CHAPTER-II

2. MATERIALS AND METHODS

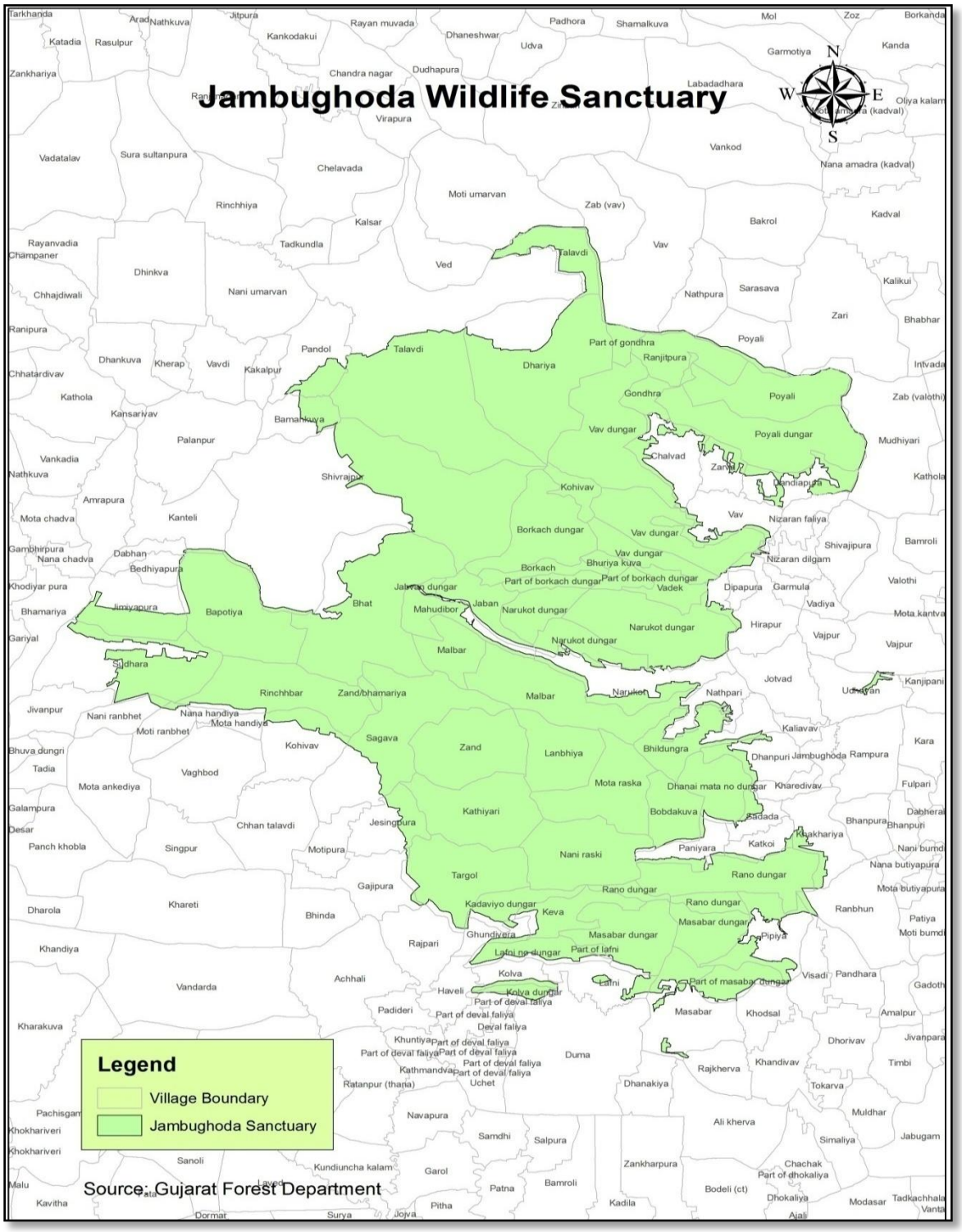
2.1 Study Area

2.1.1 Geographical Location of Study Site

The Southern Dry Deciduous Forests of Gujarat is depected by Jambughoda Wildlife Sanctuary which is located between latitudes 22º20'-20º33' N and longitudes 73º35'-73º45' E in the Panchmahal and Vadodara districts of Gujarat State, India (Figure 13). The Sanctuary area extends over 130.38 sq. km. and is covered by three forest ranges, *viz.*, Halol, Jambughoda and Vadodara. Altitudes ranging from 230 to 354 m above mean sea level. The Jambughoda Wildlife Sanctuary was notified as a protected area in May 1990 (Gazette Notification). Also the sanctuary finds a place in the 1993 United Nations List of National Parks and Protected areas prepared by the World Conservation Monitoring Centre (WCMC) and the IUCN Commission on National Parks and Protected Areas (CNPPA, since 1994 renamed as World Commission on Protected Areas [WCPA]). The Sanctuary is listed in Category IV as per Guidelines for Protected Area Management Categories (IUCN, 1994a; IUCN, 1994b).

The Sanctuary is well connected by road there are no railway lines which crossways the sanctuary. It is situated 11 kms north of Bodeli, 30 kms east of Halol and approximately 72 kms north-east of Vadodara.

Figure 13. Map of Jambughoda Wildlife Sanctury, Panchmahal District, Gujarat



According to the revised classification of the forest types of India by (Champion & Seth, 1968), the forests of Jambughoda Wildlife Sanctuary is 5A-type i.e. Southern Tropical Dry Deciduous type forest, which is further divided into two sub-types namely, 5A/ CLB - Dry teak forests and 5A/C3 - Southern dry mixed deciduous forests.

(I) 5 A/CLB- (TYPE-5A SOUTHERN TROPICAL DRY DECIDUOUS FORESTS SUB-TYPE – CLB – DRY TEAK FORESTS)

These forests are distributed in various patches at Zand, Targol, Laphni, Kada and Ranjitpura i.e., North-East region. It consists of mixed dry deciduous forest with majority of the portion covered with teak. The entire vegetation comprises of three main strata namely over-wood, under-wood and ground cover. The over-wood strata consists of trees *viz., Tectona grandis, Anogeissus latifolia, Diospyros melanoxylon, Hardwickia binata, Dalbergia latifolia, Cassia fistula, Butea monosperma, Adina cordifolia, Mitragyna parvifolia, Bridelia retusa, Aegle marmelos, Lagerstroemia parviflora, Wrightia tinctoria,* etc. whereas under-wood strata includes *Flacourtia indica, Helicteres isora, Xeromphis spinosa, Indigofera oblongifolia, Holarrhena antidysenterica,* etc. In ground cover mostly herbs like *Cassia tora, Xanthium strumarium* and *Euphorbia sp.