CHAPTER 3

MATERIALS AND METHODS

3.1. Study area

Chirang Reserve Forest (CRF) falls under Haltugaon Forest Division. This division was created after the bifurcation of the old Goalpara West Division in the year 1934 and known as East Goalpara Division with Divisional Headquater at Kokrajhar (Assam Forest Department, unpublished). These Resrve Forest (RF) falls within the Kokrajhar district under the administrative control of Bodoland Territorial Areas District (BTAD) also known as BTR (Bodoland Territorial Region). The entire RF is situated in the northern tract at the foothills of the sub-Himalayan region. Additionally, part of this Reserve Forest extends into a distinctive ecological zone known as the Terai (Assam Forest Department, unpublished). The RF is bounded on the north by an international boundary with Bhutan, on the south by the river Brahmaputra, on the east by the river Bhur and the west by the river Saralbhanga River (Das et al., 2012), and is contiguous with Raimona National Park on the western side. It is an integral part of Manas Biosphere Reserve and is one of the oldest reserve forests located in the Western part of Assam constituted in 20th November 1875 (Bhattacharjee et al, 2014). It was declared as Elephant Reserve on 6th March 2003 (Bhattacharjee et al., 2014). Rides and Parallels were introduced by Perree (1905) in these RF where comparments were made by cutting rides lines numbering 1 to 17, as well as parallel lines numbered 1 to 9 which was later extended from 10 to 14 by Das (1966). The forest type of the reserve can be divided into Sal Forest, evergreen and semi-evergreen forest, deciduous forest, savannah/grassland, and riverine forest (Bhattacharjee et.al. 2014). For the year 2017-2021, the average temperature varies from 27.87 (max) to 22.09 (min), average humidity varied from 82.64 % (max) to 67.73 % (min) and the average rainfall was 0.92 cm. The Jharbari Forest Range is an admisitrative setup of Chirang RF under Haltugaon Forest Division, lying at latitude 26º38´ to 26º43´ N and longitude 90º14´ to 90º16´ E with a total area jurisdiction of 68 sq. kms. Floral study at Chirang Reserve Forest has revealed a total of 728 species of plants belonging to 492 genera under 138 families (Basumatary,

2023). These RF is home to one of the endangered primate species i.e. Golden langur, including many bird species, mammal such as Tiger, Leopard, Clouded leopard, Hispd rare, as well as reptiles (BirdLife International, 2024). The forests is also well known for its varied diversity of butterflies.

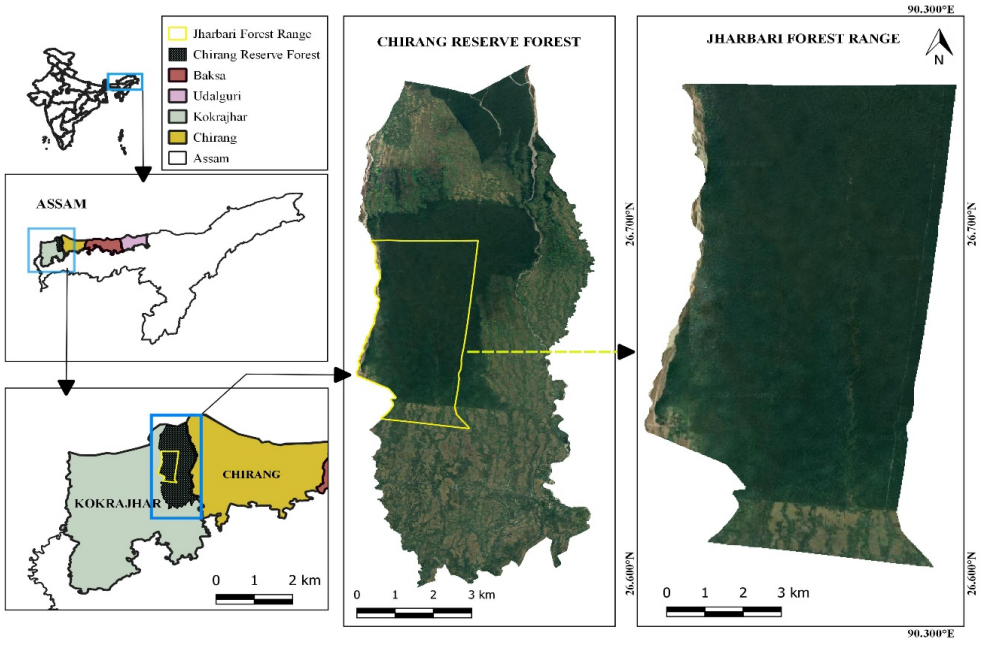


Figure 3.1. Map showing the location of Chirang Reserve Forest (CRF)

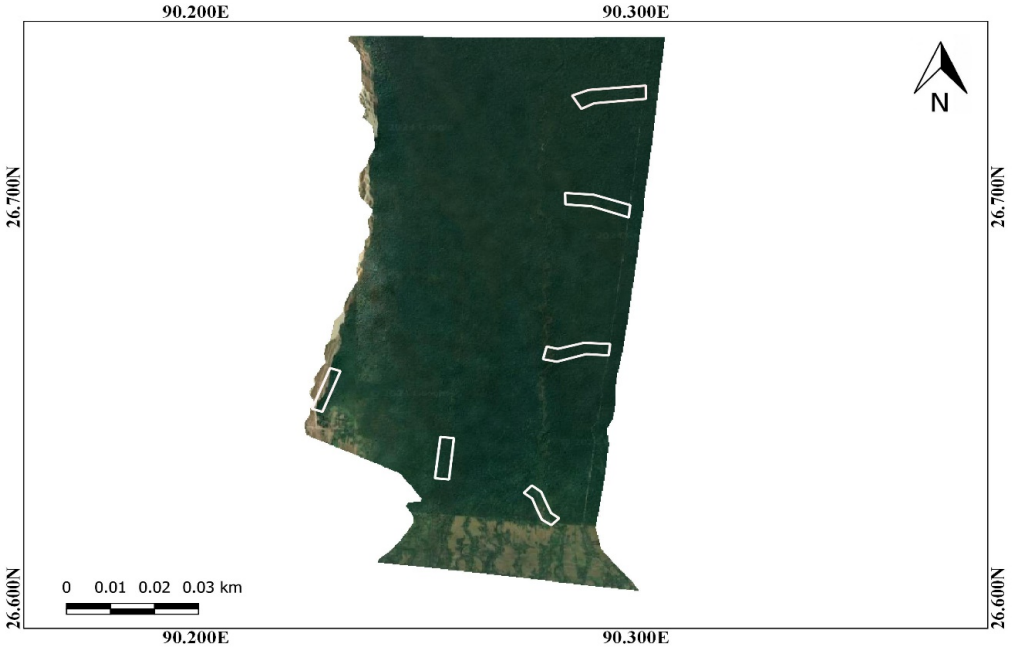


Figure 3.2. Map showing the survey sites in Jharbari Forest Range of CRF

PLATE 1

Habitat: A. Open patches of woodland**; B.** Moist open patch of grassland**; C. D**ense patch of woodland**; D.** Slow-moving perennial forest stream**; C.** Fast-flowing perennial forest stream**; F.** Open patch of dry grassland**; G.** Dense herb vegetation**; H.** Ground leaf litter**.**

3.2. Methodology

3.2.1. Spider sampling:

The study was conducted in the Jharbari Forest Range of Chirang Reserve Forest, Assam, India. Prior permission was obtained from the forest department (FGW/WL/35/Chakrasila/588) (Annexure III) before commencing the fieldwork. A total of six fixed trails, each 1.5 km long with a width of 30 meters on each side (1.5 km x 30 m), were established for the study. Each trail was traversed at a slow pace, conducting visual search from side to side to examine the vegetation and waterways. Thorough inspections were carried out at each search interval, focusing on leaf litter, tree bark, rock crevices, soil and foliage. Fieldwork was conducted three days a week, resulting in a total of 12 surveys per month (2 surveys per trail). The study was carried out in the morning hours between 08:00 and 12:00 hrs.

