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Contents

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Taxonomic study on praying mantids (Insecta: Mantodea) of Goodrical range forest, Kerala, India, with the description of a new species

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ABSTRACT: Mantid fauna of the forested tracts of different localities within the Goodrical forest range of the southern Western Ghats, Ranni forest division, Kerala, India were surveyed. During the Rapid Biodiversity Assessment (RBA) a total of 13 mantid specimens belonging to eight species under six families were collected. A new species *Caliris mukherjeei* sp. nov. (Haaniidae: Caliridinae) and two new records of rare mantids to Kerala viz., *Ceratomantis ghatei* Roy & Svenson, 2007 (Hymenopodidae: Oxypilinae) and *Dysaulophthalma nathani* Stiewe, 2009 (Eremiaphilidae: Iridinae) are reported with description and redescription.

KEY WORDS: Mantid fauna, new records, Caliridinae, Oxypilinae, Iridinae

INTRODUCTION

Goodrical range is the largest reserve forest under the Ranni forest division of Pathanamthitta district of Kerala, India. It is represented by mainly evergreen and semi-evergreen forests with an area of 505.967 km^2 situated in the eastern side of Pathanamthitta district adjacent to Periyar Tiger Reserve. Goodrical forest is well known for its scenic beauty and biodiversity and, due to its vicinity to the Periyar Tiger Reserve, it is the habitat for many wild animals of southern Western Ghats. Praying mantids are a group of ambush predatory insects which act as biocontrol agents. Currently, over 2400 species of mantises under 439 genera and 29 families are known worldwide (Schwarz and Roy, 2019). Based on the classification of Ehrmann and Roy (2002) 170 species, 71 genera in 11 families are currently reported from India (Mukherjee *et al*., 2014; Chatterjee *et al*., 2019). Mantid fauna of Western Ghats are not completely explored and many rare species were described earlier from the forested tracts of southern Western Ghats. For the Rapid Biodiversity Assessment (RBA) of the area, a faunal survey was conducted by Zoological Survey of India, with the joint participation of various organizations in Kerala, during the months of October – November, 2021. The present paper deals with the description of a new species of preying mantids and reports of some rare and interesting species from Goodrical range forest collected during the survey.

MATERIALS AND METHODS

The mantid specimens were collected from various localities of Goodrical range forest of Pathanamthitta district, Kerala, India during a RBA sponsored by

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Kerala Forest Department in collaboration with Zoological Survey of India. The area lies between 14° 45' and 15° 15' N latitude and 76° 45' and 77° 30' E longitude, at an altitudinal range of 100 to 1400 m (Menon, 2006). The specimens were collected by hand picking and sweep netting and preserved in ethanol (70%), then pinned, dried and examined under Labomed CZM6 Binocular Zoom Stereo-microscope, and the photographs were taken with Leica DFC 500 camera and Canon EOS M50 camera. Images taken at varying depths were stacked using Leica Auto Montage Software V3.80. The final illustrations were post-processed for contrast and brightness using Adobe® Photoshop® CS6 software. The specimens are deposited in the 'National Zoological Collections' of the Zoological Survey of India, Western Ghat Regional Centre, Kozhikode (ZSIK). The classification of Schwarz and Roy (2019) is followed in the manuscript and terminology mostly follows Brannoch *et al.* (2017). Various localities within the Goodrical range forest where the specimens collected are (Fig. A) —

1. Gavi: 9° 26' 10'' N, 77° 09' 57'' E Alt 1184 m
2. Kaattadikunnu: 9° 19' 16.4892'' N, 77° 7' 30.4788'' E Alt 1176 m
3. Kakki Dam site: 9° 19' 40'' N, 77° 08' 36'' E Alt 986 m
4. Kochupampa: 9° 23' 44'' N, 77° 09' 37'' E Alt 1017 m
5. Pannikunnu, Angamoozhi: 9° 19' 6.1032'' N, 77° 1' 45.0624'' E Alt 76 m

Abbreviations used: AvS - anteroventral spines; **DS -** discoidal spines; **F -** femur; **PvS -** posteroventral spines; **T -** tibia; **ZSIK -** National Zoological Collections of the Western Ghat Regional Centre, Zoological Survey of India, Kozhikode, Kerala, India.

RESULTS AND DISCUSSION

Family: Haaniidae; Subfamily: Caliridinae

Genus *Caliris* Giglio-Tos, 1915

Caliris Giglio-Tos, 1915. *Bull. Soc. Ent. Ital.*, 46: 82. Type species: *Iris masoni* Westwood, 1889.

Beesoniella Werner, 1935. *Proc. Zool. Soc. Lond.*, 498. Type species: *Beesoniella pallida* Werner, 1935.

Beesonula Uvarov, 1939. *Ann. Mag. Nat. Hist.*, 3(11): 458. (Preoccupied by *Beesoniella* Werner, 1935)

Diagnosis: Medium-sized, green body with round eyes. Lower frons transverse. Vertex arched, higher than eyes. Pronotum with oval supra-coxal dilation, Metazona finely keeled. Fore coxae as long as metazona, delicately toothed, with divergent forecoxal lobes. Fore femora with 4 posteroventral and 4 discoidal spines. Fore tibiae with 6 posteroventral spines. Hind metatarsus much longer than the other segments taken together. Fore wings sub-opaque. Hind wings with coloured patches in females; hyaline or smoky in males.

***1. Caliris mukherjeei* sp. nov. (Figs. 1-6)**

Diagnosis: Hind wings opaque, ivory coloured with two identical oval black-yellowish brown patches in discoidal area followed by 8-10 irregular black stripes towards anal area. Superior edge of fore coxae with 7-9 small spines and few spinules.

Description:

Female: *Body* (Figs. 1, 2). Medium-sized, green body in live, yellowish brown in dried condition.

Head (Fig. 3) - Eyes round. Vertex 4-grooved, juxta-ocular lobes round, prominent. Lower frons wider than high, superior edge arched in middle. Ocelli minute. Antennae simple.

Thorax (Fig. 1, 2) - Pronotum with granular disc, lateral edges denticulate, supra-coxal dilation oval; metazona at least two times longer than prozona; metazona highly constricted after dilation; prosternum with a black midline behind coxal junction.