* and grasses like *Apluda mutica, Aristida histricula, Iseilema laxum, Heteropogon contortus, Themeda quadrivalvis, Dichanthium annulatum,* etc. are included.

(II) 5A/C 3 –SOUTHERN DRY MIXED DECIDUOUS FORESTS

This particular sub-type of forest is observed at Kada, Malabar, Mahudibor, Dolimar and Targol areas, which lies in the South-East region of the sanctuary. These forests are mainly found on dry and undulating terrain. The dominant tree species found in these forests is *Anogeissus latifolia* and occurrence of teak is sporadic. These forests are prone to forest fires during summer seasons due to its dry nature. This sub-type comprises of two main strata namely over-wood and under-wood. The over-wood vegetation mainly consists of *Anogeissus latifolia, Diospyros melanoxylon, Hardwickia binata, Boswellia serrata, Soymida febrifuga, Acacia catechu, Terminalia Tomentosa, Mitragyna parvifolia, Buchanania lanzan, Lagerstroemia parviflora, Phyllanthus emblica, Cassia fistula, Aegle marmelos, Butea monosperma, Albizia lebbeck,* and *Sterculia urens*

whereas under-wood vegetation consists of *Wrightia tinctoria*, *Nyctanthes arbor-tristis*, *Ziziphus xylopyrus*, *Helicteres isora*, *Justicia adhatoda*, *Xeromphis spinosa*, *Flacourtia indica*, *Holarrhena antidysenterica*, *Careya arborea* etc.

On the basis of percentage covered by teak in the forest area, these sub-types were further sub divided into three sub-sub-types in the working plan 1996, for the forests of Panchmahal district. According to this working plan the forests, where teak is dominant species and is estimated to be more than 50% of the total vegetation will be considered as Teak type of forest; whereas in the forests where the teak is fairly present but its population is less than 50% of the total vegetation then it will be considered as mixed type of forest and where the occurrence of teak is insignificant or it is devoid of teak, will be considered as mixed miscellaneous type of forest.

