *Arachnura*, *Bijoaraneus*, *Araneilla*, and *Plebs* was confirmed as they showed steady relationships across the trees. Similarly, a paraphyletic relationship between *Bijoaraneus* and *Aoaraneus* was also confirmed, which supports the findings of Tanikawa et al. (2021). Although the genus *Perilla* was represented by a single species, its placement in the ingroup was congruent throughout the analysis, which accepts its placement by Kuntner (2002).

The members of *Neoscona* were highly represented in COI gene trees, and they exhibited a polyphyletic relationship. However, in the H3 gene trees and concatenated trees, they grouped in a monophyletic clade with limited taxa. This discrepancy can be solved through a multigene approach towards the genus, thereby increasing the taxa for the analysis. In H3 gene trees and concatenated trees, the paraphyly of *Neoscona* and *Poltys* was congruent, even it cannot be confirmed as *Poltys* was underrepresented in the analysis. Schraff & Coddington (1997) suggested a monophyly for *Cyrtarachne*, however, in the present analysis, they appeared to be paraphyletic. The analysis also provides additional support to the polyphyletic nature of *Cyclosa* (Scharff & Coddington, 1997; Scharff et al, 2020. Yu et al. (2022) suggested a clade combining *Cyphalonotus* and *Poltys*, but no such relationship was observed in none of the trees. Even so, it could not be confirmed that there is no relationship, as both genera were underrepresented in the analysis. It should also be noted that the monophyly of araneids in Kerala, even a particular genus, is not supported by any of the trees.

By analysing trees from three datasets, it is clear that, higher the number of taxa, the better the resolution of phylogenetic relationships. To understand the evolutionary relationships of a highly divergent group like Araneidae, a multigene approach is more appropriate. The resolution of the phylogenetic status of each taxon has increased while the individual genes were partitioned. Similarly, Maximum Likelihood and Bayesian Analysis will be more suitable to elucidate the evolutionary history using the molecular data than the other two methods. However, Maximum Parsimony is the best method when the morphological character matrix and molecular data are compiled.

5.5. Divergence time of araneids

A time-calibrated phylogenetic tree was constructed based on 83 extant araneid species using a relaxed clock log-normal model. The time tree was calibrated with two points, *Mesozygiella dunlopi* (115 – 121 Mya) (Magalhaes et al., 2020) and *Nephila* stem (16 Mya) (Wunderlich, 1986). The araneid ingroup clade was constrained based on the results obtained during the phylogenetic analysis. Similarly, the Nephilinae clade was also constrained.

The resulting ultrametric tree retrieved most of the monophyletic clades from the phylogenetic analysis. The stem age of the family was estimated as 115.39 mya (95% HPD: 113.4 – 117.3). This estimated age is congruent with the fossil records from the Cretaceous amber (Penney & Ortuño, 2006). Wunderlich (2004c) suggested the earliest fossil of *Nephila* from 43-47 mya, and Kuntner et al. (2013) proposed the nephilid ancestral age as 40-60 mya. However, the estimated crown age of the subfamily Nephilinae is 18.50 mya (95% HPD: 16.56 – 20.39). Representatives of *Nephila* were not included in this analysis rather, other members such as *Herennia*, *Nephilengys*, and *Indoetra* were included. Including the *Nephila* might improve the crown age, which will be congruent with Wunderlich's (2004c) findings. The stem age of the Nephilinae clade was estimated as 64.08 mya, which suggests the ancestral age of nephilids as Kuntner et al. (2013). The results of the analysis also confirm that the above-mentioned members of Nephilinae as the recently diverged lineages.

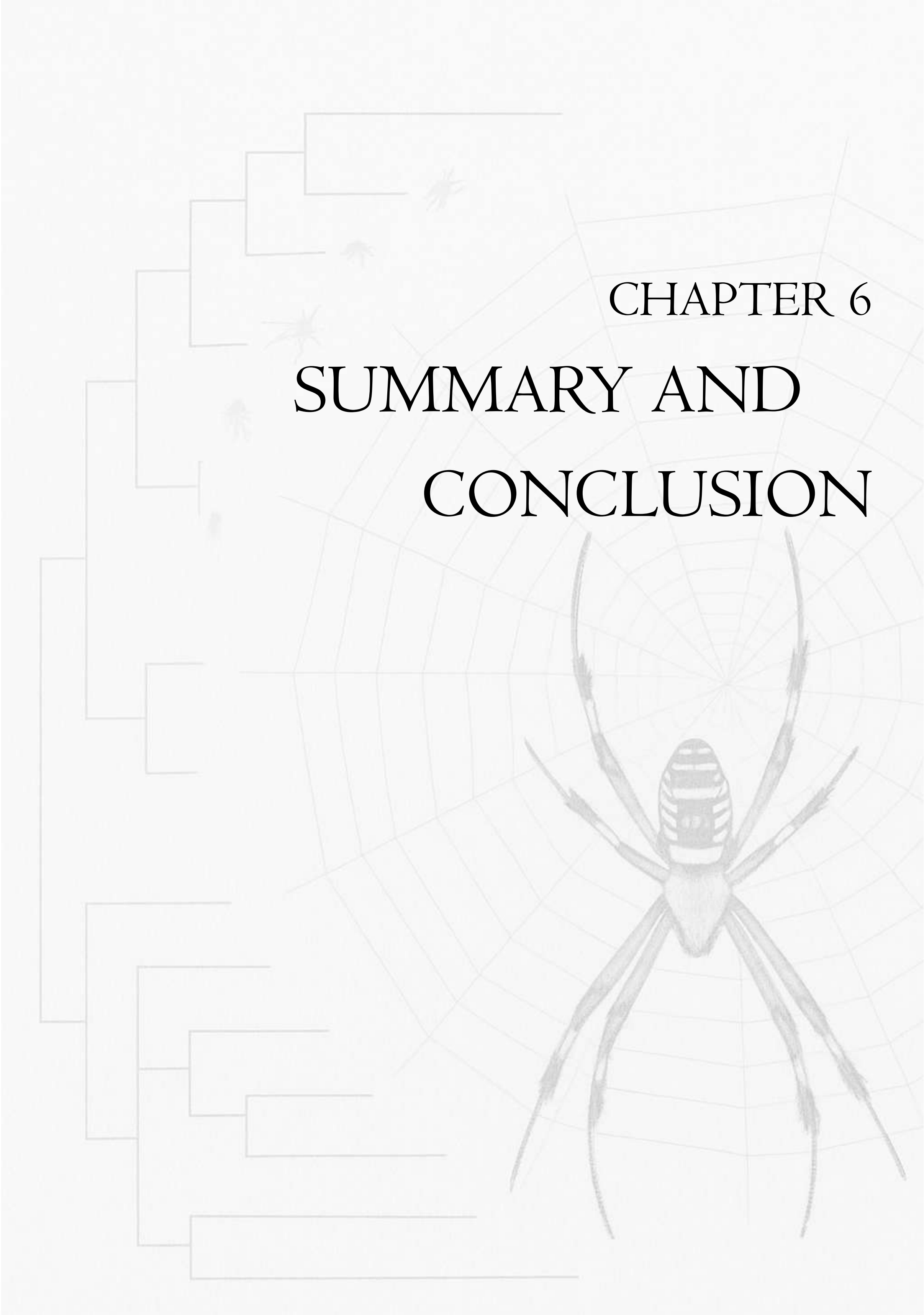
The stem age of the ARA clade (Scharff et al., 2020) was estimated as 76.66 (95% HPD: 49.89 – 104.73). Hong (1985) reported a fossil of *Argiope* from the Neogene, and the time-calibrated tree estimates the crown age of Argiopines as 39.78 mya (95% HPD: 18.09 – 64.67), suggesting the origin of the subfamily in the Paleogene. The tree also suggests some recently diverged lineages within the subfamily Argiopinae. Similarly, the crown ages of Gasteracanthinae, *Archnura*, and *Cyrtophora* date back to the Paleogene. The tree suggests that the divergence of *Cyclosa* has occurred during 30.71 – 13.84 mya. The time-calibrated tree suggests some recently diverged lineages in the groups such as *Acusilas*, *Bijoaraneus*, *Cyrtarachne*, *Cyrtophora*, *Eriovixia*, Gasteracanthinae, and *Neoscona*.

A comprehensive study exclusively to understand the evolutionary time scale of the divergence of araneids has not been reported yet. Schraff et al. (2020) attempted to understand the divergence of araneids using uncorrelated log-normal and uncorrelated exponential clock models. Even after trying different models and parameters, the MCMC generations were not converging effectively. They often suggested using the uncorrelated log-normal clock model for dating the divergence of araneids. In the present study, a relaxed log-normal model was utilised as Scharff et al. (2020) suggested.

Therefore, this study represents the first comprehensive and successful attempt to date the divergence time of the family Araneidae. However, the results of this analysis are biased as the analysis includes only representatives from the Oriental and Palaearctic regions, while taxa from other realms were excluded. Considering the cosmopolitan distribution and high diversity of the family, compiling complete global data within a limited time frame is unattainable. Hence, this study serves as a foundational framework and is recommended for future expansion by including representatives from other biogeographical landmasses.

CHAPTER 6

SUMMARY AND CONCLUSION



“***What we know is a drop, what we don’t know is an ocean***”

- ***Isaac Newton***

6.1. Summary

The present study aimed to compile an account of araneids, the orb-weaving spiders, in Kerala through both morphological and molecular approaches. The study also aimed to understand the phylogenetic relationships between araneid taxa and to plot them in an evolutionary time scale. A classical taxonomic approach was employed using advanced microscopic techniques to describe the species recorded in this study. Molecular barcoding was performed through DNA extraction and sequencing, and the resulting sequences were analysed using phylogenetic methods including Neighbour-Joining, Maximum Parsimony, Maximum Likelihood, and Bayesian Inference. Bioinformatics tools such as MEGA, RAxML, MrBayes, and BEAST were used to construct phylogenetic trees and to assess evolutionary relationships and divergence time estimation.

Through the study, a checklist of araneids, including 60 species from 23 genera, was prepared. These statistics represent the presence of 32% of Indian araneid species and 68% of Indian araneid genera in Kerala. The study reported two species, *Leviaraneus viridiventris* and *Chorizopesoides orientalis*, from India for the first time. With a detailed account on the morphological characterisation and molecular support, the study recommends the transfer of *Araneus panchganiensis* to the genus *Neoscona*.

Barcoding of araneids in Kerala resulted in the development of 76 mitochondrial COI and nuclear H3 gene barcodes. All the developed barcodes were submitted to GenBank and were accepted by providing accession numbers for each entry. The present study was able to develop 28 COI barcodes and 10 H3 barcodes as the first sequence representative of the particular species in GenBank. The COI barcoding of *Nephila pilipes* also confirmed the existence of colour morphs in Kerala. Combining the morphological and molecular data revealed the presence of three pairs of cryptic species, *Argiope pulchella* - *Argiope versicolor*, *Cyclosa hexatuberculata* - *Cyclosa spirifera*, and *Eriovixia laglaizei* - *Eriovixia poonaensis*.

The phylogenetic relationships of araneids in Kerala were revealed through this study. The monophyly of the subfamily Nephilidae and its inclusion in the araneid clade at a basal position were confirmed. Similarly, the monophyly of the subfamily Argiopinae and the polyphyly of Gateracanthinae were revealed. *Cyclosa* and *Araneus* were found to be highly polyphyletic. Paraphyly of *Cyrtophora*, *Eriovixia* and *Neoscona* were congruent throughout the analysis.

By including the araneid representatives from the Oriental and Palearctic regions, the phylogeny of the family has been resolved better. The family itself exhibited monophyly. The results highly support the placement of nephilines and zygiellines in the family Araneidae, giving a subfamily status. The discrepancies exhibited by the subfamily Gasteracanthinae were supported by the literature. The monophyly of the subfamily Argiopinae and its sister relationship to *Cyrtophora* were evident. The monophyly of *Acusilas*, *Arachnura*, *Bijoaraneus*, *Araneilla*, and *Plebs* was proved undoubtedly. Similarly, the results support the polyphyly of *Cyclosa* and *Neoscona*. Additionally, none of the results exhibited a monophyletic clade of araneids in Kerala.

This study provides the first comprehensive successful attempt to date the divergence of araneids. It was estimated that the divergence of areneids occurred at 115.39 mya (mean value), which supports the fossil evidence. Similarly, the diversification times of Nephilinae, Argiopinae, Gasteracanthinae, *Archnura*, and *Cyrtophora* date back to the Paleogene. The analysis also suggests the lineages of *Acusilas*, *Bijoaraneus*, *Cyrtarachne*, *Cyrtophora*, *Eriovixia*, and *Neoscona* as recently diverged.

6.2. Conclusion

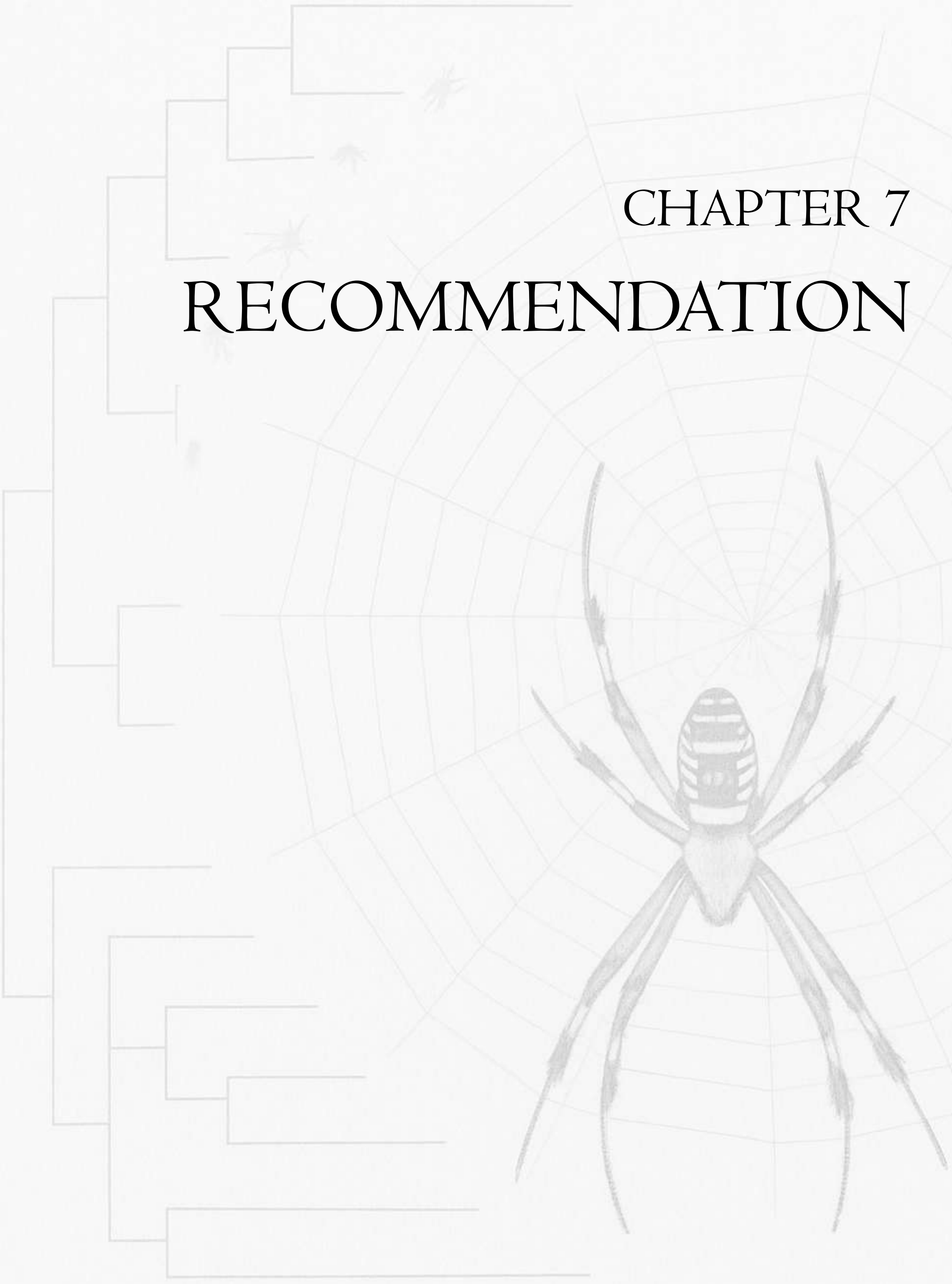
The present study demonstrates the successful integration of traditional morphology and modern molecular tools in understanding the biodiversity of this pristine planet. The findings reaffirm that molecular approaches complement rather than replace classical taxonomy, countering the long-standing concern that DNA-based techniques may overshadow the Linnaean system. The combined evidence from morphology and barcodes improved species identification and provided clearer understanding of phylogenetic relationships among araneids in Kerala.

Certain challenges in the classical taxonomic approach towards the classification of araneids have been addressed through this study. The results strongly support Maximum Likelihood and Bayesian Inference as the most robust approaches for reconstructing araneid phylogeny, and confirm the high efficacy of COI barcoding in resolving taxonomic ambiguities. This study therefore asserts that concatenated multi-gene datasets are essential for addressing inconsistencies in spider phylogeny. However, limited species representation in certain genera constrained deeper phylogenetic resolution, emphasizing the need for broader sampling.

Overall, the study contributes novel insights into the evolutionary history of Araneidae and highlights that a combined morphological–molecular framework should be adopted as a standard practice in spider taxonomy. Future research incorporating wider geographic sampling and additional molecular markers will be crucial for establishing a more comprehensive phylogeny of araneids.

CHAPTER 7

RECOMMENDATION



“***The important thing is not to stop questioning. Curiosity has its own reason for existing***”

\- ***Albert Einstein***

Araneidae, the third-largest spider family in the world, holds significant ecological and scientific importance. The present study was an attempt to provide a checklist of araneid species in a biodiversity-rich state like Kerala, resolving taxonomic ambiguities by utilising molecular data. Additionally, it also aimed to provide insights into the phylogenetic relationships of araneids and present them within an evolutionary time scale. Even though several studies on Araneidae have been conducted globally, notable gaps persist, paving the way for future research. The following recommendations will enrich the robustness of our understanding of this significant group of arthropods.

- Araneidae, being cosmopolitan, can be found in all kinds of ecosystems. In a biodiversity-rich state like Kerala, the number of species reported is quite low, and yet more to discover. Hence, studies focusing exclusively on different habitat types throughout different seasons will increase the statistics of araneids.
- It is recommended to utilise the modern visualising techniques such as Scanning Electron Microscope (SEM), high resolution stereomicroscopes, etc., in detailing the morphological characterisation of a species, which will aid in resolving the taxonomic ambiguities, such as cryptic species.
- Barcoding multiple markers of all species is recommended so that it could help in resolving taxonomic confusions and cryptic species identification. Also, it could be utilised for further phylogeny, biogeography and population studies.
- By incorporating barcode data with morphological characterisation, cryptic species could be identified. From the present study, three pairs of cryptic species were identified, combining

both approaches. Being a highly diverse group, Araneidae possess several other cryptic species, which are still unknown.

- Generic revisions of some commonly found araneid groups, such as *Cyclosa*, *Gasteracantha*, *Eriovixia*, and *Neoscona,* need to be done with high priority. The combination of both morphological and molecular data would increase the robustness of the generic revision.
- Although the phylogenetic status of several araneid taxa is resolved, there is a lot more to unwind. This could only be possible by including taxa from different geographical regions along with multiple barcode data.
- In the present study, only a limited number of taxa were utilised for the divergence time analysis. More insights could be achieved by including taxa from different geographical regions.
- Araneidae, being a cosmopolitan group, can be utilised in biogeographical studies and has the potential to reveal the biogeographic distribution across the landmasses and thereby unveil the mask of unknown truths regarding the continental formation.
- Further investigations on the web-building behaviour of araneids should be carried out, especially in highly populated cities, as they have the potential to indicate air pollution.

REFERENCE

Adobe Inc. (2020). *Adobe Photoshop* (Version 2020). [Computer software]. https://www.adobe.com/photoshop

Ahmed, J., Khalap, R., & Sumukha, J. N. (2016). A new species of dry foliage mimicking *Eriovixia* Archer, 1951 from central Western Ghats, India (Araneae: Araneidae). *Indian Journal of Arachnology*, *5*, 24-27.

Alonso, J., Arillo, A., Barrón, E., Corral, J. C., Grimalt, J., López, J. F., López, R., Mart´ınez-Delclos, X., Ortu˜no, V., Pe˜nalver, E., & Trincão, P. R. (2000). A new fossil resin with biological inclusions in Lower Cretaceous deposits from Álava (Northern Spain, Basque-Cantabrian Basin). *Journal of Paleontology*, *74*(1), 158-178. https://doi.org/10.1666/0022-3360(2000)074<0158:ANFRWB>2.0.CO;2

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Anderson, J. S. (2012). Fossils, molecules, divergence times, and the origin of Salamandroidea. *Proceedings of the National Academy of Sciences*, *109*(15), 5557-5558. https://doi.org/10.1073/pnas.1202491109

Archer, A. F. (1951). Studies in the orb weaving spiders (Argiopidae). 1. *American Museum Novitates*, *1487*, 1-52.

Asalatha, P., & Prasadan, P. K. (2019). Two new records of genus *Eriovixia* Archer, 1951 (Araneae: Araneidae) from the Western Ghats, Wayanad region. *Serket*, *16*(4), 166-169.

Asalatha, P., & Prasadan, P. K. (2020). First record of the spiders *Neoscona usbonga* Barrion & Litsinger, 1995 and *Hamataliwa pentagona* Tang & Li, 2012 (Araneae: Araneidae & Oxyopidae) from India. *Serket*, *17*(2), 97-100.

Ashfaq, M., Blagoev, G., Tahir, H. M., Khan, A. M., Mukhtar, M. K., Akhtar, S., Butt, A., Mansoor, S., & Hebert, P. D. (2019). Assembling a DNA barcode reference library for the spiders (Arachnida: Araneae) of Pakistan. *PLoS One*, *14*(5), e0217086. https://doi.org/10.1371/journal.pone.0217086

Astrin, J. J., Huber, B. A., Misof, B., & Kluetsch, C. F. (2006). Molecular taxonomy in pholcid spiders (Pholcidae, Araneae): evaluation of species identification methods using CO1 and 16S rRNA. *Zoologica Scripta*, *35*(5), 441-457. https://doi.org/10.1111/j.1463-6409.2006.00239.x

Astrin, J. J., Höfer, H., Spelda, J., Holstein, J., Bayer, S., Hendrich, L., Huber, B. A., Kielhorn, K., Krammer, H., Lemke, M., Monje, J. C., Morinière, J., Rulik, B., Petersen, M., Janssen, H., & Muster, C. (2016). Towards a DNA barcode reference database for spiders and harvestmen of Germany. *PloS one*, *11*(9), e0162624. https://doi.org/10.1371/journal.pone.0162624

Audouin, V. (1826). Explication sommaire des planches d'arachnides de l'Égypte et de la Syrie. In M. J. C. L. de Savigny (Ed.), *Description de l'Égypte, ou recueil des observations et des recherches qui ont été faites en Égypte pendant l'expédition de l'armée française, publié par les ordres de sa Majesté l'Empereur Napoléon le Grand. Histoire Naturelle* (Vol. 1, No. 4).

Baehr, B., & Baehr, M. (1987). The Australian Hersiliidae (Arachnida: Araneae): taxonomy, phylogeny, zoogeography. *Invertebrate Taxonomy*, *1*(4), 351-437. https://doi.org/10.1071/IT9870351

Ballesteros, J. A., & Hormiga, G. (2021). Molecular phylogeny of the orb-weaving spider genus *Leucauge* and the intergeneric relationships of Leucauginae (Araneae, Tetragnathidae). *Invertebrate Systematics*, *35*(8), 922-939. https://doi.org/10.1071/IS21029

Barcode of Life Data Systems. (2025). *BOLD: The Barcode of Life Data System.* Araneae | Taxonomy Browser | BOLDSYSTEMS. Accessed on 10 February 2025.

Barrett, R. D., & Hebert, P. D. (2005). Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, *83*(3), 481-491. https://doi.org/10.1139/z05-024

Barrion, A. A., Casal, C. V., Taylo, L. D., & Amalin, D. M. (1988). Two orb-weaving spiders (Araneae: Araneidae) in the Philippines causing araneidism. *Philippine Journal of Science*, *116*, 245-254.

Barrion, A. T., & Litsinger, J. A. (1995). *Riceland spiders of South and Southeast Asia*. Wallingford, UK: CAB International.

Barthel, M., & Hetzer, H. (1982). Bernstein-Inklusen aus dem Mioz¨an des Bitterfelder Raums. *Zeitschrift f¨ur Angewandte Geologie*, *28*, 314–336.

Bartoleti, L. F. D. M., Peres, E. A., Fontes, F. V. H. M., da Silva, M. J., & Solferini, V. N. (2018). Phylogeography of the widespread spider *Nephila clavipes* (Araneae: Araneidae) in South America indicates geologically and climatically driven lineage diversification. *Journal of Biogeography*, *45*(6), 1246-1260. https://doi.org/10.1111/jbi.13217

Basumatary, P., Chanda, D., Das, S., Kalita, J., Brahma, D., Basumatary, T., Basumatary, B. K., & Daimary, S. (2019). On a new species of the orb-weaving spider genus *Eriovixia* (Araneae: Araneidae) from India. *Arachnology*, *18*(1), 24-27. https://doi.org/10.13156/arac.2018.18.1.24

Benoit, P. L. G. (1964). New contribution to the knowledge of the Araneidae-Gasteracanthinae of Africa and Madagascar (Araneae). *Publicações Culturais da Companhia de Diamantes de Angola*, *69*, 41-52.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2013). GenBank. *Nucleic Acids Research, 41*(D1), D36–D42. https://doi.org/10.1093/nar/gks1195

Berman, J. D., & Levi, H. W. (1971). The orb weaver genus *Neoscona* in North America (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology*, 141(8), 465-500.

Bidegaray-Batista, L., & Arnedo, M. A. (2011). Gone with the plate: the opening of the Western Mediterranean basin drove the diversification of ground-dweller spiders. *BMC Evolutionary Biology*, *11*, 1-15. https://doi.org/10.1186/1471-2148-11-317

Biswas, B., & Biswas, K. (2007). Araneae: Spiders. *Fauna of Mizoram, State Fauna Series. Zoological Survey of India, Kolkata*, *14*, 455-475.Blackwall, J. (1865). Descriptions of recently discovered species and characters of a new genus, of Araneida from the east of Central Africa. *Annals and Magazine of Natural History*, *16*(95), 336-352. https://doi.org/10.1080/00222936508679441

Blackwall, J. (1865). Descriptions of recently discovered species, and characters of a new genus, of Araneidae from the East of Central Africa. *Annals and Magazine of Natural History*, *16*(95), 336-352. https://doi.org/10.1080/00222936508679441

Blagoev, G. A., Nikolova, N. I., Sobel, C. N., Hebert, P. D., & Adamowicz, S. J. (2013). Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. *BMC Ecology*, *13*, 1-17. https://doi.org/10.1186/1472-6785-13-44

Blaxter, M., Elsworth, B., & Daub, J. (2004). DNA taxonomy of a neglected animal phylum: an unexpected diversity of tardigrades. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(suppl_4), S189-S192. https://doi.org/10.1098/rsbl.2003.0130

Boldsystems.org. (2025). *Bold Systems v4*. [Online] Available at: http://www.boldsystems.org /index.php/Public_SearchTerms. accessed on February 20, 2025.

Bolzern, A., Burckhardt, D., & Hänggi, A. (2013). Phylogeny and taxonomy of European funnel-web spiders of the *Tegenaria–Malthonica* complex (Araneae: Agelenidae) based upon morphological and molecular data. *Zoological Journal of the Linnean Society*, *168*(4), 723-848. https://doi.org/10.1111/zoj.12040

Booysen, R., & Haddad, C. R. (2021). Revision and molecular phylogeny of the spider genus *Micaria* Westring, 1851 (Araneae: Gnaphosidae) in the Afrotropical Region. *Zootaxa*, *4940*(1), 1-82. https://doi.org/10.11646/zootaxa.4940.1.1

Bösenberg, W., & Strand, E. (1906). Japanische Spinnen. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, *30*, 93–422.

Bosmans, R., & Hervé, C. (2021). Less is more: eight new synonyms in Mediterranean spiders (Araneae), with a new *Pelecopsis* species from Tunisia (Linyphiidae). *Arachnologische Mitteilungen*, *61*, 58-64. https://doi.org/10.30963/aramit6109

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., Maio, N. De., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., Plessis, L. du., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., & Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology, 15*(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Boudinot, B. E., Probst, R. S., Brandao, C. R. F., Feitosa, R. M., & Ward, P. S. (2016). Out of the Neotropics: newly discovered relictual species sheds light on the biogeographical history of spider ants (Leptomyrmex, Dolichoderinae, Formicidae). *Systematic Entomology*, *41*(3), 658-671. https://doi.org/10.1111/syen.12181

Brown, G. R. & Henderson, C. L. (2019). First record of the Grass Cross Spider (*Argiope catenulata*) of the family Araneidae in Australia. Northern Territory Naturalist 29: 98-102.

Cabra-García, J., & Hormiga, G. (2020). Exploring the impact of morphology, multiple sequence alignment and choice of optimality criteria in phylogenetic inference: a case study with the Neotropical orb-weaving spider genus *Wagneriana* (Araneae: Araneidae). *Zoological Journal of the Linnean Society*, *188*(4), 976-1151. doi:10.1093/zoolinnean/zlz088

Caleb, J. T. D., & Sankaran, P. M. (2025). *Araneae of India*. Version 2025, Retrieved June 24, 2025, from http://www.indianspiders.in.

Čandek, K., & Kuntner, M. (2015). DNA barcoding gap: reliable species identification over morphological and geographical scales. *Molecular ecology resources*, *15*(2), 268-277. https://doi.org/10.1111/1755-0998.12304

Cao, X., Liu, J., Chen, J., Zheng, G., Kuntner, M., & Agnarsson, I. (2016). Rapid dissemination of taxonomic discoveries based on DNA barcoding and morphology. *Scientific Reports*, *6*(1), 37066. https://doi.org/10.1038/srep37066

Castanheira, P. de S., Didham, R. K., Vink, C. J., & Framenau, V. W. (2019). The scorpion-tailed orb-weaving spiders (Araneae, Araneidae, Arachnura) in Australia and New Zealand. *Zootaxa*, *4706*(1), 147-170.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution, 17*(4), 540–552. https://doi.org/10.1093/oxfordjournals.molbev.a026334

Ceccarelli, F. S., Opell, B. D., Haddad, C. R., Raven, R. J., Soto, E. M., & Ramírez, M. J. (2016). Around the world in eight million years: historical biogeography and evolution of the spray zone spider Amaurobioides (Araneae: Anyphaenidae). *PLoS One*, *11*(10), e0163740. https://doi.org/10.1371/journal.pone.0163740

Chakrabarti, S. (2009). Gea spinipes C. L. Koch 1843 (Araneae: Araneidae) found in western Himalaya, India. *Turkish Journal of Arachnology*, *1*(2), 128-132.

Cheng, R. C., & Kuntner, M. (2014). Phylogeny suggests nondirectional and isometric evolution of sexual size dimorphism in Argiopine spiders. *Evolution*, *68*(10), 2861-2872. https://doi.org/10.1111/evo.12504

Chrysanthus, P. (1961). Die Gattung *Anepsion* Strand 1929 (Arach., Araneae: Araneidae-Araneinae). *Senckenbergiana Biologica*, *42*, 463-477.

Chrysanthus, P. (1969). Additional remarks on the genus *Anepsion* Strand, 1929 (Araneae, Argyopidae). *Zoologische Mededelingen*, *44*, 31-39.