Forelegs (Figs. 5, 6) - Fore coxae with 7-9 small spines on superior and inferior margins; fore coxal lobes divergent. Fore femora dorsally with long

groove, both anteriorly and posteriorly; 4 long posteroventral spines with sides and apex black, base with black spot; 4 discoidal spines, 3rd longest, all black at apex, 1st and 3rddorsally and ventrally with black base, 2nd ventrally with black base, 1st spine ventrally with a black dot; 14 anteroventral spines (6 long, 8 small), all black at apex, base of long spines ventrally with black dots, more clear in 4th and 5th long spines; tibial spur groove near base; genicular spur prominent. Tibiae with 6 posteroventral spines; 13 anteroventral spines; all tibial spines black at apex only, gradually increasing in size towards apex. Basitarsus longer than other segments taken together; black spot at distal part of tarsomeres gradually fade towards apex.

Spination formula of fore legs;

F= 4 DS/ 14 AvS/ 4 PvS. T= 13 AvS/ 6 PvS.

Mid and hind legs (Figs. 1, 2) - Simple, with femoral genicular spine. Meso-basitarsus a little shorter, meta-basitarsus a little longer than other segments taken together.

Abdomen (Figs. 1, 2) - Green. Cerci 17 segmented, highly ciliate.

Wings (Figs. 1, 2, 4) - Reach up to 4th abdominal segment. Fore wings green (in live), broad, opaque, costal and discoidal area highly reticulate; anal area yellowish green, lower discoidal area with yellowish brown round patch. Hind wings opaque, ivory colour; discoidal area with two identical oval patches that are with 60% black area and remaining part yellowish brown followed by 8-10 irregular black stripes towards anal area.

Male: Unknown. Beier

Material examined: Holotype 1 Female, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Kaattadikunnu, Coll. No. 24266, Lat9° 19' 16.4892'' N, Long 77° 7' 30.4788'' E, Alt 1176 m, 30. x. 2021, P. M. Sureshan and Party, ZSI/WGRC/ IR/INV 20259[ZSIK].

Distribution: India- Kerala.

Measurements (mm): Body length 41, Pronotum 11.2, Prozona 3.6, Metazona 7.6 Foreleg-Coxa 8.07, Trochanter 2.35, Femur 10.3, Tibia 5.7, Basitarsus 3.46, Other tarsal segments together 2.7, Mid/hind legs - Coxa 3.5/2.9, Trochanter 1.23/1.33, Femur 9.56/11.77, Tibia 7.74/10.16, Basitarsus 2.33/3.5, Other tarsal segments together 2.88/3.03, Forewing 16.42, Hindwing 13.61.

Etymology: This species is named after Tushar Kanti Mukherjee, in recognition of his valuable work on Indian Mantodea.

Remarks: Five species of mantids are currently present in the genus *Caliris*; *C. elegans* Giglio-Tos, 1915, *C. masoni* Westwood, 1889, *C. melli* Beier, 1933, *C. mukherjeei* **sp. nov.**, *C. pallens* Wang, 1993 and *C. pallida* (Werner, 1935). All species can be distinguished from each other by their colour pattern on the wings of females. Only *C. pallida* was reported earlier from Kerala which has yellow hind wings apically with two black patches distally with pinkish tint, nine series of 3-4 black spots concentrically arranged towards anal area (Fig. 7). Whereas, hind wings of *C. mukherjeei* **sp. nov.** are ivory coloured discoidal area is with two identical oval black yellow patches followed by 8-10 irregular black stripes towards anal area (Fig. 4). The new species exhibits close affinity to *C. masoni* which was reported from north-east India; especially in the shape of fore wings and the colour of the hind wings but not in the pattern of patches .

2. Caliris pallida (Werner, 1935) (Figs. 7, 8, 15)

Beesoniella pallida Werner, 1935. *Proc. Zool. Soc. Lond.*, 498.

Iris keralensis Vyjayandi, Narendran & Mukherjee, 2006. *Orient. Insects.*, 40: 285.

Caliris pallida (Werner, 1935). In: Schwarz & Roy, 2018. *Bull. Soc. Entomol. Fr*., 123(4): 455-456.

Brief redescription: Small, green mantis (in live) with round eyes. Vertex 4-grooved, juxta-ocular lobes of vertex round, prominent, with few hairs. Lower frons wider than high, superior edge angular in middle, disc smooth. Ocelli small. Pronotum short, supra-coxal dilation oval, lateral edges not

denticulate, but a little serrate, metazona longer than prozona, metazona a little constricted after dilation. Fore coxae shorter than metazona, upper margin with 4-5 small spines and few spinules; fore-coxal lobe divergent. Fore femora with 4 posteroventral spines, a pit in between first two proximal spines, apex and lateral sides black; 4 discoidal, all black at apex, first and third ventrally with a black spot at base; 14 anteroventral spines (6 long, 8 short), all black at apex, base of long spines ventrally with a black spot which is more visible on last three longer spines. Tibial spur groove near base. Fore tibiae with 6 posteroventral spines, a gap between first two proximal spines; 13 anteroventral spines gradually increase in length towards apex, all spines black at apex only. Fore basitarsus with a reddish tint, longer than other segments together; tarsomeres black at apex. Mid and hind legs simple, with femoral genicular spines; meso-basitarsus almost as long as and meta-basitarsus longer than other segments taken together. Macropterous, costal and adjacent area of fore wings reticulate, green, sub-opaque; other areas hyaline, earth brown in colour. Hind wings brown, hyaline; costal area with a little greenish tinge; anal area with a reddish tinge. Third and fourth abdominal segments dorsally brown.

Male genitalia: paa of left phallomere long, broad, hairy tip, curved upward; afa long, finger-like, posteriorly with minute denticles. sdpm of ventral phallomere serrated. Right phallomere narrow.

Material examined: 1 Male, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Pannikunnu, Angamoozhi, Coll. No. 24265, Lat 9° 19' 6.1032'' N, Long 77° 1' 45.0624'' E, Alt 76 m. 30. x. 2021, P. M. Sureshan and party, ZSI/WGRC/IR/INV 19452 [ZSIK].

Measurements (mm): Body length 26.7, Pronotum 8, Prozona 2.5, Metazona 5.4, Foreleg-Coxa 4.6, Trochanter 1.7, Femur 7.2, Tibia 3.6, Basitarsus 2.67, Other tarsal segments together 2.13, Mid/hind legs-Coxa 1.9/2.37, Trochanter 0.93/0.86, Femur 8.05/9.16, Tibia 5.9/8.8, Basitarsus 1.9/3.2, Other tarsal segments together 2.34/2.58, Forewing 18.25, Hindwing 15.17.

