VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 1344-1354 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Morphology of Male Pedipalp in Some Spider Species of Western Ghats in Goa, India

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Received: 25th October, 2023; **Accepted:** 20th December, 2023; **Published online:** 25th December, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.151

Abstract: Western Ghats, an Indian biodiversity hotspot is a treasure trove of varied life forms, among which spiders despite their rich diversity have received less attention. Their cryptic behavior, sexual dimorphism, and unavailability of taxonomic keys for juveniles pose a major challenge to their documentation. Conventional taxonomy is limited by morphological similarities between some species, while molecular taxonomy is a laboratory-intensive and prohibitively expensive approach. Adult spiders show a clear sexual dimorphism, with males being smaller than females and/or more colourful in some families. However, one consistent distinguishing feature between the sexes is the shape of their paired pedipalps, which are situated anterior to the first pair of walking legs and lateral to the chelicerae. There is also an established functional difference in these structures; which in females aid in probing substrate and prey, while additionally serve as intromittent structures in males. The construct of the male pedipalp is species-specific and these morphological variations can offer a valuable clue in taxonomic studies. The present investigation reports structural variations in pedipalpal morphology of seven spider species occurring in Western Ghats of Goa; belonging to five families, namely Sparassidae, Theraphosidae, Nephilidae, Salticidae and Hersiliidae. This work if scaled up, can help establish a 'referral database' of species-specific palpal morphology that can aid in spider taxonomy, as also help understand selection pressures and trajectory of evolution in this group.

Keywords: Spiders, Morphology, Male Pedipalps, Taxonomy, Western Ghats, Goa

Citation: Borkar Manoj Ramakant and Fernandes Enida Mary: Morphology of male pedipalp in some spider species of Western Ghats in Goa, India. Intern. J. Zool. Invest. 9(2): 1344-1354, 2023.

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Introduction

The most comprehensive listing of Indian spiders so far is 2344 species described under 596 genera under 65 families (Singh and Singh 2021, 2022; Singh *et al*., 2023). As for the Western Ghats state of Goa, first systematic aranaeological surveys are attributed to Tikader and Bal (1981), Sethi and

Tikader (1988) and Majumder and Tikader (1991). However, the first systematic inventory of spiders from Goa was published by Bastawade and Borkar (2008). Recently, Pandit and Pai (2017), Halarnkar and Pai (2018), Borkar and Seth (2020) and Pandit and Dharwadkar (2020) have expanded the species list further. Notwithstanding these sporadic efforts, spider faunal diversity through the Western Ghats remains to be fully assessed and documented.

Animal systematics has conventionally laid emphasis on gross variations in morphology and morphometry, besides behavior; while advances in Molecular Biology with protocols such as DNA Fingerprinting and Molecular Bar-codes address overlaps between two distinct species despite their striking morphological similarities. However, these advanced techniques impose limitations of time and sophisticated equipment that is prohibitively expensive and requires substantive hands-on exposure and experience. The spider taxonomic literature, like that of many other hyper-diverse taxa and 'non-charismatic microbiota', appears to be largely dominated by traditional morphological taxonomy (Bond *et al*., 2022), though recently the Zoological Survey of India has used DNA Bar coding for rapid species identification and resolving the taxonomic ambiguities among spiders (Tyagi *et al*., 2019).

Rather than the whole animal; detailed investigation of the morphology, sculpture and morphometry of specific body parts could yield valuable insights, particularly if such a body part is implicated in sexual selection. The rationale behind this approach is that this structure being a part of the sexual assembly would be unique to that species with very little repeatability in other species, and further it's morphology will be anatomically compatible with that part of the female conspecifics, with which it aligns for the purpose of 'genital coupling'. (Comstock, 1910, 1920; Mouginot *et al*., 2015). Such a design is clearly seen in spiders with reference to the male pedipalps and the female epigyne in a species. Morphology of genitalia, genital coupling and its relevance for systematics is a promising field for the application of biosemiotic principles in scientific practice (Schult *et al*., 2021).