Clerck, C. (1757). *Svenska Spindlar, det är beskrifning öfwer de i Sverige funne spindlarne, samt deras widunderlige lägrade sätt att fånga och döda flugor.* Stockholm. doi:10.5962/bhl.title.119890

Coddington, J. A. (1990). Ontogeny and homology in the male palpus of orb-weaving spiders and their relatives, with comments on phylogeny (Araneoclada: Araneoidea, Deinopoidea). *Smithsonian Contributions to Zoology*, *496*, 1-52.

Coddington, J. A. (2005). Phylogeny and classification of spiders. In D. Ubick, P. Paquin, P. E. Cushing, & V. Roth (Eds.), *Spiders of North America: An identification manual*. American Arachnological Society.

Coddington, J. A., Griswold, C. E., Silva, D., Peñaranda, E., & Larcher, S. F. (1991). Designing and testing sampling protocols to estimate biodiversity in tropical ecosystems. In *The Unity of Evolutionary Biology: Proceedings of the Fourth International Congress of Systematic and Evolutionary Biology, 2 vols*.

Coddington, J. A., & Levi, H. W. (1991). Systematics and evolution of spiders (Araneae). *Annual Review of Ecology and Systematics*, 565-592. http://www.jstor.org/stable/2097274

Coddington, J. A., Agnarsson, I., Cheng, R. C., Čandek, K., Driskell, A., Frick, H., Gregorič, M., Kostanjšek, R., Kropf, C., Kweskin, M., Lokovšek, T., Pipan, M., Vidergar, N., & Kuntner, M. (2016). DNA barcode data accurately assign higher spider taxa. *PeerJ*, *4*, e2201. https://doi.org/10.7717/peerj.2201

Convention on Biological Diversity. (1995). *Report of the second meeting of the Conference of the Parties to the Convention on Biological Diversity (COP 2, Jakarta, 6–17 November 1995)*. UNEP/CBD/COP/2/19. United Nations Environment Programme. https://www.cbd.int/doc/meetings/cop/cop-02/official/cop-02-19-en.pdf

Dahl, F. (1914). The Gasteracanthen of the Berlin Zoological Museum and their Geographical Distribution. *Mitteilungen aus dem Zoologische Museum in Berlin*, *7*, 235-301.

Darwin, C. R. (1837-1838). *Notebook B: Transmutation of species*. [Page 36]. Retrieved December 15, 2024, from Darwin Online: The Complete Work of Charles Darwin Online

Darwin, C. R. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. Retrieved December 15, 2024, from Darwin Online: The Complete Work of Charles Darwin Online

DeSalle, R. O. B. (2006). Species discovery versus species identification in DNA barcoding efforts: response to Rubinoff. *Conservation Biology*, *20*(5), 1545-1547. http://www.jstor.org/stable/3879146

DeSalle, R., & Goldstein, P. (2019). Review and interpretation of trends in DNA barcoding. *Frontiers in Ecology and Evolution*, *7*, 302. https://doi.org/10.3389/fevo.2019.00302

Dharmaraj, J., Gunasekaran, C., & Rajkumar, V. (2018). Diversity and plethora of spider fauna at different habitats of the Nilgiris, Tamilnadu south India. *International Journal of Recent Scientific Research*, *9*(3A), 24634-24637.

Dimitrov, D., Benavides, L. R., Arnedo, M. A., Giribet, G., Griswold, C. E., Scharff, N., & Hormiga, G. (2017). Rounding up the usual suspects: a standard target gene approach for resolving the interfamilial phylogenetic relationships of ecribellate orb weaving spiders with a new family rank classification (Araneae, Araneoidea). *Cladistics*, *33*(3), 221-250. https://doi.org/10.1111/cla.12165

Dippenaar-Schoeman, A. S., & Jocqué, R. (1997). *African spiders: an identification manual* (Vol. 9). Pretoria: ARC-Plant Protection Research Institute.

Dippenaar-Schoeman, A. S., Haddad, C. R., Foord, S. H., Lotz, L. N., & Webb, P. (2022a). *South African National Survey of Arachnida photo identification guide: The Araneidae of South Africa (Version 2, Part 1: A–C)*. Zenodo. https://doi.org/10.5281/bzenodo.6326922

Dippenaar-Schoeman, A. S., Haddad, C. R., Foord, S. H., & Lotz, L. N. (2022b). *South African National Survey of Arachnida photo identification guide: Araneidae (Version 2, Part 2: E–N)*. Zenodo. https://doi.org/10.5281/zenodo.6619195

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*(Special Issue 2), 945-953.

Dobzhansky, T. (1973). Nothing in Biology Makes Sense except in the Light of Evolution. *The American Biology Teacher*, *35*(3), 125–129. https://doi.org/10.2307/4444260

Doleschall, L. (1857). Bijdrage tot de kennis der Arachniden van den Indischen Archipel. *Natuurkundig Tijdschrift voor Nederlandsch-Indie*, *13*, 399-434. https://doi.org/10.5962/bhl.title.66068

Doleschall, L. (1859). Tweede Bijdrage tot de kennis der Arachniden van den Indischen Archipel. *Acta Societatis Scientiarum Indo-Neerlandicae*, *5*(5), 1-60.

Dondale, C. D., Redner, J. H., Paquin, P., & Levi, H. W. (2003). *The insects and arachnids of Canada. Part 23. The orb-weaving spiders of Canada and Alaska (Araneae: Uloboridae, Tetragnathidae, Araneidae, Theridiosomatidae)*. Ottawa: NRC Research Press.

Dunlop, J. A., Penney, D., & Jekel, D. (2023). *A summary list of fossil spiders and their relatives*. In World Spider Catalog. Natural History Museum Bern, online at http://wsc.nmbe.ch, version 23.5, accessed on 11. 02.2025.

Ebach, M. C., & Holdrege, C. (2005). DNA barcoding is no substitute for taxonomy. *Nature*, *434*(7034), 697-697. https://doi.org/10.1038/434697b

Elgar, M. A., & Nash, D. R. (1988). Sexual cannibalism in the garden spider *Araneus diadematus*. *Animal Behaviour*, *36*(5), 1511-1517. https://doi.org/10.1016/S0003-3472(88)80221-5

Elizabeth, V. M. (2019). *Taxonomy of Spiders Belonging to the Genus Cyrtophora Simon 1864 Araneae Araneidae of the Western Ghats using Morphological and Molecular Data*. [Doctoral dissertation, Mahatma Gandhi University]. The Shodhganga@INFLIBNET Centre. http://hdl.handle.net/10603/285161

Emerit, M. (1974). Arachnides araignées Araneidae Gasteracanthinae. *Faune Madagascar*, *38*, 1-215.

Eskov, K. (1984). A new fossil spider family from the Jurassic of Transbaikalia (Araneae: Chelicerata). *Neues Jahrbuch für Geologie und Paläontologie. Monatshefte*, (11), 645-653.

Eskov, K. Y., & Wunderlich, J. (1995). On the spiders from Taimyr ambers, Siberia, with the description of a new family and with general notes on the spiders from the Cretaceous resins. *Beiträge zur Araneologie*, *4*, 95-107.

Environmental Systems Research Institute (ESRI) (2020). *ArcGIS Desktop (Version 10.8)* [Computer software]. https://www.esri.com/en-us/arcgis/products/arcgis-desktop/overview

Fabricius, J. C. (1793). *Entomologiae systematica emendata et aucta, secundum classes, ordines, genera, species adjectis synonimis, locis, observationibus, descriptionibus.* Copenhagen. https://doi.org/10.5962/bhl.title.122153

Fabricius, J. C. (1798). *Supplementum entomologiae systematicae*. Christian Gottlieb Proft.

Fang, S. G., Wan, Q. H., & Fujihara, N. (2002). Formalin removal from archival tissue by critical point drying. *Biotechniques*, *33*(3), 604-611. https://doi.org/10.2144/02333rr03

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution, 17*(6), 368–376. https://doi.org/10.1007/BF01734359

Field, K. G., Olsen, G. J., Lane, D. J., Giovannoni, S. J., Ghiselin, M. T., Raff, E. C., Pace, N. R., & Raff, R. A. (1988). Molecular phylogeny of the animal kingdom. *Science*, *239*(4841), 748-753. https://doi.org/10.1126/science.32772

Fišer Pečnikar, Ž., & Buzan, E. V. (2014). 20 years since the introduction of DNA barcoding: from theory to application. *Journal of Applied Genetics*, *55*, 43-52. https://doi.org/10.1007/s13353-013-0180-y

Fitch, W. M. (1971). Toward defining the course of evolution: Minimum change for a specific tree topology. *Systematic Biology, 20*(4), 406–416. https://doi.org/10.1093/sysbio/20.4.406

Forest Survey of India. (2019). *India State of Forest Report 2019: Volume II – Kerala*. Ministry of Environment, Forest and Climate Change, Government of India. https://fsi.nic.in/isfr19/vol2/isfr-2019-vol-ii-kerala.pdf

Forskål, P. (1775). *Descriptiones animalium, avium, amphibiorum, piscium, insectorum, vermium: quae in itinere orientali observavit*. ex officina Mölleri: Hauniae

Framenau, V. W. (2019). Generic and family transfers, and nomina dubia for orb-weaving spiders (Araneae, Araneidae) in the Australasian, Oriental and Pacific regions. *Evolutionary Systematics*, *3*, 1-27. https://doi.org/10.3897/evolsyst.3.33454

Framenau, V. W., Scharff, N., & Harvey, M. S. (2010a). Systematics of the Australian orb-weaving spider genus *Demadiana* with comments on the generic classification of the Arkyinae (Araneae: Araneidae). *Invertebrate Systematics*, *24*(2), 139-171. https://doi.org/10.1071/IS10005

Framenau, V. W., Duperre, N., Blackledge, T., & Vink, C. (2010b). Systematics of the new Australasian orb-weaving spider genus *Backobourkia* (Araneae: Araneidae: Araneinae). *Arthropod Systematics and Phylogeny*, *68*, 79-111.

Framenau, V. W., Castanheira, P. de S., & Vink, C. J. (2022). Taxonomy and systematics of the new Australo-Pacific orb-weaving spider genus *Socca* (Araneae: Araneidae). *New Zealand Journal of Zoology*, *49*(4), 263-334. https://doi.org/10.1080/03014223.2021.2014899

Franzini, P. Z. N., Dippenaar-Schoeman, A. S., Yessoufou, K., & Van der Bank, F. H. (2013). Combined analyses of genetic and morphological data indicate more than one species of *Cyrtophora* (Araneae: Araneidae) in South Africa. *International Journal of Modern Biology Research*, *1*, 21-34.

Gaikwad, S., Warudkar, A., & Shouche, Y. (2017). Efficacy of DNA barcoding for the species identification of spiders from Western Ghats of India. *Mitochondrial DNA Part A*, *28*(5), 638-644. https://doi.org/10.3109/24701394.2016.1166219

Gajbe, P. U. (2004). Spiders of Jabalpur, Madhya Pradesh (Arachnida: Araneae). *Records of the Zoological Survey of India, Occasional Paper*, *227*, 1-154.

Gajbe, U. A. (2005). Studies on some spiders of the family Araneidae (Araneae: Arachnida) from Madhya Pradesh, India. *Records of the Zoological Survey of India*, *105*(1-2), 45-60.

Gajbe, U. A., & Gajbe, P. (1999). A new *Cyrtophora* spider (Araneae: Araneidae) from Jabalpur, Madhya Pradesh, India. *Records of the Zoological Survey of India*, *97*(4), 29-31.

Garland Jr, T., Bennett, A. F., & Rezende, E. L. (2005). Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology*, *208*(16), 3015-3035. https://doi.org/10.1242/jeb.01745

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research, 31*(13), 3784–3788. https://doi.org/10.1093/nar/gkg563

George, M. E., Babu, N., Sheeja, A. D., Neermunda, N., & Prasad, G. (2024). Ant and spider diversity of Karuvatta, a coastal island in Vembanad, Kerala, India. *Entomon*, *49*(1), 35-46. https://doi.org/10.33307/entomon.v49i1.1036

Giebel, C. G. A. (1856). *Fauna der Vorwelt mit steter Berücksichtigung der lebenden Thiere, Bd. 2, Abth. I. Die Insecten und Spinnen der Vorwelt, mit steter Berücksichtigung der lebenden Insecten und Spinnen, monographisch dargestellt.* Brockhaus.

Grasshoff, M. (1986). Die Radnetzspinnen-Gattung *Neoscona* in Afrika (Arachnida: Araneae). Annalen Zoologische Wetenschappen 250: 1-123.

Grasshoff, M., & van Harten, A. (2007). Orb-weaving spiders of the family Araneidae (Araneae) from mainland Yemen and the Socotra Archipelago. *Fauna of Arabia*, *23*, 151-162.

Gravely, F. H. (1921). The spiders and scorpions of Barkuda Island. *Records of the Indian Museum, Calcutta*, *22*, 399–421.

Greenstone, M. H., Rowley, D. L., Heimbach, U., Lundgren, J. G., Pfannenstiel, R. S., & Rehner, S. A. (2005). Barcoding generalist predators by polymerase chain reaction: carabids and spiders. *Molecular Ecology*, *14*(10), 3247-3266. https://doi.org/10.1111/j.1365-294X.2005.02628.x

Gregorič, M., Blackledge, T. A., Agnarsson, I., & Kuntner, M. (2015). A molecular phylogeny of bark spiders reveals new species from Africa and Madagascar (Araneae: Araneidae: *Caerostris*). *Journal of Arachnology*, *43*(3), 293-312. https://doi.org/10.1636/0161-8202-43.3.293

Gregorič, M., Yu, K.-P., Rojas Velez, W., & Garb, J. E. (2025). *Caerostris* (Araneidae: Araneae) cryptic diversity highlights the need for taxonomic expertise in the genomic era. *European Journal of Taxonomy*, *989*(1), 1–23. https://doi.org/10.5852/ejt.2025.989.2877

Griswold, C. E., Coddington, J. A., Hormiga, G., & Scharff, N. (1998). Phylogeny of the orb-web building spiders (Araneae, Orbiculariae: Deinopoidea, Araneoidea). *Zoological Journal of the Linnean Society*, *123*(1), 1-99. https://doi.org/10.1111/j.1096-3642.1998.tb01290.x

Groves, C. R., Jensen, D. B., Valutis, L. L., Redford, K. H., Shaffer, M. L., Scott, J. M., & Anderson, M. G. (2002). Planning for biodiversity conservation: Putting conservation science into practice: a seven-step framework for developing regional plans to conserve biological diversity, based upon principles of conservation biology and ecology, is being used extensively by the nature conservancy to identify priority areas for conservation. *BioScience, 52*(6), 499–512. https://doi.org/10.1641/0006-3568(2002)052[0499:PFBCPC]2.0.CO;2

Hake, S. B., & Allis, C. D. (2006). Histone H3 variants and their potential role in indexing mammalian genomes: the "H3 barcode hypothesis". *Proceedings of the National Academy of Sciences*, *103*(17), 6428-6435. https://doi.org/10.1073/pnas.0600803103

Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series, 41*, 95–98.

Han, G. X., & Zhu, M. S. (2010). Taxonomy and biogeography of the spider genus *Eriovixia* (Araneae: Araneidae) from Hainan Island, China. *Journal of Natural History*, *44*(43-44), 2609-2635. https://doi.org/10.1080/00222933.2010.507315

Harms, D., & Dunlop, J. A. (2009). A revision of the fossil pirate spiders (Arachnida: Araneae: Mimetidae). *Palaeontology*, *52*, 779-802. https://doi.org/10.1111/j.1475-4983.2009.00890.x

Harrod, J. C., Levi, H. W., & Leibensperger, L. B., (1991). The Neotropical orbweavers of the genus *Larinia* (Araneae: Araneidae). *Psyche, Cambridge*, *97*(3-4), 241-265. https://doi.org/10.1155/1990/25941

Harvey, M. S., Austin, A. D., & Adams, M. (2007). The systematics and biology of the spider genus *Nephila* (Araneae: Nephilidae) in the Australasian region. *Invertebrate Systematics*, *21*(5), 407-451. https://doi.org/10.1071/IS05016

Haupt, J. (2003). The Mesothelae -- a monograph of an exceptional group of spiders (Aaneae: Mesothelae): (Morphology, behaviour, ecology, taxonomy, distribution and phylogeny). *Zoologica*, *154*, 1-102.

Hazzi, N. A., & Hormiga, G. (2023). Molecular phylogeny of the tropical wandering spiders (Araneae, Ctenidae) and the evolution of eye conformation in the RTA clade. *Cladistics*, *39*(1), 18-42. https://doi.org/10.1111/cla.12518

Hazzi, N. A., Wood, H. M., & Hormiga, G. (2025). Reassessing the evolutionary relationships of tropical wandering spiders using phylogenomics: a UCE-based phylogeny of Ctenidae (Araneae) with the discovery of a new lycosoid family. *Molecular Phylogenetics and Evolution*, *203*(108245), 1-11. https://doi.org/10.1016/j.ympev.2024.108245

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313-321. https://doi.org/10.1098/rspb.2002.2218

Hebert, P. D., Ratnasingham, S., & De Waard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(suppl_1), S96-S99. https://doi.org/10.1098/rsbl.2003.0025

Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S., & Francis, C. M. (2004). Identification of birds through DNA barcodes. *PLoS biology*, *2*(10), e312. https://doi.org/10.1371/journal.pbio.0020312

Heimer, S., & Nentwig, W. (1983). Thoughts on the phylogeny of the Araneoidea Latreille, 1806 (Arachnida, Araneae). *Journal of Zoological Systematics and Evolutionary Research*, *20*(4), 284-295. https://doi.org/10.1111/j.1439-0469.1983.tb00554.x

Heyden, C. von (1859). Fossile Insekten aus der Rheinischen Braunkohle. *Palaeontographica*, *8*, 1–15.

Heymons, R. (1901). Beiträge zur Morphologie und Entwicklungsgeschichte der Spinnentiere. I. Die Entwicklungsgeschichte von *Ixodes ricinus* L. *Zeitschrift für Wissenschaftliche Zoologie*, *69*, 519–543.

Hillis, D. M. (1987). Molecular versus morphological approaches to systematics. *Annual Review of Ecology and Systematics*, 23-42. http://www.jstor.org/stable/2097123

Hillis, D. M. (1997). Phylogenetic analysis. *Current Biology*, *7*(3), R129-R131. https://doi.org/10.1016/S0960-9822(97)70070-8

Hines, H. M. (2008). Historical biogeography, divergence times, and diversification patterns of bumble bees (Hymenoptera: Apidae: *Bombus*). *Systematic biology*, *57*(1), 58-75. https://doi.org/10.1080/10635150801898912

Hogg, I. D., & Hebert, P. D. (2004). Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology*, *82*(5), 749-754. https://doi.org/10.1139/z04-041

Hong, Y. (1985). *Fossil Insects, scorpionids and araneids in the diatoms of Shanwang*. Geological Publishing House, Beijing.

Hong-Chun, P., Kai-Ya, Z., Da-Xiang, S., & Yang, Q. (2004). Phylogenetic placement of the spider genus *Nephila* (Araneae: Araneoidea) inferred from rRNA and MaSp1 gene sequences. *Zoological Science*, *21*(3), 343-351. https://doi.org/10.2108/zsj.21.343

Horiike, T. (2016). An introduction to molecular phylogenetic analysis. *Reviews in Agricultural Science*, *4*, 36-45. https://doi.org/10.7831/ras.4.0_36

Hormiga, G., Eberhard, W. G., & Coddington, J. A. (1995). Web-construction behaviour in Australian *Phonognatha* and the phylogeny of nephiline and tetragnathid spiders (Araneae: Tetragnathidae). *Australian Journal of Zoology*, *43*(4), 313-364. https://doi.org/10.1071/ZO9950313

Hormiga, G., Kulkarni, S., Arnedo, M. A., Dimitrov, D., Giribet, G., Kallal, R. J., & Scharff, N. (2023). Genitalic morphology and phylogenomic placement of the Australian spider *Paraplectanoides crassipes* Keyserling, 1886 (Araneae, Araneidae) with a discussion on the classification of the family Araneidae. *Invertebrate Systematics*, *37*(12), 797-818. https://doi.org/10.1071/IS23050

Hu, J. L. (1984). *The Chinese spiders collected from the fields and the forests*. Tianjin Science and Technology Press.

Huber, B. A., & Wunderlich, J. (2006). Fossil and extant species of the genus *Leptopholcus* in the Dominican Republic, with the first cases of egg-parasitism in pholcid spiders (Araneae: Pholcidae). *Journal of Natural History*, *40*(41-43), 2341-2360. https://doi.org/10.1080/00222930601051196

Huber, B. A., Eberle, J., & Dimitrov, D. (2018). The phylogeny of pholcid spiders: a critical evaluation of relationships suggested by molecular data (Araneae, Pholcidae). *ZooKeys*, *789*, 51-101. https://doi.org/10.3897/zookeys.789.22781

Hubert, N., & Hanner, R. (2015). DNA barcoding, species delineation and taxonomy: a historical perspective. *DNA barcodes*, *3*(1). https://doi.org/10.1515/dna-2015-0006

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics, 17*(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Ibrahimi, K., Islami, B., Kastrati, B., Geci, D., Bilalli, A., & Ibrahimi, H. (2024). New findings and an updated checklist of Araneidae (Arachnida: Araneae) from Kosovo. *Journal of Insect Biodiversity and Systematics*, 547-555. https://doi.org/10.61186/jibs.10.3.547

Jäger, P. (2012). A review on the spider genus *Argiope* Audouin 1826 with special emphasis on broken emboli in female epigynes (Araneae: Araneidae: Argiopinae). *Beiträge zur Araneologie*, *7*, 272-331, 358-362.

Jeyaprakash, A., & Hoy, M. A. (2009). First divergence time estimate of spiders, scorpions, mites and ticks (subphylum: Chelicerata) inferred from mitochondrial phylogeny. *Experimental and Applied Acarology*, *47*, 1-18. https://doi.org/10.1007/s10493-008-9203-5

JGraph Ltd. (2023). *draw.io (Version 26.2.14)* [Online platform]. https://www.drawio.com/

Jose, A. C., Sudhin, P. P., Prasad, P. M., & Sreejith, K. A. (2018). Spider diversity in Kavvayi River Basin, Kerala, Southern India. *Current World Environment*, *13*(1), 100.

Jose, K. S. (2011). A new species of the genus *Cyrtarachne* (Araneae: Araneidae) from western Ghats, India. *Munis Entomology and Zoology*, *6*, 321-324.

Joseph, M. M., & Framenau, V. W. (2012). Systematic review of a new orb-weaving spider genus (Araneae: Araneidae), with special reference to the Australasian-Pacific and South-East Asian fauna. *Zoological Journal of the Linnean Society*, *166*(2), 279-341. https://doi.org/10.1111/j.1096-3642.2012.00845.x

Kallal, R. J., & Hormiga, G. (2018a). An expanded molecular phylogeny of metaine spiders (Araneae, Tetragnathidae) with description of new taxa from Taiwan and the Philippines. *Invertebrate Systematics*, *32*(2), 400-422. https://doi.org/10.1071/IS17058

Kallal, R. J., & Hormiga, G. (2018b). Systematics, phylogeny and biogeography of the Australasian leaf-curling orb-weaving spiders (Araneae: Araneidae: Zygiellinae), with a comparative analysis of retreat evolution. *Zoological Journal of the Linnean Society*, *184*(4), 1055-1141. https://doi.org/10.1093/zoolinnean/zly014

Kallal, R. J., & Hormiga, G. (2019). Evolution of the male palp morphology of the orb-weaver hunting spider *Chorizopes* (Araneae: Araneidae) revisited on a new phylogeny of Araneidae, and description of a third species from Madagascar. *Invertebrate Systematics*, *33*(3), 473-487. https://doi.org/10.1071/IS18061

Kallal, R. J., Fernández, R., Giribet, G., & Hormiga, G. (2018). A phylotranscriptomic backbone of the orb-weaving spider family Araneidae (Arachnida, Araneae) supported by multiple methodological approaches. *Molecular Phylogenetics and Evolution*, *126*, 129-140. https://doi.org/10.1016/j.ympev.2018.04.007

Kerala Forest Department (2025). *Special habitats*. Government of Kerala. https://forest.kerala.gov.in/en/special-habitats/

Kerala Soil Survey (2024). *Kerala at a Glance*. Department of Soil Survey & Soil Conservation. Government of Kerala. Retrieved December 27, 2024 from https://www.keralasoils.gov.in/en/kerala-glance

Keswani, S. (2013). Revision of spiders from the genus *Cyclosa* (Araneae: Araneidae) with description of two new species and the first record of male of *C. moonduensis* Tikader, 1963 from India. *Indian Journal of Arachnology*, *2*(1), 61-80.

Kim, S. T., & Lee, S. Y. (2012). Arthropoda: Arachnida: Araneae: Araneidae. Araneid spiders. *Invertebrate Fauna of Korea*. *21*(16), 1-146.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111-120. https://doi.org/10.1007/BF01731581

Koch, C. L. (1837). *Die Arachniden* (Vol. 3–4). C. H. Zeh'sche Buchhandlung.

Koch, C. L. (1843). *Die Arachniden* (Vol. 10). C. H. Zeh'sche Buchhandlung. https://doi.org/10.5962/bhl.title.43744

Koch, C. L. (1850). Übersicht des Arachnidensystems. Vol. 5. Nürnberg: Heft 1-6.

Koch, C. L., & Berendt, G. C. (1854). Die im Bernstein befindlichen Crustaceen, Myriapoden, Arachniden und Apteren der Vorwelt. In G. C. Berendt (Ed.), *Die im Bernstein befindlichen organischen Reste der Vorwelt* (Vol. 1, Part II). Nicholaische Buchhandlung.

Koch, L. (1871). *Die Arachniden Australiens, nach der Natur beschrieben und abgebildet* [Erster Theil, Lieferung 1–2]. Bauer & Raspe.

Koch, L. (1872). *Die Arachniden Australiens, nach der Natur beschrieben und abgebildet* (Erster Theil, Lieferung 3–7). Bauer & Raspe. https://doi.org/10.5962/bhl.title.121660

Koch, L. (1875). *Aegyptische und abyssinische Arachniden gesammelt von Herrn C. Jickeli*. Bauer & Raspe.

Kulkarni, S., & Smith, H. (2013). First record of *Poltys columnaris* Thorell, 1890 (Araneae: Araneidae) from Western Ghats, India. *Journal of Threatened Taxa*, *5*(10), 4524-4526.

Kulkarni, S., Wood, H. M., & Hormiga, G. (2023). Advances in the reconstruction of the spider tree of life: a roadmap for spider systematics and comparative studies. *Cladistics*, *39*(6), 479-532. https://doi.org/10.1111/cla.12557

Kundmann, J. C. (1737). *Rariora naturae et artis, item in re medica, oder Seltenheiten der Natur und Kunst des Kundmannischen Naturalien Cabinets, wie auch in der Artzeney-Wissenschaft.* Breslau & Leipzig.

Kuntner, M. (2002). The placement of *Perilla* (Araneae, Araneidae) with comments on araneid phylogeny. *Journal of Arachnology*, *30*(2), 281-287. https://doi.org/10.1636/0161-8202(2002)030[0281:TPOPAA]2.0.CO;2

Kuntner, M. (2005). A revision of *Herennia* (Araneae: Nephilidae: Nephilinae), the Australasian 'coin spiders'. *Invertebrate Systematics*, *19*(5), 391-436. https://doi.org/10.1071/IS05024

Kuntner, M. (2007). A monograph of *Nephilengys*, the pantropical 'hermit spiders' (Araneae, Nephilidae, Nephilinae). *Systematic Entomology*, *32*(1), 95-135. https://doi.org/10.1111/j.1365-3113.2006.00348.x

Kuntner, M., Coddington, J. A., & Hormiga, G. (2008). Phylogeny of extant nephilid orb-weaving spiders (Araneae, Nephilidae): testing morphological and ethological homologies. *Cladistics*, *24*(2), 147-217. https://doi.org/10.1111/j.1096-0031.2007.00176.x

Kuntner, M., Kralj-Fišer, S., Schneider, J. M., & Li, D. Q. (2009). Mate plugging via genital mutilation in nephilid spiders: an evolutionary hypothesis. *Journal of Zoology, 277*: 257-266. https://doi.org/10.1111/j.1469-7998.2008.00533.x

Kuntner, M., Arnedo, M. A., Trontelj, P., Lokovšek, T., & Agnarsson, I. (2013). A molecular phylogeny of nephilid spiders: evolutionary history of a model lineage. *Molecular Phylogenetics and Evolution*, *69*(3), 961-979. https://doi.org/10.1016/j.ympev.2013.06.008

Kuntner, M., Čandek, K., Gregorič, M., Turk, E., Hamilton, C. A., Chamberland, L., Starrett, J., Cheng, R. C., Coddington, J. A., Agnarsson, I., & Bond, J. E. (2023). Increasing information content and diagnosability in family-level classifications. *Systematic Biology*, *72*(4), 964-971. https://doi.org/10.1093/sysbio/syad021

Labarque, F. M., Pérez-González, A., & Griswold, C. E. (2018). Molecular phylogeny and revision of the false violin spiders (Araneae: Drymusidae) of Africa. *Zoological Journal of the Linnean Society*, *183*(2), 390-430. https://doi.org/10.1093/zoolinnean/zlx088

Lamarck, J. B. (1801). *Système des animaux sans vertèbres, ou Tableau général des classes, des ordres et des genres de ces animaux.* Paris.

Latreille, P. A. (1806). *Genera crustaceorum et insectorum secundum ordinem naturalem in familias disposita, iconibus exemplisque plurimis explicata.* Amand Koenig, Paris.

Leach, W. E. (1815). *Zoological miscellany; being descriptions of new and interesting animals*. London

Lecigne, S. (2021). Preliminary list of spiders (Araneae) of the marsh of Villiers (Saint-Josse, Pas-de-Calais), *Larinia jeskovi* Marusik, 1987 new for the fauna of France (Araneidae) and rediscovery of *Satilatlas britteni* (Jackson, 1913) in the Hauts-de-France (Linyphiidae). *Revue Arachnologique*. *8*(2), 26-37.

Levi, H. W. (1964). Redescription of the type species of the spider genera *Artonis, Chorizopes,* and *Mecynidis* (Araneae: Araneidae and Linyphiidae). *Annals of the Natal Museum, 16*, 209-214.

Levi, H. W. (1974). The orb-weaver genus *Zygiella* (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology*, *146*(5), 267-290.

Levi, H. W. (1975). The American orb-weaver genera *Larinia, Cercidia,* and *Mangora* north of Mexico (Araneae, Araneidae). *Bulletin of the Museum of Comparative Zoology, 147*, 101-135.

Levi, H. W. (1978). Orb-webs and phylogeny of orb-weavers. *Symposia of the Zoological Society of London, 42*, 1-15.

Levi, H. W. (1983). The orb-weaver genera *Argiope, Gea,* and *Neogea* from the western Pacific region (Araneae: Araneidae, Argiopinae). *Bulletin of the Museum of Comparative Zoology, 150*(5), 247-338.

Levi, H. W. (1991). The Neotropical and Mexican species of the orb-weaver genera *Araneus, Dubiepeira,* and *Aculepeira* (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology, 152*(4), 167-315.

Levi, H. W. (1992). Spiders of the orb-weaver genus *Parawixia* in America (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology, 153*, 1-46.

Levi, H. W. (1999). The Neotropical and Mexican orb-weavers of the genera *Cyclosa* and *Allocyclosa* (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology, 155*, 299-379.