Distribution: India; Kerala (Vyjayandi *et al.*, 2006), Tamil Nadu (Mukherjee *et al.*, 2017).

Remarks: In 2006, Vyjayandi *et al.* had described a new species named *Iris keralensis* which was later re-designated as *C. keralensis* by Stiewe (2009) and it was recently synonymized with *C. pallida* (Schwarz & Roy, 2018).

Family: Gonypetidae; Subfamily: Iridopteryginae; Tribe: Amantini

Genus *Amantis* Giglio-Tos, 1915

Amantis Giglio-Tos, 1915. *Bull. Soc. Ent. Ital.*, 46: 151. Type species: *Mantis (Oxypilus) reticulata* De Haan, 1842.

Cimantis Giglio-Tos, 1915. *Bull. Soc. Ent. Ital.*, 46: 154. Type species: *Cimantis fumosa* Giglio-Tos, 1915.

Shirakia Beier, 1935. *Gen. Ins.*, 47. Type species: *Gonypeta maculata* Shiraki, 1911.

Diagnosis: Small, bark coloured body. Lower frons slightly wider than high, upper edge feebly round. Antennae ciliate in males. Pronotum short, diamond shaped. Metazona a little longer than prozona. Fore femora with 4 posteroventral, margins granulated, and 4 discoidal spines, not arranged in a row. Hind metatarsus longer than other segments taken together. Costal margin of fore wing ciliated in males.

***3. Amantis saussurei* (Bolivar, 1897) (Fig. 9, 16)**

Iridopteryx saussurei Bolivar, 1897. *Ann. Soc. Ent. Fr.*, 66: 305.

Amantis saussurei (Bolivar, 1897). In: Giglio-Tos, 1927. *Tierreich* 50:171.

Brief redescription: Small, brownish body with scattered small black dots. Eyes round. Vertex with scattered black patches. Lower frons about as long as wide, with two black dots on either side; superior edge arched in middle; a long black midline end at labrum. Ocelli pale yellowish, round, prominent. Antennae ciliate. Pronotum short, with a median

black line; supra-coxal dilation prominent; metazona, longer than prozona, posteriorly with two robust tubercles in middle. Fore coxa longer than pronotum; edges with ciliated spinules. Fore femora with 4 posteroventral, 4 discoidal, 10-11 anteroventral spines. Tibial spur groove a little proximal to middle. Fore tibia with 10 posteroventral, 11 anteroventral spines. Basitarsus anteriorly brown, posteriorly black; all other tarsomeres black at posterior end. Mid and hind legs simple. Wings smoky brown; anterior edge of forewings ciliate; stigma with a black dot.

Male genitalia: paa of left phallomere finger-like, curved upward; afa long, narrow. sdp sclerotized; sdpm triangular with pointed tip; sdpl round, with minute spines. pva of horse shoe-shaped, posteriorly sclerotized; pia small with minute tubercles.

Materials examined: 1 Male, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Pannikunnu, Angamoozhi, Coll. No. 24265, Lat 9° 19' 6.1032'' N, Long 77° 1' 45.0624'' E, Alt 76 m. 30. x. 2021, P. M. Sureshan and Party, ZSI/WGRC/IR/INV 19451 [ZSIK]. 1 Male, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Gavi, Coll. No. 24264, Lat 9° 26' 10'' N, Long 77° 09' 57'' E, Alt 1184 m, 01. xi. 2021, P. M. Sureshan and Party, ZSI/WGRC/IR/INV 19453 [ZSIK].

Measurements (mm): Body length 17.5, Pronotum4.9, Prozona 2.1, Metazona 2.8, Foreleg-Coxa 5.3, Trochanter 1.6, Femur 5.8, Tibia 2.8, Basitarsus 3.2, Other tarsal segments together 2.7, Mid/hind legs-Coxa 3.0/2.8, Trochanter 0.95/0.93, Femur 7.2/8.4, Tibia 5.7/8.8, Basitarsus 2.1/3.7, Other tarsal segments together 2.4/3.0, Forewing 13.54, Hindwing 11.81.

Distribution: India- Andhra Pradesh (Mukherjee *et al.*, 1995), Chhattisgarh (Majumder *et al.*, 2015), Goa (Vyjayandi *et al.*, 2010), Karnataka (Mukherjee *et al.*, 2014), Kerala (Mukherjee *et al.*, 1995), Maharashtra (Ghate *et al.*, 2012), Tamil Nadu (Bolivar, 1897).

Remarks: This species was first reported as *Iridopteryx saussurei* from Madurai and Kodaikanal, Tamil Nadu, India by Bolivar (1897). In 1904, Kirby transferred the species to the genus *Gonypeta* and finally the genus *Amantis* (Giglio-Tos, 1927).

Family: Hymenopodidae;
Subfamily: Hymenopodinae;
Tribe: Anaxarchini

Genus *Anaxarcha* Stål, 1877

Anaxarcha Stål, 1877. *Bih. K. Svenska. Vetensk. Akad. Handl.*, 4(10): 81. Type species: *Anaxarcha graminea* Stål, 1877.

Anaxandra Kirby, 1904. *Syn. Cat. Orth.*, 1: 223. Type species: *Anaxandra grammica* Stål, 1877.

Parastatilia Werner, 1922. *Zool. Mededeel. Mus. Leiden.*, 7: 119. Type species: *Parastatilia pulchra* Werner, 1922.

Diagnosis: Round eyes. Lower frons transverse, with two lateral carinae, superior angle with a projecting point. Vertex simple with prominent juxta-ocular lobes. Pronotum slender, denticulate borders, slightly longer than fore coxae, with oval supra-coxal dilation. Fore coxae with small marginal spines. Fore femora with 4 posteroventral and 4 discoidal spines. Fore tibiae with densely placed posteroventral spines. Mid and hind legs simple. Fore wings narrow, green, mostly with yellow costal stripes. Hind wings coloured.

4. Anaxarcha limbata Giglio-Tos, 1915 (Fig. 10, 17)

Anaxarcha limbata Giglio-Tos, 1915. *Boll. Musei Zool. Anat. Comp. R. Univ. Torino.*, 30(702): 1.

Oligomantis parallela Werner, 1930. *Ark. Zool.*, 21A (34): 6.

Brief redescription: Medium-sized, greenish body. Eyes round. 4-grooved vertex with prominent juxta-ocular lobes. Lower frons transverse, superior mid angle with a projecting point. Pronotum slender, constricted after dilation; lateral borders black, denticulate; oval supra-coxal dilation; metazona at least 2.5 times longer than prozona. Fore coxa shorter than pronotum. Fore femora with 4 posteroventral, 4 discoidal, 14 anteroventral spines.