Given the rich diversity and skewed sex ratios of spider species populations, a comprehensive study of male pedipalpal morphology is a daunting task requiring active exploratory surveys and a long time-line. The current data is based on analysis of only seven species of spiders belonging to five families, including an endemic Theraphosidae spider or Tarantula. The other families being Sparassidae, Nephilidae, Salticidae and Hersiliidae. The species examined here have been randomly collected.

The archetypical male palpal tibia is relatively shorter than that of the female. On its outer side, the male tibia has a short spur/spine- the tibial/radial apophysis, which is evidently absent in females. The prominent difference lies in the modification of the tarsus; which in males is modified into a spoon shaped structure called 'Cymbium' that carries the 'Palpal Bulb' (Sebastian and Peter, 2009). This investigation is significant though preliminary, since it brings to the fore evidence of species-specific variations in pedipalpal morphology of male spiders.

Materials and Methods

The required permission was taken from the first author's institutional in-house committee for the use of live animals as specimen. The foliage of trees, walls of houses and dark damp areas were scanned visually to look out for spiders and collect them. This method was preferred since it accounts for keen observation and active search of the habitat. The specimens were randomly collected and due care was taken to avoid excessive collection or collection of species with high conservation value (IUCN and IWPA, categories). The collection was kept to a minimum. A pre-capture examination of the sex of the individual was carried out *in-situ* to avoid capturing females. However, any inadvertent captures were subsequently released without extended captivity. The individuals were collected in separate

Table 1: Comparative morphometry of the 'Palpal Bulb' of male spiders of seven species occurring in Western Ghats

S. No.	Species	Area (mm^2)	Max. Width (mm)	Max. Length (mm)	Perimeter (mm)	Length of Embolus (mm)
1	*Heteropoda venatoria*	0.31	0.53	1.08	2.14	0.48
2	*Olios lamarcki*	4.04	1.32	1.78	7.45	1.33
3	*Annandaliella travancorica*	0.36	0.64	0.72	2.20	1.31
4	*Nephila pilipes*	0.21	0.49	0.50	1.70	1.16
5	*Carrhotus sannio*	1.30	1.18	1.35	4.12	1.26
6	*Plexippus petersi*	0.29	0.57	0.66	2.03	1.38
7	*Hersilia savignyi*	0.56	0.87	0.69	2.76	0.53

containers designed for necessary aeration and quickly transported to the laboratory. After separating the males, they were sacrificed by cold narcosis. The male pedipalp was carefully dissected and observed under Meiji EMZ-5TR Stereo Zoom Trinocular Microscope with image analysis software (Figs. 1, 2). Measurements were taken of pedipalp bulb of all species for morphometry and the data was computed (Table 1).

Results

While some species were collected from human dwellings, others were picked from outdoors. Unless where the colour patterns differ between sexes of a species, it is difficult to sex spiders. One consistent observation was the difficulty in obtaining male spiders due to skewed female-biased sex ratios.

The following is the species wise account of male palpal structure (Figs. 1, 2) Morphometry of the palpal bulb of all the examined species is tabulated elsewhere (Table 1).

1. *Heteropoda venatoria* (*Linnaeus, 1767*) (Figs. 1A, B):

These are common cosmotropical Huntsman spiders belonging to family Sparassidae. These seek refuge in human dwellings and are known for their effective bio-control on insect pests. The flat brown body with long legs is typical. In the examined specimen, the male palpi are conspicuously long; the cymbium has closely set dense setae, is tawny in colour and not heavily sclerotized as compared to the bulb. The distal tip of the cymbium has characteristically different setae. The bulb stops short of extending beyond $2/3^{rd}$ of the length of the cymbium. The basal portion of the bulb has a secondary bulge towards the proximal end. The bulb and the embolus are not visualized on the dorsal aspect of the cymbium. The pedipalps are oriented towards the chelicerae and the curvature is discrete at the articulation between femur and patella. A spine is seen arising from the dorso-lateral aspect of femur. The distal end of tibia shows raised sclerotized regions with sharp points, as also it shows a tibial apophysis.