The methods for various objectives are outlined as follows:

3.2.1.1. OBJECTIVE 1: To know the overall status of the spider from Jharbari Forest Range.

3.2.1.1.1. Field data collection:

Data were collected by direct search technique (Sutherland, 1996). During the preliminary survey, various spider habitats were identified and categorized accordingly: under rocks, grasses, forest litter, burrow, forest streams, herbs, shrubs and tree bark (Table 3.1).

3.2.1.1.2. Photographic documentation:

Each individual was photographed in the field with Nikon COOLPIX L310 and Sony Cyber-shot DSC-HX90V. Whenever possible, images were taken of each individual

from above to capture the leg and body general form, along the dorsal plane to depict the shape of the carapace and abdomen, and from the front to document the ocular pattern.

Table 3.1. Characteristics of habitats.

Sl. No.	Habitats	Habitat characteristics
1	Under rocks	Space beneath rocks measuring from 10 to 30 cms in length.
2	Grasses	Area covered with patches of grasses viz, *Imperata cylindrica* (Cogon grass), *Saccharum spontaneum* with a height about 2 to 3 meters.
3	Forest Litter	Surface layer on the forest floor of loose non-living organic debris consisting of freshly fallen or slightly decomposed plant parts, such as leaves, bark, twigs, flowers, fruits and other plant matter.
4	Burrow	Abundant burrows of ground dwelling insects with a diameter ranging from 1 to 1.5 cms.
5	Forest stream	Moderate and fast flowing streams with mud and sandy bottom substrate sourrounded by dense shrub vegetation alongwith with aquatic plants viz., *Floscopa scandens*, *Enhydra fluctuant*, *Ipomoea aquatic*, *Cryptocoryne spiralis*, *Impatiens tripitala*, *Ludwigia octovalvis*, *Ludwigia hyssopifolia*, etc.
6	Herbs	Herbs (< 1 meter) consisted of following plant species viz., *Azeratum conizoides*, *Mikania micrantha*, *Persicaria hydropiper*, *Leucas lavandifolia*, *Diplazium esculentum*, etc.
7	Shrubs	Shrubs (>1 meter) composed of viz., *Melastoma malabathricum*, *Clerodendrum imfortunatum*, *Chromalaena odorata*, *Maesa indica*, *Dendrochnide sinuata*, etc.
8	Tree Bark	Outer surface of tree barks alongwith gaps on the bark of the following trees *Stereospermum chelonoides*, *Bombax ceiba*, *Dillenia indica*, *Dillenia pentagyna*, *Toona cialiata*, *Syzygium formosum*, *Glochidion ellipticum*, *Ficus racemosa*, *Litsea glutinosa*, *Litsea monopetala*, etc.

3.2.1.2. OBJECTIVE 2: To prepare a checklist of spiders from the Jharbari Forest Range.

3.2.1.2.1. Collection and preservation:

The spider specimens were collected using the direct collection technique (Zschokke and Herberstein 2014) after obtaining prior approval from the Assam State Biodiversity Board (ABB/Permission/2012/505) (Annexure III). Additionally, net sweeping and beat sheeting methods were employed when needed. Following collection, the specimens were euthanized using acetone and stored in 80% ethanol.

3.2.1.2.2. Dissection:

The epigyne and palp were initially dissected, then submerged in lactic acid for 24 hours, followed by treatment with KOH for four to five hours, and finally preserved in 80% ethanol in a separate vial. Subsequently, the habitus of the indivdiuals alongwith its genitalia were microphotographed and measured using a Leica DFC500 HD camera attached to a Leica M205A stereo microscope with Leica Application Suite (LAS) version 3.8 at the Zoological Survey of India, Shillong, with prior authorization from the Officer-in-Charge. The specimens were then stored in the National Museum at the North Eastern Regional Centre, Zoological Survey of India (NERC-ZSI), Shillong, where accession numbers were assigned to each donated specimen (Annexure V-VI). Measurements were recorded in millimeters, and line drawings of the epigyne and palp were prepared.

3.2.1.2.3. Identification:

The collected specimens were compared to known species for identification upto the species level using taxonomic keys and catalouges provided by Pocock (1895, 1899 a, b; 1900 a, b; 1901), Tikader (1987), Sebastian and Peter (2009), Majumder and Talukdar (2013), as well as other relevant publications from the WSC (2024). Taxonomy and nomenclature were followed according to the WSC (2024). Guild types were assigned to each family based on Dias et al. 2010 and Cardoso et al. 2011. The identified species were compiled, and a checklist was prepared.

3.2.1.2.4. Description and illustrations:

Detailed descriptions alongwith line drawings and photohraphic plates have been prepared for new species, new records and for some interesting species. Diagnostic characteristics of families as well as genera have been presented. Species previously reported with comprehensive descriptions have been provided with taxonomic accounts, concise descriptions, note on specimens examined and distributional information alongwith brief notes on their natural history. The scientific line diagram was prepared on Adobe Photoshop CC 2023 with XP Deco Pro-Graphic tablet.

3.2.1.3. OBJECTIVE 3: To study the abundance of spiders along with seasonal variation.

3.2.1.3.1. Data collection:

The study was conducted in the Jharbari Forest Range over a span of four years, encompassing Year 1 (December 2017–November 2018), Year 2 (December 2018–November 2019), Year 3 (December 2019–November 2020), and Year 4 (December 2020–November 2021). Seasons were categorized as pre-monsoon (March-May), monsoon (June-September), post-monsoon (October-November), and winter (December-February), following the classification by Saikia and Saikia (2015). The number of individuals and their occurrences during each season were documented.

3.2.1.3.2. Statistical analysis:

Species accumulation curves were generated using Microsoft Excel 2011. Significance tests were conducted using the Chi-square test in R Studio (V.2022.02.0+443).

3.2.1.4. OBJECTIVE 4: To study the diversity and guild types of spiders in different microhabitats.

3.2.1.4.1. Statistical analysis:

Biodiversity indices were used to determine species diversity across different habitats. Specifically, the Shannon-Wiener diversity index (H′), Pielou's evenness index (J′), and Simpson's dominance index were calculated using PAST version 4.

3.2.1.5. OBJECTIVE 5: To study the perception of the local community towards spiders on fringe villages of JharbariForest Range.

3.2.1.5.1. Schedule survey:

Villagers from the fringe forest villages of the Jharbari Forest Range, namely, Kalachi, Genduguri, Bodopur, Ronnagar, 9 No. Mahendrapur, and Diglipara, have been interviewed randomly using semi-structured questionnaires (Annexure II) in the local languages, Bodo and Assamese.

3.2.1.5.2. Analysis:

The data obtained from these interviews, reflecting people's perceptions towards spiders and sightings of various spider families along with their occurrence in fringe habitats have been compiled and analysed using Microsoft Excel 2011. The results were presented in the form of pie and bar charts. Based on the villagers' attitudes and perceptions, a conservation strategy was suggested.

Table 5. Demographic Data of Interviewee.

Characteristics of participants	Percentage (%)
1. Gender	
Male	46
Female	54
2. Average age	42.2 years (range 19–90)
3. Educational qualification	
Below 10	70
HSLC	20
HSSLC	7
Graduate	3
Others	0
4.Occupation	

Cultivator	29
Self-employed	28
Govt. employee	5
Housewife	26
Student	12
5. Ethnicity	
Boro	65
Nepali	20
Santhal	15
6. Average family size	
2–3 yrs.	23
4 –5 yrs.	52
6 –7 yrs.	22
8 –9 yrs.	3

3.2.2. Map and photoplates

The KML file of Chirang Reserve Forest and Jharbari Forest Range area have been collected from Haltugaon Forest Division. Study area have been prepared using QGIS 3.4, and the photoplate was created using Photoshop 7.0.

3.2.3. Abbreviations

The list containing abbreviations employed within the text is provided in Annexure I.

3.3. General morphology of spiders

The body of spiders is divided into two main regions: the cephalothorax and the abdomen. These two regions are connected by a narrow segment called the pedicel.

Cephalothorax: The cephalothorax is covered by a hard cuticular plate known as the carapace. It bears the eyes, fovea, chelicerae, pedipalps, and legs.