2.1.2 Sampling Sites

In order to carry out spider survey in Jambughoda Wildlife Sanctuary the entire forest area was divided into four sub-sites based on vegetation type and habitat structure of the area. GPS locations of all the sub-sites were taken using a hand unit of Global Positioning System (GPS: Garmin Oregon 550). The details of these four sub-sites are as follows:

Natural Forest: About 50% of the total flora present in the sanctuary composed of Teak trees (*Tectona grandis*) (Figure 14). Teak is one of the economically important trees for the triblals inhabiting the sanctuary area as they get timber from these trees. In association with teak there are other trees which are found to be abundant in the near vicinity namely, Mahuda (*Madhuca longifolia*), Sadad (*Terminalia crenulata*), Shisham (*Dalbergia latifolia*), kher (*Acacia chundra*) and Timru (*Diospyros melanoxylon*) which are also been used by the locals for various purposes.

Riparian Habitat: There are no perennial sources of water in the sanctuary but canals from Sukhi Dam connect Kada-Targol-Dev Dam, which provides water throughout the year. Other than these man-made canals there are few seasonal springs present at Jhand, Jabban and Ranjitnagar (Figure 19). The major vegetation present near the forest streams

and kotars are *Pongamia pinnata*, *Syzygium cumini*, *Alangium salvifolium*, *Miliusa tomentosa*, *Albizia lebbeck* and *ficus sp.*

Agricultural Fields: At various places within the sanctuary area, the tribals have removed patches of natural vegetation for the purpose of cultivation. The major agricultural crops cultivated in the area are Castor, Maize, Cotton (Figure 16), Paddy (Figure 17) and Pigeonpea (Figure 18) (Table 1) with few varieties of pulses. The agricultural harvest is stored in containers for entire year and used for household purpose only. The quality and quantity of the agricultural harvest solely depends on the amount of rains as there are very less man-made sources of irrigation in the sanctuary area.

Table 1. Major agricultural crops of Jambughoda Wildlife Sanctuary, Gujarat

Name of Crop	**Month of Sowing**	**Month of Harvesting**
Castor	July – October	October – December
Maize	June – November	September – February
Cotton	June	December – March
Paddy	June – July	September – October
Pigeonpea	June	March

Forest Plantations: Due to Illicit cutting of natural vegetation, over grazing, forest fires and clearing of forest for cultivation, the natural species of the trees are getting vanished. Hence in order to replenish the empty patches of forest, plantations have been done at various places under different schemes. These plantations are mainly of Eucalyptus (Figure 15), Khair, Bamboo and Babul. At places where there is vast reduction of natural teak, the plantation of teak is also been done to maintain the natural vegetation of the sanctuary area.

Figure 14. Natural Forest

Figure 15. Forest Plantation

Figure 16. Cotton Field

Figure 17. Paddy Field

Figure 18. Pigeonpea Field

Figure 19. Riparian Habitat

2.2 Sampling Methods

All four sub-sites were visited monthly and surveyed for faunal diversity of spiders and their habitat characteristics. The field visits were scheduled diurnal and nocturnal to get maximum diversity and to minimize the risk of skipping nocturnal spider diversity. The sampling was carried out for more than three years from July, 2011 till April, 2015.

To explore the spider diversity of Jambughoda WLS, applying single sampling technique is not efficient for examining the entire group of spiders hence there is a need to use a combination of sampling methods (Prasifka et al., 2007). A combination of seven sampling methods namely, Pitfall sampling, Cryptic search, Sweep netting, Ground Hand Collection, Arial Hand Collection, Vegetation Beating and Leaf Litter Sampling were applied. All the sampling methods were performed twice in each season except for pitfall traps which were not laid in months of monsoon season due to water flooding issues. The pitfall traps were kept under observation for a week in the field after which the samples were collected. The other semi-quantitative sampling methods were applied for one hour of active, continuous collection of samples (i.e. including time required to transfer the specimens to a vial, but excluding interruptions) as described by (Coddington et al., 1991) with modifications (Sørensen et al., 2002; Scharff et al., 2003; Cardoso et al., 2008). For all the four sub-sites sampling method applied was same.