Fore tibia with closely placed, decumbent posteroventral spines, 14 anteroventral spines. Fore basitarsus longer than other segments taken together. Mid and hind legs simple, slender. Fore wings narrow, greenish, sub-opaque, densely reticulated. Hind wings hyaline, costal area green, other areas with pinkish veins and veinlets. Abdomen dorsally pink, ventrally green.

Male genitalia - paa of left phallomere short, broad; afa sclerotized, round. sdp on ventral phallomere absent. fda of right phallomere with hairy apex; pva horse shoe-shaped, sclerotized, curved upward.

Material examined: 1 Male, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Kochupampa, Coll. No. 24267, Lat 9° 23' 44'' N, Long 77° 09' 37'' E, Alt 1017 m, 31. x. 2021, P. M. Sureshan and Party, ZSI/WGRC/IR/INV 19450[ZSIK].

Measurements (mm): Body length 28.4, Pronotum 7.5, Prozona 2.1, Metazona 5.5, Foreleg-Coxa 4.7, Trochanter 1.1, Femur 6.2, Tibia 2.5, Basitarsus 2.4, Other tarsal segments together 1.7, Mid/hind legs-Coxa2.6/2.3, Trochanter 0.53/0.56, Femur 5.9/6.4, Tibia 4.0/6.0, Basitarsus 1.5/2.7, Other tarsal segments together 1.7/2.1, Forewing 21.5, Hindwing 18.

Distribution: India; Arunachal Pradesh (Mukherjee *et al.*, 1995), Kerala (Mukherjee *et al.*, 1995), Meghalaya (Mukherjee *et al.*, 1995); Borneo (Giglio-Tos, 1915), Indonesia, Malaysia (Beier, 1937), Thailand (Mukherjee *et al.*, 2014).

Remarks: First Indian record of this species was from Kerala in 1995. This species shows closer affinity to *A. graminea* Stål, 1877 than *A. acuta* Beier, 1963 (Mukherjee *et al*., 1995).

Subfamily: Oxypilinae; Tribe: Oxypilini

Genus *Ceratomantis* Wood-Mason, 1876

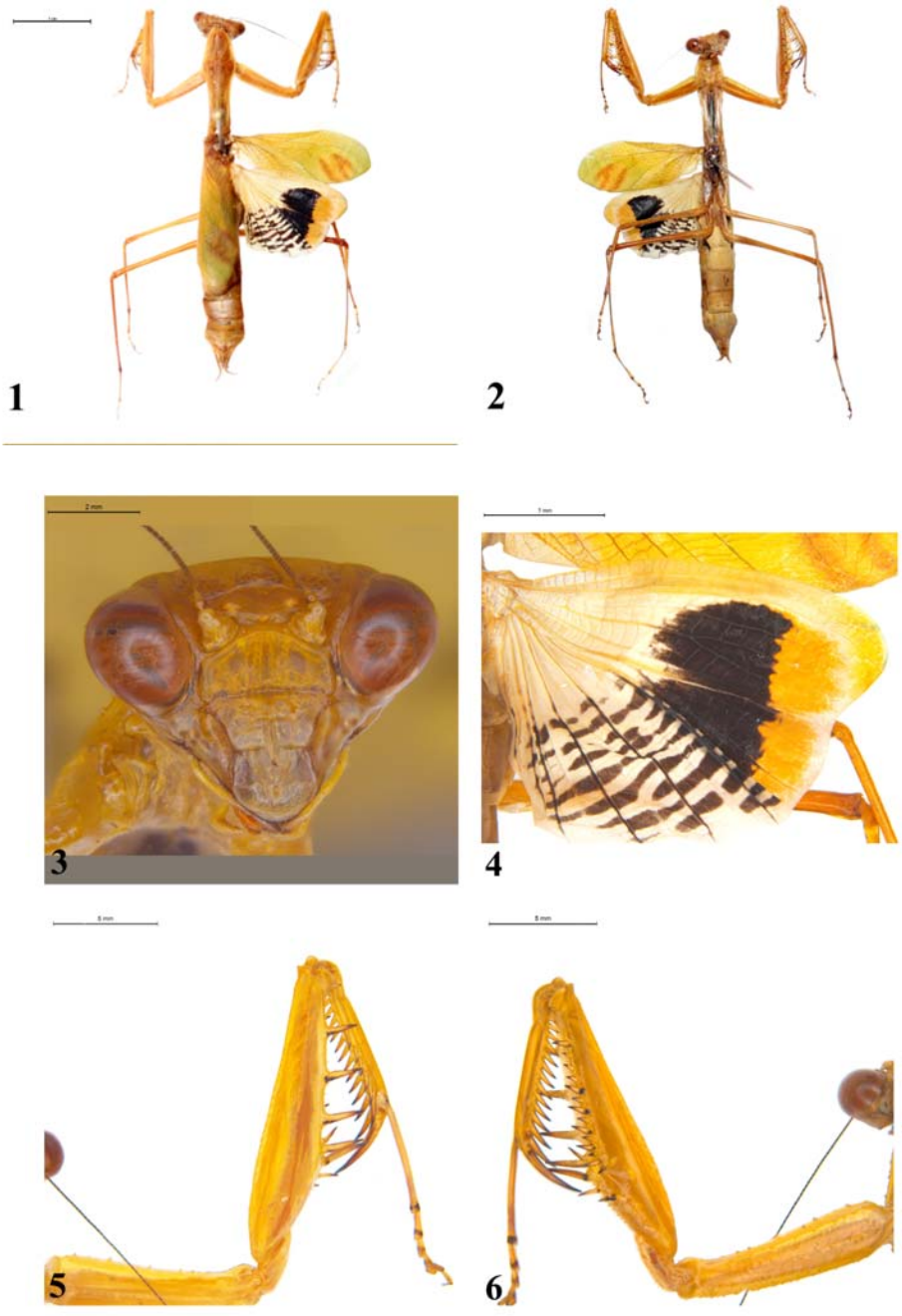
Ceratomantis Wood-Mason, 1876. *Bull. Soc. Entomol. Fr*., 112(4): 175. *Ceratomantis saussurii* Wood-Mason, 1876.