2. *Olios lamarcki* (*Latreille, 1806*) (Figs. 1C, D):

This species belongs to the family Sparassidae which is a moderately large family of spiders commonly known as Huntsman spiders, or Giant Crab spiders. The pedipalps in the male of *Olios lamarcki* appear like "boxer's gloves" and are curved towards the chelicerae. The pedipalps are short and one fourth the length of the ambulatory appendages. The cymbium is darker than and twice as broad as the rest of the palp. The tibia shows the presence of long and sparse setae, while the cymbium shows short and dense gold-coloured setae. Lateral to the bulb, the cymbium shows absence of sensilla. On the retro-lateral side of the palp is an undulate tibial apophysis with a light brownish base that gradually darkens

Fig. 1: (A, B): *Heteropoda venatoria* (♂), stereomicrograph of its pedipalp; (C, D): *Olios lamarcki* (♂), stereomicrograph of its pedipalp; (E, F): *Annadaliella travancorica* (♂), stereomicrograph of its pedipalp.

towards the distal end. The heavily sclerotized bulb is contained within the alveolus of the cymbium. The palpal bulb is black and occupies three fourth of the cymbium. A stout, pointy embolus arises just above the base of the bulb.

3. *Annandaliella travancorica* (*Hirst, 1909*) (Figs. 1E, F):

This is a large spider of family Theraphosidae, which lives in burrows in the trees or ground. The genus *Annandaliella* is endemic to Western Ghats of India, and represented by only three species: *A. travancorica* Hirst, 1909; *A. pectinifera* Gravely, 1935 and *A. ernakulamensis* Jose & Sebastian, 2008. They are mostly sluggish spiders which live under stones or fallen tree trunks. *A. travancorica* is the most known species of this genera. The length of the pedipalp of male *Annandaliella travancorica* measures almost half of its first walking appendages. The dorsal aspect of the pedipalp is brown in colour similar to the dorsal body surface. However, on the ventral surface, it is light in colour with a tinge of golden brown. From the proximal base to its distal tip, the pedipalp is clothed in dense hair. The femur at its distal end shows a light creamish band at the femur-patella junction. The pedipalp terminates with a modified trilobed cymbium. The pedipalpal tip (the cymbium) has a peculiar configuration i.e. it shows in-folding and out-pocketing. The lobes look similar to the bulb, except that the latter is large and devoid of hairs. The long, curved and tapering embolus emerges from posterior ventral area of tegulum, and points downwards. The femur is observed to be the longest article. The knocked-knee configuration of the palpal femur gives a V-shape to the distal portion of the pedipalp from the patella upwards. Tarsi are divided distally, but not deep.

4. *Nephila pilipes* (*Fabricius, 1793*) (Figs. 2A, B):

Affiliate of family Nephilidae, in the examined male specimen of Giant Wood spider (*Nephila pilipes*) the terminal palpal bulb is much enlarged as compared to the rest of the palp. Its appearance is typically as 'boxer's gloves' with the entire distal end of the pedipalp occupied by the globose bulb arising from which a single embolus points downwards towards the oral assembly. The bulbs, have dorsal lengths 0.52 mm and a dorsal breadth of 0.49 mm, and are hardened with chitin. Embolus length measuring 1.16 mm, is thrice the length of the bulb. The tip of the long embolus almost reaches the coxae of the first pair of ambulatory legs on that side. There is scanty distribution of setae on the cymbium. The colour of the palp as also of the body is reddish orange and that of the tip of the cymbium is creamish-white; whereas the bulb is intensely black. Wherein much of the palp is devoid of hair, the distal tip of the cymbium shows presence of moderately elongate setae, though sparse in distribution. When viewed dorsally the palps are present in a typical 'Swan' configuration due to the peculiar disposition of the bulb vis-à-vis the axis of the palp.

5. *Carrhotus sannio* (*Thorell 1877*) (Figs. 2C, D):

The pedipalp of this Salticidae species appears reddish-maroon in colour with a black cymbium. The articles of the pedipalp can be clearly differentiated because the inter-articular limits are not obliterated by presence of any setae. On the dorso-lateral side of the pedipalp, there is a characteristic presence of creamish patchy encrustations. Differential clothing of the pedipalp is evident, in that the dorsal side being covered in dense hair as compared to the naked surface of the ventral side. Tibial apophysis is stub-like. The bulb is blackish-brown in colour and occupies about $3/4^{th}$ of the cymbium. The distinctly curved embolus appears to arise from the base of the bulb and arches around to reach the tip of the bulb. The bulb does not protrude out of the cymbium and therefore is not visible from the dorso-lateral side.