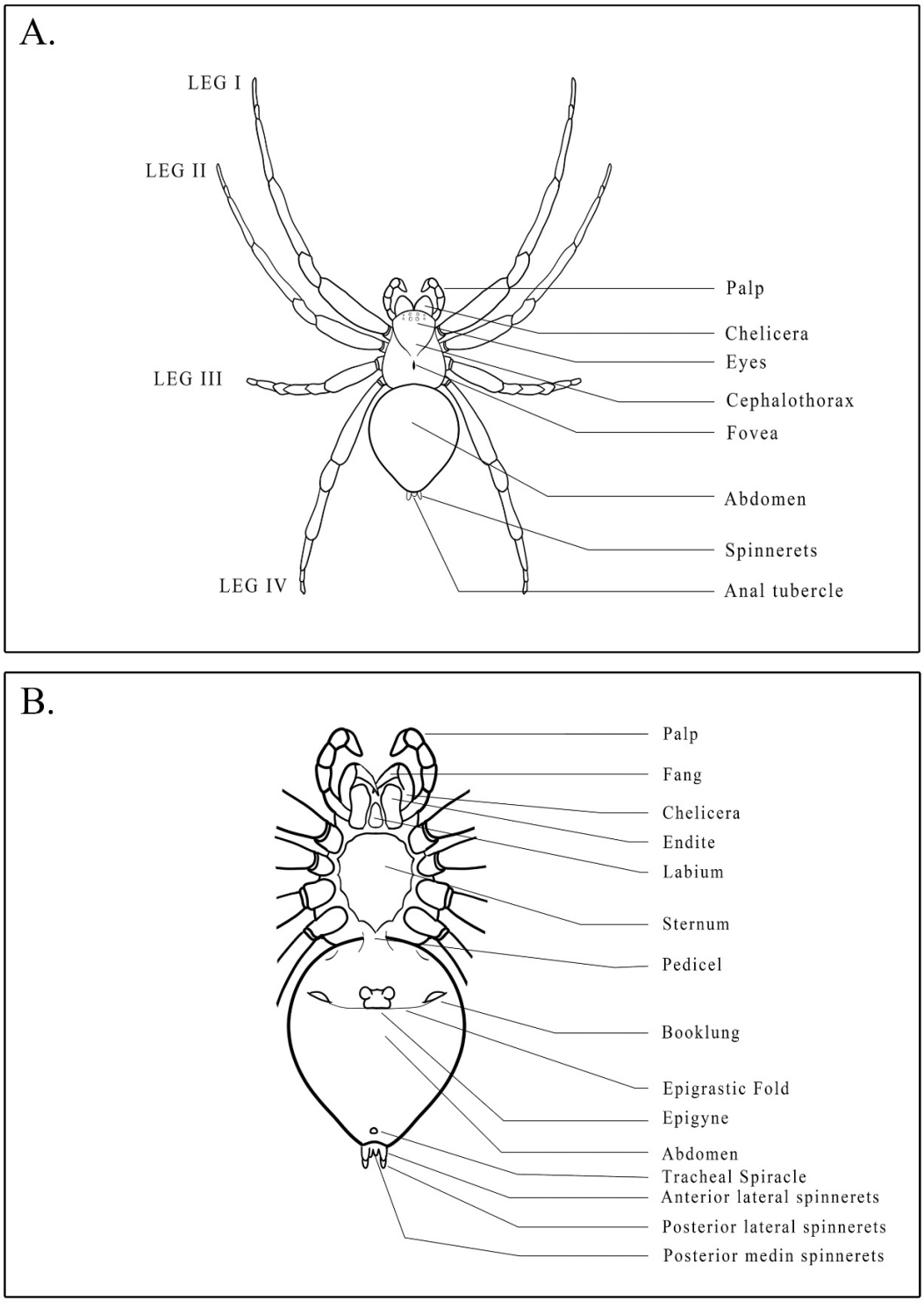
Eyes: Spiders typically have simple eyes, with most species possessing eight eyes, though some have six, four, or even none. The eyes are classified based on their position as

anterior median eyes (AME), posterior median eyes (PME), anterior lateral eyes (ALE), and posterior lateral eyes (PLE). This classification system is crucial for the taxonomic description of spider species.

Chelicerae: Chelicerae are hardened, broad structures equipped with tooth-like formations known as cheliceral teeth, which are species-specific and assist in identification. Each chelicera also has a fang that contains venom glands.

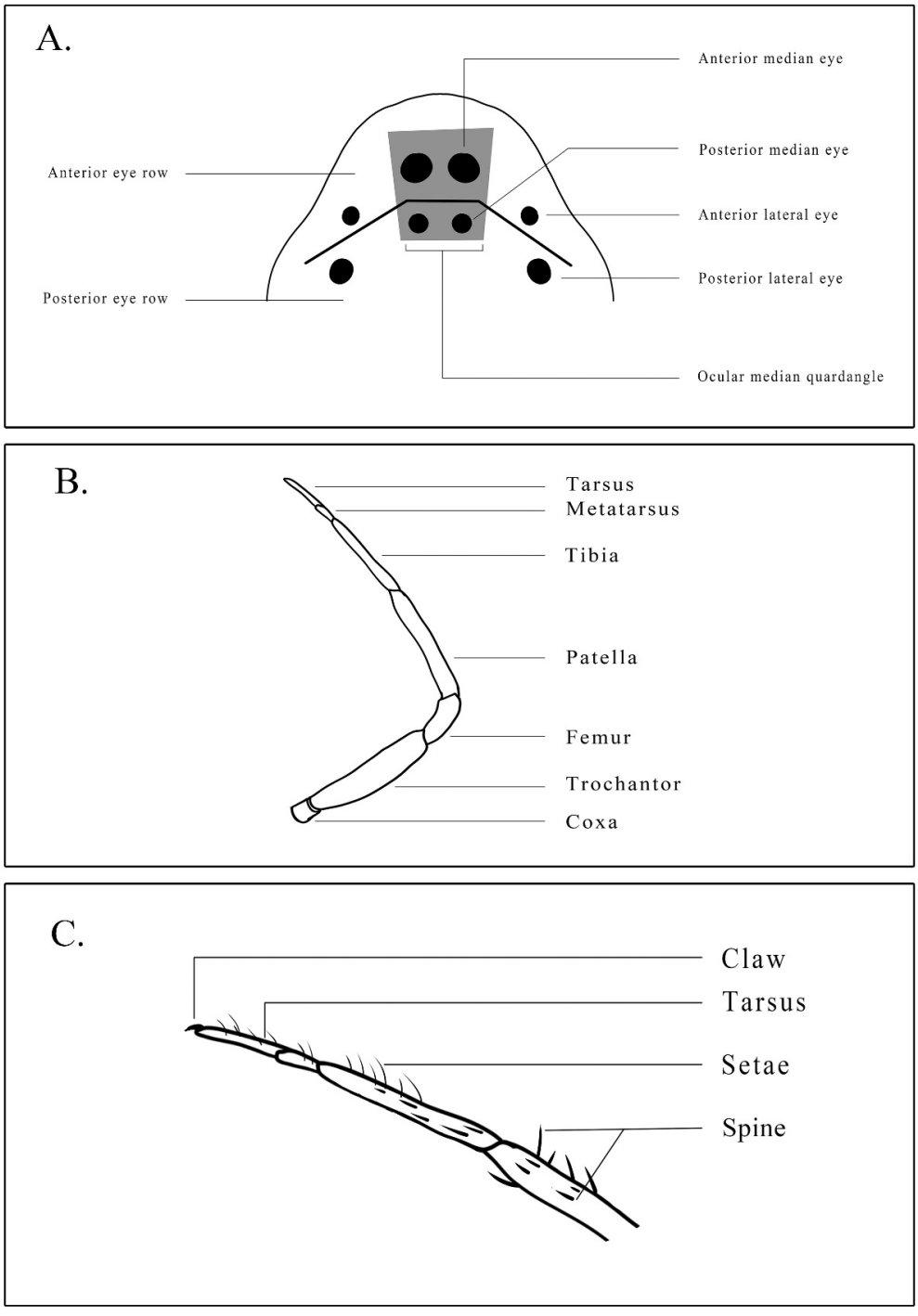
Pedipalps: Pedipalps consist of six segments: the coxa, trochanter, femur, short patella, tibia, and tarsus. In spiders, these segments are same as the segmentation of the legs. Male spiders use their pedipalps to transfer sperm to the female during mating. Additionally, pedipalps also play a crucial role in courtship displays.

