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Identity of cavity nesting honey bees of the Indian subcontinent with a description of a new species (Hymenoptera, Apidae, Apinae, Apini, *Apis*)

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ABSTRACT: A new species of cavity nesting honey bees, *Apis karinjodian* **n. sp.,** endemic to the Western Ghats biodiversity hotspot is described and illustrated. *Apis indica* Fabricius, 1798 status restored is resurrected from synonymy with *Apis cerana* Fabricius, 1793. Key to distinguish the three native cavity nesting honey bee species occurring in the Indian subcontinent *viz*., *Apis cerana* Fabricius, 1793, *Apis indica* Fabricius, 1798 and *Apis karinjodian* **n. sp.** is provided. Distribution map is given for the native cavity nesting *Apis* species of the Indian subcontinent. The morphological description of the new species is supplemented with molecular and behavioral information. Radio-Medial Index (RMI), a new measure for species discrimination in *Apis,* is proposed. South India is proposed as the center of origin of the European honeybee, *Apis mellifera* Linnaeus, 1758.
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KEY WORDS: *Apis indica, A. cerana, A. karinjodian* **n. sp**., distribution, DNA barcode, Radio-Medial Index

INTRODUCTION

The honey bees of the genus *Apis* Linnaeus, 1758 are far the most famous of all insects owing to their production of honey, pollination of crop plants and advanced eusocial behaviour, which has attracted much attention from biologists. Unfortunately, the systematics of this small and highly visible group is not clearly understood, partly owing to the high levels of intraspecific variation as well as the recent divergence of taxa (Engel, 2002).

Beekeeping has been practiced since time immemorial in India. The honey bees (Apini) occurring in India include the single comb building giant honey bees (subgenus *Megapis* Ashmead, 1904): *A. dorsata* Fabricius, 1793 and *A. laboriosa* Smith, 1871; dwarf honey bees (subgenus *Micrapis* Ashmead, 1904): *A. florea* Fabricius, 1787 and *A. andreniformis* Smith, 1857; multiple parallel comb building cavity-nesting honey bees (subgenus *Apis* Ashmead, 1904): *Apis mellifera* Linnaeus,

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1758, *A. cerana* Fabricius, 1793; *A. indica* Fabricius, 1798 and *A. karinjodian* **n. sp.**

Several species of the stingless honey bee (Meliponini) genera such as *Lepidotrigona* Schwarz, 1939, *Lisotrigona* Moure, 1961, and *Tetragonula* Moure, 1961 also occur in India (Rasmussen, 2013; Shanas and Faseeh, 2019). The European honey bee *A. mellifera* was introduced and successfully established in India during the 1960's (Mishra, 1995).

Two distinct colour morphs, yellow in plains and black in hills have been recognised among cavity nesting honey bees from India by several workers (Smith and Hagen, 1996; Oldroyd *et al.*, 2006; Chalapathy *et al.* 2014a, b; Baskaran, 2016; Gaikwad *et al.*, 2019 and the references therein). Oldroyd *et al.* (2006) provided evidence for the reproductive isolation between the yellow plain and black hill colour morphs in south India and concluded that the yellow plain bees of India could be regarded as a separate species from *A. cerana* based on non-overlap of drone flight times and occurrence of consistently different mitochondrial haplotypes. Lo *et al.* (2010) also supported the recognition of *Apis indica*, the Plains Honey Bee of south India, as a separate species from *A.cerana*.

The natural range of *A. cerana* is spread across temperate and tropical Asia from Afghanistan to Japan, north into the foothills of the Himalayas and south through Indonesia (Koetz, 2013). Radloff *et al.* (2010) revised the taxonomy of *A. cerana* and divided the *Apis cerana* complex into six morphocluster groups based on physiographic and climatic factors, wherein the "Indian Plains cerana" (Morphocluster III) was mentioned to occur across the plains of central and southern India and Sri Lanka as a '*fairly uniform population*' in the Indian subcontinent. They also gave detailed summary of 40 synonymous specific and infra-specific names and pointed out that the former subspecies trinomials such as *Apis cerana indica* no longer have any official, nomenclatural standing in *Apis* classification under the rules of the International Code of Zoological Nomenclature (ICZN, 4th Edition, 1999). However, the subspecific epithet *Apis cerana indica* is still in vogue for want of clarity on the species status of *Apis indica* Fabricius, 1798 (Otis and Smith, 2021).

The present study is an attempt to streamline the taxonomy of native cavity nesting honey bees of the Indian subcontinent.

MATERIALS AND METHODS

The study is based on a collection of native cavity nesting honey bees from different locations in India. Permanent microscope slides of wings were prepared. The wings were separated from dried specimens, passed through ethyl alcohol series (70-100%), dipped in clove oil for 30 minutes and mounted in DPX mountant. Images of wings were processed using Adobe Photoshop and images of body features were processed using Adobe Photoshop and Zerene stacker. Habitus images (Fig. 1) were taken using a Nikon D200 camera and processed using Adobe Photoshop.

The type specimen of *A. indica* is lodged at the Copenhagen collection (NHMD). Zimsen (1964) mentioned one specimen in the "Kiel collection" and two specimens in "Copenhagen collection". The first specimen (NHMD 308727), headless, which carries a label reading "*indica*" is the original Fabrician type in "Kiel collection" which is presently lodged in "Copenhagen collection" (Figs. 2A, B). Among the two non-types of *A. indica*, probably from the "Sehested-Tønder Lund collection" lodged in the Copenhagen collection, the first (NHMD308728) bears a label: *a: indica* / ex ind: or: ed lap / b: fro: Daldorff (Fig. 2C) and the second (NHMD308729) bears no label (Fig. 2D). The Lectotype of *Apis cerana* Fabricius, 1793 from China, designated by Moure, 1958 in Zimsen, 1964 (ZMUC 00241552), is also lodged at the Copenhagen collection (Figs. 2E–F). Images of both the name bearing types were examined.

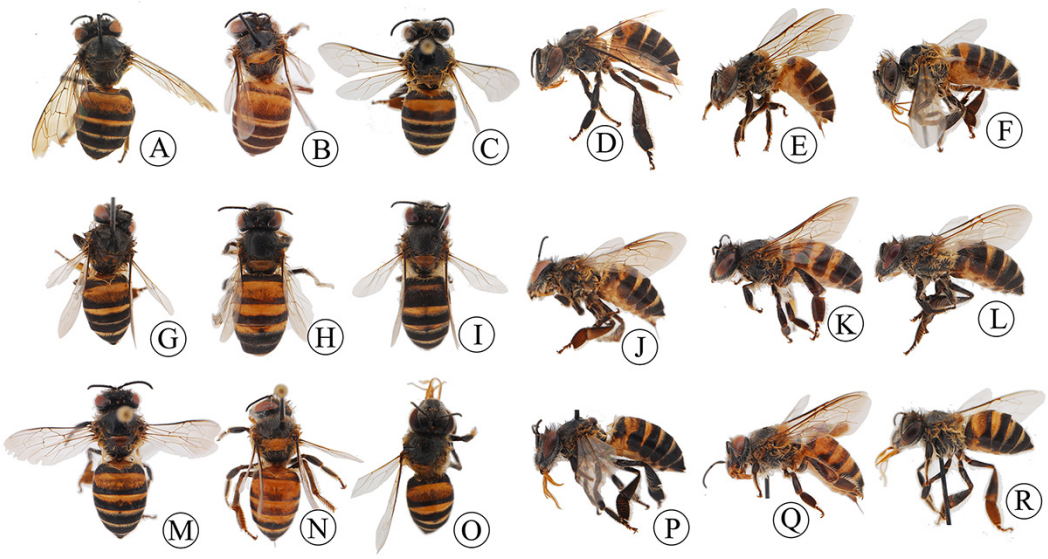


Fig. 1 Workers: A – C dorsal habitus and D – F lateral habitus of *Apis cerana* Fabricius, 1793; G – I dorsal habitus and J – L lateral habitus of *Apis karinjodian* **n. sp**.; M – O dorsal habitus and P – R lateral habitus of *Apis indica* Fabricius, 1798

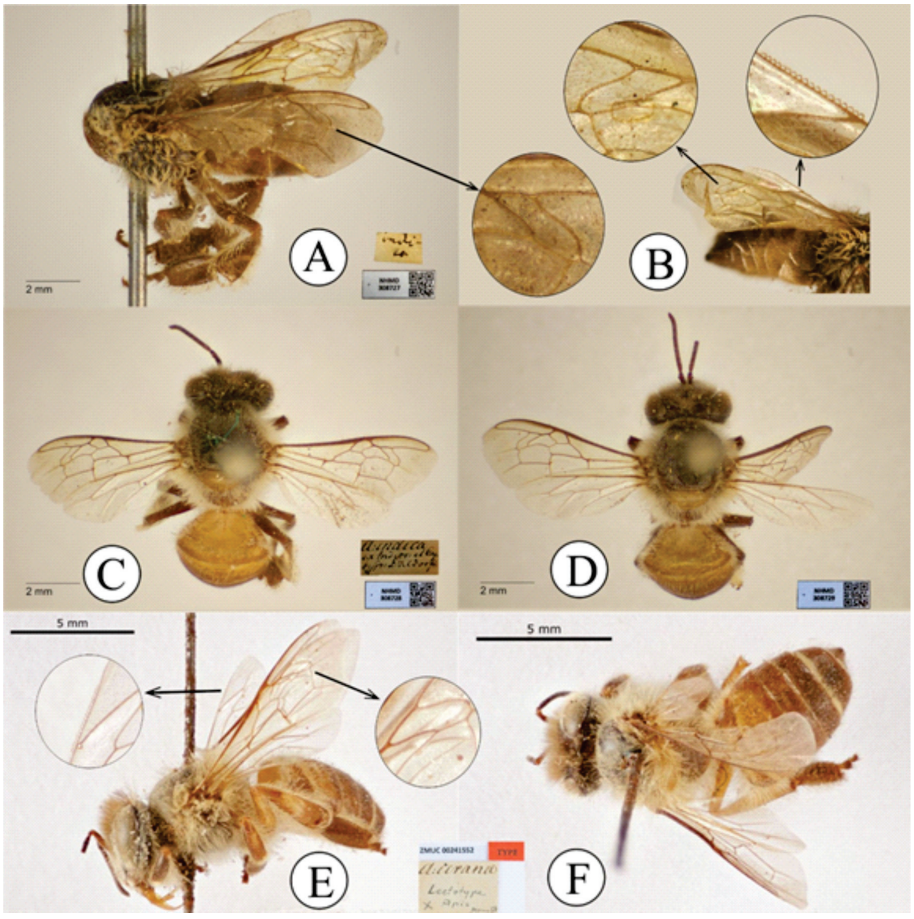


Fig. 2 Worker: A, B. type of *Apis indica* Fabricius, 1798, lateral habitus; C, D. non-types of *A. indica* Fabricius, 1798, dorsal habitus; E, F - lectotype of *A. cerana* Fabricius, 1793 lateral and dorsal habitus; inserts illustrating third submarginal cell and wing hamuli

Uneverted endophallus was dissected and isolated from drones preserved in ethanol (90%). Species identifications were confirmed using morphological characters and molecular genes. Baseline distribution maps were prepared based on collected specimens, published records, DNA barcode sequences from collected specimens as well as NCBI GenBank public database (Table 2).

The images of the type specimens of *A. indica* (Figs. 2A, B) and *A. cerana* (Figs. 2E, F) were studied. The Radio-Medial index (RMI), based on the ratio of veins c/b of the forewing is proposed as a new measure for species discrimination in addition to the widely used Cubital Index a/b (Fig. 3H). The RMI was found robust and foolproof in discriminating populations of *A. indica* and *A. cerana* and the same has been here used to resurrect *Apis indica* Fabricius, 1798 from synonymy with *A. cerana* Fabricius, 1793. The RMI and CI (Table 1) were determined from the type image of *A. indica* and *A. cerana* by superimposing a fine micrometer scale on the image of forewings. It was observed that, although the wing image may get distorted to some extent due to the imaging angle, the values of RMI takes care of such minor distortions [eg: Table 1; 4.0 (RW) and 3.8 (LW) for *A. indica*].

To determine the RMI and CI ratios, forewing of workers of *A. indica* from different states *viz*. Karnataka, Kerala, Nagaland, Odisha, West Bengal

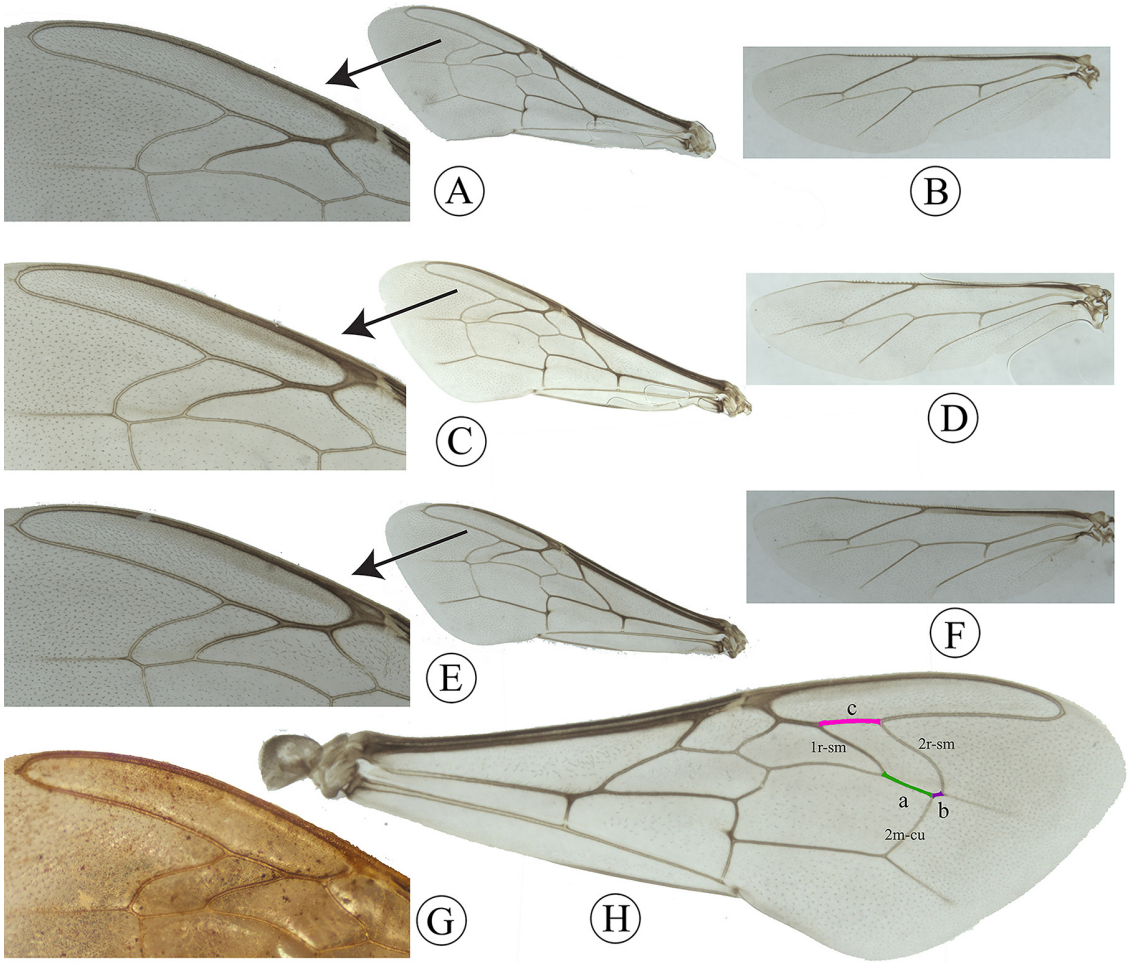


Fig. 3 Wings of worker: 3A forewing and 3B hindwing of *Apis karinjodian* **n. sp**.; 3C forewing and 3D hindwing of *A. cerana* Fabricius, 1793; 3E forewing and 3F hindwing of *A. indica* Fabricius, 1798; 3G forewing of *A. indica* Fabricius, 1798 (type); 3H forewing of *A. karinjodian* **n. sp**., illustrating veins of RMI and CI forming third submarginal cell

and Tamil Nadu (n=60 individuals from 19 locations) were measured; forewing of workers of *A. cerana* from north India (New Delhi, Maharashtra, Uttarakhand), north-east India (Assam, Nagaland, West Bengal) and south India (Tamil Nadu) were measured (n=41 individuals from eight locations); forewing of workers of *A. karinjodian* **n. sp.** from Tamil Nadu and different parts of Kerala (n=35 individuals from five locations) were measured and forewing of *A. mellifera* (n=5 individuals from two colonies in a single location) were also measured.

The veins of forewing *viz*. a, b and c, defining RMI and CI, are defined as follows: 'a' is defined as the segment of median vein laying between distal end of cross vein 1rs-m and proximal end of cross vein 2m-cu. 'b' is defined as the segment of median vein laying between proximal end of the cross vein 2m-cu and the distal end of the 2rs-m and 'c' is defined as the segment of radial sector laying between proximal end of the cross veins 1rs-m and 2rs-m (Fig. 3H).

The distribution map (Fig. 13) was prepared by correlating the information available from material examined for this study, sequence data (540 nos) available at NCBI-GenBank (Table 2) and by consulting several topographic maps and maps of maximum and minimum temperature limits that helped to demarcate the species boundary. The species are categorised as per annotation provided in the IUCN Red List Categories and Criteria (IUCN, 2022).

Morphological terminology follows Ruttner (1988), Koeniger *et al.* (1991), Engel (2001) and Michener (2007).

Phylogenetic Analysis

The PCR amplification and sequencing of the CO1 genes (Table 3) were performed at the Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, Kerala, India. In addition to the new sequences, the remaining sequences used in this study (Table 2) were obtained from the GenBank of National Center for Biotechnology Information (NCBI). The sequences were aligned with the CLUSTAL omega to depict the intraspecific conserved sites. The phylogenetic analysis was performed and average nucleotide composition of each species was determined using MEGA11 software (Tamura *et al*., 2021). Phylogenetic trees were constructed using the Neighbor-Joining (Saitou and Nei, 1987; Tamura et al., 2004), Maximum Likelihood, Tamura-Nei model (Tamura and Nei, 1993), Minimum Evolution (Rzhetsky and Nei, 1992) and UPGMA (Sneath and Sokal, 1973) methods. *Apis florea* was chosen as the out-group for the evolutionary studies.

The topology of the Neighbor-Joining (NJ) tree was congruent with that of the tree topology obtained from Maximum Likelihood (MCL), Tamura-Nei model, Minimum Evolution method and UPGMA. Hence, only the NJ tree (Fig. 12A) is presented. The percentage of repeat trees wherein the connected taxa huddled together in the bootstrap test (1000 replicates) are shown near the branches. The phylogenetic tree was generated with the branch lengths expressed in units equivalent to those of the evolutionary distances by which the evolutionary tree is inferred. The evolutionary distances, which are measured in terms of the number of base substitutions per site, were calculated using the MCL approach. Two separate phylogenetic trees for the Western population (Fig. 12B) and the Eastern population (Fig. 12C) were prepared for better interpretation of the phylogenetic affinity.

The holotype (Accession no. NIM/NBAIR/HYM/API/15922-H) and three paratypes (Accession nos NIM/NBAIR/HYM/API/15922-P1/P2–♀; P3–♂) of the new species are deposited in the National Bureau of Agricultural Insect Resources, Bengaluru (NBAIR). Paratypes will be deposited in the National Pusa Collection, Indian Agricultural Research Institute (NPC) and Zoological Survey of India, Kolkata (ZSI).

RESULTS

Systematics

Apis karinjodian **Shanas, Anju & Mashhoor, new species**

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(Figs 1 G–L; 3A, 3B, 3H; 4 A–P; 5 A–F; 6A–B; 7A–D; 8A–D)

Diagnosis: The new species is relatively large in size (10.8–11.6 mm) and darker in general appearance. The female worker is characterized by a prominent 'V' shaped projection on the propodeum (Figs. 4D–F); dark yellow scutellum, with or without a black patch (Figs. 1G–I) and abdominal terga I-IV prominently black-banded (Figs. 4G–I). Rugosely reticulate irregular sculptures were rarely observed on the frons of drones (Fig. 7B).

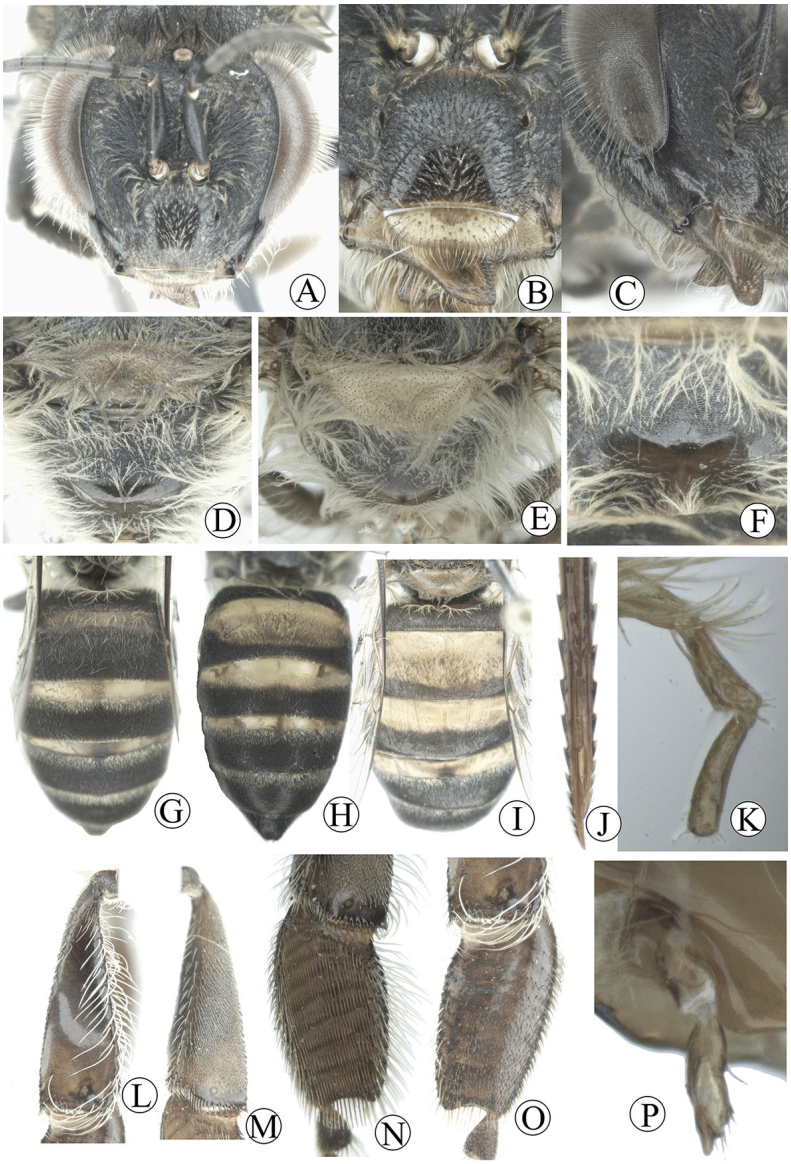


Fig. 4 Workers of *Apis karinjodian* **n. sp.:** A. frontal view of head; B. clypeus and labrum; C. malar area of face; D–F. propodeum; G–I. abdomen; J. sting; K. labial palp; L. outer surface of hind tibia; M. inner surface of hind tibia; N. inner surface of hind basitarsus; O. outer surface of hind basitarsus; P. maxillary palp

Description: Female (worker): Wings clearly hyaline; abdominal terga I with proximal part prominently black-banded (Fig. 4G–I); body length (10.8–11.6 mm); forewing length (7.31– 8.16 mm); 2.8x longer than broad; hind wing length (5.1–5.6 mm); 3.2x longer than broad; head length from anterior margin of clypeus to summit of vertex, in facial view 2.84 mm; head width 3.34 mm; length of compound eye 2.12 mm; inter antennal distance (0.19–0.27 mm); length of scape 1.16 mm; length of 2nd flagellomere 0.13 mm; length of 3rd flagellomere 0.28 mm; length of metatibia 2.82 mm; length of metatarsus 2.82 mm (n=3).

Compound eye 2.41x longer than wide; length of compound eye/length of scape ratio 1.82; interocellar distance/ocellar diameter ratio 1.1; ocelloorbital distance/interocellar distance 1.54; width/length of head ratio 1.18; length/width of scape ratio 5.7; length/width of 3rd tibia ratio 3.14;

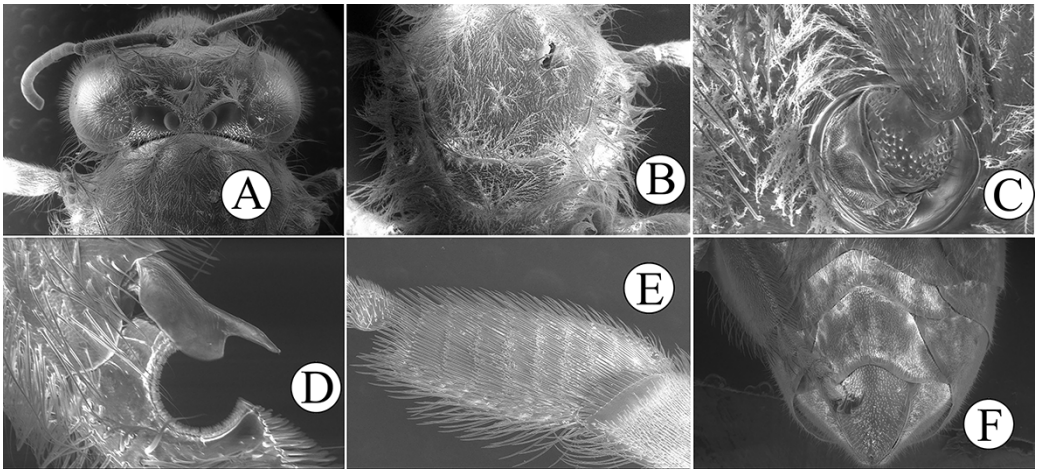


Fig. 5 Workers of *Apis karinjodian* **n. sp.:** A. dorsal view of head; B. mesothorax; C. antennal socket; D. notch and velum of foreleg; E. inner surface of hind basitarsus; F. posrero-ventral view of abdomen

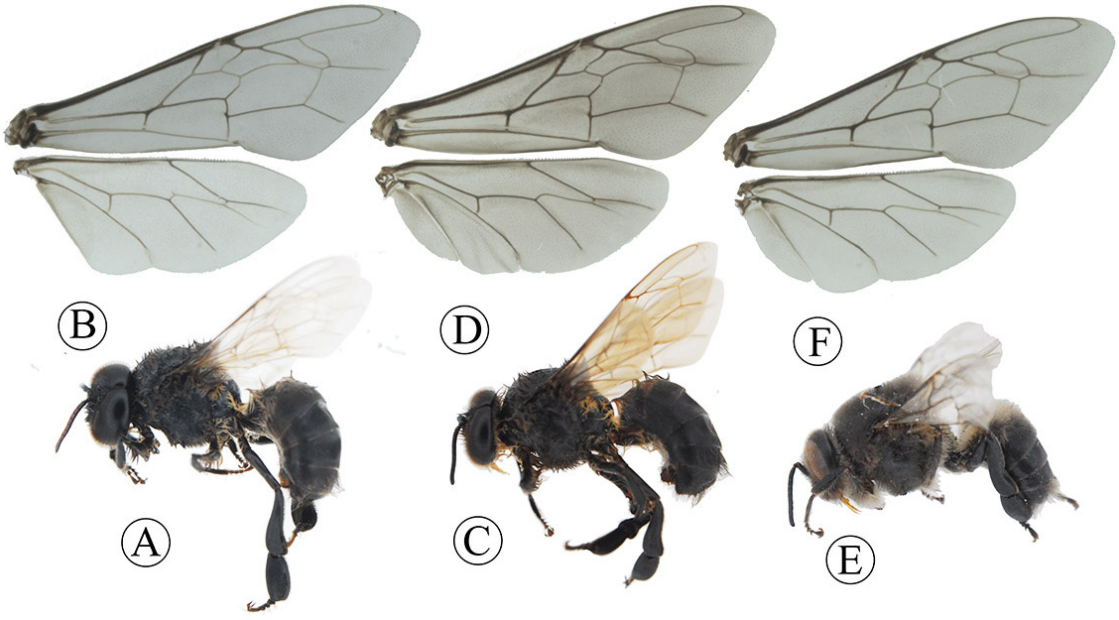


Fig. 6 Drone: A – *Apis karinjodian* **n. sp**., B – forewings and hindwing. C – *Apis indica* Fabricius, 1798, D – forewings and hindwing. E – *Apis cerana* Fabricius, 1793. F–forewing and hindwing

head width/length of metatarsus ratio 1.9; malar space/F3 ratio 2.26; malar length: 0.63–0.65 mm; interalveolar distance/interantennal space (0.19–0.27 mm; maxillary palps sometimes pointed (Fig. 4P). Wings hyaline, forewing with Radio-Medial index 5.4–6.4; Cubital index 4.6–5.4; Hindwing with 17–19 hamuli.

Male (drone): Wings mostly hyaline (Fig 6B), very feebly stained brown (Fig. 6 A–B); scutellum black (Fig. 7C); body black; body length (10.3–11.3 mm); lateral ocellar line (LOL) 0.07mm, posterior ocellar line (POL) 0.3mm, POL/LOL ratio 4.2; forewing (length 9.35 mm); 3.1x longer than broad; hind wing (length 3.1 mm); 3.1x longer than broad; head length from anterior margin of clypeus to summit of vertex, in facial view 1.92 mm; head width 3.52 mm; length of compound eye 2.88 mm; length of metatibia 3.2 mm; length of metatarsus 2.0 mm. Forewing with

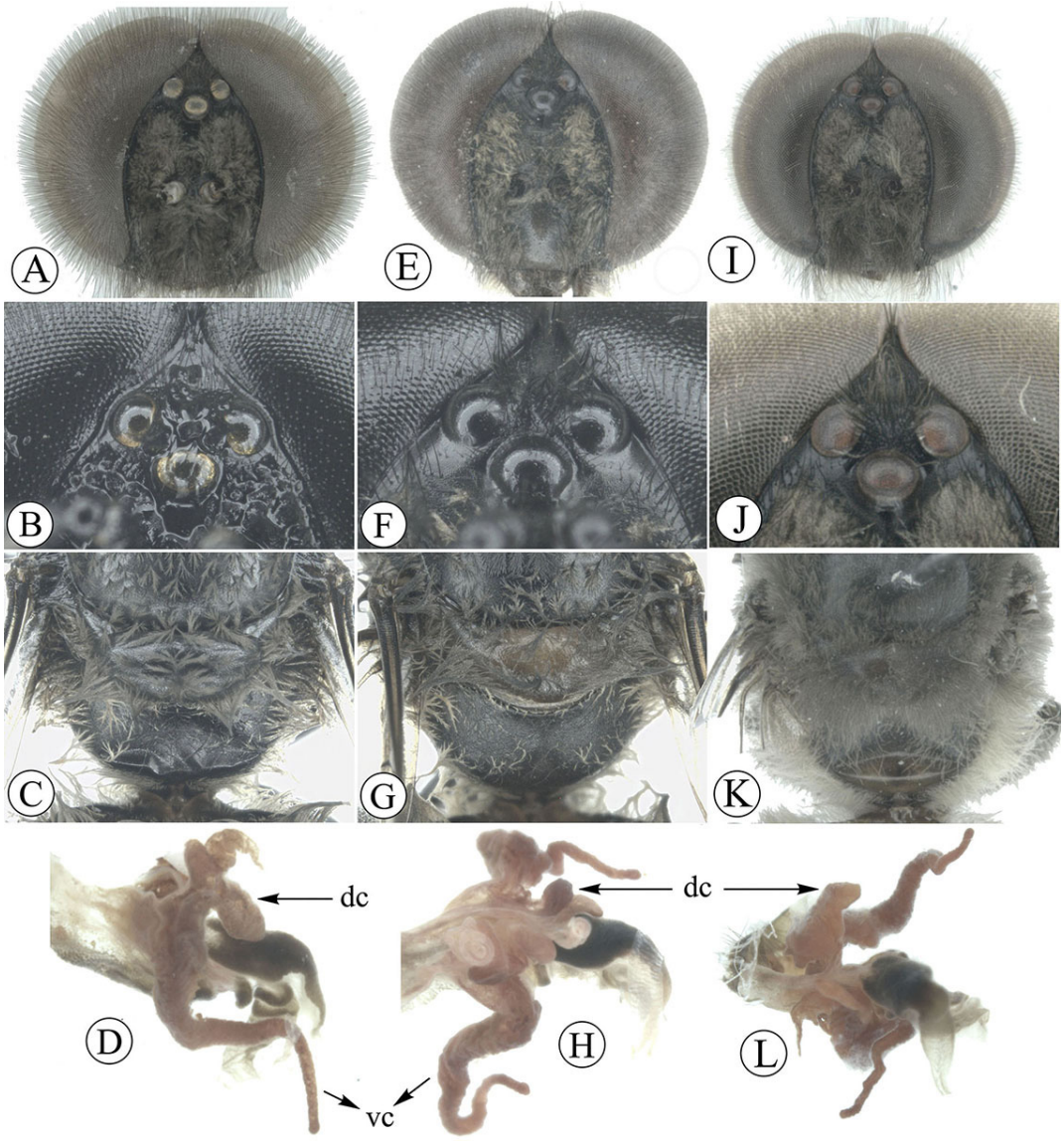


Fig. 7 Drone: A–D. *Apis karinjodian* **n. sp**. A. frontal view of head; B. vertex; C. dorsal view of thorax; D. endophallus. E–H. *Apis indica* Fabricius, 1798. E. frontal view of head; F. vertex; G. dorsal view of thorax; H. endophallus. I–L. *Apis cerana* Fabricius, 1793. I. frontal view of head; J. vertex; K. dorsal view of thorax; L. endophallus. Abbreviations: dc: dorsal cornua; vc: ventral cornua

Radio-Medial index (4.44–5.50); Cubital index (3.22–3.25); Hindwing with 14–20 hamuli (n=2); uneverted endophallus with a prominently large round lobe (0.8mm, Fig. 7D) on the three lobed dorsal cornua.

Material examined: Holotype: ♀ (worker): INDIA, KERALA, Wayanad, Shanas, S. coll, 22-XII-2019 (NBAIR); **Paratypes** [94 nos] : 30 ♀ (worker): Same data as that of Holotype; 10 ♀ (worker): Idukki, Thoppipala, Shanas, S. coll, 14-II-2019; 10 ♀ (worker): Idukki, Marayoor, Shanas, S. coll, 14-II-2019; 30 ♀ (worker): Thiruvananthapuram, Attingal, Shanas, S. coll.25-X-2021; 5 ♀ (worker): TAMIL NADU, Coimbatore, Shanas, S. coll. 20-V-2007; 8 ♂ (drone): Kerala, Thiruvanantha- puram, Maruthankuzhy, Shanas, S. coll. 20-II-2022; 1♂: Kerala, Idukki, Mattupetty, Shanas, S. coll. 14-V-2022: (3 NBAIR).

Distribution: INDIA (Goa, Karnataka, Kerala and Tamil Nadu). An analysis of the sequences (Table2) reveals the presence of *A. karinjodian* **sp. n.** in Goa (KF497586, KF497587); Karnataka (KF497265 to KF497275; KF497293 to KF497296; KF497298), Kerala (KF497550) and Tamil Nadu (KF497510). The distribution ranges from the central Western Ghats and Nilgiris to the southern Western Ghats, covering the states of Goa, Karnataka, Kerala and parts of Tamil Nadu. From its restricted distribution extending from Goa to Kerala, it is inferred that this secluded population is endemic and distributed mainly along the Western Ghats biodiversity hotspot of southern India (Fig.13).

Conservation status: Near threatened (NT) in Kerala: This species is only occasionally encountered in managed as well as feral colonies in Western Ghats region of Kerala. Data Deficient (DD) in remaining places.

Remarks: The workers of the new species resemble *A. cerana* Fabricius, 1793 by most other diagnostic characters. However, they can be distinguished by the narrow size of the median vein

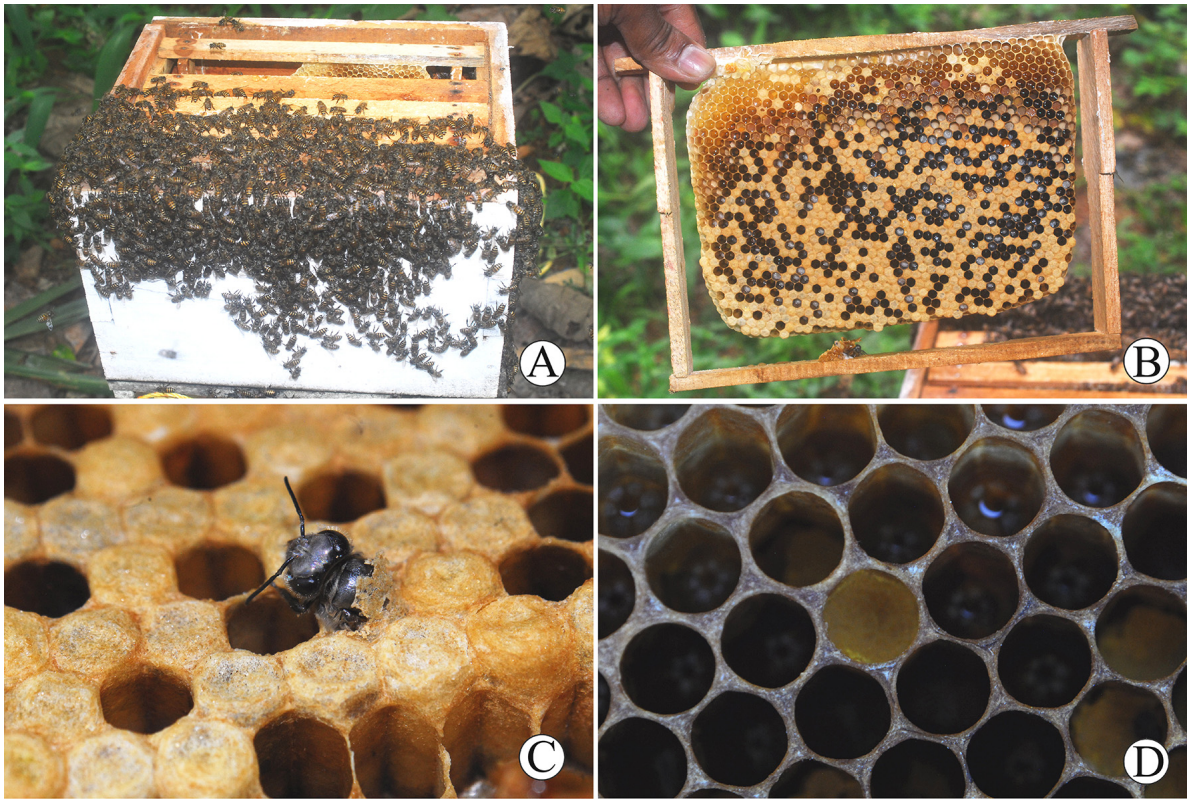


Fig. 8 *Apis karinjodian* **n. sp.** A. hive; B. brood comb; C. emerging worker; D. pollen and honey chamber

segment 'b' (0.1 mm, Figs. 3A, H) and the prominent 'V' shaped projection on propodeum (Figs. 4D–F) which are absent in *A. cerana* (Fig. 10E) and *Apis indica* (Fig. 9H). Rugosely reticulate irregular sculptures are observed rarely on frons of the drone (Fig. 7B).

Note on identity and behaviour: The distinct identity of this species was recognized by the bee-keepers in Kerala who coined the term 'karinjodian' for these visibly dark bees. According to bee-keepers, *A. karinjodian* **n. sp**. gnaws and dismantles the combs of *A. indica* when the combs are exchanged between colonies. The hive cleaning behaviour is superior to that of *A. indica* as the wax debris falling on the bottom of the hive are regularly removed, which prevents wax moth infestation during lean season. It is observed that the *A. karinjodian* **n. sp.** colonies are generally strong even during the monsoon season. They also produce more honey which is thicker in consistency compared to that of *A. indica.* However, they are not preferred for beekeeping as swarming and absconding are more during the honey flow season and they sting more profusely and their stings are more painful than that of *A. indica*. Due to these undesirable traits, bee keepers generally do not utilise this species for beekeeping. The ability of *A. karinjodian* **n. sp.** to produce higher quantities of honey, which is thicker in consistency, has been noted by bee-keepers. This could be due to the ability of *A. karinjodian* **n. sp.** to exploit diverse floral resources and their stronger fanning ability that ripens honey. When a sufficiently strong beehive is opened, the bees get disturbed and their restless movement inside and outside the colony can be easily noticed (Fig. 8A). Since the honey produced seems to be of better quality, the potential of beekeeping with *A. karinjodian* **n. sp.** should be explored.

Etymology: The specific epithet '*karinjodian*' literally means black honey bee in the vernacular local language, Malayalam. The species name is a noun in apposition. The common name, 'Indian black honey bee' is coined for the new species.

Apis indica Fabricius, 1798 Status Restored

(Figs. 1 M–R; 2 A, B; 3 E–G; 6 C, D; 7 E–H; 9 A–L)

Fabricius, 1798: 274.

Lindauer and Kerr (1960) gave systematic priority to *A. cerana* Fabricius, 1793 and treated the Indian bee as *A. cerana indica* which, according to them, is a valid subspecies, along with the conspecific nominotypical subspecies *A. cerana cerana*. The same synonymy as well as subspecific epithet has been followed by most workers (Ruttner, 1988; Engel, 1999; Radloff *et al.,* 2010) for *A. indica* Fabricius, 1798 till date.

Based on the Radio-Medial index (RMI) of 4.0 on the right forewing and 3.8 on the left forewing of the Fabrician type of *A. indica* (Figs. 2 A, B) and the calculated value (1.9–4.2) from the material examined, the name *A. indica* Fabricius, 1798 is here resurrected.

Type material: Type image: ♀(worker): Labels: (1) indi-/ca; (2) NHMD 308727 Figs. 2 A–B (NHMD, Copenhagen).

Type locality: INDIA: Tamil Nadu, Tharangambadi.

Material examined [112 nos] : 3♀(worker) each: Kerala, Ernakulam, Kannur, Kasargod, Kottayam, Kozhikode, Malappuram, Palakkad, Pathanamthitta, Thrissur, Mashhoor, K. coll. 1-III-2022 to 5-III-2022. 9♀(worker): Kerala, Wayanad, Shanas. S. coll, 22-XII-2019; 10♀(worker): Kerala, Idukki, Thoppipala, Shanas, S. coll, 14-II-2019; 10♀ (worker): Kerala, Idukki, Marayoor, Shanas, S. coll, 14-II-2019; 30♀ (worker): Kerala, Thiruvananthapuram, Attingal, Shanas, S. coll. 25-X-2021; 10 ♂: Kerala, Thiruvananthapuram, Attingal, Shanas, S. coll. 20-II-2022; 1♀(worker): Nagaland, Nagaland University, SASRD, Medziphema, Shanas, S. coll. 9-III-2019; 2♀ (worker): Odisha, Bhubaneswar, Shanas, S. coll. 12-III-2019; 1♀ (worker) Type image: INDIA: Tamil Nadu, Tharangambadi: Label: NHMD 308727; 2♀(worker): Tamil Nadu, Madurai, Anju

Krishnan, G coll. 14-IV-2022; 10♀(worker): West Bengal, Jayanagar (Nr. Sundarban), Shanas, S. coll. 11-III-2019.

Distribution: INDIA: Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, Telangana, Odisha, West Bengal, Nagaland, Andaman and Nicobar Islands; Sri Lanka.

The distribution range has been estimated based on the material examined, mt.DNA sequences (Table 2) and the estimated range.

Conservation status: Data Deficient (DD)

Remarks: Fabricius (1798) mentions the collector as Daldorff. According to Holthuis (1986), the collector Ingobert Karl Daldorff, a Danish officer, was stationed in Tranquebar (S.E. India, 11°02'N; 79°51'E) from 1790 to 1793. The former Danish colony, "Tranquebar" refers to Tharangambadi in Tamil Nadu, India. Hence, the type locality is here fixed as Tharangambadi in Tamil Nadu.

Apis cerana Fabricius, 1793

(Figs. 1. A–F; 2. E, F; 3. C, D; 6. E–F; 7. I–L; 10. A–L)

Type material: Lectotype image: ♀ (worker): Labels: (1) *a: cerana* / Lectotype/ x *Apis* Moure 58; (2) TYPE; (3) ZMUC00241552 (Fig. 2 E–F) (ZMUC).

Material examined [50 nos]: INDIA: 6♀ (worker): Assam, Digant, K. coll, 22-II-2022; 1♀ (worker): New Delhi, Shanas, S. coll, 25-III-2018; 8♀ (worker), 9♂: Uttarakhand, Tanakpur, Bablu, P. coll, 14-II-2022; 2♀ (worker): Maharashtra, Pune, Shanas, S. coll, 2-II-2019; 12♀ (worker): Maharashtra, Mumbai, Johnson. coll, 18-II-2022; 7♀ (worker): Nagaland, Nagaland University, SASRD, Medziphema, Shanas, S. coll. 9-III-2019; 3♀(worker): Tamil Nadu, Madurai, Anju Krishnan, G coll. 14-IV-2022; 2♀ (worker): West Bengal, Mohanpur, Nadia, Shanas, S. coll. 11-III-2019 (S. Shanas personal collection).

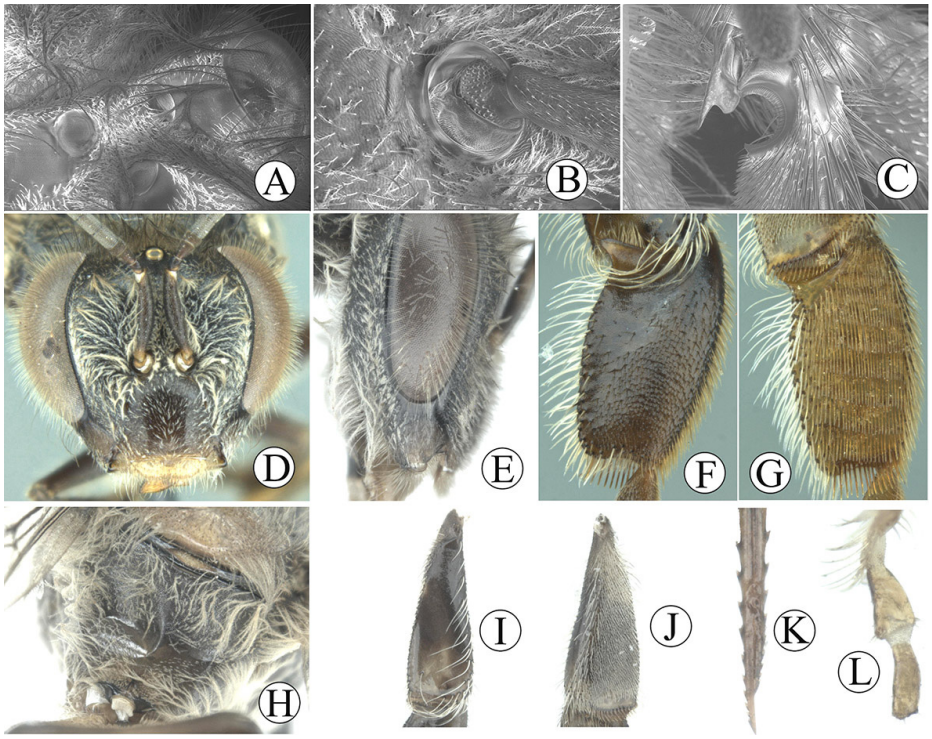


Fig. 9 Workers of *Apis indica* Fabricius, 1798: A. vertex of head; B. antennal socket; C. notch and velum of foreleg; D. frontal view of head; E. malar area of face; F. outer surface of hind basitarsus; G. inner surface of hind basitarsus; H. Propodeum; I. outer surface of hind tibia; J. inner surface of hind tibia; K. sting; L. labial palp

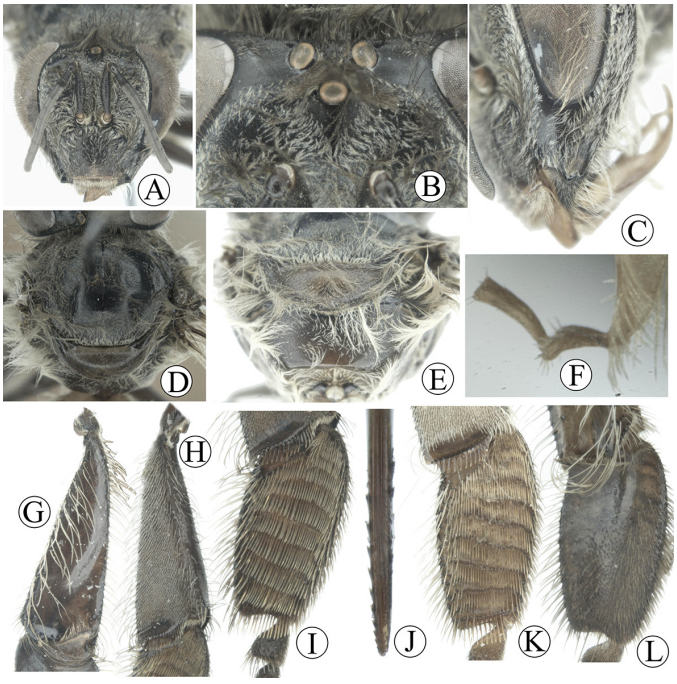


Fig. 10 Workers of *Apis cerana* Fabricius, 1793: A. frontal view of head; B. vertex of head; C. malar area of face; D. dorsal view of mesoscutellum and scutellum; E. Propodeum; F. labial palp; G. outer surface of hind tibia; H. inner surface of hind tibia; I. inner surface of hind basitarsus; J. sting; K. inner surface of hind basitarsus; L. outer surface of hind basitarsus

Distribution: Asia (South-West, South, East, South-East), Russian Far-east, Australia

Conservation status: Least Concern (LC)

Apis mellifera Linnaeus, 1758

(Figs. 11 A–D)

Material examined [5 nos] : 3♀ (worker): Kerala, Trivandrum, Rajan Nadar, K. C coll. 15-III-2022; 2♀ (worker): Nagaland, Nagaland University, SASRD, Medziphema, Shanas, S. coll. 9-III-2019 (S. Shanas Personal Collection).

Key to the species of native cavity nesting honey bees of the Indian Subcontinent

1. RMI of worker forewing = 1.9–4.2 (Figs 3E, 3G); Drone: Scutellum yellowish black (Fig 7G) [COI barcode: GenBank Accession Number. OP168348] .....……*Apis indica* Fabricius, 1798.

— RMI of worker forewing = 4.7–6.4 (Figs 3A, 3C, 3H); Drone: Scutellum black (Figs 7C, 7K) ……2

2. Drone: POL < 4x LOL; Worker: Propodeum without prominent 'V' shaped projection (Fig 10E) [COI barcode: GenBank Accession Number. OP168351]...…...… *Apis cerana* Fabricius, 1793.

— Drone: POL > 4x LOL; Worker: Propodeum with prominent 'V' shaped projection (Figs 4D–F) [COI barcode: GenBank Accession Number. OP071087]…..……. *Apis karinjodian* **sp. n.**

DISCUSSION

The calculated RMI value of the right and left forewings of the Fabrician Type NHMD308727 (Figs. 2A, B) is 4.0 and 3.8 respectively (Table 1), which confirms the specimen as *A. indica* Fabricius, 1798. The calculated RMI value obtained for the right wing of non-type NHMD308728 (Fig. 2C) and the left wing of non-type NHMD308729 (Fig. 2D) is 3.8, which also confirms the non-type specimens as *A. indica* Fabricius, 1798.