6. *Plexippus petersi* (*Karsch, 1878*) (Figs. 2E, F):

This is a native Salticid species of Asia and a household spider. The entire pedipalp measures the same as the length of the femur of the first walking leg. Dorsally it is difficult to differentiate between palpal articles due to the presence of setae. The pedipalp is slightly curved at the

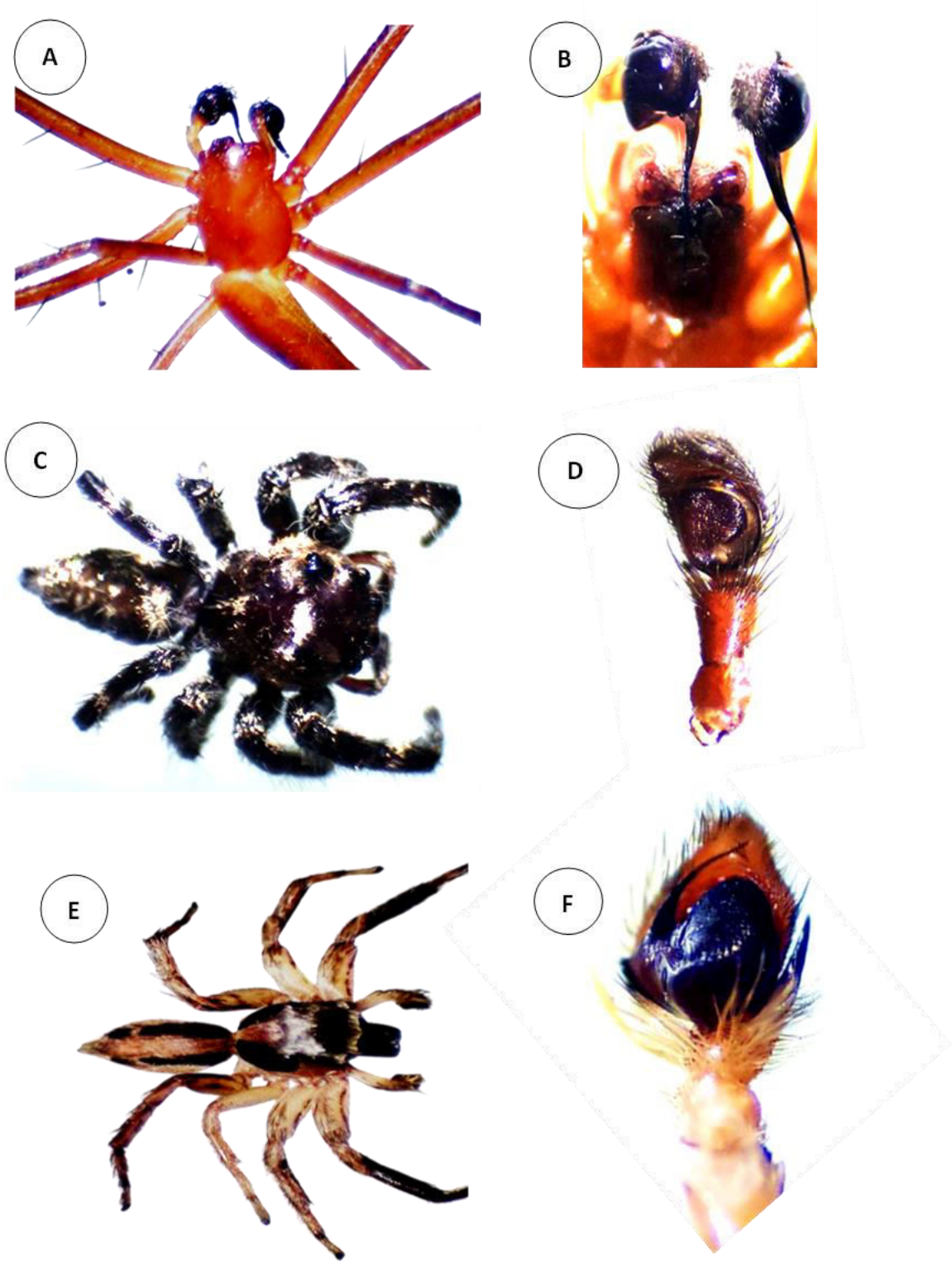

Fig. 2: (A, B): *Nephila pilipes* (♂), stereomicrograph of its pedipalp; (C, D): *Carrhotus sannio* (♂), stereomicrograph of its pedipalp; (E, F): *Plexippus petersi* (♂),stereomicrograph of its pedipalp.

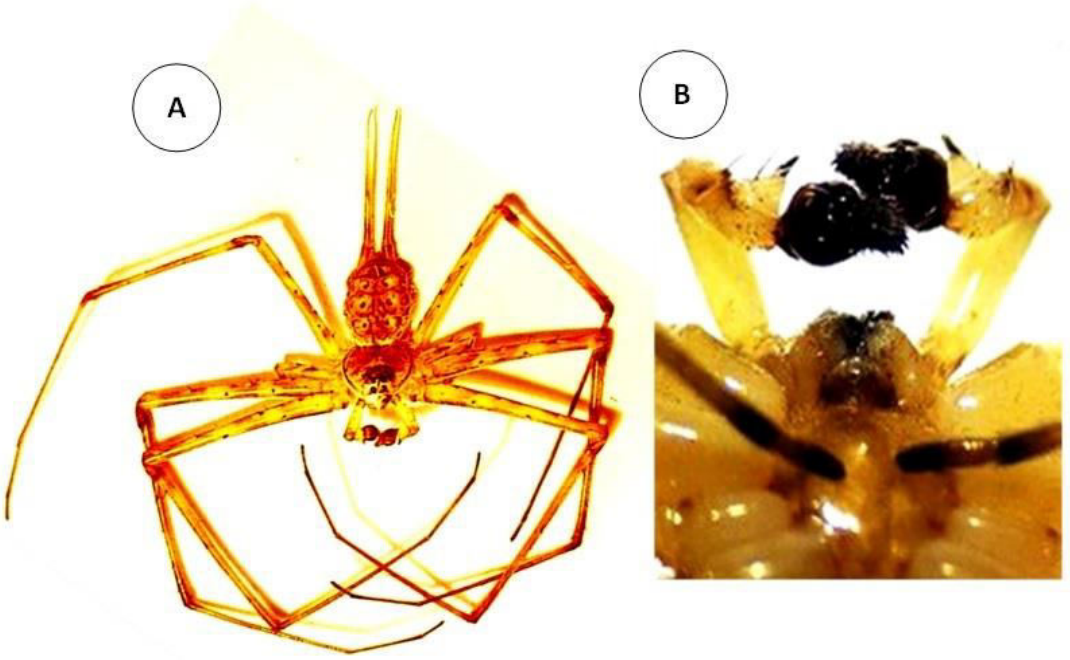

Fig. 3: (A, B): *Hersilia savignyi* (♂), stereomicrograph of its pedipalp.

terminal article. The bulb is sub terminal and is a little over half the length of the cymbium. The base of the cymbium is covered in a whorl of creamish-white stiff setae. The cymbium is little expanded and gently tapering on the tip, with closely arranged setae all along its dorso-lateral border. The lengths of the sensillae covering the cymbium, from the base to the tip are relatively short. The relatively long embolus is accommodated in the flattened expanse of the cymbium and follows its dorso-lateral curvature and is not projected out. The pedipalps in this specimen are distinguished by a dagger like tibial apophysis, whose tip colour matches with that of the distal part of the palpal bulb. The patella is typically swollen and annular in appearance. The longest hairs are found on the tibia, some even being as long as the tibial apophysis.