PLATE 2



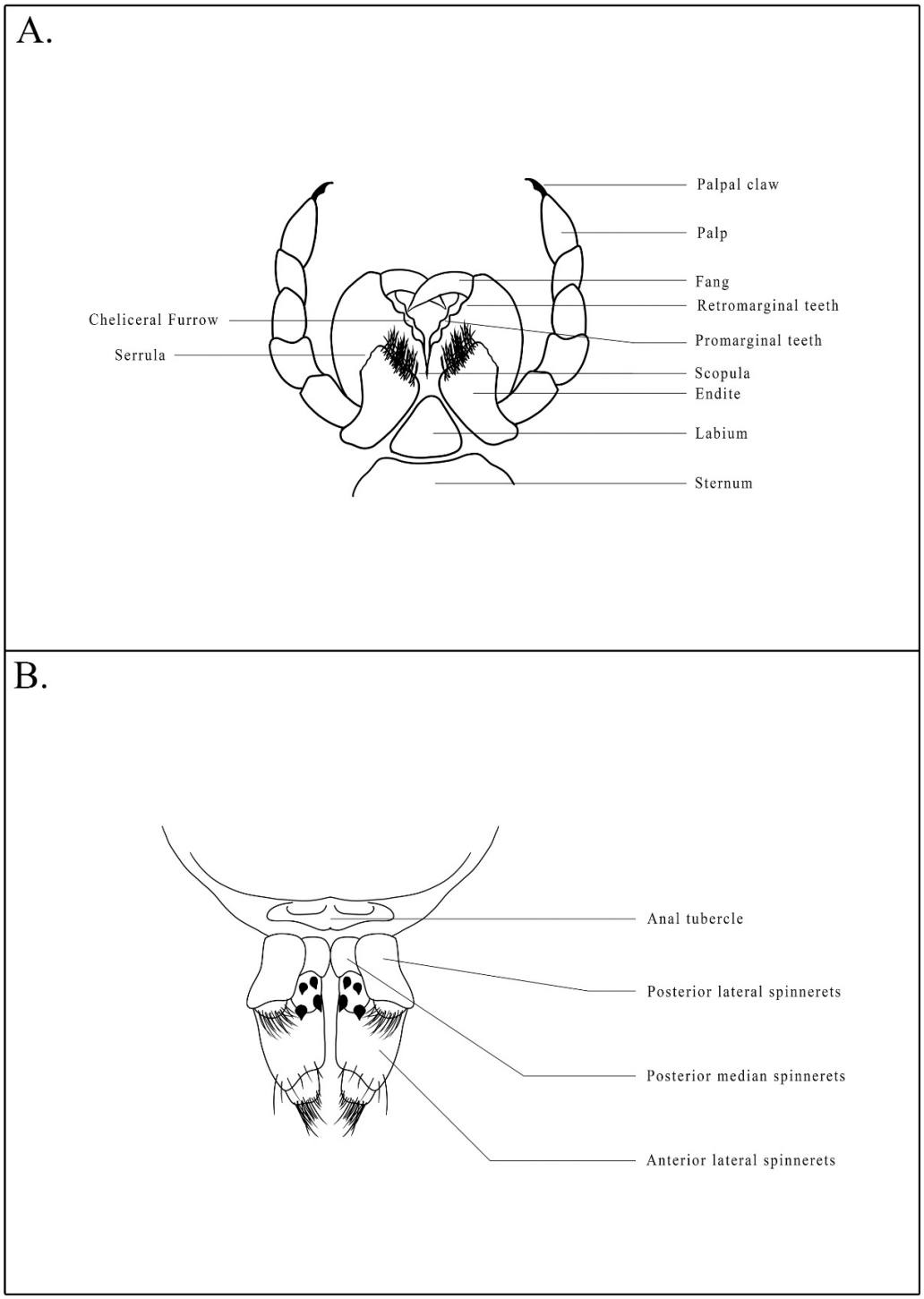
General morphology: A. Dorsal view of spider**; B.** Ventral view of spider**.**

PLATE 3



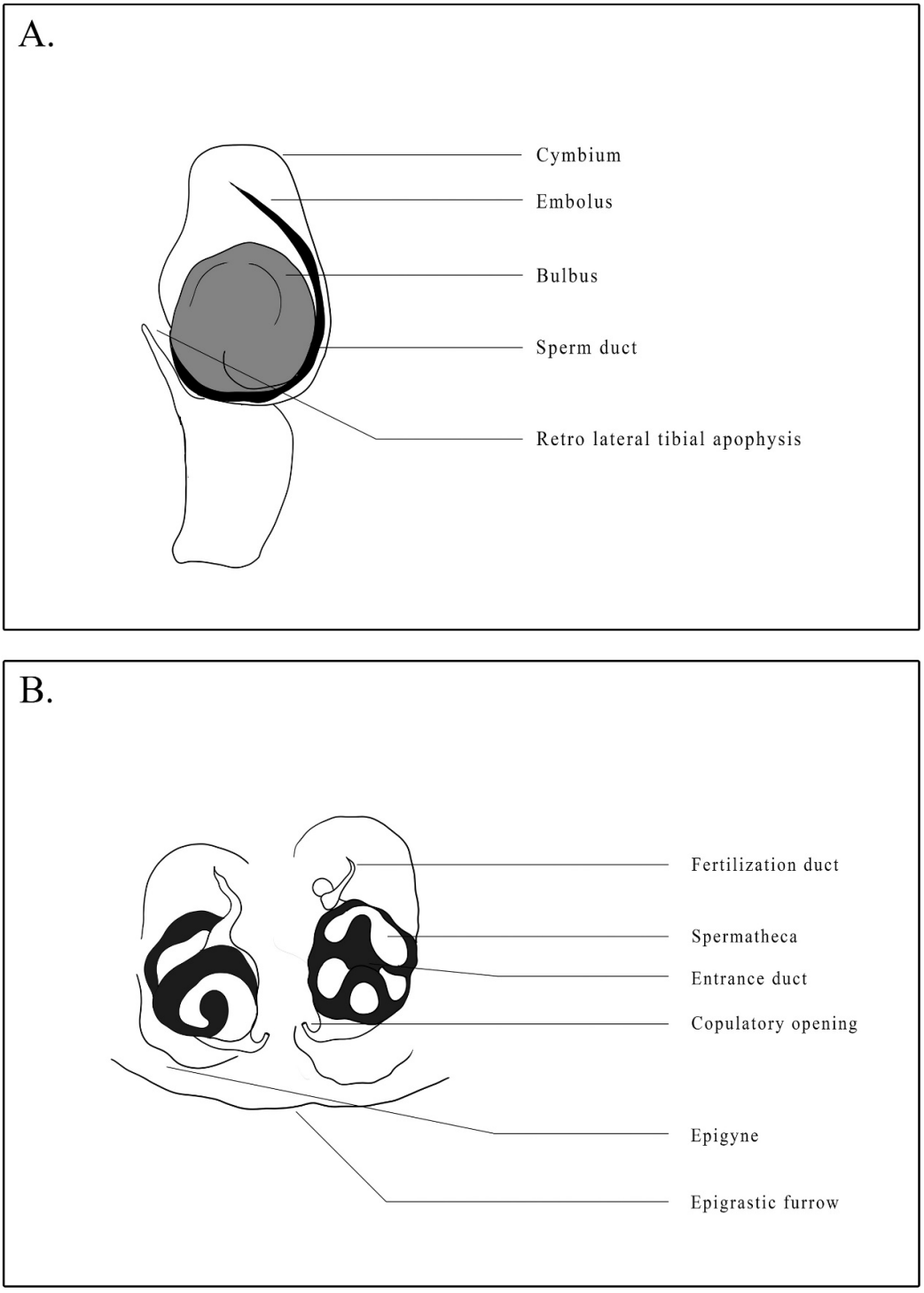
Eye arrangements and leg segments: A. Anterio-dorsal view of cephalothorax showing eye arrangement**; B.** Leg segments of a spider**; C.** Typical arrangement of spines, setae, tarsus and claws on the leg of spider.

PLATE 4



Mouth Parts and spinnerets: A. Ventral view of anterior view of the cephalothorax showing the mouth parts**; B.** Spinneret of a typical cribellate spider.