Levi, H. W. (2002). Keys to the genera of araneid orbweavers (Araneae, Araneidae) of the Americas. *Journal of Arachnology*, *30*(3), 527-562. https://doi.org/10.1636/0161-8202(2002)030[0527:KTTGOA]2.0.CO;2

Levi, H. W., & Coddington, J. A. (1983). Progress report on the phylogeny of the orb-weaving family Araneidae and the superfamily Araneoidea (Arachnida: Araneae). *Abhandlungen und Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg.*

Li, F., Jiang, T., Zhang, W., & Li, S. (2024). Ecological trait divergence over evolutionary time underlies the origin and maintenance of tropical spider diversity. *Ecography*, e07586. https://doi.org/10.1111/ecog.07586

Li, S. (2020). Spider taxonomy for an advanced China. *动物分类学报*, *45*(2), 73-77.

Linnaeus, C. (1735). *Systema naturæ, sive regna tria naturæ systematice proposita per classes, ordines, genera, & species*. – pp. [1–12]. Lugduni Batavorum. (Haak). https://www.biodiversitylibrary.org/item/15373

Linnaeus, C. (1758). *Systema Naturae per Regna Tria Naturae, Secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, Synonymis, Locis.* 10th Edition. pp. [1–4], 1–824 Laurentius Salvius: Holmiae (Stockholm). https://www.biodiversitylibrary.org/item/10277

Lorenz, J. G., Jackson, W. E., Beck, J. C., & Hanner, R. (2005). The problems and promise of DNA barcodes for species diagnosis of primate biomaterials. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1869-1877. https://doi.org/10.1098/rstb.2005.1718

Lovejoy, T. E. (1997). Biodiversity: Why is it important? In M. L. Reaka-Kudla, D. E. Wilson, & E. O. Wilson (Eds.), *Biodiversity II: Understanding and protecting our biological resources* (pp. 15–24). Washington, DC: Joseph Henry Press.

Macharoenboon, K., Siriwut, W., & Jeratthitikul, E. (2021). A review of the taxonomy of spiny-backed orb-weaving spiders of the subfamily Gasteracanthinae (Araneae, Araneidae) in Thailand. *ZooKeys*, *1032*, 17-62. doi:10.3897/zookeys.1032.62001

Maddison, W. P., Li, D. Q., Bodner, M., Zhang, J. X., Xu, X., Liu, Q. Q., & Liu, F. X. (2014). The deep phylogeny of jumping spiders (Araneae, Salticidae). *ZooKeys*, *440*, 57-87. https://doi.org/10.3897/zookeys.440.7891

Maddison, W. P., Evans, S. C., Hamilton, C. A., Bond, J. E., Lemmon, A. R., & Lemmon, E. M. (2017). A genome-wide phylogeny of jumping spiders (Araneae, Salticidae), using anchored hybrid enrichment. *ZooKeys*, *695*, 89-101. https://doi.org/10.3897/zookeys.695.13852

Maddison, W. P., & Maddison, D. R. (2023). *Mesquite: A modular system for evolutionary analysis*. Version 3.81. [Computer Software]. http://www.mesquiteproject.org

Magalhaes, I. L. F., & Santos, A. J. (2012). Phylogenetic analysis of *Micrathena* and *Chaetacis* spiders (Araneae: Araneidae) reveals multiple origins of extreme sexual size dimorphism and long abdominal spines. *Zoological Journal of the Linnean Society*, *166*(1): 14-53. https://doi.org/10.1111/j.1096-3642.2012.00831.x

Magalhaes, I. L. F., 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. https://doi.org/10.1071/IS15062

Magalhaes, I. L. F., Azevedo, G. H. F., Michalik, P., & Ramírez, M. J. (2020). The fossil record of spiders revisited: implications for calibrating trees and evidence for a major faunal turnover since the Mesozoic. *Biological Reviews*, *95*(1), 184-217. https://doi.org/10.1111/brv.12559

Magalhaes, I. L. F., Porta, A. O., Wunderlich, J., Proud, D. N., Ramírez, M. J., & Pérez-González, A. (2021). Taxonomic revision of fossil Psilodercidae and Ochyroceratidae spiders (Araneae: Synspermiata), with a new species of *Priscaleclercera* from mid-Cretaceous Burmese amber, northern Myanmar. *Cretaceous Research*, *121*(104751), 1-17. https://doi.org/10.1016/j.cretres.2020.104751

Magalhaes, I. L. F., Pérez-González, A., Labarque, F. M., Hammel, J. U., Kunz, R., Ramírez, M. J., & Solórzano-Kraemer, M. M. (2022). Revision of recluse spiders (Araneae: Sicariidae: *Loxosceles*) preserved in Dominican amber and a total-evidence phylogeny of Scytodoidea reveal the first fossil Drymusidae. *Arthropod Systematics and Phylogeny*, *80*, 541-559. https://doi.org/10.3897/asp.80.e86008

Malamel, J. J. (2018). Species increments to the Indian Araneofauna (Arachnida, Araneae) from Pathiramanal Island. *Revista Ibérica de Aracnología*, *32*, 112-116.

Malamel, J. J., Sankaran, P. M., Joseph, M. M., & Sebastian, P. A. (2015). *Cyrtarachne keralensis* Jose, 2011 is a junior synonym of *Anepsion maritatum* (O. Pickard-Cambridge, 1877) (Araneae, Araneidae). *Zootaxa*, *4039*(3), 478-482. https://doi.org/10.11646/zootaxa.4039.3.8

Martinez-Goss, M. R., & Arguelles, E. (2020). Classical Taxonomy. *Philippine Science Letters*, *15*, 145-153.

Marusik, Y. M., & Wunderlich, J. (2008). A survey of fossil Oonopidae (Arachnida: Aranei). *Arthropoda Selecta*, *17*, 65-79.

May, R. M. (1988). How many species are there on earth? *Science*, *241*(4872), 1441-1449.

McAlpine, J. F., &Martin, J. E. H. (1969). Canadian amber–a paleontological treasure chest. *Canadian Entomologist*, *101*, 819–838. https://doi.org/10.4039/Ent101819-8

Menge, A. (1866). Preussische Spinnen. Erste Abtheilung. *Schriften der Naturforschenden Gesellschaft in Danzig (N.F.)*, *1*, 1–152.

Mi, X. Q., Peng, X. J., & Yin, C. M. (2010). The orb-weaving spider genus *Eriovixia* (Araneae: Araneidae) in the Gaoligong mountains, China. *Zootaxa*, *2488*, 39-51.

Mi, X. Q., & Wang, C. (2016). First description on the female of *Eriovixia huwena* and the male of *E. poonaensis* (Araneae, Araneidae). *Sichuan Journal of Zoology*, *35*(5), 728-733.

Mi, X. Q., & Wang, C. (2018). *Chorizopesoides*, a new genus of orb-weaver spider from China (Araneae: Araneidae). *Oriental Insects*, *52*(1), 79-87.

Mi, X. Q., & Li, S. Q. (2021). On nine species of the spider genus *Eriovixia* (Araneae, Araneidae) from Xishuangbanna, China. *ZooKeys*, *1034*, 199-236. https://doi.org/10.3897/zookeys.1034.60411

Mi, X. Q., Liu, F., Wang, C., Gan, J. H., & Wu, Y. B. (2024a). Revision of the orb-weaver spider genus *Gea* C.L. Koch, 1843 (Araneae, Araneidae) from China. *ZooKeys*, *1191*, 75-88. https://doi.org/10.3897/zookeys.1191.117592

Mi, X. Q., Wang, C., & Li, S. Q. (2024b). Description of six new genera and twenty species of the orb-weaver spider family Araneidae (Araneae, Araneoidea) from Xishuangbanna, Yunnan, China. *Zoological Research: Diversity and Conservation*, *1*(4), 290-341. https://doi.org/10.24272/j.issn.2097-3772.2024.023

Miller, S. E. (2007). DNA barcoding and the renaissance of taxonomy. *Proceedings of the National Academy of Sciences*, *104*(12), 4775-4776.

Miller, J. A., Beentjes, K. K., van Helsdingen, P., & IJland, S. (2013). Which specimens from a museum collection will yield DNA barcodes? A time series study of spiders in alcohol. *ZooKeys*, (365), 245. https://doi.org/10.3897/zookeys.365.5787

Morano, E. (2023). The family Araneidae Clerck, 1757 (Arachnida: Araneae) in the Iberian-Balearic area. *Revista Ibérica de Aracnología, 42*, 67-118.

Morlon, H. (2014). Phylogenetic approaches for studying diversification. *Ecology Letters*, *17*(4), 508-525. https://doi.org/10.1111/ele.12251

Morton, S. R., & Hill, R. (2014). What is biodiversity, and why is it important? In *Biodiversity: Science and solutions for Australia* (pp. 1–12). Collingwood, Melbourne: CSIRO Publishing.

Mukhtar, M. K. (2012). Spiders of the genus *Neoscona* (Araneae: Araneidae) from Punjab, Pakistan. *Pakistan Journal of Zoology*, *44*(6), 1711-1720.

Naderinejad, M., Junge, K., & Hughes, J. (2023). Exploration of the Design of Spiderweb-Inspired Structures for Vibration-Driven Sensing. *Biomimetics*, *8*(1), 111. https://doi.org/10.3390/biomimetics8010111

Nafin, K. S. (2022). *Diversity and ecology of spiders (order Araneae) in Muriyad Kol Wetlands of Kerala* [Doctoral dissertation, University of Calicut]. The Shodhganga@INFLIBNET Centre. http://hdl.handle.net/10603/480194

Namkung, J. (1964). Spiders from Chungjoo, Korea. Atypus 33-34: 31-50.

National Forum on Biodiversity. (1986). *Proceedings of the National Forum on Biodiversity*. Washington, D.C. Retrieved December 8, 2024, from https://siarchives.si.edu/collections/siris_arc_254229

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences*, *76*(10), 5269-5273. https://doi.org/10.1073/pnas.76.10.5269.

Nentwig, W., Blick, T., Gloor, D., Jäger, P., & Kropf, C. (2019). Tackling taxonomic redundancy in spiders: the infraspecific spider taxa described by Embrik Strand (Arachnida: Araneae). *Arachnologische Mitteilungen*, *58*, 29-51.

Oh, J. H., Kim, S., & Lee, S. (2022). DNA barcodes reveal population-dependent cryptic diversity and various cases of sympatry of Korean leptonetid spiders (Araneae: Leptonetidae). *Scientific reports*, *12*(1), 15528. https://doi.org/10.1038/s41598-022-18666-y

Ohno, M., & Yaginuma, T. (1969). The spider fauna of the Goto islands belonging to Kyushu, Japan. *Journal of Tokyo University for General Education (nat. Sci.)*, *12*, 7-24.

Palem, H., Kanike, S., & Purushottam, V. R. S. (2017). Diversity of spider fauna (Arachnida: Araneae) in different ecosystems, Eastern Ghats, Southern Andhra Pradesh, India. *South Asian Journal of Life Sciences*, *4*(2), 51-60.

Patel, B. H. (1975). Studies on some spiders of the family Argiopidae (Arachnida: Araneae) from Gujarat, India. *Vidya*, *18*, 153-167.

Patel, B. H. (2003). Fauna of protected areas - 2: A preliminary list of spiders from Parambikulam Wildlife Sanctuary, Kerala, with descriptions of three new species. *Zoos'print Journal*, *18*(10), 1207-1212.

Patel, B. H., & Reddy, T. S. (1993). Two new species of the genera *Meta* C. L. Koch and *Neoscona* Simon of the family Araneidae (Arachnida: Araneae) from coastal Andhra Pradesh, India. *Records of the Zoological Survey of India*, *90*, 1-6.

Patel, S. K. (1988). A new species of spider (family: Araneidae) from Gujarat, India. *Current Science*, *57*, 1029-1030.

Patrick, R. (1997). Biodiversity: Why is it important? In M. L. Reaka-Kudla, D. E. Wilson, & E. O. Wilson (Eds.), *Biodiversity II: Understanding and protecting our biological resources* (pp. 15–24). Washington, DC: Joseph Henry Press.

Patwardhan, A., Ray, S., & Roy, A. (2014). Molecular markers in phylogenetic studies-a review. *Journal of Phylogenetics & Evolutionary Biology*, *2*(2), 131.

Paul, J. (2021). *Taxonomy and ecology of spiders in tropical montane cloud forests of Western Ghats* [Doctoral dissertation, Mahatma Gandhi University]. The Shodhganga@INFLIBNET Centre. http://hdl.handle.net/10603/595276

Paul, J., Sankaran, P. M., Joseph, M. M., & Sebastian, P. A. (2016). The male of the orb-weaving spider *Plebs mitratus* (Simon, 1895) and a redescription of the female (Araneae, Araneidae). *Zootaxa*, *4179*(1), 103-106.

Penney, D. (2000). Miocene spiders in Dominican amber (Oonopidae, Mysmenidae). *Palaeontology*, *43*(2), 343-357. https://doi.org/10.1111/1475-4983.00130

Penney, D., & Ortuño, V. M. (2006). Oldest true orb-weaving spider (Araneae: Araneidae). *Biology Letters*, *2*(3), 447-450. https://doi.org/10.1098/rsbl.2006.0506

Penney, D., & Selden, P. A. (2011). *Fossil spiders: the evolutionary history of a mega-diverse order* (Vol. 1). Manchester: Siri Scientific Press.

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.

Petersen, S. D., Mason, T., Akber, S., West, R., White, B., & Wilson, P. (2007). Species identification of tarantulas using exuviae for international wildlife law enforcement. *Conservation Genetics*, *8*, 497-502. https://doi.org/10.1007/s10592-006-9173-2

Petrunkevitch, A. (1930). The spiders of Porto Rico. Part two. *Transactions of the Connecticut Academy of Arts and Sciences*, *30*, 159-356.

Petrunkevitch, A. I. (1942). A study of amber spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, *34*, 119–464.

Petrunkevitch, A. I. (1958). Amber spiders in European collections. *Transactions of the Connecticut Academy of Arts and Sciences*, *41*, 97–400.

Piacentini, L. N., & Ramírez, M. J. (2019). Hunting the wolf: a molecular phylogeny of the wolf spiders (Araneae, Lycosidae). *Molecular Phylogenetics and Evolution*, *136,* 227-240. https://doi.org/10.1016/j.ympev.2019.04.004

Pickard-Cambridge, F. O. (1904). Arachnida - Araneida and Opiliones. In *Biologia Centrali-Americana, Zoology*. London.

Pickard-Cambridge, O. (1871). On some new genera and species of Araneida. *Proceedings of the Zoological Society of London*, *38*(3), 728-747. https://doi.org/10.1111/j.1469-7998.1870.tb00467.x

Pickard-Cambridge, O. (1877). On some new genera and species of Araneidea. *Annals and Magazine of Natural History*, *19*(109), 26-39. https://doi.org/10.1080/00222937708682093

Platnick, N. I. (1990). Spinneret morphology and the phylogeny of ground spiders (Araneae, Gnaphosoidea). *American Museum Novitates*, *2978*, 1-42.

Platnick, N. I. (2020). *Spiders of the world: a natural history*. United States: Princeton University Press.

Platnick, N. I., & Raven, R. J. (2013). Spider systematics: past and future. *Zootaxa*, *3683*(5), 595-600. https://doi.org/10.11646/zootaxa.3683.5.8

Pocock, R. I. (1892). *Liphistius* and its bearing upon the classification of spiders. *Annals and Magazine of Natural History*, 10(6), 306-314.

Pocock, R. I. (1900). *The fauna of British India, including Ceylon and Burma: Arachnida*. Taylor and Francis. London.

Poinar Jr, G. (2015). *Pulchellaranea pedunculata* n. gen. n. sp. (Araneae: Araneidae), a new genus of spiders with a review of araneid spiders in Cenozoic Dominican amber. *Historical Biology*, *27*(1), 103-108. https://doi.org/10.1080/08912963.2013.869799

Posada, D. (2008). jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution, 25*(7), 1253–1256. https://doi.org/10.1093/molbev/msn083

Prendini, L. (2005). Comment on" Identifying spiders through DNA barcodes". *Canadian Journal of Zoology*, *83*(3), 498-504. https://doi.org/10.1139/z05-025

Presl, J. S. (1822). Additamenta ad Faunam protogaeam, sistens descriptiones aliquot animalium in succino inclusorum. *Deliciae pragenses*, *1*, 191–210.

Priyadarshini, N., Kumari, R., Pathak, R. N., & Pandey, A. K. (2015). Biodiversity and community structure of spiders in Saran, part of Indo-Gangetic Plain, India. *Asian Journal of Conservation Biology*, *4*(2), 121-129.

Purcell, W. F. (1910). The phylogeny of the tracheae in Araneae. *Quarterly Journal of Microscopical Science*, *54*, 519-564. https://doi.org/10.1242/jcs.s2-54.216.519

Purty, R. S., & Chatterjee, S. (2016). DNA barcoding: an effective technique in molecular taxonomy. *Austin Journal Biotechnology & Bioengineering*, *3*(1), 1059.

Purvis, A. (2008). Phylogenetic approaches to the study of extinction. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 301-319. https://doi.org/10.1146/annurev-ecolsys-063008-102010

Quasin, S., & Uniyal, V. P. (2010). Preliminary investigation of spider diversity in Kedarnath Wildlife Sanctuary, Uttarakhand, India. *Indian Forester*, *136*(10), 1340.

Rajeevan, S., Kunnath, S. M., Varghese, T., & Kandambeth, P. P. (2019). Spider diversity (Arachnida: Araneae) in different ecosystems of the Western Ghats, Wayanad region, India. *South Asian Journal of Life Science*, *7*(2), 29-39.

Rambaut, A. (2012). *FigTree*. Version 1.4.4. [Computer Software]. http://tree.bio.ed.ac.uk/software/figtree/

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology, 67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Ramos, M., Coddingtion, J. A., Christenson, T. E., & Irschick, D. J. (2005). Have male and female genitalia coevolved? A phylogenetic analysis of genitalic morphology and sexual size dimorphism in web building spiders (Araneae: Araneoidea). *Evolution*, *59*(9), 1989-1999. https://doi.org/10.1111/j.0014-3820.2005.tb01068.x

Rasnitsyn, A. P., & Ross, A. J. (2000). A preliminary list of arthropod families present in the Burmese amber collection at The Natural History Museum, London. *Bulletin-Natural History Museum Geology Series*, *56*(1), 21-24.

Reddy, T. S. & Patel, B. H. (1992). A new species of *Neoscona* Simon (Araneae: Araneida) from coastal Andhra Pradesh, India. *Entomon*, *17*, 129-130.

Regassa, Y., Lemu, H. G., Sirabizuh, B., & Rahimeto, S. (2021). Studies on the Geometrical Design of Spider Webs for Reinforced Composite Structures. *Journal of Composites Science*, *5*(2), 57. https://doi.org/10.3390/jcs5020057

Remigio, E. A., & Hebert, P. D. (2003). Testing the utility of partial COI sequences for phylogenetic estimates of gastropod relationships. *Molecular Phylogenetics and Evolution*, *29*(3), 641-647. https://doi.org/10.1016/S1055-7903(03)00140-4

Robinson, M. H., & Robinson, B. (1980). Comparative studies of the courtship and mating behavior of tropical araneid spiders. In *Pacific Insects Monograph*.

Rodrigues, E. N. L., & Mendonça Jr, M. D. S. (2011). Araneid orb-weavers (Araneae, Araneidae) associated with riparian forests in southern Brazil: a new species, complementary descriptions and new records. *Zootaxa*, *2759*(1), 60-68. https://doi.org/10.11646/zootaxa.2759.1.3

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology, 61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029

Roy, T. K., Saha, S., & Raychaudhuri, D. (2017). On the araneid fauna (Araneae: Araneidae) of the tea estates of Dooars, West Bengal, India. *World Scientific News*, *67*(1), 1-67.

Saaristo, M. I. (2010). Araneae. In J. Gerlach & Y. M. Marusik (Eds.), *Arachnida and Myriapoda of the Seychelles Islands* (pp. 8–306). Siri Scientific Press. Manchester.

Saha, S., & Raychaudhuri, D. (2004). A survey of spiders (Araneae: Araneidae) of Jaldapara Wildlife Sanctuary, West Bengal, with description of a new *Zilla* species. *Entomon*, *29*, 245-252.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sankaran, P. M., Jobi, M. J., & Sebastian, P. A. (2015). Redescription of the orb-weaving spider *Gasteracantha geminata* (Fabricius, 1798) (Araneae, Araneidae). *Zootaxa*, *3915*(1), 147-150.

Sankaran, P. M., & Sebastian, P. A. (2018). *Phonognatha vicitra* Sherriffs, 1928—a taxonomic misidentification of the orb-weaving spider *Acusilas coccineus* Simon, 1895 (Araneae, Araneidae). *Zootaxa*, *4434*(2), 391-395.

Sankaran, P. M., Caleb, J. T. D., Joseph, M. M., & Sebastian, P. A. (2020). On a new synonymy in the spider genus *Nephila* Leach, 1815 (Araneidae, Nephilinae) from India with supplementary notes on colour polymorphism in the genus. *Zootaxa*, *4786*(4), 592-596. doi:10.11646/zootaxa.4786.4.11

Saupe, E. E., Selden, P. A., & Penney, D. (2010). First fossil *Molinaranea* Mello-Leitão, 1940 (Araneae: Araneidae), from middle Miocene Dominican amber, with a phylogenetic and palaeobiogeographical analysis of the genus. *Zoological Journal of the Linnean Society*, *158*(4), 711-725. https://doi.org/10.1111/j.1096-3642.2009.00581.x

Scharff, N., & Coddington, J. A. (1997). A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). *Zoological Journal of the Linnean Society*, *120*(4), 355-434. https://doi.org/10.1111/j.1096-3642.1997.tb01281.x

Scharff, N., Coddington, J. A., Blackledge, T. A., Agnarsson, I., Framenau, V. W., Szűts, T., Hayashi, C. Y., & Dimitrov, D. (2020). Phylogeny of the orb-weaving spider family Araneidae (Araneae: Araneoidea). *Cladistics*, *36*(1), 1-21. https://doi.org/10.1111/cla.12382

Schindel, D. E., & Miller, S. E. (2005). DNA barcoding a useful tool for taxonomists. *Nature*, *435*.

Schlüter, T. (1978). On the systematics and palecology of resin-preserved Arthropoda of a taphocenosis from the Cenomanian of NW-France. *Berliner Geowissenschaftliche Abhandlungen*, *A 9*, 1–150. https://doi.org/10.23689/fidgeo-3142

Schmidt, J. B., & Scharff, N. (2008). A taxonomic revision of the orb-weaving spider genus *Acusilas* Simon, 1895 (Araneae, Araneidae). *Insect Systematics and Evolution*, *39*(1), 1-38. https://doi.org/10.1163/187631208788784147

Sebastian, P. A., & Peter, K. V. (Eds.). (2009). *Spiders of India*. Universities Press.

Sebastian, P. A., Mathew, M. J., Beevi, S. P., Joseph, J., & Biju, C. R. (2005a). The spider fauna of the irrigated rice ecosystem in central Kerala, India across different elevational ranges. *Journal of Arachnology*, *33*(2), 247-255. https://doi.org/10.1636/05-08.1

Sebastian, P. A., Murugesan, S., Mathew, M. J., Sudhikumar, A. V., & Sunish, E. (2005b). Spiders in Mangalavanam, an ecosensitive mangrove forest in Cochin, Kerala, India (Araneae). *European Arachnology-Acta Zoologica Bulgarica*, *1*, 315-318.

Selden, P. A. (1989). Orb-web weaving spiders in the early Cretaceous. *Nature*, *340*, 711–713. https://doi.org/10.1038/340711a0

Selden, P. A., & Penney, D. (2010). Fossil spiders. *Biological Reviews*, *85*(1): 171-206. https://doi.org/10.1111/j.1469-185X.2009.00099.x

Sen, S., Roy, T. K., Dhali, D. C., Saha, S., & Raychaudhuri, D. (2011). First record of the genus *Tukaraneus* Barrion and Litsinger and *Neoscona yptinika* Barrion and Litsinger (Araneae: Araneidae) from India. *Journal of Asia-Pacific Entomology*, *14*, 367-371.

Sen, S., & Sureshan, P. M. (2020). Rediscovery of the scorpion tailed orb-weaver, *Arachnura melanura* Simon 1867 (Araneae: Araneidae) from India. *Records of the Zoological Survey of India*, *120*(3), 285-288. https://doi.org/10.26515/rzsi/v120/i3/2020/150690

Sen, S., Caleb, J. T. D., & Acharya, S. (2021). First record of the orb-weaving spider *Araneus tubabdominus* Zhu & Zhang, 1993 (Araneae: Araneidae) from India. *Journal of Threatened Taxa*, *13*(12), 19864-19866.

Shabnam, F. P., Smija, M. K., Rajeevan, S., Prasadan, P. K., & Sudhikumar, A. V. (2021). Spider diversity (Arachnida; Araneae) in different plantations of Western Ghats, Wayanad region, India: spider diversity in Western Ghats, Wayanad. *European journal of Ecology*, *7*(1). https://doi.org/10.17161/eurojecol.v7i1.14612

Sherriffs, W. R. (1928). South Indian Arachnology. Part III. *Annals and Magazine of Natural History*, *2*(8), 177-192. https://doi.org/10.1080/00222932808672866

Sherwood, D. (2021). On the nomenclatural and taxonomic status of the spider taxa in James Barbut's 'Genera Insectorum of Linnaeus' (Araneae). *Arachnologische Mitteilungen 62*, 7-10.

Shilpa, K. R., Anis, K. V., & Sudhikumar, A. V. (2023). First record of *Araneus viridiventris* Yaginuma, 1969 (Araneae: Araneidae) from India with redescription of the female. *Serket*, *19*(2).

Simon, E. (1864). *Histoire naturelle des araignées (aranéides)*. Paris: Librairie Encyclopédique de Roret. https://doi.org/10.5962/bhl.title.47654

Simon, E. (1867). On three new spiders. *Journal and Store of Pure and Applied Zoology, 19*(2), 15-24.

Simon, E. (1874). *Les arachnides de France*. Paris: Librairie Encyclopédique de Roret.

Simon, E. (1877). Études arachnologiques. 5e mémoire. IX. Arachnides recueillis aux îles Philippines par MM. G.-A. Baer et Laglaise. *Annales de la Société Entomologique de France*, *5*(7), 53–96.

Simon, E. (1886). Arachnids collected by M. A. Pavie in the kingdom of Siam, Cambodia and Cochin-China. *Actes de la Société Linnéenne de Bordeaux*, *40*, 137–166.

Simon, E. (1889). Arachnides de l'Himalaya, recueillis par MM. Oldham et Wood-Mason, et faisant partie des collections de l'Indian Museum. Première partie. *Journal of the Asiatic Society of Bengal, part II (Natural History)*, *58*(4), 334–344.

Simon, E. (1894). *Histoire naturelle des araignées* (2nd ed., Vol. 1). Paris: Roret.

Simon, E. (1895). *Histoire naturelle des araignées* (2nd ed.). Paris: Roret. https://doi.org/10.5962/bhl.title.51973

Simon, E. (1905). Arachnides de Java, recueillis par le Prof. K. Kraepelin en 1904. *Mitteilungen aus dem Naturhistorischen Museum in Hamburg*, *22*, 49–73.

Simon, E. (1909). Étude sur les arachnides du Tonkin (1re partie). *Bulletin Scientifique de la France et de la Belgique*, *42*, 69–147.

Simpson, G. G. (1961). *Principles of animal taxonomy*. Columbia University Press.

Sivayyapram, V., Kunsete, C., Xu, X., Smith, D. R., Traiyasut, P., Deowanish, S., Li, D. Q., & Warrit, N. (2024). Molecular phylogeny, biogeography, and species delimitation of segmented spider genus *Liphistius* (Araneae: Liphistiidae) in Thailand. *Zoological Journal of the Linnean Society*, *201*(2), 339-357. doi:10.1093/zoolinnean/zlad149

Smith, H. M. (2006). A revision of the genus *Poltys* in Australasia (Araneae: Araneidae). *Records of the Australian Museum*, *58*, 43-96.

Smitha, M. S., & Sudhikumar, A. V. (2020). A diversity of spiders (Arachnida: Araneae) from a cashew ecosystem in Kerala, India. *Journal of Threatened Taxa*, *12*(13), 16879-16884. https://doi.org/10.11609/jott.5973.12.13.16879-16884

Sousa, P. (2006). Spider records from Serra da Estrela Natural Park (Portugal): families Araneidae and Tetragnathidae (Arachnida, Araneae). *Boletín Sociedad Entomológica Aragonesa*, *39*(2006), 245-250.

Spasojevic, T., Kropf, C., Nentwig, W., & Lasut, L. (2016). Combining morphology, DNA sequences, and morphometrics: revising closely related species in the orb-weaving spider genus *Araniella* (Araneae, Araneidae). *Zootaxa*, *4111*(4): 448-470. doi:10.11646/zootaxa.4111.4.6

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics, 30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Starrett, J., Hedin, M., Ayoub, N., & Hayashi, C. Y. (2013). Hemocyanin gene family evolution in spiders (Araneae), with implications for phylogenetic relationships and divergence times in the infraorder Mygalomorphae. *Gene*, *524*(2), 175-186. https://doi.org/10.1016/j.gene.2013.04.037

Stoeckle, M. (2003). Taxonomy, DNA, and the bar code of life. *BioScience*, *53*(9), 796-797.

Stoliczka, F. (1869). Contribution towards the knowledge of Indian Arachnoidea. *Journal of the Asiatic Society of Bengal, part II (Physical Science)*, *38*(4), 201-251.

Strand, E. (1929). Zoological and palaeontological nomenclatorical notes. *Acta Universitatis Latviensis, 20*, 1-29.

Sudhikumar, A. V. (2021). *Keralathile chilanthikal*. Thiruvananthapuram, Kerala: State Institute of Languages.

Sudhikumar, A. V., Mathew, M. J., Sunish, E., & Sebastian, P. A. (2005a). Seasonal variation in spider abundance in Kuttanad rice agroecosystem, Kerala, India (Araneae). *European Arachnology*, *1*, 181-190.

Sudhikumar, A. V., Mathew, M. J., Sunish, E., Murugesan, S., & Sebastian, P. A. (2005b). Preliminary studies on the spider fauna in Mannavan shola forest, Kerala, India (Araneae). *European Arachnology Supplement*, *1*, 319-327.

Sudhin P. P. (2022). *Diversity and ecology of spiders in Wayanad wildlife sanctuary* [Doctoral dissertation, University of Calicut].

Sudhin, P. P., & Sen, S. (2023). Spiders (Arachnida: Araneae) of the Shendurney Wildlife Sanctuary, Kerala, India. *Records of the Zoological Survey of India*, 409-420.

Sumesh, N. V., & Sudhikumar, A. V. (2020). Checklist of spiders from the sacred groves of Northern Kerala, India. *Uttar Pradesh Journal of Zoology*, *41*(9), 104-115.

Sundevall, C. J. (1833). *Conspectus Arachnidum*. C. F. Berling.

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.

Tahir, H. M., Summer, M., Mehmood, S., Ashraf, S., & Naseem, S. (2019). DNA barcoding of spiders from agricultural fields. *Mitochondrial DNA Part B*, *4*(2), 4144-4151. https://doi.org/10.1080/23802359.2019.1693283

Tamura K, Stecher G, and Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Molecular Biology and Evolution* 38:3022-3027. https://doi.org/10.1093/molbev/msab120

Tan, J. (2018). *Argiope hoiseni*, a new species of the spider genus *Argiope* (Araneae, Araneidae) from Peninsular Malaysia based on morphology and molecular analyses. *Zootaxa*, *4457*(1): 129-142. https://doi.org/10.11646/zootaxa.4457.1.6

Tan, J., Chan, Z. J., Ong, C. A., & Yong, H. S. (2019a). Phylogenetic relationships of *Actinacantha* Simon, *Gasteracantha* Sundevall, *Macracantha* Hasselt and *Thelacantha* Simon spiny orb weavers (Araneae: Araneidae) in Peninsular Malaysia. *Raffles Bulletin of Zoology*, *67*: 32-55. https://doi.org/10.26107/RBZ-2019-0003

Tan, J., Chan, Z. Y., Wong, C. X., Koh, J. K. H., & Yong, H. S. (2019b). Morphological and molecular evidence supports *Argiope chloreis* Thorell 1877 and *A. chloreides* Chrysanthus 1961 (Araneidae: Argiopinae) as distinct species. *Acta Arachnologica*, *68*(2), 41-58. https://doi.org/10.2476/asjaa.68.41

Tanikawa, A. (1991). New record of *Arachnura melanura* Simon, 1867 (Araneae: Araneidae), from Japan, with the first description of the male. *Acta Arachnologica*, *40*(1), 11-15.