The detailed description of all the seven sampling methods is as given below:

Pitfall Sampling: Pitfall traps are efficient for collecting surface active, ground-dwelling and leaf litter inhabiting spiders (Abraham, 1983; Coddington et al., 1991; Clark & Samways, 1997; Churchill & Arthur, 1999; Lange et al., 2011). The other advantage with this sampling is it acts continuously and allows both diurnal and nocturnal species to be caught in the samples (Gil, 2009). The output of pitfall capture depends on the design of traps, arrangement of traps and selection of site where these traps are placed (Winder et al., 2001).

The pitfall traps used in the study was a plastic bowl with a diameter of 15 cm and depth of 6.5 cm (Figure 20). These pitfall traps were arranged in the 1 m^2 area (one plot) at a distance of 5 m from each other (Samu & Lovei, 1995; Hovemeyer & Stippich, 2000).

Such 50 plots are marked in each sub-sites. Trap locations are usually best determined by a stratified-random type method depending upon the environmental gradient (Uetz & Unzicker, 1976). Pitfall traps were placed by clearing the leaf litter, within the soil by digging the soil and the upper lip of the bowl was kept in level with the ground surface (Sørensen et al., 2002; Buddle & Hammond, 2003). These bowls were half-filled with a 4:1 mixture of water and ethylene glycol, with a little detergent (Curtis, 1980; Topping & Sunderland, 1992; Green, 1999; Prasifka et al., 2007). After placing the pitfall traps the leaf litter was placed back around the bowl so as to maintain the uniformity of the area. The content of pitfall tarps were collected after every 24 hours and all the traps were replenished with new solution or added to the older ones (Nobre et al., 2000; Kapoor, 2006). This process was continued for two weeks and was carried out twice in one season of the year (Bergthaler & Rėlys, 2000; Cardoso et al., 2008).

Cryptic Search: This particular sampling method was applied for those spiders which prefers to inhabit in cryptic habitats namely, under rotten logs of woods, under tree bark, in holes of trees and rocks, under rocks, bark crevices etc. For this extensive visual search was done for an hour in all the cryptic habitats in each sub-sites and all the spiders were collected by handpick method (Fujii, 1998; Sørensen et al., 2002; Scharff et al., 2003).

Sweep Netting: Spiders inhabiting in grasses, small shrubs, low herbaceous and shrubby vegetation were sampled with sweep net (Coddington et al., 1991; Scharff et al., 2003). For sweep netting, a round sweep net made of nylon mesh (1.5 mm); having a diameter of 28 cm (aluminum ring) and 1 m long handle was used (Churchill & Arthur, 1999; Cardoso et al., 2008). In order to reduce damage to the specimens, the spiders were removed from net after every five sweeps and are placed in vial containing 70% alcohol (Sørensen et al., 2002). Each sub-site was sampled by 50 sweeps separated by 2.5 meters intervals and the sampling time was kept as one hour for each sub-site (Abraham, 1983; Clark & Samways, 1997).

Ground Hand Collection: This sampling method is used for collection of spiders inhabiting on ground, for this we explored places like leaf litter, forest floor debris, plant surface, under logs of wood and stones that were below knee level (Cardoso et al., 2008; Scharff et al., 2003). Ground hand collection is also known as “Looking down” method

of sampling (Coddington et al., 1991; Coddington et al., 1996). It is also one of the types of hand collection method done with the help of paintbrush moistened with alcohol (Gil, 2009). All the spiders visible on the ground i.e. below knee level were collected in the collecting vials (Sørensen et al., 2002). This method was employed for one hour in each sub-site. Ground hand collection method is also one of the important sampling methods as by applying this method we were able to collect data on spider species natural history (Figure 21).

Arial Hand Collecting: The Arial hand collection method was applied for collection of web-building spiders and free-living foliage dwellers. In this method the collector has to walk along the area while searching on vegetation and collect the spiders visible from knee level to as high as a person can reach (Sørensen et al., 2002; Scharff et al., 2003). It is similar to the sampling method *viz.*, "Looking up" described by (Coddington et al., 1991; Coddington et al., 1996). It is one of the sub-types of hand collection method done with the help of forceps, brush and vial (Cardoso et al., 2008). A total of 50 samples were taken from each sub-site. This method is also valuable in collecting natural information on spider's microhabitat, its biological aspects and other activities (Gil, 2009).