7. *Hersilia savignyi* (*Lucas*, *1836*) (Figs. 3A, B):

This is a Hersiliid spider found commonly on tree trunks in Western Ghats and they are known as 'two tailed spiders'. The pedipalpal sculpture and construct is unmistakable due to alignment of intromittent and non-intromittent articles of the pedipalp. Cymbium is beset with multiple stiff sensillae and terminal portion has spurs hardened with chitin. The compact patella and tibia are aligned in a straight line but the two together are perpendicular to the femur. The tibia is triangular with a sharp apophysis at one angle. The entire pedipalp with exception of the cymbium and bulb is translucent with very scanty setae present. The embolus is very short. The femur is quite stout and devoid of setae.

Discussion

The consistently low encounter of males in the collection is corroborated by observations that all quasi-social species appear to have female-biased sex ratios, while congeneric species with less advanced forms of sociality have 1:1 sex ratio (Frank, 1987). Production of more female than male-determining sperms as the likely mechanism underlying female-biased sex ratio has been advanced at least in some social spider species

(Vanthournout *et al*., 2018). As a counter to sexual cannibalism, males of some species are known to select one of the two palps containing more sperm for their first copulation in what is dubbed as the "better charged palp" hypothesis (Zhang *et al*., 2022).

Literature on the spider taxonomy, like that of many other hyper-diverse and 'non-charismatic' taxa, appears to be largely dominated by traditional morphological approach (Bond *et al*., 2022) that accounts for external shape, size and colours of the body; leaving scope for ambiguity and error in identification. The use of DNA bar-coding to complement traditional morphological methods has refined resolution of many taxa that are too difficult to identify only by microscopy and morphometry (Tyagi *et al*., 2019)

Globally, spiders present a rich, varied species assemblage posing a challenge to a conformist taxonomist. However, morphology of genitals, genital coupling and its relevance for systematics is being increasingly realized. Spider gender roles are conservative, but their sexual biology is unique (Eberhard, 2004).

Pedipalp, the anterolateral non-ambulatory appendage lying between their chelicerae and the first leg on either side of the prosoma was the focus of this investigation. While the pedipalps are leg-like structures in females that are used to probe substrate and prey, they serve as secondary sexual organs in males that often have the appearance of enlarged boxing gloves (Foelix, 2011). Male pedipalps of some neo-tropical wolf spiders have also been known to have stridulatory devices that can aid in Lycosid systematic (Fernández-Montraveta and Simó, 2002). It is also suggested that palps may be implicated in stridulation during pre-copulatory behaviour in some species like *H. venatoria* (Ewunkem *et al*., 2016).

The male pedipalps are structurally and positionally oral appendages, which have no connect with the male gonads and associated ducts. The pedipalp is in fact a copulatory organ serving the crucial purpose of inseminating the females. Sperm production occurs in the testes, but all males first ejaculate on sperm webs and suck up the sperm into their paired, uniquely modified hypodermic-like pedipalps, which are inserted into the female gonopore during insemination (Eberhard, 2004).

The construct of the male pedipalps of a species is commensurate with two functions namely: (i) Juxtaposition and alignment with the female epigynum, and (ii) coupling, insertion and dilatation of the epigynum to ensure the transfer of sperm into spermathecae.

Within the conspecifics of a spider species, the male pedipalps must be structurally compatible with the female's epigynum to allow 'genital coupling' and intromission. Before copulation, the palpal organ is inflated by hydraulic pressure, which causes complex shifts of the sclerites (Huber, 2004). Most of these sclerites function as locking or bracing devices that interact with genital structures of the female (Mouginot, 2015). This further implies that the male pedipalps must have species-specific morphology, and therefore can serve as a valuable tool for spider taxonomy. The premise of this investigation is that there are very discrete interspecific structural variations in the male pedipalps of all the spider species, and the proof are the observed differences and species-specific nuances in the morphology and sculpture of this intromittent appendage across the seven species of spiders belonging to five families, examined randomly.