PLATE 5



Genitalia of Enetelegyne spiders: A. Ventral view of palp**; B.** Ventral view of Epigyne.

Legs: Spider legs typically consist of four pairs, totaling eight legs, each with seven segments: coxa, trochanter, femur, patella, tibia, metatarsus, and tarsus. All legs are equipped with hook-like sclerotized claws, aiding spiders in moving across smooth surfaces or crawling along their webs. Spider legs are incredibly versatile, facilitating various actions such as walking, jumping, climbing, and web spinning. Covered in numerous sensory hairs or setae, spider legs enable the detection of environmental changes like vibrations, air currents, and chemical signals. Additionally, spider legs play critical roles in prey capture and manipulation, mating behaviors, and defense mechanisms.

Abdomen: The spider abdomen is positioned at the rear of the spiders' body, following the cephalothorax. It typically appears larger and more rounded compared to the cephalothorax. This region accommodates numerous internal organs vital for the spider's survival, including the digestive system, reproductive organs, respiratory system, and silk glands known as spinnerets. In certain spider species, the abdomen may feature defensive structures such as spines or hairs, which serve to deter predators. Variations in the size, shape, and coloration of the abdomen are prominent across different spider species. These differences contribute to camouflage, communication, or thermoregulation strategies. Furthermore, specific abdominal markings or patterns is used in species identification.

Genitalia

Palp: Male spiders have specialized genital organs called palpal bulbs in their pedipalps. These bulbs contain structures called emboli, which help transfer sperm during mating. Additionally, the palp also possesses structures such as the tibia, cymbium, and an apophysis. These components contribute to the overall structure and function of the palp, aiding in various activities such as sensing, grasping, and mating behavior.

Epigyne: The epigyne is a sclerotized external genital structure situated on the underside of the abdomen. Within the female's abdomen, there are internal structures called spermathecae, where sperm is stored after mating. Paired ovaries located within the abdomen produce eggs, which are fertilized by sperm stored in the spermathecae.

Following fertilization, female spiders produce silk to construct an egg sac, where they deposit their eggs.

Both the male palp and female epigyne are utilized in the identification of spiders because they are species-specific.

3.4. Web structure

The structure of spider webs differs significantly among various species. Here are some notable types of webs:

Orb web: It is a circular, wheel-shaped web characterized by its radial spokes and concentric spiral design, typically used for catching prey. They vary in shape and size depending on families and genera. Examples: Araneidae, Tetragnathidae.

Sheet web: It is a flat, horizontal web featuring a dense, closely woven surface and consisting of numerous silk lines extended in all directions. Examples: Amaurobiidae.

Tent web: It is a web type built by certain spiders, featuring a tent-like structure with multiple layers of silk. The web is suspended by supporting strands of silk attached to tree branches. These webs are constructed in close proximity to form communal structures that cover extensive areas. Numerous kleptoparasites can be observed on the web. Example: Araneidae.

Net casting web: It is a unique type of web created by Deinopid spiders where they weave a small, rectangular, net-like web which they hold with their front legs, ready to quickly ensnare prey. Example: Deinopidae.

Silk tube: Cylindrical in structure, it incorporates a sheet web with a tubular extension originating from one edge. This tube acts as a protective retreat or shelter where the spider can reside and lay eggs. Example: Agelenidae.

Irregular web: It is a web structure characterized by non-uniform and tangled mass of silk threads. Example: Theridiidae.

Single line snare: It haracterized by a single horizontal strand of silk stretched between anchoring points. Example: Theridiidae.

Tubular silk line burrow: It is a cylindrical structure formed by using silk threads, serving as a tunnel or burrow where the spider resides or seeks refuge. Example: Ctenidae.

3.5. Life history

Mating in spiders involves a range of behaviors and interactions between males and females, which can vary greatly between species but often incorporate visual displays, vibrational signals and chemical cues. Male spiders showcase elaborate courtship rituals to entice females by employing dances, rhythmic movements and gifts like prey or silk. After successful courtship and mating, females may lay eggs, often depositing them in silk egg sacs for protection and may subsequently guard them until hatching. They go through three main stages of life cycle i.e., eggs, spiderlings (juvenile) and adult.

Eggs: Eggs are typically laid in clusters and enveloped in egg sacs for protection, varying in shape, color and size across species. In many species, females guard the egg sacs by constructing a silken retreat over them, while others conceal the sacs in rolled leaves, tree barks or debris. Some spiders even carry their egg sacs until hatching.

Spiderlings: The eggs hatch directly into tiny spiderlings, typically emerging within a few weeks after being laid. These spiderlings then undergo multiple molts as they grow to reach the size of an adult. The young spiders disperse from their retreat to other locations using an efficient dispersal mechanism called ballooning, where they release a long silk dragline that is caught by the wind, carrying them to another area.

Adult: Adult spiders are fully developed individuals that have reached maturity in their life cycle. They typically have fully developed reproductive organs and are capable of mating and producing offspring.

3.6. Mimicry and camouflage

Many spiders employ cryptic coloration, utilizing body patterns to blend into their surroundings. Thomisidae members can change color based on the flowers they inhabit. Araneidae spiders, like *Cyrtophora unicolor* resembling dried leaves and *Caerostris sumatrana* mimicking tree bark, also demonstrate cryptic coloration. Additionally, some spiders mimic other organisms or non-palatable objects for camouflage or protection; for instance, *Phrynarachne decipiens* mimics bird droppings, *Paraplectana mamoniae* resembles ladybird beetles and certain *Myrmaplata* species mimic ants.

3.7. Adaptations for fishing spiders (Pisauridae): The Role of Hairs and Setae

The body of the Pisaurids, like other Arachnids, are covered with numerous hairs which are highly hydrophobic. This presence of hydrophobic hairs creates a physical lungs for submerged spiders by trapping air, which allows fishing spiders such as Nilus albocinc to respire while submerged underwater.

The setae present on the legs of the fishing spider Hygropoda higenaga helps it in sensing the vibrations caused on water by potential prey or predator and thus aiding in prey capture or hide in its retreat.

3.8. Natural enemies of spiders

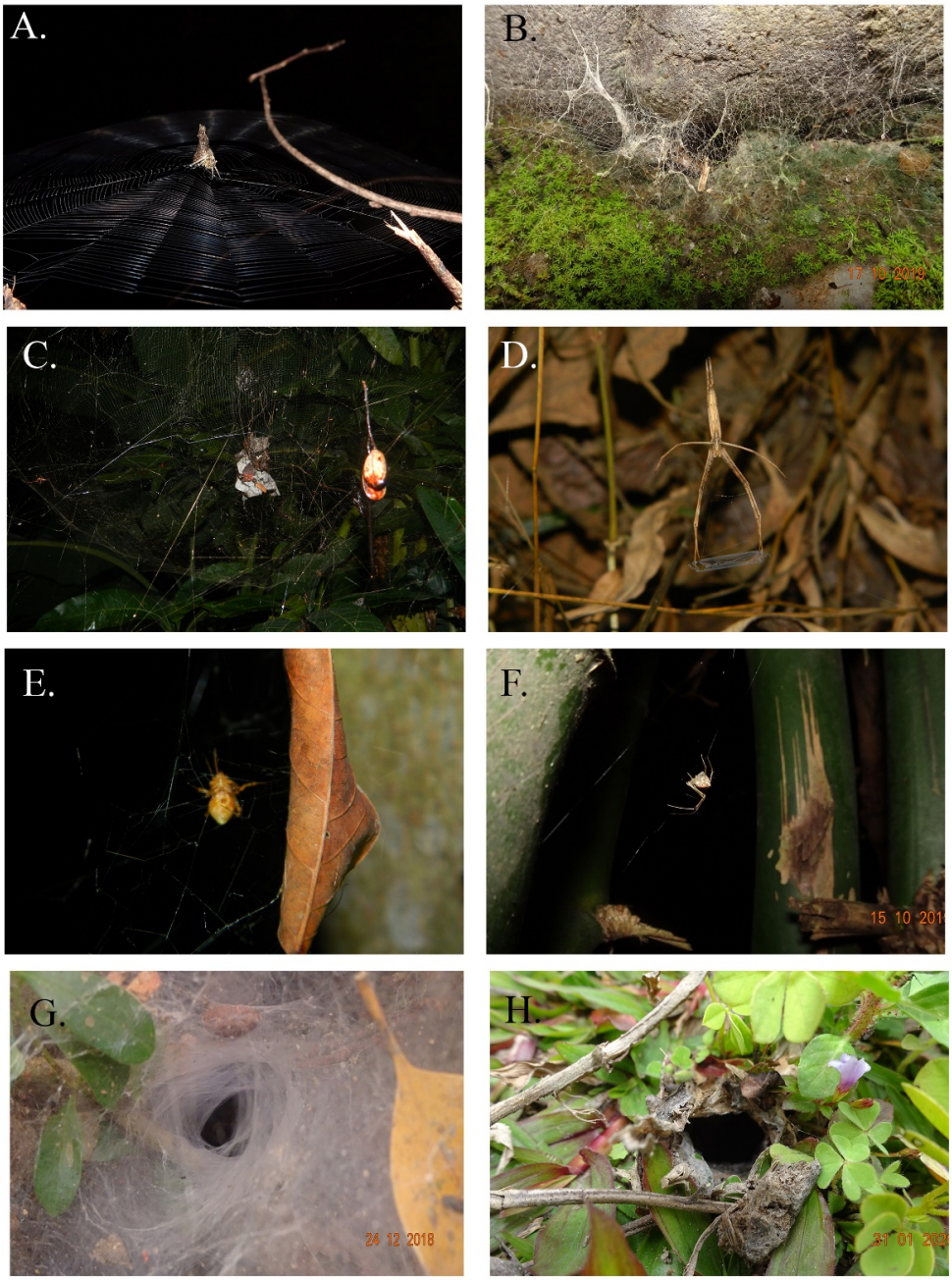
Spiders have a variety of predators, including other spiders, wasps, birds, reptiles, amphibians, mammals and even some fish. Spiders are also susceptible to Cordyceps, a genus of fungi known for its parasitic infection towards insects and spiders where it manipulates the host body, kills it and new fruiting body emerges from the hosts' body.