Tanikawa, A. (1999). Japanese spiders of the genus *Eriovixia* (Araneae: Araneidae). *Acta Arachnologica*, *48*(1), 41-48.

Tanikawa, A. (2007). An identification guide to the Japanese spiders of the families Araneidae, Nephilidae and Tetragnathidae. *Arachnological Society of Japan*, 1-121.

Tanikawa, A. (2009). Hersiliidae. Nephilidae, Tetragnathidae, Araneidae. In H. Ono (Ed.), *The spiders of Japan with keys to the families and genera and illustrations of the species*, *149*. Kanagawa: Tokai University Press.

Tanikawa, A., & Ono, H. (1993). Spiders of the genus Cyclosa (Araneae, Araneidae) from Taiwan. *Bulletin of the National Museum of Nature and Science Tokyo (A)*, *19*, 51-64.

Tanikawa, A., Chang, Y. H., & Tso, I. M. (2006). Identity of a Japanese spider species recorded as "*Pasilobus bufoninus*" (Araneae: Araneidae), with a description of the male considering the sequence of mtDNA. *Acta Arachnologica*, *55*(1): 45-49. https://doi.org/10.2476/asjaa.55.45

Tanikawa, A., Chang, Y. H., & Tso, I. M. (2010). Taxonomic revision of Taiwanese and Japanese *Cyrtophora* spiders hitherto identified with *C. moluccensis* (Arachnida, Araneae), using molecular and morphological data. *Acta Arachnologica*, *59*(1): 31-38. https://doi.org/10.2476/asjaa.59.31

Tanikawa, A., & Yamasaki, T. (2019). *Anepsion japonicum* (Bösenberg & Strand 1906) is a junior synonym of *A. maritatum* (O. Pickard-Cambridge 1877). *Acta Arachnologica*, *68*(1), 11-13.

Tanikawa, A., Yamasaki, T., & Petcharad, B. (2021). Two new genera of Araneidae (Arachnida: Araneae). *Acta Arachnologica*, *70*(2), 87-101. https://doi.org/10.2476/asjaa.70.87

Tanikawa, A., & Petcharad, B. (2023). *Leviaraneus*, a new genus of Araneidae (Arachnida: Araneae) from Asia. *Acta Arachnologica*, *72*(2): 119-127. https://doi.org/10.2476/asjaa.72.119

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., & Vogler, A. P. (2003). A plea for DNA taxonomy. *Trends in Ecology and Evolution*, *18*(2), 70-74.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic acids research*, *25*(24), 4876-4882. https://doi.org/10.1093/nar/25.24.4876

Thorell, T. (1859). Nya exotiska Epeirider. *Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar*, *16*, 299-304.

Thorell, T. (1877). Studi sui Ragni Malesi e Papuani. I. Ragni di Selebes raccolti nel 1874 dal Dott. O. Beccari. *Annali del Museo Civico di Storia Naturale di Genova*, *10*, 341-637.

Thorell, T. (1878). Studi sui ragni Malesi e Papuani. II. Ragni di Amboina raccolti Prof. O. Beccari. *Annali del Museo Civico di Storia Naturale di Genova*, *13*, 5-317.

Thorell, T. (1881). Studi sui Ragni Malesi e Papuani. III. Ragni dell'Austro Malesia e del Capo York, conservati nel Museo civico di storia naturale di Genova. *Annali del Museo Civico di Storia Naturale di Genova*, *17*, 1-720.

Thorell, T. (1887). Viaggio di L. Fea in Birmania e regioni vicine. II. Primo saggio sui ragni birmani. *Annali del Museo Civico di Storia Naturale di Genova*, *25*, 5-417.

Thorell, T. (1890). Studi sui ragni Malesi e Papuani. IV, 1. *Annali del Museo Civico di Storia Naturale di Genova*, *28*, 5-421.

Thorell, T. (1893). Novae species aranearum a Cel. Th. Workman in ins. Singapore collectae. *Bullettino della Società Entomologica Italiana*, *24*(3), 209–252.

Tikader, B. K. (1961). Revision of Indian spiders of the genus *Cyrtarachne* (Argiopidae: Arachnida). *Journal of the Bombay Natural History Society*, *57*, 547-556.

Tikader, B. K. (1962). Studies on some Indian spiders (Araneae: Arachnida). *Journal of the Linnean Society of London, Zoology*, *44*(300), 561-584.

Tikader, B. K. (1963). Studies on some spider fauna of Maharashtra and Mysore states-Part I. *Journal of the University of Poona (Sci. Tech.)*, *24*, 29-54.

Tikader, B. K. (1966). Studies on spider fauna of Khasi and Jaintia Hills, Assam, India. *Journal of the Assam Science Society*, *9*, 139-154.

Tikader, B. K. (1970). Spider fauna of Sikkim. *Records of the Zoological Survey of India*

Tikader, B. K. (1977). Studies on spider fauna of Andaman and Nicobar islands, Indian Ocean. *Records of the Zoological Survey of India*, *72*, 153-212.

Tikader, B. K. (1980). Description of a new species of spider of the genus *Neoscona* (Family: Araneidae) from India and some observations on intraspecific colour variation. *Proceedings of the Indian Academy of Science (Anim. Sci.)*, *89*, 247-252.

Tikader, B. K. (1982). Part 1. Family Araneidae (= Argiopidae), Typical orb-weavers. In: *The fauna of India. Spiders: Araneae* (Vol. II). Calcutta: Zoological Survey of India.

Tikader, B. K., & Bal, A. (1981). Studies on some orb-weaving spiders of the genera Neoscona Simon and Araneus Clerck of the family Araneidae (=Argiopidae) from India. *Records of the Zoological Survey of India, Occasional Paper*, *24*, 1-60.

Tikader, B. K., & Biswas, B. (1981). Spider fauna of Calcutta and vicinity: Part-I. *Records of the Zoological Survey of India, Occasional Paper*, *30*, 1-149.

Trüper, H. G., & Krämer, J. (1981). Principles of characterization and identification of prokaryotes. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, & H. G. Schlegel (Eds.), *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria* (pp. 176-193). Berlin, Heidelberg: Springer Berlin Heidelberg.

Tso, I. M., & Tanikawa, A. (2000). New records of five orb-web spiders of the genera *Leucauge*, *Mesida*, and *Eriovixia* (Araneae: Tetragnathidae and Araneidae) from Taiwan. *Acta Arachnologica*, *49*(2), 125-131.

Turk, E., Čandek, K., Kralj Fišer, S., & Kuntner, M. (2020). Biogeographical history of golden orbweavers: Chronology of a global conquest. *Journal of Biogeography*, *47*(6), 1333-1344. https://doi.org/10.1111/jbi.13838

Türkeş, T., & Mergen, O. (2008). The orb-web weavers spiders fauna of the Central Anatolian region in Turkey with three new records for Turkey (Araneae: Araneidae). *Munis Entomology and Zoology, 3*(1), 295-302.

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. https://doi.org/10.1038/s41598-019-50510-8

UN, I. (1992). Convention on biological diversity. *Treaty Collection*. Retrieved from Convention Text.

Uno, G., Storey, R., & Moore, R. (2001). *Principles of Botany*. New York: McGraw Hill

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics, 27*(2), 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Vinson, A. (1863). *Aranéides des îles de La Réunion, Maurice et Madagascar* [Spiders of the islands of La Réunion, Mauritius, and Madagascar]. Librairie Classique Eugène Belin. https://doi.org/10.5962/bhl.title.125517

Vishnudas, E., Ajitha, V., & Sudhikumar, A. V. (2021). Diversity of spiders in Poovar mangrove ecosystem, Kerala, India. *Serket*, *8*(1), 53-58.

von Siebold, C. T. E. (1848). *Lehrbuch der vergleichenden Anatomie der wirbellosen Thiere.* Verlag von Veit und Comp, Berlin.

Walckenaer, C. A. (1841). *Histoire naturelle des insectes. Aptères* (Vol. 2). Roret.

Wang, Z. L., Yang, X. Q., Wang, T. Z., & Yu, X. (2018). Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. *Mitochondrial DNA Part A*, *29*(5), 695-702. https://doi.org/10.1080/24701394.2017.1350949

Wanntorp, H. E., Brooks, D. R., Nilsson, T., Nylin, S., Ronquist, F., Stearns, S. C., & Wedell, N. (1990). Phylogenetic approaches in ecology. *Oikos*, 119-132.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical transactions of the royal society B: biological sciences*, *360*(1462), 1847-1857. https://doi.org/10.1098/rstb.2005.1716

Wheeler, W. C., Coddington, J. A., Crowley, L. M., Dimitrov, D., Goloboff, P. A., Griswold, C. E., Hormiga, G., Prendini, L., Ramírez, M. J., Sierwald, P., Almeida-Silva, L. M., Álvarez-Padilla, F., Arnedo, M. A., Benavides, L. R., Benjamin, S. P., Bond, J. E., Grismado, C. J., Hasan, E., Hedin, M., Izquierdo, M. A., Labarque, F. M., Ledford, J., Lopardo, L., Maddison, W. P., Miller, J. A., Piacentini, L. N., Platnick, N. I., Polotow, D., Silva-Dávila, D., Scharff, N., Szűts, T., Ubick, D., Vink, C., Wood, H. M., & Zhang, J. X. (2017). The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. *Cladistics*, *33*(6), 576-616. https://doi.org/10.1111/cla.12182

Whyte, R., & Anderson, G. (2017). *A field guide to spiders of Australia*. Clayton, Australia: CSIRO Publishing.

Wood, H. M., Matzke, N. J., Gillespie, R. G., & Griswold, C. E. (2013). Treating fossils as terminal taxa in divergence time estimation reveals ancient vicariance patterns in the palpimanoid spiders. *Systematic Biology*, *62*(2), 264-284. https://doi.org/10.1093/sysbio/sys092

Workman, T. (1896). Malaysian spiders. Belfast, pp. 25-104. doi:10.5962/bhl.title.101972

World Spider Catalog (2025). *World Spider Catalog*. Version 25.5. Natural History Museum Bern, Retrieved January 10, 2025 from http://wsc.nmbe.ch. https://doi.org/10.24436/2

Wunderlich, J. (1986). *Spinnenfauna gestern und heute. 1. Fossile Spinnen in Bernstein und ihre heute lebenden Verwandten*. Bauer.

Wunderlich, J. (2004a). The fossil mygalomorph spiders (Araneae) in Baltic and Dominican amber and about extant members of the family Micromygalidae. *Beiträge zur Araneologie*, *3*, 595-631.

Wunderlich, J. (2004b). The fossil spiders (Araneae) of the families Tetragnathidae and Zygiellidae n. stat. in Baltic and Dominican amber, with notes on higher extant and fossil taxa. *Beiträge zur Araneologie*, *3*, 899-955.

Wunderlich, J. (2004c). Fossil taxa of the family Araneidae (Araneae) inclusively Nephilinae in Baltic and Dominican amber, with the description of a new extant subfamily and notes on selected extant taxa. *Beiträge zur Araneologie*, *3*, 956-997.

Wunderlich, J. (2004d). The fossil spiders of the family Anapidae s. l. (Aeaneae) [sic] in Baltic, Dominican and Mexican amber and their extant relatives, with the description of the new subfamily Comarominae. *Beiträge zur Araneologie*, *3*, 1020-1111.

Wunderlich, J. (2004e). The fossil Zoropsidae in Baltic amber with revised diagnoses of the family Zoropsidae and its fossil and extant higher taxa (Arachnida, Araneae). *Beiträge zur Araneologie*, *3*, 1489-1522.

Wunderlich, J. (2004f). The fossil spiders (Araneae) of the family Zodariidae in Baltic amber, with remarks on their subfamilies including the Cryptothelinae and the Homalonychinae. *Beiträge zur Araneologie*, *3*, 1578-1611.

Wunderlich, J. (Ed.). (2004g). *Fossil spiders in amber and copal: conclusions, revisions, new taxa and family diagnoses of fossil and extant taxa*.

Wunderlich, J. (2008). On extant and fossil (Eocene) European comb-footed spiders (Araneae: Theridiidae), with notes on their subfamilies, and with descriptions of new taxa. *Beiträge zur Araneologie*, *5*.

Wunderlich, J. (2011). On extant European spiders of the tribe Mangorini (Araneae: Araneidae) and two doubtful taxa in Baltic amber. In Extant and fossil spiders (Araneae). *Beiträge zur Araneologie*, *6*, 9-18.

Wunderlich, J. (2012). New fossil spiders (Araneae) of eight families in Eocene Baltic amber, and revisions of selected taxa. In Fifteen papers on extant and fossil spiders (Araneae). *Beiträge zur Araneologie*, *7*, 94 –149

Xu, X., Liu, F., Chen, J., Li, D., & Kuntner, M. (2015). Integrative taxonomy of the primitively segmented spider genus *Ganthela* (Araneae: Mesothelae: Liphistiidae): DNA barcoding gap agrees with morphology. *Zoological Journal of the Linnean Society*, *175*(2), 288-306. https://doi.org/10.1111/zoj.12280

Yaginuma, T., & Wen, Z. G. (1983). Chinese and Japanese spiders (II). *Faculty of Letters Revue, Otemon Gakuin University*, *17*, 187–205.

Yaginuma, T. (1955). On the Japanese spiders: genera Mangora, Neoscona, and Zilla. *Acta Arachnologica*, *14*(1), 15-24.

Yaginuma, T. (1958). Revision of Japanese spiders of family Argiopidae. II. Genus *Cyrtophora*. *Acta Arachnologica*, *16*(1), 10-17.

Yaginuma, T. (1960). *Spiders of Japan in colour*. Hoikusha.

Yaginuma, T. (1963). Spiders from Okinawa Island. *Atypus*, *29*, 18–21.

Yaginuma, T. (1968). [The spider genus *Cyrtophora* of Japan]. *Kansaishizenkagaku*, *20*, 34–38.

Yaginuma, T. (1986). *Spiders of Japan in color* (New ed.). Hoikusha Publishing Co.

Yin, C. M. (1978). A study on the general orb-weaver spiders and wolf-spiders (Araneae: Araneidae, Lycosidae) from rice fields. *Journal of Hunan Teachers College (nat. Sci. Ed.)*, *1978*(10), 1-21.

Yin, C. M., & Wang, J. F. (1982). A new species of spider of genus *Neoscona* from China (Araneae, Araneidae). *Acta Zootaxonomica Sinica*, *7*, 260-262.

Yin, C. M., Wang, J. F., Zhang, Y. J., Peng, X. J., & Chen, X. O. (1989). The study of the subfamily [sic] Argiope from China (Araneae, Araneidae). *Acta Scientiarum Naturalium Universitatis Normalis Hunanensis*, *12*, 60-69.

Yin, C. M., Wang, J. F., Xie, L. P., & Peng, X. J. (1990). New and newly recorded species of the spiders of family Araneidae from China (Arachnida, Araneae). In: *Spiders in China: one hundred new and newly recorded species of the families Araneidae and Agelenidae*. Hunan Normal University Press.

Yin, C. M., Wang, J. F., Zhu, M. S., Xie, L. P., Peng, X. J., & Bao, Y. H. (1997). *Fauna Sinica: Arachnida: Araneae: Araneidae*. Beijing: Science Press.

Yin, C. M., Peng, X. J., Yan, H. M., Bao, Y. H., Xu, X., Tang, G., Zhou, Q. S., & Liu, P. (2012). *Fauna Hunan: Araneae in Hunan, China*. Changsha: Hunan Science and Technology Press.

Yong, H. S., Song, S. L., Chua, K. O., Suana, W., Eamsobhana, P., Tan, J., Lim, P. E., & Chan, K. G. (2021). Complete mitochondrial genomes and phylogenetic relationships of the genera *Nephila* and *Trichonephila* (Araneae, Araneoidea). *Scientific Reports*, *11*(10680), 1-13. doi:10.1038/s41598-021-90162-1

Yu, K. P., Kuntner, M., & Cheng, R. C. (2022). Phylogenetic evidence for an independent origin of extreme sexual size dimorphism in a genus of araneid spiders (Araneae: Araneidae). *Invertebrate Systematics*, *36*(1), 48-62. doi:10.1071/IS21019

Yuan, T., & Zhang, Z. S. (2020). New molecular evidence for the synonymy of *Nephila laurinae* and *Trichonephila antipodiana*. *Acta Arachnologica Sinica*, *29*(2): 119-121. doi:10.3969/j.issn.1005-9628.2020.02.010

Zamani, A., Marusik, Y. M., & Šestáková, A. (2020). On *Araniella* and *Neoscona* (Araneae, Araneidae) of the Caucasus, Middle East and Central Asia. *ZooKeys*, *906*, 13-40.

Zamani, A., Enayatnia, M., Mirshekar, A., Mayvan, M. M., Kováč, L., & Marusik, Y. M. (2025). New data on the spider fauna of Iran (Arachnida: Araneae), part XII. *Zootaxa*, *5601*(1), 1-45.

Zhang, J. X., & Maddison, W. P. (2013). Molecular phylogeny, divergence times and biogeography of spiders of the subfamily Euophryinae (Araneae: Salticidae). *Molecular Phylogenetics and Evolution*, *68*(1), 81-92. https://doi.org/10.1016/j.ympev.2013.03.017

Zhu, M. S., & Zhang, Y. Q. (1993). Records of some spiders of the family Araneidae from Guangxi (Arachnida: Araneae). *Journal of the Guangxi Agricultural College*, *12*, 36-43.

Zschokke, S., Countryman, S., & Cushing, P. E. (2021). Spiders in space—orb-web-related behaviour in zero gravity. *The Science of Nature*, *108*(1). https://doi.org/10.1007/s00114-020-01708-8

Zuckerkandl, E., & Pauling, L. (1965). Evolutionary divergence and convergence in proteins. In V. Bryson & H. Vogel (Eds.), *Evolving genes and proteins*. New York: Academic Press. https://doi.org/10.1016/B978-1-4832-2734-4.50017-6

APPENDICES

Appendix I - **Table S1.** Details of voucher specimens used for COI barcoding, along with DNA quantification results based on spectrophotometer readings, summary of BLAST results showing percent identity and closest matching species and GenBank accession numbers

Appendix II - **Table S2.** Details of voucher specimens used for H3 barcoding, along with DNA quantification results based on spectrophotometer readings, summary of BLAST results showing percent identity and closest matching species and GenBank accession numbers

Appendix III - **Table S3.** Details of sequences used for the phylogenetic analysis of Oriental and Palaearctic araneids, including the GenBank accession numbers

Table S1. Details of voucher specimens used for COI barcoding, along with DNA quantification results based on spectrophotometer readings, summary of BLAST results showing percent identity and closest matching species and GenBank accession numbers

* Sample contamination, # Unsuccessful PCR, $ Further analysis required, (N) New records to the GenBank database

Sl. No.	Voucher No.	Species	Absorbance 260 nm	Absorbance 280 nm	DNA Concentration	260/280	Percent identity	Match species	Accession no.	Remarks
1	ARAMP007	*Bijoaraneus mitificus*	0.843	0.43	42.1	1.96	97.29	*Bijoaraneus mitificus*	PQ651518	
2	ARAMP008	*Chorizopesoides orientalis*	0.18	0.113	9	1.6	93.04	Chorizopesoides wulingensis	PQ651519	(N)
3	ARAMP012	Eriovixia sakeidaorum	1.637	0.779	81.8	2.1	94.88	*Eriovixia hainanensis*	PQ651564	(N)
4	ARAMP015	*Herennia multipuncta*	0.065	0.036	65	1.805	100	*Herennia multipuncta*	PQ495943	
5	ARAMP016	*Neoscona elliptica*	0.543	0.26	27.1	2.09	99.63	*Guizygiella sp.*	PQ651524	(N)
6	ARAMP017	*Nephila pilipes*	0.138	0.078	138	1.769	100	Nephila pilipes	PQ495944	
7	ARAMP018	*Plebs mitratus*	3.546	1.718	177.3	2.06	87.28	Araneus omnicolor	PQ558939	(N)
8	ARAMP019	*Poltys columnaris*	0.869	0.475	43.4	1.83	84.82	Verrucosa sp.	PQ558940	(N)
9	ARAMP020	*Thelacantha brevispina*	0.797	0.406	39.9	1.96	100	*Thelacantha brevispina*	PQ495946	
10	ARAMP021	*Acusilas coccineus*	0.183	0.095	183	1.926	98.37	*Acusilas coccineus*	PQ558941	(N)
11	ARAMP022	*Anepsion maritatum*	0.836	0.405	41.8	2.06	93.39	*Anepsion japonicum*	PQ558942	

12	ARAMP023	*Araneus tubabdominus*	0.894	0.459	44.7	1.95	90.49	*Neoscona punctigera*	PQ187441	
13	ARAMP024	*Argiope aemula*	0.925	0.463	925	1.997	97.11	*Argiope aemula*	PQ558943	
14	ARAMP025	*Argiope catenulata*	0.127	0.062	127	2.048	98.15	*Argiope catenulata*	PQ558944	
15	ARAMP026	*Cyrtophora cicatrosa*	1.289	0.665	64.4	1.94	99.47	*Cyrtophora cicatrosa*	PQ495948	
16	ARAMP027	*Cyrtophora moluccensis*	0.186	0.102	186	1.823	100	*Cyrtophora moluccensis*	PQ495949	
17	ARAMP028	*Cyrtophora unicolor*	0.113	0.058	113	1.948	98.54	*Cyrtophora unicolor*	PQ558946	
18	ARAMP029	*Arachnura melanura*	0.529	0.274	26.4	1.93	99.64	*Arachnura melanura*	PQ651520	
19	ARAMP030	*Arachnura melanura*	0.014	0.009	14	1.555	97.3	*Arachnura melanura*		*
20	ARAMP031	*Arachnura melanura*	1.004	0.664	50.2	1.51	99.64	*Arachnura melanura*	PQ651521	
21	ARAMP032	*Arachnura melanura*	0.572	0.274	28.6	2.08	99.64	*Arachnura melanura*	PQ651522	
22	ARAMP033	*Argiope anasuja*	0.351	0.179	351	1.96	99.21	*Argiope anasuja*	PQ558945	
23	ARAMP034	*Argiope pulchella*	0.111	0.06	111	1.85	100	*Argiope pulchella*	PQ651537	
24	ARAMP035	*Argiope pulchella*	0.131	0.07	131	1.871	99.81	*Argiope versicolor*	PQ651538	
25	ARAMP036	*Argiope versicolor*	0.052	0.029	52	1.793	97.38	*Argiope versicolor*	PQ651539	
26	ARAMP037	*Argiope versicolor*	0.019	0.011	19	1.727	98.34	*Argiope versicolor*	PQ651540	

27	ARAMP038	*Argiope pulchella*	0.332	0.175	16.6	1.9	99.63	*Argiope versicolor*	PQ651541	
28	ARAMP039	*Araneus sp*	0.666	0.345	33.3	1.93	90.42	*Neoscona sp.*	PQ651536	(N)
29	ARAMP041	*Cyclosa bifida*	0.612	0.314	30.6	1.95	100	*Cyclosa bifida*	PQ495950	
30	ARAMP042	*Cyclosa bifida*	0.257	0.133	12.8	1.92	99.42	*Cyclosa bifida*	PQ187442	
31	ARAMP043	*Cyclosa bifida*	0.187	0.108	9.4	1.73	99.1	*Cyclosa bifida*	PQ187443	
32	ARAMP044	*Cyclosa confraga*	0.198	0.11	9.9	1.81	93.05	*Cyclosa chichawatniensis*	PQ558948	(N)
33	ARAMP045	*Cyclosa hexatuberculata*	0.016	0.009	16	1.777	100	*Cyclosa hexatuberculata*	PQ651542	
34	ARAMP046	*Cyclosa hexatuberculata*	0.11	0.07	5.5	1.57	100	*Cyclosa hexatuberculata*	PQ651543	
35	ARAMP047	*Cyclosa hexatuberculata*	0.019	0.011	19	1.727	100	*Cyclosa hexatuberculata*	PQ651544	
36	ARAMP048	*Cyclosa spirifera*	0.411	0.211	20.6	1.94	99.79	*Cyclosa spirifera*	PQ651545	
37	ARAMP049	*Cyclosa mulmeinensis*	0.286	0.15	14.3	1.9	98.84	*Cyclosa mulmeinensis*	PQ558947	
38	ARAMP050	*Cyclosa spirifera*	0.065	0.034	65	1.911	100	*Cyclosa spirifera*	PQ651546	
39	ARAMP051	*Cyclosa purnai*	0.012	0.007	12	1.714	99.8	*Cyclosa spirifera*	PQ651549	(N)
40	ARAMP052	*Cyclosa quinquegutteta*	0.008	0.005	8	1.6	90.37	*Cyclosa turbinata*	PQ558949	

41	ARAMP053	*Cyclosa spirifera*	0.033	0.017	33	1.941	99.63	*Cyclosa spirifera*	PQ651550	
42	ARAMP054	*Cyclosa spirifera*	0.014	0.008	14	1.75	100	*Cyclosa spirifera*	PQ651547	
43	ARAMP055	*Cyclosa spirifera*	0.128	0.076	6.4	1.69	100	*Cyclosa spirifera*	PQ651548	
44	ARAMP056	*Cyclosa sp*	0.364	0.174	18.2	2.1	95.07	*Cyclosa alba*	PQ651551	(N)
45	ARAMP057	*Cyclosa sp*	0.122	0.056	6.1	2.2	97.63	*Cyclosa bifida*	PQ651552	(N)
46	ARAMP058	*Cyclosa sp*	0.204	0.107	10.2	1.9	90.23	*Cyrtarachne nagasakiensis*	PQ651553	(N)
47	ARAMP059	Cyclosa sp	0.042	0.013	2.1	3.29				#
48	ARAMP060	*Parawixia dehaani*	0.036	0.022	36	1.636	98.76	*Parawixia dehaani*	PQ558950	
49	ARAMP061	*Eriovixia gryffindori*	0.213	0.098	10.7	2.17	88.85	*Araneus pentagrammicus*	PQ651554	(N)
50	ARAMP062	*Eriovixia excelsa*	0.033	0.013	1.7	2.5				#
51	ARAMP063	*Eriovixia sp*	0.141	0.068	7	2.07	88.32	*Aranea sps*	PQ651555	(N)
52	ARAMP064	Eriovixia sp	0.275	0.139	13.8	1.98				#
53	ARAMP065	*Eriovixia poonaensis*	0.253	0.136	12.7	1.87	97.77	*Eriovixia poonaensis*	PQ651557	
54	ARAMP066	*Eriovixia sp*	0.321	0.208	16	1.54	86.2	*Austracantha minax*	PQ651556	(N)
55	ARAMP067	*Eriovixia poonaensis*	0.432	0.194	21.6	2.23	99.58	*Eriovixia poonaensis*	PQ651558	

56	ARAMP068	*Eriovixia sp*	0.29	0.113	14.5	2.58	82.15	*Araniella coreana*	PQ651559	(N)
57	ARAMP069	*Eriovixia sp*	0.41	0.165	20.5	2.49	82.54	*Eustella sps*	PQ651560	(N)
58	ARAMP070	*Gasteracantha dalyi*	0.323	0.128	16.1	2.53	95.55	*Macracantha hasselti*	PQ187444	(N)
59	ARAMP071	*Gasteracantha geminata*	0.497	0.215	24.8	2.31	94.86	*Gasteracantha kuhli*	PQ558952	(N)
60	ARAMP072	*Macracantha hasselti*	4.249	1.986	212.5	2.14				#
61	ARAMP073	*Gasteracantha dalyi*	0.252	0.086	12.6	2.92	99.81	*Gasteracantha dalyi*	PQ495951	
62	ARAMP074	*Thelacantha brevispina*	0.601	0.269	30	2.24	99.64	*Thelacantha brevispina*	PQ495947	
63	ARAMP075	*Gea sp*	0.353	0.155	17.7	2.28	93.87	*Gea spinipes*	PQ651562	
64	ARAMP076	*Gea sp*	1.216	0.575	60.8	2.11	97.52	*Gea spinipes*	PQ651563	(N)
65	ARAMP077	*Nephila pilipes*	0.168	0.084	168	2	99.46	*Nephila pilipes*	PQ495945	
66	ARAMP078	*Nephilengys malabarensis*	0.113	0.07	5.6	1.61	89.76	*Nephilengys malabarensis*	PQ558953	
67	ARAMP079	*Leviaraneus viridiventris*	1.115	0.491	55.7	2.27	100	Leviaraneus noegeatus	PQ651523	
68	ARAMP080	*Neoscona sp*	0.678	0.31	33.9	2.19	90.49 90.13	*Paraplectana sakaguchii* *Neoscona vigilans*	PQ651525	(N)
69	ARAMP081	*Neoscona vigilans*	11.235	5.913	561.7	1.9				#

70	ARAMP082	*Neoscona vigilans*	0.56	0.255	28	2.2	100	*Neoscona vigilans*	PQ651532	
71	ARAMP083	*Neoscona bengalensis*	0.35	0.159	17.5	2.2	91.55	*Neoscona vigilans*	PQ651526	(N)
72	ARAMP084	*Neoscona bengalensis*	0.233	0.099	11.7	2	91.9	*Neoscona vigilans*	PQ651527	(N)
73	ARAMP085	*Neoscona bengalensis*	0.509	0.23	25.4	2.21	91.64	*Neoscona vigilans*	PQ651528	(N)
74	ARAMP086	*Neoscona nautica*	0.366	0.147	18.3	2.49	99.82	*Neoscona nautica*	PQ558951	
75	ARAMP087	*Neoscona punctigera*	0.029	0.018	29	1.6	99.27	*Neoscona punctigera*	PQ651530	
76	ARAMP088	*Neoscona punctigera*	0.488	0.199	24.4	2.45	98.9	*Neoscona punctigera*	PQ651531	
77	ARAMP089	*Neoscona sp*	0.52	0.222	26	2.34	91.94	*Neoscona nautica*	PQ651534	(N)
78	ARAMP090	*Neoscona sp*	0.008	0.004	8	2	91.34	*Neoscona polyspinipes*	PQ651535	(N)
79	ARAMP091	*Neoscona yptinika*	0.861	0.391	43.1	2.2	91.75	*Neoscona vigilans*	PQ651529	(N)
80	ARAMP092	*Neoscona vigilans*	0.459	0.196	22.9	2.34	100	*Neoscona vigilans*	PQ651533	
81	ARAMP093	*Eriovixia sp*	0.843	0.382	42.1	2.21	87	*Austracantha minax*	PQ651561	(N)
82	ARAMP094	Genus 1	1.036	0.484	51.8	2.14				#
83	ARAMP095	Genus 2	0.214	0.08	10.7	2.66	88.87	Argiope trifasciata		$
84	ARAMP096	*Eriovixia excelsa*	0.583	0.247	29.1	2.36	100	*Eriovixia excelsa*	PQ558954	

Table S2. Details of voucher specimens used for H3 barcoding, along with DNA quantification results based on spectrophotometer readings, summary of BLAST results showing percent identity and closest matching species and GenBank accession numbers

* Sample contamination, # Unsuccessful PCR, (N) New records to the GenBank database