Out of the 40 synonyms of *A. cerana* enlisted by Radloff *et al.* (2010), five type localities are from

India (excluding the Himalayan region): *A. indica* Fabricius, 1798 ('Tharangambadi' in Tamil Nadu); *A. socialis* Latreille, 1804 (Bengal), *A. perrottetii* Guérin-Méneville, 1844 ('Neelgherries', Tamil Nadu); and *Apis delessertii* Guérin-Méneville, 1844 (Pondicherry). Among these, only *A. perrottetii* Guérin-Méneville, 1844 has been reported to inhabit the Western Ghats (Nilgiris), which falls inside the geographic range of *A. karinjodian* **n. sp.**

Guérin-Méneville (1844) described *A. perrottetii* and *A. delessertii* without comparing with *A. indica*, Fabricius, 1798. Guérin-Méneville briefly mentioned about *A. indica* Fabricius, seen in Bosc's collection being similar to *A. zonata* (currently treated as a synonym of *A. dorsata*). Smith (1857) considered the specimen from Borneo (Sarawak) though paler, to be *A. perrottetii* Guérin-Méneville, 1844. He later synonymized *A. perrottetii* with *A. indica* Fabricius, 1798 (Smith, 1865). In the description of *A. perrottetii* Guérin-Méneville, 1844, the species is reported to have its entire front of first segment of abdomen yellow and its protruding part entirely brown. However, the proximal part of first segment of the abdomen appears black in all observed specimens of *A. karinjodian* **n. sp.** (Figs. 1G–I, 4G–I); which clearly distinguishes it from *A. perrottetii* Guérin-Méneville, 1844.

Although *A. cerana* Fabricius, 1793 and *Apis indica* Fabricius, 1798 are being treated as synonyms (Lindauer and Kerr, 1960), confusion still persists as the widely accepted name, *Apis indica*/ *Apis cerana indica*/ *Apis cerana* based on which, most publications are authored in India. Engel recognized two subspecies of *Apis cerana* which occur in India, of which, the plains bee taxonomically corresponds to the subspecies *Apis cerana indica* Fabricius, 1798 while the hills bee appears to be *A. cerana cerana* Fabricius, 1793 (Engel, 1999, 2002).

Several indices of wing venation were introduced (Louis 1963; Goetze 1964). All of these indices, except the very important Cubital Index (CI), became obsolete since the introduction of venation angles (Ruttner, 1988).

Cubital Index (Fig. 3H) in this work, is calculated as per Ruttner (Fig. 6.8, 1988). It was observed that, the cubital index was not useful to discriminate between representative populations of *A. indica* (CI=2.1–4.2) and *A. cerana* (CI=3.1–5.2) effectively. However, the c/b ratio (Fig. 3H), defined herein as the Radio-Medial Index (RMI), gave an accurate measure for discriminating populations of *A. indica* and *A. cerana* + *A. karinjodian* **n. sp**. The RMI, to the best of our knowledge, has never been used earlier for morphometric discrimination of *Apis* species. The closest index to RMI ever used as per literature, is the dumb-bell index (Fig. 4.7, Goetze 1964). The calculated dumb-bell index for *A. indica* (0.5–1.2) and *A. cerana* (1.0-1.2) did not help in discriminating these species.

The RMI, in our opinion, provides higher resolution and may be used along with the Cubital Index (CI) for discriminating sufficiently diverged *Apis* species confined to the subcontinental boundaries. The RMI

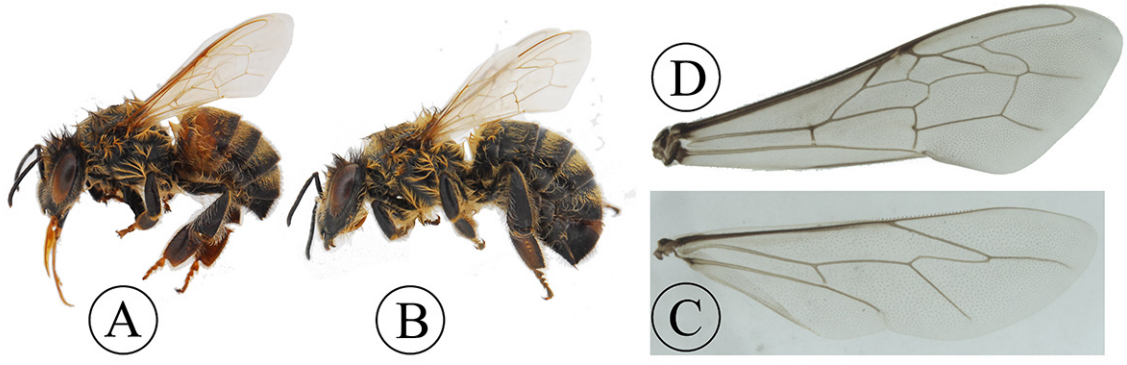


Fig. 11 Worker. A–*Apis mellifera;* B–*A. mellifera carnica,* C– hindwing, D–forewing

Table 1**. Forewing index ratios of cavity nesting honeybees of the Indian subcontinent**

Forewing Index (Fig 3H)	*A. indica* (Type)	*A. cerana* (Lectotype)	*A. indica*	*A. cerana*	*A. karinjodian*	*A. mellifera*
Cubital index CI= a/b	4.2 (RW) 3.4 (LW)	3.4 (LW)	2.1-4.2	3.1-5.2	4.6-5.4	2.3-2.9
Radio-Medial index RMI= c/b	4.0 (RW) 3.8 (LW)	5.0 (LW)	1.9-4.2	4.7-6.4	5.4-6.4	3.5-3.8

RW= Right wing; LW= Left wing (Figs 2A, 2B)

Table. 2. The COI and COII region of the mtDNA examined in this study from NCBI Genbank.

COI			COII		
Apis cerana Fabricius 1793	*Apis indica* Fabricius 1798	*Apis karinjodian* n. sp	*Apis cerana* Fabricius 1793	*Apis indica* Fabricius 1798	*Apis karinjodian* n. sp
KF760518(IN)KA	KC414930(IN)KA	MH588669(IN)KA	KF497250(IN)KA	KF497249(IN)KA	KF497265(IN)KA
KF760521(IN)KA	KF760519(IN)KA	MH588653(IN)KA	KF497251(IN)KA	KF497252(IN)KA	to
KF861941(IN)KA	KF760523(IN)KA	MH588675(IN)KA	KF497261(IN)KA	to	KF497275(IN)KA
KM495732(IN)KA	to	KU963189(IN)KA	KF497277(IN)KA	KF497260(IN)KA	KF497293(IN)KA
KM495733(IN)KA	KF760527(IN)KA	KR010696(IN)KA	to	KF497262(IN)KA	to
KM591907(IN)KA	KJ139456(IN)KA	KF760522(IN)KA	KF497292(IN)KA	to	KF497296(IN)KA
KM591908(IN)KA	KM230116(IN)KL	KF760520(IN)KA	KF497297(IN)KA	KF497264(IN)KA	KF497298(IN)KA
KM591909(IN)KA	KM495728(IN)KA	**7**	KF497299(IN)KA	KF497276(IN)KA	KF497510(IN)TN
KM610315(IN)KA	KM495730(IN)KA	OK465105(IN)HP	KF497300(IN)KA	KF497301(IN)KA	KF497550(IN)KL
KM610318(IN)KA	KM495731(IN)KA	OK483361(IN)HP	KF497306(IN)KA	to	KF497586(IN)GA
KM610319(IN)KA	KM593931(IN)KA	OK602702(IN)HP	KF497307(IN)KA	KF497305(IN)KA	KF497587(IN)GA
KM610320(IN)KA	to	OK626675(IN)HP	KF497309(IN)JK	KF497308(IN)KA	**20**
KP255460(IN)MH	KM593939(IN)KA	OK626676(IN)HP	to	KF497425(IN)AP	
to	KM610316(IN)KA	OK626762(IN)HP	KF497368(IN)JK	to	KF497509(IN)TN
KP255467(IN)MH	KM610317(IN)KA	OK626764(IN)HP	KF497369(IN)AS	KF497432(IN)AP	KF497511(IN)TN
KT960839(IN)PB	KU963191(IN)KA	OK626778(IN)HP	to	KF497434(IN)AP	to
KU212336(IN)MI	KX587509(IN)KL	OK626780(IN)HP	KF497377(IN)AS	KF497436(IN)AP	KF497534(IN)TN
to	MH331013(IN)KL	OK632479(IN)HP	KF497380(IN)AS	KF497442(IN)AP	KF497545(IN)KL
KU212341(IN)MI	MH588650(IN)KA	OL436247(IN)HP	to	to	to
KU963187(IN)KA	to	OL457389(IN)HP	KF497392(IN)AS	KF497478(IN)AP	KF497549(IN)KL
KU963188(IN)KA	MH588652(IN)KA	OL468548(IN)HP	KF497393(IN)ME	KF497480(IN)AP	KF497551(IN)KL
KU963190(IN)KA	MH588654(IN)KA	OL589569(IN)HP	to	KF497485(IN)TN	to
MH588658(IN)KA	to	OL589591(IN)HP	KF497396(IN)ME	to	KF497560(IN)KL
MH588661(IN)KA	MH588657(IN)KA	OL639224(IN)HP	KF497397(IN)AR	KF497496(IN)TN	KF497562(IN)KL
to	MH588659(IN)KA	OM319700(IN)HP	to	KF497500(IN)TN	to
MH588668(IN)KA	MH588660(IN)KA	OM320364(IN)HP	KF497404(IN)AR	KF497505(IN)TN	KF497585(IN)KL
MH588670(IN)KA	MH588671(IN)KA	OM320444(IN)HP	KF497405(IN)AS	to	KF497588(IN)GA
MH588672(IN)KA	MH588673(IN)KA	OM321429(IN)HP	to	**150**	
MH588674(IN)KA	MH682148(IN)KA	OM766175(IN)MI	KF497412(IN)AS	KF497479(IN)AP	KF497544(IN)TN
MK904657(IN)WB	MW093739(IN)TN	OM766178(IN)MI	KF497413(IN)ME	KF497481(IN)AP	KF497561(IN)KL
MK904727(IN)WB	**39**	ON331706(IN)PB	to	to	KF497589(IN)MH
MK904728(IN)WB	MT027905(IN)HP	ON506013(IN)UT	KF497420(IN)ME	KF497484(IN)AP	to
MK904731(IN)WB	MT027915(IN)HP	M6Z558042(BD)	KF497421(IN)AS	KF497497(IN)TN	KF497648(IN)MH
to	to	M7Z558043(BD)	to	to	
MK904735(IN)WB	MT027917(IN)HP	MZ558037(BD)	KF497424(IN)AS	KF497499(IN)TN	
MK904739(IN)WB	MT027919(IN)HP	MZ558038(BD)	KF497433(IN)AP	KF497501(IN)TN	
MK904756(IN)WB	to	MZ558039(BD)	KF497435(IN)AP	to	
MK904774(IN)WB	MT027922(IN)HP	MZ558040(BD)	KF497437(IN)AP	KF497504(IN)TN	
MN242984(IN)KA	OK287086(IN)HP	MZ558041(BD)	to	KF497535(IN)TN	
MT027904(IN)HP	OK310864(IN)HP	KY834222(PK)	KF497440(IN)AP	to	
97			**227**		
TOTAL: 97+39+7+227+150+20 = **540**					

Country Abbreviations: India (IN); Bangladesh (BD); Pakistan (PK)

India State Codes: AP: Andhra Pradesh, AR: Arunachal Pradesh, AS: Assam, GA: Goa, HP: Himachal Pradesh, JK: Jammu and Kashmir, KA: Karnataka, KL: Kerala, ME: Meghalaya, MH: Maharashtra, MI: Mizoram, PB: Punjab, TN: Tamil Nadu, UT: Uttarakhand, WB: West Bengal.

NCBI Genbank access date: until 20/6/2022

and CI values should always be given as ranges for accuracy rather than a single average value that may lead to misidentifications.

With regard to occurrence of colour morphs of yellow and black bees in south India, especially in Bengaluru, contrary to the popular belief that the occurrence of intermediate colour morphs, suggest the absence of mating barrier among both colours (Viraktamath *et al.,* 2013) and migratory beekeeping being an exclusive reason for the merger of black and yellow strains, which could have led to genetic recombination between the strains (Chalapathy *et al.,* 2014a), it is evident that black as well as yellow colour morphs are present in both *A. cerana* and *A. indica* populations in Karnataka (in Chalapathy *et al.,* 2014a) as well as Tamil Nadu (in Chalapathy *et al.,* 2014b). In a study undertaken by Chalapathy *et al*. (2014a) in Karnataka, ACBLR COIB refers to black *A.cerana* from Bengaluru (Table 2, KF760518) and ACBLR COIY refers to yellow *A. indica* from Bengaluru (Table 2, KF760519). The presence of black *A. indica* (Table 2, KF760523) and yellow *A. indica* (Table 2, KF760524) too is evident elsewhere in Karnataka (Madikeri), which is not too far from Bengaluru, in terms of the species range. Similarly, in a study by Chalapathy *et al.* (2014b) from the Nilgiri Biosphere Reserve, spread over regions of Karnataka, Kerala and Tamil Nadu, presence of black *A. indica* (ACCNRCOI_B) from Tamil Nadu (Coonor) along with the Yellow strains (ACCKHCOI_Y and ACVZTCOI_Y) from Chokkanahalli and Vazhaithottam is evident. Also, presence of yellow *A. cerana* (ACBNGCOI_Y) in Banagudi and Black strains (ACKTGCOI_B and ACOTYCOI_B) in Kotagiri and Ooty in Tamil Nadu are evident.

Light (yellow) and dark (black) colour morphs occur among *A. cerana* and *A. indica* populations in south India (Fig. 1). They appear yellow or black to the unaided human eye due to the light yellow (Figs. 1B, 1N) as well as dark colours (Figs. 1A, 1G, 1M) on their scutellum and abdomen. Hence, we cannot distinguish species only based on body colouration. In the south Indian states of Kerala, Tamil Nadu and Karnataka, the presence of the Indian black bee (*A. karinjodian* **n. sp**.) in the Western Ghats region adds to this conundrum. The black and yellow bees seen in Bengaluru are exclusively a mixture of *A. cerana* and *A. indica* populations. The Indian black honey bee (*A.karinjodian* **n. sp**.) may not be present in Bengaluru. The bees encountered there are mostly *A. cerana* with a dark scutellum (Fig 1A) or light yellow scutellum (Fig. 1B) and *A. indica* with a dark scutellum (Fig. 1M) or light yellow scutellum (Fig. 1A).

Lo *et al.* (2010) had supported recognition of *A. indica*, the plains honey bee of south India, as a separate species from *A. cerana*. Extensive differentiation between two forms of honey bees, not physically separated by any substantial barriers, almost certainly indicates that they are reproductively isolated and consequently distinct species and the only data available to address this question other than morphological differences are the timing of mating flights of drones (Otis, 1996). Oldroyd *et al.* (2006) proved that the yellow plain bees of India could be regarded as a separate species from *A. cerana* based on non-overlap of drone flight times and occurrence of consistently different mitochondrial haplotypes. A similar study by Hadisoesilo and Otis (1996) confirmed the species status of *A. nigrocincta* Smith, 1860, a species distinct from *A. cerana* F., 1793, by drone flight times in Sulawesi, Indonesia.

Hence it is emphasized that *A. cerana* Fabricius, 1793 and *A. indica* Fabricius, 1798 are distinct valid species and they do not interbreed in nature.

Male genitalia

The anatomy of uneverted endophallus of drones were studied. It was observed that the endophallus of *A. karinjodian* **n. sp.** displayed three lobed dorsal cornua with a prominently large round lobe (0.8mm, Fig.7D). *Apis cerana* Fabricius, 1793 seems to possess a comparatively smaller lobe on dorsal cornua (0.5mm, Fig. 7L). It was also observed that the endophallus of *A. indica* Fabricius , 1798 had a comparatively small dorsal cornua (0.3mm, Fig. 7H).

Table 3. Details of *Apis* species and its CO1 partial coding sequence generated in the study

No	Species	Voucher No.	GenBank Accession No.	Collection location
1	*A. karinjodian* **n. sp**.	ACKWD1	OP071087	Wayanad, Kerala, India
2	*A. karinjodian* **n. sp**.	ACKWD2	OP068196	Wayanad, Kerala, India
3	*A. karinjodian* **n. sp**.	ACKID1	OP071086	Idukki, Kerala, India
4	*A. karinjodian* **n. sp**.	ACKID2	OP161981	Idukki, Kerala, India
5	*A. karinjodian* **n. sp**.	ACKID3	OP161980	Idukki, Kerala, India
6	*A. indica* F., 1798	ACIWB1	OP168188	West Bengal, India
7	*A. indica* F., 1798	ACITVM1	OP168315	Trivandrum, Kerala, India
8	*A. indica* F., 1798	ACIID4	OP168348	Idukki, Kerala, India
9	*A. indica* F., 1798	ACIOR1	OP168349	Odisha, India
10	*A. cerana* F., 1793	ACCNG1	OP168351	Nagaland, India
11	*A. cerana* F., 1793	ACCNG2	OP168371	Nagaland, India

Table 4. Nucleotide frequencies of CO1 sequence of *A. karinjodian* **n. sp.**, *A. cerana* and *A. indica*

Name of Species	T%	C%	A%	G%
A. karinjodian **n. sp**.	40.90	15.80	33.70	9.60
A. cerana Fab., 1793	41.70	15.10	33.70	9.50
A. indica Fab., 1798	43.20	13.60	33.70	9.50

Viraktamath (2015) undertook a pioneering study of comparative morphometry of drones of all the three species of honey bees (*Apis cerana*, *A. dorsata* and *A. florea*) known to occur in India from seven states and concluded that, the genitalia of drone of each species of honey bee are distinct but the genitalial structures within the species varied. The results of scatter plot (Fig. 1 in Viraktamath, 2015) indicates that, the cluster 1, 2 and 3 containing drones of *A.cerana* from Jammu & Kashmir, Assam and Karnataka seems to denote *A. cerana* Fabricius, 1793 and the cluster 4 containing drones of *A.cerana* from Andhra Pradesh, Karnataka, Kerala, Maharashtra and Tamil Nadu seems to denote *Apis indica* Fabricius,1798.

Molecular Analysis

The CO1 sequences generated in this study were submitted to the NCBI- GenBank and the accession numbers are given in Table 3.

Table 5. Evaluation of evolutionary divergence between CO1 partial coding sequences of native cavity-nesting honey bees *Apis* spp.

No	Name of Species	1	2	3	4	5	6	7	8	9	10
1.	*A. karinjodian* ACKWD1										
2.	*A. karinjodian* ACKWD2	0.006									
3.	*A. karinjodian* ACKID1	0.016	0.010								
4.	*A. karinjodian* ACKID2	0.006	0.000	0.010							
5.	*A. karinjodian* ACKID3	0.012	0.010	0.012	0.010						
6.	*A. cerana* ACCNG1	0.030	0.028	0.022	0.028	0.028					
7.	*A. cerana* ACCNG2	0.030	0.028	0.022	0.028	0.028	0.004				
8.	*A. indica* ACIWB1	0.056	0.054	0.050	0.054	0.059	0.056	0.057			
9.	*A. indica* ACIID4	0.054	0.052	0.048	0.052	0.056	0.054	0.054	0.006		
10.	*A. indica* ACIOR1	0.065	0.063	0.059	0.063	0.068	0.066	0.066	0.008	0.014	
11.	*A. indica* ACITVM1	0.054	0.052	0.048	0.052	0.056	0.054	0.054	0.006	0.000	0.014

Multiple sequence alignment using CLUSTAL omega revealed the percentage of intraspecific conserved sites in the COI gene of Indian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica*. The COI conserved nucleotide sites observed in *A. karinjodian* **n. sp**., *A. cerana* and *A. indica* are 95.42, 92.37 and 97.53 per cent respectively.

The COI sequence of *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica* exhibited bias to nucleotides A and T (Table 4). *Apis indica* has high AT content (76.90%) followed by *A. cerana* (75.40%) while *A. karinjodian* **n. sp.** has less AT content (74.60%) when compared to the other two species.

Evolutionary divergence estimation (Table 5) clearly depicts the degree of divergence between the Indian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica*. The overall average divergence within populations of *A. karinjodian* **n. sp.** was 0.009. The degree of divergence of the CO1 partial coding sequence of *A. karinjodian* **n. sp.** was high with *A. indica* than with *A. cerana*. The mean divergence of *A. karinjodian* **n. sp.** with *A. cerana* was 0.0275 and with *A. indica* it was recorded at 0.0557. *Apis cerana* exhibited 0.0578 mean divergence with *A. indica*.

The phylogenetic trees (Figs. 12A-C) depict the phylogenetic relationship of the Asian cavity-nesting honey bees and the phylogenetic position of *A. karinjodian* **n. sp**. The out group, *A. florea* was placed at the base of the tree and the Asian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana, A. indica, A. koschevnikovi, A. nigrocincta and A. nuluensis* formed separate clads in the phylogenetic tree (Fig. 12A). The trees confirm that all cavity nesting honey bees analyzed here are monophyletic and it is also noted that the Indian cavity-nesting honey bees *A. karinjodian* **n. sp**., *A. cerana,* and *A. indica* diverged from a common ancestor. The new species *A. karinjodian* **n. sp.** formed a sister clad to *A. cerana* with a strong support of bootstrap value 99 per cent.

The Indian black bee, *A. karinjodian* **n. sp.** has evolved from *A. cerana* morphotypes which got acclimatized to the hot and humid environments surrounding the Western Ghats. Apparently, the sympatric origin of the species could have been facilitated by the lower temperature and the abundant untapped floral resources in the humid and moderate environments surrounding the Western Ghats range of mountains. The high humidity and moderate temperatures appear to be the limiting factors those confine *A. karinjodian* **n. sp.** to the Western Ghats region, while low humidity seems to be the favoring factor restricting *A. cerana* populations from spreading into the Western Ghats, whereas, extremely low winter temperatures seem to be the limiting factor preventing the spread of *A. indica* towards the central and northern India.

It is interesting to note that the RMI of the European honey bee *A. mellifera* (3.45–3.8), falls within the RMI range of *A. indica* (1.9–4.2), suggesting a possible synapomorphy. This indicates that the ancestral population of *A. mellifera* could possibly have descended from ancestral *A. indica,* rather than ancestral *A. cerana*. Hence, based on this single synapomorphy, phylogenetic affinity (Fig.12B) and the occurrence of *A. indica* populations spread over most of south India, it is proposed that the center of origin of the ancestral clade of the European honey bee *A. mellifera*, could possibly be south India. The origin of *A. mellifera* seems to be from the ancestral

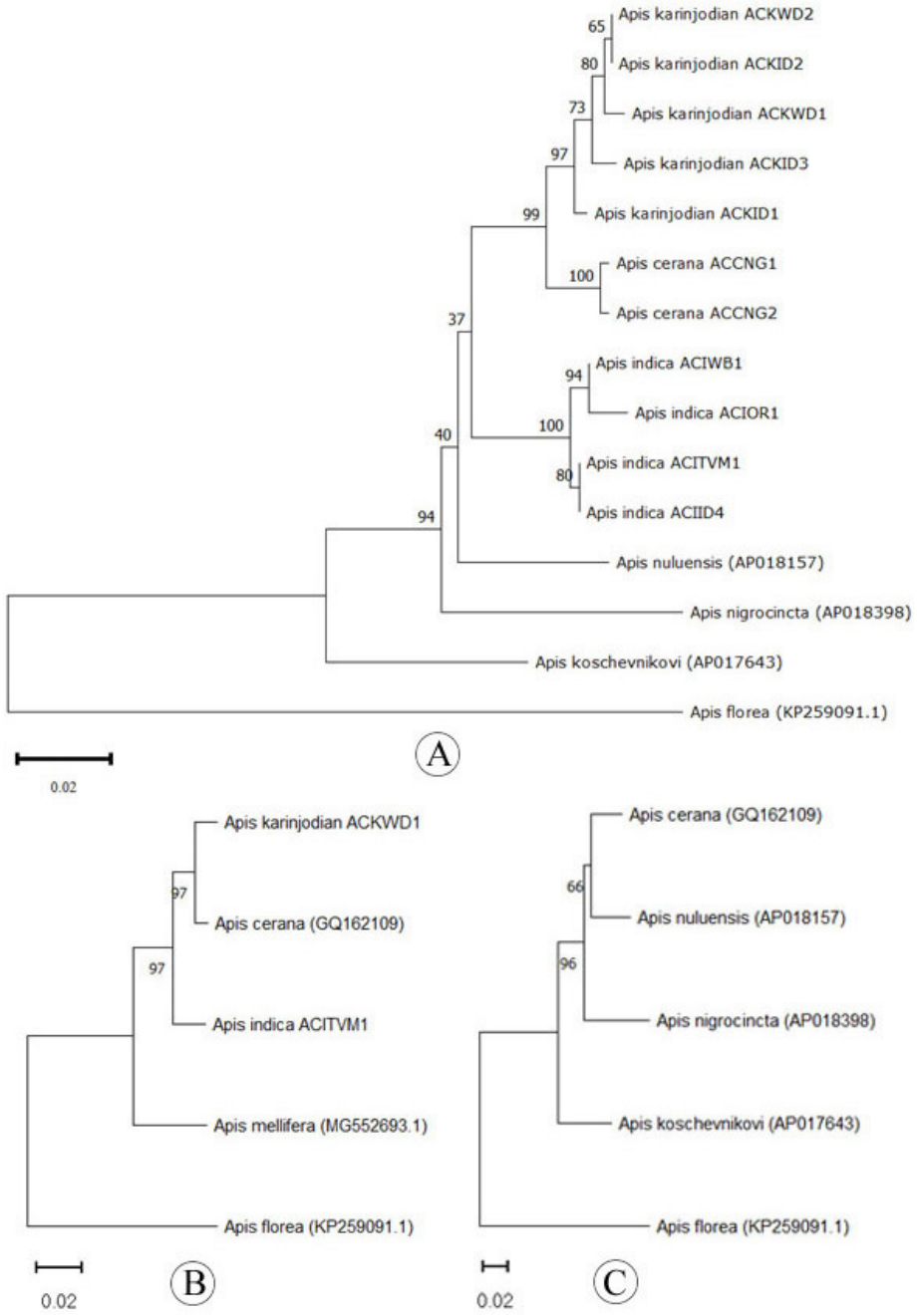


Fig. 12 Neighbor-joining trees (A–C) depicting the evolutionary relationship of cavity nesting honey bees. B: western population, C: eastern population. NJ bootstrap values are shown near the branches

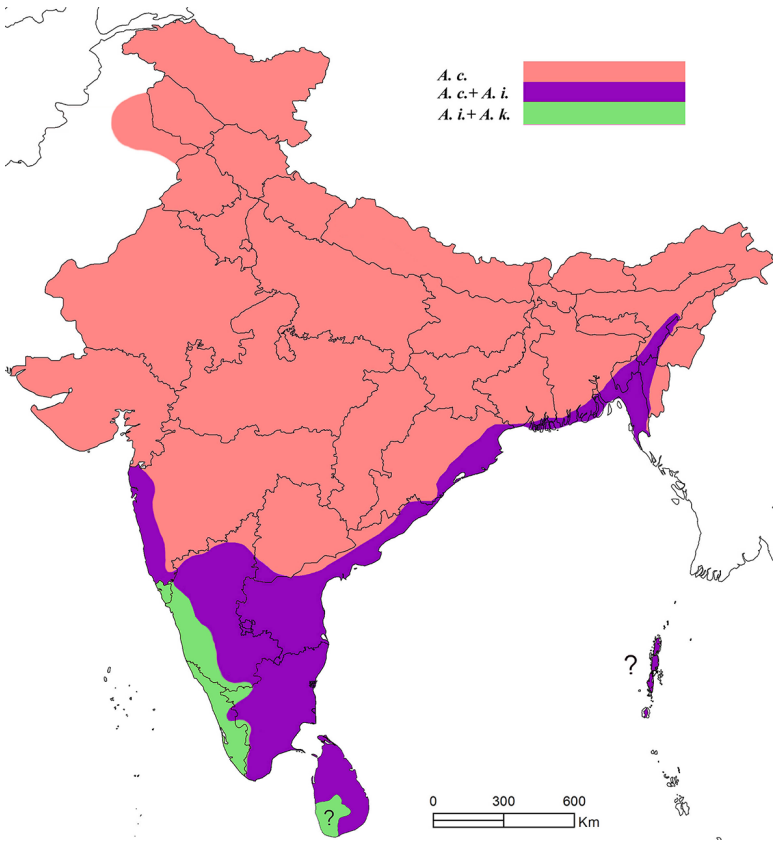


Fig. 13 Distribution of the cavity nesting honey bees of the Indian subcontinent. Abbreviations: *Ac*: *Apis cerana* Fabricius, 1793; *Ai*: *Apis indica* Fabricius, 1798; *Ak*: *Apis karinjodian* **n. sp**

A. indica + *A. mellifera* morphotypes (IM morphotypes) inhabiting the moist evergreen forests of Peninsular India during the late Miocene. The IM morphotypes from peninsular India could have reached the present day Iran and the Arabian Peninsula taking the coastal route along the Arabian Sea. As the ancestral origin of contemporary *A. mellifera* lineage remains unresolved (Dogantzis *et al.*, 2021), the findings narrow down to the hypothesis of an Indian origin of *A.mellifera*.

Ecological segregation has played the major role in the origin of *A. indica* and *A. karinjodian* **n. sp.** in south India whereas allopatric speciation seems to be the dominant factor responsible for the orgin of *A. koschevnikovi* and *A. nigrocincta* in south east Asia.

Distribution

The distribution map (Fig. 13), being the first baseline map of cavity nesting honey bees of India, will serve the purpose of future ground surveys for demarcating the accurate species limits in south, south-east and north east India. Most of the sequence data available through NCBI GenBank (Table 2) only mentions the particular state due to which, the exact species location inside the state could not be plotted. The first confirmed reports of *Apis indica* from Nagaland, Odisha and West Bengal are based on specimens obtained by field collection. Its distribution along the eastern coastal planes could be due to the moderate range of mean temperature prevailing in the coastal region.

Apis cerana, the eastern honey bee, is the most widespread among the cavity nesting honey bees occurring in the Oriental region (Radloff *et al.*, 2010). It is omnipresent in India (Fig. 13) as, out of 324 COI sequences of *Apis cerana* analysed (Table 2), it is inferred that its distribution ranges from Pakistan (Islamabad) in West, to Bangladesh and Assam in the East and Jammu and Kashmir in the North to Kerala and Tamil Nadu in south India. The sequence KF497561 (Table 2) confirms the

presence of *Apis cerana* in Kerala. In a study by Baskaran (2016), based on sequence analysis of intergenic region between CO1 and CO II of mitochondrial DNA, the specimens obtained from Perambalur, Pichavaram, Paramakudi and Mayiladuturai in Tamil Nadu appears to be *A. indica*; specimens from Kodaikanal and Mudhumalai belongs to *A. cerana* and a specimen from Udhagai seems to be *A. karinjodian* **n. sp.**, thus confirming the presence of all three species in Tamil Nadu and also the presence of *A. cerana* near to borders of Kerala state.

It is interesting to note that, all 68 sequences available from Maharashtra (Table 2) belong to *Apis cerana* and a study by Gaikwad *et al.* (2019), wherein the sampling was carried out from Bhimashankar in northern Maharashtra to Mahabaleshwar and Wai in the southern part of Maharashtra (Table 2 KP255460 to KP255467), has confirmed *Apis cerana* as the only species present. These results confirm that, *A. cerana* is the only species encountered in Maharashtra state and it is the only species encountered in the northern Western Ghats region beyond Goa as well. The sequence KF497588 confirms the presence of *A. indica* in Goa. Hence, there is all possibility of *A. indica* inhabiting the Northern Western Ghats region of Maharashtra from Goa, along the coastal stretch up to northern limits of Maharashtra bordering Gujarat. The confirmed presence of "western" form in Andaman Islands (Smith and Hagen, 1996), which was probably introduced from Mumbai ("Bombay"), also points to the presence of *A. indica* in Maharashtra. The species complex occurring in the Andaman Islands is presently unknown.

The northern range limit of *A. indica* seems to lay at upper state boundary limits of Karnataka which is inferred from the study undertaken by Chalapathy *et al.* (2014a, Fig. 3) where ACRCH_COIY from Raichur pools together with ACMLR_COIY: KF760525 and ACBLR_COIY: KF760519 which are confirmed as *A. indica* sequences.

Apis indica is the dominant species occurring in Kerala followed by *A. karinjodian* **n. sp.** and *A. cerana*. The three species are present in Tamil Nadu and Karnataka as well. Further, statewide distribution of any species is not being attempted here for the lack of clarity on the specimens collected (managed / feral / field collected) and lack of details on exact place of collected samples in the NCBI GenBank database (Table 2).

Based on non-coding region of mitochondrial DNA sequence data, Smith and Hagen (1996) divided *A.cerana* into "western" form which was found in India, Sri Lanka and the Andaman Islands, and the "eastern" form found in all other localities. The western form refers to *A. indica* Fabricius, 1798, which confirms its presence in Sri Lanka and the Andaman Islands. Lo *et al.* (2010) indicated the possibility of existence of *A. indica* in Sri Lanka as well. The presence of *A. cerana* in Sri Lanka is also evident from Tan *et al.* (2008, Fig. 1), wherein the wing RMI index can be calculated as 4.8 which falls within the range of *A.cerana* (Table 1). It is highly possible that *A.indica* and *A. cerana* occur as sympatric populations in Sri Lanka as well as *A. karinjodian* **n. sp.** present in the south west evergreen forests of Sri Lanka since the two land masses were connected during the Pleistocene.

The mention by Smith and Hagen (1996) about the presence of "eastern" form of *A. cerana* in Nepal and sequences KT174434, KT174435, KT174436 and KT174437 (Tan *et al.,* 2016) confirm the presence of *A. cerana* in Nepal. Nidup and Dorji (2016) reported *A. cerana* to be very common in Bhutan as well. A study in Bangladesh (Riaz *et al.*, 2021) reported only sequences of *A. cerana* and all sequences obtained from West Bengal and the north eastern states of Arunachal Pradesh, Assam, Meghalaya and Mizoram confirm the presence of only one species, *A. cerana* in the north eastern states (Table 2).

Rajkumari *et al.* (2020, Table 4), indicate that sympatric populations of *A. cerana* and *A. indica* could be present in the south eastern hill tract and Barak valley since the reported CI values for the region are 3.46±0.26 and 3.63±0.16 respectively, which fall near CI values for *A. indica* (Table 1).

The range of *A. indica* towards the eastern borders has shown to encompass the eastern coastal plains

and the mangrove areas of West Bengal and Bangladesh, since these are the probable areas with moderate temperature fluctuations. Proper collections and study have to be carried out in the eastern coastal states of Andhra Pradesh, Odisha, West Bengal and the Barak valley of Assam, Nagaland, Manipur, Mizoram and Tripura to demarcate species range of *A. indica*.

Bee-keepers select only the less aggressive bees for Apiculture. Hence, the more aggressive and wild populations are left out during routine collection surveys. All the results presently obtained from NCBI GenBank database (Table 2) are probably from specimens collected from managed colonies as these specimens are the most easily obtainable. Only this can explain the stark biased absence of *A. indica* sequences in GenBank from Maharashtra and North Eastern states. This could also be the reason for *A. cerana* as the only species obtained from Maharashtra, especially the sides bordering Western Ghats and Karnataka border. The states of central India and eastern coastal belt are already data deficient.

Hence it is cautioned that, specimen collection during surveys should only be based on field collected material, ideally from honey bees foraging on different flowering plants. This method alone can give the exact species distribution in each locality leading to an authentic distribution mapping for the whole country. Collections based on managed colonies or few feral colonies from anywhere can lead to biased results that may not be useful for accurate distribution mapping and delimiting population ranges thereafter.

It is also emphasized that only reproductively isolated, valid species can coexist as sympatric populations whereas sympatric subspecies can never exist. Hence, designating any valid species as sympatric subspecies is erroneous. Cavity nesting honey bees should ideally be treated as two distinct species groups *viz.* "cerana species group" and "mellifera species group" and sufficiently diverged populations among these species groups which do not display any intermediate haplotypes should be treated as valid species instead of subspecies. Thirty-three distinct honey bee subspecies of *A. mellifera* (Ilyasov *et al.*, 2020) should ideally be reduced to distinct valid species based on this approach or a combination of molecular and morphometric approaches.

The current study has added two more species to the honey bee fauna of the world thus, bringing the total number of valid species to 11 *viz*. the cavity-nesting honey bees: *A. cerana* Fabricius, 1793; *A. indica* Fabricius, 1798; *A. karinjodian* **n. sp**.; *A. koschevnikovi* Enderlein, 1906; *A. mellifera* Linnaeus, 1758; *A. nigrocincta* Smith, 1860; *A. nuluensis* Tingek, Koeniger and Koeniger, 1996; the dwarf honey bees: *A. florea* Fabricius, 1787; *A. andreniformis* Smith, 1857; the giant honey bees: *A. dorsata* Fabricius, 1793 and *A. laboriosa* Smith, 1871.

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Do aphids maintain differential densities on plant parts? A case study with *Aphis craccivora* Koch (Hemiptera, Aphididae)

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ABSTRACT: Differential aggregation of aphids on host plant parts may indicate site-specific optimal densities for efficient utilization of plant parts, besides the usual inference of preferential colonization. Differential densities of aphids within the plant were studied using the legume aphid *Aphis craccivora* Koch in cowpea *Vigna unguiculata* (L.) Walp. ssp. *unguiculata*. In field-collected infested sample stems and pods (n=60), colonies were demarcated, aphid colony size, length and circumference measured, and colony area and density calculated. The results indicated that colony dimensions and colony size were significantly higher in pod than in stem whereas colony density did not differ significantly between the two plant parts. Colony density was significantly higher in leaflets of top most leaf than in leaflets of top 2nd or 3rd leaf. Overall, the four plant parts could be graded in the descending order as stem>pod>leaflets of top most leaf> leaflets of top 2nd or 3rd leaf for colony density. Significant positive curvilinear and linear relationship between colony size and colony density in both stem and pod indicated that *A. craccivora* showed a propensity to spread out colonies at low populations but tended to compact them with a rise in population levels. Identical colony density in stem and pod suggested that the aphid may not require differential densities to overcome host defenses or utilize food from these two plant parts. In top most leaf and top 2nd or 3rd leaf, finite leaflet size apparently limits proliferation of the aphid. Higher density on top immature leaves could be more an outcome of nutritional suitability than the need to overcome host defenses. Variable colony densities on the four parts of *V. unguiculata* indicated differential optimal densities. © 2022 Association for Advancement of Entomology

KEY WORDS: *A*ggregation, cowpea, colony dimensions, optimal density

INTRODUCTION

The concept of optimal density range of organisms on exhaustible units of food resource (Peters and Barbosa, 1977) revolves around the premise that organisms maintain their densities between a minimum and maximum to offset the disadvantages of undercrowding and overcrowding, respectively (Prokopy, 1981). Although proposed for laboratory insect cultures (Peters and Barbosa, 1977), the concept has direct relevance to field populations of several groups of insects (Prokopy, 1981). Limited capability for successful dispersal among resource patches and other causes were suggested to subject

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a species to less selection pressure for early establishment of an optimal density range ultimately favoring a high degree of population clumping (Prokopy, 1981).

As *r*-strategists, the Sternorrhyncha, *e.g.* Aphidoidea, form aggregations on annual plants in short-lived crop systems and show increased fitness within optimal density ranges on host plants (Way and Banks, 1967; Dixon and Wratten, 1971). Aggregation of aphids confers benefits of greater ability to divert host nutrients from other leaves (Way and Cammell, 1970), besides enhanced protection from predators by aphid-tending ants (Nault *et al.,* 1976). Changes in the phloem amino acid composition of host plants induced by two species of aphids appeared to affect systemically at least the whole leaf they were feeding on with likely nutritional advantages for the aphids (Sandström *et al.,* 2000). Aphids are known to display preferences for different organs of the same plant. For example, *Aphis gossypii* G. preferred older leaves of tender plants of aubergine *Solanum melongena* L. (Banerjee and Raychaudhuri, 1985) and was most abundant on the basal part of cantaloupe vines *Cucumis melo* L. (Edelson, 1986). Besides preferential colonization governed by nutritional differences, differential colony size of aphids may indicate maintenance of site-specific optimal density ranges for utilization of plant parts and enhancement of survival and reproduction.

The legume aphid *Aphis craccivora* Koch (Hemiptera, Aphididae) maintains large aggregations (Srikanth and Lakkundi, 1988a; Srikanth, 2001) on individual plant parts of preferred annual plants like cowpea *Vigna unguiculata* (L.) Walp. ssp. *unguiculata* (Family: Fabaceae) (Srikanth and Lakkundi, 1988b), with the overall populations growing rapidly from vegetative stage to reproductive stage of the crop (Srikanth and Lakkundi, 1990). It is likely that optimal densities also change along with growth in aphid populations through different phenological stages of the host. Within-plant characterization of aphid colonies is likely to reveal the occurrence of differential densities which, in turn, would allow further studies to infer on feeding site preferences and optimal densities. Therefore, in the present study, the within-plant colonization pattern of *A. craccivora* in *V. unguiculata* was explored to hypothesize on the occurrence or maintenance of optimal densities of the aphid on different plant organs.

MATERIALS AND METHODS

Inter-organ variability in the colonies of *A. craccivora* was examined in the locally popular and aphid-susceptible cultivar C 152 of *V. unguiculata* raised in the field. While stem, pod, leaflets of top most leaf and leaflets of top 2nd or 3rd leaf hosting dense colonies were considered, middle and lower leaves were ignored as they harbored sparse aphid colonies. Colonies were treated as dense when the aphids were in close physical contact with one another, whose visual counting would surely be erroneous. On the other hand, sparse colonies were those whose members maintained some distance from their neighbors and could be easily counted visually. Samples of the four plant parts (n=60) were collected from pod formation to pod-filling stages as these phenological stages ensured abundant aphid populations and colonies on all parts of the plant.

Infested sample stems and pods of varying size and aphid intensity were detached carefully from the plant using a sharp razor blade, placed in plastic containers of suitable size and brought to the laboratory. In stems, since aphid aggregations extended across leaf nodes with gaps, two adjacent aggregations that were separated distinctly were regarded as independent colonies. Aggregations that covered young pods completely were taken as a single colony but those that covered mature pods with distinct gaps were considered independent colonies. After marking both ends of the colonized portion on stem or pod as colony boundaries, aphids from the colonized portion were dislodged on a sheet of white paper by tapping and brushing the aphids off the stem or pod using a fine camel hair brush, and their number was counted (hereafter colony size). The length (hereafter colony length) and circumference (hereafter colony circumference) of marked, i.e. colonized portion of stem or pod were measured and the surface area (hereafter colony area) of colonized portion was calculated; together, these three parameters constituted colony

dimensions. From colony size and colony area, aphid density per unit area (number cm^{-2}) (hereafter colony density) was estimated.

For characterization of colonies on leaves, aphid number (colony size) on leaflets of top most leaf and leaflets of top 2nd or 3rd leaf was counted (n = 60 leaflets) by dislodging the aphids on a sheet of white paper as described above. Since the leaflets of top most leaf could be covered easily by a 1 cm^2 window cut on paper, their area was assumed to be 1 cm^2. Consequently, colony density cm^{-2} on these leaflets was same as the colony size. Similarly, area of leaflets of the top 2nd or 3rd leaf was assumed to be 4 cm^2 after verifying that these leaflets could be covered by a window of 2 cm x 2 cm. Consequently, colony density per unit area (number cm^{-2}) was estimated as one-fourth of the colony size.

In preliminary examination of data, normal probability plots of the five colony parameters, namely colony length, circumference, area, size and density from 60 sample units for stem and pod indicated deviation from normality; the confirmatory Shapiro-Wilk W test established the same. Similar analysis of data of colony density on leaflets of top most leaf and leaflets of top 2nd or 3rd leaf also showed deviation from normality. In view of the non-normality of data, the non-parametric Mann-Whitney U test was used to compare the five colony parameters between stem and pod, and colony density between leaflets of top most leaf and leaflets of top 2nd or 3rd leaf. The colony parameter data from the four plant organs were also examined for skewness. Colony density on stem, pod, leaflets of top most leaf and leaflets of top 2nd or 3rd leaf was compared by non-parametric Kruskal-Wallis analysis of variance (ANOVA) and multiple comparison tests. The inter-relationships among colony length, colony circumference, colony area, colony size and colony density for stem and pod were examined by correlation and regression analysis. Normality and non-parametric tests were performed in StatSoft Inc. (2004) and correlation and regression analyses were carried out in TableCurve 2D (2002).

RESULTS AND DISCUSSION

Colony parameters of *A. craccivora*, with the exception of colony length, deviated significantly from normality in both stem and pod, as confirmed by Shapiro-Wilk W test (Table 1). The distribution of colony length and colony circumference was not skewed whereas colony area, colony size and colony density displayed significant positive skewness in stem and pod (Fig. 1, 2). Mean colony length, colony circumference, colony area and colony size were significantly higher in pod than in stem by Mann-Whitney U test (Table 2). Colony density on stem and pod, however, did not differ significantly despite the slightly higher mean and range in the former.

Colony size was, in general, significantly and positively correlated with colony dimensions in stem and pod with minor variations and a few exceptions

Table 1. Normality and skewness of colony characteristics of *Aphis craccivora* in *Vigna unguiculata* stem and pod

Parameter	Shapiro-Wilk W@		Skewness z#	
	Stem	Pod	Stem	Pod
Colony length (cm)	0.962^{ns}	0.978^{ns}	1.67^{ns}	1.11^{ns}
Colony circumference (cm)	0.954^{*}	0.940^{**}	0.26^{ns}	1.92^{ns}
Colony area (cm^2)	0.948^{*}	0.943^{**}	1.97	3.40
Colony size (no.)	0.875^{****}	0.884^{****}	4.68	4.34
Colony density (no./cm^2)	0.909^{***}	0.934^{**}	2.37	2.28

@ Shapiro-Wilk W: significance at $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$; ^{ns} not significant $P > 0.05$;
Skewness/SE of skewness; ^{ns} not significant ($z < \pm 1.96$), the rest are significant (Cramer and Howitt, 2004)

(Table 3). While colony length lacked significance in stem, colony circumference was not significant in pod. In contrast, colony density was negatively correlated with colony dimensions in both stem and pod with the exceptions of colony circumference in stem and colony length in pod. Colony density was significantly and positively correlated with colony size in stem and pod considered separately; the relationship was slightly stronger for stem than for pod (Table 3). The overall colony density vs. colony size relationship for the data pooled for stem and pod was also positive and significant ($r = 0.535$; $n = 120$; $P < 0.0001$). Colony density vs. log colony size linear relationship (Fig. 3) was stronger than

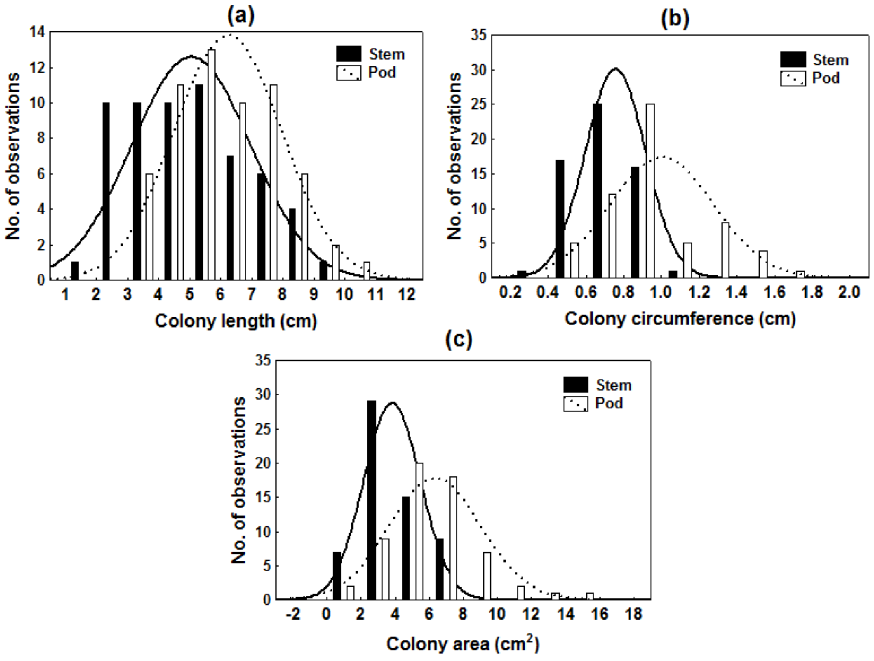


Fig. 1 Frequency distribution of *Aphis craccivora* colony dimensions in *Vigna unguiculata* stem and pod: (a) colony length (b) colony circumference (c) colony area

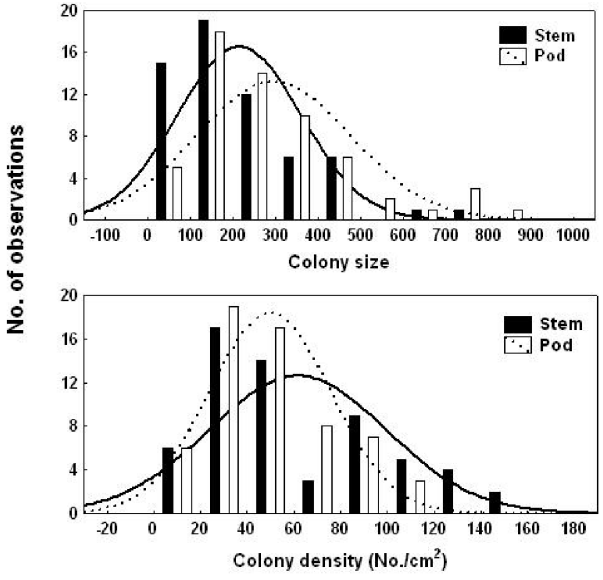


Fig. 2 Frequency distribution of *Aphis craccivora* colony size (top) and colony density (bottom) in *Vigna unguiculata* stem and pod

Table 2. Comparative colony characteristics of *Aphis craccivora* in *Vigna unguiculata* stem and pod

Parameter	Stem	Pod	Z value[@]
Colony length (cm)	5.03 ± 1.90[#] (2.00 - 9.80)[!]	6.21 ± 1.74 (3.20 - 10.60)	3.422***
Colony circumference (cm)	0.75 ± 0.16 (0.40 - 1.10)	1.00 ± 0.28 (0.50 - 1.70)	5.243****
Colony area (cm^2)	3.80 ± 1.66 (1.32 - 7.84)	6.29 ± 2.69 (1.60 - 15.90)	5.571****
Colon size (no.)	212.62 ± 144.67 (51.00 - 744.00)	293.08 ± 181.25 (71.00 - 878.00)	2.858**
Colony density (no./cm^2)	61.10 ± 37.93 (11.11 - 147.10)	49.45 ± 26.07 (14.12 - 111.87)	1.302ns

[#] Mean ± SD; n=60; [!]figures in parentheses are ranges[@] Mann-Whitney U test - Significance at ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; ns $P > 0.05$

colony density vs. colony size relationship (Fig. 4) for both stem and pod. Colony density on leaflets of both top most and top 2nd or 3rd leaves deviated significantly from normality (Table 4) but did not skew significantly in both (Fig. 5). Colony density was significantly higher on leaflets of top most leaf than on leaflets of top 2nd or 3rd leaf. ANOVA comparison of colony density on all the four plant parts graded them in the descending order as stem, pod, leaflets of top most leaf and leaflets of top 2nd or 3rd leaf with some overlapping differences (Table 5). While colony density on stem was significantly higher than those on leaflets of both leaves, colony density on pod was significantly higher than that on leaflets of top 2nd or 3rd leaf alone.