Vegetation Beating: This method was applied for spiders which inhabit branches of trees, bushes, shrubs, small trees and other lower vegetation (Abraham, 1983; Cardoso et al., 2008; Sørensen et al., 2002). A white sheet of cloth measuring 1 m square was spread horizontally below the vegetation and spiders were dislodged by beating shrub with a stout wooden stick. The beating with stick was done with a jerk and continued until no more spiders fell down on the cloth (Coddington et al., 1991; Coddington et al., 1996; Costello & Daane, 1997; Kapoor, 2006; Gil, 2009; Nobre et al., 2000; Scharff et al., 2003). This method was employed for 30 minutes at each sample plot (vegetation). All the spiders on the cloth were collected and transferred to the sampling vials filled with 70% alcohol. A total of 50 shrubs were sampled by vegetation beating method from each sub-site.

Litter Sampling: Litter sampling was done for the collection of spiders inhabiting on the forest floor, hiding under the leaves and in the upper humus layer of the soil. For this leaf litter was gathered by hand from a 2 m^2 area and was placed in large garbage plastic bags.

These bags were then sealed from the mouth so that the leaf litter and spiders stays in the bag till it was transported to lab. Sorting of the spiders from the leaf litter was done by placing the litter on a white sheet of paper (Coddington et al., 1991; Coddington et al., 1996). Headlights were used to spot spiders form the litter spread on white sheet of paper. These spiders were handled with brush dipped in alcohol and the specimens were directly placed in 70% alcohol. Five litter samples were collected from each plot of 10 m X 10 m area in all the sub-sites except in the riparian habitat.

Methodology for observing spider web structure and other ecological parameters:

Production of silk is one of the unique characteristics of spiders. It's a fibrous protein produced by silk gland through spinnerets which are situated in the posterior part of the abdomen. Different spider species use different variety of silk to sustain in the environment and to perform various functions. Majority of the orb-weaving spiders in the Jambughoda WLS starts constructing their webs during dusk and consumes them in the following morning. They are basically nocturnal and relay on prey entangled in their web during night hours. In order to observe their web pattern whenever the spider web was spotted, observations were made on spider orientation (upward facing, sideways or downwards facing) and web symmetry (symmetrical or asymmetrical). Latter adult spiders and prey exuviae were collected by hand pick method from each web before taking the morphometry of web. For prey preference studies, the insects which were discarded by spider were not considered, only those insects were included which were fed by spiders during the observation time. Regarding web morphometry parameters, standard Stanley non-magnetic measuring tape was used; all measurements for web morphometry studies were taken in centimeters. Web characteristics like web diameter, web height from the ground surface, capture area, mesh size, number of radii, number of spirals and time required to complete a web were also noted. For height of web, measurements were taken from the ground level to the centre of hub. Diameter of the webs were taken four times at 90 degrees to each other; for mesh size the mean distance between two consecutive sticky spirals were taken into consideration. For comparing different observations Pearson's Correlation was used and the graphs were prepared using Microsoft Excel.

Other than the web morphometry studies regarding orb-weaving spiders and their prey preferences, the ecological parameters for non-weaver spiders were also observed on field which includes habitat preferences, prey preferences, mating, cocoon formation and parental care. While applying the above described sampling methods it was taken care to observe the natural habitat from where the spider is collected, their activities like feeding, resting in retreat, wandering, taking care of cocoon, mating, egg laying, etc. were also noted before it was collected. Along with this predators of spiders were also enlisted during the study in order to see the ecological role of spiders in an ecosystem.

2.3 Specimen Preservation and Deposition

All the collected specimens were preserved in 70-80% ethanol (ethyl alcohol) and stored separately in clear tarsons polypropylene (PP) sampling containers (50 ml) (Figure 22) for further identification and measurements. Each identified specimen was accompanied with a label that includes the specific identity number, family, scientific name, sex, date of collection, exact name of the site of collection, broader area name and collector's name. All permanent labels were written with true non-fading carbon ink (India ink) and allowed to dry properly. These labels were then placed in their respective specimen vials filled with alcohol (Gregg, 1968). All the described specimens were deposited at the Public Museum of Wildlife Information Liaison Development Society, Coimbatore, Tamil Nadu, India, tagged with unque tag number for deposited specimens e.g WILD-14-ARA-1277. Rest all the specimens were deposited in the Zoology Museum of Department of Zoology, Faculty of Science, The M.S. University of Baroda, Vadodara, Gujarat, and is in the charge of guiding teacher (Dolly Kumar) later on these specimens will be deposited in a National Repository.