Sl. No.	Voucher No.	Species	Absorbance 260 nm	Absorbance 280 nm	DNA Concentration	260/ 280	Percent identity	Match species	Accession no.	Remarks
1	ARAMP007	*Bijoaraneus mitificus*	0.843	0.43	42.1	1.96	99.65	*Bijoaraneus legonensis*	PQ654379	
2	ARAMP008	*Chorizopesoides orientalis*	0.18	0.113	9	1.6	95.36	*Araneus pentagrammicus*	PQ654389	(N)
3	ARAMP012	*Eriovixia sakeidaorum*	0.036	0.018	36	2	97.84	*Eriovixia laglaizei*	PQ654396	(N)
4	ARAMP015	*Herennia multipuncta*	0.065	0.036	65	1.805	99.64	*Herennia multipuncta*	PQ654395	
5	ARAMP016	*Neoscona elliptica*	0.543	0.26	27.1	2.09	97.94	*Neoscona domiciliorum*	PQ654382	(N)
6	ARAMP017	*Nephila pilipes*	0.138	0.078	138	1.769	91.67	*Stegodyphus dumicola*		*
7	ARAMP018	*Plebs mitratus*	0.111	0.067	111	1.656	97.74	*Plebs cyphoxis*	PQ654405	(N)
8	ARAMP019	*Poltys columnaris*	0.869	0.475	43.4	1.83	97.25	*Neoscona domiciliorum*	PQ654385	(N)
9	ARAMP020	*Thelacantha brevispina*	0.797	0.406	39.9	1.96	99.32	*Thelacantha brevispina*	PQ654386	
10	ARAMP021	*Acusilas coccineus*	0.183	0.095	183	1.926	97.93	*Acusilas coccineus*	PQ654388	
11	ARAMP022	*Anepsion maritatum*	0.046	0.026	46	1.769	96.74	*Anepsion sp*	PQ654391	

12	ARAMP023	*Araneus tubabdominus*	0.894	0.459	44.7	1.95	95.38	*Araneus angulatus*	PQ654378	(N)
13	ARAMP024	*Argiope aemula*	0.925	0.463	925	1.997	99.33	*Argiope aemula*	PQ629468	
14	ARAMP025	*Argiope catenulata*	0.127	0.062	127	2.048	99.66	*Argiope catenulata*	PQ629469	
15	ARAMP026	*Cyrtophora cicatrosa*	1.289	0.665	64.4	1.94	93.73	*Cyrtophora citricola*	PQ629467	
16	ARAMP027	*Cyrtophora moluccensis*	0.186	0.102	186	1.823	99.64	*Cyrtophora moluccensis*	PQ629465	
17	ARAMP028	*Cyrtophora unicolor*	0.113	0.058	113	1.948	99.64	*Cyrtophora unicolor*	PQ629466	
18	ARAMP032	*Arachnura melanura*	0.572	0.274	28.6	2.08	95.4	*Arachnura logio*	PQ654392	
19	ARAMP034	*Argiope pulchella*	0.111	0.06	111	1.85	100	*Argiope pulchella*	PQ654393	
20	ARAMP043	*Cyclosa bifida*	0.187	0.108	9.4	1.73	95.52	*Wagneriana taim*	PQ654401	
21	ARAMP047	*Cyclosa hexatuberculata*	0.019	0.011	19	1.727				#
22	ARAMP049	*Cyclosa mulmeinensis*	0.286	0.15	14.3	1.9	95.17	*Cyclosa walckenaeri*	PQ654402	
23	ARAMP052	*Cyclosa quinquegutteta*	0.008	0.005	8	1.6	93.33	*Cyclosa walckenaeri*	PQ654403	
24	ARAMP054	*Cyclosa spirifera*	0.014	0.008	14	1.75	94.93	*Wagneriana atuna*	PQ654387	
25	ARAMP060	*Parawixia dehaani*	0.036	0.022	36	1.636	99.28	*Parawixia dehaani*	PQ654404	
26	ARAMP061	*Eriovixia gryffindori*	-0.008	-0.011	-8	0.727	97.48	*Eriovixia laglaizei*	PQ654397	(N)

27	ARAMP067	*Eriovixia poonaensis*	0.432	0.194	21.6	2.23	98.92	*Eriovixia laglaizei*	PQ654399	
28	ARAMP070	*Gasteracantha dalyi*	0.323	0.128	16.1	2.53	98.19	*Eriovixia laglaizei*	PQ654381	(N)
29	ARAMP071	*Gasteracantha geminata*	0.497	0.215	24.8	2.31	99.65	*Gasteracantha kuhlii*	PQ654380	(N)
30	ARAMP078	*Nephilengys malabarensis*	0.113	0.07	5.6	1.61	89.22	*Nephilengys malabarensis*	PQ654390	
31	ARAMP079*	*Leviaraneus viridiventris*	1.115	0.491	55.7	2.27				#
32	ARAMP082	*Neoscona vigilans*	0.56	0.255	28	2.2	96.36	*Neoscona sp*	PQ654384	
33	ARAMP084	*Neoscona bengalensis*	0.233	0.099	11.7	2	95.65	*Neoscona domiciliorum*	PQ654383	(N)
34	ARAMP086	*Neoscona nautica*	0.366	0.147	18.3	2.49	98.61	*Neoscona domiciliorum*	PQ654400	
35	ARAMP096	*Eriovixia excelsa*	0.583	0.247	29.1	2.36	97.84	*Eriovixia laglaizei*	PQ654398	
36	ARAMP097	*Argiope anasuja*	0.25	0.12	250	2.08	98.6	*Argiope anasuja*	PQ654394	

Table S3. Details of sequences used for the phylogenetic analysis of Oriental and Palaearctic araneids, including the GenBank accession numbers

Sequences used for COI gene tree, ^ Sequences used for H3 gene tree, $ Sequences used for concatenation, * Sequences developed in the present study, + Sequences used in divergence time analysis using BEAST

Sl. No.	Species	COI GenBank accession no.	Region	H3 GenBank accession no.	Region	Remarks
1	*Aculepeira ceropegia*	KY269201	Germany			#
2	*Acusilas coccineus*	MK420054	Thailand	MK420289	Thailand	# ^ $
3	*Acusilas malaccensis*	KR526560	China	KR526603	China	# ^ $ +
4	*Agalenatea redii*	MK420055	Denmark	MK420290	Denmark	# ^ $ +
5	*Anepsion depressum*	AB808480	Thailand			#
6	*Aoaraneus amabilis*	LC637705	Japan	LC637723	Japan	# ^ $ +
7	*Aoaraneus pentagrammicus*	LC637704	Japan	LC637722	Japan	# ^ $ +
8	*Arachnura logio*	KJ957944	Taiwan	KJ957997	Taiwan	# ^ $ +
9	*Arachnura melanura*	KJ957945	Taiwan			#
10	*Araneus affinis*	MK153828	Pakistan			#
11	*Araneus alsine*	KY270365	Germany			#
12	*Araneus angulatus*	KC849063	Spain	KC849022	Spain	# ^ $ +
13	*Araneus diadematus*	KC849064	Slovenia	KC849023	Slovenia	# ^ $ +
14	*Araneus ejusmodi*	KY467241	China			#
15	*Araneus ishisawai*	LC735301	Japan			#
16	*Araneus macacus*	LC516597	Japan			#
17	*Araneus marmoreus*	KY269225	Germany			#
18	*Araneus matsumotoi*	LC630881	Japan			#
19	*Araneus nordmanni*	MZ627839	Finland			#
20	*Araneus ogatai*	LC735300	Japan			#

21	*Araneus quadratus*	KY270313	Germany			#
22	*Araneus reizan*	LC516592	Japan			#
23	*Araneus saevus*	MZ609192	Finland			#
24	*Araneus seminiger*	LC516601	Japan			#
25	*Araneus stella*	JN817142	Korea			#
26	*Araneus sturmi*	KY269954	Germany			#
27	*Araneus triguttatus*	KX536845	Germany			#
28	*Araneus uyemurai*	LC516599	Japan			#
29	*Araneus variegatus*	LC735310	Japan			#
30	*Araneus ventricosus*	KY467249	China			#
31	*Araniella alpica*	KT250379	France			#
32	*Araniella coreana*	JN817162	Korea			#
33	*Araniella cucurbitina*	MK420074	Portugal	MK420304	Portugal	# ^ $ +
34	*Araniella displicata*	KY270007	Germany			#
35	*Araniella inconspicua*	KT250421	Greece			#
36	*Araniella opisthographa*	KT250452	Czech Republic			#
37	*Araniella proxima*	MZ610503	Finland			#
38	*Araniella yaginumai*	JN817163	Korea			#
39	*Argiope aemula*	KJ957938	Taiwan	KJ957993	Taiwan	# ^ $
40	*Argiope aetherea*	GU353201	Taiwan			#
41	*Argiope aetheroides*	KJ957940	Taiwan	KJ957995	Taiwan	# ^ $ +
42	*Argiope ahngeri*	KJ957948	Turkey	KJ957999	Turkey	# ^ $ +
43	*Argiope amoena*	GU353199	Taiwan			#
44	*Argiope amoena*	KJ957941	Japan			#
45	*Argiope anasuja*	MK154555	Pakistan	KJ957996	India	# ^ $
46	*Argiope appensa*	GU353203	Taiwan			#

47	*Argiope boesenbergi*	KJ957953	Japan			#
48	*Argiope bruennichi*	KC849062	Slovenia	KC849021	Slovenia	# ^ $ +
49	*Argiope caesarea*	PP808888	China			#
50	*Argiope dang*	KU055770	Cambodia	KU055865	Cambodia	# ^ $ +
51	*Argiope jinghongensis*	KJ957967	China	KJ958015	China	# ^ $ +
52	*Argiope lobata*	MK154282	Pakistan			#
53	*Argiope lobata*	KJ957971	Spain	KJ958019	Spain	# ^ $ +
54	*Argiope macrochoera*	KJ957973	China	KJ958021	China	# ^ $ +
55	*Argiope minuta*	KY467229	China			#
56	*Argiope ocula*	KJ957979	Taiwan			#
57	*Argiope pulchella*	MK154452	Pakistan			#
58	*Argiope pulchelloides*	GU353196	Taiwan			#
59	*Argiope trifasciata*	MT454231	Spain	MT467429	Spain	# ^ $ +
60	*Argiope trifasciata*	MT454202	India	MT467391	India	# ^ $
61	*Argiope trifasciata*	MK154862				#
62	*Argiope versicolor*	PP864022	Vietnam			#
63	*Bijoaraneus komachi*	LC637707	Japan	LC637725	Japan	# ^ $ +
64	*Bijoaraneus legonensis*	LC637709	Thailand	LC637727	Thailand	# ^ $ +
65	*Bijoaraneus mitificus*	LC637706	Thailand	LC637724	Thailand	# ^ $
66	*Bijoaraneus postilena*	LC637710	Thailand	LC637728	Thailand	# ^ $ +
67	*Caerostris sumatrana*	KT267113	Laos	KR526613	China	# ^ $ +
68	*Cercidia prominens*	MK420087	Denmark	MK420312	Denmark	# ^ $ +
69	*Chorizopes nipponicus*	JN817165	Korea			#
70	*Chorizopes quadrituberculata*	MK392787	India			#
71	*Chorizopesoides wulingensis*	MK154593	Pakistan			#
72	*Cyclosa alba*	AB453390	Japan			#
73	*Cyclosa argentata*	MN202156	China			#

74	*Cyclosa argenteoalba*	KY467238	China			#
75	*Cyclosa bianchoria*	MK392666	India			#
76	*Cyclosa bifida*	LC415136	Thailand			#
77	*Cyclosa bulla*	LC415141	Thailand			#
78	*Cyclosa chichawatniensis*	MK155009	Pakistan			#
79	*Cyclosa confusa*	JN817170	Korea			#
80	*Cyclosa conica*	EU003282	Denmark	EU003316	Denmark	# ^ $ +
81	*Cyclosa hexatuberculata*	MK154120	Pakistan			#
82	*Cyclosa japonica*	JN817171	Korea			#
83	*Cyclosa kumadai*	JN817173	Korea			#
84	*Cyclosa laticauda*	KY467254	China			#
85	*Cyclosa maritima*	AB453763	Japan			#
86	*Cyclosa monticola*	JN817168	Korea			#
87	*Cyclosa moonduensis*	MK153883	Pakistan			#
88	*Cyclosa mulmeinensis*	AB453766	Japan			#
89	*Cyclosa octotuberculata*	JN817167	Korea			#
90	*Cyclosa oculata*	KX537258	Germany			#
91	*Cyclosa omonaga*	LC387835	Japan			#
92	*Cyclosa quinqueguttata*	MK392786	India			#
93	*Cyclosa sachikoae*	AB453770	Japan			#
94	*Cyclosa sedeculata*	JN817166	Korea			#
95	*Cyclosa spirifera*	MK392677	India			#
96	*Cyclosa vallata*	AB453771	Japan			#
97	*Cyphalonotus assuliformis*	MZ673432	China	MZ666941	China	# ^ $ +
98	*Cyphalonotus variabilis*	MZ673436	Taiwan	MZ666940	Taiwan	# ^ $ +
99	*Cyrtarachne akirai*	AB910432	Japan			#
100	*Cyrtarachne bufo*	MK420094	Japan	MK420319	Japan	# ^ $ +

101	*Cyrtarachne inaequalis*	MK420095	Japan	MK420320	Japan	# ^ $ +
102	*Cyrtarachne jucunda*	AB820895	Japan			#
103	*Cyrtarachne nagasakiensis*	MK420096	Japan	MK420321	Japan	# ^ $ +
104	*Cyrtarachne yunoharuensis*	AB910437	Japan			#
105	*Cyrtophora cicatrosa*	MK392747	India			#
106	*Cyrtophora citricola*	MK155014	Pakistan			#
107	*Cyrtophora citricola*	KC849071	Spain	KC849030	Spain	# ^ $ +
108	*Cyrtophora exanthematica*	KJ957957	Taiwan	KJ958005	Taiwan	# ^ $ +
109	*Cyrtophora moluccensis*	KY467257	China			#
110	*Cyrtophora unicolor*	KJ957959	Taiwan	KJ958007	Taiwan	# ^ $
111	*Eriovixia excelsa*	MK155027	Pakistan			#
112	*Eriovixia hainanensis*	MN204479	India			#
113	*Eriovixia laglaizei*	OQ821698	India			#
114	*Eriovixia poonaensis*	MK392777	India			#
115	*Gasteracantha diadesmia*	MT584895	Thailand	MT584955	Thailand	# ^ $ +
116	*Gasteracantha diardi*	MT584900	Thailand	MT584957	Thailand	# ^ $ +
117	*Gasteracantha doriae*	MT584902	Thailand	MT584959	Thailand	# ^ $ +
118	*Gasteracantha kuhlii*	MT584913	Thailand	MT584963	Thailand	# ^ $ +
119	*Gasteracantha kuhlii*	DQ518416	Japan			#
120	*Gea spinipes*	KJ957965	Taiwan	KJ958013	Taiwan	# ^ $ +
121	*Gea subarmata*	MK154788	Pakistan			#
122	*Gibbaranea bituberculata*	KY268919	Germany			#
123	*Gibbaranea gibbosa*	KY270274	Germany			#
124	*Gibbaranea omoeda*	MZ628257	Finland			#
125	*Guizygiella guangxiensis*	KR526576	China			#
126	*Guizygiella indica*	MK154875	Pakistan			#
127	*Guizygiella nadleri*	KR526577	China	KR526616	China	# ^ $ +

128	*Guizygiella salta*	KR526578	China	KR526617	China	# ^ $ +
129	*Herennia multipuncta*	OK017123	Sri Lanka	MK420331	Laos	# ^ $
130	*Hypsosinga alboria*	MK154904	Pakistan			#
131	*Hypsosinga albovittata*	KY268435	Germany			#
132	*Hypsosinga heri*	MK420115	Hungary	MK420334	Hungary	# ^ $ +
133	*Hypsosinga pygmaea*	KY467232	China			#
134	*Hypsosinga sanguinea*	MZ608637	Finland			#
135	*Hypsosinga wanica*	MK154987	Pakistan			#
136	*Indoetra thisbe*	KC849070	Sri Lanka	KC849029	Sri Lanka	# ^ $ +
137	*Larinia bonneti*	MK420119	Hungary			#
138	*Larinia jeskovi*	MK420120	Hungary	MK420337	Hungary	# ^ $ +
139	*Larinia joei*	LC597526	Thailand			#
140	*Larinia phosop*	LC756460	Thailand			#
141	*Larinia phthisica*	JN306172	Pakistan			#
142	*Lariniaria argiopiformis*	JN817161	Korea			#
143	*Larinioides cornutus*	KY270298	Germany			#
144	*Larinioides ixobolus*	KY270281	Germany			#
145	*Larinioides patagiatus*	KY268472	Germany			#
146	*Larinioides sclopetarius*	KC849077	Slovenia	KC849036	Slovenia	# ^ $
147	*Leviaraneus noegeatus*	LC761862	Thailand			#
148	*Leviaraneus viridiventris*	LC761858	Japan	LC761885	Japan	# ^ $ +
149	*Leviellus inconveniens*	KR526579	Israel	KR526618	Israel	# ^ $ +
150	*Leviellus kochi*	MW997986	Spain			#
151	*Leviellus thorelli*	KR526580	Macedonia	KR526619	Macedonia	# ^ $ +
152	*Lipocrea fusiformis*	LC597529	Thailand			#
153	*Macracantha arcuata*	OQ150141	United Kingdom			#

154	*Macracantha arcuata*	MT584917	Thailand	MT584967	Thailand	# ^ $ +
155	*Macracantha hasselti*	OQ150143	United Kingdom			#
156	*Macracantha hasselti*	MT584908	Thailand	MT584961	Thailand	# ^ $ +
157	*Mangora acalypha*	KY270137	Germany			#
158	*Neoscona adianta*	KY467218	China			#
159	*Neoscona mellotteei*	PQ530534	China			#
160	*Neoscona mukerjei*	KT383761	India			#
161	*Neoscona multiplicans*	JN817150	Korea			#
162	*Neoscona nautica*	KY467215	China			#
163	*Neoscona polyspinipes*	MK950518	Oman			#
164	*Neoscona polyspinipes*	MK155010	Pakistan			#
165	*Neoscona pseudonautica*	JN817148	Korea			#
166	*Neoscona punctigera*	JN817151	Korea			#
167	*Neoscona scylla*	MK155031	Pakistan			#
168	*Neoscona scylloides*	JN817153	Korea			#
169	*Neoscona subfusca*	MK950519	Oman			#
170	*Neoscona subfusca*	JN306160	Pakistan			#
171	*Neoscona subpullata*	JN817156	Korea			#
172	*Neoscona theisi*	MK155020	Pakistan			#
173	*Neoscona theisi*	KY467221	China			#
174	*Neoscona tianmenensis*	JN817154	Korea			#
175	*Neoscona vigilans*	MK155003	Pakistan			#
176	*Nephila pilipes*	HQ441937	Taiwan	OM850328	India	# ^ $
177	*Nephila pilipes*	JN032337	France			#
178	*Nephilengys malabarensis*	MK420143	Thailand	MK420355	Thailand	# ^ $
179	*Nuctenea silvicultrix*	MZ610833	Finland			#

180	*Nuctenea umbratica*	MK420145	Denmark	MK420357	Denmark	# ^ $ +
181	*Ordgarius hobsoni*	DQ518417	Japan			#
182	*Ordgarius sexspinosus*	DQ518418	Japan			#
183	*Paraplectana sakaguchii*	AB546976	Japan			#
184	*Paraplectana tsushimensis*	MK420148	Japan	MK420360	Japan	# ^ $ +
185	*Parawixia dehaani*	MK392966	India			#
186	*Parazygiella montana*	KX039308	Switzerland			#
187	*Pasilobus hupingensis*	AB910443	Japan			#
188	*Perilla teres*	KC849102	China	KC849058	China	# ^ $ +
189	*Plebs himalayaensis*	MK154077	Pakistan			#
190	*Singa hamata*	KY270019	Germany			#
191	*Singa nitidula*	MK420162	Hungary			#
192	*Thelacantha brevispina*	MT584921	Thailand	MT584971	Thailand	# ^ $
193	*Trichonephila antipodiana*	PP864025	Vietnam			#
194	*Trichonephila clavata*	KY467131	China			#
195	*Yaginumia sia*	JN817174	Korea	KR526629	China	# ^ $ +
196	*Zilla diodia*	KY268818	Germany			#
197	*Zygiella atrica*	KR526594	Czech Republic	KR526630	Czech Republic	# ^ $ +
198	*Zygiella keyserlingi*	KR526595	Macedonia	KR526631	Macedonia	# ^ $ +
199	*Acusilas coccineus*	PQ558941	India	PQ654388	India	* # ^ $ +
200	*Anepsion maritatum*	PQ558942	India	PQ654391	India	* # ^ $ +
201	*Arachnura melanura*	PQ651522	India	PQ654392	India	* # ^ $ +
202	*Araneus sp*	PQ651536	India			* #
203	*Araneus tubabdominus*	PQ187441	India	PQ654378	India	* # ^ $ +
204	*Argiope aemula*	PQ558943	India	PQ629468	India	* # ^ $ +
205	*Argiope anasuja*	PQ558945	India	PQ654394	India	* # ^ $ +

206	*Argiope catenulata*	PQ558944	India	PQ629469	India	* # ^ $ +
207	*Argiope pulchella*	PQ651537	India	PQ654393	India	* # ^ $ +
208	*Argiope versicolor*	PQ651539	India			* #
209	*Bijoaraneus mitificus*	PQ651518	India	PQ654379	India	* # ^ $ +
210	*Chorizopesoides orientalis*	PQ651519	India	PQ654389	India	* # ^ $ +
211	*Cyclosa bifida*	PQ187443	India	PQ654401	India	* # ^ $ +
212	*Cyclosa confraga*	PQ558948	India			* #
213	*Cyclosa hexatuberculata*	PQ651543	India			* #
214	*Cyclosa mulmeinensis*	PQ558947	India	PQ654402	India	* # ^ $ +
215	*Cyclosa purnai*	PQ651549	India			* #
216	*Cyclosa quinquegutteta*	PQ558949	India	PQ654403	India	* # ^ $ +
217	*Cyclosa sp*	PQ651551	India			* #
218	*Cyclosa sp*	PQ651552	India			* #
219	*Cyclosa spirifera*	PQ651550	India	PQ654387	India	* # ^ $ +
220	*Cyclosa spirifera*	PQ651547	India			* #
221	*Cyrtophora cicatrosa*	PQ495948	India	PQ629467	India	* # ^ $ +
222	*Cyrtophora moluccensis*	PQ495949	India	PQ629465	India	* # ^ $ +
223	*Cyrtophora unicolor*	PQ558946	India	PQ629466	India	* # ^ $ +
224	*Eriovixia excelsa*	PQ558954	India	PQ654398	India	* # ^ $ +
225	*Eriovixia gryffindori*	PQ651554	India	PQ654397	India	* # ^ $ +
226	*Eriovixia poonaensis*	PQ651557	India	PQ654399	India	* # ^ $ +
227	*Eriovixia poonaensis*	PQ651558	India			* #
228	*Eriovixia sakeidaorum*	PQ651564	India	PQ654396	India	* # ^ $ +
229	*Eriovixia sp*	PQ651555	India			* #
230	*Eriovixia sp*	PQ651556	India			* #
231	*Eriovixia sp*	PQ651559	India			* #
232	*Gasteracantha dalyi*	PQ187444	India	PQ654381	India	* # ^ $ +

233	*Gasteracantha geminata*	PQ558952	India	PQ654380	India	* # ^ $ +
234	*Gea sp*	PQ651562	India			* #
235	*Herennia multipuncta*	PQ495943	India	PQ654395	India	* # ^ $ +
236	*Leviaraneus viridiventris*	PQ651523	India			* #
237	*Neoscona bengalensis*	PQ651526	India	PQ654383	India	* # ^ $ +
238	*Neoscona chrysanthusi*	PQ651528	India			* #
239	*Neoscona elliptica*	PQ651524	India	PQ654382	India	* # ^ $ +
240	*Neoscona nautica*	PQ558951	India	PQ654400	India	* # ^ $ +
241	*Neoscona punctigera*	PQ651531	India			* #
242	*Neoscona sp*	PQ651525	India			* #
243	*Neoscona sp*	PQ651534	India			* #
244	*Neoscona sp*	PQ651535	India			* #
245	*Neoscona vigilans*	PQ651532	India	PQ654384	India	* # ^ $ +
246	*Neoscona yptinika*	PQ651529	India			* #
247	*Nephila pilipes*	PQ495944	India			* #
248	*Nephila pilipes*	PQ495945	India			* #
249	*Nephilengys malabarensis*	PQ558953	India	PQ654390	India	* # ^ $ +
250	*Parawixia dehaani*	PQ558950	India	PQ654404	India	* # ^ $ +
251	*Plebs mitratus*	PQ558939	India	PQ654405	India	* # ^ $ +
252	*Poltys columnaris*	PQ558940	India	PQ654385	India	* # ^ $ +
253	*Thelacantha brevispina*	PQ495946	India	PQ654386	India	* # ^ $ +

LIST OF PUBLICATIONS

Journal articles:

- **Shilpa, K. R.**, Anis, K.V., & Sudhikumar, A. V. (2025). Transfer of *Araneus panchganiensis* Tikader & Bal, 1981 to *Neoscona* (Araneae, Araneidae). *Arachnology, 20*(3), 448-453. https://doi.org/10.13156/arac.2024.20.3.12
- **Shilpa, K. R.**, Anis, K. V., & Sudhikumar, A. V. (2023). First record of *Araneus viridiventris* Yaginuma, 1969 (Araneae: Araneidae) from India with redescription of the female. *Serket, 19*(2), 132-139.
- **Shilpa, K. R.**, & Sudhikumar, A. V. (2022). A new spider species of the genus *Philoponella* Mello-Leitão 1917 (Araneae: Uloboridae) from Western Ghats of India. *Acta Arachnologica, 71*(1), 21-26. https://doi.org/10.2476/asjaa.71.21

Articles in conference proceedings:

- **Shilpa, K. R.**, & Sudhikumar, A. V. (2021). Diversity of orb web spiders (Araneae: Araneidae) in Peruvanamuzhi Forest, Calicut, India. In *Current status of biological research: An overview* (pp. 51–54). Vimala College (Autonomous), Thrissur. ISBN: 978-81-7255-134-6.

PRESENTATIONS

- Presented a paper titled “Taxonomic dilemmas in the Indian Araneids (Araneae: Araneidae)” at the **Multidisciplinary National Conference & Research Expo** organised by Christ College (Autonomous), Irinjalakuda, Thrissur, Kerala in February 2024.
- Presented a paper titled “Indian *Cyclosa* Menge 1866 (Araneae: Araneidae): an example of taxonomic redundant group” at the **International Conference on Advance Research, INSIGHT 2024,** organised by MES Keveeyam College, Valanchery, Malappuram, Kerala in February 2024.
- Presented a paper titled “Indian *Neoscona* Simon, 1864 (Araneae: Araneidae): an example of taxonomic redundant group” in the **International Seminar on Advanced Techniques**

in Biological Research (ATBR – 2023) organised by KKTM Government College, Pullut, Thrissur, Kerala in November 2023.

AWARDS

- Awarded the **Vincent Roth Fund for Systematics Research** by the American Arachnological Society (AAS) for the project entitled "Molecular taxonomy and phylogeny of the genus Araneus Clerck, 1757 and Neoscona Simon, 1864 (Araneae: Araneidae) in Western Ghats, India" in May 2023.

PARTICIPATIONS & TRAININGS

- Participated in the **International workshop on: Evolutionary Biology of Arachnids in the Western Ghats of India**, organized by the Department of Zoology, Bharata Matha College (Autonomous), Thrikkakara, Ernakulam in association with the University of Central Oklahoma, USA, the Museum of Nature, Hamburg, Germany, and the European Society of Evolutionary Biology (ESEB) in June 2025.
- Participated in the international workshop on **Molecular Phylogenetics: From Theory to Practice**, organised by Quaxon Bio & IT Solutions, India, in December 2024.
- Participated in the **SCIENTIA-2, Multidisciplinary International Conference and Research Expo 2024**, organised by Christ College (Autonomous), Irinjalakuda, Kerala, in November 2024.
- Participated in the hands-on training in **DNA Sequence Analysis: A Comprehensive Guide**, organised by MYTOSCIpro, Marigenome, Matsyasevanakendra, the Fisheries Department of Kerala and PMMSY in June 2024
- Completed a short-term course on **Basics of Map Preparation using Geographic Information System (GIS),** jointly organised by Teaching Learning Centre, ScholarsConnect – Research's Forum of Christ College and Department of Geology and Environmental Science, Christ College (Autonomous), Irinjalakuda in November 2023.

- Participated in the **National Faculty Development Program on Research and Publication Ethics (RPE)**, organised by Christ College (Autonomous), Irinjalakuda, Thrissur, Kerala, in association with the Kerala State Higher Education Council and Directorate of Research, University of Calicut, in October 2023.
- Participated in the hands on workshop on R Programming for Data Analysis" organised by the Department of Statitics, Christ College (Autonomous), Irinjalakuda, Thrissur, Kerala in August 2023.
- Participated in the **International short-term workshop on** "**Arachnid Diversity: Hands-on training in Molecular Phylogenetics and Basic Taxonomy**" organised by the Division of Arachnology, Department of Zoology, Sacred Heart College (Autonomous), Thevara, Kochi, in association with Museum of Nature Hamburg-Zoology, Germany, in August 2023.
- Completed a hands-on training on **Isolation of DNA (Manual and Kit method), Primer Designing, Amplification of DNA (PCR), Purification of DNA (Gel elution/Column pass), Sequence Analysis (Phylogeny, SNP detection, InDel detection),** under the guidance of Dr. Beena P. S. at OmicsGen LifeSciences Pvt. Ltd, Kochi in June 2023.
- Participated in DST-SERB sponsored **National Seminar on Spiders of Western Ghats**, organised by Deva Matha College, Kuravilangad, Kerala in February 2021.

Transfer of *Araneus panchganiensis* Tikader & Bal, 1981 to *Neoscona* (Araneae: Araneidae)

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Abstract

We conducted phylogenetic analyses using Maximum Likelihood and Bayesian Inference methods to infer phylogenetic relationships for the species *Araneus panchganiensis* Tikader & Bal, 1981, and this study presents the first record of the species beyond its type locality. The female of the species is redescribed with detailed illustrations, while the male remains undescribed. Our results support the transferring of the species to the genus *Neoscona* based on morphological examination and mitochondrial COI gene sequence analysis, forming the new combination *Neoscona panchganiensis* **comb. nov.**

Keywords: molecular taxonomy • spider • Western Ghats

Introduction

With over 3157 species across 198 genera, the family Araneidae Clerck, 1757 is the third-largest spider family in the world (World Spider Catalog 2025). *Araneus* Clerck, 1757, the largest genus in the family, includes 550 species, while *Neoscona* Simon, 1864 comprises 123 species (World Spider Catalog 2025). Members of both genera are cosmopolitan and easily recognizable in their natural habitat, having been studied since the early periods of Arachnology. Identifying species within both genera can be challenging due to the lack of proper illustrations in the available literature and the absence of well-defined diagnostic characteristics.

Several species have been placed in *Araneus* without appropriate diagnosis or characterization, hence the genus is often considered a dumping ground (Scharff & Coddington 1997) for species that do not fit into other groups. The wide morphological spectrum of the speciose genus led to its polyphyletic nature, consisting of multiple monophyletic clades (Scharff & Coddington 1997; Breitling 2019). Scharff *et al.* (2020) revealed the polyphyletic nature of the genus *Araneus*, indicating that its current classification does not accurately represent evolutionary relationships. However, a monophyletic and well-supported clade of Holarctic species, including *A. diadematus*, *A. marmoreus*, *A. cavaticus*, and *A. gemmoides*, was identified. The study did not provide a coherent phylogenetic insight into the position of *Neoscona* due to limited taxa representation. Since *Araneus* has proved to be a polyphyletic group (Scharff & Coddington 1997; Scharff *et al.* 2020), diagnostic characterization of the genus as a whole will definitely lead to confusions and erroneous placements, rather each monophyletic clades within the genus should be focused and characterized in detail. Detailed investigations of morphological characters using advanced technologies, have opened doors for revising the group (Berman & Levi 1971; Grasshoff 1986; Yin *et al*. 1997; Joseph & Framenau 2012; Framenau 2019) and sometimes led to the establishment of new genera (Tanikawa, Yamasaki & Petcharad 2021; Framenau & Castanheira 2022a,b; Framenau & Kuntner 2022; Framenau, Castanheira & Vink. 2022; Castanheira & Framenau 2022, 2023; Tanikawa & Petcharad 2023; Mi, Wang & Li 2024).