Examination of colony characters of *A. craccivora* in *V. unguiculata* in the present study revealed interesting trends. The lack of skewness for colony length and colony circumference suggested that the aphid colonized and exploited stems and pods of diverse size or age and maturity randomly, under the pressure of growing populations at pod formation stage. On the contrary, non-normality and significant positive skewness of colony area, colony size and colony density indicated the tendency of the aphid

Table 3. Correlations among colony characteristics of *Aphis craccivora* in *Vigna unguiculata* stem and pod

Parameter	Size	Density
Stem		
Colony length	0.141ns	-0.468***
Colony circumference	0.530***	0.175ns
Colony area	0.377**	-0.312*
Colony size	-	0.690***
Pod		
Colony length	0.594***	-0.094ns
Colony circumference	0.242ns	-0.418**
Colony area	0.585***	-0.263*
Colony size	-	0.566***

Significance at * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns Not significant $P > 0.05$

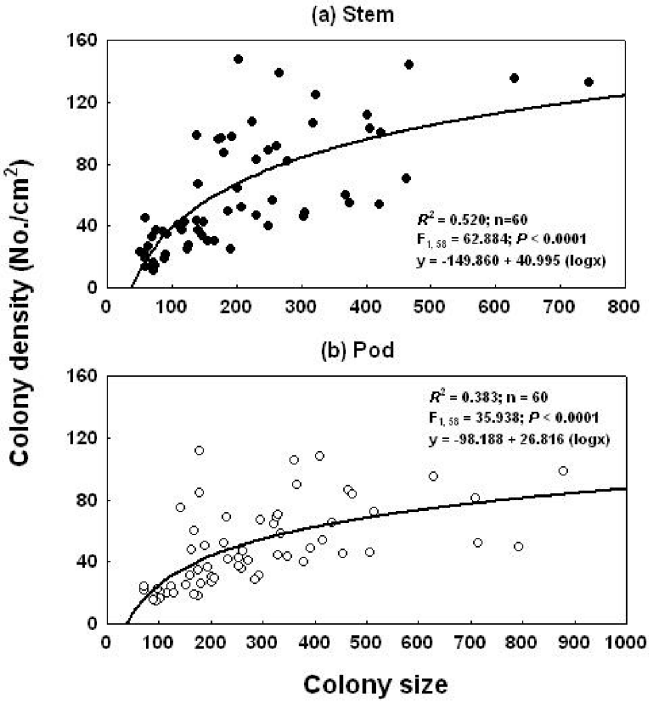


Fig. 3 *Aphis craccivora* colony density vs. log colony size linear relationship for *Vigna unguiculata* stem (a) and pod (b)

to optimize colonization of both stem and pod surface representing the food resource available. The significant positive correlations between colony area and colony size for stem and pod further supported the idea of optimal utilization of food resource. The lack of significance for colony size vs. colony length relationship in stem and colony size vs. colony circumference relationship in pod could be related to structural differences in the plant organs. While stems of a given age or position have more or less uniform circumference but are interspersed with leaves which break the linear colony contiguity, pods of any age display some variation in circumference yet provide uninterrupted linear dimension for colonization. The general negative colony density vs. colony dimension relationships pointed out that with increased availability of food resource, the aphid tended to spread out its colonies to avoid intra-specific competition and vice-versa. However, the positive correlation between colony density and colony size indicated that with a rise in population and consequent increase in colony size on resource units, i.e. stem and pod, the aphid showed a tendency to compact its colonies. The slightly better fit of linear-log relationship than the simple linear model between colony density and colony size suggested that density increases at a decreasing rate, probably mediated by reduced fecundity with colony growth (Way, 1968), and reaches an upper limit at saturation of the resource unit.

While significantly higher colony length, colony circumference and colony area in pod than in stem indicates that the aphid is more expansive on pod than on stem, governed probably by the contiguous surface area available in pod, significantly higher colony size in pod points out the possibility of preferential colonization. On the other hand, similar colony density in stem and pod seemed to suggest that the aphid does not require differential densities to overcome host defenses or utilize food from these two resource types representing source and sink, respectively. It is possible that during reproductive phase of the crop when stems and pods are available abundantly, the aphid exploits the two resource types equally by maintaining uniform densities on them.

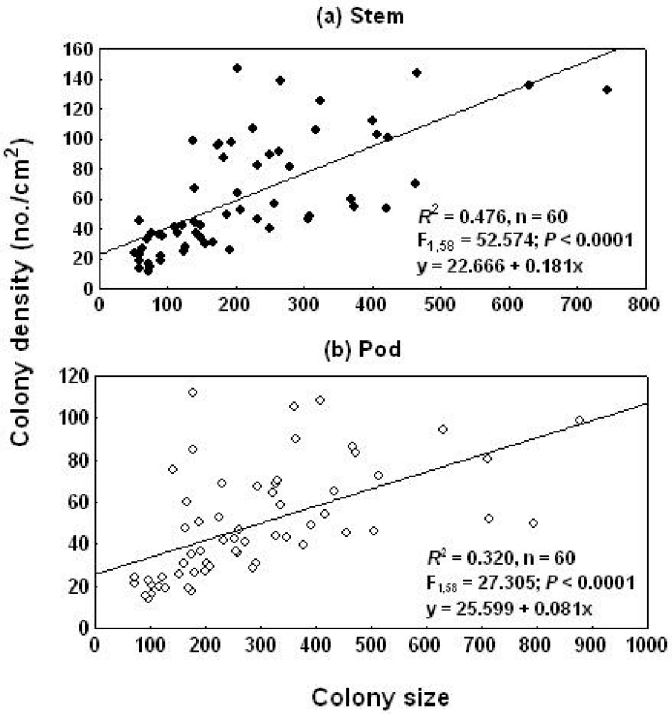


Fig. 4 *Aphis craccivora* colony density vs. colony size linear relationship for *Vigna unguiculata* stem (a) and pod (b)

Lack of skewness in colony density on leaflets of top most leaf and leaflets of top 2nd or 3rd leaf appears to be a case of finite resource size limiting proliferation of *A. craccivora*. Nevertheless, higher density on leaflets of top most leaf than on leaflets of top 2nd or 3rd leaf suggested some possibilities. For example, Ibbotson and Kennedy (1951) suggested that unequal distribution of *Aphis fabae* among leaves primarily reflected intrinsic differences between leaves, aided by gregariousness, whereas aggregation on leaves primarily reflected gregariousness and only secondarily differences between portions of the leaf. Within plants, leaf stage selection and colonization by aphids were related to differential leaf toughness and phloem phytochemistry, despite the possibility of higher concentrations of defensive metabolites in phloem sap of young leaves than in that of old leaves (Gould *et al.,* 2007; Jakobs *et al.,* 2019). Higher density of *A. craccivora* on top immature

Table 4. Comparative colony density statistics of *Aphis craccivora* on leaflets of top most leaf and leaflets of top 2nd or 3rd leaf in *Vigna unguiculata*

Resource type	Shapiro-Wilk W@	Skewness z#	Colony density (no./cm²)
Leaflets of top most leaf	0.956*	1.265ns	38.98 ± 19.63$ (9.0 - 81.0)
Leaflets of top 2nd or 3rd leaf	0.926**	1.565ns	19.35 ± 9.62 (6.25 - 38.75)
Mann-Whitney U test (Z# value)	-	-	5.76****

@ Shapiro-Wilk W: significance at $^{*}P < 0.05$; $^{**}P < 0.01$ # Skewness/SE of skewness; ns not significant ($z < \pm 1.96$) (Cramer and Howitt, 2004); $ Mean ± SD; n=60; Figures in parentheses are range; Significance at **** $P < 0.0001$

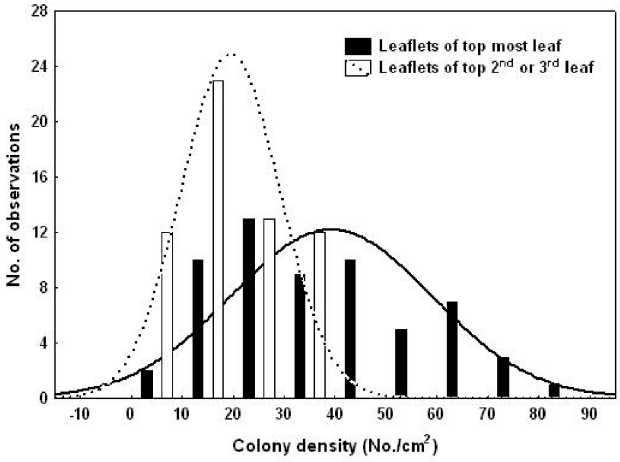


Fig. 5 Frequency distribution of *Aphis craccivora* colony density on leaflets of top most leaf and leaflets of top 2nd or 3rd leaf of *Vigna unguiculata*

leaves of *V. unguiculata* could be due to both nutritional suitability and lack of metabolites, besides the possible ability of the aphid to divert food from other leaves through phloem and acting somewhat like a sink (Way and Cammell, 1970).

The gradation of stem, pod, leaflets of top most leaf and leaflets of top 2nd or 3rd leaf in the decreasing order for *A. craccivora* colony density reinforced the trends observed in stem vs. pod and leaflets of top most leaf vs. leaflets of top 2nd or 3rd leaf comparisons. In earlier studies on alfalfa, higher proportions of *A. craccivora* (Berberet *et al.,* 2009) and *Acyrthosiphon kondoi* (Zarrabi *et al.,* 2005) were found on stems than on leaf blades. Similarly, populations were higher and aphid development period was shorter for *Aphis glycines* on stems than adaxial and abaxial leaf surfaces of soybean (Nalam *et al.,* 2021). Aggregations aid in overcoming host defenses and mobilizing or utilizing host nutrients (Prokopy, 1981; Sandström *et al.,* 2000), since aphids probe often and remain with their stylets inserted longer in the presence of colony members (Ibbotson and Kennedy, 1951). Variable aggregation size or colony density of *A. craccivora* in the four organs of *V. unguiculata* may be an adaptation to serve these two functions. If these organ-specific aggregations represent optimal densities, their ranges are likely to change with growth of aphid populations through different phenological stages of the host plant, as the positive correlations between colony size and colony density in stem and pod indicated.

Aphid species are known to be constrained chemically to certain plant species or even parts of the plants governed by chemical profile and trichome arrays (Loxdale *et al.,* 2019). Both between and within-plant differences in phloem sap chemistry are known to affect the performance and

Table 5. Comparison of *Aphis craccivora* colony density on different resource types in cow pea

Resource type	Colony density (no./cm^2)
Stem	61.10 (157.87)^a
Pod	49.45 (145.62)^{ab}
Leaflets of top most leaf	38.98 (122.70)^b
Leaflets of top 2nd or 3rd leaf	19.35 (55.82)^c

Figures in parentheses are mean rank values; Mean ranks followed by the same letter are not significantly different ($P > 0.05$) by multiple comparison, z values of Kruskal-Wallis test ($1/2^2 = 77.377$, df=3, n=240, $P < 0.001$)

abundance of aphids in relation to their nutritional needs, and behavioral or physiological responses (reviewed in Jakob *et al.,* 2019). Performance of *A. glycines* on a specific location of soybean plant is primarily driven by accessibility and the quality of phloem composition rather than structural traits (Nalam *et al.,* 2021). The preferential attack of different host plants (Srikanth and Lakkundi, 1988b) with differential rates of reproduction (Srikanth and Lakkundi, 1988c) by *A. craccivora* could be due to the relative suitability of hosts determined by morphological, anatomical, physiological and nutritional factors. On the other hand, among different organs of *V. unguiculata*, although higher colony dimensions and colony size of *A. craccivora* in pod (sink) than in stem (source) seem to support the idea of preferential colonization of these plant parts, similar colony density on these two organs appears to reject the possibility of different optimal densities. Systematic simulation studies to assess the fitness or reproductive performance of the aphid at different densities on the four organs, in relation to within-plant phytochemical composition (Enyiukwu *et al.,* 2018), would shed some light on the occurrence of optimal densities to either maximize nutrient utilization or overcome host defenses. Since optimal densities are likely to change with crop growth and the consequent aphid population buildup, such studies need to be carried out at different phenological stages of the crop to reveal their temporal dynamics.

From applied ecology point of view, within-plant variation in colonization pattern of *A. craccivora* has implications for sampling accuracy. Based on the concentration of a high percentage of the aphid population in the middle and lower portions of alfalfa canopy, Berberet *et al.* (2009) suggested sampling by removal of stems for accurate population estimate. Differential colonization rates in *V. unguiculata* in the present study indicated that sampling stems, leaflets of top most leaf and leaflets of top 2nd or 3rd leaf in the vegetative phase, and pods in the reproductive phase would be ideal for estimating *A. craccivora* populations in studies on population dynamics of the aphid and natural enemies (Srikanth and Lakkundi, 1990).

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Metabolites in galls induced on the leaves of *Trewia nudiflora* (L.) (Euphorbiaceae) by *Trioza fletcheri* Crawford (Hemiptera, Triozidae)

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ABSTRACT: *Trioza fletcheri* Crawford is a sap-sucking psyllid that induces galls on *Trewia nudiflora* leaves. Early stages of *T. fletcheri* feed on parenchyma, whereas late-stages and adults feed on phloem, causing galls which arise in an isolated, agglomerated mass and rosette form only on the abaxial surface of *T. nudiflora* leaves. The feeding action of immature stages induces changes in metabolites of host tissue and creates a nutrition sink for feeding. The biochemical study revealed that galled tissues had higher levels of metabolites (total soluble sugars, reducing sugars, total protein and free amino acids) than ungalled tissues, with average values measuring 3.4±0.09, 1.4±0.1, 0.63±0.03, 1.9±0.23, 3.0±0.72mg/gdw in ungalled leaves; 4.3±0.02, 2.9±0.3, 1.9±0.47, 3.7±0.36, 4.7±0.53 mg/g dw in young galls; 3.8±0.50, 3.7±0.3, 1.03±0.04, 2.9±0.35, 5.4±0.31 mg/g dw in mature galls; and 2.7±0.23, 2.4±0.3, 0.83±0.03, 2.6±0.34 and, 4.3±0.22mg/g dw in old galls, respectively. Enhanced activities of IAA-oxidase, α-amylase, peroxidase, and invertase were observed in galled infested leaves than in ungalled leaves, and their values were measured to be 2.45±0.53, 2.4±0.3, 0.9±0.2, and 3.7±0.5 in ungalled leaves, 2.92±0.32, 3.2±0.2, 1.9±0.5 and 4.5±0.3in young galls, 3.7±0.43, 3.6±0.4, 1.4±0.4, 4.3±0.2 in mature galls, and 2.51±0.03, 2.9±0.4, 1.4±0.4, 3.8±0.1 in old galls respectively. © 2022 Association for Advancement of Entomology

KEYWORDS: Psyllid, infested tissue, biochemical changes, metabolites, enzyme activities

INTRODUCTION

Trioza fletcheri Crawford is a sap-sucking insect that belongs to the family Triozidae and subfamily Psylloidea (Bodlah *et al*., 2012). Life stages of the Psylloidea, *T. fletcheri,* include adult stage (Fig.1), egg (Fig. 2) and five immature stages (Sharma *et al*., 2013, 2014, 2015). Early stages (Fig. 3) feed on parenchyma, whereas late stages (Fig. 4) and adults feed on phloem. Gall initiation is triggered by the feeding action and salivary enzymes released by first instars (Burckhardt, 2005; Raman, 2011, 2016; Sharma and Raman, 2022). The galls are the modified, invariably symmetrical, naturally developing plant structures that arise because of messages from the inducing insects (Mani, 1964; Raman, 2011), and develop as an extension of the host plant phenotype (Weis *et al.,* 1988; Chen *et al.,* 2015). The galls provide nutrition and shelter to the inducing insects. The insect activates a perturbation in growth mechanisms and alters the differentiation processes in the plant, modifying the plant's architecture to its advantage (Raman, 2003). *T. fletcheri* induces pimples and pouches like galls

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that arise in an isolated (Fig. 6), agglomerated mass (Fig. 7) and rosette form (Fig. 8) only on the abaxial surface of *T. nudiflora* leaves.

The medicinal plant *Trewia nudiflora (L.) (Euphorbiaceae)* (Fig. 5) is an important in the Indian medicine in Ayurveda and Siddha (Balakrishnan *et al*., 2013). The root decoction of *T. nudiflora* is used as a stomachic therapy for flatulence, gout, rheumatism, cancer, particularly leukaemia and hepatobiliary affections, while, a decoction of shoots and leaves is used to treat edoema, flatulence, excess bile and sputum (Balakrishnan *et al*., 2013). It is a host for *T. fletcheri* that induces galls on its leaves (Crawford, 1924; Yang and Raman, 2007). The present study aims to describe the metabolite changes in leaf galls of *T. nudiflora* induced by *T. fletcheri.*

MATERIALS AND METHODS

Collection of samples: Samples for the study were collected from the Haridwar district, a small historic city in the state of Uttarakhand located on the banks of the Ganges River at 29.945690 latitude and 78.164246 longitude.

Biochemical analysis: For biochemical analysis, fresh samples from the same study area were collected from the uninfested and infested plant of *T. nudiflora* plant during 2019-2020. For convenience the collected samples of leaves were categorized into: (A) Ungalled leaves of uninfested plants; (B) galled leaves of infested plant with young, mature, and old galls. Fifty samples in each category were chosen for the study.

The samples were washed in distilled water and then dried in the air on Whatman's filter paper for 25-30 minutes. Gall samples were trimmed with the help of a sharp razor and residing nymphs were removed by partial slitting of the galls. A sub sample of each of ungalled leaves, gall infested leaves, young, mature and old galls was prepared and used for each biochemical assay.

The samples were ground in a mortar and boiled in ethanol (80%) to obtain an extract, which was then filtered through Whatman's (#1) filter paper.The extract was used for assaying total sugars following Plummer (1971), total phenols following Bray and Thorpe (1951), free amino acids following Moore and Stein (1948), total proteins following Lowry *et al*. (1951), reducing sugars following Miller (1972), and total soluble sugars following Dubois *et al.* (1951). Tests were repeated for 4-5 duplicates, and five values of each biochemical assay of ungalled and galled tissue were observed to obtain the mean data and standard error. Obtained values were expressed as mg g^{-1}.

pH analysis: For pH analysis,both ungalled and galled infested leaves were collected from the study area. From the gall infested leaves, young, mature, and old galls were picked. The young and mature galls were carefully opened to remove the nymphs residing in the galls. A solutionof the ungalled leaves, galled leaves, young, mature, and old galls was prepared by crushing in distilled water, and the pH value of each sample was measured using BDH paper strips and a digital pH meter (BST-PT13 manufactured by Bionics Scientific Technologies (P). Ltd, India).

Estimation of enzyme activity: One gram plant sample was crushed in 3 ml of cold sodium phosphate buffer (0.02M, pH 6.0). The concentrate was centrifuged at 12298 'G' values (Remi Refrigerated Centrifuges, C-24 Plus) for 15 min in a refrigerated centrifuge at 4^0C. The supernatant was mixed in a two fold quantity of cold acetone and allowed to incubate at 5^0C for half an hour to precipitate the proteins. The solution was centrifuged again at 4^0C for 10 min at 3600 rpm. The supernatant was disposed, while the pellet was re-suspended in 10 ml of phosphate buffer (0.02M, pH 6.4) and used as the enzyme source. The extract was used for assaying peroxidase following Birecka *et al*. (1973), Alpha-amylase following Bernfeld (1955), Invertase following Harris and Jeffcott (1974) and IAA-oxidase following Mahadevan and Sridhar (1986). The experiments were repeated four to five times to obtain the mean values with standard errors.

The experiments were conducted in randomized

design and the data expressed in average of replications were analysed statistically.

RESULTS AND DISCUSSION

The study showed that gall inducing on the leaves of *T. nudiflora* by *T. fletcheri* leads to considerable changes in major metabolites of plant's tissue.

Total soluble sugars

Higher values of total soluble sugars were recorded in galled tissues as compared to ungalled tissues. The soluble sugars were higher in young galls than in mature and old galls, with mean values of3.4±0.09, 4.3±0.02, 3.8±0.50, 2.7±0.23 mg g^{-1} dw in ungalled leaves, young, mature, and old galls respectively (Table1). The total sugar content might be higher in young and mature galls because of the enhanced metabolic activity of nymphs during feeding stress. Mukharjee *et al*. (2016) and Biswas *et al*. (2018) also reported enhanced total sugar content in mature and perforated psyllid galls of *T. tomentosa* and *T. arjuna.*.

Total reducing sugars

Enhanced value of total reducing sugars was observed in galled tissues in comparison to ungalled leaves. Highest value of reducing sugars was observed in mature galls. The mean values of total reducing sugars were recorded to be 1.4±0.1, 2.9±0.3, 3.7±0.3, 2.4±0.3 mg g^{-1} dw in ungalled leaves, young, mature and old galls respectively (Table 1). Galled tissues showed higher level of reducing sugars than ungalled leaves, which is supported by previous reports of Dsouza and Ravishankar (2014) and Kumar *et al*. (2015).

Total phenols

Total phenol content was higher in galled tissues as compared to ungalled tissues.The highest phenol content was observed in young galls, especially during early cecidogenesis, and declined later in the mature and old galls. Average values of the total phenol were 0.63±0.03, 1.9±0.47, 1.03±0.04 and 0.83±0.03 mg g^{-1} in ungalled leaves, young, mature and old galls respectively (Table1). In winters, quantity of phenol increases in the galled tissues to trap the solar heat and to maintain the optimum temperature inside the gall. Elevation of phenol compounds in psyllid galls is a common phenomenon (Balakrishna and Raman,1992). As a defence against invading pests and pathogens, plants synthesize a variety of secondary chemicals and phenols depending on their genotype (Kar *et al*., 2013). Various groups of phenolic compounds defend the plant against microbes and herbivores (Kraus and Spiteller,1997). Plants develop resistance to pathogen-caused disease when high levels of phenol are present (Mehrotra and Aggarwal, 2003). Thus, the high amounts of phenolic compounds in the galled tissue of *T. nudiflora* provide resistance to insect infestation. Dsouza and Ravishankar (2014), Mukharjee *et al*. (2016) and Mujahid *et al*. (2019) also reported higher phenol contents in psyllid galls.

Total protein

Total protein content increased in galled tissues as compared to ungalled tissues. The highest protein content was observed in young galls that declined gradually in mature and old galls. The total protein contents were 1.9±0.23, 3.7±0.36, 2.9±0.35, 2.6±0.34 mg g^{-1} in ungalled leaves, young, mature, and old galls respectively (Table1). The galled tissues had higher total protein levels than the ungalled leaf tissues, which is supported by the previous studies (Arora and Patni, 2001; El-Akkad, 2004; Scareli Santos and Varanda, 2003; Mukharjee *et al*., 2016). Plant protein synthesis play an important role in defence (Reinbothe *et al*., 1994). Higher protein levels in the leaves during gall infestation are due to enhanced secretion of defensive proteins by the host plant, which inhibits the activity of insect's proteolytic enzymes.These proteins are proteinase inhibitors that are quickly stored throughout the plant and ungalled areas as a result of insect feeding, even in those areas that are far from the early feeding location (Ananthakrishnan, 2001). Besides this, decline in protein content in mature and old galls may be due to less need of protein or the protein secretion has reached satiation in the host-plant (Raman *et al.*, 1997).

Free amino acids

The quantity of free amino acid was equal in ungalled leaves and young galls; whereas, mature galls showed the higher concentration of free amino acids. The quantity of free amino acid decreased in old gall tissue. The mean values of free amino acids measured to be 3.0±0.72, 3.0±0.72, 5.4±0.31, 4.3±0.22 mg g^{-1} dw in ungalled leaves, young, mature and old galls respectively (Table1). An increase in amino acid in galled tissues is due to the breakdown of protein into usable molecules by protease secreted by the insects' salivary glands (Miles and Lloyd,1967; Miles, 1968). In gall-inducing insects, the amino acids in galls serve as building blocks for protein synthesis (Birch, 1974). The galled leaves of *T. nudiflora* had a higher concentration of free amino acids than the ungalled leaves. The gall-inducing insects manipulate the host plants for their own advantage (Hartley and Lawton, 1992).

IAA-oxidase activity

Enhanced activity of IAA-oxidase was observed in galled tissues than in ungalled tissues and it was highest in mature galls. The mean values of IAA-oxidase were 2.45±0.53, 2.92±0.32, 3.7±0.43, and 2.51±0.03 mg in ungalled leaves, young, mature, and old galls respectively (Table1). IAA is the main auxin in higher plants, which has profound effects on plant growth and development. Both plants and some plant pathogens can produce IAA to modulate plant growth (Zhao, 2010). The high level of phenol adversely affects the IAA-oxidase activity in plant tissue, resulting in a higher level of IAA, thus leading to hyperauxinity and gall induction. A high level of IAA supports cell expansion (hypertrophy) and cell division (hyperplasia).

α-amylase activity

Increased α-amylase activity was observed in galled tissues as compared to ungalled tissues and was maximum in mature galls. The mean values of α-amylase activity were measured to be 2.4±0.3, 3.2±0.2, 3.6±0.4, and 2.9±0.4 mg m^{-1} dw in ungalled leaves, young, mature, and old galls, respectively (Table1). Enhanced activity of α-amylase in mature galls of *T. nudiflora* is triggered by the feeding stress of immatures of *T. fletcheri*, which increases the metabolic activity of galled tissues, stimulating sugar formation. The depletion of starch accumulates carbohydrates due to activated α-amylase activity and other enzymes. Increased activity of α-amylase in gall-infested leaves along with increased sugar levels was also reported by Garg and Mandhar (1975), Shekhawat (1980), Rao (1989), Purohit (1980) and Dsouza and Ravishankar (2014).

Peroxidase activity

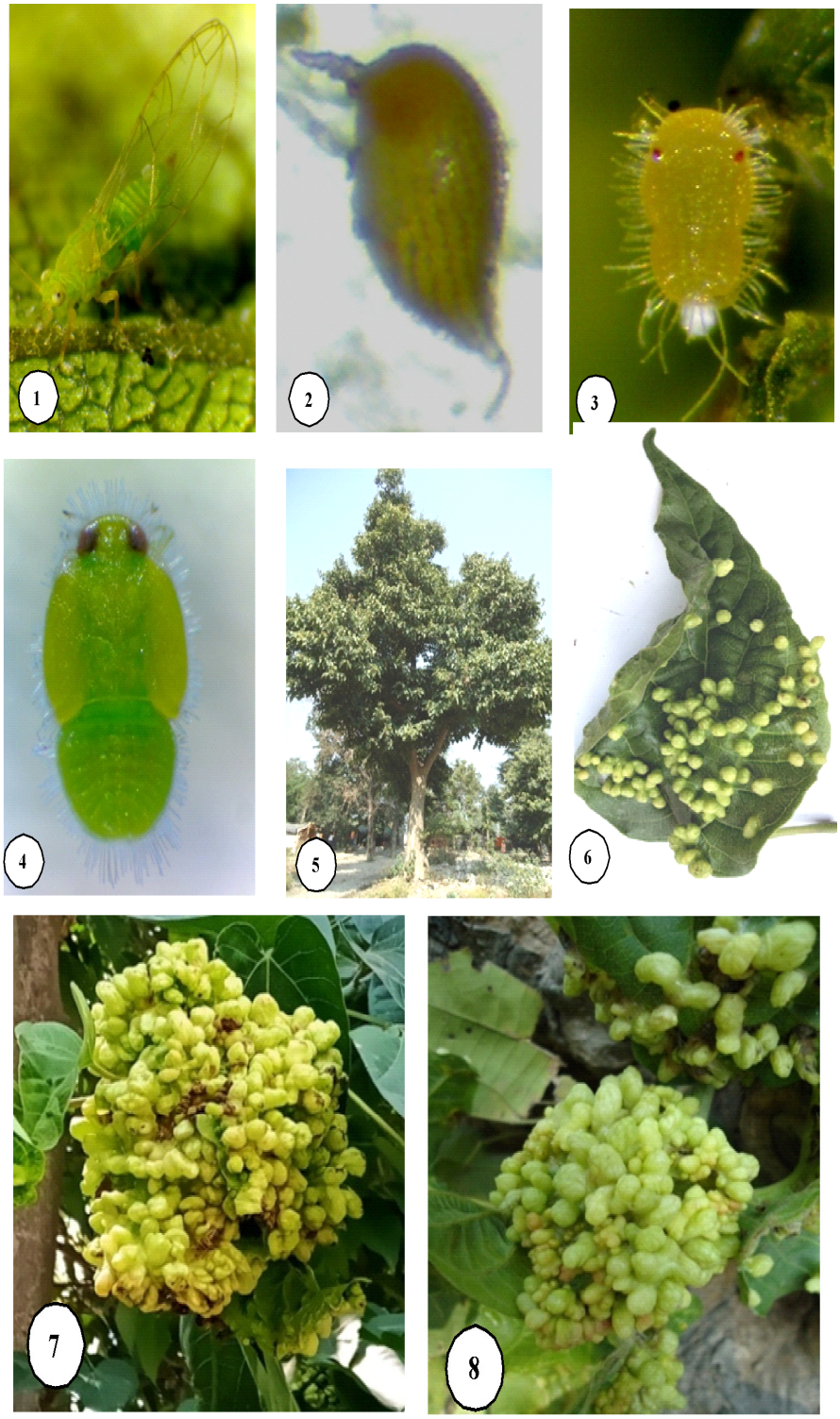
Enhanced activity of peroxidase was recorded in galled tissues as compared to ungalled leaf tissues. The maximum activity was observed in mature galls. The average values measured were 0.9±0.2, 1.9±0.5, 1.4±0.4, 1.4±0.4 mg m^{-1} dw in ungalled leaves, young, mature and old galls respectively (Table1). Peroxidases are a ubiquitous enzyme cluster, responsible to the catalytic reduction of peroxide and generate reactive oxygen species (ROS). Usually infestation by any arthropod will stimulate peroxidase activity in cell sap and enhance total soluble protein levels. In any such activity, peroxidases measured to indicate ROS production, a key stress indicator. The study also showed increased peroxidase activity in *T. nudiflora* galls, which is supported by the findings of Biswas *et al*. (2018).

Invertase activity

Increased invertase activity was highest in young galls.Average values are 3.7±0.5, 4.5±0.3, 4.3±0.2, 3.8±0.1 mg/min dw in ungalled leaves, young, mature, and old galls, respectively (Table1). Invertase is a sucrose-hydrolyzing enzyme found in plant tissues, serving as a physiological sink. Enhanced invertase activity was also reported by (Dsouza and Ravishankar, 2014) in psyllid galls of *Ficus glomerata* Roxb induced by *Pauropsylla depressa* Crawford, which is consistent with the present observations.

pH changes

The pH of ungalled leaves was observed to be slightly acidic, while, it was more acidic in galled tissues. The mean pH values observed were 6.052



Figs. (1) Adult - *T. fletcheri,* (2) Egg, (3) I instar, (4) V instar of *T. fletcheri,* (5) *T. nudiflora* plant, (6) isolated galls, (7) agglomerated mass of galls, (8) rosette galls (Photo courtesy: Dr. Om Datta)

Table 1. Metabolites in ungalled leaves and young, mature and old leaf galls of *Trewia nudiflora* induced by *Trioza fletcheri* on dry weight basis

Metabolites/ Plant parts	Ungalled	Young gall	Mature gall	Old gall
Total soluble sugars (mg g^{-1})	3.40±0.09	4.3±0.02	3.80±0.50	2.70±0.23
Total reducing sugar (mg g^{-1})	1.40±0.10	2.9±0.30	3.70±0.30	2.40±0.30
Total phenol (mg g^{-1})	0.63±0.03	1.9±0.47	1.03±0.04	0.83±0.03
Total protein (mg g^{-1})	1.90±0.23	3.7±0.36	2.90±0.35	2.60±0.34
Total free amino acids (mg g^{-1})	3.00±0.72	4.7±0.53	5.40±0.31	4.30±0.22
IAA-Oxidase	2.45±0.53	2.92±0.32	3.70±0.43	2.51±0.03
α-Amylase starch (mg/min)	2.40±0.30	3.2±0.20	3.60±0.40	2.90±0.40
Peroxidase (Δ A/g /min)	0.90±0.20	1.90±0.50	1.40±0.40	1.40±0.40
Invertase sucrose (mg/min)	3.70±0.50	4.50±0.30	4.30±0.20	3.80±0.10

±0.17, 5.916±0.10, 5.1±0.1, 5.58±0.09 and 5.95±0.12 in ungalled and gall-infested leaves, young, mature and old galls respectively. The pH of mature galls was recorded to be more acidic than that of ungalled leaves. Immature stages of *T. fletcheri* inject saliva into leaf tissues during feeding, which contains some chemicals that decrease the pH of galled tissues. The pH level of old gall tissues increases as the fifth instar immature comes out of the gall.

The study concludes that due to interaction between gall-inducing immature stages of *T. fletcheri* and plant tissue, certain biochemical and physiological changes occur in the leaves. Since the cells require a high amount of protein, the young and mature galled tissue show a major difference in protein concentration when compared to normal leaf tissue. When gall-inducing insects attack a plant, they inject elicitors that cause the plant to produce large numbers of various types of enzymes and metabolites as a defence mechanism against the biotic stress. Insects activate the plant's defence system, which starts several biochemical reactions and physiological processes that lead to the development of gall. Until the immature stages of gall-inducing insects live within the host, its metabolism gets altered to that extent that the galls show varying levels of metabolites. The study shows that feeding of immature stages of *T. fletcheri* leads to significant changes in metabolites such as total soluble sugars, reducing sugars, total protein, free amino acids, and increased activity of IAA-oxidase, α-amylase, peroxidase, and invertase in galled tissues of *T. nudiflora.*

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Intraguild predation of inferior larval instars of two ladybirds *Menochilus sexmaculatus* (Fabricius) and *Propylea dissecta* (Mulsant) (Coleoptera, Coccinellidae)

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ABSTRACT: Ladybird beetles are predatory insects, which consume several insect pests and have immense biocontrol potential, particularly against aphids. In prey scarcity, they resort to intraguild predation (IGP) by consuming immature stages of other heterospecific ladybirds. A laboratory experiment was performed to quantify the incidence of IGP of first and second larval instars by older instars and adults of two co-occurring ladybird species, *Propylea dissecta* and *Menochilus sexmaculatus*. IGP of first and second larval instars increased significantly with increase in the larval stages followed by adult males and females. Predatory stages of *M. sexmaculatus* were more potential intraguild predators than those of *P. dissecta*. Among adults, the female consumed a greater number of early and weaker heterospecific instars. The presence of dorsal spines and hair on the larva of *M. sexmaculatus* provided aposematism that helps in defense against superior heterospecific larvae thereby enabling its successful establishment and distribution. *M. sexmaculatus* could act as an intraguild predator in the fields and may contribute in declining the population density of *P. dissecta* during aphid-prey scarcity.
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KEY WORDS: Population density, heterospecific instars, aposematism, aphid-prey scarcity

INTRODUCTION

The majority of predaceous ladybirds (Coleoptera, Coccinellidae) are economically important for their biocontrol potential against several phytophagous insect pests, *viz*. aphids, coccids, diaspidids and aleyrodids (Hodek *et al.,* 2012). Older larval instars used to prey upon eggs and the early instars of heterospecific ladybirds during aphid scarcity (Pervez *et al.,* 2021). Such interactions are referred to as intraguild predation (IGP), where two predators compete for a common prey resource, which are frequent in ladybirds (Polis *et al.,* 1989; Khan and Yoldas, 2018). The dominant predator (IG predator) attacks and consumes the inferior one (IG prey) sharing common prey resource (extraguild prey) (Lucas, 2012). The terms, 'superior' and 'inferior' are used when one species (Reitz and Trumble, 2002; Putra *et al.*, 2009), competitor (Inouye, 2005), larval stage (Pervez *et al.*, 2021) or aphid-prey (Bilu and Coll, 2009) dominates the other one. IGP may disrupt biocontrol programmes (Mansfield, 2019) and can endanger biodiversity (Smith and Gardiner, 2013) by attacking

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inferior and native coccinellid species of different agroecosystems.

Exotic invading ladybirds may dominate the agro-ecosystems by competing for a shared prey, declining native coccinellid populations by IGP and overlapping niche (van Lenteren *et al.,* 2006). IGP in ladybirds is generally bidirectional and asymmetrical (Rondoni *et al.,* 2014; Rocca *et al.,* 2017). Usually, adults and higher coccinellid instars attack immobile and poorly-defended vulnerable life stages like eggs and lower instars (Lucas, 2005; Hemptinne *et al.,* 2011). Eggs and inferior larvae of conspecifics (Pervez *et al.,* 2006; Roy *et al.,* 2007) and heterospecifics (de Clercq *et al.,* 2005; Michaud and Jyoti, 2008) are even more nutritious than aphids to ladybirds in certain cases.

Propylea dissecta (Mulsant) and *Menochilus sexmaculatus* (Fabricius) are oriental ladybirds, which abundantly occur in the agricultural fields preying on aphids (Omkar and Pervez, 2004). The coccinelid *P. dissecta* can consume aphids raised on both nutritious and toxic hosts, which reveals its sustainability in the aphid available agro-ecosystems (Pervez and Omkar, 2011) and possesses immense biocontrol potential (Bhoopathi *et al.,* 2020). It co-occurs with many coccinellid species, including *M. sexmaculatus*. Similarly, *M. sexmaculatus* feeds on a vast range of aphid-prey (Pervez and Chandra, 2018), however little is known on its intraguild interactions. Singh *et al.* (2020) found that it may resort to oophagy with deteriorating fitness during aphid scarcity. The two ladybird species can easily be mass-reared for the augmentative release as aphid biocontrol agents. Singh *et al.* (2016) emphasized on the slow and fast developing individuals in these two ladybird species in a common habitat. Despite their co-occurrence, *M.sexmaculatus* seems to have a better compensatory ability than *P. dissecta* to overcome the stress of fluctuating prey-resource (Chaudhary *et al.,* 2016). Prescott and Andow (2019) suggested ineffective avoidance mechanisms to be the possible reason for the co-occurrence of IG predators. However, the question on the possible impact of these ladybirds on each other's abundance during prey-scarcity is still unaddressed. An investigation was undertaken to assess the potential intraguild predator and the intraguild prey when the two ladybird species co-occur in the absence of natural aphid prey and the stage-specific larval interaction during intraguild combat between the two ladybird species.

MATERIALS AND METHODS

Stock Culture

Adults of *M. sexmaculatus* and *P. dissecta* were collected from the agricultural fields near the suburbs of Kashipur, Uttarakhand (29°2104′N; 78°9619′E), and brought to the laboratory. These adults were paired in the Petri dishes (9.0 × 2.0 cm) consisting of *ad libitum* supply of aphids, *Aphis craccivora,* infested on the bean (*Dolichos lablab* L.) twigs along with moist filter paper under constant conditions (27 ± 2°C; 65 ± 5% RH; 14L: 10D) in an Environmental Test Chamber (REMI, Remi Instruments). The adults mated and laid F_1 eggs, which were reared from egg-hatch to adult emergence in the above mentioned abiotic and biotic conditions (using the above prey). The emerging F_1 adults were again sexed, isolated, paired, and allowed to mate. The F_2 eggs laid by the F_1 adult females after mating, were collected and isolated. These F_2 eggs were used to develop the larval instars needed for the experimental design.

Experimental Design

Propylea dissecta as an intraguild predator

Ten first-instars (10 h old) of *M. sexmaculatus* were released as intraguild prey in a Petri dish). Thereafter, one-day-old second instar of *P. dissecta* was released as an intraguild predator in the same Petri dish and allowed to consume the first instars. After 12 h, the second-instar predator was removed from the Petri dish and the numbers of remaining first instars were counted to determine the number of first instars consumed. The experiment was replicated ten times (n =10). The same experiment was repeated using third instar, fourth instar, adult male and female *P. dissecta,* as intraguild predator(s). The above experiment was repeated by using ten second-instars (10 h old) of

M. sexmaculatus as intraguild prey in a Petri dish. Thereafter, a 1-day-old third-instar *P. dissecta* was released in it as an intraguild predator and the observation on the larval IGP was taken, after 12 hours(n =10). The experiment was repeated using fourth instar, adult male and female of *P. dissecta*, as intraguild predator(s).

Menochilus sexmaculatus as an intraguild predator

The above experiment (as in *P. dissecta* as an intraguild predator) was repeated by switching ten first instars (10 h old) of *P. dissecta* as intraguild prey and second, third, fourth instar, adult male, and female of *M. sexmaculatus* as an intraguild predator(s). Ten numbers of second instars (10 h old) of *P. dissecta* as intraguild prey with second, third, fourth instar larvae, adult male and female of *M. sexmaculatus* as intraguild predator(s).

The data on the IGP of larvae by the two-ladybird species were tested for normality using Kolmogorov – Smirnoff Test and homogeneity of variance using Bartlett's Test on statistical software, SAS 9.0 (SAS, 2002). The larval IGP by the different predatory stages were subjected to one-way ANOVA and the data were compared using Tukey's Test using SAS 9.0. IGP by the two species at a particular predatory stage was compared using a two-sample t-test. The data were further subjected to Two-way ANOVA using (i) 'species' and (ii) 'predatory stage' as independent variables and 'IGP' as the dependent variable.

List of abbreviations used: IGP - Intraguild Predation; IG Predator - Intraguild predator; IG prey- Intraguild prey; Ms-*Menochilussexmaculatus*; Pd-*Propy leadissecta*

RESULTS AND DISCUSSION

All the predatory stages of both ladybird species resorted to IGP of larvae for their survival. However, the intensity of IGP differed significantly between the species. The number of first and second instars consumed by their superior larval stages increased significantly with the larval instars (Table1). IGP of first ($F=4.54$; $P<0.01$; d.f. = 4, 49) and second ($F=13.75$; $P<0.001$; d.f. = 3, 39) instars of *M. sexmaculatus* by predatory stages of *P. dissecta* differed significantly. Similarly, IGP of second instars *P. dissecta* by the older larvae and adults of *M. sexmaculatus* also varied significantly ($F=4.91$; $P<0.01$; d.f. = 3, 39). However, IGP of the first instar *P. dissecta* by the predatory stages of *M. sexmaculatus* did not vary significantly ($F = 2.33$; N.S.; d.f. = 4, 49). Older larval instars and adults of both species consumed the heterospecific inferior larvae, which indicates that perhaps in the fields, older instars might use the early heterospecific instars as food for their survival and development during aphid scarcity. When the IGP was compared at the species level, it was noted that second instar ($t = -3.09$; $P<0.01$; d.f. = 12), third instar ($t =-2.40$; $P<0.05$; d.f. = 12), adult male ($t = -6.50$; $P<0.001$; d.f. = 12) and female ($t = -2.60$; $P<0.05$; d.f. = 12), *M. sexmaculatus* consumed significantly greater number of first-instars than those consumed by *P. dissecta* (Fig. 1). Similarly, in the case of third instar ($t = -3.48$; $P<0.01$; d.f. = 17), fourth-instar ($t = -2.54$; $P<0.05$; d.f. = 17), and adult male ($t = -4.37$; $P<0.001$; d.f. = 17), *M. sexmaculatus* consumed significantly greater number of second-instars than those consumed by *P. dissecta* (Fig. 2). IGP of second instars by the adult heterospecific females did not differ significantly ($t = -1.28$; $P = 0.219$; d.f. = 15). These results support our hypothesis that *M. sexmaculatus* will dominate the agroecosystems, where *P. dissecta* co-occur and will reduce the abundance of latter in the prey absence.

The larval morphology of *M. sexmaculatus* with the presence of spines and hair provides aposematic security against IGP. Dorsal spines of the second instar *M. sexmaculatus* provide physical protection against intraguild predators, as also is the case in *Harmonia axyridis* (Pallas), where spineless larvae were more attacked by superior IG predators than the spined larvae (Hautier *et al.,* 2017). Morphological anti-predator traits and adaptations provide strong selection pressure and defense to the developing stages (Lima and Dill, 1990). Spines are highly conspicuous and offer physical defense in both plants and animals (Inbar and Lev-Yadun, 2005). Defense mechanism due to the presence of

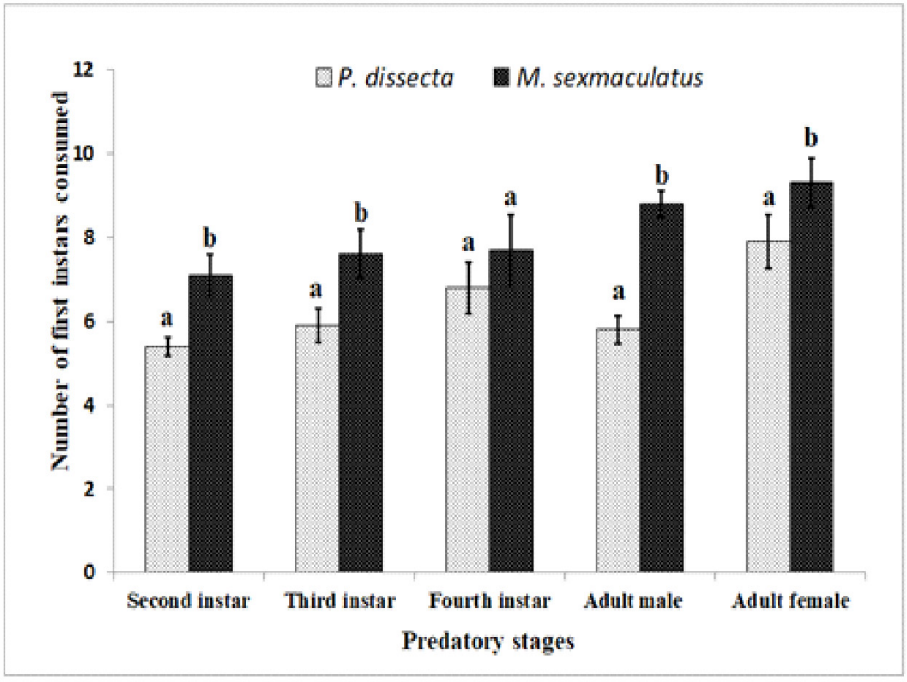


Fig.1 Intraguild predation of the first instar *P. dissecta and M. sexmaculatus* by different predatory stages of two ladybirds. Data is Mean ± S.E. Different letters denoted in the columns indicate that the data are statistically significant

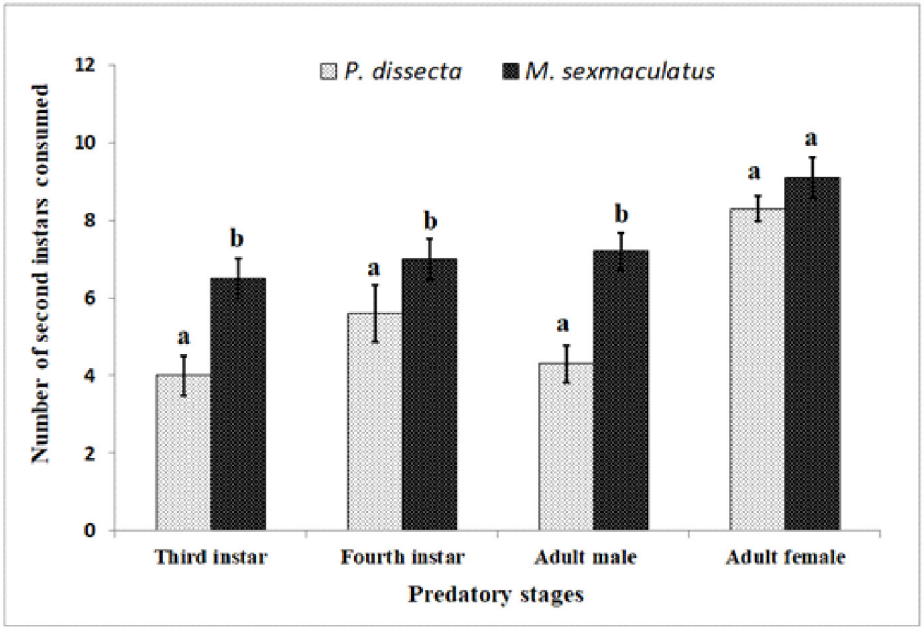


Fig.2 Intraguild predation of the second instar *P. dissecta and M. sexmaculatus* by different predatory stages of two ladybirds. Data is Mean ± S.E. Different letters in the columns denote that the data are satistically significant

Table1. Consumption of different predatory stages of ladybirds, *P. dissecta (Pd)* and *M. sexmaculatus (Ms)* on first and second instars

Prey stage Predator stage	I instar *Ms* *Pd*	I instar *Pd* *Ms*	II instar *Ms* *Pd*	II instar *Pd* *Ms*
Second instar	5.4 ± 0.70 a	7.1 ± 1.59 a	-	-
Third instar	5.9 ± 1.28 ab	7.6 ± 1.83 ab	4.0 ± 1.56 a	6.5 ± 1.65 a
Fourth instar	6.8 ± 1.93 bc	7.7 ± 2.71 ab	5.6 ± 2.3 b	7.0 ± 1.7 ab
Adult male	5.8 ± 1.03 ab	8.8 ± 1.03 bc	4.3 ± 1.49 a	7.2 ± 1.47 ab
Adult female	7.90± 2.02 c	9.3 ± 1.88 bc	8.3 ± 1.05 c	9.10 ± 1.66 c
F-value	F=4.54;P<0.01*	F=2.33; N.S.*	F=13.75;P<0.001**	F=4.91;P<0.01**

Data represents Mean ± S.D.; *d.f. = 4, 49 and Turkey's range =4.02; ** d.f. = 3, 39; Turkey's range =3.81 estimated using one-way ANOVA. Different letters in the same column denote that the data are statistically significant

dorsal spines perhaps is more needed by the first and second instars, whose smaller size makes them more vulnerable to IG predators. It was noted that dorsal spines perhaps protect larvae against IGP by decreasing the probability of being attacked and increasing the opportunities of biting back or escaping from the IG predators. This could be one of the reasons for the increase in population density and the dominance of *M. sexmaculatus* among the coccinellid fauna in the Oriental region (Omkar and Pervez, 2004). Inferior larval stages of *H. axyridis* secrete noxious reflex blood, harmonine, as a larval antipredator response against the IG predator's attack (Grill and Moore, 1998).

Zarei *et al.* (2020) suggested that size-disparity and hunger level of the IG predators trigger IGP by coccinellids. IGP in favour of *M. sexmaculatus* may be ascribed to its greater body-size and increased activity than *P. dissecta*. However, the fourth instar *M. sexmaculatus* seemed to be sluggish in attacking first-instars *P. dissecta*. This might be due to its critical weight, which might have been achieved before pupation. *Propylea dissecta* also co-occurs with *Coccinella transversalis* Fabricius in the agricultural fields and generally acts as an intraguild prey due to the larger size of the latter (Omkar *et al.,* 2006; Pervez *et al.,* 2006). *Propylea japonica* (Thunberg) was treated as IG prey by the co-occurring *H. axyridis* and *Coccinella septempunctata* L. (Yang *et al.,* 2017). *Harmonia axyridis* had a higher relative growth rate and faster developmental time during its second and third instar compared to *Coleomegilla maculata* (Labrie *et al.,* 2006). By attaining larger body size, *H. axyridis* is likely to be the successful intraguild predator than *C. maculata*.