Diagnosis: Body small, eyes round. Lower frons transverse, pentagonal, upper margin with a point in the middle. Vertex with a long, conical tubercle above the ocelli. Prominent juxta-ocular lobes. Pronotum short, strongly arched, with prominent dilation. Metazona almost as long as prozona, each with a pair of conical tubercles. Lateral borders of pronotum with long tubercles. Fore femora dilated, with 4 posteroventral and 4 discoidal spines. Mid and hind legs simple. Wings longer than abdomen in males, shorter in females.

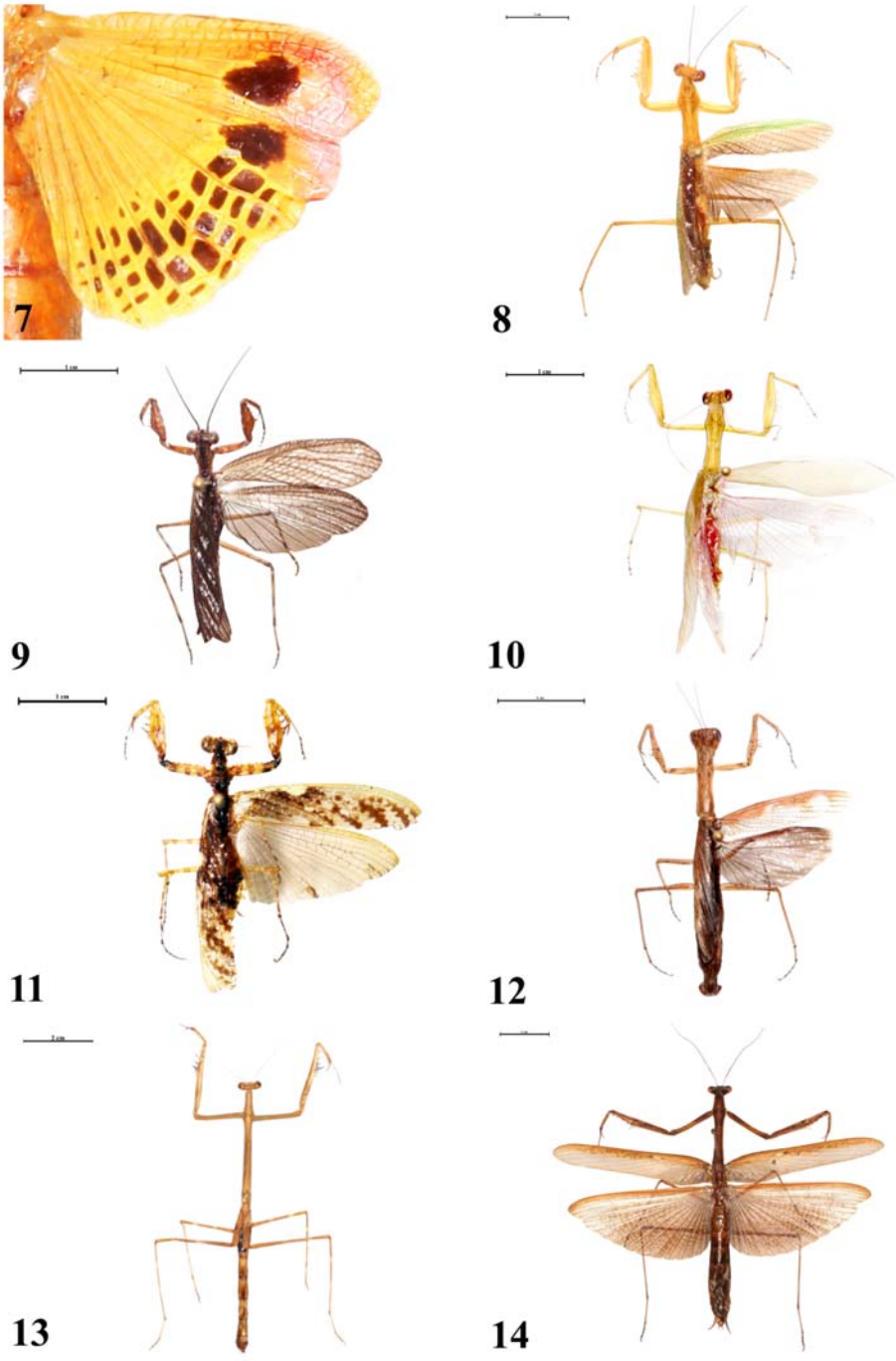
***5. Ceratomantis ghatei* Roy & Svenson, 2007 (Fig. 11, 18)**

Ceratomantis ghatei Roy & Svenson, 2007. *Bull. Soc. Entomol. Fr*., 112(4): 433.

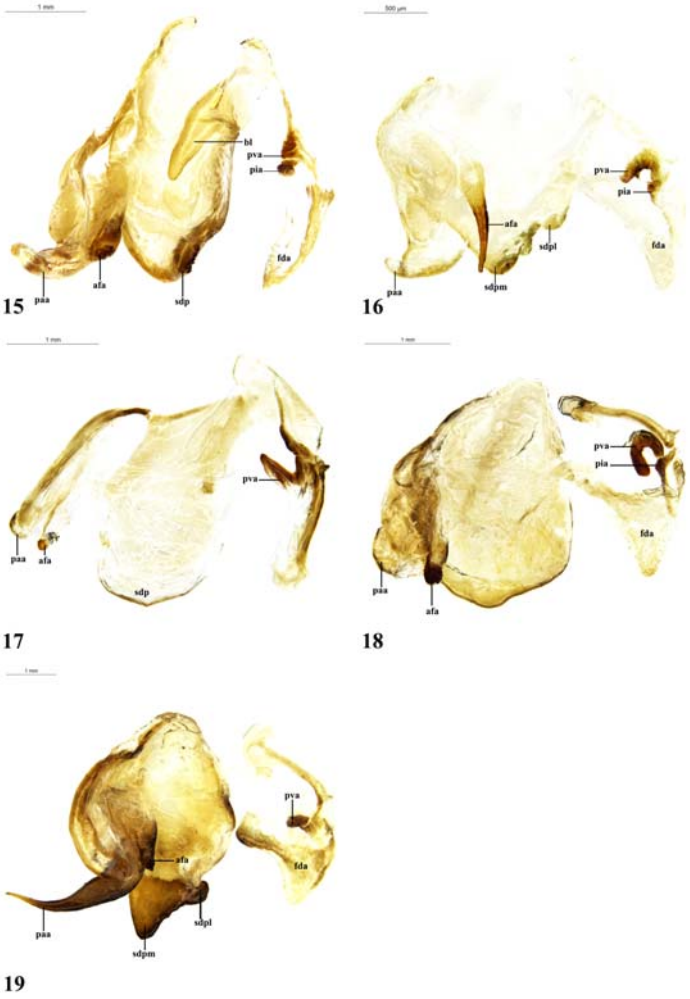
Brief redescription: Medium-sized body. Round, bulging eyes. Lower frons pentagonal with a tubercle. Process of vertex bifid at terminus with two strikingly pronounced carinae; mid and basal lateral projections pronounced, diverging strongly from process. Juxta-ocular lobes prominent with a strong tubercle. Pronotum short, robust, tuberculated with a pair of dorsally projecting spines each on prozona and metazona; lateral edges tuberculate; supra-coxal dilation spinous; prozona almost as long as metazona. Superior margin of fore coxa with strong denticles, inferior margin with many tubercles; internal fore coxae apically with black patch around small pale spot on lower side which continues to trochanter. Fore femora with superior hump near apex; 4 posteroventral, 4 discoidal, 9-10 anteroventral spines; tibial spur groove near base; all longer anteroventral spines completely brown, all other femoral spines black at apex only. Fore tibiae with 11 posteroventral, 7 anteroventral spines. First and second tarsomeres brownish with a pale band in distal half, apically black; all other tarsomeres completely black. Femora of mid and hind legs with sub-apical lobe. Abdomen brown with all lateral lobes pale tan except on third segment. Fore wings with greenish costal area; discoidal area mostly brown with scattered hyaline spots; base and area proximal to middle with a whitish, opaque patch. Hind wings hyaline; costal area green.