Although *Araneus* and *Neoscona* share similarities, they can be distinguished by some morphological characters. The thoracic fovea is transverse in *Araneus* females and longitudinal in *Neoscona* females (Berman & Levi 1971; Tikader 1982; Grasshoff 1986). The lateral eyes are on prominent tubercles in the former whereas not so in the latter (Tikader 1982). The epigynal scapes of *Araneus* are either long or short and mostly wrinkled while the latter bear a simple tongue, completely fused to the base with one or two pair of lateral lobes (Berman & Levi 1971; Grasshoff 1986; Framenau 2019) either inflexible or flexible (Zamani, Marusik & Šestáková 2020). Copulatory ducts of *Neoscona* females are simple, without much convolutions, and are the other way in *Araneus* (Grasshoff 1986). Copulatory openings are in grooves on either side of the scape in *Araneus* and underneath the scape in *Neoscona* (Berman & Levi 1971). *Araneus* males have a narrow, strip-like cymbium whereas *Neoscona* males have a broader cymbium (Tikader 1982).

Material and methods

Taxon sampling and morphological examination: specimen were collected by visual searching and hand-picking methods, and were transferred to small plastic vials containing 100% ethanol. A detailed morphological examination was done carefully using a Leica M205C stereomicroscope equipped with a Leica DMC4500 digital camera. Image stacking was performed using the Leica Application Suite (LAS) software package, version 4.3.0, with the LAS montage facility. All measurements were recorded in millimetres. The identification of the specimens was confirmed using the key provided by Tikader (1982) and by comparing the photographs of the type specimen deposited in the National Zoological Collection portal (Zoological Survey of India 2025). The examined specimen has been deposited in the reference collection of the Centre for Animal Taxonomy and Ecology (CATE), Department of Zoology, Christ College, Irinjalakuda, Kerala, India. The map was generated using ArcGIS version 10.8 (Esri, 2020).

Abbreviations: ALE = anterior lateral eye, AME = anterior median eye, CD = copulatory duct, CO = copulatory opening, FD = fertilization duct, LL = lateral lobe, PME =

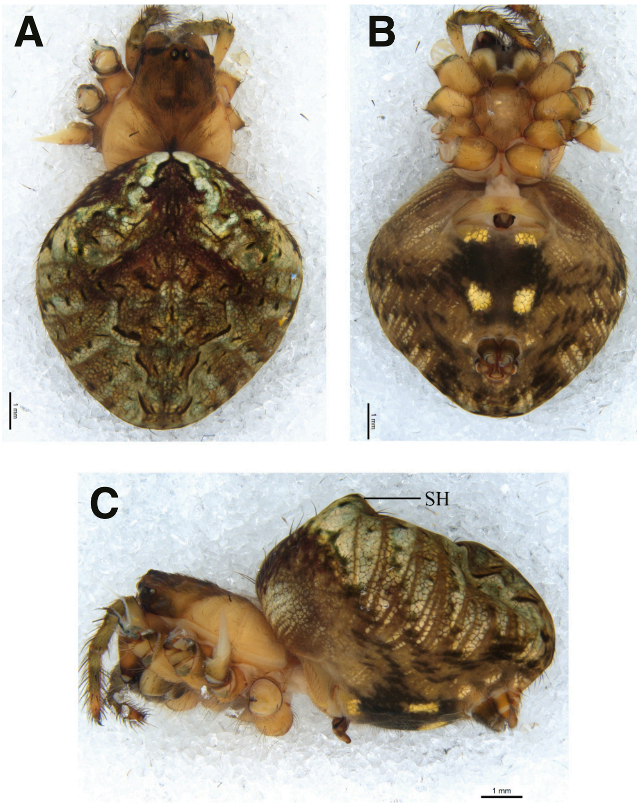

Fig. 1: *Neoscona panchganiensis* comb. nov., female from Kerala, habitus. **A** dorsal view; **B** ventral view; **C** lateral view. Scale bars = 1 mm.

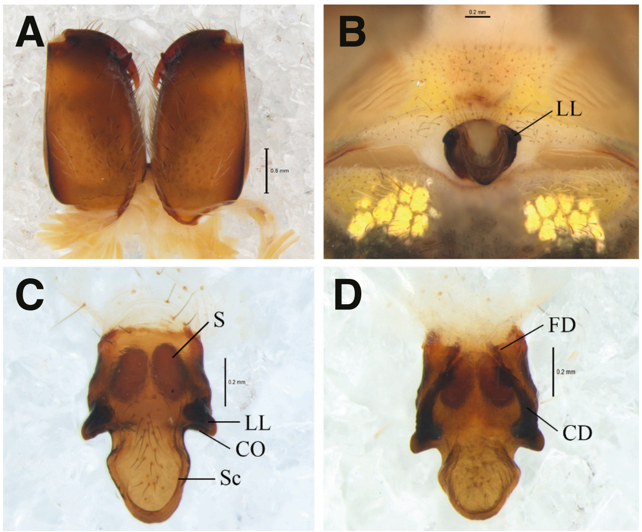

Fig. 2: *Neoscona panchganiensis* comb. nov., female from Kerala. **A** chelicera, frontal view; **B** epigyne, in situ; **C** same, ventral view; **D** same, dorsal view. Scale bars = 0.5 mm (A), 0.2 mm (B–D).

posterior median eye, PLE = posterior lateral eye, S = spermatheca, Sc = scape, SH = shoulder hump.

DNA extraction and sequencing: Four legs, along with the coxa, from one side of a preserved specimen, were used for genomic DNA extraction, which was performed using the DNeasy Blood and Tissue Kit (Qiagen). Fragment of the mitochondrial cytochrome oxidase I (COI) gene was sequenced using the forward primer LCO1490 (GGTCAACAAATCATAAAGATATTGG) (Folmer et al. 1994) and the reverse primer Chelicerate-R2 (GGATGGCCAAAAAATCAAAATAAATG) (Barrett & Hebert 2005). The PCR reaction (25 µL) consisted of 8 µL of genomic DNA, 12.5 µL of Taq PCR master mix, 1 µL of each primer and 2.5 µL of distilled water. A touch-up protocol was employed (Cheng & Kuntner 2014) including an initial denaturation at 94°C for 2 minutes followed by 20 cycles of touch-up sequence amplification (DNA denaturation at 94°C for 50 seconds, primer annealing at 42 to 52°C for 1 minute, and sequence extension at 72°C for 50 seconds), followed by 15 cycles of sequence amplification (DNA denaturation at 94°C for 50 seconds, primer annealing at 52°C to for 1 minute, and sequence extension at 72°C for 50 seconds) and a final extension at 72°C for 7 minutes. The PCR product was then subjected to Sanger sequencing, which was an outsourced service.

Genetic divergence and phylogenetic analysis: The sequence was manually edited using BioEdit 7.0.9. and a BLAST search was performed. The sequence was translated to amino acids as an additional quality control step and checked for stop codons. Reference sequences of the representatives from both genera and the outgroup were retrieved from NCBI for phylogenetic analysis (Table S2). The sequences were aligned using Clustal X 1.81. MEGA 11.0.13. was used to compute pairwise p-distance. Best-fit substitution model was found using jModelTest and Maximum Likelihood (ML) analysis was done in RAxML-GUI 2.0.15. Additionally, Bayesian Inference (BI) was performed using Mr. Bayes and the cladograms visualised with FigTree v1.4.4z.

Supplementary material is available at: 10.5281/zenodo.17259336.

Araneidae Clerck, 1757

Neoscona Simon, 1864

Neoscona panchganiensis (Tikader & Bal, 1981) comb. nov.

Araneus panchganiensis Tikader & Bal, 1981: 48, Figs. 103−102 (♀)

Type material: Holotype ♀, deposited at Zoological Survey of India, Calcutta, India, Reg. No. 4950/18. Date of Reg. 01 February 1980, Locality: Panchgani, Satara, Maharashtra (Tikader & Bal 1981). The type material has not been examined by the authors. The conclusions are made based on the descriptions and illustrations (Supplementary Fig. S1) of the type material provided by the Zoological Survey of India (2025).

Additional material examined: 1♀ (CATE9.121.75) from India: Kerala: Ranipuram, 12°24′45″N 75°21′24″E, 915 m, 28 September 2022, E. H. Vishnudas, from retreat, by hand.

Diagnosis: Females of *Neoscona panchganiensis* comb. nov. share the paired dorsal shoulder humps seen in *N. bihumpi* Patel, 1988, *N. kisangani* Grasshoff, 1986, *N. novella* (Simon, 1907), and *N. quincasea* Roberts, 1983 yet they possess a distinctive, small, tongue-like scape with a

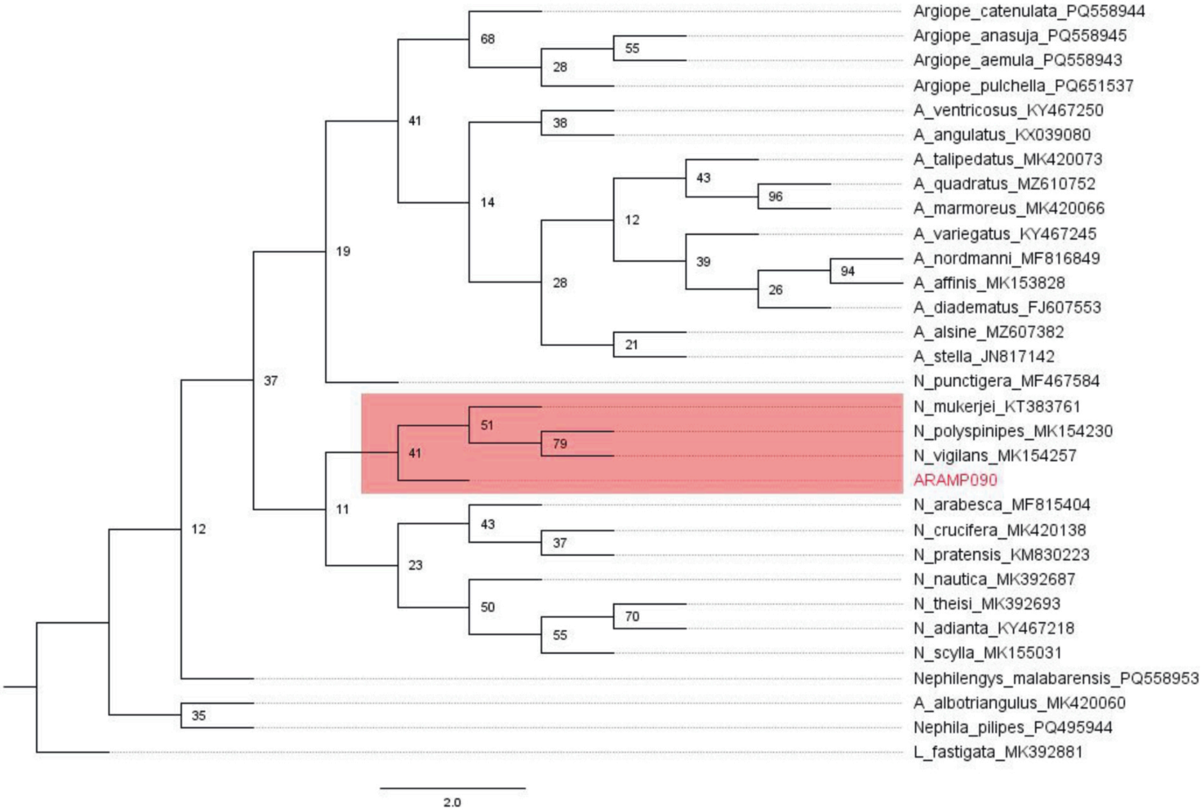

Fig. 3: Phylogenetic tree reconstructed based on ML analysis.

central depression and a pair of horn-like lateral projections flanking the copulatory openings. Similarly, *N. panchganiensis* comb. nov. shares the prominent lateral epigynal lobes with *N. hirta* (C. L. Koch, 1844), *N. menghaiensis* Yin, Wang, Xie & Peng, 1990, and *N. piaoyi* Mi, Wang & Li, 2024; however, they lack the shoulder humps. It further differs from *N. menghaiensis* and *N. piaoyi* in having a short scape and horn-like lateral lobes conspicuous in ventral view (long scape and bulging lateral lobes conspicous in lateral view in *N. menghaiensis* and *N. piaoyi*). *N. panchganiensis* comb. nov. can also be distinguished from *N. hirta* by two features: 1) *N. panchganiensis* comb. nov. have a continuous transition between the epigynal base and scape with lateral projection at the junction (in *N. hirta* it is a clear transition with lateral projection in the epigynal base), 2) copulatory openings at the base of the projection (in *N. hirta* on the projection).

Description of female (Figs. 1A–C, 2A–D): Total length 11.01 mm; carapace length 3.96 mm, width (at the widest) 3.54 mm; abdomen length 7.49 mm, width (at the widest) 7.28 mm. Cephalothorax pear-shaped, with pale brown thoracic region and darker cephalic area. Dark brown anchor-shaped patch extending from the posterior median eyes to the thoracic fovea (Fig. 1A). Cephalic region covered with pale and dark brown pubescence. AME largest, PME close and ringed with black, ALE and PLE adjacent on less distinct tubercle (Fig. 1A). Eye rows recurved; area between AME and lateral eyes darker. Chelicera dark brown with a distinct boss, 4 promarginal and 3 retromarginal teeth (Fig. 2A). Sternum heart-shaped, brown, narrowing between coxae IV, with pubescence (Fig. 1B). Labium wider than long, maxilla longer than wide with distinct scopulae, both brown with pale margins. Legs yellowish, long, robust, annulated, with two dark brown femoral bands, leg formula 1243. Abdomen nearly orbicular with brownish yellow green dorsum and a pair of lateral shoulder humps (Fig. 1C), a dark mid-folium extends posteriorly (Fig. 1A,C). Venter brown with pale markings and dark spinnerets, a black patch bordered by four white spots between epigastric furrow and spinnerets (Fig. 1B). Epigyne subtriangular in ventral view, with a short tongue-like scape having a central depression and paired horn-like lateral lobes (Fig. 2B, C). Spermathecae oval, short diverging copulatory ducts, short fertilization ducts (Fig. 2D); copulatory openings at the base of horn-like projections (Fig. 2C).

Model	BIC	AICc
GTR+G+I	15289.205	14925.332
TIM1+I+G	15292.200	14938.723
TIM3+I+G	15293.539	14940.062
GTR+G	15294.830	14936.155
TIM1+G	15298.846	14950.568

Table 1: Bayesian Information Criterion (BIC) and Akaike Information Criterion (AICc) values of first five nucleotide substitution models, based on 31 nucleotide sequences used in the study.

Male unknown.

Distribution: Western Ghats, India (Fig. 5).

Genetic divergence and phylogenetic analysis

The newly sequenced data from this study, hereinafter referred to as the query sequence, is submitted to GenBank

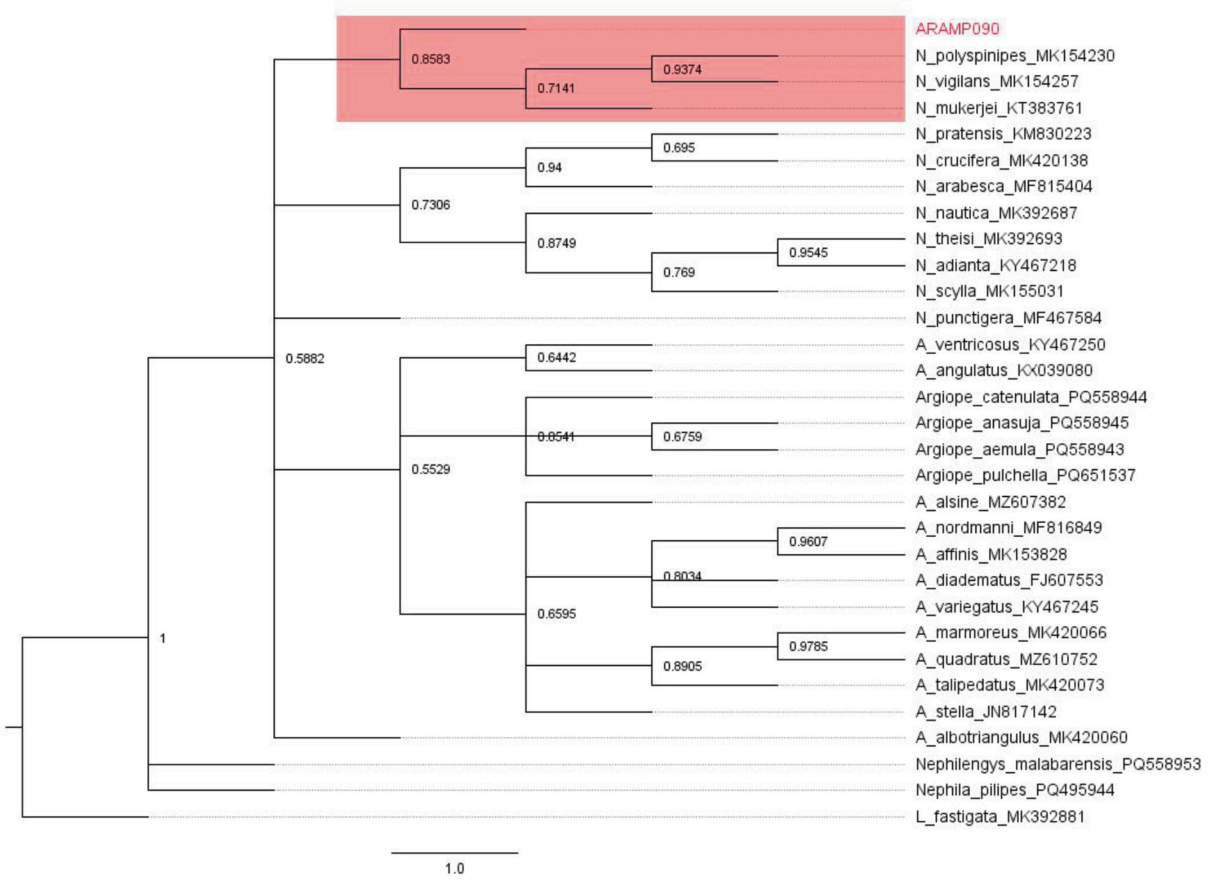

Fig. 4: Phylogenetic tree reconstructed based on BI analysis.

(Table S2). Model testing revealed that the best-fit substitution model for the given dataset is the General Time Reversible (GTR) model incorporating Gamma-distributed rate heterogeneity (+G) and a proportion of Invariant sites (+I) (Table 1). The average intra-generic genetic distance between *Neoscona* species was 0.124 and between *Araneus* species was 0.705 (Table 2). The query sequence exhibited an average genetic distance of 0.130 from the *Neoscona* species and 0.488 from the *Araneus* species (Table 2). The p-distance matrix has been provided as a supplementary data (Supplementary Table S1). Phylogenetic analysis based on the Maximum Likelihood with 1000 bootstraps and Bayesian Inference with 3 million MCMC generations, placed the query species in the Neoscona clade, along with *Neoscona mukerjei* Tikader, 1980, *Neoscona nautica* (L. Koch, 1875), and *Neoscona vigilans* (Blackwall, 1865) (bootstrap support = 41, posterior probability = 0.85) (Figs. 3–4).

Average p-distance	Mean p-distance	Min–Max p-Distance
Intra-genetic distance (within *Neoscona*)	0.124	0.057 – 0.180
Intra-genetic distance (within *Araneus*)	0.705	0.051 – 3.980
N. panchganiensis and *Neoscona*	0.130	0.107 – 0.170
N. panchganiensis and *Araneus*	0.488	0.057 – 0.180

Table 2: Average genetic distance calculated using the p-distance analysis in MEGA11.

Discussion

The examined species was originally assigned to the genus *Araneus* by Tikader & Bal (1981), primarily based on the thoracic fovea. However, the proposed transfer to *Neoscona* is justified by the features of epigyne and COI barcode analysis. The longitudinal thoracic fovea of *Neoscona* females is less distinct than the males (Berman & Levi, 1971), especially in the Asian species (Grasshoff, 1986), hence, it cannot be considered as a diagnostic feature of the genus rather the focus should be given to the genitalia.

The query species share similarities in the ventral abdominal pattern and certain epigynal features with the type species of the genus, *Neoscona arabesca* (Walckenaer, 1841). The examined specimen (Figs. 1A–C, 2A–D) and the photographs (Supplementary Fig. S1) of type specimen has a distinctive black patch between the epigastric furrow and spinnerets, bordered by two pairs of white spots, which is a characteristic feature of *Neoscona* (Berman & Levi 1971). By examining the specimen and illustrations of the type specimen, certain epigynal features of the genus *Neoscona* are evident. Tongue-like scape completely fused to the base, short copulatory duct, dorsal copulatory opening are some synapomorphies of the genus *Neoscona* (Berman & Levi 1971; Grasshoff 1986), which is also observed in the query species. The scape is simple tongue-like with a central depression, possess a pair of distinctive horn like lateral lobe and the copulatory ducts are short with slight curves

Fig. 5: Distribution of *Neoscona panchganiensis* in India.

from the openings to the spermatheca (Grasshoff 1986). The scape completely fused to the base with the copulatory openings positioned on the dorsal side of the epigyne (Berman & Levi 1971; Levi 2002).

The genetic divergence analysis showed an average distance of 0.130 between *Neoscona panchganeinesis* comb. nov. and other *Neoscona* species, while 0.488 with *Araneus* species (Supplemantary Table 2, S1), suggesting a closer evolutionary relationship with the genus *Neoscona*. Also, the pairwise genetic distance between the query species and the type species of *Neoscona*, *N. arabesca*, is 0.125, while with *Araneus* type species, *A. angulatus* is 0.2004. The phylogenetic analysis based on the COI barcodes using Maximum Likelihood and Bayesian Inference methods clearly shows the polyphyletic nature of both *Araneus* and *Neoscona*. The polyphyly of *Araneus* was already confirmed by Scharff & Coddington (1997) and Scharff *et al.* (2020); however, the phylogenetic status of *Neoscona* has not been studied thoroughly yet. In the current analysis, more representatives of the genus *Neoscona* have included and showed a polyphyly. This could only be confirmed by adding all the available species from different geographical regions. The internal node support of both trees was low, though the runs were conducted with different alignments, numbers of bootstrap replicates or MCMC generations. Although with lower supports (bootstrap = 41, posterior probability = 0.85), *Neoscona panchganeinesis* comb. nov. always positioned in the *Neoscona* clade along with *N. mukerjei* Tikader, 1980, *N. nautica* (L. Koch, 1875), and *N. vigilans* (Blackwall, 1865). Thus, analysing the p-distance calculations and phylogenetic trees, it is clear that the *Neoscona panchganeinesis* comb. nov. is closer to genus *Neoscona* than *Araneus*. The morphological evidences also support the closer affinities of the query species with *Neoscona*. Hence, we are suggesting a taxonomic placement that better reflects its phylogenetic relationships. However, the nodal supports should be improved, which can attain by incorporating multiple barcodes.

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References

BARRETT, R. D. & HEBERT, P. D. 2005: Identifying spiders through DNA barcodes. *Canadian Journal of Zoology* **83**: 481–491.

BERMAN, J. D. & LEVI, H. W. 1971: The orb weaver genus *Neoscona* in North America (Araneae: Araneidae). *Bulletin of the Museum of Comparative Zoology* **141**: 465–500.

BLACKWALL, J. 1865: Descriptions of recently discovered species and characters of a new genus, of Araneida from the east of Central Africa. *Annals and Magazine of Natural History, decade 3* **16**: 336–352.

BREITLING, R. 2019: Barcode taxonomy at the genus level. *Ecologica Montenegrina* **21**: 17–37.

CASTANHEIRA, P. DE S. & FRAMENAU, V. W. 2022: *Quokkaraneus*, a new monotypic genus of Australian orb-weaving spider (Araneae, Araneidae). *Australian Journal of Taxonomy* **10**: 1–9.

CASTANHEIRA, P. DE S. & FRAMENAU, V. W. 2023: *Kangaraneus*, a new genus of orb-weaving spider from Australia (Araneae, Araneidae). *Zoosystematics and Evolution* **99**: 307–323.

CHENG, R. C. & KUNTNER, M. 2014: Phylogeny suggests nondirectional and isometric evolution of sexual size dimorphism in Argiopine spiders. *Evolution* **68**: 2861–2872.

CLERCK, C. 1757: *Svenska Spindlar, det är beskrifning öfwer de i Sverige funne spindlarne, samt deras widunderlige lägrade sätt att fånga och döda flugor*. Stockholm: Salvii.

ESRI 2020: *ArcGIS desktop, version 10.8*, online at https://www.esri.com/en-us/arcgis/products/arcgis-desktop/overview

FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–297.

FRAMENAU, V. W. & CASTANHEIRA, P. DE S. 2022a: Revision of the new Australasian orb-weaving spider genus *Salsa* (Araneae, Araneidae). *ZooKeys* **1102**: 107–148.

FRAMENAU, V. W. & CASTANHEIRA, P. DE S. 2022b: A new genus of Australian orb-weaving spider with extreme sexual size dimorphism (Araneae, Araneidae). *Zoosystematics and Evolution* **98**: 137–149.

FRAMENAU, V. W. & KUNTNER, M. 2022: The new Australian leaf-curling orb-weaving spider genus *Leviana* (Araneae, Araneidae). *Evolutionary Systematics* **6**: 103–133.

FRAMENAU, V. W. 2019: Generic and family transfers, and nomina dubia for orb-weaving spiders (Araneae, Araneidae) in the Australasian, Oriental and Pacific regions. *Evolutionary Systematics* **3**: 1–27.

FRAMENAU, V. W., CASTANHEIRA, P. DE S. & VINK, C. J. 2022: Taxonomy and systematics of the new Australo-Pacific orb-weaving spider genus *Socca* (Araneae: Araneidae). *New Zealand Journal of Zoology* **49**: 263–334.

GRASSHOFF, M. 1986: Die Radnetzspinnen-Gattung *Neoscona* in Afrika (Arachnida: Araneae). *Annalen Zoologische Wetenschappen* **250**: 1–123.

JOSEPH, M. M. & FRAMENAU, V. W. 2012: Systematic review of a new orb-weaving spider genus (Araneae: Araneidae), with special reference to the Australasian-Pacific and South-East Asian fauna. *Zoological Journal of the Linnean Society* **166**: 279–341.

KOCH, C. L. 1844: *Die Arachniden, Elfter Band*. Nürnberg: Zeh.

KOCH, L. 1875: *Aegyptische und abyssinische Arachniden gesammelt von Herrn C. Jickeli*. Nürnberg: Bauer & Raspe.

LEVI, H. W. 2002: Keys to the genera of araneid orbweavers (Araneae, Araneidae) of the Americas. *Journal of Arachnology* **30**: 527–562.

MI, X. Q., WANG, C. & LI, S. Q. 2024: Description of six new genera and twenty species of the orb-weaver spider family Araneidae (Araneae, Araneoidea) from Xishuangbanna, Yunnan, China. *Zoological Research*: *Diversity and Conservation* **1**: 290–341.

PATEL, S. K. 1988: A new species of spider (family: Araneidae) from Gujarat, India. *Current Science* **57**: 1029–1030.

ROBERTS, M. J. 1983: Spiders of the families Theridiidae, Tetragnathidae and Araneidae (Arachnida: Araneae) from Aldabra atoll. *Zoological Journal of the Linnean Society* **77**: 217–291.

SCHARFF, N. & CODDINGTON, J. A. 1997: A phylogenetic analysis of the orb-weaving spider family Araneidae (Arachnida, Araneae). *Zoological Journal of the Linnean Society* **120**: 355–434.

SCHARFF, N., CODDINGTON, J. A., BLACKLEDGE, T. A., AGNARSSON, I., FRAMENAU, V. W., SZŰTS, T., HAYASHI, C. Y. & DIMITROV, D. 2020: Phylogeny of the orb-weaving spider family Araneidae (Araneae: Araneoidea). *Cladistics* **36**: 1–21.

SIMON, E. 1864: *Histoire naturelle des araignées* (*aranéides*). Paris: Roret.

SIMON, E. 1907: Arachnides recueillis par L. Fea sur la côte occidentale d'Afrique. 1re partie. *Annali del Museo Civico di Storia Naturale di Genova* **43**: 218–323.

TANIKAWA, A. & PETCHARAD, B. 2023: *Leviaraneus*, a new genus of Araneidae (Arachnida: Araneae) from Asia. *Acta Arachnologica* **72**: 119–127.

TANIKAWA, A., YAMASAKI, T. & PETCHARAD, B. 2021: Two new genera of Araneidae (Arachnida: Araneae). *Acta Arachnologica* **70**: 87–101.

TIKADER, B. K. & BAL, A. 1981: Studies on some orb-weaving spiders of the genera *Neoscona* Simon and *Araneus* Clerck of the family Araneidae (Argiopidae) from India. *Records of the Zoological Survey of India, Occasional Paper* **24**: 1–60.

TIKADER, B. K. 1980: Description of a new species of spider of the genus *Neoscona* (Family: Araneidae) from India and some observations on intraspecific colour variation. *Proceedings of the Indian Academy of Science* (*Animal Science*) **89**: 247–252.

TIKADER, B. K. 1982: Part 1. Family Araneidae (Argiopidae), tTypical orb-weavers. In *The fauna of India. Spiders*: *Araneae* (*Vol. II*), Calcutta: Zoological Survey of India: 1–293.

WALCKENAER, C. A. 1841: *Histoire naturelle des Insects. Aptères. Tome deuxième*. Paris: Roret.

WORLD SPIDER CATALOG 2025: *World spider catalog, version 25.5*. Bern: Natural History Museum, online at http://wsc.nmbe.ch

YIN, C. M., WANG, J. F., XIE, L. P. & PENG, X. J. 1990: New and newly recorded species of the spiders of family Araneidae from China (Arachnida, Araneae). In: *Spiders in China: one hundred new and newly recorded species of the families Araneidae and Agelenidae*. Hunan: Hunan Normal University Press.

YIN, C. M., WANG, J. F., ZHU, M. S., XIE, L. P., PENG, X. J. & BAO, Y. H. 1997: *Fauna Sinica*: *Arachnida*: *Araneae*: *Araneidae*. Beijing: Science Press.

ZAMANI, A., MARUSIK, Yu. M. & ŠESTÁKOVÁ, A. 2020: On *Araniella* and *Neoscona* (Araneae, Araneidae) of the Caucasus, Middle East and Central Asia. *ZooKeys* **906**: 13–40.

ZOOLOGICAL SURVEY OF INDIA 2025: Specimen details: *Araneus panchganiensis*. The National Zoological Collection. Online at https://zsicollections.in/search/ZSI0000019948

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First record of *Araneus viridiventris* Yaginuma, 1969 (Araneae: Araneidae) from India with redescription of the female

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Abstract

Revisions and detailed studies on the cosmopolitan genus *Araneus* Clerck, 1757 are carrying out in different parts of the world. Light green garden spider, *Araneus viridiventris* Yaginuma, 1969 are known from China, Japan, and Taiwan. It is recorded from India for the first time. Redescription of female genitalia with detailed photographs are presented in this study. Known distribution of this species is also mapped.

Keywords: Araneidae, light green garden spider, orb-weaver, distribution, Kerala, India.