Two-way ANOVA revealed that the main effects of 'species' (F = 26.15; P< 0.0001; d.f. = 1) and 'predatory stage' (F = 5.31; P< 0.001; d.f. = 4) were statistically significant using first instars as IG prey. The interaction 'species' and 'predatory stage' was not found to be statistically significant (F = 1.04; P = 0.390; d.f. = 4). Similarly, using second-instars as IG prey, the main effects of 'species' (F = 26.58; P< 0.0001; d.f. = 1) and 'predatory stage' (F = 17.19; P< 0.0001; d.f. = 3) were found to be statistically significant. The interaction 'species' and 'predatory stage' was not found to be statistically significant (F = 1.73; P = 0.390; d.f. = 3). Amongst the predatory stage, the adult female of both the ladybird species consumed a greater number of IG prey, which may be ascribed to the bigger body-size and increased energy demands owing to egg-production (ovariole number) (Rasekh and Osawa, 2020). Thus, both species and the predatory stage have a direct impact on the outcome of IGP. Little is known regarding IGP and egg cannibalism using *M. sexmaculatus* (Agarwala *et*

al., 1998; Agarwala and Yasuda, 2001), however no information is available on its IGP at the larval level. This study seems to be the pioneer in using *M. sexmaculatus* as both intraguild predator and prey. Thus, further studies on IGP and the rate of intraguild predation between the slow and fast developing variants of the two-coccinellid species are needed to investigate its successful establishment and dominance in the agro-ecosystems.

It is concluded that larval instars and adults of *M. sexmaculatus* and *P. dissecta* can attack the lower heterospecific instars during prey scarcity and indulge in IGP. However, *M. sexmaculatus* has more IGP potential, and it could act as intraguild predator in the absence of natural prey, *i.e.* aphids, and can easily attack heterospecific larvae, which could be the reason for its successful establishment and wide distribution.

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Morphological investigations on the wing scales of four species of common Indian butterflies

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ABSTRACT: Wing scales of butterflies exhibit extreme diversity in shape, size, colour, and number of spines. They are sub microscopic with a length of 300 to 600 µm and a breadth of 150 to 400 µm. A typical scale possessed a flat body with basal pedicel and apical crown which is provided with a varying number of pointed edges called spines. Investigations were carried out on the morphology of wing scales in four species of common butterflies viz., *Pachilopta hector* (Linnaeus 1758), *Troides minos* (Cramer 1779), *Jamides celeno* (Cramer 1775) and *Eurema andersonii* (Linnaeus 1758). Wings of *P. hector* possessed nine types of scales, the crown of all are with pointed spines of varying numbers, ranging from one to five. The southern birdwing *T. minos*, possessed nine types of scales. A major portion of wings with black colour is due to black coloured scales but white bands of the forewings are due to transparent and colourless scales. The prominent yellow colour on the hind wings of this butterfly is due to the presence of a single type of scale with a round crown that is devoid of spines and is fully packed with yellow pigment. *Jamides celeno* possessed twenty different types of scales, most of them are devoid of spines and the ridges within the scales are not clear. Scales on the upper surface of the wing with ashy blue colour and scales of white bands on the lower surface of the wings are identical and are transparent and colourless. The common grass yellow *E. andersonii* possessed twenty five different types of scales, of which thirteen are on the black margins of wings and twelve are in the yellow portions of wings. Almost half of the total number of scales in the yellow portions of the wing is transparent and colourless and in coloured scales distribution of pigment is not uniform. This is the first report on the different types of wings scales in the selected butterflies. © 2022 Association for Advancement of Entomology

KEY WORDS: Morphology, scales, types, spines, distribution of pigment, bands

INTRODUCTION

Lepidopteran insects produce their wing colours in two fundamentally distinct ways; via scale pigmentation (chemical colour) and through scale morphology (physical colour) (Vértesy *et al*., 2004). Butterflies and moths belong to the order Lepidoptera. The order is classified into 46 super families (Imms *et al*., 2013; Heppner, 2010) which are subdivided into 126 families (Heppner, 2022), each one with characteristic unique features. Butterflies are adored for their striking metallic colours and varied wing patterns. Presence of thousands of microscopic scales on their wings is the reason behind the diverse coloration in the animal kingdom. The periodic nanostructures of chitin and air in the scales of the wings produce the structural colours of butterflies (Ghiradella, 1991; Vukusic *et al*., 2002). Scales possess various types

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of pigments such as melanin with varying shades of colour from brown to black, derivatives of uric acid, and flavones of larval food, which provide different shades of yellow (Nijihout and Koch, 1991; Scoble, 1995). The structural colours of butterflies and moths have been attributed to a wide range of physical processes, including multilayer interference, diffraction, Bragg scattering, Tyndall scattering and Rayleigh scattering.

The upper surface of the wing contains two types of scales called cover and basal scales; which are alternately arranged in an overlapping manner. Scales are embedded in the scale sockets of the wing membrane. Therefore, the colours are believed to be due to the functional difference among the scales and actually they are modified hairs. Scale contains photonic nanostructures that are mainly constructed with chitinous matrix, including air holes (Scoble, 1995; Kristensen *et al.*, 2007). These microscopic scales attract more and more attention because they constitute a transition between random and crystalline order (Shawkey *et al*., 2009 a, b; Liu *et al.,* 2011; Munisha Murali and Sheeba, 2022). Scales have different functions like pattern formation, pheromone dispersal, thermoregulation, mimicry, visual signalling and protection from enemies (Archana *et al*., 2022). The most relevant studies about butterfly scales and photonics were done by Ghiradella (1998) and dealt mainly with the biological aspect of the subject. Despite this, butterfly wing scales represents a less studied area and demand the immediate attention of researchers to unravel the hidden truth behind these beautiful organisms. There are no detailed studies reported from India based on butterfly wing scales (Imms *et al*., 2013; Heppner, 2022). A study was undertakenon the morphology of wing scales in four species of common butterflies viz., *Pachilopta hector* (Linnaeus 1758)*, Troides minos* (Cramer 1779), *Jamides celeno* (Cramer1775) and *Eurema andersonii* (Linnaeus 1758).

MATERIALS AND METHODS

Selection of butterfly samples

Butterfly species that are commonly present in the premises of the University College, Thiruvananthapuram, Kerala were selected for the study. The selected butterfly families are Papilioniodae, Pieridae and Lycaenidae, from which four species, *P. hector, T. minos*, *J. celeno* and *E. andersonii,* were chosen for the study. Butterflies were identified using the taxonomic keys (Lowalker and Kunte, 2020; Heppner, 2022). Common grass yellow (*E. andersonii*) belonging to family Pieridae, has a yellow coloured body with a brown border. The yellow coloured regions and black border of the wing were analysed. Common cerulean (*J. celeno*) is a member of family Lycaenidae. The upper side of the butterfly has pale ashy white colour and the lower side of the butterfly have a greyish brown colour with transverse white bands. Both sides of the wings were selected for the study. Southern bird wing (*T. minos*) and crimson rose (*P. hector*) are members of super family Papilionoidea. The southern bird wing has a black coloured forewing with white streaks; the hind wing has a yellow colour with black margins. The yellow spot on the hind wing, white streaks on the forewing, and black colour of the wing were selected for collecting scales. Crimson rose is a swallow-tailed butterfly with black-coloured forewing with white patches. The hind wing has red or crimson coloured spots with tail-like extension. The areas selected for the study include red spots, white patches, and black colour on the wing. Butterflies collected for taxonomic studies were preserved as dry specimens and such samples were used for scale morphology.

Extraction of scales

Scales were collected from the upper and lower sides of the wing of the *J. celeno* butterfly. The upper surface of the wing is ashy blue and the lower surface is brown with white streaks and black eye spots possessing pseudo antennae at the posterior margin of the hind wing. Three regions were selected from the lower side of the wing and they are black spots on the hind wing, a brown border, and white wavy lines. Three regions of the *P. hector* were selected for closer observation. They are white-coloured streaks on the forewing, red-coloured spots on the hind wing, and black colour on certain parts of the wing. The selected

regions from *T. minos* include black-coloured regions and white shades on the wing. Yellow coloured regions of the hind wing were also selected for the study. In *E. andersonii,* the yellow coloured region and black border of the wings were selected for the study. Scales were gently separated from the wings by using soaked cotton attached to the pinhead and transferred to a microscopic slide, with a drop of water and covered with a coverslip. A microscope (Labomed, USA) with a camera was used to save the images, and measurements were recorded with help of micrometry software within the microscope.

RESULTS AND DISCUSSION

A typical scale has three-parts, a crown with spines, a flat body or blade, and a pedicel or stalk for the attachment to the scale socket. The body has upper and lower lamina. Longitudinally running parallel ridges are present in the lamina (Fig. 1). Wings of *P. hector* (Fig. 2) are black coloured with white bands or streaks on the forewings and prominent crimson spots on the hindwings. Nine different types of scales were identified from this butterfly (Fig. 3). Scale crown with a variable number of spines such as one, three, four or five could be identified in the study. Scale with a single spine appeared as a transparent lancet with a length of 480µm and width of 110 µm was identified from the black region (Fig. 3b4). The other three types of scales in the black region are black coloured but their spines in the crown varied from three to five and the intensity of black shade was different in individual scales. (Fig. 3b1- b3). Four types of crimsoncoloured scales were identified from crimson spots (Fig. 3a1- a4), with differences in size, number of crown spines, distribution of crimson pigments, and on possession of parallel lines. The length of these scales ranged from 460 to 490 µm and the width ranged from 160 to 310 µm. Only one type of scale was observed from the white bands of the forewings and were transparent, colourless with four spines (Fig. 3c).

Wing colour of the *T. minos* is a mixing of black shades with white prominence at the immediate vicinity of wing veins in forewings and bright yellow coloured with prominent black margins in hind wings. Nine different types of scales were identified from *T. minos* (Fig. 4). Some of the black coloured scales in their wings are very large and their length was up to 600 µm. White patches of forewing showed five types of scales, differing in shape, size and number of spines on the crown. The number of spines ranged from three to five and all of them were transparent and colourless. Scales of the white patches exhibited a length of 400 to 460 µm and width of 170 to 280 µm and they are the smallest scales in these butterflies (Fig. 5a1-a5). The yellow region exhibited only one type of scale which has a different morphology when compared with other scales. The yellow scale is devoid of spines and the scale appeared like a spatula (Fig. 5b). The black region of the hind wings and forewings showed three types of scales (Fig. 5c1- c3). One type of black scale has three spines and the others are broad with six spines on the crown.

The ashy blue colour on the upper surface of wings in *J. celeno* (Fig. 6) possessed six different types of scales (Fig.7). All these scales are transparent and colourless. The crown of all these scales is either devoid of spines or spines with smooth undifferentiated spines (Fig. 7a1 - a6). The length of the transparent scales ranged between 370 and 580µm and the breadth is between 180 and 200µm. Scales under the surface of the wings with brown shades of colour exhibited nine different types of scales (Fig. 8) and their pigmentation is not uniform. Some of the scales in the brown region possessed granulated ridges (Fig. 8b1), others are devoid of such granules. Crown of all these scales are smooth and spines are undifferentiated. Some of the scales in the brown regions are lancet like with length ranges between 560 and 580 µm and width ranges between 40 and 50 µm (Fig. 8b5). Black eye spot on the lower surface of the wing *J. celeno* possessed four different types of scales (Fig. 8c1 to c4). Unlike the scales in other regions of the wing, scales of the black eye spot possessed well-differentiated spines on the crown, the number of which varied from three to six (Fig. 8c1, c4). Among these four different types of scales one group of scales are narrow and long with length up to 600 µm and breadth up to 120 µm (Fig. 8c1 and

c2), and the other group of scales are broad with length up to 500 µm and breadth up to 400 µm (Fig. 8c3, c4). All the seven white bands seen as traversed by the underside of the forewing exhibited only one type of transparent and colourless scale (Fig. 8 d1). Due to their extremely transparent nature, the scales were nearly invisible under the microscopic field. In total, 20 different types of scales are identified in *J. celeno*. Compared to the scales of *T. minos* and *P. hector* scales of *J. celeno* are thin and scale ridges are not visible.

Common grass yellow *E. andersonii* is very common butterfly with a wingspan of 40-50mm. It is recognized by their bright yellow wings with a black margin on the upper side of the wings. Both pairs of wings (Fig. 9) are yellow with black margins. Twelve different types of scales were identified from the black margins and they exhibited significant variation in size, shape, colour and the number of spines (Fig.10). Majority of scales were with three or four spines and all of them are small with 320 to 350 µm length and 300 to 320 µm width. Another small proportion of black scales with two spines and among them one type of long narrow and broad triangular with the tapering basal end (Fig. 10a6) were detected.Yellow portions of wings in *E. andersonii* exhibited thirteen different types of scales (Fig. 11). Among them almost half the proportion exhibited yellow pigmentation (Fig. 11 b1- b6) but the body is not filled with pigment. Crown of these scales exhibited variation in shapes such as flat, round, and round spines. The other half proportion of scales are completely transparent and colourless (Fig. 11 b7 - b13). A total of 25 different types of scales were identified from the wings of *E. andersonii.*

Ghiradella (1991, 1998) has extensively studied the structure of the Lepidopteran wing using TEM micrographs and reported that the chitinous wing scale has different parts, and they are organized into the upper and lower lamina. The lamina has numerous rows of parallel ridges with cross ribs in between them, are called as trabeculae. It has now been proposed that all structurally coloured butterfly scale nanostructures, regardless of anatomical position (on the surface or within the scale) or spatial organization (multilayer or crystal-like), share the same physical process for producing colour. The mechanism is called as coherent scattering, which means the differential reinforcement and interference of visible wavelengths by light scattered within the nanostructureswhich differed in refractive index (Prum *et al.,* 2006). Transparent scales were obtained from all the white regions, irrespective of the butterfly taken. Transparent scales reflect all the incident light resulting in white colour as seen in the case of southern bird wing and crimson rose. Papilionids exhibit more structurally coloured scales than other families. This group represents all the structurally coloured scale types reported so far in all other families (Ghiradella, 1985). Transparent, yellow, red and dark-coloured scales are observed in the papilionid butterflies examined.

Pachliopta hector is one of the spectacular species of swallow tails, with a red body and wing span of 90-120 mm. The upper side of the fore wings is bluish-black and fore wings carry interrupted, and irregular discal and apical white bands, and the hind wings bear nearly round and marginal rows of bright crimson crescent spots (Ramana *et al*., 2004). Nine different types of scales could be identified in this butterfly. Eventhough the background colour of wings is black the colour of scales in this region of wings ranged with different shades of brown and the colour variation is due to variation in the melanin pigment deposited in them. Among the four species of the studied butterflies, *P. hector* possessed wing scales with clear and sharp ridges in the scale lamina.

Commonly known as southern birdwing *T. minos* is one of the largest Indian butterflies with a wing span ranging between 140-190 mm, is considered as least concern on IUCN red list (Sharmila and Thatheyus, 2014; Jiji *et al*., 2015). Now, it is described as the state butterfly of Karnataka state in India, and this species is endemic to the Western Ghats of south India (Jiji *et al*., 2015; Shawkey *et al*., 2009a, b). The forewings of are black coloured with white shades along their cellular disc regions. The hind wings have a bright yellow colour with black coloured veins and black markings at their

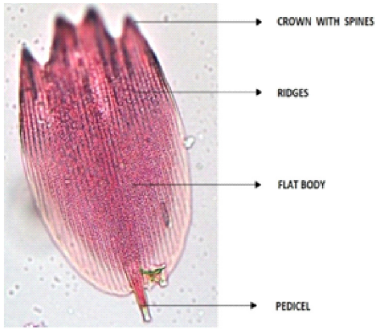


Fig. 1

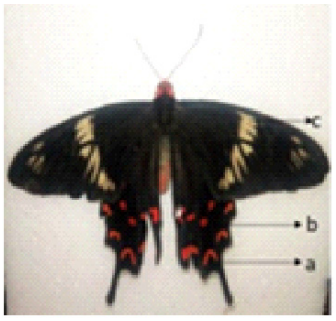


Fig. 2

Fig. 1 Structure of a typical wing scale
Fig. 2 *Pachliopta hector* L.- Three regions of the butterfly selected for the study are marked as a, b & c

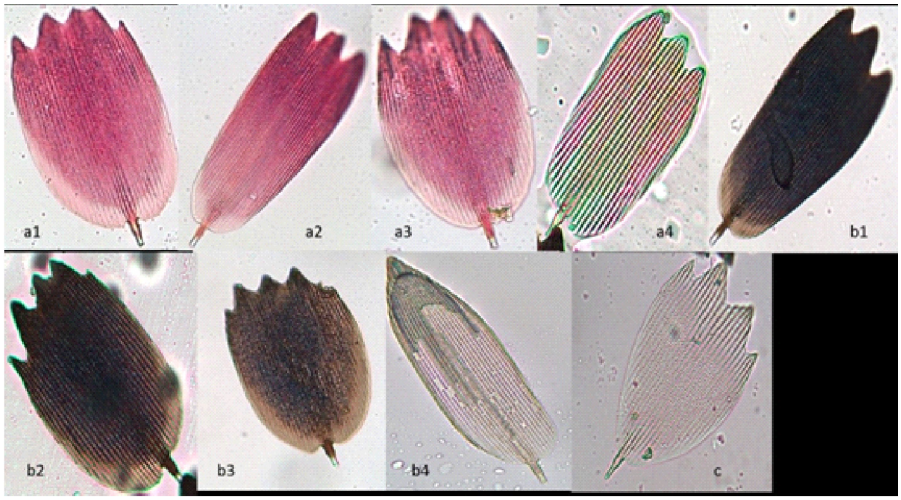


Fig. 3 Wing scale diversity of *Pachliopta hector* L. obtained from regions which are marked as a, b and c.Length ranged from 460 to 490 µm and width ranged from 160 to 310 µm



Fig. 4

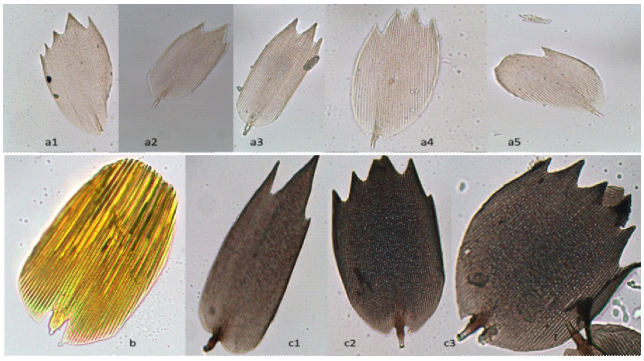


Fig. 5

Fig. 4 *Troides minos* -Three regions of the butterfly selected for the study are marked as a,b & c Fig. 5 Wing scale diversity of scales obtained from *Troides minos* -Scales of the white patches exhibited length of 400 to 460 µm and width of 170 to 280 µm and are the smallest scales in these butterflies



Fig. 6 *Jamedes celeno*-Upper and lower sides of the butterfly scales removed from the marked regions are indicated as a,b,c and d

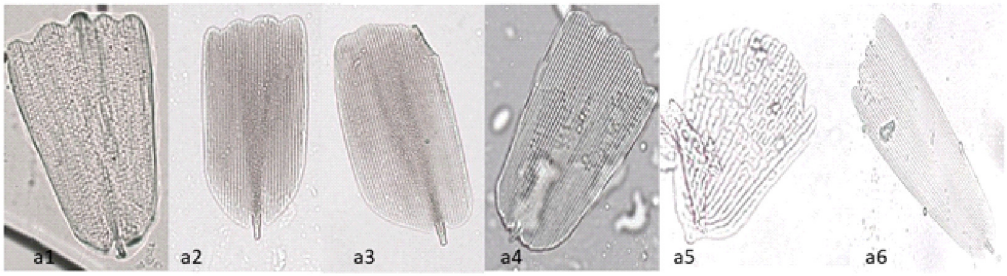


Fig. 7 Scales of ashy blue colour in *Jamedes celeno*- Six different types of scales are identified from ashy blue side of wing (Fig. 7a1 to a6). Length ranged between 370 and 580 µm and breadth between 180 and 200 µm

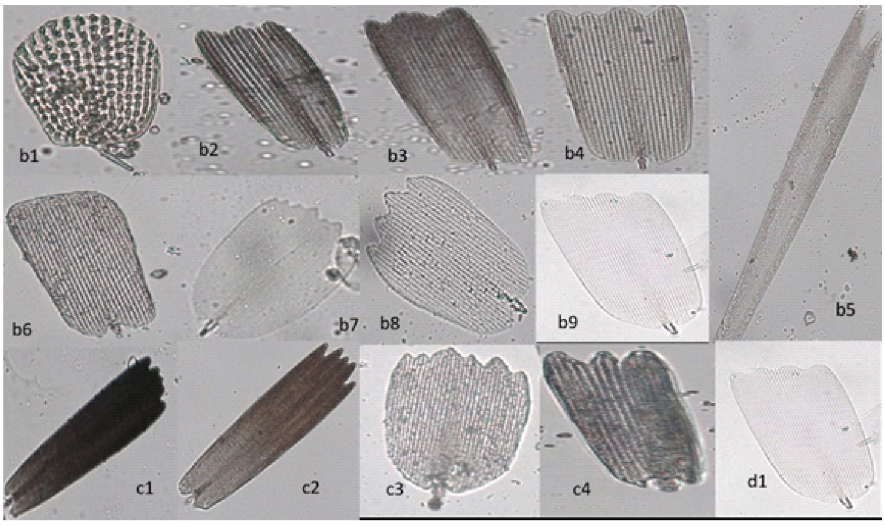


Fig. 8 Scales of brown region, black eye spot and white lines in *Jamedes celeno* (rod like with length between 560 and 580 µm and width between 40 and 50 µm (Fig. 8 b5). Two types of scales in the eye spot are black, narrow and long with length upto 600 µm and breadth upto 120 µm (Fig. 8 c1 and c2), the other two group of scales are broad with length upto 500 µm and breadth upto 400 µm (Fig. 8c3 and c4). Scales of the white bands are transparent (Fig. 8 d1)

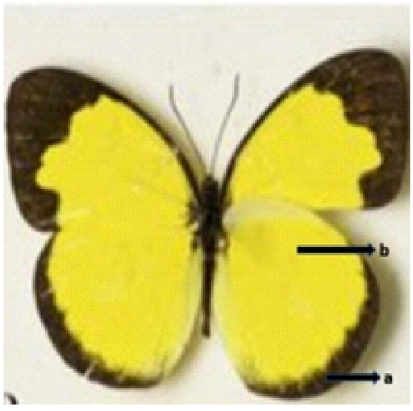


Fig. 9

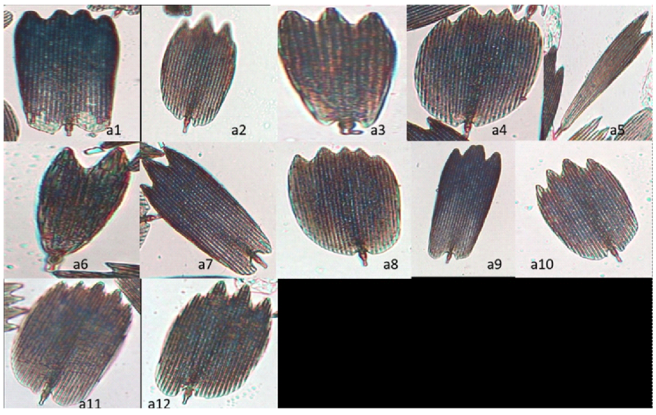


Fig. 10

Fig. 9 *Eurema hecabe* Fig. 10 Scales on the black margins of wings in *Eurema hecabe* - Majority of scales are with three or four spines with 320 to 350 µm in length and 300 to 320 µm width

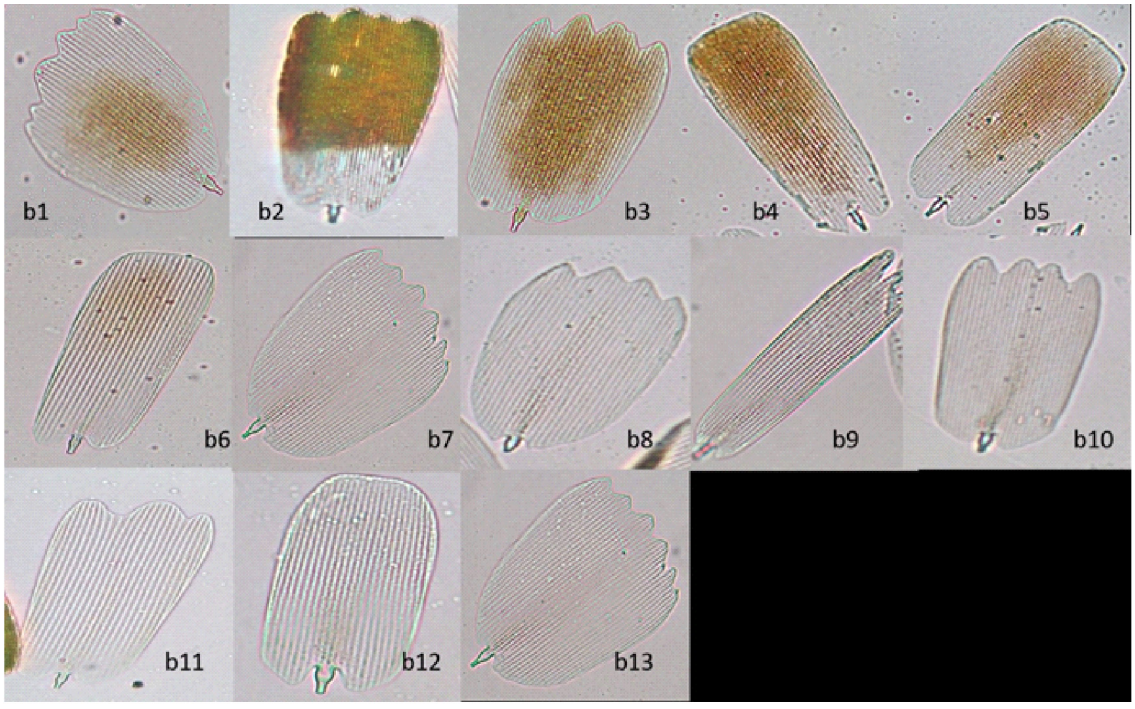


Fig. 11 Scales on the yellow regions of wings in *Eurema hecabe* - Among the scales, almost half the population exhibited yellow pigmentation (b1 to b6) but the body is not completely filled with pigment. Crown of the scales exhibited variation in shape such as flat, round and with round spines. Other scales are completely transparent and colourless (b7 to b13)

margins. A black spot was observed between the hind wing's 5th and 6th disc cellular veins (Ghiradella, 1991). Among the four species of the studied butterflies, *T. minos* exhibited largest of all scales with length upto 600 µm.

In *J. celeno* mostly transparent and brown-coloured scales observed. The vivid colours on the upper surface of wings in the lycaenids, normally ranges between the blues and violets but extends to the ultraviolet and greens, arise from microstructural components of the undifferentiated structures embedded withinthe transparent scales. This will enable the white light falling upon the scales to interfere one with another; the resulting a species specific colour which is a cumulative action of physical effects and not due to pigmentation (Onslow, 1921; Mason, 1926). Extensive anatomical studies of lycaenid wing scales have revealed three different spatial organizations that give rise to structural colours. Lycaenid butterflies produce iridescent colours via a multilayer elaboration within the lumen of the scale into a Urania-type microstructure (Tilley and Eliot, 2002). Wilts *et al.* (2009, 2011) suggested that lycaenids apparently can modulate their coloration by subtle changes in their scale structures. *J.celeno* is a sexually dimorphic species with wide distribution in the south and East Asia (Eastwood *et al*., 2005) and is a tailed bluish-white butterfly with the terminal margin of the forewing having a narrow border. When the wings are kept extended, the upper surface appeared as ashy blue but in the folded state, it has a whitish appearance. The lower surface of the wings is greyish brown traversed by seven white lines. At the folded state hind wings possessed a pair of pseudo antennae and prominent black eye spots. The characteristic feature observed in this butterfly is the difference on the arrangement of transparent and colourless scales on either side of wings. Scales arranged on the upper surface of wingsare in such a way to cause diffraction of light, leading to ashy blue colour and identical scales on the lower surface of wings which differ on the pattern of arrangement, resulting reflection of lightleads to white colour.

Transparent and yellow coloured scales were obtained from pierid butterflies. Previous work demonstrated that the pigments of pierid wings belong to the class of pterins (Wilts *et al*., 2009, 2011; Archana *et al*., 2022). Various pterins have different absorption spectra, which can be restricted to the ultraviolet or can extend into the yellow or green wave length range. Another study reports that the scales of pierid butterflies have usually numerous pigmented beads, which absorb light at short wavelengths and enhance light scattering at long wavelengths. Males of many species of the pierid subfamily Coliadinae have ultraviolet-iridescent wings because the scale ridges are structured into a multilayer reflector. The iridescence is combined with a yellow or orange-brown coloration, causing the common name of the subfamily, the yellows or sulfurs (Stavengaen and Leertouwer, 2007). In the present study E. *andersonii* showed 25 different types of scales. Conspicuous yellow colour in both pairs of wings in *E. andersonii*is due to packed arrangement of completely colourless and transparent scales and another group of scales with their distal end is yellow but proximal end is colourless.The study demonstrates that the structure of the scale is different in different species of butterflies and the butterflies under the same family also showed different types of scales. There were transparent and colourless scales with differences in scale morphology from the all-white regions examined, irrespective of the butterfly species selected. From this, it is evident that scales play a fundamental role in the colour production and pattern formation of butterfly wings.

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Field evaluation of management strategies against *Lipaphis erysimi* (Kaltenbach) (Homoptera, Aphididae) infesting Indian mustard in Haryana, India

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ABSTRACT: Effectiveness of diverse eco-safe strategies against mustard aphid, *Lipaphis erysimi* (Kaltenbach) infesting Indian mustard was evaluated for two years with 11 treatments viz., *Beauveria bassiana* @ 10^8 CS ml^{-1}, neem seed kernel extract (NSKE) @ 5 per cent, neem oil @ 5 per cent, *B. bassiana* @ 10^8 CS ml^{-1} after clipping of infested twigs (CIT), nimbecidine @ 0.03 per cent, NSKE @ 5 per cent after CIT, neem oil @ 5 per cent after CIT, nimbecidine @ 0.03 per cent after CIT, clipping of infested twigs alone, dimethoate 30 EC @ 625 ml ha^{-1} and control. The pooled data revealed that dimethoate contributed maximum efficacy in reducing *L. erysimi* population over control (89.74 %), followed by *B. bassiana* after CIT (83.16 %) and nimbecidine @ 0.03 per cent after CIT (80.51%). Seed yield (1716 kg ha^{-1}) was maximum in dimethoate, followed by treatments *B. bassiana* @ 10^8 CS ml^{-1} after CIT (1636.5 kg ha^{-1}) and nimbecidine @ 0.03per cent after CIT (1608 kg ha^{-1}), whereas minimum (1211 kg ha^{-1}) in the control. The gross income (Rs 64350 ha^{-1}) and net return (Rs 18017 ha^{-1}) were highest in dimethoate, followed by *B. bassiana* @ 10^8 CS ml^{-1} after CIT with gross income of Rs 61388 ha^{-1} and net return of Rs 13865 ha^{-1}. The incremental cost-benefit ratio was also maximum in dimethoate (1: 19.58), followed by *B. bassiana,* nimbecidine and NSKE treatments (1: 6.33 to 7.27). Results suggest that *B. bassiana* @ 10^8 CS ml^{-1} after CIT and nimbecidine @ 0.03 per cent after CIT can be used as a non- chemical control option as a substitute to chemical control. © 2022 Association for Advancement of Entomology

KEY WORDS: *Beauveria bassiana*, clipping, bio-intensive IPM, cost-benefit ratio

INTRODUCTION

Aphids are nefarious, sap-sucking, soft-bodied insect pests of Brassicaceae members (Guerrieri and Digilio, 2008; Blackman and Eastop, 2017). The mustard aphid, *Lipaphis erysimi* (Kaltenbach) (Homoptera, Aphididae) is the most overwhelming sucking insect pest of rapeseed-mustard in India. Worldwide, India placed 1st in the case of the rapeseed-mustard area and occupied 2nd position in production after China. Rapeseed-mustard is economically important as it provides vegetables, animal feed and edible oils (Khavse *et al*., 2014; Jat *et al*., 2019). Mustard has very good nutritional value due to its seed containing proteins (17-25%), fibres (8-10%), and oil (30-33%) (Sudhir *et al*., 2013). Indian mustard (*Brassica juncea* L.) oil contains a high amount of oleic, eicosenoic, and erucic acids (70%) and linoleic and linolenic acids (22 %), and a low amount of palmitic and stearic

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acids (8%) (Kumar, 2015). In India, rapeseed-mustard crops are grown in the area of land 6.69 million hectares with a production of 10.11 million tonnes and productivity of 1511 kg ha^{-1}. In Haryana, it was cultivated on 0.63 million hectares with production and productivity of 1.28 million tonnes and 2027 kg ha^{-1} respectively (Anonymous, 2021).

Mustard aphid suck the cell sap from different parts of the plant viz., leaves, inflorescence, tender stem, and pods. Its substantial attack causes curling of leaves, weak pod formation and undersized grains. Honeydew secreted by this insect pest was liable for the development of sooty mould and reduces the photosynthetic rate (Bakhetia and Sekhon, 1989). Heavy infestation of *L. erysimi* (Fig. 1) causes seed yield loss ranging from 32.62 to 100 per cent (Singh and Sachan, 1999; Sahoo, 2012; Sharma *et al*., 2019; Shrestha *et al*., 2020). Patel *et al*. (2017), Maurya *et al*. (2018), Kumar and Sharma (2020) and Kumar (2021) concluded that the chemical insecticides such as thiamethoxam 25 WG, fenvalerate 20 EC, malathion 50 EC, dimethoate 30 EC, quinalphos 25 EC, chlorpyriphos 20 EC, imidacloprid 17.8 SL, acephate 75 SP, pymetrozine 50 WG, clothianidine 50 WDG and acetamiprid 20 SP were effective in the management of *L. erysimi* in diffrent regions of the country.

The main concern in chemical control is that it causes environmental pollution, adverse effects on human health, resurgence, and toxicity to pollinators and natural enemies (Singh, 2001). This has demanded the use of substitute non- chemical (eco-friendly) strategies viz., Aloe vera leaf extract @10 per cent, neem oil @ 2% followed by *Chilomenes septempunctata* @ 5,000 beetles ha^{-1}, *Verticilium lecanii* @ 10 ^{8} CS ml^{-1} + clipping of infested twigs, *Beauveria bassiana* @ 10^{8} CS ml^{-1} + NSKE @ 5 per cent, tobacco leaf extract @ 10 per cent, eucalyptus leaf extract @ 10 per cent and azadirachtin 1500 ppm @ 1.0 ml litre^{-1} of water for the management of *L. erysimi* on mustard (Yadav and Singh, 2015; Sharma *et al*., 2017; Kumar *et al*., 2020). The present investigation was carried out to evaluate the efficacy of certain environmentally friendly strategies against *L. erysimi* in the mustard ecosystem.

MATERIALS AND METHODS

Field experiment was undertaken in 2019-20 and 2020-21 during *rabi* season in the farmer's field, Kolana village, Aravalli Hills Region, Rewari, Haryana, India. It is located in the south western area of Haryana at 28°12'24.7"N latitude, 76°21'11.0"E longitude, and an altitude of 296 m above sea level. This region falls under semi-arid zones of the country with dry and hot summer as well as severe cold in winter. The surface soils textures at the experimental area are sandy loam. *Brassica juncea* genotype RH 725 taken as the host plant for *L. erysimi* was obtained from the Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Regional Research Station (RRS), Bawal, Rewari, Haryana, India. Experiments were conducted under randomized block design with three replications and 11 treatments (Table 1).

Preparation of Neem seed kernel Extract (NSKE): One kg of dried neem seed kernels crushed and soaked overnight in 10 litres water. Soaked material was filtered through muslin cloth and the volume of the filtrate was made to 10 litres. Dilute to 5 per cent (50 ml decanted solution in one litre of water) and 1 per cent Teepol (10 ml litre^{-1} of water) was added at the time of spraying (Anonymous, 2008).

Preparation of Neem oil: Neem seeds were picked from neem plants, and these seeds were dried and extracted in the oil expeller machine. Crude oil was filtered through muslin cloth and that oil was used as per the requirement of the experiment.

The seeds of genotype *B. juncea* RH 725 were sown in the field in each plot of 4.2×3 m size at 30×10 cm spacing. The crop was raised following standard recommended agronomic practices and irrigations. The treatments were imposed one time at the pod formation stage, when the target pest reached the economic threshold level. The population counts of the aphids in the field were recorded from 10 cm main apical shoot of 10 randomly selected and tagged plants in each plot. Pre-treatment counts refer to the pest population

was undertaken one day before the treatments, whereas post-treatment interpretations were made on the first, third, seventh, tenth, and fifteenth days after spray of treatments (Sharma *et al.*, 2017). For calculating the per cent reduction in pest population over control, the following formula was used.

$$\frac{\text{Population in control - Population after spray}}{\text{Population in control}} \text{ X } 100$$

After harvesting the crop, seed yield from each plot was weighed, and then converted into kg ha^{-1}. To determine the economic viability of different treatments, gross income was computed by multiplying seed yield (kg ha^{-1}) with the price of mustard seed @ Rs 37.50 kg^{-1}. The net return over control was worked out by subtracting total cost of treatment from incomes obtained from increased seed yield over control. Total cost of treatment comprised cost of treatment and labour charge. The incremental cost-benefit ratio was calculated by dividing net return over control with total cost of treatment.

The data on pest population was statistically analysed after square root transformation. The critical difference (CD) at 5 per cent level of probability was computed to assess the significant difference amid treatment means by proper method using online statistical software OPSTAT developed by Sheoran *et al*. (1998).

RESULTS AND DISCUSSION

Pre-treatment *L. erysimi* population: The pooled data of two years (*Rabi*, 2019-20 and 2020-21) showed that in pre- treatment (before spray), the pest population (17.34 to 19.88 aphids plant^{-1}) scattered non- significantly (P>0.05) (Table 1).

Effect of different treatments on population: The pooled data revealed that all the treatments were significantly ($p< 0.05$) superior in decreasing the infestation of *L. erysimi* over control (Table 1). At 1st day after spray (DAS), treatment T_{10}- dimethoate 30 EC @ 625 ml ha^{-1} was most effective with minimum population of 9.29 aphids plant^{-1}, followed by T_4- *B. bassiana* @ 10^8 CS ml^{-1} after CIT (11.05 aphids plant^{-1}) and T_8- nimbecidine @ 0.03 per cent after CIT (12.72 aphids plant^{-1}), which were statistically at par with each other. After the treatment, T_1- *B. bassiana* @ 10^8 CS ml^{-1} (12.94 aphids plant^{-1}) was statistically at par with T_6- NSKE @ 5 per cent after CIT (13.18 aphids), T_7- neem oil @ 5 per cent after CIT (13.52 aphids) and T_5- nimbecidine @ 0.03 per cent (14.23 aphids). Following the next order of efficiency, T_9- CIT (15.15 aphids), T_2- NSKE @ 5 per cent (15.94 aphids) and T_3- neem oil @ 5 per cent (16.54 aphids) treatments were statistically at par with one another. The maximum pest population was recorded in T_{11}- control (21.32 aphids). At 3rd DAS, the minimum pest population (5.30 aphids) was observed in dimethoate 30 EC @ 625 ml ha^{-1} against control (24.14 aphids). Next promising treatments were *B. bassiana* @ 10^8 CS ml^{-1} after CIT (8.30 aphids), nimbecidine @ 0.03 per cent after CIT (9.28 aphids) and *B. bassiana* @ 10^8 CS ml^{-1} (9.55 aphids) and all were statistically at par with one another. There after, treatment NSKE @ 5 per cent after CIT (10.43 aphids) was noted statistically at par with neem oil @ 5 per cent after CIT (11.28 aphids). It was followed by nimbecidine @ 0.03 per cent (12.44 aphids) and NSKE @ 5 per cent (13.79 aphids) and are statistically at par with each other, followed by neem oil @ 5 per cent (14.60 aphids). Treatment CIT was observed least effective (17.40 aphids). Outcomes obtained on the seventh and tenth days after spray showed the same order of effectiveness of various treatments against *L. erysimi*. Dimethoate was the most effective treatment among all the tested, followed by *B. bassiana* @ 10^8 CS ml^{-1} after CIT and nimbecidine @ 0.03 per cent after CIT, while CIT alone was least effective (Table 1).

Observing the inclusive efficacy of different treatments against *L. erysimi* revealed that at the 15th DAS, the lowest number of pest population (4.02 aphids) was observed in dimethoate and was superior to all the remaining treatments. The subsequent promising treatments were *B. bassiana* @ 10^8 CS ml^{-1} after CIT (6.60 aphids) and nimbecidine @ 0.03 percent after CIT (7.64 aphids) and both were statistically at par with each other. Treatment *B. bassiana* @ 10^8 CS ml^{-1} (8.19 aphids) was found statistically at par with NSKE @ 5 per

cent after CIT (9.23 aphids plant^{-1}); followed by neem oil @ 5 per cent after CIT (9.95 aphids) and nimbecidine @ 0.03 per cent (10.82 aphids), which were statistically at par with each other. The next effective treatment NSKE @ 5 per cent (11.67 aphids) was statistically at par with neem oil @ 5 per cent (12.97 aphids), however CIT alone was detected least effective (29.28 aphids). The maximum pest population (39.19 aphids plant^{-1}) was registered in control (Table 1).

Pest reduction: Combined results of both the years revealed treatment at 15 DAS, the population reduction was maximum (89.74 %) in dimethoate and was paramount treatment in managing the pest. This is in accordance with Meena *et al*. (2013), Singh *et al*. (2014), Sharma *et al*. (2017), Kumar *et al*. (2020), Kumar and Sharma (2020) and Yadav *et al*. (2021). In the present study *B. bassiana* @ 10^8 CS ml^{-1} after CIT gave 83.16 per cent pest reduction, followed by nimbecidine @ 0.03% after CIT (80.51 %), *B. bassiana* @ 10^8 CS ml^{-1} (79.10%), NSKE @ 5 per cent after CIT(76.45%), neem oil @ 5 per cent after CIT (74.61%), nimbecidine @ 0.03 per cent (72.39%), NSKE @ 5 per cent (70.22%) and neem oil @ 5 per cent (66.90%), whereas CIT was reported least

Table 1. Field efficacy of different treatments against *L. erysimi* in *B. juncea* (Pooled data of *Rabi*, 2019-20 and 2020-21)

Treatments	Population of *L. erysimi* plant^{-1}						Pest reduction (%)
	Before spray	1 DAS	3 DAS	7 DAS	10 DAS	15 DAS	
T_1- *Beauveria bassiana* @ 10^8 CS ml^{-1}	19.88 (4.57)	12.94 (3.73)	9.55 (3.24)	9.07 (3.17)	8.82 (3.13)	8.19 (3.02)	79.10
T_2- Neem seed kernel extract (NSKE) @ 5%	18.80 (4.45)	15.94 (4.11)	13.79 (3.85)	12.90 (3.73)	12.19 (3.63)	11.67 (3.56)	70.22
T_3- Neem oil @ 5%	19.35 (4.51)	16.54 (4.19)	14.60 (3.95)	13.82 (3.85)	13.30 (3.78)	12.97 (3.74)	66.90
T_4-*Beauveria bassiana* @ 10^8 CS ml^{-1} after CIT	17.57 (4.31)	11.05 (3.47)	8.30 (3.05)	7.25 (2.87)	6.83 (2.80)	6.60 (2.76)	83.16
T_5- Nimbecidine @ 0.03%	17.52 (4.30)	14.23 (3.90)	12.44 (3.66)	11.62 (3.55)	11.15 (3.48)	10.82 (3.44)	72.39
T_6- NSKE @ 5% after CIT	17.89 (4.34)	13.18 (3.76)	10.43 (3.38)	10.12 (3.33)	9.62 (3.26)	9.23 (3.20)	76.45
T_7- Neem oil @ 5% after CIT	17.34 (4.28)	13.52 (3.81)	11.28 (3.50)	10.84 (3.44)	10.47 (3.39)	9.95 (3.31)	74.61
T_8- Nimbecidine @ 0.03% after CIT	18.77 (4.45)	12.72 (3.70)	9.28 (3.21)	8.45 (3.07)	7.95 (2.99)	7.64 (2.94)	80.51
T_9- Clipping of infested twigs (CIT)	18.10 (4.37)	15.15 (4.02)	17.40 (4.29)	19.82 (4.56)	23.75 (4.97)	29.28 (5.50)	25.29
T_{10}- Dimethoate 30 EC @ 625 ml ha^{-1}	19.00 (4.47)	9.29 (3.21)	5.30 (2.51)	4.62 (2.37)	4.15 (2.27)	4.02 (2.24)	89.74
T_{11}- Control (unsprayed)	18.52 (4.42)	21.32 (4.72)	24.14 (5.01)	29.82 (5.55)	31.78 (5.73)	39.19 (6.34)	-
CD at 5%		0.24	0.25	0.23	0.23	0.24	-
SE(m)	0.06	0.08	0.08	0.08	0.08	0.08	-

Figures in parentheses are square root transformed value; DAS- Day after spray ; CIT - clipping of infested twigs

Table 2. Comparative economic analysis of different treatments against *L. erysimi* in *B. juncea* (Pooled data of *Rabi*, 2019-20 and 2020-21)

Treatments	Total cost (Rs. ha^{-1})	Seed yield (kg ha^{-1})	Gross income (Rs. ha^{-1})	Income over control (Rs. ha^{-1})	Net returns over control (Rs. ha^{-1})	cost-benefit ratio
T_1- *Beauveria bassiana* @ 10^8 CS ml^{-1}	1810	1587.5	59550	14137	12327	1: 6.81
T_2- Neem seed kernel extract (NSKE) @ 5%	1350	1475	55313	9900	8550	1: 6.33
T_3- Neem oil @ 5%	2000	1460.5	54788	9375	7375	1: 3.69
T_4-*Beauveria bassiana* @ 10^8 CS ml^{-1} after CIT	2110	1636.5	61388	15975	13865	1: 6.57
T_5- Nimbecidine @ 0.03%	1590	1528.5	57338	11925	10335	1: 6.50
T_6- NSKE @ 5% after CIT	1650	1574.5	59063	13650	12000	1: 7.27
T_7- Neem oil @ 5% after CIT	2300	1552	58200	12787	10487	1: 4.56
T_8- Nimbecidine @ 0.03% after CIT	1890	1608	60300	14887	12997	1: 6.88
T_9- Clipping of infested twigs (CIT)	300	1235.5	46350	937	637	1: 2.12
T_{10}- Dimethoate 30 EC @ 625 ml ha^{-1}	920	1716	64350	18937	18017	1:19.58
T_{11}- Control (unsprayed)	-	1211	45413	-	-	-

Note: Labour charge for the spray of each insecticide= Rs. 450 ha^{-1}; Labour charge for applying treatment Clipping of infested twigs (cultural method) = Rs. 300 ha^{-1}; Price of mustard = Rs. 37.50 kg^{-1}.



Fig. 1 *L. erysimi* infested plants of *B. juncea*

effective (25.29%). The results of Sharma *et al*. (2017) and Yadav *et al*. (2021) corroborated with present findings, who suggested that treatments viz., *B.bassiana* @ 10^8 CS ml^{-1} (79.44 and 82.42%), *B. bassiana* @ 10^8 CS ml^{-1}+ clipping infested twigs (84.09 and 85.88 %), NSKE @ 5 per cent (82.63 and 80.19 %) and NSKE @ 5 per cent+clipping infested twigs (87.77 and 83.74 %) controlled *L. erysimi* infestation. Chanchal and Lal (2009) and Kumar *et al*. (2020) reported that NSKE 5, neem oil 2 and 3 per cent reduced the population of *L. erysimi* effectively.

Seed yield and economics: Maximum seed yield (1716 kg ha^{-1}) was noted in dimethoate treatment as compared to the control (1211 kg ha^{-1}). It was followed by *B. bassiana* @ 10^8 CS ml^{-1} after CIT (1636.5 kg ha^{-1}), nimbecidine @ 0.03 per cent after CIT (1608 kg ha^{-1}), *B. bassiana* @ 10^8 CS ml^{-1} (1587.5 kg ha^{-1}), NSKE @ 5 per cent after CIT (1574.5 kg ha^{-1}), neem oil @ 5 per cent after CIT (1552 kg ha^{-1}), nimbecidine @ 0.03 per cent (1528.5 kg ha^{-1}), NSKE @ 5 per cent (1475 kg ha^{-1}) and neem oil @ 5 per cent (1460.5 kg ha^{-1}), whereas minimum was registered in CIT alone (1235.5 kg ha^{-1}) (Table 2). Sharma *et al*. (2017), Kumar *et al*. (2020) and Yadav *et al*. (2021) observed maximum seed yield in dimethoate 30 EC @ 625 ml ha^{-1}.

Highest gross income (Rs 64350 ha^{-1}) and maximum net return over control (Rs 18017 ha^{-1}) was obtained in treatment dimethoate with the maximum incremental cost-benefit ratio (ICBR) of 1:19.58. Among non-chemical treatments, treatment *B.bassiana* @ 10^8 CS ml^{-1} after CIT recorded maximum gross income (Rs 61388 ha^{-1}) and net return over control (Rs 13865 ha^{-1}) but ICBR with 1:6.57 ICBR, whereas NSKE @ 5 per cent after CIT gave 1:7.27 ICBR. The next promising treatments were nimbecidine @ 0.03 per cent after CIT (Rs 60300 and 12997 ha^{-1}), *B. bassiana* @ 10^8 CS ml^{-1} (Rs 59550 and 12327 ha^{-1}) and NSKE @ 5 per cent after CIT (Rs 59063 and 12000 ha^{-1}), whereas minimum was noted in treatment CIT alone (Rs 46350 and 637 ha^{-1}), respectively. ICBR in the treatment nimbecidine @ 0.03 per cent after CIT was 1: 6.88 and found economically viable after treatment NSKE @ 5 per cent after CIT, followed by treatment *B. bassiana* @ 10^8 CS ml^{-1} (1: 6.81), while treatment CIT alone was computed least economical (1: 2.12) (Table 2). Sharma *et al*. (2017) also reported highest cost-benefit ratio in dimethoate (1:14.92), followed by NSKE @ 5 per cent + clipping of infested twig (1:13.81). Sahoo (2012), Meena *et al*. (2013) and Kumar *et al*. (2020) reported dimethoate as economical treatment.

Conclusively, the chemical treatment, dimethoate 30 EC @ 625 ml ha^{-1}demonstrated high efficacy in comparison to non- chemical treatments in managing *L. erysimi* population. However, non- chemical treatments such as *B. bassiana* @ 10^8 CS ml^{-1} after CIT and nimbecidine @ 0.03 per cent after CIT were efficacious in suppressing the aphid infestation and recommended as an excellent substitute to chemical controls for the aphid management as a result avoids detrimental effects of chemical insecticides on human health, non- target organisms and environment.