Figs. 1-6 *Caliris mukherjeei* Kamila & Sureshan sp. nov. 1. Dorsal view, 2. Ventral view, 3. Head frontal view, 4. Hind wing dorsal view, 5. Fore leg dorsal view, 6. Fore leg ventral view



Figs. 7-14: 7. *Caliris pallida* (Werner, 1935) female hind wing dorsal view, 8. *Caliris pallida* (Werner, 1935) male, 9. *Amantis saussurei* (Bolivar, 1897), 10. *Anaxarcha limbata* Giglio-Tos, 1915, 11. *Ceratomantis ghatei* Roy & Svenson, 2007, 12. *Dysaulophthalma nathani* Stiewe, 2009, 13. *Indomenella indica* (Ghate & Mukherjee, 2004), 14. *Statilia maculata* (Thunberg, 1784)



Figs. 15-19 Male genitalia Dorsal view. 15. *Caliris pallida* (Werner, 1935), 16. *Amantis saussurei* (Bolivar, 1897), 17. *Anaxarcha limbata* Giglio-Tos, 1915, 18. *Ceratomantis ghatei* Roy & Svenson, 2007, 19. *Statilia maculata* (Thunberg, 1784)

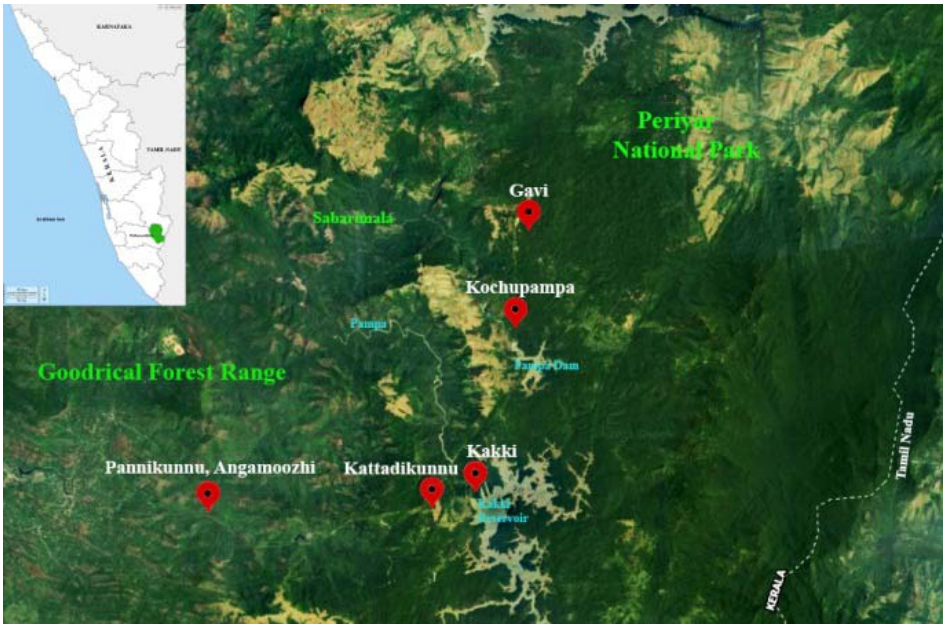
Male genitalia: paa broad, semi-circular with a median groove. afa globular with granules. Ventral phallomere terminating with a widely rounded structure. fda of right phallomere posteriorly with hair-like structures; pva sclerotized, cane-shaped; pia small, sclerotized with granules.

Material examined:1 Male, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Kakki dam site, Lat 9° 19' 40'' N, Long 77° 08' 36'' E, Alt 986 m, 31. x. 2021, Jafer Palot, ZSI/WGRC/IR/INV 19457[ZSIK].

Measurements (mm): Body length 25.3, Pronotum 4.5, Prozona 2, Metazona 2.5, Foreleg-Coxa 5, Trochanter 1.3, Femur 5.9, Tibia 1.9, Basitarsus 2.6, Other tarsal segments together 2.1, Mid/hind legs-Coxa 2.3/1.9, Trochanter 0.5/0.7, Femur 4.9/5.6, Tibia 4.2/4.7, Basitarsus 2.1/2.4, Other tarsal segments together 2.8/3.1, Forewing 20.1, Hindwing 17.9.

Distribution: India- Kerala (New record), Karnataka (Roy and Svenson, 2007).

Remarks: This is the first report of *C. ghatei* from Kerala. This species was described by Roy and Svenson with only one male specimen from Agumbe forest of Karnataka, India in 2007. Female unknown.



Area of specimens collection: Gooderical range forest, Pathanamthitta, Kerala

Family: Eremiaphilidae; Subfamily: Iridinae; Tribe: Dysaulini

Genus *Dysaulophthalma* Stiewe, 2009

Dysaulophthalma Stiewe, 2009. *Ent. Mon. Mag.*, 145: 52. Type species: *Dysaulophthalma nathani* Stiewe, 2009.