2.4 Taxonomic Identification

All the preserved specimens of spiders were identified under a stereomicroscope (WILD^{TM} and Leica MPS) with magnification from 20X to 75X (Figure 23). Taxonomic identification of spiders till species level can be confirmed only by observing the internal structures of epigynum in case of female spiders and padipalp structures in case of male spiders. For all the mature females in the collection, epigynum were dissected and

cleaned in concentrated lactic acid for 15–20 minutes and further compared the epigymun structures with the available literature, after which the these dissected epigyne's were stored in 1 inch long glass microvials plugged with cotton and placed in the respective spider species vial. Similarly in case of mature male spiders the padipalp of left hand side were plucked and its structures were compared with the available literature. Spiders were identified upto species level using standard monographs (Almeida-Silva et al., 2010; Baehr & Baehr, 1993; Gajbe, 2007; Gajbe, 2008; Huber, 2011; Jocqué, 1991; Jocqué & Dippenaar-Schoeman, 2006; Javed et al., 2010; Kraus & Kraus, 1989; Levi & Levi, 1962) (Majumder & Tikader, 1991; Patel, 2003; Patel & Reddy, 1989; Reddy & Patel, 1994; Sethi & Tikader, 1988; Srinivasulu et al., 2013; Platnick et al., 2011; Pocock, 1900; Tikader, 1982) and various other relevant literature. Immature spiders were classified up to the genus or family level. For prey preference studies, all the collected prey exuviae were identified upto order level using standard monographs (Richards & Davies, 1977 ; Borror et al., 1989; Ananthkrishnan & David, 2004). All measurements were done with a calibrated ocular micrometer and Mitutoyo™ Dial Caliper and are in mm. Identification of all the collected specimens till species level was done by me and further its confirmation was done by well known arachnologist Dr. Manju Siliwal, Research Associate, Wildlife Information Liaison Development Society, Coimbatore, Tamil Nadu, India.

2.5 Photography

The photographs were clicked using different cameras in accordance with the requirements of the subtle objectives of this research. Mostly spiders were photographed in live condition in order to study their feeding behavior and other ecology related behaviors. On field photography of spiders was preferred wherever possible, and these on field photographs were taken by SONY DSC-HX400V; CANON EOS 550D and SONY DSC-HX10V. In case of preserved specimens photography was done by SONY DSC-WX50 camera, which was attached to stereomicroscope. All illustrations were prepared with the help of camera lucida attached to a CETII™ stereomicroscope. Scanning Electron Microscope (SEM) images for epigynum, padipalp and other micro-structures of

spider's body were taken through SEM-Zeiss EVO-40EP at the Wadia Institute of Himalayan Geology, Dehradun.

2.6 Abbreviations

ALE–anterior lateral eyes; AME–anterior median eyes; BS–base of spermatheca; C–cymbium; CD–copulatory duct; DA–distal apophysis; ER–epigynal rim; F–female; GP–giant pore; HS–head of spermatheca; J–Juvenile; JWLS–Jambughoda Wildlife Sanctuary; M–male; MA–median apophysis; MF–median field; PLE–posterior lateral eyes; PLT–prolateral lobe of tibialapophysis; PME–posterior median eyes; PP–primary pore; RLT–retrolateral lobe of tibialapophysis; S–spermathecae; SEM–Sacnning Electron Microscope; T–tegulum; WILD–Wildlife Information Liaison Development Society; WLS–Wildlife Sanctuary. Abbreviations used for spine counts are: d–dorsal; fe–femur; mt–metatarsus; p–prolateral; pa–patella; r–retrolateral; ti–tibia; v–ventral.

Figure 20. Pitfall Trap

Figure 21. Ground Hand Collection

Figure 22. Preservation of Spider Samples

Figure 23. Steriomicroscope