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A new species of *Protosticta* Selys, 1885 (Odonata, Zygoptera, Platystictidae) from the Brahmagiri Hills, Kerala, India

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ABSTRACT: A new species of *Protosticta* Selys, 1885 is described from Brahmagiri hills of Coorg landscape of the Western Ghats in Peninsular India. The new species *Protosticta francyi* **sp. nov**., is a congener of *P. antelopoides* Fraser, 1931 and *P. ponmudiensis* Kiran, Kalesh & Kunte, 2015, occupying a similar microhabitat, but distributed north of the major biogeographical divide, the Palghat Gap. The new taxon is distinguished from all other *Protosticta* of Western Ghats by the presence of long prothoracic spines in the males, the structure of the tip of the male cerci and genital ligula. A revised key to the species of *Protosticta* of Western Ghats is provided based on mature males.
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KEYWORDS: Damselfly, hill stream ecology, endemic species, new taxon, revised key

INTRODUCTION

The genus *Protosticta* consists of slender built damselflies commonly called reed-tails or shadow-damsels inhabiting hill streams of tropical, subtropical, and temperate jungles. The genus has 50 extant species distributed from Pakistan, through the Indian subcontinent to Indo-China and Southeast Asian Islands (van Tol, 2009). Indian region has 16 species of *Protosticta* and of them, 13 species are known from Western Ghats (WG) (Sadasivan *et al.,* 2022). The characters useful for species identification are the synthoracic and abdominal markings, the structure of the prothorax and anal appendages in the males (van Tol, 2000). The currently known species of this genus from WG

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are *Protosticta gravelyi* Laidlaw, 1915; *P. hearseyi* Fraser, 1922; *P. sanguinostigma* Fraser, 1922; *P.antelopoides* Fraser, 1924; *P. mortoni* Fraser, 1924; *P. davenporti* Fraser, 1931; *P. rufostigma* Kimmins, 1958; *P. ponmudiensis* Kiran, Kalesh & Kunte, 2015; *P. monticola* Emiliyamma & Palot, 2016; *P. myristicaensis* Joshi & Kunte, 2020; *P. sholai* Subramanian & Babu, 2020; *P. cyanofemora* Joshi, Subramanian, Babu & Kunte, 2020 and *P. anamalica* Sadasivan, Nair & Samuel 2022 (Fraser, 1933; Kiran *et al.,* 2015; Tiple and Koparde, 2015; Emiliyamma and Palot, 2016; Joshi *et al.,* 2020; Nair *et al.,* 2021; Sadasivan *et al.*, 2022). Of these, two species – *P. antelopoides* and *P. ponmudiensis* are relatively robust and large and the males have very prominent prothoracic spines (Nair *et al.,* 2021).

During the explorations for odonates of Kerala state, the authors came across a distinct and robust *Protosticta* species from the foothills of WG, near Aaralam Wildlife Sanctuary (WLS), Kannur District from the Brahmagiri Hills of Coorg landscape. The species appeared superficially similar to *P. antelopoides* and *P. ponmudiensis* but differed significantly in the structure of prothorax, male genitalia and the tip of cerci and hence described here as a new species. A revised key to *Protosticta* of WG is provided based on males, modified from Joshi *et al.* (2020) and Sadasivan *et al.*(2022).

MATERIALS AND METHODS

The morphological description follows Garrison *et al.* (2010). Nomenclature follows Paulson *et al.* (2022). Taxonomic keys to the species are modified based on Fraser (1933), Joshi *et al.* (2020) and Sadasivan *et al.* (2022). The known distribution of the species follows Joshi *et al.* (2020) and Nair *et al.* (2021). The wing venation terminology follows Riek and Kukalová-Peck (1984). Photographs of the specimens were taken with Canon EOS 70D DSLR camera fitted with a 180mm macro lens and MPE 65 f 2.8 1–5x lens. Damselflies were collected in the field with an insect net and preserved in absolute ethanol as wet specimens. The anal appendages were studied using a stereo-zoom microscope (HEADZ Model HD81). Measurements, morphological details, illustrations and comparison of caudal appendages were done from the specimens in voucher collections of TORG. The male prothorax, cerci and genital ligula were hand drawn and digitalized.

Abbreviations used:

Ax	Antenodal crossveins
FW	Forewing
HW	Hindwing
Px	Postnodal crossveins
Pt	Pterostigma
S1–10	Segments of the abdomen
TL	Total length of the specimen including appendages
AL	Abdominal length
FWL	Forewing length
HWL	Hindwing length
TNHS	Travancore Nature History Society
TORG	TNHS Odonate Research Group

RESULTS AND DISCUSSION

Protosticta francyi Sadasivan, Vibhu, Nair & Palot sp. nov.

LSIDurn:lsid:zoobank.org:act:0EA8A75E-853C-4FB2-991D-45FD0CC2F1FD

(Figs. 1, 2B, 3, 4, 5, 6A, 6D, 6G)

Types: Holotype-Male, TORG 1012. Elapeedika, Kanichar, Near Aaralam Wildlife Sanctuary, Kannur District, Kerala, India. 29.vii.2022, 450 m a.s.l., coll. Vibhu V&Vinayan P Nair; currently with TORG collections, Trivandrum, Kerala; wet specimen in alcohol; will be deposited in the insect collection facility of National Centre for Biological Sciences, Bengaluru.

Paratypes:1) Male, TORG 1013, bearing the same collection data as on the holotype; wet specimen in alcohol; will be deposited in the insect collection facility of Zoological Survey of India (ZSI), Pune, Maharashtra.

2) Female, TORG 1014 and Male TORG 1022, bearing the same collection data as on the holotype; wet specimen in alcohol; will be deposited in the insect collection facility of Zoological Survey of India (ZSI), Kozhikode, Kerala.

Etymology: The species is named after Dr. Francy K. Kakkassery (Retired Professor of Zoology, St. Thomas College, Thrissur), the pioneer in odonate studies in Kerala, for his contribution to odonate conservation and popularization of the subject in the state.

Suggested Common Name: Francy's Reed-tail.

Description of the male holotype
(Figs. 2B, 3, 4, 5A, 5D, 5G)

Head (Figs. 2B, 3). Eyes (in live insect) anteriorly green, anterosuperiorly dark green, postero-inferiorly pale greenish white, and inferolaterally greenish-yellow (in life). Labium pale honey-yellow and the anterior border with amber brown hairs and tooth; mandible bluish-white and inferior half bordered in black; labrum pale bluish-white, with almost a half of its free margin bordered thickly in black; anteclypeus paler bluish; postclypeus jet black; genae brownish-black; antefrons, postfrons black with faint bronze reflex; vertex black with blush reflex; occiput jet black; post-ocular lobe jet black, ocelli translucent white; antennae basal segment and half of the first segment brownish, first joint pale blush white, rest of the segments pale brown, with the color fading distally; sparse brownish hairs on lateral aspect of the anteclypeus and free edge of the labrum; longer pale brown hairs along the inferior border of anteclypeus and on the labium. Occipital bar with long brown paradorsal group of hairs; posterior border of head behind the occipital bar sinuous and having mid-dorsal convexity.

Prothorax (Fig. 3F, 6A). Anterior lobe almost half the middle lobe, posterior lobe almost same size as the middle lobe; in life the general color of prothorax bluish-white, anterior lobe with a yellowish collar like paradorsal posterior expansion on each side, middle lobe blue, posterior lobe bluish-black with collar and spines pale bluish-black; notopleural suture and adjoining aspect of the anterior lobe pale yellow; propleuron yellowish blue; pronotal collar bears a pair of medial and lateral spines; medial spines slender, sharp, straight and its tip turned outwards extending just beyond the mesostigmal plate; Lateral spines small, triangular and rudimentary almost like an angular extension of the collar directed inferolaterally. Prolegs coloured as in the synthorax.

Synthorax (Figs. 2A, B, D). General color in life is black marked with pale yellowish-white and pale blue. In dorsal view, mesostigmal plate jet black; mid-dorsal carina black. In lateral view, the mesepisternum shiny dark green with bronze reflex; mesepimeron dorsal half black and inferiorly bluish-white, and borders with the interpleural suture black; mesinfraepisternum superiorly dark and inferior fourth yellowish-white; metepisternum superior half of the middle half bordering the interpleural suture black, rest of it pale bluish-white; metin fraepisternum pale brownish-white, metepimeron bluish-white; metathoracic spiracle brownish. In ventral view, the venter of metathorax pale yellowish-white, mid-ventrum of prothorax and synthorax are black. The coxae and trochanter of all legs yellowish-white; femora pale brownish, with tibio-femoral joint region suffused in blue; tibiae pale brownish, with its lateral aspect suffused in blue; spines of the tibial comb, tarsus and the claws brownish.

Wings (Figs. 4A, C). Hyaline; Pt of both wings brown occupying less than one and one-fourth cells, trapezoidal; anterior border straight slanting anteriorly; posterior border convexslanting posteriorly, thus making the superior border shorter than the inferior; inferior border convex. Pt length at its middle is equal to its breadth. Anal bridge absent. Ax–2 in all wings. Px–FW 15-16 and HW15.

Abdomen (Figs. 2B, 3A, 4B, 4D). General color is brownish-black and marked in pale yellowish to bluish-white as follows: S1 laterally pale bluish-white, dorsally brown; S2 below a diagonal connecting the anterosuperior to the posteroinferior edges bluish-white, rest of it brown; S3–7 marked with basal annuli, that increases in thickness towards the posterior segments, ventral part of each of them extends posteriorly for some distance; the posterior extension on annuli in S2–S3 almost reaching the posterior-fifth of its length; the annuli slightly increases in thickness in S4–S6; S6 annulus,

including its posterior extension on ventral side reaching almost one-fourth length of S6; mark on S7, largest of the annuli, extends laterally reaching mid-lateral aspect of S7, distal half of this mark suffused, gradually merging with ground colour; dorsal aspect of S7 basal annulus occupies basal third, and rest of the segment is black, and this black extends dorsally as a narrow triangular part into posterior half of annulus splitting it laterally; basal annulus on S8 incomplete dorsally it extends posterolaterally to reach the anterior thirds, and then thinly extends ventrally to cross in to its posterior-thirds but stopping short of joint with S9; S8 basal annulus half the thickness of that on S5; crescent marks on membranes between S8-9 and S9-10 colored yellowish blue dorsally. Annuli of S3 conspicuously thinner (reduced to almost a third) than on rest of segments S4–6. S8 annulus is largest of annulus and on ventrolateral aspect stops just short of its distal margin. Segments 9 and10 are fully black.

Male ligula (Fig. 6G). Basal region of ligula sinuous, tip thick and curved, general structure as illustrated in the figure

Caudal appendages (Figs. 4E–G). Coloured dark brownish-black, except basal half of paraprocts which is coloured pale honey-yellow with a bluish hue; length of cerci three times that of S10 in lateral view. Cerci long thin and sinuous, furnished with a small blunt tooth directed postero-dorsally at junction of its basal third and middle third; middle third uniformly tapering and curved inwards, and distal-third spatulate. Spatulate tip of cerci with proximal end narrow and distal end expanded. Paraprocts uniformly curved inwards; long, ending just short of cerci; tip of paraprocts curved inwards; paraprocts bear a long thin spine like lamella directed posteromedially at its distal fourth, this lamella with a concavity posteriorly and its tip is notched superiorly.

Measurements (mm).TL- 54, AL- 45, FWL-27, HWL- 26.

Description of female paratype (Figs. 2C, 5)

Head (Figs. 5B–D)**.** Colored exactly as male. Eyes and labium as in males; mandible bluish-white and inferior half black; labrum pale bluish-white, a little more than lower third bordered thickly in black; anteclypeus paler bluish; postclypeus jet black; genae black; antefrons, postfrons, vertex, occiput and post-ocular lobe black as in males; ocelli translucent pinkish-white; antennae basal segment and half of first segment brownish, around first joint pale blush white, rest of segments pale brown; setae and hairs on labium and occiput as in males.

Prothorax (Fig. 5C, 5E) as in males, but spines on posterior lobes are shorter and broader.

Synthorax (Figs. 5A-C) Colour and structure as in males.

Wings (Fig. 5A). Hyaline; Pt of both wings brown occupying less than one and one-fourth cells, trapezoidal; anterior border convex and slanting anteriorly; posterior border convex and slanting posteriorly thus making superior border shorter than inferior; Pt length at its middle as longas its breadth. Anal bridge absent. Ax–2 in all wings. Px–FW 16 and HW 15.

Abdomen (Fig. 4A). Segments S1–S7 coloured as in male, but basal bluish-white rings are much shorter and almost half their size as that on corresponding segments of males.

Segment 7 bears triangular bluish-white patch, dorsolateral part of this extends till half of S7, while in ventral aspect it extends till end of S7. S8 has small inferolateral blue spot on anteroventral aspect of segment on each side. Segment 9 and 10 unmarked black, but membranes connecting them pale blue. S8 is four times length of S10, while S9 is three-fourths of length of S10.

Caudal appendages (Figs. 5F, G). Cerci dark brown, as long as length of S10, triangular in lateral view with a superior border slightly convex, tip blunt and directed posteroinferiorly; paraprocts reduced, rounded brown, half-length of cerci; valve of ovipositor black; ovipositor black, ending in a brownish-black flat-tipped style reaching well beyond level of cerci and valve.

Measurements (mm).TL- 47, AL-39, FWL-28, HWL-27.

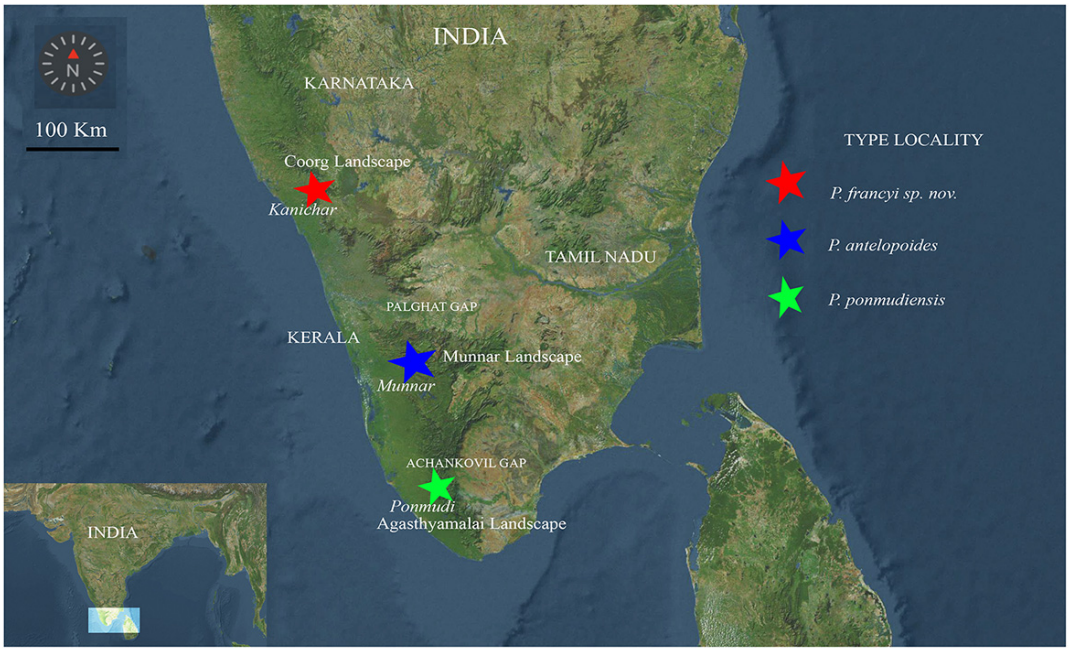


Fig. 1 Map showing the type localities of *Protosticta francyi* **sp. nov.**, *P. antelopoides* and *P. ponmudiensis*, and biogeographical gaps in the Western Ghats

Distribution, habitat and ecology: They inhabit dark forest streams at low-mid elevations (<500m). The species, as far as known, is restricted to the western slopes of Aaralam WLS, Kottiyoor WLS and the forests of north Wayanad forest division in Brahmagiri hills of Coorg landscape in central WG. This taxon is restricted north of Palghat Gap. The vegetation of the area is mainly of semi-evergreen or evergreen type with good canopy cover. All the streams are perennial with boulders and well-covered riparian vegetation. The adults spend the day perched on low-hanging shrubs and fern leaves of marginal vegetation in shady hill streams. They undertake short flights to hunt flying insects and return to their perches. Flight period as is May to October. Observed many specimens from Kottiyoor WLS, Chavachi part of Aralam WLS, foothills of Suryamudi near Kottiyoor during these periods.

Remarks: The previous records of *P. antelopoides,* in published literature from Thusharagiri, Kozhikode (Palot and Emilyamma, 2015) and Wayanad (MJP in Nair *et al.*, 2021), possibly pertains to this new species.

The three species have peculiar distribution in WG; *P. ponmudiensis* is found in Agasthyamala is south of Achankovil Gap, *P. antelopoides* in the Anamalais are seen south of Palghat Gap, north of Achankovil Gap and *P. francyi* **sp. nov.,** in the Brahmagiri Hills (Coorg landscape), north of Palghat Gap (Fig.1). In addition to the geographical distribution, the three closely similar species *P. antelopoides, P. ponmudiensis* and *P. francyi* **sp. nov.,** are distinguished from other *Protosticta* of Western Ghats by the long prominent prothoracic spines, the spatulate tip of male cerci, and large size (TL > 50 mm) of the males. Superficially these three species appear similar but are distinguishable based on the length of the prothoracic spines, the structure of the spatulate process on tip of the cerci of males, and the basal portion of the male ligula and also coloration of S7.

Lateral spines on prothorax are well-developed in both *P. antelopoides* and *P. ponmudiensis*, while rudimentary in *P. francyi* **sp. nov**. In dorsal view, the tip of the lateral spine is directed laterally in *P. francyi* **sp. nov.,**while it is directed anteriorly in *P. ponmudiensis.* Medial spines on prothorax are



Fig. 2 A–Image showing the habitat at the type locality of *Protosticta francyi* **sp. nov.**, in Brahmagiri Hills © Vinayan P Nair; B–Live male *Protosticta francyi* **sp. nov.** © Vibhu V; C–Live female *Protosticta francyi* **sp. nov.** © Vibhu V.

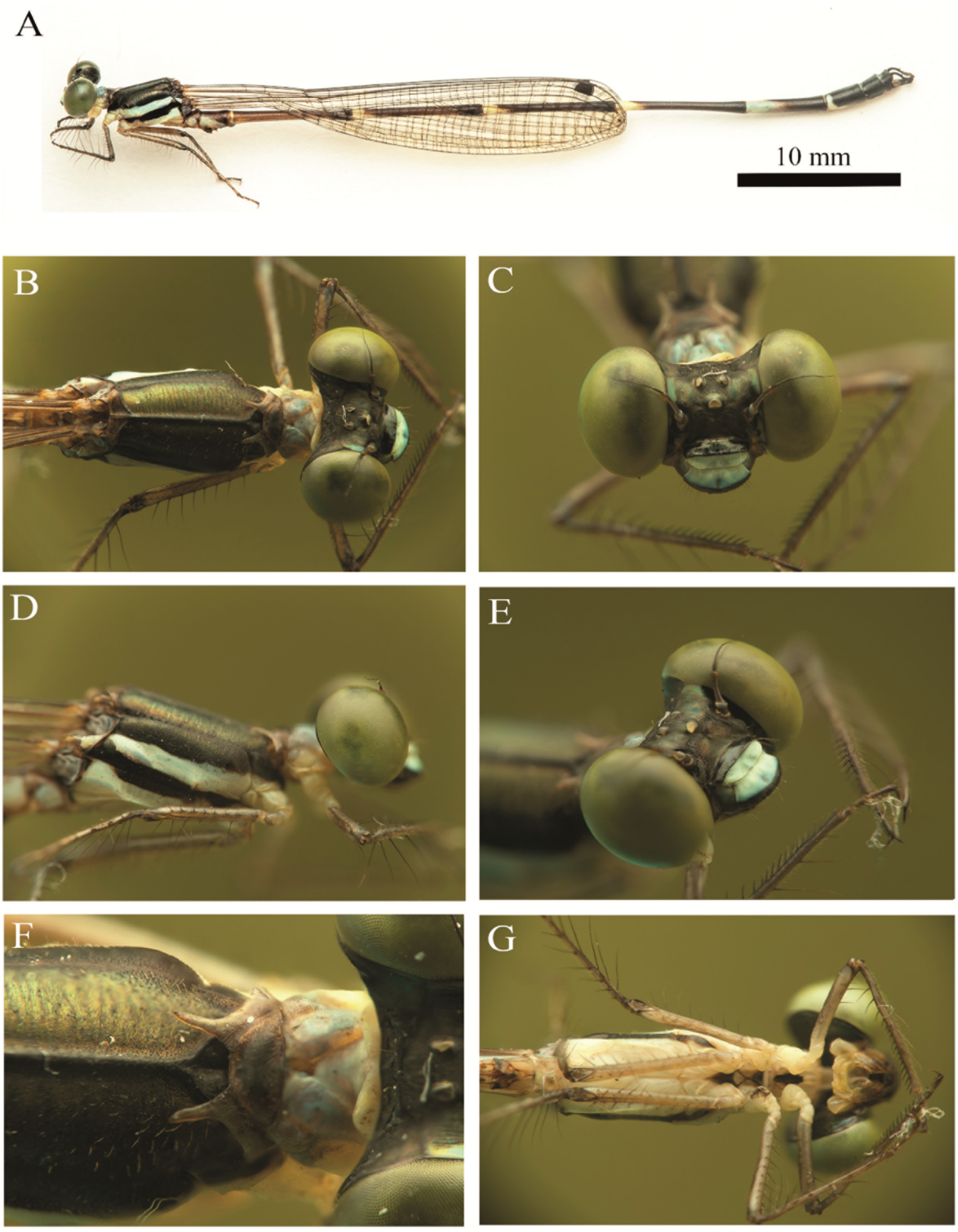


Fig. 3 *Protosticta francyi* **sp. nov.** Holotype male (TORG 1012). A–Whole insect lateral view; B–Dorsal view of the head, prothorax, and synthorax; C–Close-up of head anterior view; D–Lateral view of synthorax; E–Close-up of head anterio-lateral view; F–Dorsal close-up of prothoracic spines; G–Ventral view of synthorax and head © Kalesh Sadasivan.

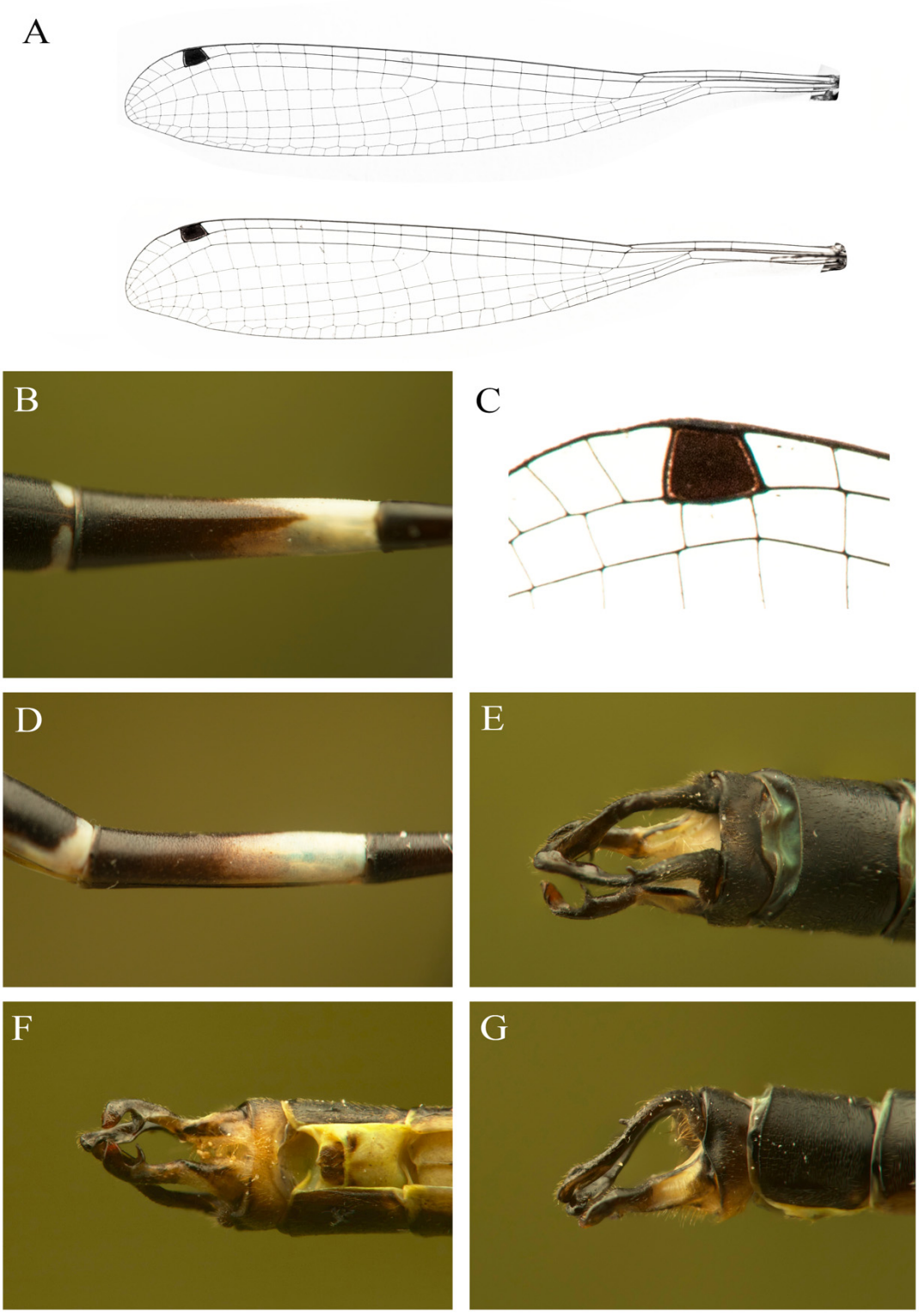


Fig. 4 *Protosticta francyi* **sp. nov.** Holotype male (TORG 1012).
A–Venation, forewing (top) and hindwing (bottom); B–Dorsal view of S7; C–Close-up of pterostigma of forewing; D–Lateral view of S7; E–Anteromedial view of anal appendages; F–Ventral view of anal appendages; G–Lateral view of anal appendages © Kalesh Sadasivan.

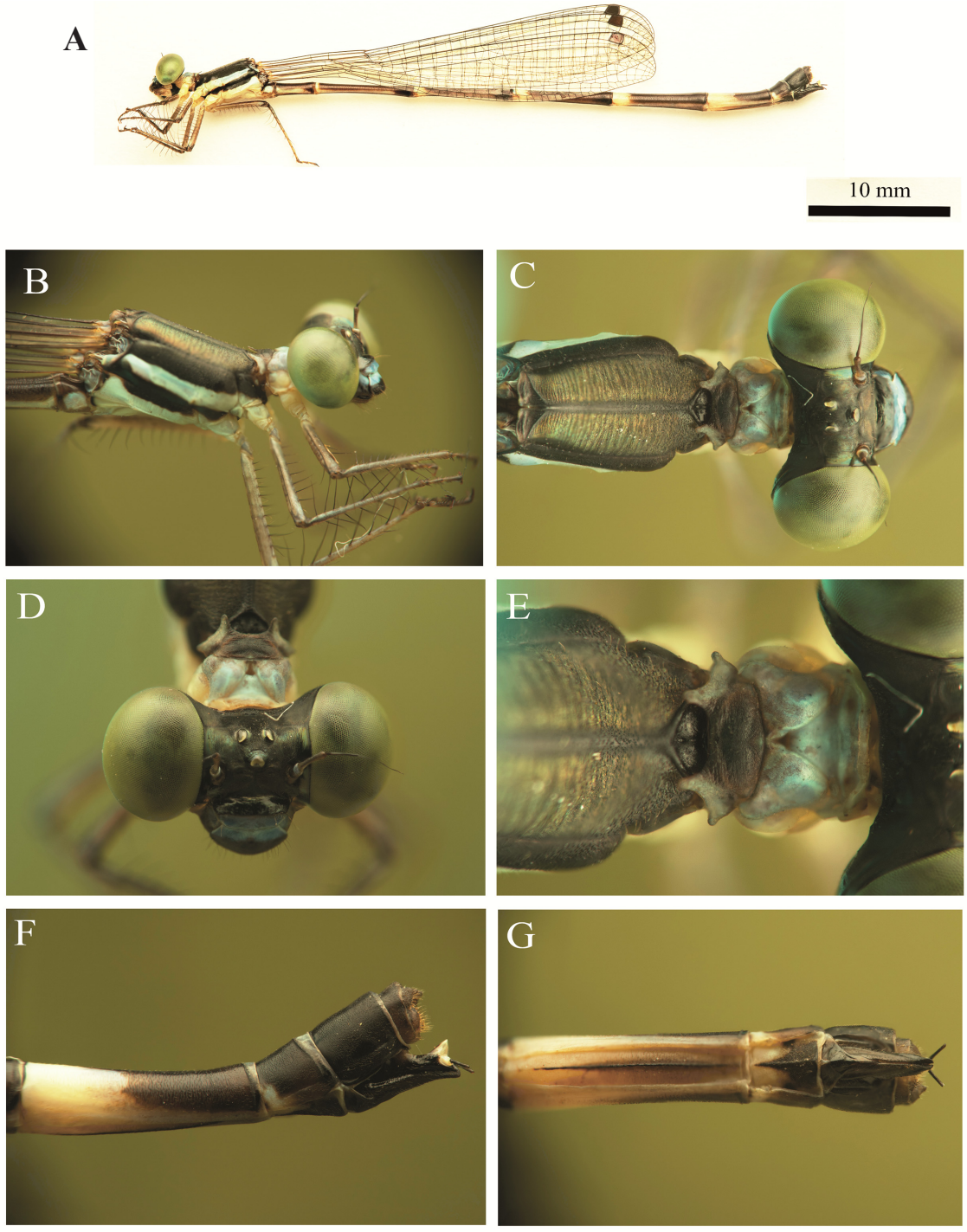


Fig. 5 *Protosticta francyi* **sp. nov.** Paratype female (TORG 1014). A–Whole insect lateral view; B–Lateral view of the head, prothorax, and synthorax; C–Dorsal view of the head, prothorax, and synthorax; D–Close-up of head anterior view; E–Dorsal close-up of prothoracic spines; F–Lateral view of S7-S10 and anal appendages; G–Lateral view of S7-S10 and anal appendages © Kalesh Sadasivan.

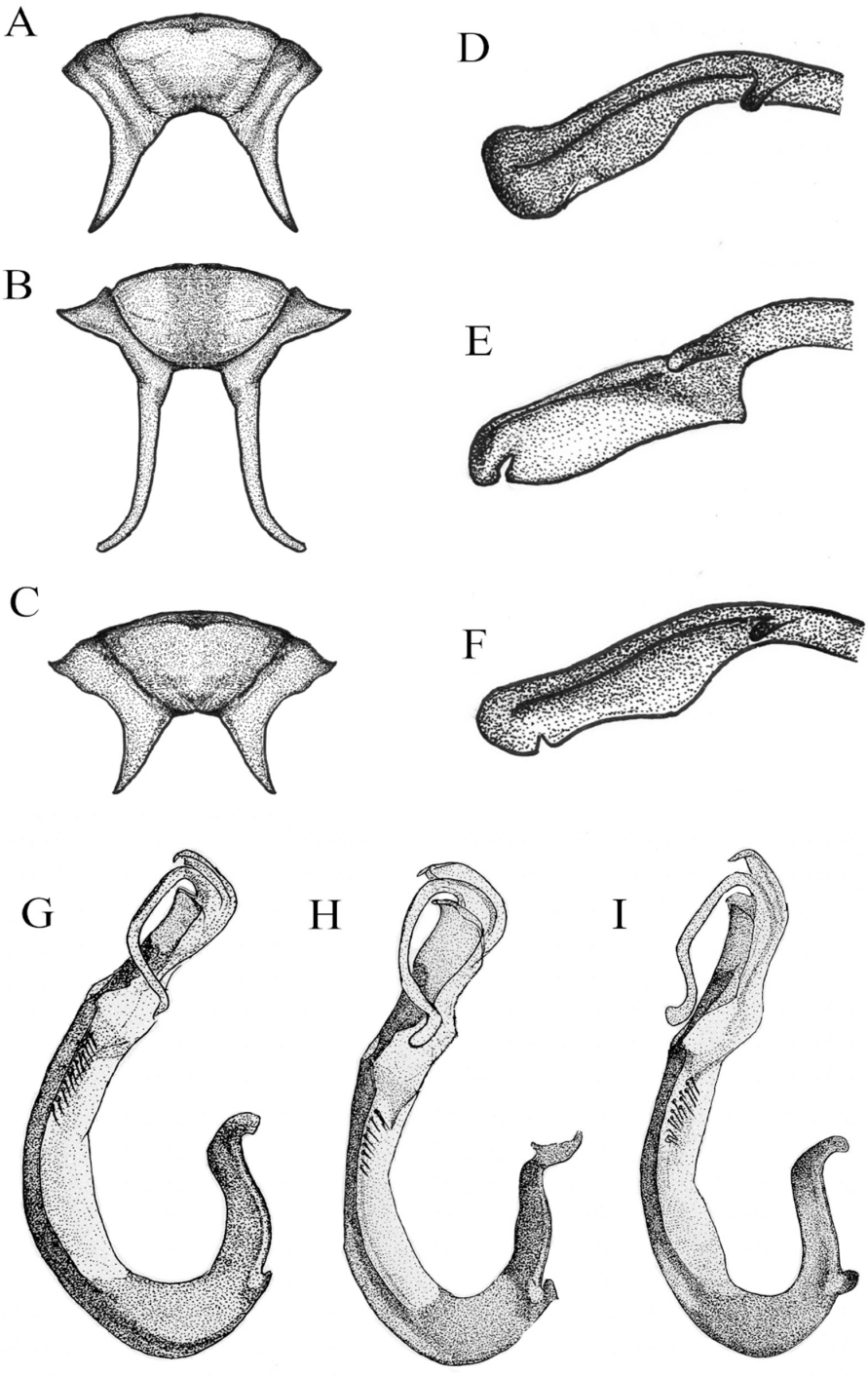


Fig. 6 Comparison of prothoracic spines, spatulate tip of cerci of males, and ligula of *Protosticta francyi* **sp. nov.**, *P. antelopoides* and *P. ponmudiensis*. A–Prothoracic spines of *Protosticta francyi* **sp. nov;** B–Prothoracic spines of *P. antelopoides*; C–Prothoracic spines of *P. ponmudiensis*; D–Spatulate tip of cerci of males of *Protosticta francyi* **sp. nov.**; E–Spatulate tip of cerci of males of *P. antelopoides*; F–Spatulate tip of cerci of males of *P. ponmudiensis*; G–Ligula of *Protosticta francyi* **sp. nov.**; H–Ligula of *P. antelopoides*; I–Ligula of males of *P. ponmudiensis* © Kalesh Sadasivan.

very long, narrow-based, and elaborate extending posteriorly well beyond twice the length of the mesostigmal plate in *P. antelopoides*. Medial spines areshort, broad-based, and never extend posteriorly beyond the apex of the mesostigmal plate in *P. ponmudiensis*; while it is short, narrow-based, and extend posteriorly just beyond the apex of the mesostigmal plate in *P. francyi* **sp. nov.**

The anal appendages of the three species are apparently similar, however, differ in the fine structure of the cerci and the disposition of spines on it. The location and structure of the dorsal spine on cerci in lateral view are different in the three species. While it is beyond the mid-cerci and directed posteriorly in *P. antelopoides*, it is located just before the middle in *P. ponmudiensis* and directed posteromedially, while it is located at the junction of proximal and middle third, and directed posterodorsally in *P. francyi* **sp. nov**. The structure of the spatulate tip of the cerci in the dorsomedial view is very different in the three species. The spatulate tip is broader at the base and tapers into the bifid apex, with the proximoventral angle being acute and extending downwards like a tooth in *P. antelopoides* (Fig. 6E). The spatulate tip is broader at the base and tapers into the rounded and notched apex and the proximoventral angle is obtuse and rounded in *P. ponmudiensis* (Fig. 6F). The spatulate tip is narrow at the base and broadens into the expanded apex and the proximoventral angle is absent in *P. francyi* **sp. nov.** (Fig. 6D). The location of the medial spine like lamina of paraprocts in ventral view in *P. antelopoides* is at the distal-most end, however, in the other two species are more proximally at the junction of middle and distal thirds. The direction of this medial spine like lamina is posteromedial in *P. antelopoides,* while it is medial at right angles to the long axis of paraprocts in *P. ponmudiensis* and *P. francyi* **sp. nov.** (Fig. 4F).

The new species has synthorax with paradorsal yellowish bronze-green metallic reflux, while *P. ponmudiensis* and *P. antelopoides* have their dark green metallic reflex. The lateral marking on S7 is useful in distinguishing the three species, in *P. antelopoides* this is yellow and extends ventrally diagonally along the inferior half of the whole segment from the anterosuperior aspect of S7 to itsposteroinferior angle; in *P. ponmudiensis* the marking is bluish-white and extends more dorsally occupying four-fifth of S7 dorsum and only half of the ventrum; while in *P. francyi* **sp. nov.**, the colour is bluish-white and on the dorsum it is restricted to the basal third and never extends beyond half of the lateral aspect of S7. S8 blue annulus is variable and hence not useful in distinguishing the three species (Table 1).

Table1. Comparison of morphometric characters of *P. antelopoides*, *P. ponmudiensis* and *P. francyi* sp. nov. based on males

Character	*P. francyi* **sp. nov.**	*P. ponmudiensis*	*P. antelopoides*
Total Length	55mm	55mm	59mm
Abdominal length	46mm	47mm	50mm
FW length	29mm	28mm	30mm
HW Length	28mm	27mm	29mm
Post nodal count FW and HW	16, 15	17–18, 16-–17	18, 17

Mean values in mm and postnodal count in Range; FW- Forewing; HW- Hindwing

Key to species of *Protosticta* Selys, 1885 of Western Ghats based on males modified from Joshi *et al.* (2020) and Sadasivan *et al.* (2022)

1. The posterior lobe of prothorax with a pair of long, divaricate horn-like medial spines…….....……2

- The posterior lobe of prothorax without such long spines.....4

2. The posterior lobe of prothorax with very long medial spines (Fig. 6B), extending posteriorly to a distance more than twice the length of the mesostigmal plate.....*P. antelopoides*

- Posterior lobe of prothorax short medialspines extending posteriorly till the apex of the mesostigmal plate or shorter.....3

3. Lateral spines on prothorax well developed, short triangular with tip directed anterolaterally; medial spines short never extending posteriorly beyond the apex of the mesostigmal plate (Fig. 6C); tip of cerci broad at base and tapering distally (Fig. 6F).....*P. ponmudiensis*

- Lateral spines on prothorax rudimentary, reduced to an angular projection on the prothoracic collar, and its tip directed laterally; medial prothoracic spine long, thin, extending posteriorly just beyond the apex of the mesostigmal plate (Fig. 6A); tip of cerci narrow at base and expanding distally (Fig. 6D) *P. francyi* **sp. nov.**

4. Anterior 1/3rd or more of S8 bright turquoise-blue connected dorsally….....5

- Anterior 1/3rd of S8 yellow or blue, not connected dorsally.....10

5. Apical fork of cerci deeply incised more than 1/3rd of the total length….....6

- Apical fork of cerci shallow incised, much less than 1/3rd of total length ….....8

6. Cerci with a small tubercle at middle of the apical fork; length of abdomen + caudal appendages < 25 mm…….....*P. myristicaensis*

- Cerci without such a tubercle at its center; length of abdomen + caudal appendages >25 mm.....7

7. Prothorax with a hexagonal black marking covering central portion of posterior lobe and a small portion of the middle lobe; cerci with a prominent laterally pointed basal spine; the paraprocts with an inner stout spine at base ….....*P. gravelyi*

- Anterior & middle lobes of prothorax colored blue, no hexagonal black mark; cerci with a small laterally pointed basal spine; paraprocts without an inner spine at base.....*P. mortoni*

8. Prothorax completely blue; length of abdomen +caudal appendages <30 mm; inferior lobe of cerci very short, superior lobe not expanded .....*P. hearseyi*

- Anterior and middle lobes of prothorax pale yellow, posterior lobe partially or completely black; length of abdomen + caudal appendages > 30 mm; inferior lobe of cerci more than 1/3rd length of superior lobe, the latter expanded .....…9

9. Dorsum of middle portion of posterior lobe of prothorax completely black extending as two points to the dorsum of middle lobe; inner fork of cerci thin and small, superior lobe rounded at apices and more than twice the length of inferior.....….*P. davenporti*

- Dorsum of posterior lobe of prothorax black, laterally brown; middle lobe of prothorax with a small dorsal faint black spot; inner fork of cerci thick, superior lobe ending in a quadrangle, less than twice the length of inferior .....*P. rufostigma*

10. S9 completely black or marked only at ventral border; posterior border of prothorax not expanded; paraprocts not clubbed at apices….....11

- S9 laterally marked with a large yellow spot at anterior border, reaching more than 2/3rd of the

segment, not connected apically in both sexes; posterior border of prothorax expanded; paraprocts thin, long and clubbed at apices….....*P. sholai*

11. Pt red; cerci with a prominent and robust basal spine; tip of superior lobe of cerci bilobed.....*P. sanguinostigma*

- Pt black or brown; cerci with a small blunt basal protuberance, inwardly pointed; tip of outer fork of cerci not bilobed….....12

12. Pt rectangular with length more than twice the breadth; Px in all wings always 11 or more; caudal appendages thrice the length of S10; outer margin of cerci including the superior lobe comparatively straighter on dorsal view .....….....….....13

- Pt trapezoid with length twice the breadth; Px in all wings 10 or less; caudal appendages only twice the length of S10; outer margin of cerci sinuous; tip of superior lobe of cerci straight on dorsal view….....*P. anamalaica*

13. Eyes blue; femur bright blue internally; S8 with a bright blue annule extended laterally 2/3rd of its length; tip of superior lobe of cerci straight .....*P. cyanofemora*

- Eyes grey and brown; femur pale yellow internally; S8 black dorsally, ventrolaterally yellow extends to its distal end; tip of the superior lobe of cerci bent inward at the apices ..... *P. monticola*

Hill streams and the invertebrates associated are the most vulnerable in the wake of climate change (Rogers *et al.,* 2020), thus highlighting the importance of these stream-associated indicator species. The discovery of this endemic *Protosticta* from the WG raises the current number of species in this genus to 17 in India and 14 in the WG. The addition of *P. francyi* **sp. nov**. raises the Odonata species diversity of the Western Ghats to 209 species with 82 endemics, and that of Kerala to 183 species with 70 endemics.

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SPECIAL

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New synonymy and redescription of two species from the Pseudoscorpion genus *Olpium* L. Koch, 1873 (Arachnida, Pseudoscorpiones, Olpiidae) in India

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ABSTRACT: *Olpium digitum* Murthy and Ananthakrishnan, 1977 is redescribed with first description of the female and updating its distribution in India. A new subjective synonymy is being proposed; *Olpium tibium* Sivaraman, 1980=*O. digitum,* Murthy and Ananthakrishnan, 1977. Supplementary description for *O. gladiatum* Murthy and Ananthakrishnan, 1977 is reported.
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KEY WORDS: Morphology, redescription, variation, Western Ghats, distribution

INTRODUCTION

The genus *Olpium* L. Koch, 1873 has a total of 12 species identified till date in India and includes *Olpium jacobsoni* (Tullgren 1908), *O. lindbergi* (Beier,1952, 1959), *O. indicum* (Beier, 1967), *O. asiaticum*, *O. crypticum*, *O. digitum*, *O. gladiatum*, *O. graminum*, *O. granulatum*, *O. robustum*, *O. tropicum* (Murthy and Ananthakrishnan, 1977) and *O. tibium* (Sivaraman, 1980). Though the genus is numerically rich, species from India are known only from original descriptions, which lack detailed descriptions and illustrations. The type specimens of V.A. Murthy collections (VAM colls.) and Dr. S. Sivaraman which are deposited in the museum of Department of Zoology, Loyola College, Chennai are missing and were confirmed with personal observation and communication with Dr. Sivaraman and Dr. D. Sudarsanam. Thus, the revision of topotypes will end the ambiguity in the identity of the species. In the present paper, a proposal to synonymise *O. tibium* with *O. digitum* and a supplementary description of *O. gladiatum* is presented.

MATERIALS AND METHODS

Specimens were preserved in ethanol (70%) and studied under Leica M205C (Kerala) and Nikon SMZ25 (NHMW) stereomicroscopes, as well as Leica DM2000 (Kerala) and Nikon ECLIPSE Ni (NHMW) compound microscopes. Drawings were made by the aid of a drawing tube attached to the microscope. All measurements are in millimeters. Specimens are deposited in the Division of Arachnology, Department of Zoology, Sacred Heart College, Thevara, Cochin, Kerala, India (ADSH).

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Morphological terminology and mensuration follow Chamberlin (1931), Harvey (1992) and Judson (2007).

The following trichobothrial abbreviations were used: *eb*= external basal; *esb* = external sub-basal; *ib*= internal basal; *isb* = internal sub-basal; *ist* = internal sub-terminal; *est* = external sub-terminal; *it* = internal terminal; *et* = external terminal; *t* = terminal; *st*= sub-terminal; *b* = basal; *sb*= sub-basal.

RESULTS AND DISCUSSION

Taxonomy -

Family Olpiidae Banks, 1895

Genus *Olpium* L. Koch, 1873

Diagnosis: For description and diagnosis of the genus, see Dashdamirov & Schawaller (1993)

Type species- *Obisium pallipes* Lucas, 1849, by subsequent designation of International Commission of Zoological Nomenclature, 1987: 53

Olpium digitum Murthy and Ananthakrishnan, 1977 (Figs. 13 A–B)

Olpium digitum Murthy and Ananthakrishnan, 1977: 47, Figs. 13 a–b.

Olpium tibium Sivaraman, 1980: 335, Figs. 5 a–b. NEW SYNONYMY

Type material- Holotype (♂) of *O. digitum* from INDIA: Tamil Nadu, Madras from Neem tree log. Murthy V.A, 8 January 1961, repository VAM colls, not examined (lost from the repository; personal communication with Dr. Sivaraman).

Holotype (♂) of *O. tibium* from INDIA: Tamil Nadu, Avadi from waro house leg. Sivaraman S. 15 October 1976, repository museum of the Zoology Department, Loyola College, Madras, Tamil Nadu, India, not examined (lost from the repository; personal communication with Dr. Sivaraman).

Topotype examined: INDIA, Tamil Nadu: 5 ♂♂ (ADSH PS0101) Avadi, Chennai [13°8′8½ N; 80°6′10½ E], 30m, 12 December 2018, 21 January 2019 & 03 April 2019 M.V. Aneesh leg., from bark of *Tamarindus indicus*, 2 ♂♂ (ADSH PS0102), 1 ♀ (ADSH PS0103) Nungambakkam, Chennai [13°3′54½ N; 80°14′1½ E], 10m, 11 December 2019 & 22 January 2019 M.V. Aneesh leg., from bark of Neem tree.

Diagnosis - *O. digitum* is very similar to *O. lindbergi* in having equally sized femur and patella. It can be distinguished from *O. lindbergi* by its stouter chela and lesser number of palpal teeth. *O. digitum* can be distinguished from *O. indicum* by its stouter patella and having two

Redescription

Adults (Figs. 1A–D) chitinized regions are dark brown in colour.

Carapace (Fig. 2A): 1.32–1.40 (♂), 1.38 (♀) x longer than broad; both pairs of eyes well developed and corneate, tapetum present; 22 setae in the formula 4–6–4–4–2–2.

Chelicera (Figs. 2B, G). All the five setae well developed. Fixed finger with 5 triangular teeth. Movable finger with well–developed apical tooth and two subapically divided lobes (Fig. 2G); Serrula exterior with 17 (♂), 18 (♀) blades; rallum with 3 blades, distal blade longest and widest with serrations (Fig. 2G). Galea with 2 terminals and 1 sub-terminal rami in both males and females. Galeal seta shorter than galea.

Pedipalps (Figs. 3A, B). Dark brown in colour, smooth. Trochanter without tubercle, 1.65 (♂), 1.97(♀) x longer than broad. Femur with 2 long setae on the dorsal, 3.03–3.04 (♂), 2.98 (♀) x longer than broad. Patella 2.55–2.59 (♂), 2.54 (♀) x longer than broad. Chela 3.10–3.17 (♂), 3.09 (♀) x longer than broad. Trichobothrial pattern (Fig. 3B): *eb*, *esb*, *isb* are situated in the exterior aspect. *t* is situated proximal to the middle, at a distance more than *sb* from *st*; *ib* located basally; *it* much distal to est; *ist* sub–basal in position, nearer to *ib* than to it; distance between *t* and *st* is twice the distance between *sb* and *st*; *t* is proximal to *est*; fixed finger with 27–28 (♂), 27 (♀) and movable finger with 24 -27 (♂), 26 (♀) teeth, proximal 1/3rd with flattened teeth; venom tooth well developed in both fingers; venom ducts proximal to *et* of the fixed finger.