All the spiders studied here (except *Annandaliella travancorica*) affiliate with Group Entelegynae of the Infra-Order Araneomorphae. These groups have been distinguished based on the female genitalia (epigynum) (Hormiga and Griswold, 2014). In all the Entelegyne spiders, the female epigynum is known to be heavily sclerotized; which is why the pedipalp of the males in species examined here viz., *Heteropoda venatoria*, *Olios lamarcki*, *Carrhotus sannio*, *Plexippus petersi* and *Hersilia savignyi* have evolved to be bewilderingly complex. As observed

the palpal bulb has two or three divisions, sometimes with an elaborate embolic division. The tegulum usually has two apophyses, in addition to the embolus. Our observations are in agreement with those of Coddington (2005) that any or all of these pedipalps in entelegynes can be wonderfully complex; with knobs, levers, grooves, hooks, serrations, sinuous filaments, and spiraling parts. Contrastingly in Haplogyne species (not represented in this study) the males are known to have comparatively simpler pedipalps due to the unsclerotized female epigynum (Elias *et al*., 2011).

In so far as entelegyne spiders are concerned, scattered data are available on pedipalpal construct and copulatory mechanism (Gering, 1953; Van Helsdingen, 1969; Blest and Pomeroy, 1978; Huber, 1993).

Some of the examined species viz. *Nephila pilipes*, *Carrhotus sannio*, *Plexippus petersi* and *Hersilia savignyi* show sparse distribution of setae on their pedipalps. Perhaps this decrease in setae is an adaptation that facilitates easy and firm genital coupling and avoid damage that would otherwise result from friction with the female during copulation (Eberhard and Huber, 2010).

Perusal of literature indicates that sperm induction and involvement of male pedipalps in intromission has been a fairly well researched subject (Buffet and Viera, 2016). The exact mechanism of sperm web construction has been investigated in a few spiders such as *Tetragnatha*, *Theridion*, *Kukulcania*, *Anelosimus* and *Pholcus* (Costa and Pérez, 2002; Barrantes and Ramírez, 2013). However, there is paucity of understanding on the pedipalpal involvement and mechanism of sperm web construction in Indian species.

In the present study, the examination of male pedipalps of *Nephila pilipes* underpins some interesting observations and inferences. This species is characterized by interplay of female-biased sexual size dimorphism (SSD). Nephilid spiders perhaps demonstrate the highest degree of SSD among terrestrial animals, with sedentary (low-risk) females and dwarf, roving (high-risk) males.

Because most morphological characters increase with size, species like the *Nephila pilipes* that exhibit extreme SSD may experience a functional conflict due to the different sizes of the reproductive organs of males and females. While most morphological features tend to scale with total somatic size, this does not necessarily hold for genitalia, because divergent evolution in somatic size between the sexes would cause genital size mismatches.

That extreme size divergence in genitalia could impair effective mating, especially in species with hard or sclerotized genitalia like *Nephila pilipes* (Ramos *et al*., 2005) is a view that has been contested recently by the evidence that genital and somatic size dimorphism are phylogenetically decoupled, i.e. traits composing genital versus somatic size dimorphism evolve independently (Lupše *et al*., 2016).

Conclusion

From our observations and corroborations, we conclude the following: The field collection reaffirms that the sex ratios in social spiders are heavily skewed in favour of females, limiting the sample size for investigation of male pedipalps. Further, it is established that male pedipalps in spiders are sexually dimorphic structures within a species. There are interspecific variations in the morphology, micro-sculpture and morphometry of male pedipalps within and between families, offering an effective scheme for taxonomy. The size, shape and construct of the bulb are species-specific and also differs between conspecifics depending on their reproductive status. Cymbium articulates with genital bulb at varying angles, at times projecting beyond it or in some cases being in line with the bulb and the embolus.

Character of the tibial apophysis, an important retro-lateral spur is varied and species-specific and can thus serve as a taxonomic clue. However, in some species it is greatly reduced or even absent. The length of the embolus grossly correlates with the sexual size of the female

conspecific, despite incompatible somatic size. Stereomicroscopy limits elucidation of internal character of the genital bulb, which if investigated could lend further confidence to the work. Scanning Electron Microscopy could further resolve the micromorphology of the male pedipalps allowing charting of subtle differences in the pedipalps across species.

Acknowledgements

Dr. D. B. Bastawade (Retd. Zoologist, ZSI, Pune) confirmed species identification. BMR acknowledges the assistance rendered in this investigation by his undergraduate students Briana Barbosa, Mabel Alves, Queency Barreto and Rosha Crasto.

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