Introduction

The angulate orb-weavers are coming under genus *Araneus* Clerck, 1757 that is one among the genera that have been firstly described during the initial period of araneofaunal studies. Genus *Araneus* has been marked its presence over the whole world except Antarctica. Currently 541 species (+15 subspecies) are included in this genus worldwide (World Spider Catalog, 2023). Of these, 18 species have been recorded from India (Caleb & Sankaran, 2023). Since the genus is one among the initially described genera, many confusions have been raised in species level classification as new techniques and methods in taxonomy have evolved. Detailed revisions and molecular studies are carrying out in different parts of the world.

Araneus viridiventris Yaginuma, 1969, commonly called as light green garden spider, was first reported from Japan (Ohno & Yaginuma, 1969). Later the species distribution was updated from China (Yin *et al.*, 1990) and Taiwan (Chang & Tso, 2004). Even though few photographs assumed to be *A. viridiventris* have been published in iNaturalist Research-grade Observations, an authentic record of the species from India has not been reported yet. Hence this study can be considered as the first record of *A. viridiventris* from India. Detailed descriptions on the somatic features of the species are available in the literature (Ohno & Yaginuma, 1969; Yin *et al.*, 1990; Chang & Tso, 2004; Tanikawa, 2007). But a detailed description and photographs of the female and male genitalia are not available. Hence, we are redescribing the female of this species with detailed photographs. We could not be able to give a detailed description of the male genitalia since we were able to collect only the subadult one.

Material and Methods

The specimens of *A. viridiventris* were handpicked. The live adult female specimen was photographed in a laboratory platform within an hour after collecting. Then the specimens were transferred to small 5 ml plastic vials having 70% ethanol. Leica M205C stereomicroscope was used to explore the morphological features. Detailed photographs of the genitalia and other features were taken with the help of Leica DMC4500 digital camera attached to Leica M205C stereomicroscope. Stacking of images and measurements were taken using the software package Leica Application Suite (LAS) Windows version 4.3.0. Epigyne was dissected and internal genitalia were cleared in 10% potassium hydroxide (KOH) solution.

All measurements are in millimetres. Measurements of legs and pedipalp were taken from the proximal to distal position of each segment and recorded as follows: total length [femur, patella, tibia, metatarsus (except palp), tarsus]. After the examination, the specimens were deposited in the reference collection at the Centre for Animal Taxonomy and Ecology (CATE), Department of Zoology, Christ College, Irinjalakuda, Kerala, India.

Abbreviations used in the text and figure plates: AER = anterior eyes row, ALE = anterior lateral eye, AME = anterior median eye, CD = copulatory duct, CO = copulatory opening, d = dorsal, FD = fertilization duct, H = hood, LC = lateral condyle, MOA = median ocular area, PER = posterior eyes row, pl = prolateral, PLE = posterior lateral eye, PME = posterior median eye, PT = patellar tubercle, rl = retrolateral, S = spermatheca.

Taxonomy

Family **Araneidae** Clerck, 1757
Genus ***Araneus*** Clerck, 1757
Araneus viridiventris Yaginuma, 1969
(Figs. 1, 2A-E, 3A-E, 4A-C)

Araneus viridiventris Yaginuma, in Ohno & Yaginuma, 1969: 21-24, figs. 3a-f (♂♀).
Araneus viridiventris Yin *et al.*, 1990: 22, figs. 51-57 (♂♀).
Araneus viridiventris Chang & Tso, 2004: 27-28, figs. 1-4 (♂♀).
Araneus viridiventris Tanikawa, 2007: 83, figs. 245, 689-691 (♂♀).

Material examined: India. Kerala. 1♀ (CATE823530a) from college garden, Christ College (Autonomous), Irinjalakuda, Thrissur District, 10°21'20"N, 76°12'48"E, 25m asl, coll. E.H. Vishnudas, 8 December, 2022. 1 subadult ♂ (CATE823530b) from Ranipuram forest, Kasaragod District, 12°24'45"N, 75°21'24"E, 915m asl, coll. K.R. Shilpa, 28 September, 2022.

Fig. 1. *Araneus viridiventris* Yaginuma, 1969 ♀, Habitus, dorsal view (alive).

Diagnosis: *Araneus viridiventris* can be diagnosed by the following characters: presence of distinct lateral condyle, subequal median eyes, subequal and contiguous lateral eyes which are not on tubercles, wider than longer abdomen. Females can be distinguished by a well sclerotized epigynum with U-shaped hood, globular spermatheca and copulatory opening which is at both sides of the hood. Males are diagnosed by the presence of large filamentous embolus, apex bifurcated median apophysis and a long bristle on the palpal patella (Ohno & Yaginuma, 1969: 23, figs. 3e-f).

Description of female (Figs. 1, 2A-E, 3A-E): Total body length 5.03. Cephalothorax length 2.01, width (at the widest portion) 1.89. pear shaped, longer than wide, broad thoracic area, narrow roundish cephalic region (Fig. 2A). Raised cephalic region which slants down towards the thoracic area (Fig. 2C). Brown carapace with brownish black cephalic area in the habitus. The colour fades to orange brown in alcohol. Cephalic area possesses black hairs. Few black hairs on the MOA and clypeus. Thoracic region sparsely haired. Distinct transverse fovea. Eye diameter and interdistances: AME 0.16, ALE 0.11, PME 0.14, PLE 0.10, AME-AME 0.17, AME-ALE 0.20, PME-PME 0.15, PME-PLE 0.25, AME-PME 0.13. Eight eyes in two rows. AER slightly recurved and PER procurved. Subequal median eyes. Lateral eyes small, subequal and contiguous. Lateral eyes not on a tubercle. Each eye is surrounded by a black patch. MOA nearly square. Sternum length 0.89, width 0.73. Nearly triangular, yellowish brown, barely covered with black and grey hairs. Maxilla rectangular with distinct scopulae, orange brown with pale white inner margin densely covered with black hairs. Labium pale white coloured, triangular with few black hairs on the apex (Fig. 2B). Chelicera length 0.78, width (at base) 0.51. Short and robust with distinct boss and lateral condyle (Fig. 2D) with 3 promarginal and 3 retromarginal teeth. Black hairs on the inner margin of paturon. In the ventral view short black hairs scattered in a row towards the base of paturon. Legs orange brown where the intensity increases from femur to tarsus, covered with hairs. Leg I 8.35 [2.61, 0.99, 2.14, 1.99, 0.62], Leg II 7.66 [2.31, 0.95, 1.96, 1.89, 0.55], Leg III 3.83 [1.31, 0.49, 0.81, 0.82, 0.40], Leg IV 3.85 [1.33, 0.62, 0.70, 0.75, 0.45]. Palp 2.18 [0.61, 0.29, 0.44, 0.84]. Leg formula 1243. Spination: Leg I: femur d 2 pl3 rl 1, patella pl 1 rl 1, tibia pl 2 rl 2, metatarsus spineless, Leg II: femur d 2 pl 1 rl 1, patella rl 1, tibia pl 1 rl 1, metatarsus spineless, Leg III: femur d 2, patella d 1, tibia d 1 pl 1 rl 1, metatarsus d 3, pl 3, Leg IV: femur d 2, patella d 2, tibia d 1, metatarsus spineless. Tarsus of all legs have many short spines and hairs. Short patellar basal tubercle on the ventral side of legs I and IV (Fig. 2E).

Abdomen length 2.96, width (at the shoulder) 3.86. inverted triangle with blunt apex and slightly convex base. Bright green dorsum in the habitus and pale greyish yellow in alcohol. Dark brown pigmentation in the anterior part. Random arc shaped dark brown spots surrounded by white patches arranged in the lateral margin from anterior to posterior (Figs. 1, 2A, 2C). Four distinct pairs of dark coloured sigilla. Randomly patterned greyish transverse and vertical lines. Dark brown ventrum with four pairs of small sigilla in between epigyne and spinnerets (Fig. 2B). Deep brown spinnerets and epigyne. Highly sclerotized epigyne, heart shaped with a short U-shaped hood in the ventral view (Fig. 3C). Base of the hood is fully fused with the atrium (Figs. 3C, 3E). Hood has a median depression. CO is situated in the depressions on the sides of the hood (Fig. 3B). Highly sclerotized and globular spermatheca. Less sclerotized and foliose like FD starting from the anterior end of spermatheca in the dorsal view (Fig. 3D). CD also starts from the same point as FD. Before opening into CO, CD takes many turns which makes the path convoluted.

Description of subadult male (Figs. 4A-C): Total body length 2.61. Cephalothorax length 0.91, width (at the widest portion) 1.02. Shape and colour similar to female. Cephalic area, MOA and clypeus possess black hairs. Fovea similar to female. Eye diameter and interdistances AME 0.08, ALE 0.07, PME 0.10, PLE 0.06, AME-AME 0.13, AME-ALE 0.10, PME-PME 0.12, PME-PLE 0.16, AME-PME 0.08. Eye pattern and arrangements similar to female except AER slightly procurved. Sternum length 0.61, width 0.49, oblong, covered by grey hairs throughout the sternum. Pale yellowish-brown sternum, maxilla, and labium. Rest of the features similar to female. Chelicera length 0.47, width (at base) 0.24, similar to female. Leg I 4.25 [1.29, 0.53, 1.11, 0.93, 0.39], Leg II 3.81[1.14, 0.52, 0.90, 0.86, 0.39], Leg III 1.97 [0.68, 0.26, 0.37, 0.38, 0.28], Leg IV 2.85 [1.0, 0.36, 0.52, 0.66, 0.31]. Spination: Leg I: femur pl 1, tibia pl 1, Leg II: femur d 1, Leg III and leg IV were spineless. Leg formula and colour same as female.

Abdomen length 1.76, width (at the shoulder) 1.98. Shape, colour and markings similar to female except the strongly convex anterior portion (Fig. 4A). Three distinct pairs of sigilla are visible. Dark brown ventrum with deep brown spinnerets. Pedipalp is covered by numerous hairs and spines. It was not possible to describe the male genitalia as the collected specimen was subadult male.

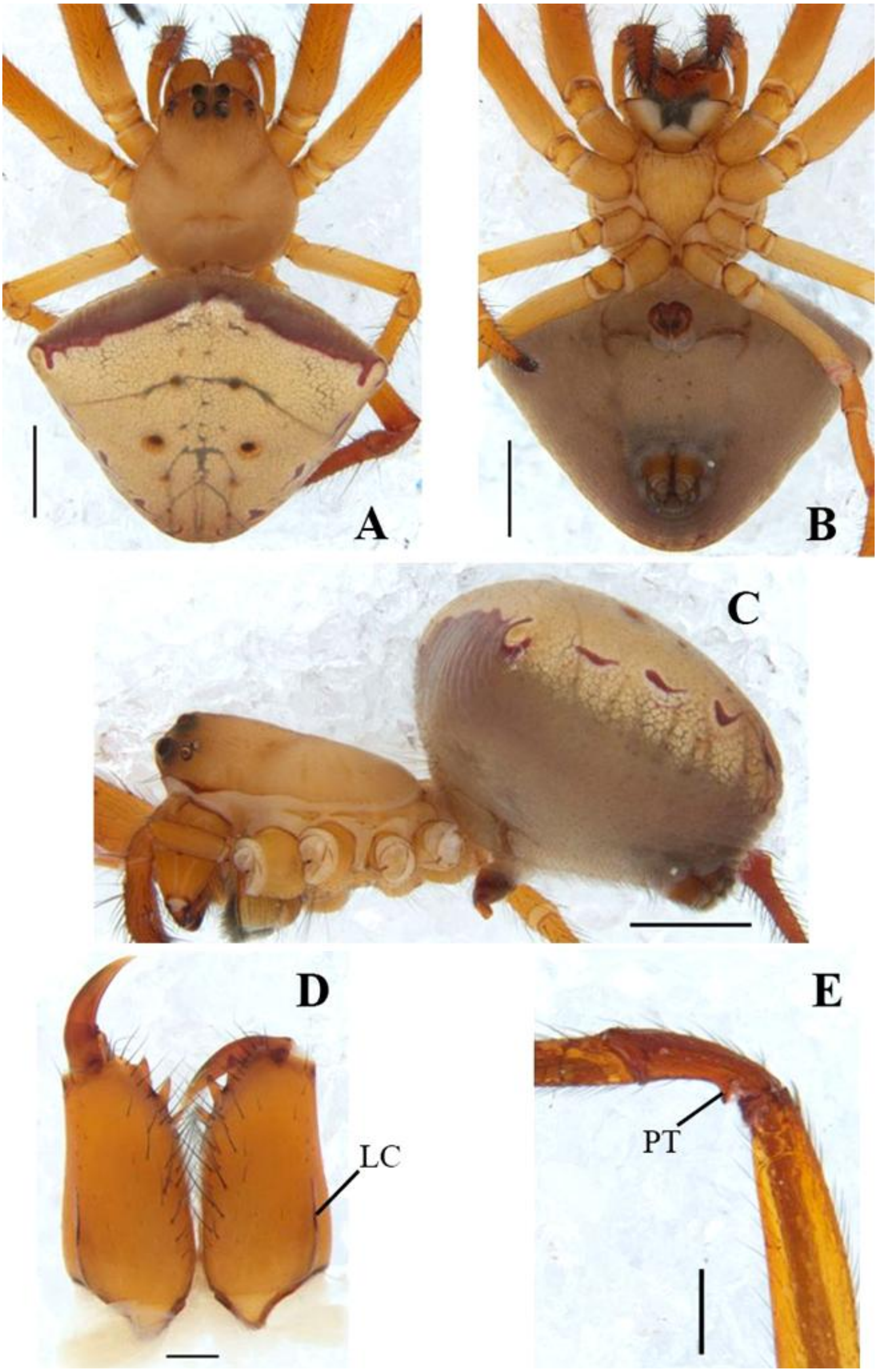

Fig. 2. *Araneus viridiventris* Yaginuma, 1969 ♀. A-C. General appearance in alcohol. A. dorsal view. B. ventral view. C. lateral view. D. Chelicerae, dorsal view. E. Leg I. (Scale bars: A-C. 1 mm, D. 0.2 mm, E. 0.5 mm).

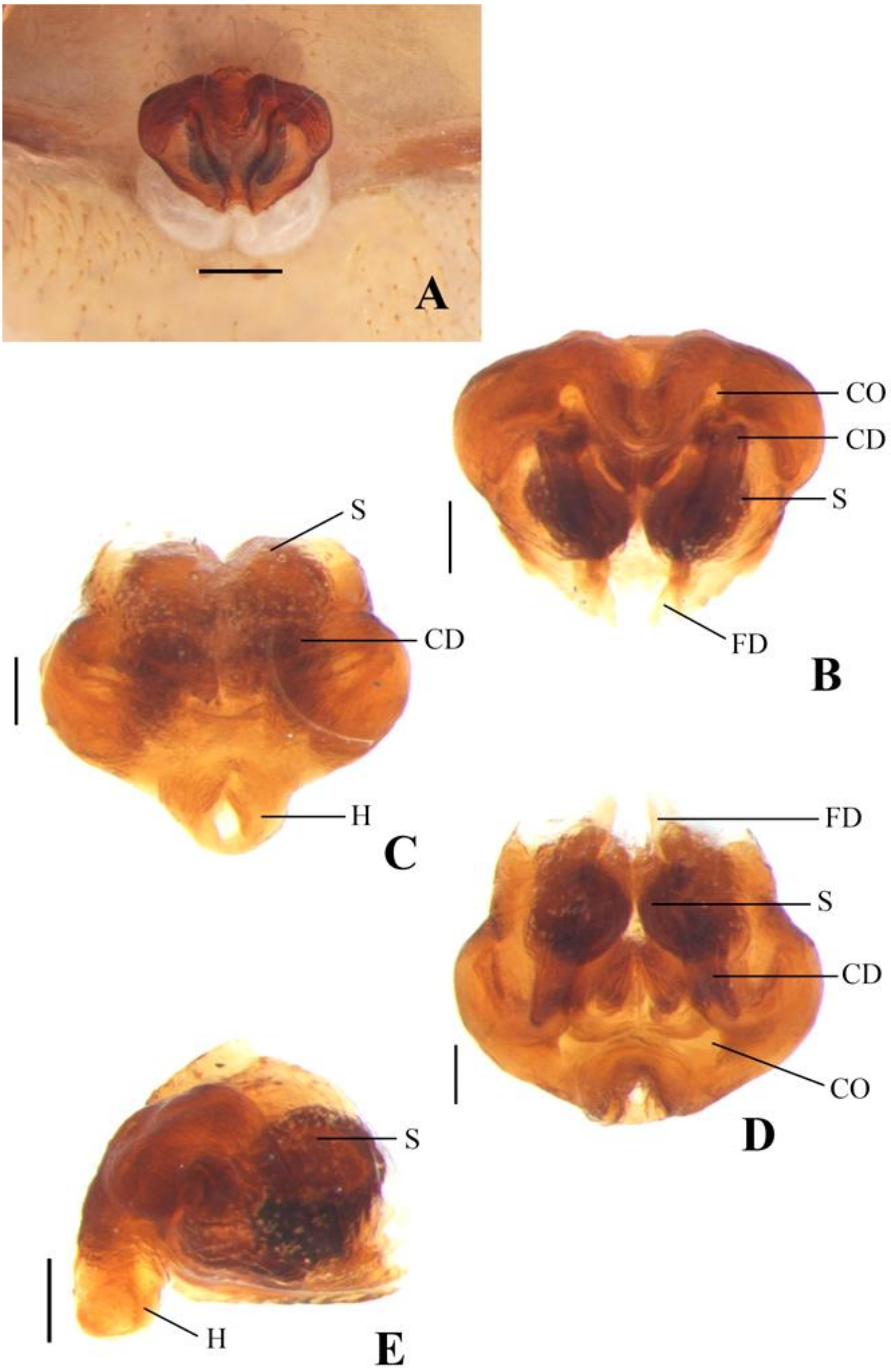

Fig. 3. *Araneus viridiventris* Yaginuma, 1969 ♀. A-E. Epigyne and internal genitalia. A. *in situ* view. B. posterior view. C. ventral view. D. dorsal view. E. lateral view. (Scale bar: A-E. 0.1 mm).

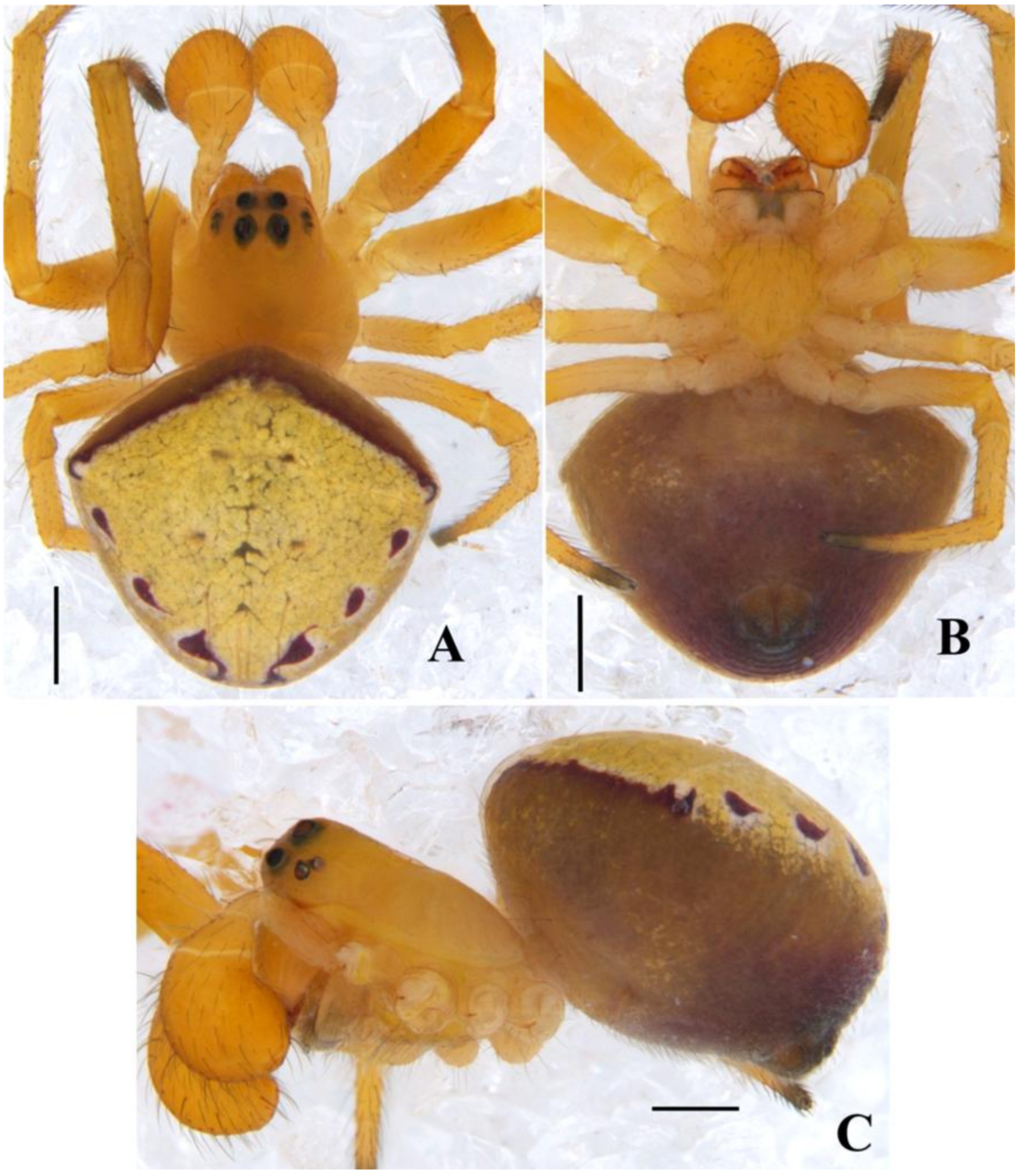

Fig. 4. *Araneus viridiventris* Yaginuma, 1969 s♂. A-C. General appearance in alcohol. A. dorsal view. B. ventral view. C. lateral view. (Scale bar: A-C. 0.5 mm).

Distribution: Japan, China, Taiwan (Fig. 5) and India (new record).

Remarks: All the morphological features mentioned in the literature were matching with the current specimen and hence confirmed the species. But an exception was there with the height of cephalic and thoracic regions. In the first description of *A. viridiventris*, Yaginuma (1969) mentioned the statement ‘head as high as thorax’ (Ohno & Yaginuma, 1969: 21). This feature was not mentioned in rest of the literature. In our specimen of *A. viridiventris* the cephalic region is higher than the thoracic region and the cephalic region slants down towards the thoracic region (Figs. 2C, 4C).

Natural history: Both the male and female specimens were found in the retreat near the slight horizontal web. Male was found in a tropical forest habitat whereas the female was found in man-made garden. Both habitats were humid and specimens were collected in sunny days. This concludes that the species prefers a complex habitat with humid and warm climate.

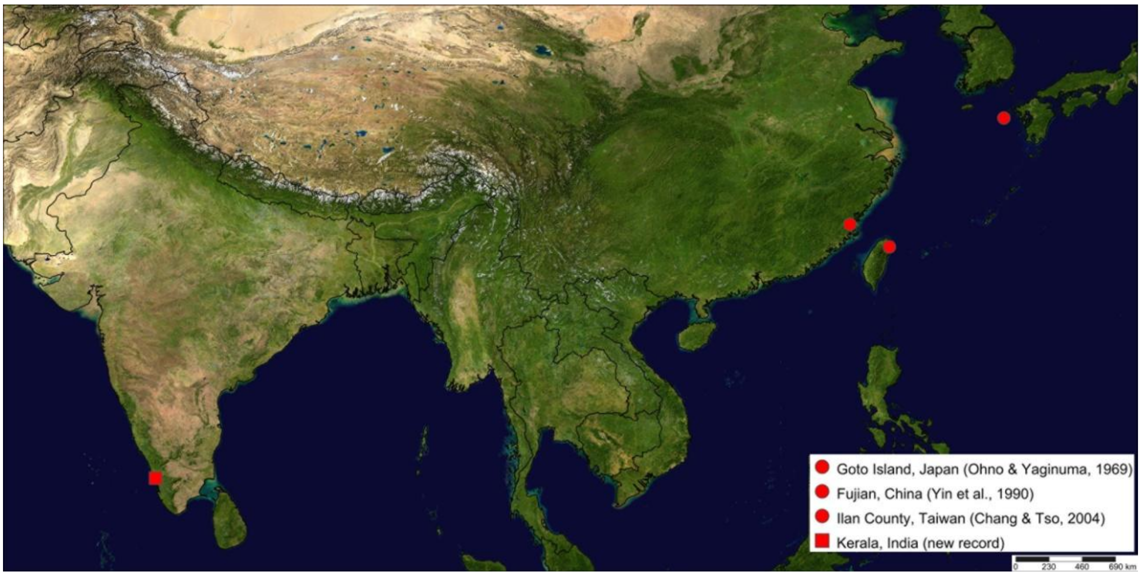

Fig. 5. Distribution map of *Araneus viridiventris* Yaginuma, 1969.

Acknowledgments

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References

Caleb, J.T.D. & Sankaran, P.M. 2023. *Araneae of India*, version 2023, online at https://indianspiders.in, accessed on 29.01.2023.

Chang, Y.H. & Tso, I.M. 2004. Six newly recorded spiders of the genera *Araneus*, *Larinia*, *Eriophora*, *Thanatus*, *Portia* and *Dolichognatha* (Araneae: Araneidae, Philodromidae, Salticidae and Tetragnathidae) from Taiwan. *Acta Arachnologica*, 53(1): 27-33.

Clerck, C. 1757. *Aranei Svecici. Svenska spindlar, uti sina hufvud-slågter indelte samt under några och sextio särskildte arter beskrefne och med illuminerade figurer uplyste*. Laurentius Salvius, Stockholmiae, 154 pp.

Ohno, M. & Yaginuma, T. 1969. The spider fauna of the Goto islands belonging to Kyushu, Japan. *Journal of Tokyo University for General Education* (nat. Sci.), 12: 7-24.

Tanikawa, A. 2007. *An identification guide to the Japanese spiders of the families Araneidae, Nephilidae and Tetragnathidae*. Arachnological Society of Japan, 121 pp.

World Spider Catalog 2023. *World Spider Catalog.* Version 24. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on 29.01.2023.

Yin, C.M., Wang, J.F., Xie, L.P. & Peng, X.J. 1990. New and newly recorded species of the spiders of family Araneidae from China (Arachnida, Araneae). In: Spiders in China: One Hundred New and Newly Recorded Species of the Families Araneidae and Agelenidae. Hunan Normal University Press, pp. 1-171.

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A new spider species of the genus *Philoponella* Mello-Leitão 1917 (Araneae: Uloboridae) from Western Ghats of India

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Abstract — A new species from the spider genus *Philoponella* Mello-Leitão 1917 – *Philoponella rostralis* n. sp. (♂♀) is diagnosed and described from the Kottapara Hills, Western Ghats, Kerala, India. A detailed description of its morphology, diagnostic features, photographs and the depictions of the copulatory organs of both sexes are given.

Key words — epigyne, feather legged spider, morphology, palp, taxonomy

Introduction

Family Uloboridae Thorell 1869, the only orb web spinning cribellate spiders, is composed of 290 species from 19 genera dispersed across the world (World Spider Catalog 2022). Uloborids are remarkably unique in many aspects. They lack cheliceral venom glands hence, have a distinct way of feeding.

Presently, a total of 42 species has been reported from the genus *Philoponella*. From which two species were reported from India. *Philoponella* males are characterised by having an oval carapace with a broad, transverse thoracic groove, whereas the females are distinguished by the presence of a ventral epigynal atrium whose posterior margin is composed of two lateral lobes (Opell 1979). Anterior median eyes of *Philoponella* are located on a tubercle. In the present study, we focussed on the description and illustration of *P. rostralis* n. sp. (♂♀) collected from Kottapara Hills, Western Ghats, Kerala, India.

Materials and methods

Specimens were collected by visual searching and hand picking method. Then they were transferred to small plastic vials having 70% ethanol. Morphological features were explored under a Leica M205C stereomicroscope. Leica DMC4500 digital camera attached to Leica M205C stereomicroscope, with the software package Leica Application Suite (LAS), version 4.3.0. LAS montage facility was used to record the detailed photographs. All measurements are recorded in millimetre. Measurement data for palps and legs are as follows: total length [femur, patella, tibia, metatarsus (except palp), tarsus]. Terminology used in the text and figures follows Opell 1979. Examined specimens are deposited in the reference collection at the Centre for Animal Taxonomy and Ecology (CATE), Department of Zoology, Christ College, Irinjalakuda, Kerala, India.

Abbreviations used in the text and figure plates: A – atrium, AER – anterior eyes row, ALE – anterior lateral eye, AME – anterior median eye, CBL – conductor basal lobe, CD – copulatory ducts, CO – copulatory opening, CS – conductor spike, CY – cymbium, do – dorsal, E – embolus, FD – fertilization duct, FT – femoral tubercle, MAB – median apophysis bulb, MAS – median apophysis spur, MH – middle hematodocha, MOA – median ocular area, PER – posterior eyes row, pl – prolateral, PLE – posterior lateral eye, PME – posterior median eye, rl – retrolateral, S – spermatheca, ST - subtegulum, T – tegulum, v – ventral.

Taxonomy

Family: Uloboridae Thorell 1869

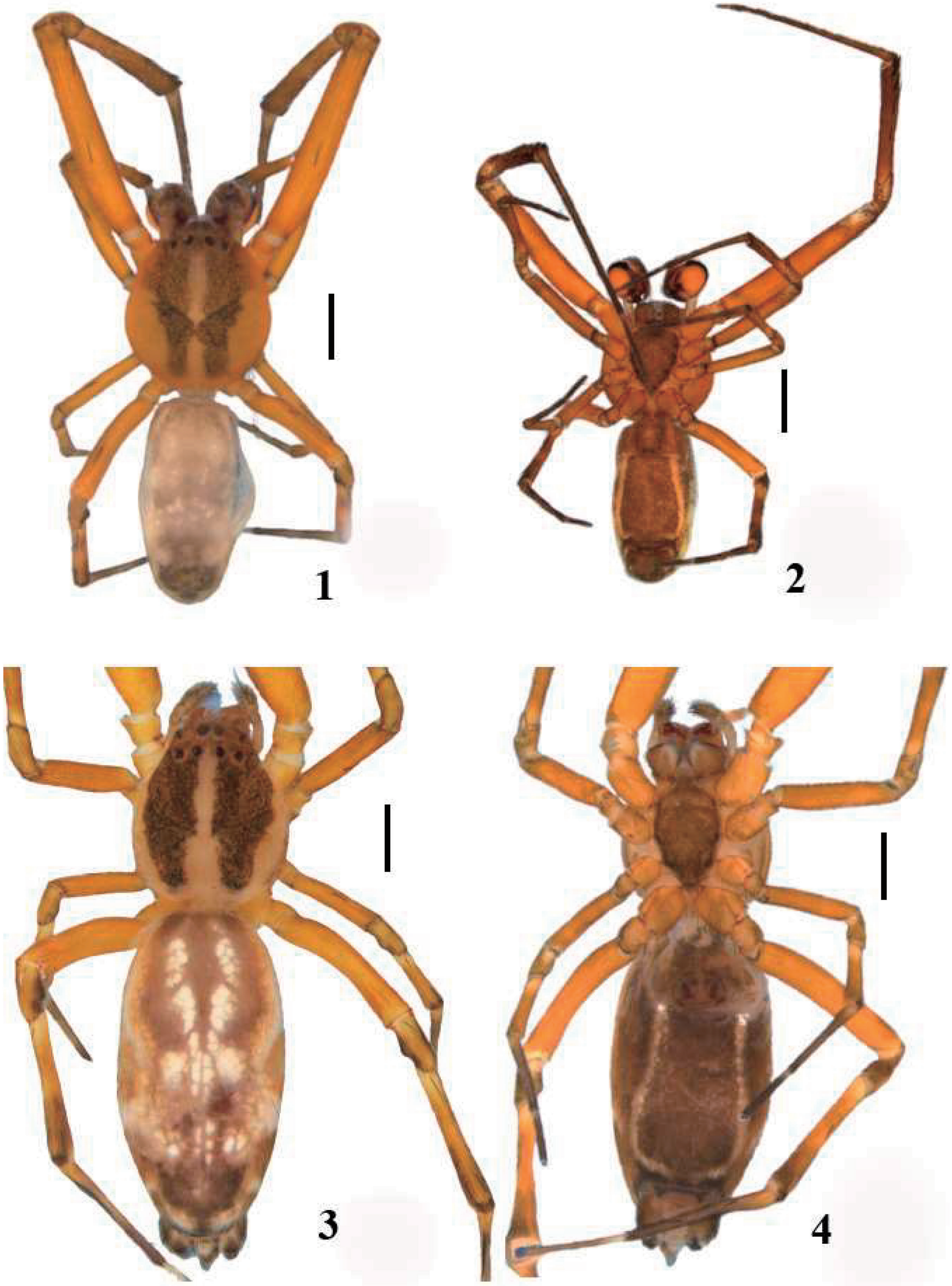
Philoponella Mello-Leitão 1917
Type species: *Philoponella republicana* (Simon 1891)

Philoponella rostralis n. sp.