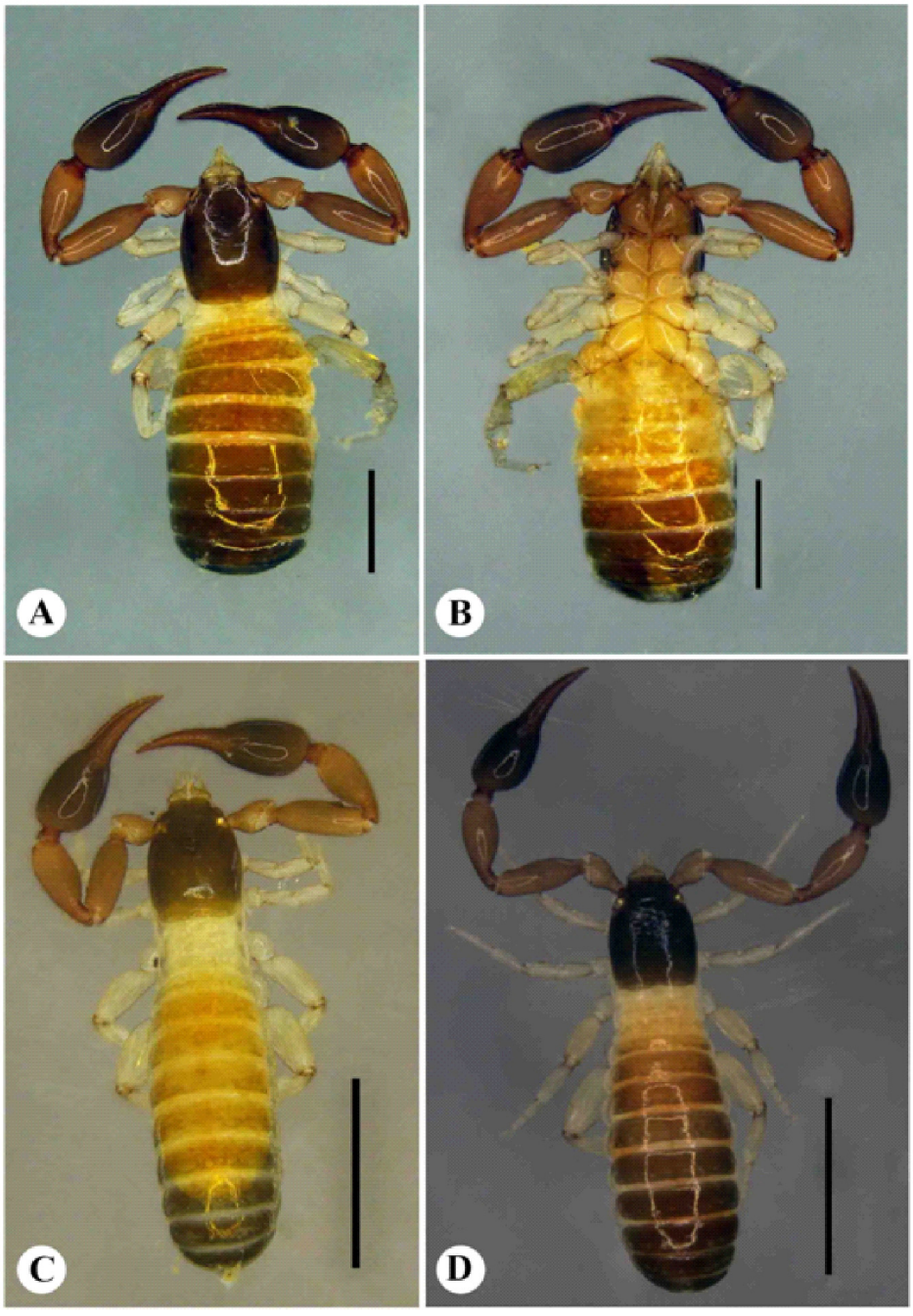


Fig. 1 *Olpium digitum*, A - male dorsal view; B - male ventral view; C - male (paratype) dorsal; D - male (paratype) dorsal. Scale bars: A–B = 0.5 mm, C–D = 1 mm

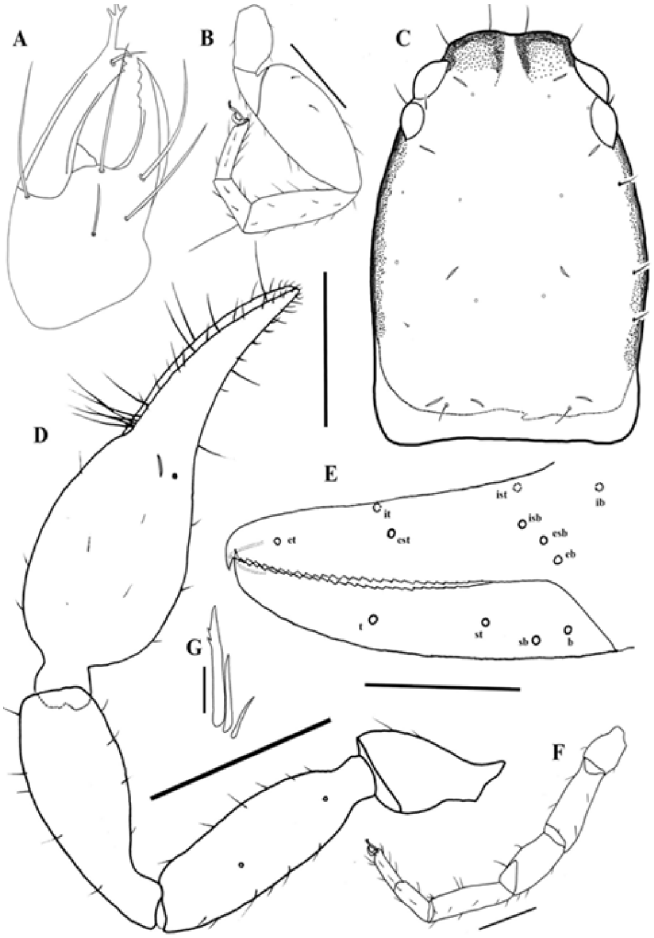


Fig. 2 *Olpium digitum*, A- chelicera; B- leg IV; C- carapace I; D- Pedipalp; E- Chela; F- Leg I; G- Rallum.
Scale bars: B–C, E, F = 0.2 mm, D = 0.5 mm, G = 0.02 mm, A = not to scale.

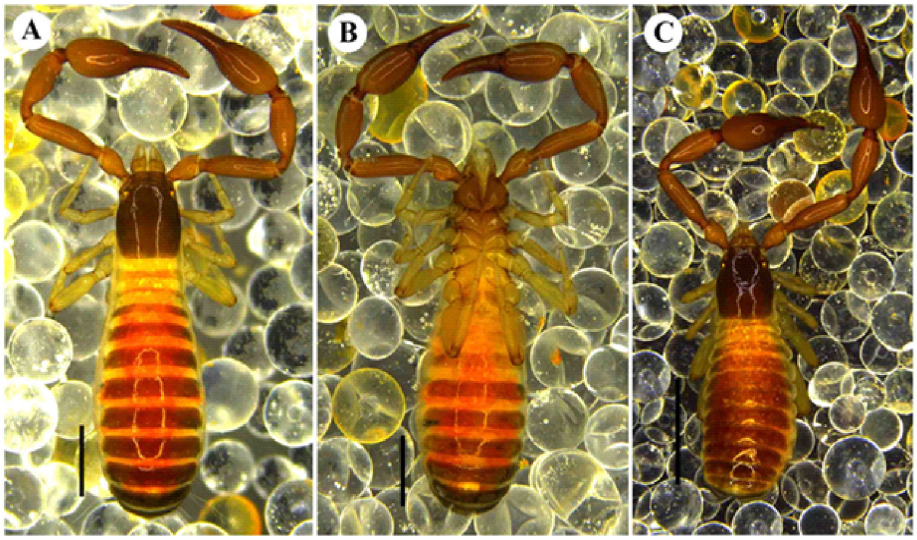


Fig. 3 *Olpium gladiatum* : A - male dorsal view; B - male ventral view; C- female dorsal view.
Scale bars: A–B = 0.5 mm, C = 1mm

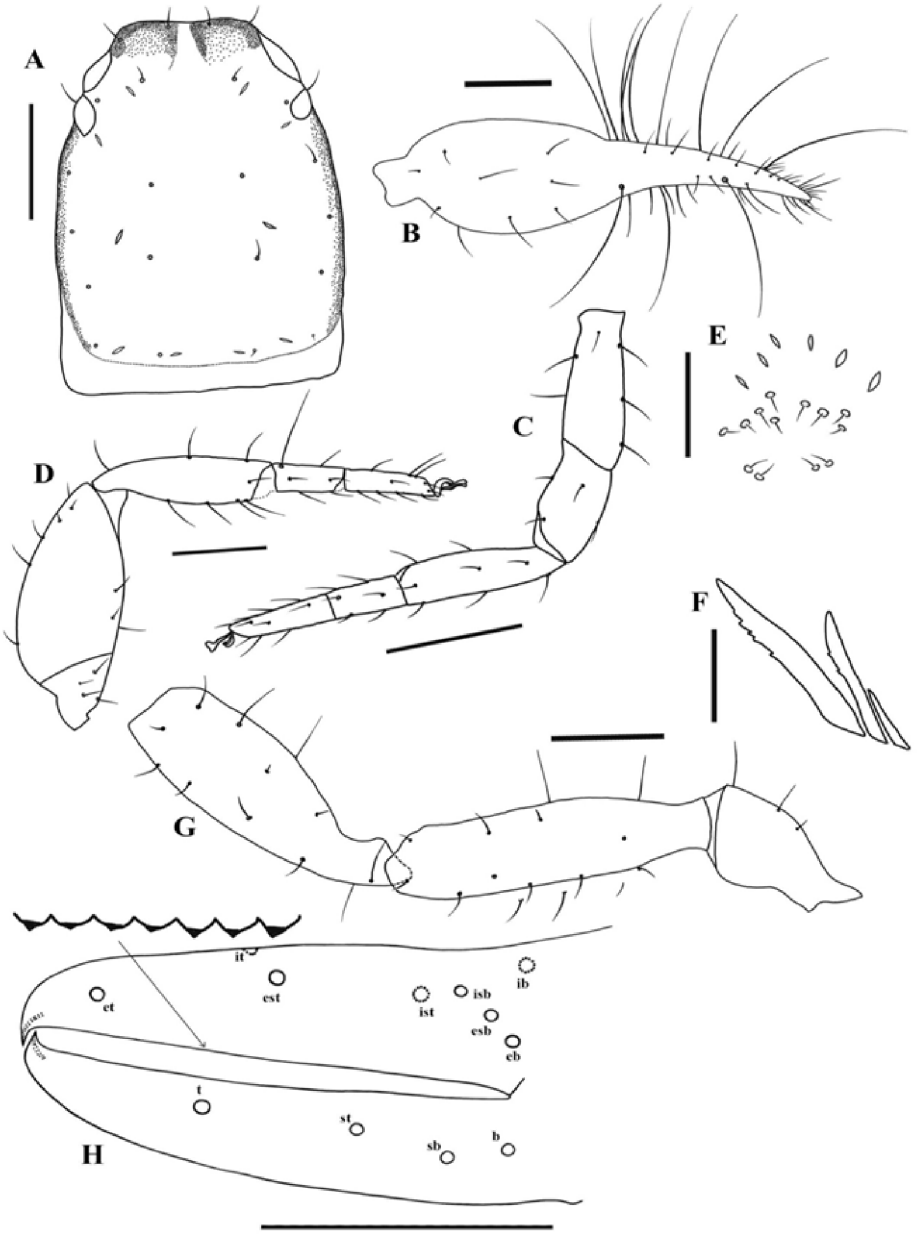


Fig. 4 *Olpium gladiatum:* A - carapace; B - chela dorsal view; C - Leg I; D - Leg IV; E -Pedipalp; F - Chela; Genital area; G - Rallum. Scale bars: A–D, G 0.2mm, E 0.05 mm, F 0.02 mm

Legs (Fig. 2C–D). Femur of leg I longer than telofemur. Femur + patella 2.38 (♂), 2.25 (♀) x longer than broad. Metatarsi of leg III and IV with pseudotactile seta.

Abdomen: Tergites II to XI highly sclerotised. Tergalchaetotaxy: ♂, 2:4:4:4:4:4:4:4:4–6:6 (including 2 tactile setae): 6 (including 2 tactile setae): 2, ♀, 2:4:4:4:4:4:4:4:6 (including 2 tactile setae):6 (including 2 tactile setae): 2. Spiracles obliquely placed in 3rdand 4th segment. Sternal chaetotaxy: ♂, 8+4:4:5:5:4:4:6:6 (including 2 tactile setae): 8 (including 2 tactile setae): 2, ♀, 5+6:4:4:6:5:4:4:6:6 (including 2 tactile setae): 8 (including 2 tactile setae):2.

Measurement. Male: body length 1.98–2.62. Carapace 0.629–0.699/0.477–0.499. Pedipalps: trochanter 0.299–0.330/0.180–0.181, femur 0.524–0.556/0.172–0.183, patella 0.510–0.535/0.20–0.206, chela (with pedicel) 0.941–0.955/0.296–0.308, chela (without pedicel) 0.891/0.296–0.308, hand 0.412–0.442, movable finger 0.456–0.474. Leg I: femur 0.222–0.237/0.092–0.093, patella.173–0.176/0.096–0.097, tibia 0.224–0.245/ 0.064–0.065), metatarsus 0.123–0.124/0.041–0.048, tarsus 0.11–0.136/ 0.032–0.039. Leg IV: femur + patella 0.512–0.525/0.215–0.242, tibia 0.267–0.440/ 0.99–0.115, metatarsus 0.177–0.179/0.059–0.066, tarsus 0.193–0.204/0.045–0.054.

Female: Body length 2.489. Carapace 0.68/0.491. Pedipalps: trochanter 0.287/0.197, femur 0.568/ 0.177, patella 0.557/0.218, chela (with pedicel) 0.992/0.319, chela (without pedicel) 0.915/0.319, hand 0.464, movable finger 0.480. Leg I: femur 0.234/0.097, patella 0.183/0.097, tibia 0.261/0.063, metatarsus 0.119/0.043, tarsus 0.142/ 0.037. Leg IV: femur + patella 0.505/0.224, tibia 0.422/0.111, metatarsus 0.162/0.060, tarsus 0.203/0.05.

Olpium gladiatum Murthy and Anantha krishnan, 1977

Olpium gladiatum Murthy and Ananthakrishnan, 1977: 57, fig. 17.

Type material-Holotype (♂) of *O. gladiatum* from INDIA: Goa from grass leg. Murthy V. A, 10 July 1966, repository VAM colls, not examined (lost from the repository; personal communication with Dr Sivaraman).

Topotype examined - INDIA, Goa: 7 ♂♂ (ADSH PS0104), 8♀♀ (ADSH PS0105) Netravali [15°3′42½ N; 74°14′35½ E], 420m, 30 January 2020 M.V. Aneesh leg., from litter.

Other materials examined- INDIA, Goa: 4 ♂♂ (ADSH PS0106), 5 ♀♀ (ADSH PS0107) Mollem [15°20′31½ N, 74°15′34½ E], 110m, 27 November 2019 M.V. Aneesh leg., from litter.

Diagnosis. *O. gladiatum* is very similar to *O.indicum* in having 4 posterior setae of carapace. It can be distinguished from *O. indicum* by its slender femur and chela. In *O. gladiatum ist* is nearer to *ib* than to *it* whereas in *O. indicum ist* is exactly in the middle of *ib* and *it. O. gladiatum* can be distinguished from *O. digitum* by its slender femur and greater number of teeth in the fixed

Redescription

Adults (Figs. 3A–C) chitinized regions are dark brown in colour.

Carapace (Fig. 4A):1.31–1.33 (♂), 1.29–1.36 (♀) x longer than broad; both pairs of eyes well developed and corneate, tapetum present; 22 setae in the formula 4–6–4–4–2–4.

Chelicera (Fig. 4F). All the five setae well developed. Fixed finger with 5 triangular teeth. Movable finger with well-developed apical tooth and two subapically divided lobes; serrula exterior with 17–19 (♂), 18 (♀) blades; rallum with 3 blades, distal blade longest and widest, two blades with serrations. Galea with 3 terminal rami in both males and females. Galeal seta shorter than galea.

Pedipalps (Figs. 4B, H). Dark brown in colour, smooth. Trochanter without tubercle, 1.86–1.96 (♂), 1.73–2.01 (♀) x longer than broad. Femur with 2 long setae on the dorsal, 3.51–3.81 (♂), 3.59–3.69 (♀) x longer than broad.Patella 2.85–3.04 (♂), 2.94–2.97 (♀) x longer than broad. Chela 3.44–3.56 (♂), 3.40–3.68 (♀) x longer than broad. Trichobothrial pattern (Fig. 4H): *eb*, *esb*, *isb* are situated in the exterior aspect. distance between *t* and *st* is 1.6 to 1.65 times the distance between *sb* and *st*; *ib* located basally; *it* much distal to est; *ist* sub–basal in position, nearer to *ib* than to it;

distance between *t* and *st* is twice the distance between *sb* and *st*; *t* is proximal to *est*; fixed finger with 26–28 (♂), 30–32 (♀) and movable finger with 29–30 (♂), 29–32 (♀) teeth; venom tooth well developed in both fingers; venom ducts proximal to *et* of the fixed finger.

Legs (Fig. 4C–D). femur of leg I longer than patella. Femur +patella 2.38 (♂), 2.25 (♀) x longer than broad. Metatarsi of leg III and IV with pseudotactile setae at the base.

Abdomen (Fig. 4E): Tergites II to XI highly sclerotised. Tergalchaetotaxy: ♂, 4:4:4:4:4:4:4:4:6 (including 2 tactile setae): 6 (including 4 tactile setae): 6 (including 4 tactile setae): 2,♀, 2:4:4:4:4:4:4:4: 6 (including 2 tactile setae): 6(including 4 tactile setae): 6(including 4 tactile setae): 2. Spiracles obliquely placed in 3rd and 4thsegment. Sternal chaetotaxy: ♂, 11–13:4:6:6:4:4:4:4:6 (including 2 tactile setae): 6 (including 2 tactile setae): 2, ♀, 7:4:4:6:5:4:4:6:6 (including 2 tactile setae): 8 (including 2 tactile setae): 2. Genital area of male with 7–9 anterior and 4 posterior setae (Fig. 4E).

Measurements. Male: Body length 1.901–2.50. Carapace 0.632–0.637/0.473–0.485. Chelicera: movable finger 0.133, palm 0.17/0.105. Pedipalps: trochanter 0.323–0.335/0.164–0.180, femur 0.568–0.587/0.156–0.164, patella 0.576–0.588/0.189–0.206, chela (with pedicel) 1.019–1.076/0.286–0.312, chela (without pedicel) 0.949–0.998, hand 0.524, movable finger 0.558–0.568. Leg I: femur 0.269/0.091, patella 0.170/0.092, tibia 0.250/0.067, metatarsus 0.128/0.049, tarsus 0.149/ 0.041. Leg IV: femur + patella 0.567/0.212, tibia 0.408/0.107, metatarsus 0.175/0.062, tarsus 0.201/0.050.

Female: Body length 2.0–2.18. Carapace 0.660–0.677/0.485–0.546. Chelicera: movable finger 0.032, palm 0.0485/0.027. Pedipalps: trochanter 0.320–0.350/0.159–0.202, femur 0.565–0.668/ 0.157–0.181, patella 0.557–0.681/0.187–0.231, chela (with pedicel) 1.016–1.009/0.276–0.361, chela (without pedicel) 0.944–1.143, hand 0.498–0.637, movable finger 0.570–0.606. Leg I: femur 0.272/0.098, patella 0.175/0.101, tibia 0.270/0.073, metatarsus 0.120/0.049, tarsus 0.162/ 0.041. Leg IV: femur + patella 0.590/0.224, tibia 0.428/0.112, metatarsus 0.174/0.062, tarsus 0.215/0.052.

Justification of synonymy

Sivaraman (1980) described *O. tibium* from Avadi, a province in Chennai, Tamil Nadu state of southern India. The original description of *O. tibium* is based on a single male holotype and is supported by two text figures. Sivaraman (1980) separated it from its *O. digitum* comparing the dimensions of carapace. But the dimensions of carapace show variation, which were not considered. The statement by Sivaraman (1980) and Murthy and Ananthakrishnan (1977) that the "proximal one third of the fixed finger without teeth" is incorrect. In our specimens, the tips of the teeth are flattened towards the proximal region of both fixed and movable fingers. Specimens collected from the type localities exhibited intra–specific variations and demands synonymy of species. The stripes on the carapace are not considered to be a valid characteristic feature to distinguish the species, as it is present in all *Olpium* species.

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Seasonal diversity, distribution and abundance of Araneae in the Thattekkad Bird Sanctuary, Kerala, India

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ABSTRACT: The aim of the present study was to analyze the diversity of spiders across various parts of the Thattekkad Bird Sanctuary, Kerala, a tropical, semi evergreen, low-land forest located between the tributaries of the Periyar river. Survey of the spider fauna was carried out for a period of twelve months. In total, 3286 individuals were collected from the sanctuary, which consist of 89 species of spiders under 59 genera and 18 families. Araneidae was the most abundant family. The most abundant species was *Hippasa agelenoides* of Lycosidae family. Spiders belonging to six feeding guilds, i.e., orb - web weavers, stalkers, ground runners, scattered line weaver foliage runners and ambushers were identified. Relative abundances of spider community strongly differed with the pre-monsoon, monsoon and post monsoon seasons. Diversity indices - Margalef richness index, Pielou's evenness index, Shannon-Wiener and Simpson index were calculated. © 2022 Association for Advancement of Entomology

KEYWORDS: Araneidae, dominance, guild, seasonal diversity indices

INTRODUCTION

Spiders are scattered everywhere and are found in almost all habitats. They are also considered indicators of ecosystem health (Mathew *et al.,* 2009). They play a key role in maintaining the ecosystem balance due to their high abundance and insectivorous feeding habits (Wise, 1993). The data on the relative abundance, distribution and richness of taxa serve as a reference for ecological studies and as a basis for conservation planning (Raven and Wilson, 1992; Magurran, 2004). For conservation planning efforts, there should be an understanding of the patterns of diversity on regional scales (Uniyal and Shrivastava, 2012). Spiders can be classified into different guilds based on the similarity in their foraging behavior (Cardo *et al*., 2011; Mansoor lone *et al.,* 2015). Spiders belonging to different foraging guilds and populations were high during the monsoon and winter seasons (Deshmukh and Raut, 2014). Prey density mainly depends on the season and the type of vegetation, which can constantly change throughout the year that, in turn affects the diversity and abundance of spiders (Deshmukh and Raut, 2014). The present work aims to examine the spider population and its

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diversity and abundance in the Thattekkad Bird Sanctuary at various seasons of the year to answer the following questions: (1) which of the spider species are more common in the study area?; (2) what is the diversity and abundance of spiders in the study area? and (3) are there seasonal effects on the spider diversity and abundance in the study area?

MATERIALS AND METHODS

Study Area: The Thattekkad Bird Sanctuary is situated at 10°08' N; 76°41' E and covers an area of 25.16 km^2 on the northern bank of Periyar River. The sanctuary borders Forest Reserve of Kuttampuzha and Neriyamangalam Range and the two rivers Periyar and the Edamalayar. The study area has diverse vegetation types, with large trees such as *Albizia lebbeck. Antidesma bunius. Calophyllum apetalum, Canarium strictum, Hydnocarpus pentandrus, Termanalia paniculate, Symplocos cochinchinensis, Oleadioica* and small plants like *Dioscorea spicata, Argyreia cymosa., Almania species, Mukia maderaspatana, Zonia diphylla, Mimosa pudica, Acacia caesia and Clerodendrum infortunatum.* Sampling was conducted over and along nearby human settlement areas and transition and buffer zones.

Sampling methods: Random quadrat sampling was done in all the seasons. Quadrat sampling is commonly used in terrestrial biodiversity monitoring, in which the observer can collects all taxa in a given area (Schoenly *et al.,* 2003; Corti *et al.,* 2013). To get statistically significant results, 5m X 5m random quadrat samples (replicate samples) were taken from each sampling site at the same time. Standard sampling techniques such as sweep netting, beating sheets, active searching and hand picking were adopted. Samples collected from each sampling plot were noted separately. Sampling was done in every month during the period from February 2017 to January 2018 (monsoon, pre-monsoon, and post-monsoon).

Preservation: The collected samples were anesthetized with chloroform and placed separately in vials containing (75%) ethyl alcohol. The collection dates, collection site, and the number of specimens were recorded on each vial. The collected specimens were studied under a Zeiss Stemi 2000-C stereomicroscope and were identified using standard taxonomic keys (Majumder, 2007; Sebastian, 2009). Identified specimens are further verified on the World Spider Catalog online version (2022). Spider photographs from the field were taken with a Canon EOS 20D camera with Canon 100mm macro photo lens.

Statistical analysis: Plymouth Routines in Multivariate Ecological Research (PRIMER 7e) software (Clarke and Gorley, 2015) was used for the multivariate analysis. Diversity indices like Shannon-Wiener index (H'), Margalef's index (d), Pielou's evenness index (J') and Simpson's dominance (λ) were estimated on the species abundance data. The multivariate procedure includes multidimensional scaling (MDS) (Clarke, 1993); Bray-Curtis coefficient (Bray and Curtis, 1957) was used to produce the ordination plots. Other PRIMER protocols used in the present study include K-dominance curve. This is a plot representing the percentage cumulative abundance against log species rank (Lambshead *et al*., 1983). It is a graphical method used for comparing diversity between samples.

RESULTS AND DISCUSSION

A total of 3286 spider specimens were collected during the study period, of which 699 number of specimens were collected during the pre-monsoon, 1580 in the monsoon and 1007 in the post monsoon seasons. There were 89 species under 59 genera and 18 families (Table 1). Among the families, Araneidae was the most abundant family (40.35%) followed by Lycosidae (20.87%), Salticidae (17.43%), Oxyopidae (5.53%), Tetragnathidae (5.38%), Corinnidae (3.86%), Clubionidae (3.59%), Gnaphosidae (1.86%), Theridiidae (1.22%), Thomicidae (1.19%) and Philodromidae (1.03%). Lowest species diversity was noted in Cheiracanthidae (0.09%), Hersilidae (0.09%), Pholcidae (0.12%), Pisauridae (0.52%), Scytodidae (0.79%), Sparassidae (0.58%) and Uloboridae (0.24%).

The collected species exhibited seasonal variations in their abundance. A total of 72 species, 48 species and 56 species were recorded during monsoon (June, July, August, September), post-monsoon (October, November, December, January) and pre-monsoon seasons (February, March, April, May) respectively. Twenty four species did not show any seasonal changes in their number throughout the year.

Six feeding guilds were identified from the collections. Orb web weavers (36%) were the most dominant guild followed by stalkers (30.3%), ground runners (10.1%), scattered line weavers (9%), foliage runners (7.9%) and ambushers (6.7%).

Season-wise diversity analysis shows that the evenness, richness, and diversity indices of spiders were higher during the monsoon season. Shannon diversity was high in the monsoon season (2.78 ± 0.107) and low in the pre-monsoon season (2.12 ± 0.174). Highest dominance of species was observed during the pre-monsoon season (0.1911 ± 0.028), followed by the post monsoon season (0.16 ± 0.027). Evenness was higher in the monsoon season followed by the post monsoon season (0.77 ± 0.027) and 0.73 ± 0.017 respectively). The maximum species richness index of 5.8 ± 0.213 was recorded during the monsoon season (Table 2).

Similarity of seasonal abundance of the spider species was established by Bray-Curtis and MDS analysis. Cluster analysis (Fig. 1) revealed that there is about 70 per cent similarity in the distribution of spiders in the summer season especially in months of February and January. Samples collected in the months of November-December and February-January showed 57.85 per cent similarity in their species composition. In the non-metric multidimensional scaling (nMDS), plot stress value, 0.1 showed a great representation of the seasonal similarity (Fig. 2). The results revealed the influence of various seasons in the diversity pattern of the spider assemblages during the study period.

Samples collected during the months of March, April and May, appeared in entirely different dimensions and exhibited different diversity compositions. Three distinct clusters were formed with 40 per cent similarity. Samples from November, December, January and February formed a cluster with 40per cent similarity, while samples from August, September and October showed 60 per cent similarity in species abundance.

In k-dominance curves, the cumulative relative abundances of species, ranked in decreasing order of their importance in terms of abundance, are plotted against species rank. The k-dominance curve measures the intrinsic diversity, and in this plot, the lower lines represent samples with higher diversity. In the K-dominance curve, the steepest and elevated curve represents a very low species diversity compared to others and it shows a state of disturbed condition. Seasonal analysis depicted relatively high dominance of spiders during the monsoon season.

The higher curve in a k-dominance plot shows lower evenness and richness in spider diversity. In the K-dominance curve of Pre monsoon season gives an information that in these months there is a disturbed state (Fig. 3).

The diversity analysis showed that, there is some seasonal influence on the distribution of spiders. Monsoon and winter seasons are favorable for their richness as compared to the summer seasons. This may be due to the low temperature and more availability of prey. Studies of Valdez-Mondragón (2006) also showed that the species richness is influenced by increased precipitation that promotes flowering and vegetation growth, providing food for insects, the primary prey of most spiders. Maximum spider diversity was recorded in the wet seasons because of the favorable temperature relative humidity and rain fall, which are suitable for the breeding of mature spiders (Khan *et al*., 2017). The present study revealed that June to October are the most suitable months for spiders compared to other months, as shown by the higher abundance of spiders. Studies by Pitta *et al.* (2019) showed that the community composition of spiders was most strongly influenced by habitat type, availability of prey and temperature. Therefore, the availability and the density of insect populations may also be one of the factors that determine the diversity of

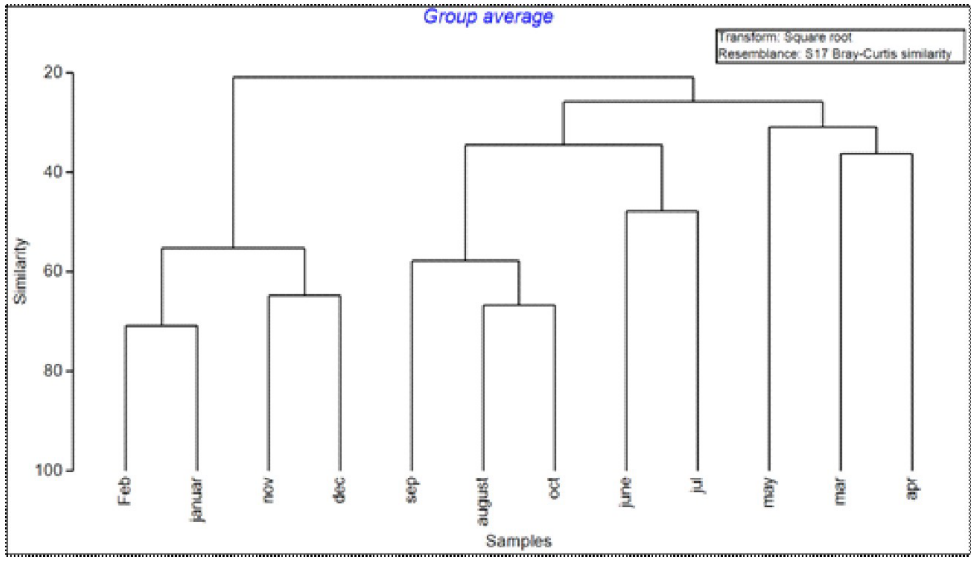


Fig. 1 Cluster diagram showing the similarity of seasonal samples

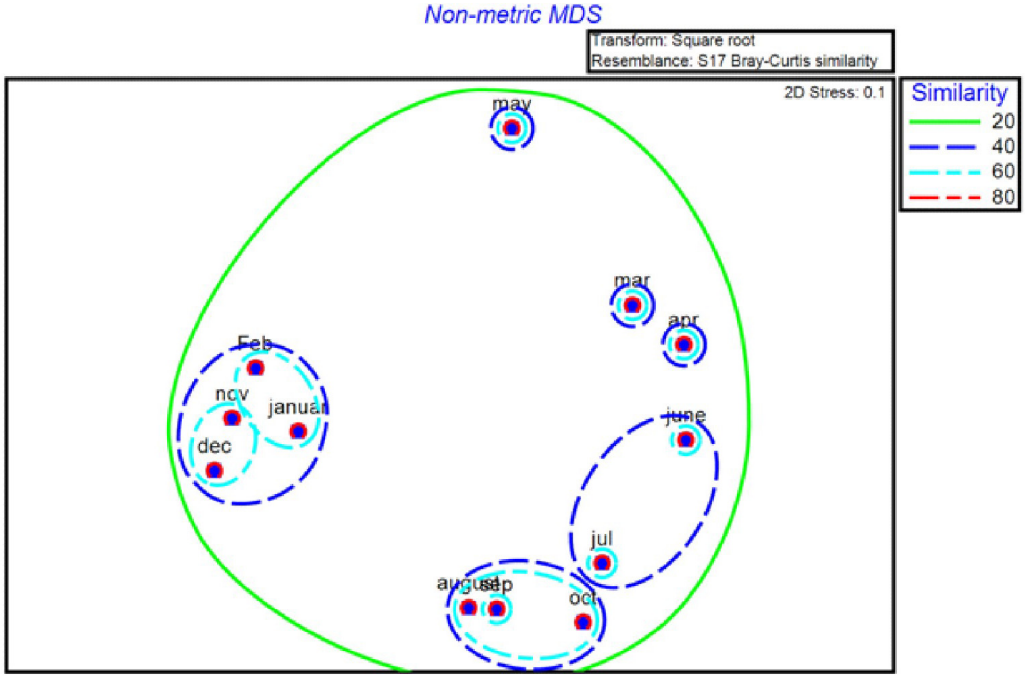


Fig. 2 n MDS plot showing the similarity of seasonal samples



Argiope pulchella (Thorell, 1881)



Anepsion maritatum
(O. Pickard-Cambridge, 1877)



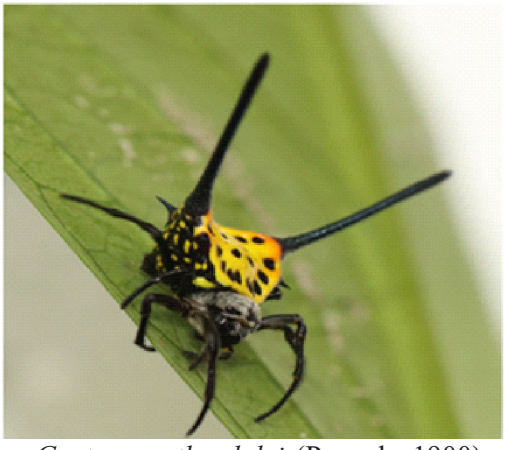
Camaricus formosus (Thorell, 1887)



Leucauge fastigata (Simon, 1877)



Thiania bhamoensis (Thorell, 1887)



Gasteracantha dalyi (Pocock, 1900)

Figs. 4 Common spiders of Thattekkad BirdSanctury, India

Table 1 Checklist of spider species found in Thattekkad Bird Sanctuary

No	Family/ Foraging Guild	Species
1	Araneidae Clerck, 1757/ Orb-web builders	*Arachnura angura* Tikader, 1970
2		*Araneus viridisomus* Gravely, 1921
3		*Argiope aemula* Walckenaer, 1841
4		*A. pulchella* Thorell, 1881
5		*Cyrtophora cicatrosa* Stoliczka, 1869
6		*C. citricola* Forsskal, 1775
7		*Eriovixia excelsa* Simon, 1889
8		*E. laglaizei* Simon, 1877
9		*Gasteracantha geminate* Fabricius, 1798
10		*G. dalyi* Pocock, 1900
11		*Geaspinipes* Koch, 1843
12		*Herennia multipuncta* Doleschall, 1859
13		*Neoscona bengalensis* Tikader&Bal, 1981
14		*N. mukerjei* Tikader, 1980
15		*N. theisi* Walckenaer, 1841
16		*N. vigilans* Blackwall, 1865
17		*Nephila pilipes* Fabricius, 1793
18		*Parawixia dehaani* Doleschall, 1859
19		*Anepsion maritatum* Pickard-Cambridge, 1877
20		*Cyclosa hexatuberculata* Tikader, 1982
21		*Araneus* sp.1
22	Cheiracanthiidae Wagner, 1887/ Foliage runner	*Cheiracanthium melanostomum* Thorell, 1895
23	Clubionidae Wagner, 1887/ Foliage runner	*Clubiona drassodes* Pickard-Cambridge, 1874
24		*C. filicata* Pickard-Cambridge, 1874
25	Corinnidae Karsch, 1880/ Ground runner	*Corinnomma severum* Thorell, 1877
26		*Castianeira zetes* Simon, 1897
27	Gnaphosidae Banks, 1892/ Ground runner	*Poecilochroa barmani* Tikader, 1982
28		*Urozelote spatulusus* Sankaran & Sebastian, 2018
29	Hersiliidae Thorell, 1869/ Foliage runner	*Hersilia savignyi* Lucas, 1836
30	Lycosidae Sundevall, 1833/ Ground runner	*Hippasa agelenoides* Simon, 1884
31		*H. greenalliae* Blackwall, 1867
32		*H. lycosina* Pocock, 1900
33		*Pardosa sumatrana* Thorell, 1890
34	Oxyopidae Thorell, 1869/ Stalkers	*Oxyopes birmanicus* Thorell, 1887
35		*O. javanus* Thorell, 1887
36		*O. bharatae* Gajbe, 1999
37		*O. salticus* Hentz, 1845
38		*O. shweta* Tikader, 1970
39		*O. sunandae* Tikader, 1970
40	Philodromidae Thorell, 1870/ Ambushers	*Thanatus elongatus* Tikader, 1960
41	Pholcidae Koch, 1850/ Scattered line weavers	*Pholcus kapuri* Tikader, 1977
42	Pisauridae Simon, 1890/ Ambushers	*Dendrolycosa gitae* Tikader, 1970
43	Salticidae Blackwall, 1841/ Stalkers	*Epeus albus* Prószynski, 1992

44		*E. sindicus,* Prószyñski, 1992
45		*E. striangulopalpis* Malamel, Nafin, Sudhikumar& Sebastian, 2019
46		*Indopadilla insularis* Malamel, Sankaran & Sebastian, 2015
47		*Brettus cingulatus* Thorell, 1895
48		*Carrhotus sannio* Thorell, 1877
49		*Chalcotropis pennata* Simon, 1902
50		*Hasarius adansoni* Audouin, 1826
51		*Hyllus semicupreus* Simon, 1885
52		*Menemerus bivittatus* Dufour, 1831
53		*Myrmaplata plataleoides* O. P.-Cambridge, 1869
54		*Myrmarachne dirangicus* Bastawade, 2002
55		*M. melanocephala* MacLeay, 1839
56		*Phintella debilis* Thorell, 1891
57		*P. vittate* Koch, 1846
58		*Plexippus paykulli* Audouin, 1826
59		*P. petersi* Karsch, 1878
60		*Telamonia dimidiate* Simon, 1899
61		*Thiania bhamoensis* Thorell, 1887
62		*Thyene bivittate* Xie & Peng, 1995
63		*Evarcha*sp.1
64	Scytodidae Blackwall, 1864/ Foliage runners	*Scytodes fusca* Walckenaer, 1837
65		*S. thoracica* Latreille, 1802
66	Sparassidae Bertkau, 1872/ Foliage runners	*Heteropoda venatoria* Linnaeus, 1767
67		*Olios milleti* Pocock, 1901
68	Tetragnathidae Menge, 1866/ Orb-web builders	*Leucauge decorate* Blackwall, 1864
69		*L. dorsotuberculata* Tikader, 1982
70		*L. granulate* Walckenaer, 1841
71		*L. fastigata* Simon, 1877
72		*L. tessellate* Thorell, 1887
73		*Tetragnatha javana* Thorell, 1890
74		*T. mandibulata* Walckenaer, 1841
75		*T. keyserlingi* Simon, 1890
76		*T. viridorufa* Gravely, 1921
77		*Tylorida ventralis* Thorell, 1877
78	Theridiidae Sundevall, 1833/ Scattered line weavers	*Argyrodes flavescens* O. Pickard-Cambridge, 1880
79		*Chikunia nigra* Pickard-Cambridge, 1880
80		*Chrysso angula* Tikader, 1970
81		*Phycosoma martinae* Roberts, 1983
82		*Theridion zonulatum* Thorell, 1890
83		*Thwaitesia margaritifera* Pickard-Cambridge, 1881
84		*Meotipa* sp.1
85	Thomisidae Sundevall, 1833/ Ambushers	*Indoxysticus minutus* Tikader, 1960
86		*Camaricus formosus* Thorell, 1887
87		*Pistius* sp. 1
88		*Strigoplus netravati* Tikader, 1963
89	Uloboridae Thorell, 1869/ Orb-web builders	*Miagrammopes extensus* Simon, 1889

Table 2. Average seasonal values (mean ± SE) of diversity indices of Thattekkad bird sanctuary during the study period

Seasons	Margalef richness (d)	Pielou'seveness (J')	Shannon Wiener - H' (log_e)	Simpson dominance index (ë)
Pre -monsoon	3.8451 ±0.678	0.711825± 0.041	2.12375±0.174	0.1911± 0.028
Monsoon	5.8730±0.213	0.779425±0.020	2.78025±0.107	0.097035±0.014
Post- monsoon	3.7025±0.720	0.737850±0.017	2.21125±0.176	0.167193±0.027

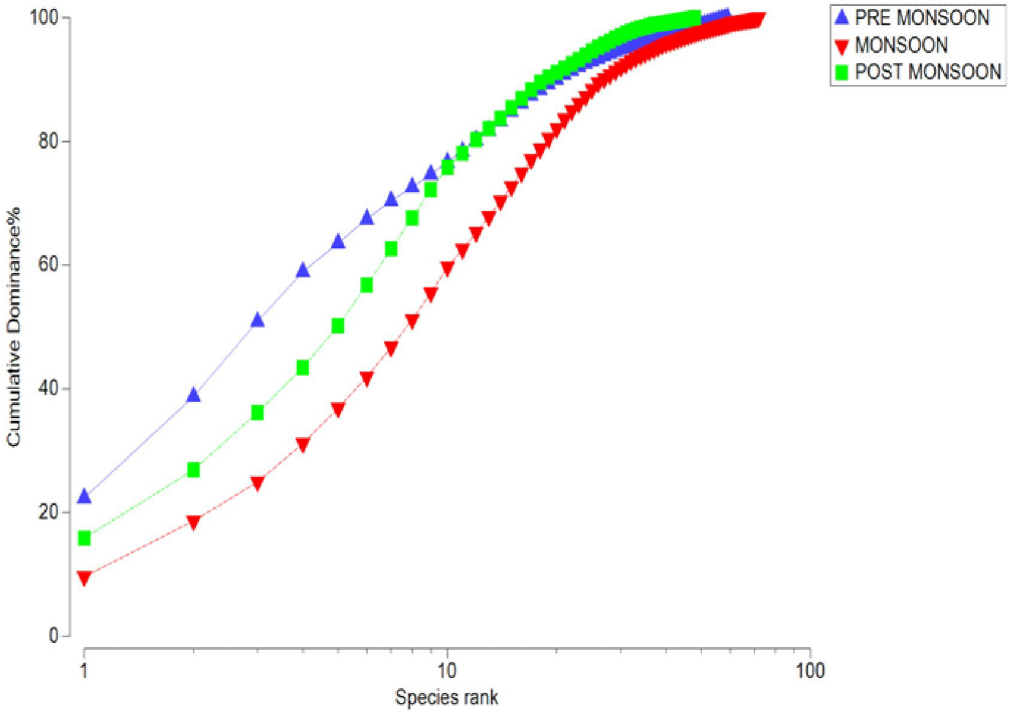


Fig. 3 K - dominance curve showing the seasonal variations

spiders. Dominance curve exhibited seasonal influence on the species composition and abundance of spider communities in Thattekkad sanctuary. The elevated K-dominance curve for the pre-monsoon season provides information that there is a disturbed state showing a low species abundance, which may be due to the environmental stress that makes many intolerant species to become rare (Clarke, 1990). Studies of Deshmukh and Raut (2014) also indicated the influence of similar seasonal variations on the occurrence and diversity of spiders in Salbardi forest, Maharashtra. Forest vegetation plays an important role in species composition and structurally more complex vegetation can sustain higher abundance and diversity of spiders (Sudhikumar *et al.,* 2005). Diverse vegetation hosts a range of insect species, which in turn leads to a large diversity of spiders (Chetia and Kalita, 2012). The floral diversity of about 163 tree species is reported in the Thattekkad Conservation area (Rijuraj *et al.,* 2017). During the monsoon season, some seasonal plants begin to flourish in the sanctuary. This can attract large numbers of insect fauna, which in turn can positively affect spider abundance. Collection of about 89 species indicated that the study area has fairly good population of

spiders and the microhabitats in the sanctuary supports rich spider diversity. So, it is essential to give more emphasis to the spider fauna conservation in these protected areas as they play an important role in the effective functioning of the ecosystem.

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Faunistic diversity of spiders (Araneae) in Peechi-Vazhani Wildlife Sanctuary, Kerala, India

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ABSTRACT: The study describes the identification of the spider assemblages with respect to their diversity within the Peechi-Vazhani Wildlife Sanctuary. A total of 106 species, from 24 families were recorded from the area, which forms baseline information of spiders of the sanctuary. Families showed varying degrees of habitat fidelity with some being abundant while others rare. Amongst these, Salticidae, Araneidae, Oxyopidae and Lycosidae were to have more species in the area. However, analyses of functional groups, e.g., ground runners (29%) showed the positive influence of structural complexity of the habitat. The presence of different species in all habitats highlights the importance of conserving a wide array of representative habitats within ecosystems.
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KEYWORDS: Identification, guild structure, functional groups, Western Ghats

INTRODUCTION

The Western Ghats region's exceptional biological richness and endemism are inherent in its inclusion among the 34 global hotspots. Although protected areas (PAs) are the most effective strategy to conserve biodiversity (Terborgh *et al.*, 2002), it is becoming increasingly recognized that they are insufficient to conserve tropical biodiversity in the long run (Rosenzweig, 2003). In the past, invertebrates were mostly overlooked when it came to conservation, and were only saved as a result of existing parks and reserves (DeWet and Schoonbee, 1991). Spiders are extremely varied arthropods, with 49,932 species classified into 4,239 taxa and 130 families worldwide. There are 1,897 species of spiders in India, divided into 488 genera and 60 families (World Spider Catalog, 2022). A comprehensive pioneering study was carried out on the alpha diversity of spider fauna in Peechi-

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Vazhani Wildlife Sanctuary and to provide species database to the Forest Department for developing a conservation action.

MATERIALS AND METHODS

Study area

Peechi-Vazhani Wildlife Sanctuary (P-VWS) situated in Thrissur district, Kerala state (76º 18' and 76º28' E ; 10º 28' and 10º 38' N) extending about 125 km^2. On the east, it is bounded by the Chimmini Wildlife Sanctuary, while on the north, it is flanked by the forests of the Palakkad division (Fig. 1). The sanctuary, which is located at 45–900m, receives 3000mm of annual precipitation. According to Champion and Seth (1986), the sanctuary's forest type is moist deciduous forest (almost 80%), followed by evergreen and semi-evergreen forest (15%), and teak and softwood plantations (5%). *Erythrina indica, Eugenia hemispheria, Dalbergia latifolia, Palanquium ellipticum, Terminalia tomentosa, Mesua ferrea, Cullenia excelsa, Cedrella toona, Bombax ceiba, Syzygium cumini, Largerstroemial anceolata, Adina cordifolia, Albizia procera, Alstonia scholaris and Xylia xylocarpa* are the common tree species. The lower canopy includes, *Ixora* spp., *Clerodendrum* sp. and *Lantana camara.*

Sampling

The study was carried out during April 2021. Study sites included Vellani, Vazhani, Vallikayam and Olakkara sections of the sanctuary (Fig. 2). Spiders were actively searched from different microhabitats such as ground, litter, undergrowth, bushes, tree trunks, foliage, and water bodies. A visual search technique using a line transect was used to make collections. The handpicking and beating method were mostly used for collection. Smaller spiders were captured by pushing them into alcohol-filled tubes using a brush soaked in alcohol. Holding the jar open beneath the spiders and tapping them into it with the lid, spiders found on leaf blades, tree trunks, and webs were captured in the container. Running and wandering species, like lycosids, were explored among leaf litter, under surface of logs, rocks, and plant surfaces and captured and transferred them to the jars (Sebastian and Peter, 2009). When a spider was noted, it was photographed and collected using Tikader's (1987) recommended handpicking approach. The specimens were preserved in ethanol (70%) and deposited in the collection of spiders, Arachnologylab, Deva Matha College, Kuravilangad (DMCK). Nomenclature follows the World Spider Catalog (2022). Adult males and females were identified up to species level while immature spiders were identified up to generic level.

RESULTS AND DISCUSSION

The spider diversity of Peechi-Vazhani Wildlife Sanctuary is found to be rich. Spiders representing 106 species coming under 68 genera and 24 families were recorded from the Sanctuary (Table 1, Plate 1 - 3). Among the twentyfour families, Salticidae (24 genera and 29 species) dominated in terms of spider diversityfollowed by Araneidae (5 genera and 19 species), Oxyopidae (4 genera and 12 species) and Lycosidae (4 genera, 5 species). Greater the variety of habitat type's available, larger is the diversity (Ried Miller 1989; Sudhikumar *et al.*, 2005; Siliwal and Molur, 2007; Adarsh and Nameer, 2015; Caleb, 2020). According to studies, habitat complexity and species richness are correlated, which shows that structurally more complex plants can support a greater variety of spider communities (Uetz,1991). Salticidae or jumping spiders, are masters of camouflage and can coexist with their surroundings which may be the probable reason for their dominance in the nature. The abundance of various spider families in terms of individual numbers, which prominently reflects Salticidae and Araneidae as more abundant through a less diverse family in comparison to Cheiracanthiidae, Ctenidae, Hersiliidae, Clubionidae, Linyphiidae, Liocranidae, Mimetidae, Philodromidae, Pholcidae, Pisauridae, Theraphosidae, Scytodidae and Zodaridae.

The spiders of Peechi-Vazhani Wildlife Sanctuary can be divided into eight feeding guilds based on the foraging behaviour (Uetz *et al*., 1999). They are the orb weavers, stalkers, ground runners, foliagerunners, foliage hunters, sheet web builders, scattered line weavers and ambushers (Table 1).