Diagnosis: Slender body with oblong eyes. Lower frons trapezoidal. Height of vertex as same as eyes. Pronotum with serrated margin. Fore femora with 4 posteroventral, first spine larger than others, and 4 discoidal spines. Metathorax and first 4 abdominal tergites metallic- black in colour. Fore wing with brown or orange colouration. Hind wing without any colouration.

***6. Dysaulophthalma nathani* Stiewe, 2009 (Fig. 12)**

Dysaulophthalma nathani Stiewe, 2009. *Ent. Mon. Mag.*, 145: 51.

Brief redescription: Medium-size, body brown with scattered brown patches. Eyes oval. Vertex of same height as eyes, juxta-ocular lobes inconspicuous. Lower frons transverse, wider than high, disc smooth. Pronotum long, somewhat rectangular, lateral margins denticulate; supra-coxal dilation oval; prozona short; metazona a little constricted behind dilation. Fore coxae dorsally with two black parallel patches near apex, ventrally with 4-5 dark granules, ventral posterior apical lobe with black patch, under side of coxa with a black midline. Trochanter dorsally with a black spot and hairs. Fore femora with 4 posteroventral, 4 discoidal and 10-12 anteroventral spines; superior border slightly concave near apex; tibial spur groove proximal to middle. Fore tibiae with 7-8 posteroventral and 8-10 anteroventral spines. Mid and hind legs simple. Wings shorter than abdomen. Fore wings anterior half brown, opaque, medially and distally with two hyaline areas; posterior half hyaline with brown veins. Hind wings smoky, sub-hyaline. First 4 abdominal tergites metallic black, others brown.

Measurements (mm): Body length 28.7, Pronotum 7.2, Prozona 2, Metazona 5.2, Foreleg- Coxa 3.5, Trochanter 0.98, Femur 4.4, Tibia 2.6, Basitarsus 2.2, Other tarsal segments together 2.4, Mid/hind legs- Coxa 1.8/1.6, Trochanter 0.65/0.6, Femur 3.7/5.9, Tibia 3.6/5.9, Basitarsus 1.0/1.4, Other tarsal segments together 2/2.65, Forewing 14.7, Hindwing 12.1.

Material examined:1 Female, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Kochupampa, Coll. No. 24267, Lat 9° 23' 44'' N,

Long 77° 09' 37'' E, Alt 1017 m, 31. x. 2021, P. M. Sureshan and Party, ZSI/WGRC/IR/INV 19493[ZSIK].

Distribution: India- Kerala (New record), Tamil Nadu (Stiewe, 2009).

Remarks: The genus *Dysaulophthalma* is endemic to South India. This species is reported for the first time from Kerala since its original description from Anamalai hills, Tamil Nadu, which was collected in 1959. Male unknown.

Family: Deroplatyidae; Subfamily: Deroplatyinae; Tribe: Euchomenellini

Genus *Indomenella* Roy, 2008

Indomenella Roy, 2008. *Bull. Soc. Entomol. Fr.*, 113(3): 330. Type species: *Euchomenella indica* Ghate & Mukherjee, 2004.

Diagnosis: Long, slender body with round, bulging eyes. Lower frons wider than high. Vertex straight. Pronotum much longer than fore coxae, lateral margins denticulated. Superior edge of fore coxae with small spines. Fore femora with 4 posteroventral and 4 discoidal spines. Females brachypterous, males macropterous.

***7. Indomenella indica* (Ghate& Mukherjee, 2004) (Fig. 13)**

Euchomenella indica Ghate & Mukherjee, 2004. *Genus.*, 15(3): 329.

Indomenella indica (Ghate & Mukherjee, 2004). In: Roy, 2008. *Bull. Soc. Entomol. Fr.*, 113(3): 330.

Brief redescription: Long, slender, body brown. Head wider with round, bulging eyes. Vertex 4-grooved with indistinct juxta-ocular lobes. Lower frons transverse, superior edge arched in middle. Pronotum long, slender, lateral edges serrate; anterior and posterior ends with two small mid lobes; oval supra-coxal dilation; metazona much longer than prozona; two small pits on either side of anterior metazona. Fore coxae shorter than metazona with spinulate edges; convergent fore coxal lobe. Fore trochanter ventrally with few granules. Fore femora ventrally with three distinct brown bands in apical half; spines distally placed; 4 posteroventral, 4 discoidal, 15 anteroventral spines (7 long, 8 short); tibial spur groove a little distal to middle. Fore tibiae with 8 posteroventral spines, all unequal in length; 14 anteroventral spines. Fore basitarsus a little longer than other segments taken together; tarsomeres dorsally black at apex; ventrally completely black. Mid and hind legs simple. Wings brachypterous; fore wings opaque, brown; costal area green; stigma with veinless dark brown patch; anal area with brown spots; ventrally reddish brown with a long black line in lower half of costal area. Hind wings metallic black except brown base; lower half with 12-14 hyaline non-continuous stripes.

Materials examined: 2 Females, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Pannikunnu, Angamoozhi, Coll. No. 24265, Lat 9° 19' 6.1032'' N, Long 77° 1' 45.0624'' E, Alt 76 m, 30. x. 2021, Dr. P.M. Sureshan and Party, ZSI/WGRC/IR/INV 19491-19492[ZSIK].

Measurements (mm): Body length 72.7, Pronotum 31.2, Prozona 6.2, Metazona 25, Foreleg-Coxa 14.8, Trochanter 3.1, Femur 16.4, Tibia 6, Basitarsus 3.8, Other tarsal segments together 3.6, Forewing 11.2, Hindwing 7.8.

Distribution: India; Kerala (Ghate and Mukherjee, 2004), Tamil Nadu (Mukherjee *et al.*, 2017).

Remarks: This species is very common in forested areas of Kerala and looks like slender twigs and branches. When disturbed, they stiffen their body by laying forelegs along the body and sham death for a long time.

Family: Mantidae; Subfamily: Mantinae

Genus *Statilia* Stal, 1877

Statilia Stal, 1877: 36. Type species: *Pseudomantis nemoralis* Saussure, 1870.

Diagnosis: Lower frons transverse, with arched or a little angular superior edge. Vertex smooth. Pronotum slender, lateral edges denticulated, with pronounced supra-coxal dilation. Fore coxae serrated. Fore femora with 4 posteroventral and 4 discoidal spines. Claw groove placed distally. Mid and hind femora without apical spines. Hind

metatarsus slightly longer than other segments taken together.