(Figs. 1–15)

Type series. Holotype: ♂ (CATE, 1251032A): Kottapara Hills, Idukki District, Kerala, India (10°01’38.56”N, 76°58’13.36”E) 1076 m a.s.l., 10 October 2021, collected by E.H. Vishnudas & A.V. Sudhikumar. Paratypes: 1 ♀ 2 ♂ (CATE, 1251032B) same data as in the holotype.

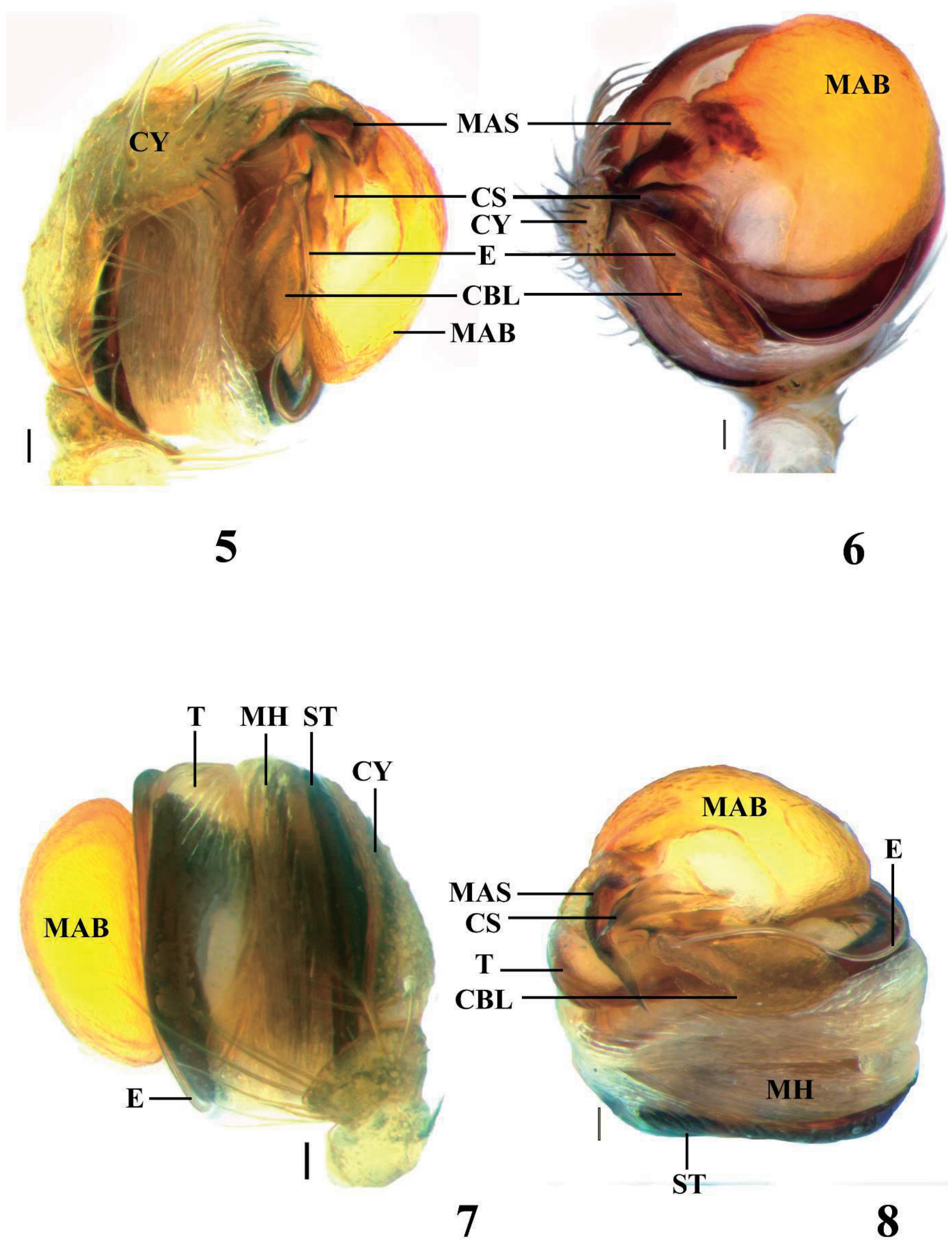
Etymology. Specific epithet of *P. rostralis* n. sp. derived from the Latin word rostrum (beak), referring to the rostri-



Figs. 1–4. *Philoponella rostralis* n. sp., male holotype (1–2) and female paratype (3–4). 1, 3, habitus, dorsal view; 2, 4, same, ventral view. Scales = 0.5 mm.

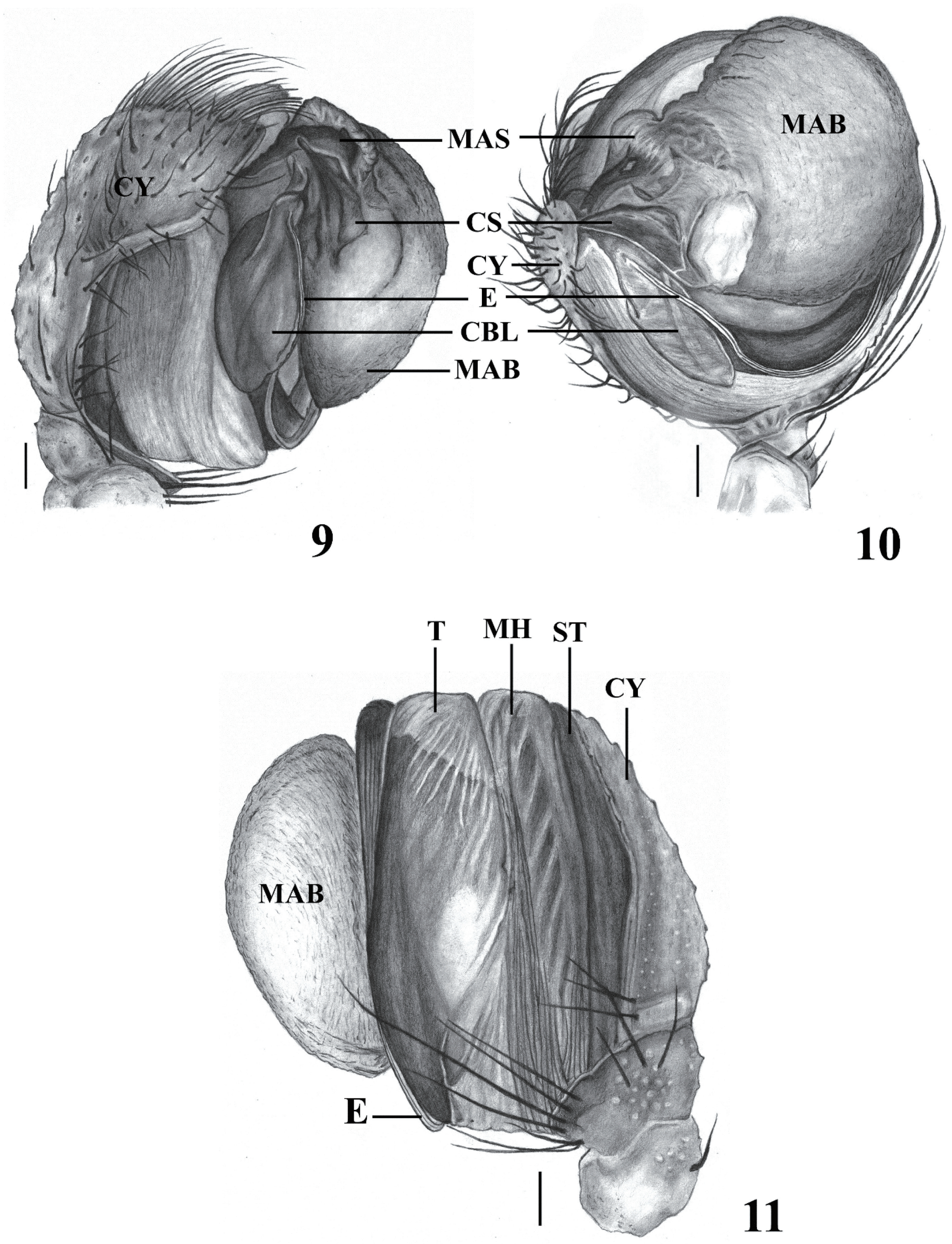
form MAS of male palp.

Diagnosis. The male copulatory organ of *P. rostralis* n. sp. is similar to that of *P. nasuta* (Thorell 1895) from China and Myanmar, but both can be distinguished by the following combination of characters. Rostriform MAS with nearly rectangular base (olecranoid in *P. nasuta*); cymbium broad at the base and narrows gradually towards the apex (cymbium narrows abruptly from the middle in *P. nasuta*); dome shaped MAB in the reterolateral view of palp (nearly triangular with blunt apex in *P. nasuta*). Epigyne of *P. rostralis* n. sp. can be compared with that of *P. nasuta*. Copulatory openings of both are on the lateral sides of atrium. Also, the fertilization duct is foliated in both species, but both can be distinguished by the following characters: copulatory ducts with two spirals (four spirals in *P. nasuta*); anteriorly joining copulatory duct to the spermatheca (posteriorly joining in *P. nasuta*) (cf. Figs. 5–15 with figs. 4–7, 11 in Zhou, Zhou & Peng 2020).



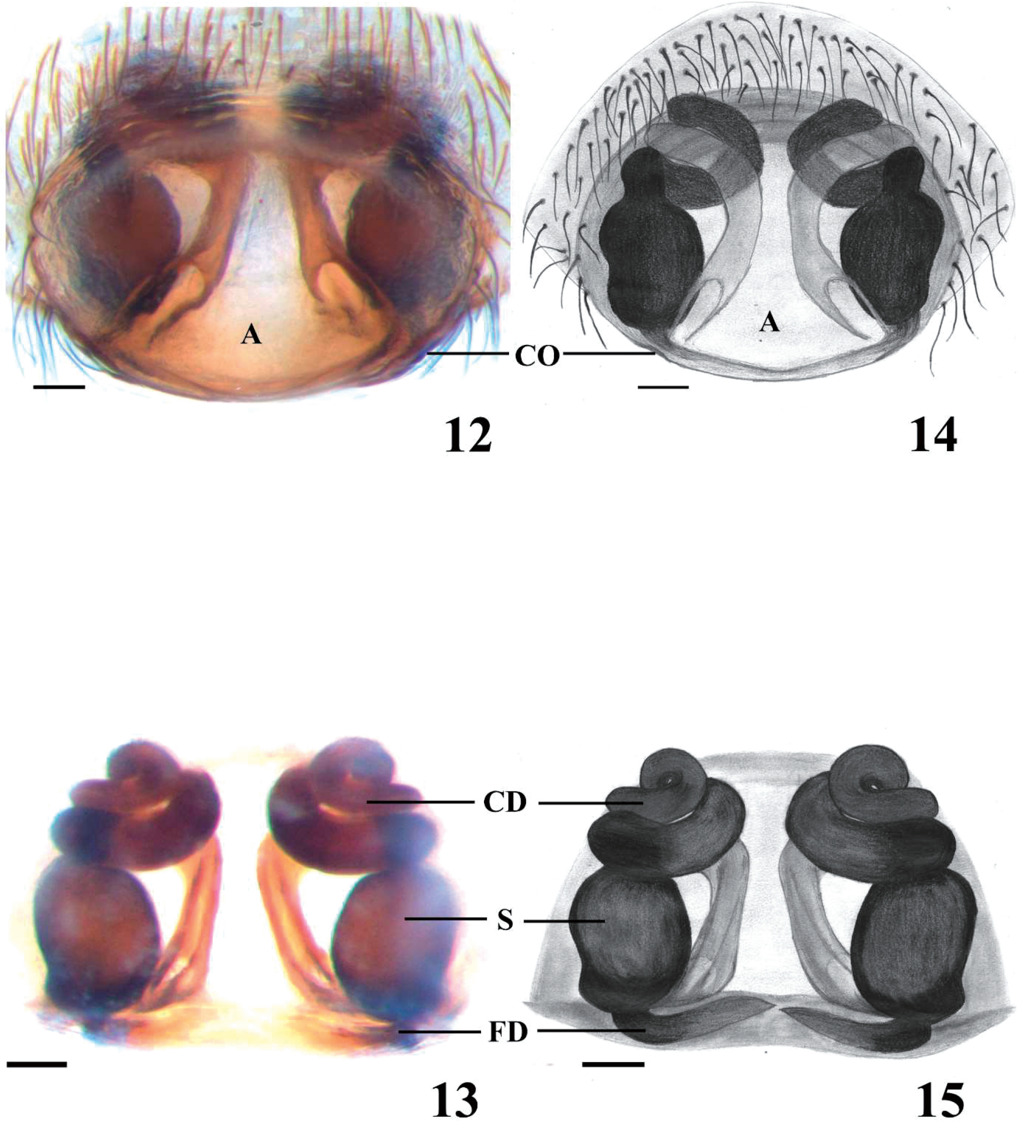
Figs. 5–8. *Philoponella rostralis* n. sp., male holotype. 5, left palp, prolateral view; 6, same, ventral view; 7, same, retrolateral view; 8, bulb, prolateral view. Scales = 0.05 mm.

Description. Male (holotype, CATE, 1251032A) (Figs. 1–2, 5–11). Measurements. Body length 3.28. Carapace length 1.40, width (at middle) 1.23, height (at thoracic groove) 0.69. Abdomen length 1.79, width (at middle) 1.02. Eye diameters: AME 0.08, ALE 0.05, PME 0.05, PLE 0.06. Eye interdistances: AME-AME 0.08, AME-ALE 0.12, PME-PME 0.15, PME-PLE 0.09, ALE-PLE 0.11. MOA nearly square. Clypeus height 0.14. Length of chelicera 0.28. Cribellum length 0.19, width (at middle) 0.12. Leg measurements: leg I 6.83 [1.87, 0.52, 1.49, 2.04, 0.91], II 3.48 [0.91, 0.35, 0.63, 0.97, 0.62], III 2.7 [0.79, 0.25, 0.49, 0.60, 0.58], IV 3.74 [1.24, 0.36, 0.98, 0.69, 0.46]. Leg formula: 1423. Spination. Legs: I femur – do 1 pl 3 rl 1, patella – do 1, tibia – do 2 pl 4 rl 5, metatarsus – pl 8 rl 4, II femur – do 1 rl 1, patella – do 1, tibia – do 1 pl 1 rl 3, metatarsus – pl 1 rl 3, III femur – do 1, patella – do 1, tibia – do 1 pl 1 rl 1, metatarsus – pl 1 rl 1, IV femur – do 1, patella – do 1, tibia – do 1 pl 2 rl 2, metatarsus – pl 3 rl 4. Carapace oval, yellowish orange with two longitudinal irregular black bands extending from the ocular area to the posterior margin of



Figs. 9–11. *Philoponella rostralis* n. sp., male holotype. 9, left palp, prolateral view; 10, same, ventral view; 11, same, retrolateral view. Scales = 0.05 mm.

carapace leaving a pale coloured light band in between the dark bands (Fig. 1). Distal end covered with yellow brown hairs. Lateral side have short yellow brown hairs. Thoracic region slightly raised than the cephalic region. Transverse fovea with two black slanting striae emerging from fovea to the either side of the fovea across the two parallel dark bands (Fig. 1). Eight eyes in two rows. AER recurved, PER slightly recurved. Black patches on each eye. AME is on slight projection. White hairs are there in between AME. Dark brown pyriform sternum narrowing posteriorly (Fig. 2). Continuous light brown margin around the sternum and black patches on the margin of sternum corresponding to each coxa. Labium wider than long, triangular with blunt corners, dark brown in colour with white patches in the anterior end. Maxillae rectangular, dark brown with white colour in the lateral side towards labium, covered with numerous black hairs, have distinct scopulae. Chelicera pale coloured paturon with 7 promarginal teeth, covered with black hairs, fang dark brown in colour. Abdomen pale greyish with short black streak on the lateral side of anterior half, white spots on the middle region and black markings on the posterior end (Fig. 1). Dark coloured anal tubercle extending beyond



Figs. 12–15. *Philoponella rostralis* n. sp., female paratype. 12, 14, epigyne, ventral view; 13, 15, vulva, dorsal view. Scales = 0.05 mm.

the posterior end of the abdomen. Oblong shape with blunt ends. Dorsum of abdomen covered with white pubescence. White U – shaped marking on the ventral side of the abdomen, which extends from the posterior margin of booklungs to the anterior margin of the cribellum (Fig. 2). Ventral side covered with brown hairs. Cribellum wider than long, nearly triangular, dark brown coloured with light brown apex, mid portion have a semi-circular black demarcation with black hairs. Anterior and posterior pair of spinnerets dark brown, the median pair is light compared to the others. Legs orange, tarsus dark. Femur of leg II, III and IV have numerous trichobothria. Calamistrum on the fourth tarsus having 14 bristles dorsally. Yellowish brown palpal segments. Cymbium broad at base and gradually narrows towards the distal end, covered with long white macrosetae, distal end has short black hairs (Figs. 5, 9). ST is thin, sclerotized and dark brown in colour. Thick, off-white MH above ST. Highly sclerotized dark coloured tegulum. Long thin tube shaped embolus which emerges reterolaterally and coiled once around MAB, posterior tip directed towards CBL and CS. Yellow coloured globular MAB resting over the tegulum. Highly sclerotized rostriform MAS with nearly rectangular base. Dark brown wedge-shaped CS which is broad at base and gets narrower towards the tip. Foliole like CBL, longer than wide and have heavily sclerotized dark coloured pointed projection and the base (Figs. 5–11). Short ventral FT.

Female (paratype; Figs. 3–4, 12–15). Measurements. Body length 4.25. Carapace length 1.42, width (at middle) 1.23. Abdomen length 2.79, width (at middle) 1.32. Eye diameters: AME 0.08, ALE 0.07, PME 0.06, PLE 0.07. Eye interdistances: AME-AME 0.06, AME-ALE 0.13, PME-PME 0.13, PME-PLE 0.09, ALE-PLE 0.05. Clypeus height 0.1. Length of chelicera 0.36. Cribellum length 0.16, width (at middle) 0.41. Palp and leg measurements: palp 0.67 [0.23, 0.07, 0.11, 0.26], leg I 6.84 [2.06, 0.54, 1.72, 1.92, 0.59], II 4.25 [1.20, 0.44, 0.78, 1.15, 0.68], III 2.72 [0.62, 0.31, 0.48, 0.76, 0.54], IV 5.53 [1.72, 0.44, 1.16, 1.30, 0.92]. Leg formula: 1423. Spination. Legs: I femur – do 1 pl 1 rl 1, patella – do 2, tibia – do 2 pl 3 rl 4, metatarsus – pl 6 rl 7, II femur – do 1 rl 1, patella – do 2, tibia – do 2, rl 1, metatarsus – do 3 rl 1 v 1, III femur – do 1 pl 1, patella – do 1, tibia – do 2, metatarsus – do 1 pl 1 rl 1, IV femur – do 1, patella – do 2, tibia – do 3 pl 2, metatarsus – pl 3. Carapace longer than wide, yellowish brown with two dark broad black longitudinal bands extending from the ocular region to the posterior end of the thoracic region (Fig. 3). Oval, where the anterior end narrower than the posterior, carapace covered with greyish white pubescence. Dark brown, slightly curved lateral line with brown hairs on the cephalothorax. Thoracic region elevated from fovea it declines to the cephalic region. Transverse thoracic groove but not prominent as in male. Eight eyes in two rows. AER recurved, PER slightly recurved. Black patches on each eye. AME on slight projection. White hairs are there in between AME. Sternum dark brown, oval, narrowing posteriorly, covered with black hairs (Fig. 4). Black continuous margin around the sternum except the anterior blunt end. Discontinuous light brown margin around the sternum extending from the labium to the posterior end. Proximal end of coxa III and IV possess dark coloured spine like projection towards the sternum (Fig. 4). Labium longer than wide, triangular with blunt corners, dark brown, white patch in the anterior end. Maxillae wider than long, dark brown with white colouration in the lateral side towards labium, covered with numerous black hairs, have distinct scopulae. Pale orange coloured chelicera with 7 promarginal teeth. Abdomen longer than wide, blunt posterior and anterior ends. Dorsum covered with greyish white pubescence and dark grey coloured with two longitudinal lines having discontinuous white patches extending from anterior end to two-thirds of the abdomen (Fig. 3). The space between these two lines gets narrowed from anterior to posterior. A pair of white patches towards the centre of the abdomen on the either side of the lines. White patches are also present on the lateral sides, dark spots on the posterior end. White U-shaped marking on the ventral side of the abdomen, which extends from the posterior margin of book lungs to the anterior margin of the cribellum (Fig. 4). Anal tubercle extending beyond the posterior end of the abdomen. Cribellum wider than long, oblong, light brown in colour, anterior half have a semi-circular black demarcation with black hairs. Anterior and posterior pair of spinnerets are dark brown in colour whereas the median pair is light compared to the others. Orange coloured legs with pale white annulation at the base of tibia, metatarsus and tarsus. Legs covered with numerous hairs and spines. Femur of legs II, III and IV have numerous trichobothria. Calamistrum composed of 25 bristles on the dorsal side of fourth metatarsus which if half long as the fourth metatarsus. Metatarsus and tarsus of leg I is very slender compared to other segments. One dorsal macro seta is present on the tibia and patella of the palp. Tip of palpal tarsus contains 7 spines around the distal end. Semi-circular epigynal atrium with anterior and posterior rim, copulatory opening is on the lateral side of the posterior rim, lateral lobes are fused, in the ventral view spermatheca and copulatory duct is covered by a semi-transparent membrane (Figs. 12, 14). In the dorsal view, the copulatory duct loops twice before joining to the kidney shaped spermatheca anteriorly. Foliole like fertilization duct linking to the posterior margin of the spermatheca (Figs. 13, 15).

Distribution. Only known from the type locality.

Natural history. The studied specimens were collected from the lower branches of the trees in scrub jungle. They build small, flimsy orb webs near the rocky areas. They prefer thick vegetation to construct web in order to resist wind. Unlike other oriental *Philoponella* species, *P. rostralis*, does not show any social behaviour.

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The authors are grateful to Rev. Fr. Dr. Jolly Andrews CMI, Principal, Christ College (Autonomous), Irinjalakuda, Kerala, India for providing all facilities for undertaking this work. We express our gratitude to Asha T. Joy, scientific illustrator and other research scholars of CATE. The authors also acknowledge the funding rendered by DST-SERB Major Research Project EEQ/2021/000453, for the facilities used in this study. We are also thankful to the Kerala State Council for Science, Technology & Environment (KSCSTE) for providing financial assistance for this study as Junior Research Fellowship (No. KSCSTE/2092/2019-FSHP- MAIN).

References

Mello-Leitão, C. F. de. 1917. Generos e especies novas de araneidos. Arch. Esc. Sup. Agric. Med. Veter., 1: 3–19.

Opell, B. D. 1979. Revision of the genera and tropical American species of the spider family Uloboridae. Bull. Mus. Comp. Zool., 14: 443–549.

Simon, E. 1891. Observations biologiques sur les arachnides. I. Araignées sociable. Ann. Soc. Ent. France, 60: 5–14.

Thorell, T. 1869. On European spiders. Part I. Review of the European genera of spiders, preceded by some observations on zoological nomenclature. Nova Acta Reg. Soc. Sci. Upsaliensis, (3) 7: 1–108.

Thorell, T. 1895. Descriptive catalogue of the spiders of Burma, based upon the collection made by Eugene W. Oates and preserved in the British Museum. London, 406 pp. doi:10.5962/bhl.title.17492

World Spider Catalog 2021. World Spider Catalog. Version 22.5. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on December 20, 2021.

Zhou, X. W., Zhou, G. C. & Peng, X. J. 2020. Redescription of *Philoponella nasuta* with the first report of the female (Araneae: Uloboridae). Acta Arachnol. Sin., 29: 30–34. doi:10.3969/j.issn.1005-9628.2020.01.008

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Current Status of Molecular Taxonomy of Spiders in India Diversity of Orb Web Spiders (Araneae: Araneidae) in Peruvanamuzhi Forest, Calicut, India

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Abstract

Araneidae is the third largest family of spiders. Family Araneidae includes the spiders that construct spiral wheel-shaped webs. Hence the members are also known as orb web spiders. This is a large cosmopolitan family often found in gardens, fields and forests. Peruvanamuzhi forest range forms the part of Malabar Wildlife Sanctuary which situates on the northwest slopes of Western Ghats. Several studies have been conducted to reveal the diversity of vertebrates, butterflies and odonates of this ecologically fragile region. But studies on spiders are very less. Hence, the present study focuses on the diversity of araneidsin Peruvanamuzhi forest range. From the study, we were able to record 15 species from 9 genera, out of 36 from India. The generic diversity is in the order of *Cyclosa* (3), *Neoscona*(3), *Eriovixia*(2), *Gasteracantha*(2), *Acusilas* (1), *Anepsion* (1), *Argiope*(1), *Nephila*(1) and *Thelacantha*(1). The present study also provides a checklist of araneids in the forest range. The abundance of each species varied between different ecosystems within the forest range.

Keywords: orb web, araneidae, Peruvanamuzhi, taxonomy, diversity.

Introduction

The most imposing facet of the planet is its diversity and the uniqueness of the life forms. Spiders are seen ubiquitous. They may look small and insignificant, but the role it plays in an ecosystem need immense appreciation. They are important predators and prey for many of other animals. They are the largest order of arachnids and rank seventh in total species diversity among all other orders of organism (Sebastian & Peter, 2009). Family Araneidae includes the spiders that construct spiral wheel-shaped webs. Hence the members are also known as orb web spiders. This is a large cosmopolitan family often found in gardens, fields and forests. The members show a broad disparity in size, colour, shape and behaviour. With around 3089 species in 180genera worldwide (WSC, 2022), Araneidae is the third largest family of spiders. With 189 species in 38 genera, family Araneidae is the second largest family in India (Caleb &Sankaran, 2022). They use the "spin-wrap-attack" method to subdue their prey (Simon, 1895). The general shape, number of radii, spirals, shape of hub and decorations of the web vary between genera and subfamilies (Simon, 1895). Most of the Indian araneids construct orb webs except the genus *Cyrtophora* which construct very complicated tent webs for prey capture (Simon, 1895). Since the members in this family are so diversified it has been a predicament in its proper diagnose. Hence the need to record the species diversity is important.

Materials and methods

Study area: Peruvanamuzhi forest range (11°36’06”N 75°49’26”E) forms the part of Malabar Wildlife Sanctuary which situates on the northwest slopes of Western Ghats. Peruvanamuzhi forest range lies in the catchment areas of Kuttiady River. Tropical rain forest, grassland, teak plantation and riparian forest are the different ecosystems which constitutes the Peruvanamuzhi forest range. The forest range is also a part of Kozhikode Eco-tourism project.

Habitats which likely to support the existence of spiders were carefully searched during the study. Specimens were collected by visual searching and hand picking method. Then they were transferred to small plastic vials having 70% ethanol. Morphological features were explored under a Leica M205C stereomicroscope. Leica DMC4500 digital camera attached to Leica M205C stereomicroscope, with the software package Leica Application Suite (LAS), version 4.3.0. LAS montage facility was used to record the detailed photographs.Details of the male and female copulatory organs were thoroughly analysed. Each specimen was identified by running available keys (Tikader, 1982) and also by comparing the photographs and morphological features with the available literature (Barrion&Litsinger, 1995; Sebastian & Peter, 2009; WSC, 2022).

Results

A total of 15 species from 9 genera were identified through this study. Highest number of species was from the genus *Cyclosa* and *Neoscona* followed by *Eriovixia* and *Gasteracantha*. From the genus *Acusilas*, *Anepsion*, *Argiope*, *Nephila* and *Thelacantha* single species were recorded. Table 1 shows the checklist of araneids.

Sl. No	Species
1	*Acusilas coccineus*
2	*Anepsion maritatum*
3	*Argiope pulchella*
4	*Cyclosa bifida*
5	*Cyclosa confraga*
6	*Cyclosa quinqueguttata*
7	*Eriovixia laglaizei*

8	*Eriovixia sakiedaorum*
9	*Gasteracantha dalyi*
10	*Gasteracantha geminata*
11	*Neoscona bengalensis*
12	*Neoscona mukerjei*
13	*Neoscona nautica*
14	*Nephila pilipes*
15	*Thelacantha brevispina*

Table 1. Checklist of araneids in Peruvanamuzhi forest range

Discussion

The study reveals that, Peruvanamuzhi forest range possess wide araneid diversity. Out of 38 genera from India, we were able to record species from 9 genera. This was the first attempt to record araneids from Peruvanamuzhi forest. The forest range is rich with different ecosystems such as tropical rain forest, teak plantation, grassland and riparian forest. The number of specimens varies between different ecosystems. *Neoscona*is the most diverse araneid genus in India followed by *Cyclosa* (Caleb & Sankaran, 2022) *Neoscona* and *Cyclosa* were the most diverse genus in the study also. *Anepsion maritatum*, *Cyclosa* species and *Neoscona* species were distributed among all types of ecosystem but the number of specimens varied.*Thelacantha brevispina* was found only in the teak plantation. *Gastercantha* species were found in both teak plantation and rain forest. *Nephila pilipes* were found all ecosystems except grassland. The collection possesses different morphs of *Nephila pilipes*. Distribution of the species among different ecosystems reveals the priority of species in choosing the ecosystem. The study reveals only the araneid diversity. Studies are not yet done to figure out the entire araneofauna. Understanding the araneofauna can greatly emphasize not only their protection but the conservation of the entire ecosystem.

References

1. Barrion, A.T.,&Litsinger, J.A. (1995).*Riceland spiders of South and Southeast Asia*. CAB International, Wallingford, UK, xix + 700 pp.
2. Caleb, J.T.D.,&Sankaran, P.M. (2022). Araneae of India. Version 2022, online at http://www.indianspiders.in, accessed on 20.01.2022
3. Sebastian, P.A., & Peter, K.V.(2009). Spiders of India. Orient Blackswan, Hyderabad. 614pp.
4. Simon, E. (1895). Histoire naturelle des araign_ees. LibrairieEncyclop_edique, Paris.
5. Tikader, B.K. (1982). The fauna of India. Spiders: Araneae. Vol. II. Part 1 Family Araneidae (Argiopidae) typical orb-weavers.
6. World Spider Catalog. (2022). World Spider Catalog. Version 23.0. Natural History Museum Bern, online at http://wsc.nmbe.ch, accessed on January 2022