Table 1. Checklist of spiders of Peechi-Vazhani Wildlife Sanctuary, Kerala

	Family: Araneidae Clerck, 1757
1.	*Gasteracantha geminata* (Fabricius, 1798)
2.	*Cyclosa spirifera* (Simon, 1889)
3.	*Cyclosa* sp 2 (Menge, 1866)
4.	*Neoscona mukerjei* (Tikader, 1980)
5.	*Araneid* sp (Clerck, 1757)
6.	*Eriovixia* sp 2 (Archer, 1951)
7.	*Cyclosa* sp (Menge, 1866)
8.	*Eriovixia laglaizei* (Simon, 1877)
9.	*Cyclosa* sp (Menge, 1866)
10.	*Gasteracantha* sp (Sundevall, 1833)
11.	*Gasteracantha kuhli* (C. L. Koch, 1837)
12.	*Araneid* sp (Clerck, 1757)
13.	*Neoscona* sp male (Simon, 1864)
14.	*Neoscona* sp1 (Simon, 1864)
15.	Neoscona sp 2 (Simon, 1864)
16.	*Neoscona* sp 3 (Simon, 1864)
17.	*Argiope pulchella* (Thorell, 1881)
18.	*Neoscona* sp (Simon, 1864)
19.	*Gasteracantha* sp (Sundevall, 1833)
	Family: Cheiracanthiidae Wagner, 1887
20.	*Cheiracanthium* sp (C. L. Koch, 1839)
	Family: Clubionidae Wagner, 1887
21.	*Clubiona* sp1 (Latreille, 1804)
22.	*Clubiona* sp 2 (Latreille, 1804
	Family: CorinnidaeKarsch, 1880
23.	*Cambalida* sp (Simon, 1909)
24.	*Castianeria* sp (Keyserling, 1879)
25.	*Cambalida deorsa* (Murthappa, Prajapati, Sankaran & Sebastian, 2016)
	Family: Ctenidae Keyserling, 1877
26.	*Ctenus cochinensis* (Gravely, 1931)
	Family: Gnaphosidae Pocock, 1898
27.	*Scotophaeus* sp (Simon, 1893)
28.	*Gnaphosid* sp (Pocock, 1898)
	Family: Hersiliidae Thorell, 1870
29.	*Hersilia* sp (Audouin, 1826)
	Family: Linyphiidae Blackwall, 1859
30.	*Oeodothorax* sp (Bertkau, 1883)
	Family: Liocranidae Simon, 1897
31.	*Oedignatha binoyii* (Reddy & Patel, 1993)
	Family: Lycosidae Sundevall, 1833
32.	*Draposa* sp (Kronestedt, 2010)
33.	*Lycosa* sp (Latreille, 1804)
34.	*Hippasa agelenoides* (Simon, 1884)
35.	*Pardosa pseudoannulata* (Bösenberg & Strand, 1906)
36.	*Pardosa* sp (C. L. Koch, 1847)
	Family: Mimetidae Simon, 1881
37.	Mimetidae sp (Simon, 1881)
	Family: Oxyopidae Thorell, 1870
38.	*Oxyopes shweta* (Tikader, 1970)
39.	*O. sunandae* (Tikader, 1970)
40.	*Hamadraus* sp (Deeleman-Reinhold, 2009)
41.	*Hamataliwa* sp1 (Keyserling, 1887)
42.	*Oxyopes javanus* (Thorell, 1887)
43.	*O. forcipiformis* (Xie& Kim, 1996)
44.	*Oxyopes* sp1 (Latreille, 1804)
45.	*Hamataliwa* sp 2 (Keyserling, 1887)
46.	*Peucetia viridans* (Hentz, 1832)
47.	*Oxyopes* sp 2 (Latreille, 1804)
48.	*Hamataliwa pentagona* (Tang & Li, 2012
49.	*Oxyopes birmanicus* (Thorell, 1887)
	Family: Philodromidae Thorell, 1870
50.	*Philodromidae* sp (Thorell, 1870)
	Family: Pholcidae C. L. Koch, 1850

51. *Pholcus* sp1 (Walckenaer, 1805)
52. *Pholcus* sp 2 (Walckenaer, 1805)

Family Pisauridae Simon, 1890

53. *Dendrolycosa* sp1 (Doleschall, 1859)
54. *Dendrolycosa* sp 2 (Doleschall, 1859)

Family: Salticidae Blackwall, 1841

55. *Tamigalesus munnaricus* (¯abka, 1988)
56. *Epeus indicus* (Prószyñski, 1992)
57. *Stenaelurillus albus* (Sebastian, Sankaran, Malamel & Joseph, 2015)
58. *Plexippus paykulli* (Audouin, 1826)
59. *Epocilla xaurantiaca* (Simon, 1885)
60. *Brettus cingulatus* (Thorell, 1895)
61. *Salticid* sp (Blackwall, 1841)
62. *Hyllus semicupreus* (Simon, 1885)
63. *Cyrba ocellata* (Kroneberg, 1875)
64. *Hasarius adansoni* (Audouin, 1826)
65. *Epeus* sp (Prószyñski, 1992)
66. *Habrocestum* sp1 (Simon, 1876)
67. *Salticid* sp (Blackwall, 1841)
68. *Asemonea tenuipes* (O. Pickard-Cambridge, 1869)
69. *Myrmaplata plataleoides* (O. Pickard Cambridge, 1869)
70. *Bianor* sp. (G.W. Peckham & E.G. Peckham, 1886)
71. *Epeus triangulopalpis* (Malamel, Nafin, Sudhikumar & Sebastian, 2019)
72. *Lyssomanes* sp (Hentz, 1845)
73. *Salticid* sp (Blackwall, 1841)
74. *Rhene flavigera* (C. L. Koch, 1846)
75. *Indopadilla insularis* (male) (Malamel, Sankaran & Sebastian, 2015)
76. *Indopadilla insularis* (female) (Malamel, Sankaran & Sebastian, 2015)
77. *Epeus* sp (G. W. Peckham & E. G. Peckham, 1886)
78. *Rhene flavicomans* (Simon, 1902)
79. *Phintella vittata* (C.L. Koch, 1846)
80. *Telemonia* sp (Thorell, 1887)
81. *Salticid* sp (Blackwall, 1841)
82. *Chalcotropis* sp (Simon, 1902)
83. *Telamonia dimitata* (male) (Simon, 1899)
84. *Telamonia dimitata* (female) (Simon, 1899)

Family Scytodidae Blackwall, 1864

85. Scytodes thoracica (Latreille, 1802)

Family: Sparassidae Bertkau, 1872

86. *Olios milleti* (Pocock, 1901)
87. *Heteropoda venetoria* (Linnaeus, 1767)
88. *Heteropoda* sp. (Latreille, 1804)

Family: Tetragnathidae Menge, 1866

89. *Leucagede corata* (Blackwall, 1864)
90. *Tetragnatha keyserlingi* (Simon, 1890)
91. *Tetragnatha* sp (Latreille, 1804)
92. *Leucauge fastigata* (Simon, 1877)
93. *Tetragnatha* sp1 (Latreille, 1804)

Family: Theridiidae Sundevall, 1833

94. *Parasteatoda celsabdomina* (Zhu, 1998)
95. *Molione* sp (Thorell, 1892)
96. *Theridiidae* sp1 (Sundevall, 1833)
97. *Theridiidae* sp 2 (Sundevall, 1833)

Family: Theraphosidae Thorell, 1869

98. *Annandaliella travancorica* (Hirst, 1909)

Family: Thomisidae Sundevall, 1833

99. *Tmarus* sp (Simon 1875)
100. *Thomisus projectus* (Tikader, 1960)
101. *Camaricus formosus* (Thorell, 1887)
102. *Indoxysticus minutus* (Tang, Yin & Peng, 2005)

Family Uloboridae Thorell, 1869

103. *Miagrammopes* sp (O.Pickard-Cambridge, 1870)
104. *Uloborus* sp (Latreille, 1806)
105. *Miagrammopes* sp1 (O.Pickard-Cambridge, 1870)

Family: Zodariidae Thorell, 1881

106. *Zodariidae* sp (Thorell, 1881)

Table 2. Number of families, genera, species and functional guilds of spiders in Peechi-Vazhani Wildlife Sanctuary

No.	Family	Genera	Species	Guild
1.	Araneidae	5	19	Orb weavers
2.	Cheiracanthiidae	1	1	Foliage hunters
3.	Clubionidae	1	2	Foliage runners
4.	Corinnidae	3	3	Ground runners
5.	Ctenidae	1	1	Ground runners
6.	Gnaphosidae	2	2	Ground runners
7.	Hersilidae	1	1	Ambushers
8.	Linyphiidae	1	1	Sheet web builders
9.	Liocranidae	1	1	Ground runners
10.	Lycosidae	4	5	Ground runners
11.	Mimetidae	1	1	Stalkers
12.	Oxyopidae	4	12	Stalkers
13.	Philodromidae	1	1	Ambushers
14.	Pholcidae	1	2	Scattered line weavers
15.	Pisauridae	1	2	Foliage hunters
16.	Salticidae	24	29	Stalkers
17.	Scytodidae	1	1	Foliage hunters
18.	Sparassidae	2	3	Foliage runners
19.	Tetragnathidae	3	5	Orb weavers
20.	Theraphosidae	1	1	Ground runners
21.	Theridiidae	3	4	Scattered line weavers
22.	Thomisidae	4	5	Ambushers
23.	Uloboridae	2	3	Orb web weavers
24.	Zodaridae	1	1	Ground runners



Fig. 1 Location map of Peechi Vazhani Wildlife Sanctuary, southern Western Ghats

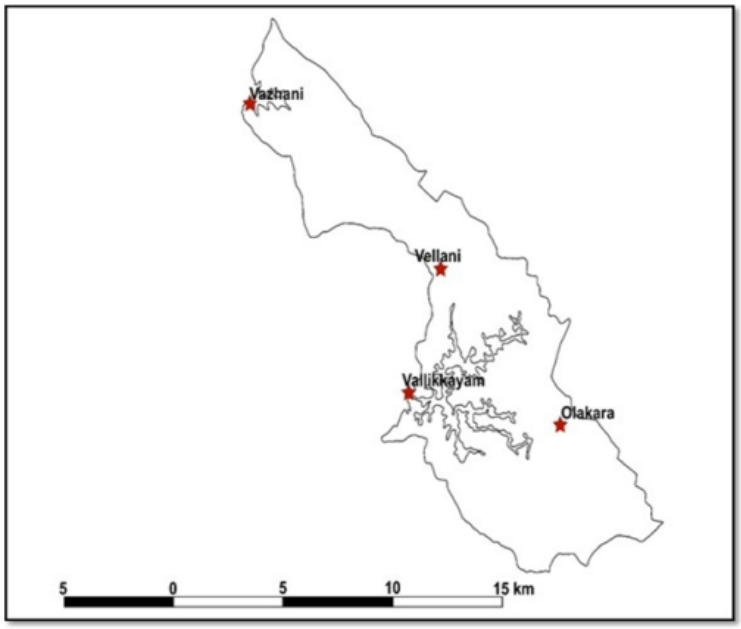


Fig. 2 Sample sites of Peechi - Vazhani Wildlife Sanctuary, Trissur, Kerala

Plate 1



Amyciaea sp.



Annandaliella travancorica



Argiope anasuja



Araneus sp.



Camaricus sp.



Cheracanthium sp.



Cyclosa sp.



Corinnomma sp.



Cyrtophora cicatrosa



Eriovixia sp.



Ctenus indicus



Gasteracantha dalyi



Dendrolycosa robusta

Plate 2



Phintella vittata



Rhene flavicomans



Oxyopes shweta



Stenaelurillus albus



Scytodes sp.



Plexippus paykulli



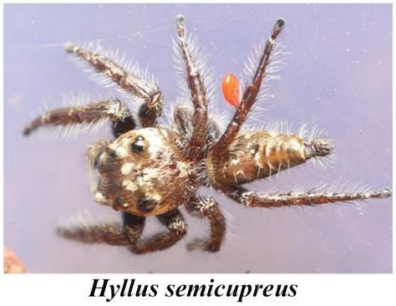
Pardosa sp.



Peucetia sp.



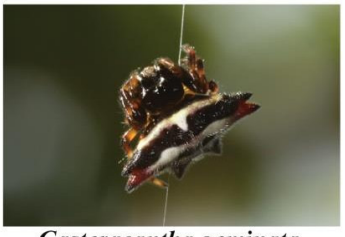
Smeringopus sp.



Hyllus semicupreus



Nephilengys malabarensis



Gasteracantha geminata



Oxyopes sp.



Leucauge fastigata



Thomisus sp.

Plate 3



Indopadilla insularis



Tetragnatha sp.



Neoscona sp.1



Miagrammopes sp.



Heteropoda sp.



Asemonea tenuipes



Telamonia dimidiata



Zelotes sp.



Hamataliwa sp.



Tmarus sp.



Neoscona sp.2



Porcataraneus sp.



Tamigalesus sp.



Anepsion maritatum



Hippasa agelenoides



Hersilia sp.1



Hersilia sp.2



Wadicosa sp.

Ground runners (29%) constitute the dominant feeding guild and are followed by stalkers (13%), ambushers (13%), foliage hunters (13%), orb weavers (12%), foliage runner (8%), scattered line weavers (8%) and sheet web builders (4%). The most probable reason for the observed pattern of spider guilds is structural diversity, micro environment, or the degree of habitat disturbance. The composition of guilds can shed information on the effect of habitat modification and disturbances on arthropod diversity.

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Spider (Arachnida, Araneae) diversity at Godrej mangroves, Vikhroli, Mumbai, Maharashtra, India

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ABSTRACT: Among the varied aquatic and terrestrial invertebrate diversity of mangrove ecosystem, spiders are considered to be an important bioindicators of ecological health. Studies on the spider diversity in the mangroves at Vikhroli, Mumbai, Maharashtra conducted resulted in the documentation of a total of 38 spider species belonging to the 33 genera under 12 families. Salticidae was found to be the dominant with 10 species from 10 genera, followed by Araneidae (9 species and 7 genera). The survey done in three mangrove zones revealed that zone II with moderate mangrove density resulted in the highest diversity (H=0.89) of spiders. Guild structure analysis revealed six different guilds. Stalkers (Salticidae and Oxyopidae) were the predominant feeding guild (34.00%). Seasonal (pre monsoon, monsoon and post monsoon) analysis showed more species diversity in the month of June to September.
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KEY WORDS: Species, bioindicators, mangrove density, guild structure, seasons

INTRODUCTION

Spiders are ubiquitous organisms belonging to order Araneae of class Arachnida under phylum Arthropoda. Worldwide there are 49,720 species of spiders belonging to 4,232 genera and 129 families. India represents about 1,799 species under 448 genera and 59 families (Caleb, 2020; World Spider Catalog, 2020). Mumbai, the financial capital of Maharashtra, has a history of luxuriant mangrove vegetation. But increasing extensive anthropogenic burden has created a threat to the mangroves and has caused depletion of about 40 per cent of all mangroves in the past, putting coastal region at risk. Despite of such pressures, today still mangrove forests are seen along the Vasai creek, Thane creek, Manori and Malad, Mahim - Bandra, Versova, Sewree, Mumbra-Diva, Vikhroli and Bhandup (Sarkar, 2017). A significant credit for the conservation and maintenance of mangrove forest of Mumbai goes to the Godrej mangroves, Vikhroli, and it acts as an ideal bionetwork and harbors around 82 butterflies, 209 birds, 13 crabs, 7 prawns and 20 fish species Spiders have proved to be good bio (Mangroves App, 2017) indicators of anthropogenic disturbance. Not much studies have been made on the spider diversity of mangrove forest of Mumbai region and its outskirts; except some valuable studies done from the Mumbai zones

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by (Mirza and Sanap, 2010; Sanap *et al.,* 2017) contributing the significant data about spider research. According to Mirza and Sanap (2010) the faunal diversity of Aarey milk colony, Mumbai includes 19 families of spiders (Infra-Order-Araneomorphae). According to Wise (1993), spiders are generalist predators playing an important role in terrestrial ecosystem. With this objective, current study has been carried out explicitly in the Godrej mangroves, Vikhroli (Mumbai), Maharashtra to understand spider status in overall invertebrate diversity of mangrove habitat.

MATERIALS AND METHODS

The study was conducted at Godrej Mangroves which is a private land owned by Godrej and Boyce Mfg. Co. Ltd and Soonabai Pirojsha Godrej Foundation located along the Eastern Express Highway at Vikhroli, Mumbai, Maharashtra ($19^0$06'37.47"N; $72^0$56'32.16"E to $19^0$03'53.39"N; 72^0 5'33.66"E). Thousands of acres of this land comprise of mangrove forest, which includes 16 species of mangroves and their associate species that are being maintained by the Godrej for the past 65 years. The survey was done from June 2017 to May 2018. The study area was divided into the three zones based on density of mangrove namely zone I - dense mangroves (1- KN post area, 2- Pond near post and 3- KN road); zone II - moderate mangroves (4- KT Post to Tower-2 road, 5- KT road, 6- Medicinal garden, 8- Butterfly garden and 9- BMC road area) and zone III - sparse mangroves (7- Jetty road, 10- RC road and 11- Link road).

The sampling methods include – all out search method involving visual searching in spider supporting microhabitats as far as, distinct vision is possible and ground search under litter, fallen or dry leaves and wood. In each zone, visual observation was done for about two hours in the morning (8.00am to 10.00am). All the individuals recorded from the above three zones were photographed using Nikon D7200 camera and dedicated macro lens: - Nikon AF micro 200mm F4. Nikon flash R1C1 used for macro shoots. Adult specimens were identified up to genus level and species wherever possible (Jocqué and Dippenaar-Schoeman, 2007; Sebastian and Peter, 2009; World Spider Catalog, 2020).

Statistical analyses were worked out as –

H_{max} = ln(S) = Maximum diversity possible (S = Sample count)

Shannon's diversity index (H) = **$H = -\Sigma (p_i \ln P_i)$**

Where,

H = General diversity index.

Pi = Proportion of the ith species such that (Pi = Ni/N)

Ni = Number of individuals in the ith species,

N= Total number of individuals of all species in the community

Evenness: $E = H/H_{max}$

RESULTS AND DISCUSSIONS

A total 38 spider species has been documented belonging to the 33 genera and 12 families from the study area; representing 20.34 per cent of total 59 families reported from India (World Spider Catalog, 2019-2021). Among all the families, Salticidae was found to be dominant (10 genera and 10 species), followed by Araneidae (7 genera and 9 species) (Table 1, Plate 1). However, in terms of abundance, Araneidae representing 32 total individuals ranked first, whereas Salticidae (22), Oxyopidae (18), Thomisidae (8) were on second, third and fourth position respectively (Table 2).

Guild analysis discovered total six different guilds; stalkers (34%), orb-web weavers (32%), ambushers (18%), ground runners (5%), space web builder (3%), and foliage hunters (8%) (Uetz *et al.,* 1999). Among stalkers Salticidae and Oxyopidae found to be the dominant. Zone-wise distribution of spiders (Table 4) showed that zone-II having moderate mangrove vegetation had the highest richness (53%) as well as abundance of spiders (57 individuals from all 12 families). Major contribution to the zone II in terms of abundance has been done by Araneidae (29.82%) and Salticidae (17.54%). Zone III showed

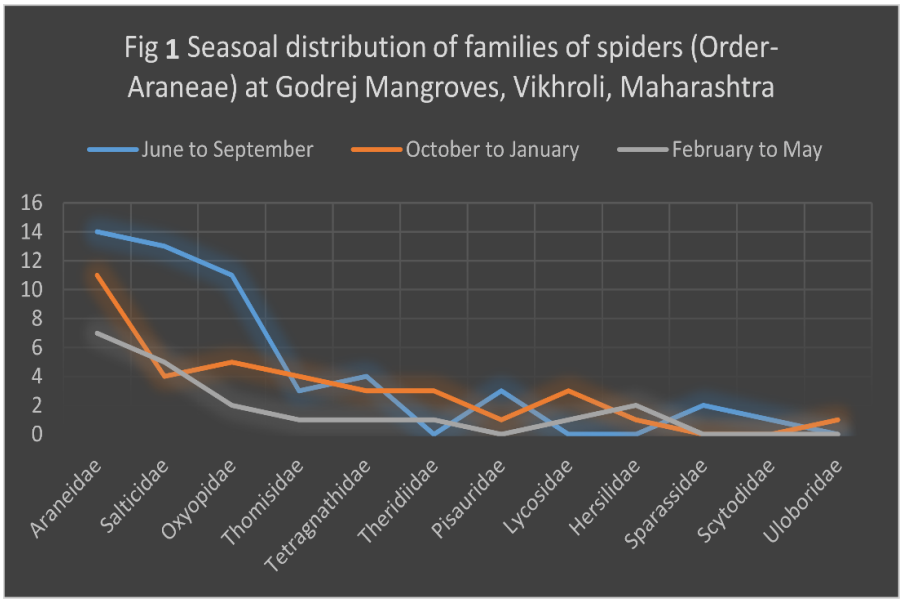
Table 1. Total number of Family, Genera and Species of spiders along with guild structure at Godrej Mangroves. Vikhroli, Maharashtra

No.	Family	Genus	Species	Guild
1	Salticidae	10	10	Stalker
2	Araneidae	07	09	Orb-web weaver
3	Thomisidae	03	04	Ambushers
4	Oxyopidae	01	03	Stalker
5	Lycosidae	02	02	Ground runners
6	Tetragnathidae	02	02	Orb-web weavers
7	Theridiidae	01	01	Space web builder
8	Sparassidae	01	01	Foliage hunters
9	Pisauridae	03	03	Ambushers
10	Uloboridae	01	01	Orb-web builder
11	Scytodidae	01	01	Foliage hunters
12	Hersiliidae	01	01	Foliage hunter
Total	**12**	**33**	**38**	

Table 2. Zone wise number of spiders at Godrej Mangroves

No.	Species	Godrej mangroves Zone			
		I	II	III	Total
Family: Araneidae					
1	*Argiope aemula*	01	04	01	06
2	*Argiope pulchella*	-	02	03	05
3	*Neoscona* sp1	-	01	-	01
4	*Neoscona* sp2	-	-	01	01
5	*Cyrtophora* sp	01	06	08	15
6	*Cyclosa* sp	-	01	-	01
7	*Thelacantha* sp	-	01	-	01
8	*Araneus* sp	-	01	-	01
9	*Eriovixia* sp	-	01	-	01
Total	09	02	17	13	32
Family: Salticidae					
1	*Rhene flavicomans*	01	01	-	02
2	*Hyllus semicupreus*	02	-	01	03
3	*Phintella vittata*	02	01	-	03
4	*Icius alboterminus*	-	-	01	01
5	*Hasarius adansoni*	-	01	01	02
6	*Carrhotus viduus*	01	02	02	05
7	*Telamonia dimidiate*	-	02	-	02

8	*Thyene imperialis*	-	01	01	02
9	*Asemonea tenuipes*	-	01	-	01
10	*Phlegra* sp	-	01	-	01
Total	10	06	10	06	22
Family: Oxyopidae					
1	*Oxyopes shweta*	02	02	-	04
2	*Oxyopes pankaji*	01	04	06	11
3	*Oxyopes javanus*	-	03	-	03
Total	03	03	09	06	18
Family: Thomisidae					
1	*Thomisus* sp 1	-	02	01	03
2	*Thomisus* sp 2	01	01	-	02
3	*Oxytate virens*	-	02	-	02
4	*Xysticus* sp	01	-	-	01
Total	04	02	05	01	08
Family: Tetragnathidae					
1	*Tetragnatha* sp	01	03	01	05
2	*Leucauge decorata*	01	02	-	03
Total	02	02	05	01	08
Family: Theridiidae					
1	*Argyrodes* sp	01	03	-	04
Total	01	01	03	00	04
Family: Pisauridae					
1	*Nilus sp*	0	02	-	02
2	*Perenethis* sp	0	01	-	01
3	*Pisaura* sp	0	01	-	01
Total	03	00	04	00	04
Family: Lycosidae					
1	*Lycosa* sp	01	02	-	03
2	*Hippasa* sp	01	00	-	01
Total	02	02	02	00	04
Family: Hersiliidae					
1	*Hersilia* sp	01	01	01	03
Family: Sparassidae					
1	*Olios lamarcki*	-	-	02	02
Family: Scytodidae					
1	*Scytodes* sp	00	00	01	01
Family: Uloboridae					
1	*Miagrammopes* sp	00	01	00	01
Total Abundance		19	57	31	107



29 per cent abundance of spiders, while zone I had only 18 per cent.

In the seasonal documentation, the species abundance was predominant during monsoon (June to September) followed by post monsoon (October to January); pre-monsoon (February to May) showed comparatively a lesser number (Fig. 5).

The maximum possible diversity is represented by H_{max} which is calculated considering all total 38 species recorded during the study. The Shannon index for zone I, zone II and zone III calculated were 2.7256, 3.2675 and 2.3548 respectively. Greater diversity was noted in zone II. Values of Evenness index suggest that species are more evenly distributed (zone I - 0.7492, zone II - 0.8982 and zone III - 0.6473).

H_{max}=ln(S) = Maximum diversity possible= 3.63758616

Godrej mangroves form a fairly productive ecosystem; extremely rich in biodiversity. According to Buchholz and Ceylan (2013) and Pearce and Venier (2006), invertebrates due to their short life span, great abundance, sensitivity towards fluctuating environment and high diversity can be utilized as bio-indicators to study the characters of the habitat within which they found. Spider without being an exception, might have its contribution in maintaining the health of an environment. Spiders are generalist predators (Wise, 1993), although insects found to form a major part of their diet, other arthropods are also happened to be preyed by them, while some species observed to be show cannibalistic behavior too (Foelix, 1996). Habitat heterogeneity supports sufficient alternative prey for them Maloney *et al.* (2013) stated that spiders can act as effective predators of certain insect pests in agricultural fields. Apart from above, during the survey, klepto-parasitic behaviour was observed in the family Theridiidae, where *Argyrodes* sp spotted in the web of *Argiope* sp and *Cyrtophora* sp (family-Araneidae) to steal the food captured and paralyzed by respective spider species (Plate 1: Image 2). Some feed on plant sap (Nyffeler *et al.,* 2016). It can be said that variation in spider population indirectly has consequences on overall food web of an ecosystem. Due to fairly high diversity, ease of collection and wide range distribution, spiders can be used as efficient bio-indicators (Pearce and Venier, 2006).

The statistical analysis worked out gives an idea about the overall diversity of Order Araneae at Godrej Mangroves, Vikhroli, Maharashtra. Zone I the dense mangroves represents inferior spider diversity (H=2.7256, E=0.7492). A dense mangrove comes under the coastal region where, vegetation is exposed to much salinity also the habitat is quite

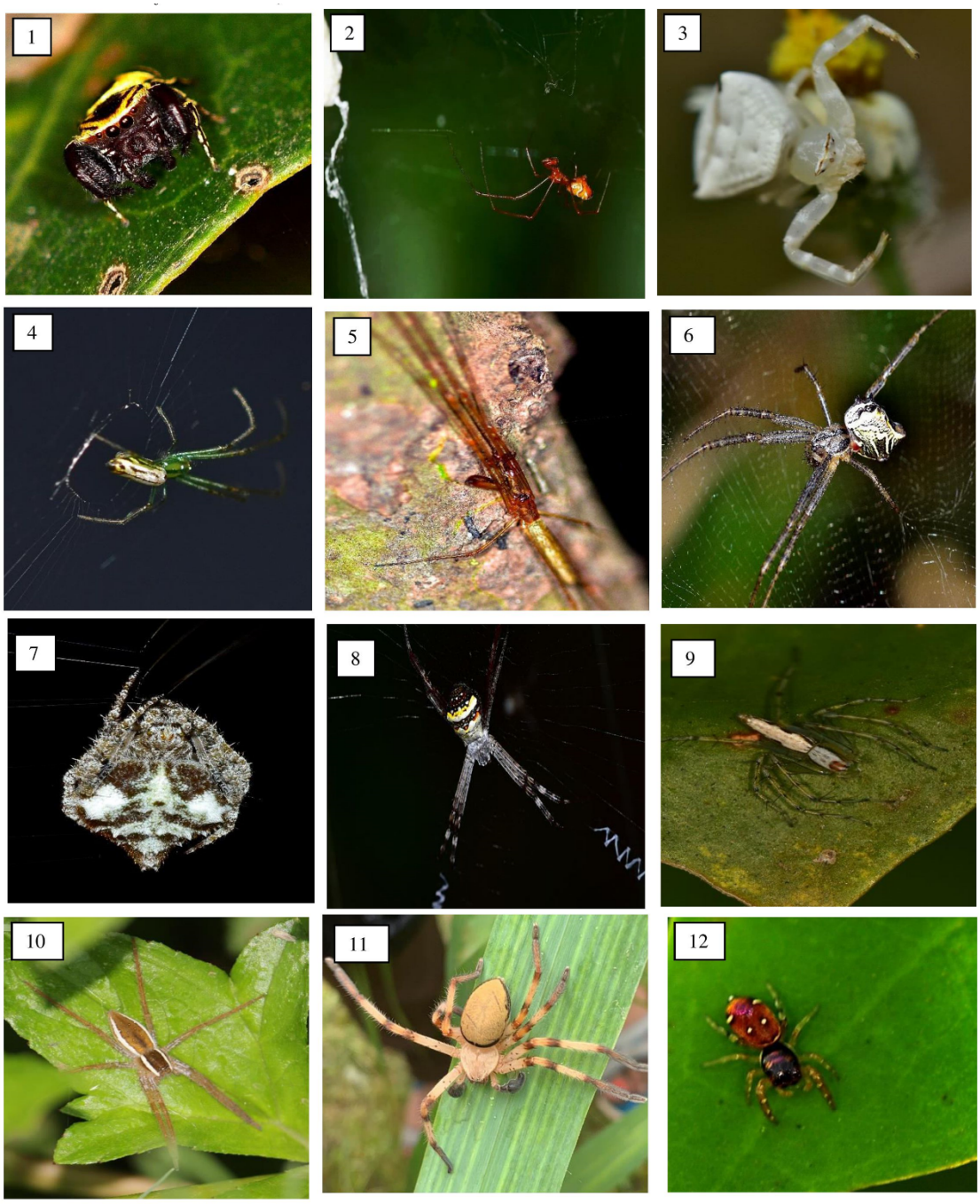


Plate 1. Spider diversity at Godrej mangroves, Vikhroli, Mumbai, Maharashtra 1. *Rhene flavicomans,* 2. *Argyrodes* sp, 3. *Thomisus* sp, 4. *Leucauge decorate*, 5. *Tetragnatha* sp, 6. *Cyrtophora* sp, 7. *Eriovixia* sp, 8. *Argiope pulchella* 9. *Oxyopes shweta*, 10. *Nilus* sp, 11. *Olios* sp, 12. *Icius alboterminus*

marshy and wet therefore it may cause difficulty for spiders to survive as it's been observed that they need a bit dry place for web development as well as for their other activities like prey catching, mating, parental care, etc. In addition, as low insect abundance can also be attributed to fewer occurrences of spiders in the zone I. The moderate mangrove zone, which can act as a connecting link between coastal and terrestrial region; has combination of halophyte and other land vegetation; forming an ideal habitat for spider population. Godrej Zone-II has blend of true mangroves like *Rhizophora apiculata* (Red Mangrove), *Rhizophora mucronata* (Red Mangrove), *Avicennia marina marina* (Gray mangrove) as well as mangroves associates such as *Acanthus ilicifolius* (Sea Holly), *Salvadora persica, Derris trifoliata, Sesuvium portulacastrum* along with other vegetation which includes, *Thespesia populnea* and *Hibiscus tiliaceus*. The occurrence of greater diversity and evenness in zone II (H=3.2675, E=0.8982) might be due to varied heterogeneous habitat which can act as an ideal environment for other invertebrates including insects; so, as the prey population is comparatively high in Zone-II spider diversity has been found to be superior. As the vegetation structure moves further reaching zone III, it mainly comprises of grassland where the diversity of spiders is comparatively low (H=2.3548, E=0.6473). In case of grassland, web construction, protection from predators, prey capture, might be fairly challenging due to lack of suitable place to retreat, or the habitat is pretty much open thus possibly making them vulnerable to predators. Evenness indices of three zones revealed that composition and properties of mangrove flora affect the distribution and abundance of spiders (Rashid *et al*., 2009).

The extensive leafy canopy provides great humidity (Sasekumar, 1974; Ross and Underwood, 1997) and shelter for spider retreat which otherwise would expose them to greater risk of desiccation. Godrej mangroves shows dominance of family Salticidae which can be attributed to their aggressive predatory approach, small size, various color-morphs; assisting them to easily camouflage within surrounding while stalking a prey. In addition, salticids have relatively a good vision and are able to distinguish colours and thus can distinguish prey from a considerable distance (Sebastian and Peter, 2009) which help in unrestricted foraging strategies from canopy to the exposed ground when tide levels are low (Macnae, 1969). However, in terms of abundance, Araneidae positioned first which is also supported by Macintosh and Ashton (2002). Least diversity at Godrej Mangrove was represented by Uloboridae, Scytodidae, Hersiliidae and Sparassidae constituting one species under each genus**.** The reason might be due to lack of preferable food or these species might not be able to tolerate such harsh environment of mangroves of study area.

Seasonal study discovered a distinct pattern in the spider abundance (Fig. 1). It indicated that the species are considerably more during monsoon due to temperature, relative humidity and abundance of food (Ghafoor and Mohamood, 2011) and further it shows a decline. After monsoon insects reduce their activity and become dormant due to lack of favorable environment; this may create a pressure on spiders while searching for food causing decreased in diversity. Variation in habitat, its structure, vegetation composition, temperature, humidity and prey availability causes clear spatial patterns of spiders in the mangrove forests (Berry, 1963). Spiders have a very significant role to play in ecology by being exclusively predatory and thereby maintaining ecological equilibrium (Sebastian and Peter, 2009). In the view of above study, spiders found to be the one of the key-indicator group of mangrove ecosystem. Understanding the dynamics of mangrove ecosystem, is a need of time especially when, anthropogenic activities taking toll of this fragile ecosystem.

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New distributional record of *Argyrodes bonadea* Karsch, 1881 and *Argyrodes nephilae* Taczanowski, 1873 (Araneae, Theridiidae) from Kerala, India

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ABSTRACT: *Argyrodes* Simon, 1864 is one of the rich genera of Theridiidae. For the first time *A. bonadea* Karsch, 1881 and *A. nephilae* Taczanowski, 1873 were reported from Kerala. Digital photographs are used to redescribe the species. © 2022 Association for Advancement of Entomology

KEYWORDS: Kleptoparasitic, Theridiidae, comb-footed, Neyyar Wild life sanctuary, Kumarakom bird sanctuary

Comb-footed spiders are one of the most well-known spider families, with 2542 species divided into 125 genera (World spider catalog, 2022). The family is diverse not only in the terms of number of species but also in terms of web styles, behavior, ecology and morphology. Even though India is a country of varying diversity, studies about Family Theridiidae Sundevall, 1833 in Indian region is lacking (Siliwal, 2009). The genus *Argyrodes* Simon, 1864 contains 98 species with a cosmopolitan distribution. About 15 species of the genus *Argyrodes* have been reported from India so far. Some members are kleptoparasitic, a reciprocal interaction in which one individual takes advantage from the foraging investments of another. During our study two kleptoparasitic species *A. bonadea* Karsch, 1881 and *A. nephilae* Taczanowski, 1873 were recorded.

The specimens were studied under a LEICA SAP0 and Luxeo 6Z stereomicroscope. All measurements are in millimeters (mm). Leg measurements are given as: Total, Femur, Patella, Tibia, Metatarsus (except palp) and Tarsus. The microphotographic images were taken by digital camera attached with Luxeo 6z stereomicroscope and Leica FLEXACAM C1 digital camera attached to a Leica LEICA SAP0 stereomicroscope with the software package Leica Application Suite X (LAS X). The specimens are deposited in a reference collection housed at the Department of Arachnology, Department of Zoology, Deva Matha College, Kuravilangadu, Kottayam, Kerala, India (DMCK).

Abbreviations used in the text: ALE-anterior lateral eye, AME-anterior median eye, PLE-posterior lateral eye, PME-posterior median eye, L-Length, W-Width, DMCK TH- Deva Matha Colege Kuravilangadu Theridiidae

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Taxonomy

Family: Theridiidae Sundevall, 1833; Genus: *Argyrodes* Simon, 1864

Diagnosis: Kleptoparasitic spiders with varying abdominal shape and colour. Reduced number of combs when compared with other Theridiid spiders. Cephalothorax flat, eye region elevated. Cheliceral teeth present. Long slender legs, usually first leg is longer. Colulus large.

***Argyrodes bonadea* Karsch, 1881 (**Figs. 1-2)

Material examined: 3 ♀ (DMCK TH-007), Kumarakom bird sanctuary, Kottayam district, Kerala State, India (9°37'38.028"N; 76°25'42. 996"E), 20 January 2020, Coll. Reshmi Sekhar.

Diagnosis: *Argyrodes bonadea* (Karsch, 1881) shows similarity with *Rhomphaea labiata* (Zhu and Song, 1991) by the structure of legs, eyes, fertilization ducts that bent and arise from the posterior margin of spermathecae and can be distinguished by the nature of spermatathecae. It is found to be closely touching each other in *A. bonadea while it is separated by half of its diameter in Rhomphaea labiata*. There is a scerotized postero-lateral arch that touches the spermathecae is found in *R. labiata* while it is absent in *A. bonadea.*

Description: Female. Overall, in a shade of reddish-brown. Silver patterns at both laterals of the abdomen join at the posterior end, ventral, dark brownish (Fig. 1A). Colourless faded in preservative. Cephalothorax longer than wide without any patterns. Ocular region elevated. Well defined transverse fovea. Total length 4.99 mm; Carapace 1.98 L; 3.01 W; Abdomen 0.63 L, 2.08 W. Eyes arranged in two rows. Ocular quadrangle wider anteriorly. PME white, prominent. ALE smaller than others. Distance between the eyes: PME-PME= PME-AME, 0.10; AME-AME, 0.14; PLE-ALE, closely placed (Fig. 2A). Sternum longer than wide, reddish-brown, heart-shaped, wider near first coxae (Fig.1B). Long, slender legs. Light yellowish shade except first. Clothed with fine hairs. Measurements of palp and legs (Femur, patella, tibia, metatarsus (except palp) and tarsus): Palp 0.57 [0.33, 0.04, 0.12, 0.08]; Leg I 9.54 [3.31, 0.41, 2.44, 2.06, 1.32], II 3.41 [1.07, 0.28, 0.95, 0.70, 0.41], III 1.96 [0.74, 0.16, 0.49, 0.33, 0.24], IV 2.96 [1.24, 0.28, 0.66, 0.45, 0.33]. Leg formula; 1243. Abdomen slightly oval, extending beyond the spinnerets. Dorsal region with fine hairs. Ventrally reddish brown. A total of 5 spots is seen around spinnerets, three are white, and 2 are light orange in the shade. Well developed between spinnerets. Colulus present. Epigyne covered with reddish-brown chitinous plate centrally. Spermatathecae, slightly oval, found close to each other. Copulatory duct short and slightly bending. Fertilization duct pointed (Fig. 2B).

Habitat: Collected from the web of a large spider.

Distribution: China, India (Delhi), Japan, Korea, Philippines, Taiwan.

Argyrodes nephilae Taczanowski, 1873
(Figs. 3-4)

Material examined: 2♀ (DMCK TH-032, DMCK TH-033), Neyyar wildlife sanctuary, Trivandrum District, Kerala State, India (8°32'59.99" N; 77°12'30.00" E), 17 October 2019, Coll. Reshmi Sekhar.

Diagnosis: *Argyrodes nephilae* (Taczanowski, 1873) shows similarity with closely related species *A. rostratus* (Blackwall, 1877) and can be separated each other by cone shaped and silvery in colour in the abdomen of *A. rostratus* (Blackwall, 1877).

Description: Female. Brownish and golden in shade. Cephalothorax brownish, abdomen golden with golden patches (Fig. 3A). Colour not much faded in preservative. Cephalothorax longer than wide, brownish shade. Ocular region elevated. Fovea absent. Total length 4.98mm; Carapace 2.52 L; 1.43 W; Abdomen 2.47 L, 1.69 W. Eyes heterogeneous, eight in number arranged in two rows. Except for PME all are black. Distance between the eyes: PME-PME 1.20; PME-PLE, 0.45; AME-AME, 1.14; AME-ALE 0.18, ALE-PLE closely placed. Eye diameter: PME 0.61, PLE 0.25, ALE 0.13, AME 0.63 (Fig. 4A). Sternum heart-shaped, wider near first coxa, color

similar to the cephalothorax with light yellowish and cream colored irregular patterns (Fig. 3B, 4B). Long slender legs, clothed with fine hairs. Light yellowish in shade, color decreases towards the end. Measurements of palp and legs (Femur, patella, tibia, metatarsus (except palp) and tarsus): Palp 1.57 [0.51, 0.27, 0.32, 0.47]; Leg I 5.29 [1.95, 0.89, 1.08, 0.98, 0.39], II 4.17 [1.74, 0.51, 0.71, 0.92, 0.29], III 1.83 [0.69, 0.17, 0.38, 0.45, 0.14], IV 3.33 [1.45, 0.39, 0.66, 0.62, 0.21]. Leg formula 1243. Abdomen triangular, extending beyond the spinnerets. Laterals golden with a black patch in the middle towards the tip dorsally. Ventral side black with fine hairs. Spinnerets not equal in size, surrounded by three golden spots which form a triangle when connected.

Epigyne: Small, spermathecae oval, separated by half of its diameter. Fertilization duct at the base of spermatheca projecting up wards (Fig. 4C).

Habitat: From an undisturbed area between plants.

Distribution: Argentina, Galapagos Island, USA, Caribbean, India (Andhra Pradesh)

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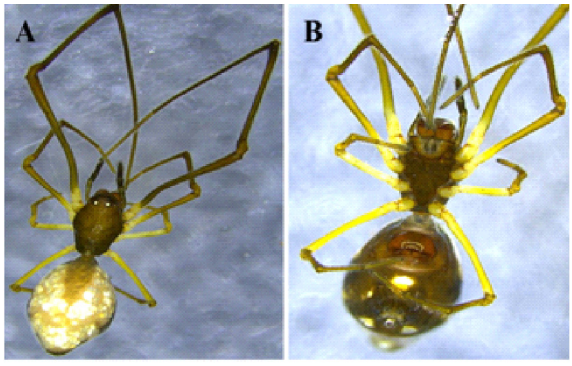


Fig. 1 *Argyrodes bonadea* Karsch, 1881
Female -A. dorsal view; B. ventral view

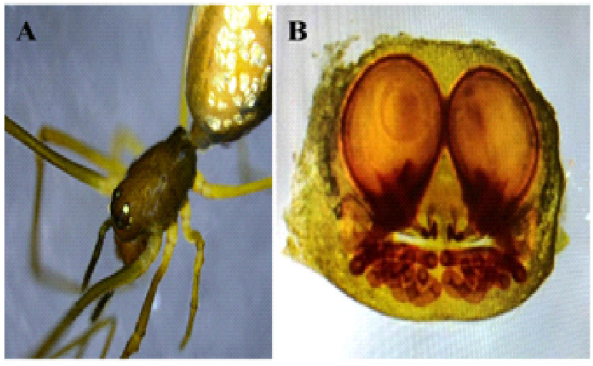


Fig. 2 *Argyrodes bonadea* Karsch, 1881 -
A. eyes; B. epigyne

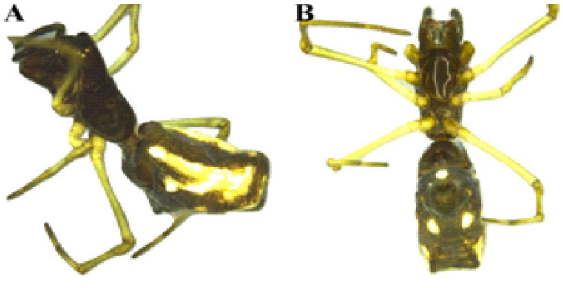


Fig. 3 *Argyrodes nephile* Taczanowski, 1873 -
A. dorsal view; B. ventral view

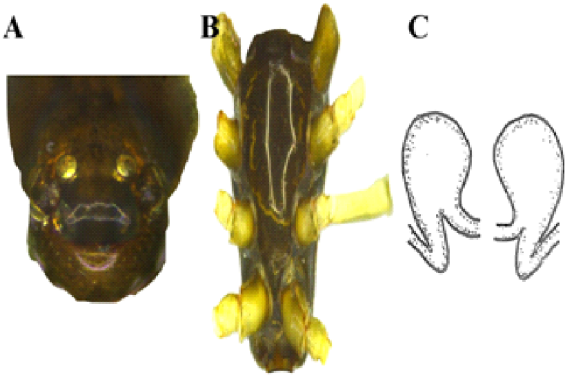


Fig. 4 *Argyrodes nephile* Taczanowski, 1873 -
A. eyes view; B. sternum; C. epigyne

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First record of *Mitrager rustica* (Tanasevitch, 2015) and *Neriene birmanica* (Thorell, 1887) (Araneae, Linyphiidae) from Kerala, India

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ABSTRACT: Two linyphiid species, *Mitrager rustica* (Tanasevitch, 2015) and *Neriene birmanica* (Thorell, 1887) are reported from Kerala. *M. rustica*, endemic to India, is making its second report after 15 years and it is first report of *N. birmanica* from Kerala. © 2022 Association for Advancement of Entomology

KEYWORDS: Kanjoor, tegulum, palp, report

Linyphiidae Blackwall, 1859 is the second largest family of spiders with a worldwide report of 4724 species under 624 genera. *Mitrager* Van Helsdingen, 1985 comes under subfamily Erigoninae and *Neriene* Blackwall, 1833 belongs to subfamily Linyphiinae. Till date, 25 species of *Mitrager* and 60 species from *Neriene* have been reported worldwide (World Spider Catalog, 2022). However, six *Mitrager* species have been reported from India namely: *M. cornuta* (Tanasevitch, 2015), *M. falciferoides* (Tanasevitch, 2015), *M. globiceps* (Thaler, 1987), *M. lopchu* (Tanasevitch, 2015), *M. rustica* (Tanasevitch, 2015) and *M. villosus* (Tanasevitch, 2015) described *M. rustica* from Tamil Nadu. *Neriene birmanica* Thorell, 1887 has been reported from Jammu & Kashmir, Karnataka and Uttarakhand.

Linyphiid specimens were collected from Central Kerala and preserved in ethyl alcohol (80%). Specimens were examined using compound microscope. Microphotographs were taken using Flexacam C1 attached to LEICA SAPO Automontage Microscope using Leica Application Suite X (LAS X) software. All measurements are in millimeters. The measurements of legs are given in the order: total [femur, patella, tibia, metatarsus (except palp), tarsus]. Epigyne was cleared by boiling in KOH (10%) for 5 minutes.

Abbreviations used in the text:

ALE- anterior lateral eye; AME- anterior median eye; DSA- distal suprategular apophysis; EM- embolic membrane; MP- median plate; PC- paracymbium; PLE- posterior lateral eye; PLP- posterior part of lamella; PME- posterior median eye; R- receptacle; T- tegulum; ST- subtegulum.

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Taxonomy

Family Linyphiidae Blackwall, 1859

Genus *Mitrager* Van Helsdingen, 1985

Type species: *Mitrager noordami* van Helsdingen, 1985

***Mitrager rustica* (Tanasevitch, 2015)** Figs. 1-4

Diagnosis: Males are characterized by slightly modified carapace, spination of paracymbium and specific structure of convector (Tanasevitch, 2015). In epigyne, lateral sides of median plate are directed inwards and receptacles are slightly curved outwards.

Material examined: 1 (DMCKLIN056), Kanjoor, Ernakulam district, Kerala (10°08'39.0"N; 76°25'04.4"E), hand-collected from leaf litter, 22 December 2020, leg. A. Domichan. 1♂ with same data (DMCKLIN035).

Description: Male - Total length 2.70. Prosoma length 1.32, width 1.22. Eye interdistances: AME-AME 0.04, AME-ALE 0.03, PME-PME 0.04, PME-PLE 0.04. Eye diameter: 0.03. Opisthosoma length 1.37, width 1.29. Measurements of legs and palp: I 3.55 [1.14, 0.30, 0.85, 1.02, 0.24], II 3.35 [1.07, 0.31, 0.80, 0.97, 0.20], III 3.26[1.08, 0.31, 0.81, 0.98, 0.24], IV 3.8 [1.17, 0.31, 0.92, 1.14, 0.26] palp: 2.05 [0.55, 0.31, 0.56, 0.63]. Leg formula 4123. Chaetotaxy 2-2-1-1. All metatarsi with a trichobothrium. TmI 0.65. Carapace yellowish. Area behind ocular region raised. Long hairs present behind posterior median eyes. Abdomen yellowish with three pairs of black patches on dorsal side. Pair of black patches on anterior and posterior lateral sides (Fig.1A). Pair of black patches on ventral sides. Sternum yellowish with dark borders (Fig.1B). Legs yellowish with white bands. Paracymbium simple with many spines. Tip of retro lateral tibial apophysis pointed. Distal suprategular apophysis narrows towards the central portion and distal portion appears rounded. Teeth like structure present at central portion of distal suprategular apophysis. Tip of distal convector apophysis pointed and twisted (Fig. 2 A-C).

Female - Total length 2.8. Prosoma length 1.3, width 0.94. Eye interdistances: PME-PME 0.03, PME-PLE 0.03. Eye diameter: 0.02. Opisthosoma length 1.5, width 0.98. Measurements of legs and palp: I 1.77 [0.53, 0.13, 0.40, 0.38, 0.33], II 1.69 [0.47, 0.13, 0.42, 0.36, 0.31], II I1.67 [0.50, 0.12, 0.41, 0.33, 0.31], IV 2.16 [0.61, 0.13, 0.51, 0.55, 0.36], palp 1.03 [0.32, 0.08, 0.29, 0.34]. Leg formula 4123. Chaetotaxy 2-2-1-1. All metatarsi with a trichobothrium. TmI 0.78. Carapace brownish. Area behind ocular region raised. Abdomen slightly pinkish with three pairs of black patches on dorsal side (Fig. 3A). Dark patches along lateral sides. Pair of black patches below epigyne. Sternum brownish with dark borders (Fig. 3B). Legs slightly yellowish with white bands. Thick lateral lines of median plate of epigyne directed inwards with slightly curved, hook like tip. Receptacles, round, slightly curved outwards (Fig.4 A-B).

Ecology: The specimens were collected from leaf litter. Due to its proximity to a moving stream, the soil and litter were slightly damp. After a mild rainfall the day before, the weather was slightly humid. The collection was done early in the day. Using a specimen vial, the running specimens were captured.

Distribution: India (Tamil Nadu, Kerala)

Genus *Neriene* Blackwall, 1833

Type species: *Neriene marginata* Blackwall, 1833

***Neriene birmanica* (Thorell, 1887)** Figs. 5-6

Diagnosis: Paracymbium possess narrow distal arm tapering to a sharp tip and hook-shaped tip of distal part of median apophysis curved in ventral direction (Helsdingen, 1969). It can be distinguished by the tiny paracymbium, sword-shaped embolic tip, terminal apophysis with about one coil (Xu *et al.,* 2010).

Material examined: 1 (DMCK LIN 072), Kumarakom Bird Sanctuary, Kottayam district, Kerala (9°37'39.8; 76°25'42.3"E), collected from sheet web, 19 February 2021, leg. A. Domichan.

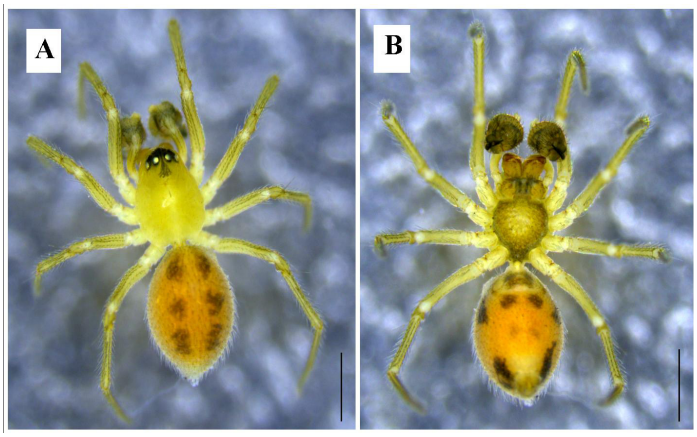


Fig. 1 *Mitrager rustica* (Tanasevitch, 2015) Male habitus; A. dorsal view; B.ventral view (Scale bars: A-B 1mm.)