They are also parasitized by nematode, where the abdomen of spiders becomes bulgy and will emrge out for next host once they had sucked all the nutrients from the spider.

3.9. Diet

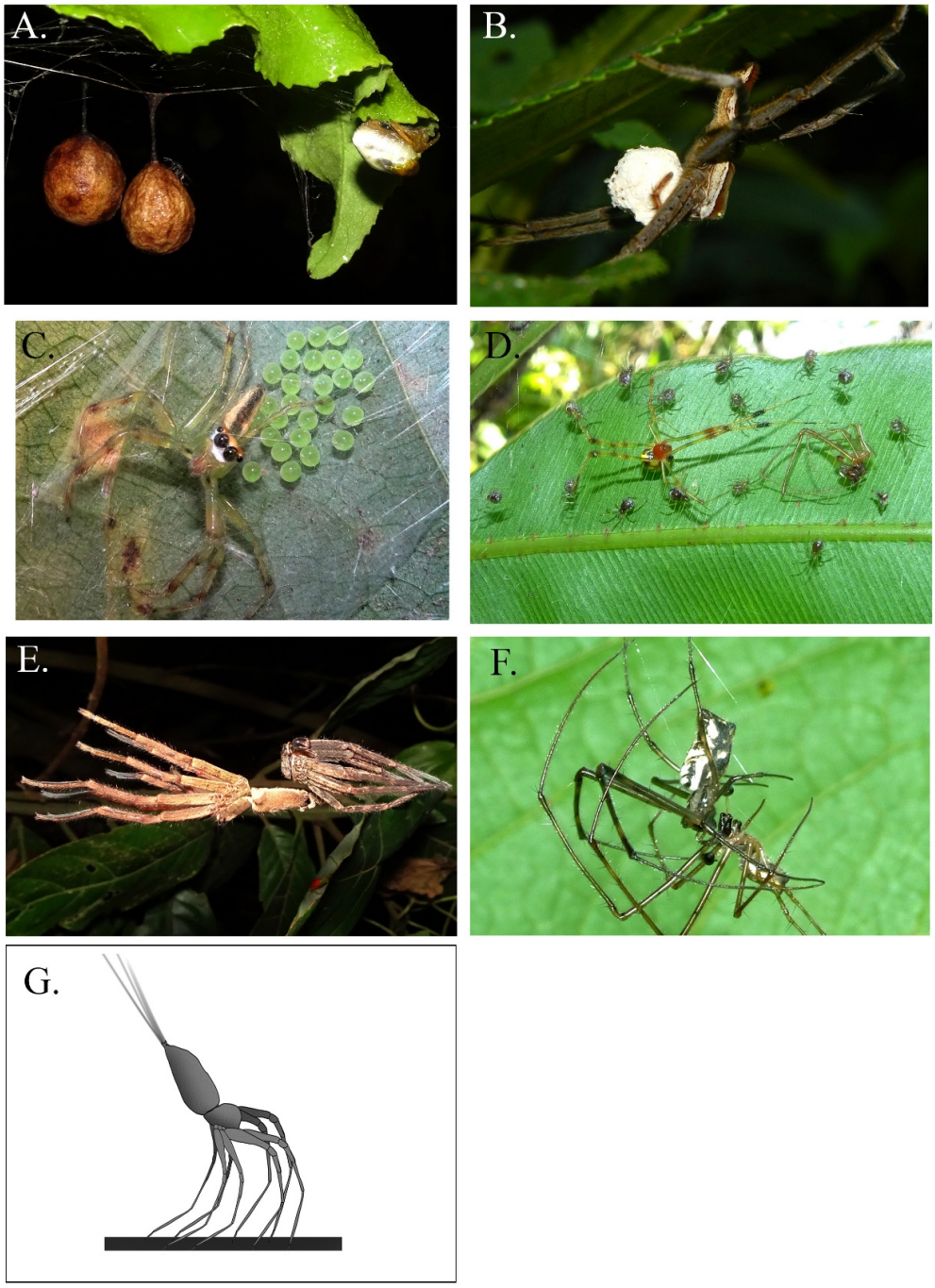
Spiders are carnivorous hunters, mainly feeding on small arthropods like insects, flies, mosquitoes, and beetles, as well as sometimes preying on other spiders. Larger spider species may even target small vertebrates such as frogs, lizards, or mice. Once prey is captured, spiders typically immobilize it with venom injected through their fangs before consuming it. The venom then digests the inerts of the prey which is then sucked up with the help of chelicerae.