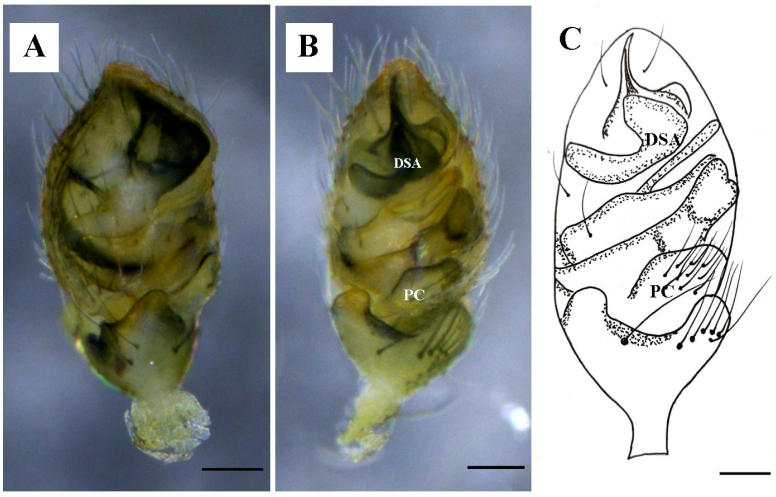


Fig. 2 *Mitrager rustica* (Tanasevitch, 2015). Male palp - A. dorso-ventral view; B-C. ventral view (Scale bars: A-C 1mm)

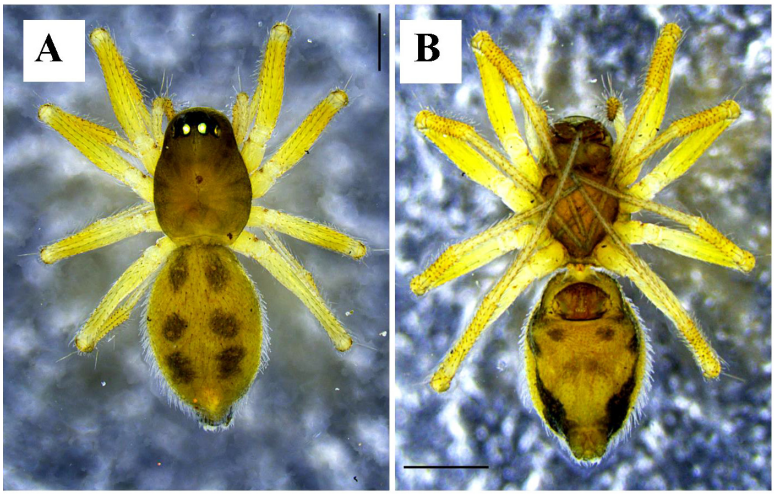


Fig. 3 *Mitrager rustica* (Tanasevitch, 2015) Female habitus - A. dorsal view; B.ventral view (Scale bars: A-B 1mm)

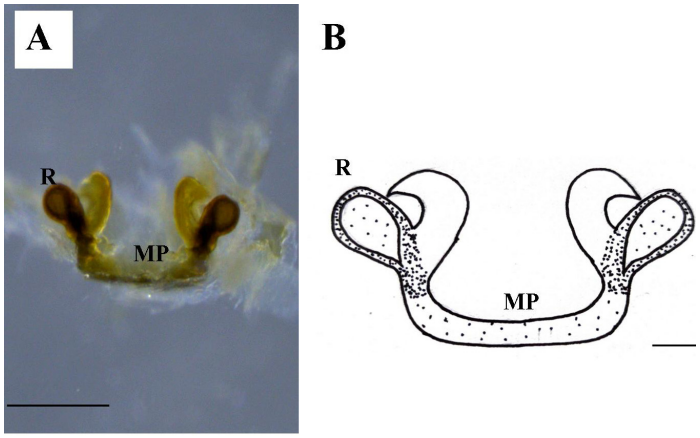


Fig. 4 *Mitrager rustica* (Tanasevitch, 2015). Female epigyne A -B. ventral view (Scale bars: A-B 0.1 mm)

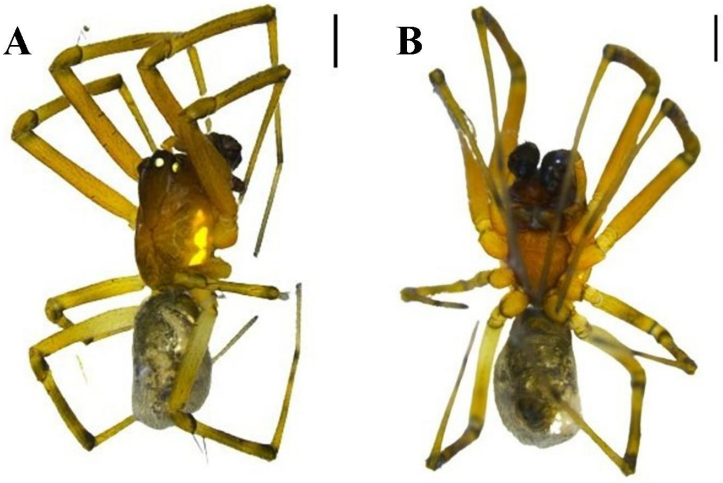


Fig. 5 *Neriene birmanica* (Thorell, 1887) Male habitus A. dorsal view; B. ventral view (Scale bars: A-B 1 mm)

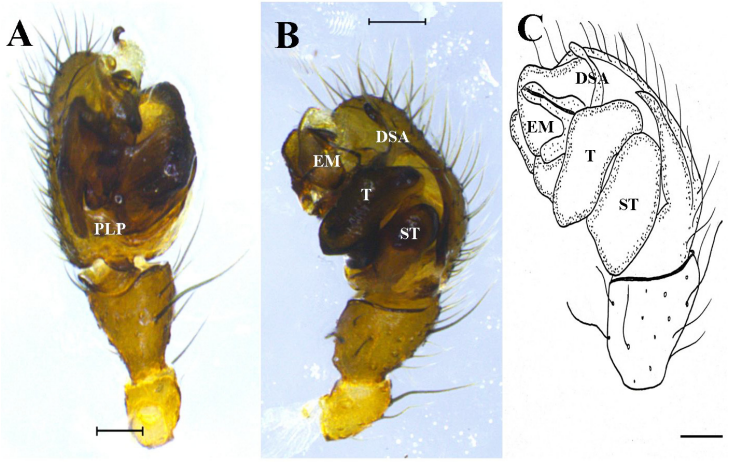


Fig. 6 *Neriene birmanica* (Thorell, 1887). Male palp A. retro-lateral view; B-C. ventro-lateral view (Scale bars: A-C 0.1 mm)

Description: Male - Total length 3.49. Prosoma length-1.73, width-1.54. Eye interdistances: AME-AME 0.04, AME-ALE 0.05, PME-PME 0.06, PME-PLE 0.08. Eye diameter: 0.07. Opisthosoma length 1.75, width 1.5. Chelicerae length 1.45. Measurements of legs and palp:I 5.18[1.62, 0.48,1.27,1.54,0.27], II 5.06 [1.58,0.45, 1.3,1.5,0.23], III 4.58[1.3, 0.43,1.18, 1.39,0.28], IV 4.97[1.48,0.47, 1.29,1.52,0.21], palp [0.66,0.52, 0.61,0.56]. Leg formula 1243. Cephalothorax brownish yellow. Ocular area raised. Heterogeneous eight eyes. Long, black hairs on ocular area. Anterior row of eyes recurved. Posterior row of eyes straight. Anterior and posterior lateral eyes juxtaposed. Chelicerae with three promarginal teeth and three retromarginal teeth. Stridulatory ridges absent. Sternum slightly brownish, wider near first coxa and darker along border (Fig. 5B). Legs yellowish, with black colouration at end of each segment. Thin legs with black long spines and short black hairs. Abdomen white with black markings on dorsal surface and greenish brown on ventral surface. Anterior part of abdomen straight. Posterior abdomen wider and slightly curved upwards. Body somewhat cylindrical in shape (Fig. 5A).

Palp: Distal end of paracymbium narrow towards end, with its pointed tip. Paracymbium U shaped with pointed proximal end. Terminal apophysis with one coil. Median apophysis with slender, hook like tip. Lamella well developed with thin, long and pointed lateral portion, broad and short anterior side. Posterior side of lamella thin, short and pointed whereas dorsal side is short and broad. Embolus thin and curved with sword-shaped tip. Tegulum broad and bulged medially. Subtegulum broad and bulging (Fig. 6 A-C).

Ecology: The specimen was collected, from sheet web, built on lower branches of a tree. Only one male specimen was found in the web. Kumarakom Bird Sanctuary had a humid weather during collection.

Distribution: India (Jammu & Kashmir, Karnataka, Uttarakhand, Kerala)

Present study identifies *M. rustica*, endemic to India, is making its second report after 15 years and it is first report of *N. birmanica* from Kerala. Despite minor differences, morphological characters like colour and pattern of abdomen is similar among most of the linyphiid litter dwellers. Thorough study of male and female genitalia is essential part of linyphiid identification. Being an understudied area, Central Kerala might be home to many linyphiids.

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Species diversity and vertical stratification of spiders of the family Tetragnathidae Menge, 1866 (Araneae) in different paddy farming practices at Kuttanad, Kerala, India

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ABSTRACT: The study aims to understand the species composition and habitat preferences of the spiders of the family Tetragnathidae in rice agroecosystem of Kuttanad. Surveys were carried out for a period of five months from July to November 2020. A total of thirteen tetragnathid species were collected from paddy fields and their nearby areas following organic way (do not use any pesticide) and a total of nine species collected from rice fields with its surroundings which use chemical pesticides. It was observed that the ecological guild structure of these spiders was based on foraging behaviour in relation to the height of the rice plant. © 2022 Association for Advancement of Entomology

KEY WORDS: Rice ecosystem, foraging behaviour, chemicals, ecological guilds

In the current scenario of using integrated pest management techniques in agroecosystems, the importance of spiders as a biological agent due to their inherent predatory nature is gaining more attention. The abundance and richness of these spiders in agricultural fields made them essential regulators of insect populations (Wise,1993). Also, studies describe spiders as one of the most potential predators in the paddy fields (Tanaka, 1989; Sudhikumar *et al*., 2005). And it has been noted that Tetragnathidae spiders are the most prevalent in cultivated fields (Vungsilabutr, 1988). Various studies examining qualitative analysis of spiders in the rice ecosystem revealed that spiders belonging to the genus Tetragnatha were found more in number (Okuma, 1979; Chatterjee and Datta, 1979, Kamal *et al*., 1990; Thakur *et al*., 1995). Sebastian *et al*. (2005) and Sudhikumar *et al*. (2005) reported Tetragnthathidae as the most dominant family, contributing up to 90 per cent of the spiders in the rice agroecosystems. *Tetragnatha mandibulata* is the most common species among tetragnathids (Bhardwaj and Pawar, 1987). The present study estimates the species composition in paddy fields and adjacent areas along with the vertical stratification of these spiders belonging to the family Tetragnathidae.

Study Area: Kuttanad is a low-lying agricultural area that spread over the Pathanamthitta, Alappuzha, and Kottayam districts of Kerala contributing nearly 20 per cent of Kerala's total rice production and is known as the "Rice Bowl of Kerala." This wetland rice agroecosystem is a

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warm, humid region with a seasonal variation in temperature ranging from 21–38 °C. The plots selected for the present study were Champakulam, Edthuva, Veeyapuram, and Kainakary situated at 9.41°N, 76.4 °E; 9.36°N, 76.45°E; 9.30°N, 76.46°E and 9.48°N, 76.37°E respectively. The experimental plots were decided on the basis of farming techniques adopted there. Champakulam and Edthuva are organic agroecosystems which do not use any pesticide, while Kainakary and Veeyapuram use chemical pesticides for pest control. The study was carried out for a period of five months, from July to November 2020.

Sampling: The sampling was done around every 30 days for a period of five months. The plots were selected and divided into ten quadrants (1m x 1m). The specimens were collected between 6 am to 9 am. Visual search and sweep net method was used for a sample collection from each quadrant. A sweep net with a handle length of 30 cm, rim diameter 10 cm, and mesh size 1 mm was used for collecting spiders. Collected specimens were preserved in 70 percent alcohol for further analyses.

Identification: Detailed examination of each spider was done using a stereo zoom microscope (Magnus, MS 24). The epigynum of female adult spider was dissected, cleared in 10% KOH, mounted on a temporary slide and observed under a compound microscope (Leica DM1000 LED) at both 10X and 20X magnifications to study the internal structures. Adult male spiders were identified by the morphology of the chelicerae and the pedipalps. Spiders were identified using World Spider Catalog (2020) and Platnick (2014) at family, genus and species level. All available published taxonomic papers and distributional records enlisted in Aranea of India (Caleb and Sankaran, 2022) were also used for identification. All the collected specimens are deposited in the Zoological Museum of the Department of Zoology, University of Kerala, Kariavattom.

Vertical stratification: Spiders belonging to family Tetragnathidae were observed to be distributed in different strata of the paddy. The specimens were observed, photographed and collected. The strata are based on habitat preferences of the spider species related to the average height of the rice plant. The height is measured in centimetres from ground level: 15-20 cm, 20-50 cm, 50-80 cm, >80 cm and at the tip of the leaf during the reproductive stage of the rice crop. The ecological stratification of these spiders in terms of constructing orb webs at different levels may reflect foraging strategies and dominance of different species.

A total of 3435 tetragnathid specimens were collected from the study areas during the surveys. Thirteen species of tetragnathid spiders were recorded during the survey. *Tetragnatha mandibulata*, *T. javana*, *Glenognatha dentata* and *Tylorida striata* were recorded in all the paddy fields and its proximate areas of Edthua, Champakulam, Kainakary and Veeyapuram in Kuttanad. All the other tetragnathid species varied in their occurrence (Table 1, Plate 1, 2). The vertical stratification of seven tetragnathid species observed during the study (Table 2).

It was observed that tetragnatha spiders sustain on a diverse range of pests which may explain their potential occurrence during the growing season of rice crops (Saksongmuang *et al*., 2020). In the present study, the most abundant spiders recorded in the rice fields of Edthuva and Champakulam using organic fertilizers included *Tetragnatha mandibulata*, *T. javana*, *T. keyserlingi*, *Tylorida striata, Glenognatha dentata* followed by *Leucauge* sp. and *L. granulata*. Few other species were also recorded from the nearby areas of these fields like *Tetragnatha ceylonica*, *T. viridorufa*, *Tetragnatha* sp., *Leucauge decorata*, *Tylorida ventralis* and *Guizygiella* sp. Whereas in rice fields of Veeyapuram and Kainakary which employ chemical fertilizers, the species found in more numbers are *T. mandibulata*, *T. javana*, *G. dentata* and *Ty. striata*. Tetragnathids collected from the proximate areas of pesticide affected farm lands were *T. viridorufa*, *L. decorata*, *Ty. ventralis* and *Guizygiella* sp. *Tetragnatha mandibulata*, *T. javana* and *T. keyserlingi* occupied the topmost part of the leaf, according to the vertical stratification of tetragnathid spiders in rice fields that was determined from soil level, while

Table 1. Tetragnathids collected from paddy fields and its proximate areas in Kuttanad

Species	Edthua		Champakulam		Kainakary		Veeyapuram	
	PA	PR	PA	PR	PA	PR	PA	PR
Tetragnatha mandibulata Walckenaer, 1841	+	+	+	+	+	+	+	+
T. javana (Thorell, 1890)	+	+	+	+	+	+	+	+
T. keyserlingi Simon, 1890	+	+	+	+	+	-	-	-
T. ceylonica O. Pickard-Cambridge, 1869	-	+	-	+	-	-	-	-
Tetragnatha sp.	-	+	-	+	-	-	-	-
T. viridorufa Gravely, 1921	-	+	-	+	-	+	-	+
Glenognatha dentate (Zhu & Wen, 1978)	+	+	+	+	+	+	+	+
Leucauge granulate (Walckenaer, 1841)	+	+	+	+	-	-	-	-
Leucauge sp.	+	+	+	+	-	-	-	-
L. decorata (Blackwall, 1864)	-	+	-	+	-	+	-	+
Tylorida striata (Thorell, 1874)	+	+	+	+	+	+	+	+
Ty. ventralis (Thorell, 1874)	-	+	-	+	-	+	-	-
Guizygiella sp.	-	+	-	+	-	-	-	+



Plate 1- Tetragnathids of Kuttanad. ©Nishi Babu. Fig. 1 *Glenognatha dentata;* Fig. 2 *Tetragnatha ceylonica;* Fig. 3 *Leucauge granulata;* Fig. 4 *T. javana;* Fig. 5. *Tetragnatha viridorufa*; Fig. 6 *Tylorida striata;* Fig. 7 *Tylorida ventralis;* Fig. 8 *T. mandibulata;* Fig. 9 *Guizygiella* sp.

Table 2. Vertical stratification of tetragnathid spiders in rice fields from soil level

No.	15-20 cm	20-50 cm	50-80 cm	>80 cm	Tip of the leaf
1.	*Glenognatha dentata*	*G. dentata*	*Leucauge* sp.	*Tylorida striata*	*Tetragnatha mandibulata*
2.		*Leucauge* sp.	*L. gegranulata*	*G. dentata*	*T. javana*
3.					*T. keyserlingi*



Plate 2 - Tetragnathids feeding on pests of paddy. ©BinishRoopas. Fig. 1 *Tetragnatha javana* feeding on brown plant hopper; Fig. 2 *T. mandibulata* feeding on green; plant hopper; Fig. 3 *Glenognatha dentata* feeding on rice pest; Fig. 4 Juvenile *Tetragnatha* sp. feeding on rice aphids

Glenognatha dentata predominated in the basal section. Other areas of the plant were colonised by *Tylorida striata*, *Leucauge granulata* and *Leucauge* sp. In the entire paddy field, the spider species that were taken from various parts of the plant were found to be identical. Spiders have been seen to create multiple guilds based on pest abundance, microhabitat, crop season and foraging technique. However, vegetation structure may also affect spiders' choices for habitat as reported by Mathew *et al*. (2014).

Tetragnthids make up a significant portion of the spider community in rice agroecosystems, hence the variety and abundance of these spiders in rice fields must be positively correlated with pest eradication and biological management (Joseph and Premila, 2016). The findings presented here show the variations in the species makeup of the Tetragnathidae family in four rice ecosystems utilizing various agricultural practices and chemical and organic fertilizers in Kuttanad. The presence of a wide variety of pests as prey species in the area due to the lack of pesticide use may explain the considerable number of tetragnathids and differences in species composition in farms using organic fertilizer. Additional research is being done to examine the importance of its predatory behaviour and the tactics employed to control the pest population in paddy fields.

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Spider fauna (Araneae: Arachnida) in different localities of Kannur District, Kerala, India

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ABSTRACT: A survey conducted to reveal on the spider diversity in different areas in Kannur District, Kerala, indicated a total of 31 species belonging to 15 families. The families Salticidae and Araneidae showed maximum species diversity. The study revealed that the selected study areas have favorable microhabitats for the spider fauna. © 2022 Association for Advancement of Entomology

KEYWORDS: Salticidae, Araneidae, diversity, microhabitats

Spiders are ubiquitous group belonging Arachnidea play an important role in controlling population of smaller invertebrates in an ecosystem (Riechert, 1974). Spiders can be used as bioindicators in environmental assessment programmes like heavy metal pollution (Maelfait and Frederik, 1998), urbanization (Mansouri *et al.,* 2019) forest fragmentation (Malvido *et al.,* 2020). One of the relevant issues in global conservation is the protection of biodiversity. Meaningful conservation cannot be possible without the knowledge on species involved. Spider fauna in India comprises 1905 species belonging to 61 families (Caleb and Sankaran, 2022). In Kerala region, very limited work has been carried on the faunal studies of spider (Sudhikumar *et al*., 2005; Sruthi *et al*., 2019; Shabnam *et al*., 2021).

The study areas are I- Kuppam,II-Trichambaram, III -Morazha, IV -Karimbam and V -Koovode in Kannur District, Kerala. Study sites were rich in vegetation including pepper plantations, grass land and home gardens. Investigation was carried from January to April. Spiders were collected at weekly intervals. For a systematic collection, specimens were collected from 4 quadrates (1m×1m) placed at four corners of 10m×10m area by visual search method between 9.30-11.30 h. A sufficient core area was left to avoid edge effect. All 4 quadrates were searched for a total of one hour. Collection was made mainly by hand picking method. Arial sampling of spiders was done by searching leaves, branches, bushes, tree trunks.Specimens were collected from knee height up to maximum overhead arm's reach and transferring them into collection bottles. Ground dwelling spiders were searched exploring leaf litter, under surface of logs, rocks, plant surface below knee. Specimens from each quadrate were preserved in alcohol (75%) and identified up to species level using literature

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Table 1. Spider species collected from different localities in Kannur District, Kerala

Family	Sl.No.	Scientific name	Study areas				
			I	II	III	IV	V
Salticidae	1	*Hyllus semicupreus* (Simon, 1885)	+				
	2	*Plexippus paykulli* (Audouin, 1826)		+	+		
	3	*P. petersi* (Karsch, 1878)	+				
	4	*Bavia kairali* (Samson & Sebastian, 2002)				+	
	5	*Telamonia dimidiata* (Simon, 1899)	+			+	
Ctenidae	6	*Ctenus cochinensis* (Gravely, 1931)					+
Miturgidae	7	*Cheiracanthium danieli* (Tikader, 1975)		+	+		
	8	*C. melanostomum* (Thorell, 1895)			+		
Araneidae	9	*Argiope anasuja* (Thorell, 1887)				+	
	10	*A. pulchella* (Thorell, 1881)	+				
	11	*Cyrtophora citricola* (Forsskal, 1775)		+			+
	12	*Gasteracantha geminata* (Fabricius, 1798)				+	
	13	*Cyclosa confraga* (Thorell, 1892)	+				
Corinnidae	14	*Castianeira zetes* (Simon, 1897)			+		
Thomisidae	15	*Thomisus pugilis* (Stoliczka, 1869)			+		
Tetragnathidae	16	*Opadometa fastigata* (Simon, 1877)				+	
	17	*Leucauge pondae* (Tikader, 1970)		+			
Theridiidae	18	*Theridion tikaderi* (Patel, 1973)				+	
	19	*Argyrodes gazedes* (Tikader, 1970)		+			
Pholcidae	20	*Crossopriza lyoni* (Blackwall, 1867)		+			
	21	*Pholcus kapuri* (Tikader, 1977)	+				
Sparassidae	22	*Heteropoda venatoria* (Linnaeus, 1767)				+	
Pisauridae	23	*Pisaura gitae* (Tikader, 1970)					+
Psecheridae	24	*Psechrus torvus* (O.Pickard-Cambridge, 1869)			+		
Hersiliidae	25	*Hersilia savignyi* (Lucas, 1836)				+	
Lycosidae	26	*Pardosa atropos* (L.Koch, 1878)					+
	27	*Hippasa agelenoides* (Simon, 1884)	+				
	28	*Trochosa punctipes* (Gravely, 1924)	+				
Oxyopidae	29	*Oxyopes birmanicus* (Thorell, 1847)				+	
	30	*O. javanus* (Thorell, 1887)				+	
	31	*Peucetia viridans* (Hentz, 1832)				+	

(Tikader, 1987; Barrion and Litsinger, 1995) and with the help of taxonomic experts.

Survey revealed that the population comprised of 31spider species belonging to Salticidae, Ctenidae, Miturgidae, Araneidae, Corinnidae, Thomisidae, Tetragnathidae, Theridiidae, Pholcidae, Sparassidae, Pisauridae, Psecheridae, Hersiliidae, Lycosidae and Oxyopidae (15 families). Maximum density of spider species was recorded in II and IV (25%) followed by III (19.64%), I (17.86%) and minimum at V (12.50%). Salticidae was the most dominant family with five species comprising of jumping spiders. Second dominant family was Araneidae (5 species) followed by Oxyopidae and Lycosidae (3 species each), Miturgidae, Tetragnathidae, Theridiidae and Pholcidae (2 species each). Ctenidae, Corinnidae, Thomisidae, Sparassidae, Pisauridae, Psecheridae and Hersilidae comprised one each (Table 1). Out of the spider species identified, 32.12 per cent is under Salticidae and 21.42 per cent under Aranaeidae. This is in accordance with the similar studies in different parts of Kerala (Marina and Tom, 2018; Asima *et al*., 2021). Salticidae in India comprises 181 species in 62 genera (Siliwal *et al*., 2005). Sudhikumar (2013) reported 27 species of predatory spiders from Nelliyampathy hill ranges in Kerala while Shabnam *et al*. (2021) reported 20 species from Wayanad region, Kerala. In the present study, *Plexippus paykulli* (Audouin, 1826), *Hyllus semicupreus* (Simon,1885) and *Telamonia dimidiata* (Simon, 1899) are observed in maximum density. *P. paykulli* is a cosmopolitan in distribution and reported to suppress the pests of agricultural crops (Rao *et al*., 1981; Tahir *et al*., 2014). Araneidae comprises of orb- weavers construct orb-web in the foliage upon trees, herbs, shrubs or grass (Gajbe, 2005) and in the present study, five species were recorded. *Cyrtophora citricola* was seen in maximum density. Abundance of orb- web species in the study sites might be attributed to the abundance of vegetation.

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Distributional record of *Annandaliella travancorica* Hirst 1909, (Araneae, Theraphosidae) from Western Ghats of Kerala, India

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ABSTRACT: Only three species of *Annandaliella* are known from the Western Ghats of Kerala, *A. travnacorica* Hirst 1909, *A. ernakulamens* is Jose and Sebastian 2018, *A. pectinifera* Gravely 1935. *A. travnacorica* is distinguished by its primary apophysis on first leg, which has a horn-like projection with a pointed black spine at the tibial end. The presence of a tibial apophysis with a thick black spine distinguishes it from *A. ernakulamensis* and *A. pectinifera*. *A. travancorica* has previously been reported in Kerala's Travancore, Kozikode, and Thrissur. This is the first report from Peechi Wildlife Sanctuary. © 2022 Association for Advancement of Entomology

KEYWORDS: Wildlife sanctuary, first report, apophysis

The genus *Annandaliella* which is endemic to India is distinguished by the presence of a stridulatory spine on the male and female chelicerae, as well as a tibial apophysis on the male tibia (World Spider Catalog, 2020). *Annandaliella travnacorica* Hirst 1909, *A. ernakulamens* is Jose and Sebastian 2018, *A. pectinifera* Gravely 1935, are the three species reported from Western Ghats of Kerala. The presence of comb-like primary apophys differentiate *A. ernakulamensis* and *A. pectinifera* from *A. travancorica* Hirst (1909) first described *A. travancorica* in 1909, but the original description appears to lack details. Sunil Jose and Prasanth (2015) provided a detailed description of *A. travancorica*.

During a survey *A. travancorica* was collected from Peechi Wild Life Sanctuary and deposited at Deva Matha College, Kuravilangad, Kerala (DMCK 21/ 395). The specimens were preserved in ethyl alcohol (70%). The whole body including legs and eye measurements and photographs were taken using LASX application suite X software and live images of the specimen were captured with Nikon d3500 digital camera. All measurements were taken in millimetres. The dorsal aspect of the leg and pedipalps were used to take measurements. The measurements of the eyes were taken using a calibrated eye micrometre and expressed in millimetres.

Material examined: Three males collected from Peechi wildlife sanctuary on 17th October 2021, 10°31'28" N; 76°21'19.9". Deposited at DMCK 21/395.

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Fig. 1 Habitat of *Annandaliella travancorica* Hirst 1909

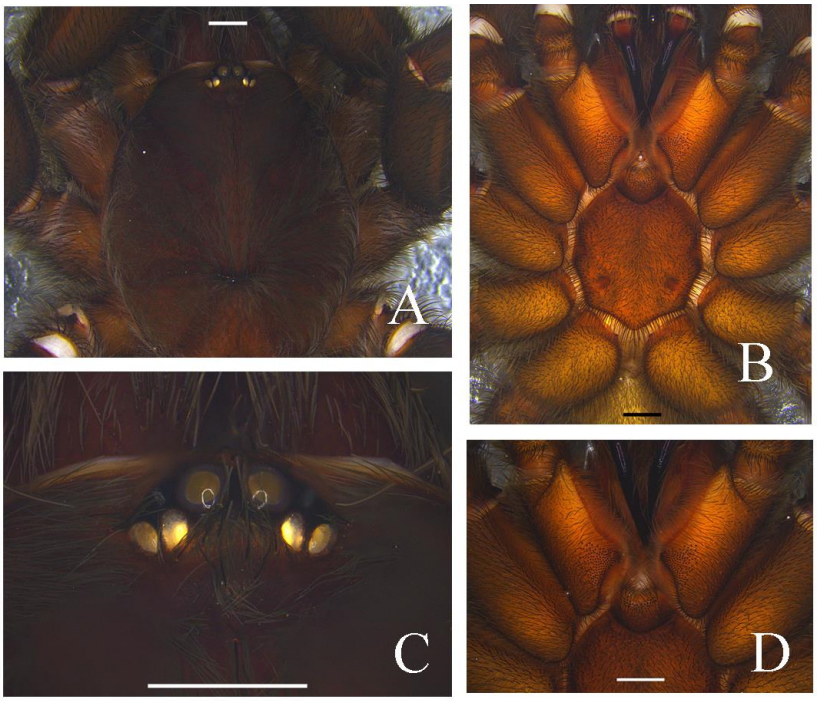


Fig. 2 A. Carapace, B. Sternum, C. Eyes, D. Maxillae and Labium

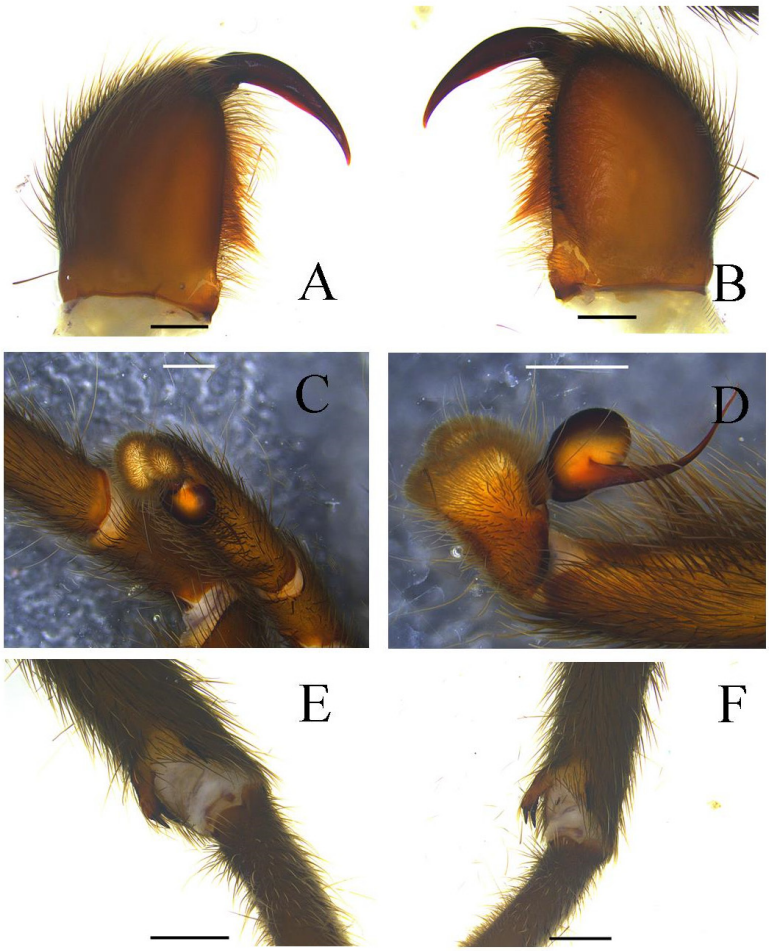


Fig. 3 A. Chelicerae Prolateral view, B. Chelicerae Retrolateral view, C. Palp Dorsal view, D. Palp Retrolateral view, E. Tibial apophysis Ventral view, F.Tibial apophysis Retrolateral view

Abbreviations used: AME: Anterior Median Eyes, PME: Posterior median eyes, PLE: Posterior lateral eyes, ALE: Anterior Lateral Eyes, PLS: Posterior lateral spinneret, PMS: Posterior median spinneret.

Taxonomy: *Annandaliella travancorica* Hirst 1908

Diagnosis: Primary apophysis is like a thick horn like projection ends with black spine. A single spine present on the base of the primary apophysis. Chelicerae of *A. travancorica* had no stridulatory spines on the inner surface, whereas *A. ernakulamensis* and *A. pectinifera* chelicerae had this stridulatory spine. A white patch along all leg tarsi with dark black body.

Description: Carapace: 7.66 long 6.53 wide. Wider than longer, oval in shape covered with pale yellow hairs. Ocular area is close to clypeus. Thoracic streaks arise from fovea. Foveais concave. Eyes: AME is larger than others. PLE is smaller. Ocular area is 0.62 long, 1.32 wide. Eye diameter; AME: 0.32, ALE: 0.10, PME: 0.16, PLE: 0.16. Eye interdistances: AME-AME-0.11, PME-PME-0.57, PME-PLE-0.06, and ALE-PLE-0.17. Maxillae: 2.31 long, 1.43 wide. A thick bush of Orangish hairs covers antero-dorsally. Maxillary cuspules distributed over anterior triangular corner. Labium: 0.95 long, cuspules covering anterior dorsal half. Sternum rounded, anterior corner is concave, posterior blunt end separating the fourth legs. Edges of sternum lined by black hairs. Three pairs of

Sigillae, Posterior 0.38 diameters, Median 0.21 diameter and Anterior Sigillae is marginal. Legs I: 3.36, 1.65, 3.82, 1.54, 1.04; II:3.03, 1.05, 2.69, 2.37, 1.64; III:2.57, 0.75, 2.30, 2.53, 1.97; IV:2.51, 4.21, 3.49, 3.32, 1.97; Palp;2.77, 1.41, 2.31,-,0.51. Abdomen is oval, longer (6.83) than wider (4.01). Spinnerets are digit form, PLS-Anterior segment 0.74 L, 0.45 W; Median segment: 0.73L, 0.39W; Posterior segment:1.01 L, 0.31 W; PMS- 0.68 L, 0.32 W. Palp having palpal bulb with thick long brownish curved spine.

Distribution: Thiruvananthapuram, Kozhikode, Kulathupzha, Thrissur, Peechi wildlife sanctuary (New report).

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Araneid spiders of Shendurney Wildlife Sanctuary in southern Western Ghats, India

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ABSTRACT: A survey of spiders conducted for a period of two seasons in Shendurney Wildlife Sanctuary revealed a total of 38 species. *Nephila pilipes, Cyclosa hexatuberculata and C. bifida* were the common species. Checklist of Araneid spiders is prepared.
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KEYWORDS: Checklist, *Nephila pilipes, Cyclosa hexatuberculata, Cyclosa bifida*

Araneae is the most diverse, female-dominated and entirely predatory order in the arthropod world. Evidently, they are key components of all ecosystems in which they live. They have, however, largely been ignored because of the human tendency to favour some organisms over others of equal importance because they lack a universal appeal (Humphries *et al*., 1995). Family Araneidae Simon, 1895 is a large family commonly known as orb weavers. This family exhibits a wide variation in their colour, size, shape and behaviour.Members of this family are small to large, ecribellate, three clawed spiders with eyes arranged in two rows with lateral eyes widely separated from the median eyes; constructs perfect orb webs with sticky spiral or a modified orb web (Sebastian and Peter, 2009). This is the second dominant diverse family in India. A total of 185 species under 36 genera has been reported from India (Caleb and Sankaran, 2021).

The present study was conducted in the Shendurney Wildlife Sanctuary located in (8^0 48'- 8^0 57'N; 77^0 4'-77^0 16'E) in the Agastyamalai Hills of the southern Western Ghats. The sanctuary lies in the catchment of the Parappar Dam (Thenmalai) constructed across the Kallada River and has an expanse of 171 km^2. The altitude ranges from 100 m above msl at the base of the hills to 1550 m on top of Alwarkurichi, the highest peak.

For the purpose of the study the sanctuary was surveyed, by dividing the area into two sites based on elevation.The details of three base camps utilized for data collection along an altitudinal gradient is as follows;

1) Kattalappara has an elevationof less than 250 m and the vegetation is formed by West Coast tropical Evergreen, Riparian vegetation, Myristica swamps, secondary forests and plantations along with the border with Thenmala forest Range. The Myristica swamps of the evergreen region, a peculiar low land freshwater swamp ecosystem with unique flora and

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Plate 1 Araneid spiders identified from Shendurney Wildlife Sanctuary

Table 1. Araneid spiders from selected habitats of Shendurney Wildlife Sanctuary

No.	Species
1.	*Acusilus coccineus* Simon,1895
2.	*Anepsion maritatum* O.Pickard-Cambridge, 1877
3.	*Arachnura angura* Tikader,1970
4.	*Araneus mitificus* Simon, 1886
5.	*A. viridisomus* Gravely, 1921
6.	*Argiope aemula* (Walckenaer, 1841)
7.	*Argiope anasuja* Thorell, 1887
8.	*Argiope catenulata* (Doleschall, 1859)
9.	*Argiope pulchella* Thorell, 1881
10.	*Chorizopes* sp. 1
11.	*Chorizopes* sp.2
12.	*Cyclosa bifida* (Doleschall, 1859)
13.	*C. confraga* (Thorell, 1892)
14.	*C. gossipiata* Keswani, 2013
15.	*C. hexatuberculata* Tikader, 1982
16.	*C. insulana* (Costa, 1834)
17.	*C. moonduensis* Tikader, 1963
18.	*C. neilensis* Tikader, 1977
19.	*C. purani* Keswani, 2013
20.	*C. simoni* Tikader, 1982
21.	*C. spirifera* Simon, 1889
22.	*Cyrtarachne* sp.1
23.	*Cyrtophora unicolor* (Doleschall, 1857)
24.	*Eriovixia excelsa* (Simon, 1889)
25.	*E. lagleizei* (Simon, 1877)
26.	*E. sakiedaorum* Tanikawa, 1999
27.	*Gasteracantha dalyi* Pocock, 1900
28.	*G. geminata* (Fabricius, 1798)
29.	*Gasteracantha* sp.1
30.	*Gea subarmata* Thorell, 1890
31.	*Herennia multipuncta* (Doleschall, 1859)
32.	*Neoscona bengalensis* Tikader & Bal, 1981
33.	*N. mukerjei* Tikader, 1980
34.	*N. nautica* (L. Koch, 1875)
35.	*Neoscona* sp.1
36.	*N. yptinika* Barrion & Litsinger, 1995
37.	*Nephila pilipes* (Fabricius, 1793)
38.	*Nephilengys malabarensis* (Walckenaer, 1841)

associated fauna. Three subsites were selected namely site 1 (semi evergreen) site 2 - (dry deciduous) and site 3 (Myristica swamps).

2) Kallar is in the mild elevation with altitude from 240-700 m, the habitat is formed by west coast tropical evergreen, southern hilltop evergreen forests, tropical semi evergreen, Ochlandra Reed patches, Secondary forests and plantations. Three subsites were selected namely, site 1 (evergreen), site 2 (semi evergreen) and site 3 (Ochlandra reed brakes).

The study was conducted for two seasons, dry summer and south-west monsoon from March 2021 to August 2021. The microhabitats that are likely to support the spiders in the study area including ground, litter tree, trunks, foliage, water bodies, undergrowth and bushes were searched for spiders. Collections were made by active searching for spiders following a line transect method. Spiders were collected by beating method and, direct handpicking method.The area around each vegetation along the transect was thoroughly examined from the top to bottom on leaf blades, flowers and dry leaves for spiders. The ground area near the plants was also searched. All the collected specimens were preserved in (70%) ethyl alcohol. World spider catalogue by Platnick (2014) and website Araneae of India, version 2021 (Caleb and Sankaran, 2021) was used for the identification of spiders.

A total 38 species of spiders belong to the family Araneidae were recorded during the period of two seasons (Table 1; Plate 1). *Nephila pilipes, Cyclosa hexatuberculata* and *C. bifida* were found as the most common species.

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Checklist of spiders from Vallakadavu Range of Western Ghats, Kerala, India

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ABSTRACT: In the preliminary study conducted to document the spider fauna in Vallakadavu range, Idukki district, Kerala, a total of 33 species of spiders belonging to 29 genera from 12 families were recorded. Among the 12 families recorded, Araneidae was the most predominant with orb web weavers. From the guild analysis, the recorded families were categorized into seven principle types of web patterns. The study extends the range of *Poecilotheria striata* Pocock, 1895 in the state to the east.
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KEYWORDS: Araneidae, Theraphosidae, web patterns, Idukki district

Spiders are among the largest and most diversified Arthropod groups. They are abundant and pervasive in nearly all environments and are one of the most essential components of global biodiversity. Thus far, 49,754 species have been recognised (World Spider Catalog, 2021). Joseph *et al.* (1997) documented 20 spider species in Periyar Tiger Reserve. This Reserve located in the Western Ghats, Idukki District of Kerala contains many unexplored regions that potentially serve as spider hotspots.

The present study was conducted in Vallakadavu range of Periyar East division, Periyar Tiger Reserve. It is located between 76^0 55' and 77^0 25' East Longitude and 9^0 18' and 9^0 41' North Latitude extended over an area of 777 km^2. The vegetation comprises of semi evergreen forests, moist deciduous forests, transitional fringe evergreen forests, grasslands,Vayals and Eucalyptus plantations.The study was conducted during December 2020. Opportunistic observations were mainly used for the study. Spiders were mainly collected by handpicking method as recommended by Tikader (1987). The collected spiders were preserved in alcohol (70%). The nomenclature followed is as per the World Spider Catalogue (2021).

During this investigation, a total of 33 spider species belonging to 30 genera and 12 families were identified. With 11 species, Araneidae was the most abundant family, followed by salticidae with five species. *Argiope anasuja* and *Nephila pilipes* were found to be the most common species in Araneidae. *Hippasa* and *Pardosa* were genera that represented the family Lycosidae. The Family Theraphosidae of Mygalomorphae is comprised of

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Table 1. Spiders from Vallakadavu Range, Western Ghats

No	Family/ Guild	Scientific name
1	Araneidae/ Orb web weavers	*Herennia multipuncta* (Doleschall, 1859)
2		*Argiope anasuja* (Thorell, 1887)
3		*Cyclosa bifida* (Doleschall, 1859)
4		*Anepsion maritatum* (O. Pickard-Cambridge, 1877)
5		*Cyrtophora cicatrosa* (Stoliczka, 1869)
6		*Eriovixia laglaizei* (Simon, 1877)
7		*Neoscona* sp 1
8		*Neoscona* sp 2
9		*Nephila kuhli* (Doleschall, 1859)
10		*N. pilipes* (Fabricius, 1793)
11	Ctenidae/ Ground runners	*Ctenus* sp
12	Hersillidae/ Ambushers	*Hersilia savignyi* (Lucas, 1836)
13	Lycosidae/ Ground runners	*Hippasa agelenoides* (Simon,1884)
14		*Pardosa atropalpis* (C.L. Koch, 1847)
15		*Pardosa* sp 1
16		*Pardosa* sp 2
17	Linyphiidae/ Sheet web weavers	*Prosoponoides* sp (Millidge & Russell-Smith,1992)
18	Oxyopidae/ Stalkers	*Oxyopes* sp (Latreille,1804)
19	Salticidae/ Stalkers	*Hyllus semicupreus* (Simon,1885)
20		*Myrmarachne* sp (MacLeay,1839)
21		*Phintella vittate* (C.L.Koch,1846)
22		*Plexipus paykulli* (Audouin,1826)
23		*Carrhotus viduus* (C.L. Koch,1846)
24	Sparrasidae/ Foliage runners	*Olios* sp (Walckenaer, 1837)
25	Tetragnathidae/ Orb web weavers	*Opadomata fastigata* (Simon, 1877)
26		*Leucauge decorata* (Blackwall, 1864)
27		*Tetragnatha mandibulata* (Walckenaer,1841)
28	Theraphosidae/ Ground runners	*Annandaliella* sp 1
29		*Annandaliella* sp 2
30		*Poecilotheria* sp
31	Theridiidae/ Space web builders	*Argyrodes* sp (Simon,1864)
32		*Parasteatoda celsabdomina* (Zhu, 1998)
33	Thomisidae/ Ambushers	*Tmarus truncates* (L. Koch, 1876)



Fig.1 *Poecilotheria striata* occurrence inVallakadavu Range

huge spiders that reside in burrows, trees and the ground or beneath rocks.

The genera *Annandaliella, Plesiophrictus* and *Poecilotheria* have been identified within the family Theraphosidae.They are endemic to the Western Ghats of India. The occurence of *Poecilotheria striata* Pocock, 1895 (Fig. 1) in Vallakadavu Range of Periyar tiger reserve is newinformation indicating the species' distribution to the east. *Annandaliella* and *Plesiophrictus* species do not belong to any of the currently recognised species.

The Peryar Tiger Reserve is home to twelve families, 30 genera, and 33 species of spiders. They are classified as orb web weavers, ground runners, ambushers, sheet web weavers, foliage hunters, space web builders and stalkers. Among these orb web weavers were more dominant. Spiders classified into 12 families, 29 genera and 33 species were categorized into seven principle types of web patterns. Orb web weavers (40%), ground runners (21%), ambushers (6%), sheet web weavers (3%), foliage hunters (6%), space web builders (6%) and stalkers (18%). Among these orb web weavers were dominant. This is a preliminary list of the spiders recorded in the Vallakadavu region in the East Division of the Periyar Tiger Reserve.

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Spider silk as a potential antibiotic substitute

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ABSTRACT: Studies were undertaken on the antibacterial activity of spider silk against bacterial strains, using egg case silk of *Parawixia dehaani* (Doleschall, 1859) and web silk of *Pholcus phalangioides* (Fuesslin, 1775). Both silk types inhibited gram negative bacteria more than gram positive bacteria. The egg case silk of *P. dehaani* showed more antimicrobial activity than the web silk of *P. phalangioides*. The egg case silk brought about 26.05 per cent inhibition against *E. coli*, compared to a 22.7 per cent inhibition for *B. subtilis*. A linear association was found between the volume of silk extract and the percentage of inhibition. The percentage of inhibition against *E. coli* increased from 5.13 to 26.05 per cent as the volume rose from 20 to 100 µl. in *P. dehaani.* © 2022 Association for Advancement of Entomology

KEYWORDS*: Parawixia dehaani*, *Pholcus phalangioides*, antibacterial, web silk, egg case silk

Spiders have long piqued people's interest due to their unique ability to weave webs with geometrical precision and artistry (Soler and Zaera, 2016; Vollrath and Krink, 2020). Spiders use their silk for a variety of purposes, including as a lifeline that allows them to safely flee from predators, as a shelter, as a snare, and also to guard their eggs (Römer and Scheibel, 2008; Iqbal *et al.,* 2019). Their capacity to produce multiple types of silk from distinct glands enables them to rely on silk as a means for survival. The cynosure here is the egg case silk and web silk produced by aciniform, tubuliform and ampullate (major and minor) glands of spiders. Web silk, including drag line, that has tensile strength greater than high tensile engineering steel (Sebastian and Peter, 2009; Kono *et al*., 2021) and egg case silk, which has its own methods of enduring environmental risks such as infections from outside sources (Vierra *et al*., 2011), are both valuable biomimetic prospects, with some of them already being actively utilized in research.

Spider silk is being studied for its antibacterial effects in addition to its use as a mechanical biomimetic. The idea of examining spider silk for antibacterial potential appears to have been sparked by historical reports of wound healing using spider silk. Amaley *et al*. (2014) using drag line silk of *Nephila pilipes* (Fabricius, 1793) (Family: Araneidae) against different bacteria including *Escherichia coli*, and Keiser *et al*. (2015) employing social spider *Stegodyphus dumicola* Pocock 1898 (Family: Eresidae) against *Bacillus thuringiensis* illustrated the antibacterial efficacy of spider silk. While several studies revealed that spider silk has antibacterial properties (Sawarkar and Sawarkar, 2017; Phartale *et al*., 2019; Deshmukh and Pansare, 2019; Sangavi *et al*., 2020) others dismissed it as a hoax or perhaps a methodological flaw (Fruergaard *et al*., 2021).

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The focus of this research is to evaluate the antibacterial properties of two types of spider silk collected from two different spider species.

Web silk of *Pholcus phalangioides* (Fuesslin, 1775) (Family: Pholcidae) and egg case silk of *Parawixia dehaani* (Doleschall, 1859) (Family: Araneidae) were collected to evaluate the antimicrobial property. Spiders were collected from Kandakkadavu wetlands (Ernakulam district, Kerala, India). Spiders were reared separately in large sterile plastic bottles of 200 ml size (100 mm tall and 56 mm in diameter). Mosquitoes were offered to spiders on daily basis. Web silk made by *P. phalangioides* inside the bottle was collected and preserved in sterile conditions. *P. dehaani* laid eggs in the bottle and the silk from its egg case was collected after the emergence of spiderlings and preserved. Gram negative *Escherichia coli* (MTCC NO 2414) and gram positive *Bacillus subtilis* (MTCC NO 443) were the bacterial strains chosen for the test. Antibacterial assay was carried out using the microtiter plate method with an ELISA reader (Thermo Scientific, USA) under aseptic conditions.

Preparation of silk extract and microbial culture: One gram of spider silk was weighed and hydrolyzed with 10 ml of acetone for one week at 30°C to make the silk sample. The hydrolyzed cobweb was centrifuged for 30 minutes at 4000 rpm. To ensure decontamination, the supernatant was then passed through a filter with a pore size of 0.4 µm and refrigerated. For the microbial culture, a single pure colony of *E. coli* and *B. subtilis* was loaded into a 10 ml nutrient broth (HiMedia) tube, sealed, and incubated at 37°C overnight (incubator used REMI). Then, using McFarland standards as a reference, the turbidity of suspensions was estimated and adjusted.

Preparation of the microtiter plates: In the current study, microtiter plates were used for the antimicrobial assay. A varied volume of silk sample (20 µl, 40 µl, 60 µl, 80 µl and 100 µl), 100 µl of nutrient broth and microbial suspension were added to each well of the microtiter plate. Control dilutions of test material were also kept. As organism control, a column was prepared with all solutions excluding the silk sample. To keep the culture from being dried, the plate was wrapped loosely in cling film. After a 24 hour incubation period at 37°C, an OD reading was taken (OD_{600}) using spectrophotometer (Thermo Scientific, USA). The experiment was repeated twice and the mean values were tabulated (Table 1 and 2).

Optical density was calculated by subtracting the control OD from the sample OD and the percentage of inhibition was calculated from the following equation,

Percentage of inhibition =
[(Control –Test) / Control] ×100

where, OD of extract is the difference between test OD and final test OD

The egg case silk of *P. dehaani* showed more antimicrobial activity than the web silk of *P. phalangioides*. Both silk samples showed more inhibition against the gram negative bacteria, *E. coli*. As the volume of extract was raised from 20 to 100 µl, a progressive increase in the percentage of inhibition was observed. However, in both silk samples, a significant percentage was detected only at 100 µl (Table 1, 2).

Only a few studies have been conducted on the antibacterial activity of egg case silk. Wright (2011) observed antibacterial properties in the egg case silk of the spider *Pityohyphante sphrygianus* (C.L. Koch, 1836) (Family: Linyphiidae) against *E. coli* and *B. subtilis*. The finding of the present investigation is consistent with this result. The presence of water-soluble, polar coating peptides isolated from egg sac silk, which have been theorized to be antibacterial, could be one rationale (Vierra *et al*., 2011).

In present investigation, antibacterial activity in the web silk of *P. phalangioides* was observed in both gram positive and gram negative bacteria, with gram negative bacteria being more prone to inhibition. Tahir *et al*. (2017) employed silk from *Cyclosa confraga* (Thorell, 1892) to reach the same conclusion. In 2018, Tahir *et al*. used silk of

P. phalangioides in a comparable study and established the vulnerability of gram negative bacteria. Gram negative bacterial strains were again found to be more susceptible than gram positive strains in an experiment where the antibacterial capabilities of spider silk against multidrug resistant bacteria were analyzed (Haq *et al*., 2019). The heightened sensitivity of gram negative bacteria can be explained by *E. coli*'s limited bacterial adhesion to the silk surface (Sharma, 2014). The thin peptidoglycan coating in gram negative bacteria could also be a possible reason. Gram positive bacteria may be more resistant to spider silk because they have a thick peptidoglycan coating and teichoic acids in their cell walls (Brown *et al*., 2013). The presence of compounds such as Gamma-aminobutyric acid (GABA) in the silk, which serves as a defense mechanism against natural enemies rather than as a silk byproduct, contributes to the bacteriostatic quality of the silk (Zhang *et al*., 2012). However, there are certain other studies thatcontradict our results. Silk inhibition was found to be more effective against gram positive bacteria than gram negative bacteria (Wright, 2011; Mirghani *et al*., 2012; Roozbahani *et al*., 2014; Al Kalifawi and Kadem, 2017). The linear relationship between the concentration of silk sample and the inhibition percentage corroborates with the study conducted by Tahir *et al*. (2018, 2019) in *Eriovixia excelsa* (Simon, 1889) where they used increasing concentrations of silk in sodium hydroxide (NaOH) solvent solution. Understanding the capabilities of spider silk has opened the door to a larger range of uses, including therapeutic ones, which are perhaps the most exciting prospects and call for further research.

Table 1 **Antimicrobial activity of egg sac silk of *Parawixia dehaani* against *B. subtilis* and *E. coli***

Bacteria	OD/ Inhibition	Concentration µl 20	40	60	80	100
B. subtilis	Final test OD	0.704	0.669	0.628	0.599	0.563
	Inhibition (%)	3.43	8.23	13.85	17.83	22.7
E. coli	Final test OD	0.998	0.948	0.876	0.844	0.778
	Inhibition (%)	5.13	9.89	16.73	19.77	26.05

Table 2 **Antimicrobial activity of web silk of *Pholcus phalangioides* against *B. subtilis* and *E. coli***

Bacteria	OD/ Inhibition	Concentration µl 20	40	60	80	100
B. subtilis	Final test OD	0.712	0.690	0.673	0.660	0.641
	Inhibition (%)	2.33	5.35	7.68	9.47	12.07
E. coli	Final test OD	1.008	0.990	0.972	0.922	0.873
	Inhibition (%)	4.18	5.89	7.60	12.36	17.02

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