PLATE 6



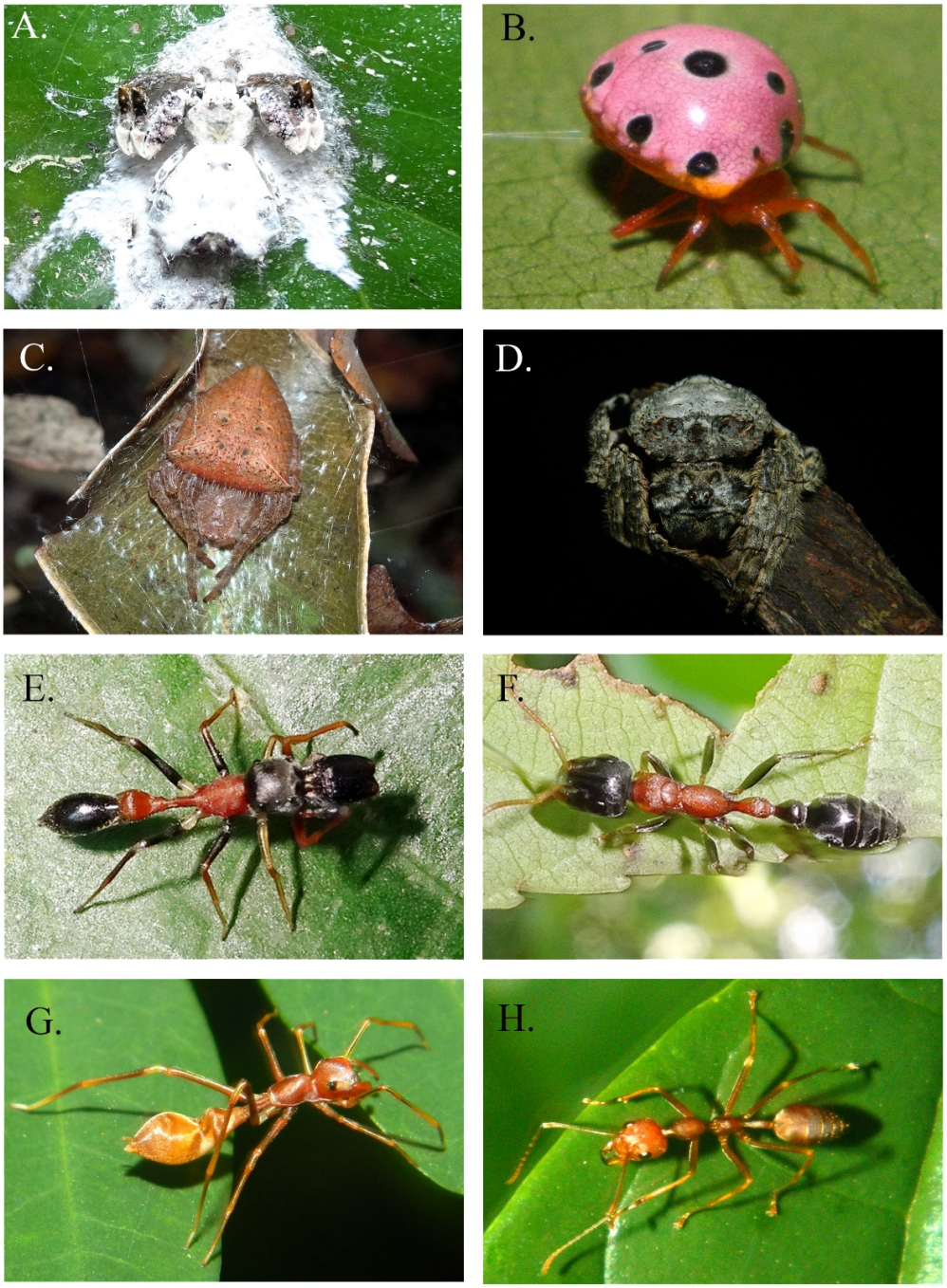
Web types of spiders from Jharbari Forest Range: A. Orb web: *Poltys columnaris* (Araneidae); **B.** Sheet web: *Himalmartensus ausobskyi* (Amaurobiidae); **C.** Tent web: *Cyrtophora unicolor* (Araneidae); **D.** Net-casting web: *Asianopis goalparaensis* (Deinopidae); **E.** Cob web: *Parasteatoda celsabdomina* (Theridiidae); **F.** Single silk dragline: *Thwaitesia margaritifera* (Theridiidae); **G.** Silk-tube: *Dendolycosa songi* (Pisauridae); **H.** Tubular silk-lined burrow: *Gravelyia boro* (Nemesiidae).

PLATE 7



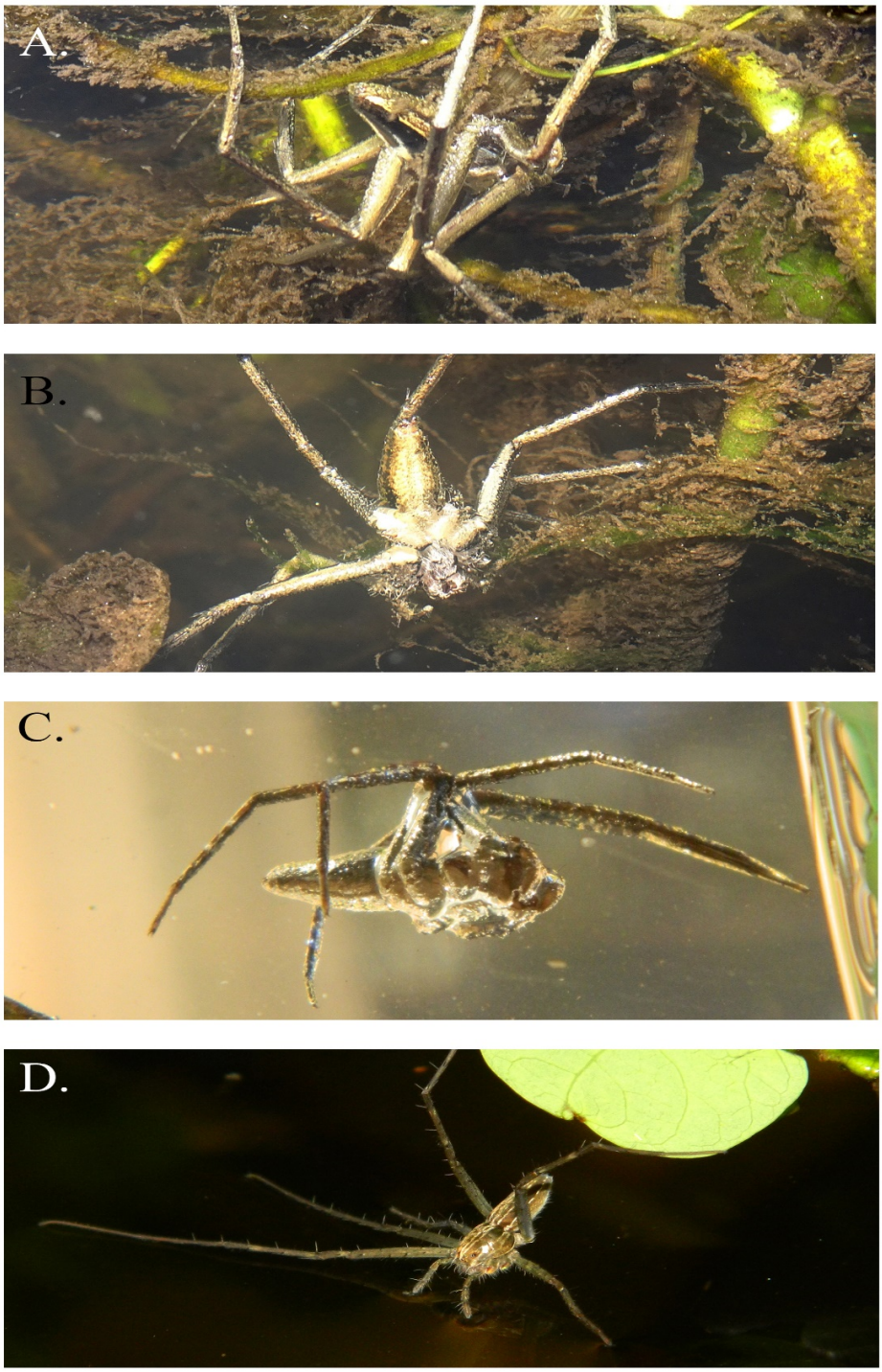
Life history: A. Female of *Cyrtarachne inequalis* with egg sac; **B.** Female of *Nilus albocicntus* with egg sac; **C.** Female of *Epeus* spp. guarding the eggs; **D.** Female of *Chrysso urbasae* guarding the spiderlings; **E.** Moulting of skin; **F.** Male and female of *Tylorida ventralis* showing courtship; **G.** Diagrammatic representation of ballooning of juvenile spiders.

PLATE 8



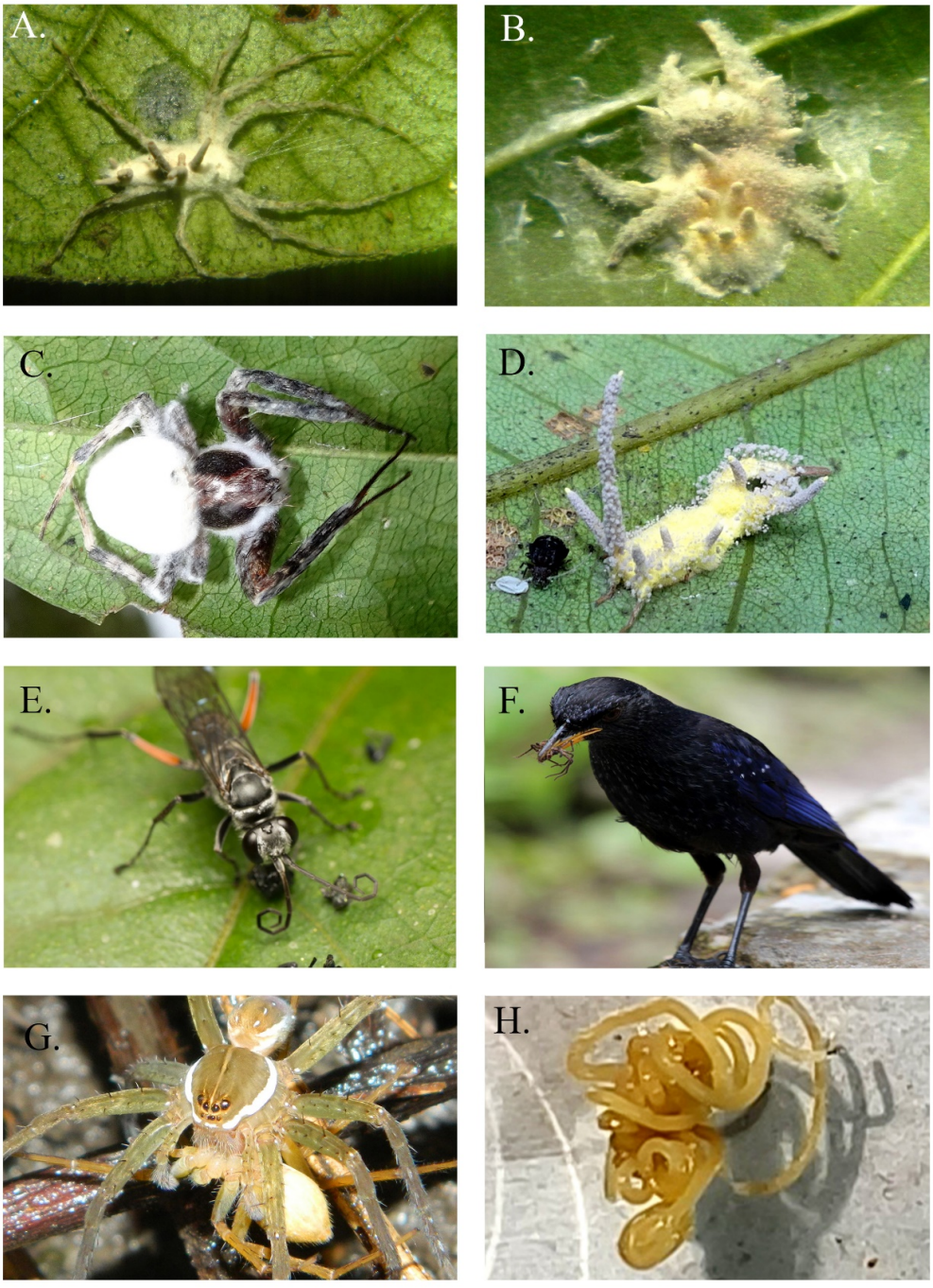
Mimicry: A. *Phrynarachne decipiens* mimicking bird dung; **B.** *Paraplectana mamoniae* mimicking lady bird beetles; **C.** *Cyrtophora unicolor* mimicking dry leaf; **D.** *Caerostris sumatrana* mimicking tree bark; **E.** *Myrmarachne melanocephala* mimicking *Tetraponera rufonigra*; **F.** *T. rufonigra*; **G.** *Myrmaplata plataleoides* mimicking *Oecophylla smaragdina*; **H.** *O. smaragdina.*

PLATE 9



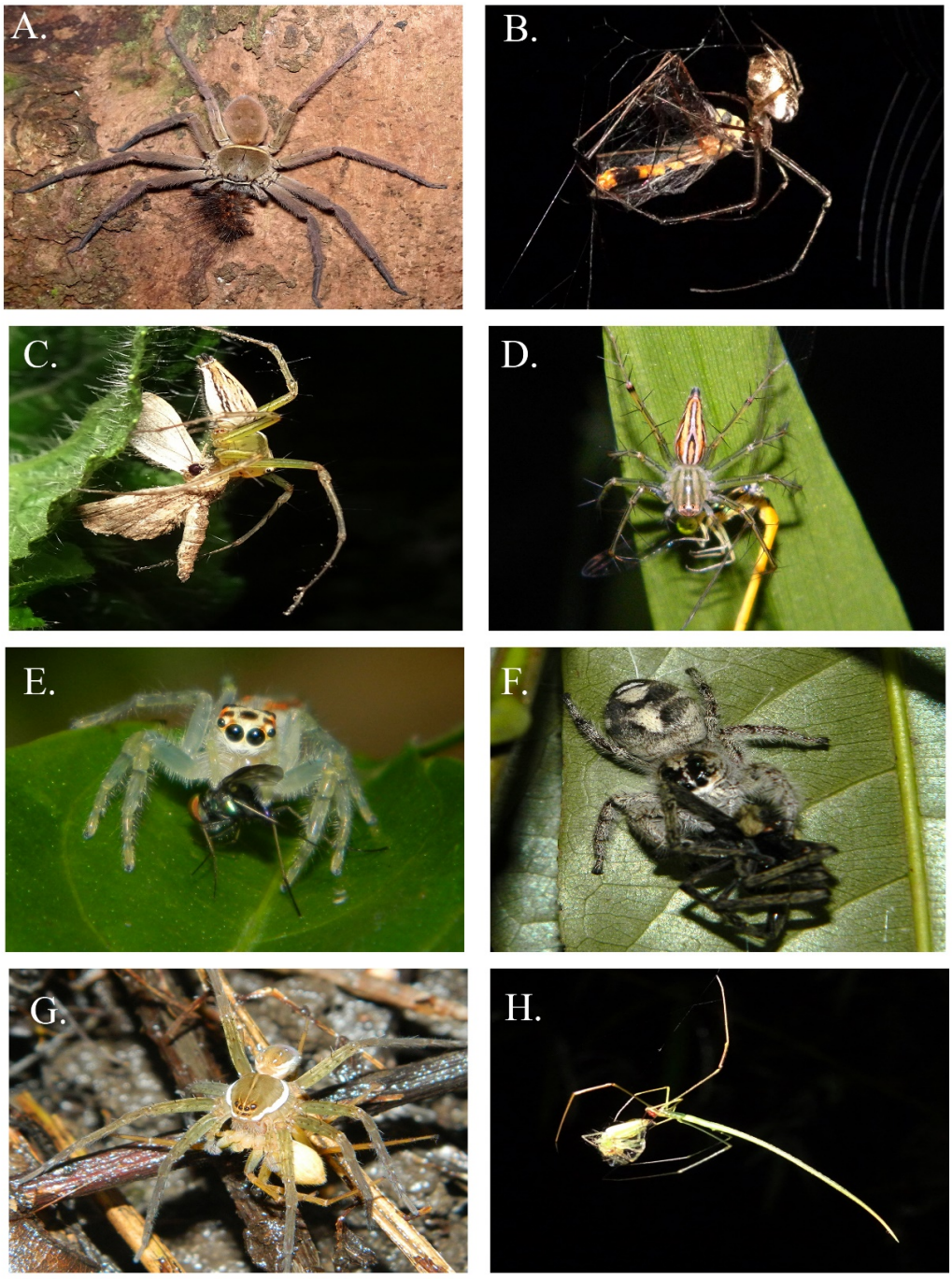
Role of hairs and setae: A and **B.** *Nilus albocicntus* observed retreating under water for about 5-6 minutes; **C.** Hairs trapped the air like a bubble; **D.** *Hygropoda higenaga* sensing for vibrations on water.

PLATE 10



Natural enemies: A, B, C and **D:** Araneopathogenic fungi, *Cordycep* spp., parasitizing the host spider body to release its pores; **E.** Spider wasps (Pompilidae) feeding on spider; **F.** Blue whistling thrush feasting on spider; **G.** Spider predating on other spiders; **H.** Nematode of unknown species remove from spider's abdomen.

PLATE 11



Diet of spiders: A. *Heteropoda venatoria* feeding on a caterpillar; **B.** *Tylorida ventralis* feeding on cranefly; **C.** *Oxyopes* spp. feeding on a moth; D. *Oxyopes sitae* feeding on damselfly; E. Salticid preying on fly; **F.** *Hyllus diardi* predating on spider; **G.** Pisaurid preying on spider; **H.** *Ariamnes cylindrogaster* wrapping up a spider with silk.