8. Statilia maculata (Thunberg, 1784) (Fig. 14, 19)

Mantis maculata Thunberg, 1784. *Nov. Ins. Spec.*, 3: 61.

Mantis orientalis Saussure, 1870. *Mitt. Schweiz. Ent. Ges.*, 3: 233.

Pseudomantis haani Saussure, 1871. *Mem. Soc. Hist. Nat. Geneve.*, 21: 185.

Statilia maculata var. *Hyalina* Giglio-Tos, 1927. *Das Tierreich.*, 50: 411.

Brief redescription: Medium-sized body with scattered black patches. 4-grooved vertex with black long patch, juxta-ocular lobes indistinct. Eyes round. Ocelli round, prominent. Lower frons wider than high, superior edge angular in middle, inferior edge arched. Pronotum long, slender, lateral margins denticulate. Prosternum with black patch at coxal joint area. Fore coxae ventrally with 6-7 small triangular whitish spines and few spinules on superior margin; internally with black patch near base. Fore femora with 4 posteroventral, 4 discoidal, 14 anteroventral spines (6 long, 8 short); all spines black at apex only; internally with shining yellow patch in tibial spur groove followed proximally with a black patch, distally with a small black patch. Bases of anteroventral spines with a black line which extend to apex of fore femora. Fore tibiae with 7 posteroventral, 11 anteroventral spines. Mid and hind legs simple, without femoral genicular spine. Wings reach beyond abdomen. Fore wings highly reticulate; costal area opaque, pale brown, anteriorly with a black line, posteriorly with few brown patches; other areas hyaline; lower part of anal area with small brown patches; stigma present. Hind wings with opaque, reddish brown costal area; discoidal area highly reticulate, with brown patch at apex; other areas hyaline, black with scattered hyaline patches and stripes.

Male genitalia - paa of left phallomere very long, curved leftwards, base broad, gradually become narrow towards apex. afa large with a pointed tip. sdp of ventral phallomere broad, strongly sclerotized; sdpm triangular; sdpl hook-shaped. pva of right phallomere sclerotized, apex paddle-shaped, curved upward; pia small with a narrow groove.

Materials examined: 2 Males, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Kochupampa, Coll. No. 24267, Lat 9° 23' 44'' N, Long 77° 09' 37'' E, Alt 1017 m, 31. X. 2021, P. M. Sureshan and Party, ZSI/WGRC/IR/INV 19454-19455 [ZSIK].1 Male, INDIA, Kerala, Pathanamthitta district, Goodrical range forest, Kakki dam, Lat 9° 19' 40'' N, Long 77° 08' 36'' E, Alt 986 m, 1. Xi. 2021, Jafer Palot, ZSI/WGRC/IR/INV 19456 [ZSIK].

Measurements (mm): Body length 50.4, Pronotum 15.4, Prozona 3.9, Metazona 11.5, Foreleg-Coxa 10.7, Trochanter 3.2, Femur 13, Tibia 4.9, Basitarsus 4.2, Other tarsal segments together 3.1, Forewing 34.6, Hindwing 30.5.

Distribution: India; Andaman Island (Srinivasan *et al.*, 2017), Andhra Pradesh (Mukherjee *et al.*, 1995), Arunachal Pradesh (Mukherjee *et al.*, 1995), Assam, Bihar (Sureshan and Sambath, 2009), Chhattisgarh (Majumder *et al.*, 2015), Himachal Pradesh, Kerala (Vyjayandi, 2007), Madhya Pradesh, Maharashtra (Ghate *et al.*, 2012), Meghalaya, Odisha (Sureshan *et al.*, 2006), Sikkim (Mukherjee *et al.*, 1995), Tamil Nadu (Mukherjee *et al.*, 2017), Telangana, Uttarakhand (Werner, 1935), Uttar Pradesh, West Bengal (Mukherjee *et al.*, 1995).

Borneo; China, Indonesia (Saussure, 1871), Japan (Thunberg, 1784), Labuan, Laos, Malaysia, Myanmar, Nepal, New Guinea, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam.

Remarks: These mantids are commonly seen on grasses and bushes and exist in brown and green morphs.

The study added three more species to the list of mantid fauna of Kerala state which updates the species list to 52 species under 30 genera and 13 families. Present study is a result of the Rapid Biodiversity Assessment (RBA) survey of Goodrical forest range of Ranni Forest Division,

Pathanamthitta district, Kerala conducted to document the biodiversity of the area and to suggest conservation management measures for the area and document the endangered, threatened and endemic species. During the field survey, 13 mantid specimens of eight species were collected from the area, which include a new species to science and two new records to Kerala state. The study points to the fact that the forested areas of Goodrical range are rich in faunal diversity evidenced by the record of some rare species of preying mantids and other invertebrates.

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Fourier transform infra-red spectrochemical analyses of Pieridae butterfly wings

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ABSTRACT: Fourier transform infrared (FTIR) spectroscopic technique was carried out in wings of nine different Pierid butterflies to deduce the functional groups and properties of cuticular hydrocarbons which play a pivotal role in exhibiting charismatic colour patterns, controlling body temperature, attracting potential mates and camouflaging against predators. There were three major types of hydrocarbons present in all butterfly wings including alkanes, alkenes and methyl hydrocarbons. The second predominant compounds found in the butterfly wing region include alkyl halides, alcohols and phenols. There were no significant differences in the functional groups among the wings of butterflies. FTIR analysis of nine different Pierid butterfly wings showed many relative sizes of peaks. There were no significant differences in the chemical composition of wings but the colour differences were observed among the nine different butterflies. It was inferred that not majorly due to the differences in the cuticular hydrocarbons and may be due to the presence of different microstructures like scales, ridges and grooves.

KEY WORDS: Cuticular hydrocarbons, microstructures, functional groups, pterins

INTRODUCTION

Butterflies are celebrated widely because of their aesthetic appeal and are often considered as a flagship group of insects in the field of insect biology and conservation (Aarti and Arya, 2021; Kunte *et al*., 2020). The majority of butterflies have pigmented phenotypes. The color patterns exhibited among butterflies are classified as structural colors and pigmented colors (Kaspar *et al*., 2019). Colorations of butterfly wings are due to the presence of microstructures and specific pigments. These pigments appeared colorful because they absorb and reflect light in a certain wavelength, and the remaining is dissipated as heat (Sharmila *et al*., 2020; Shamim *et al.*, 2014). This phenotypic plasticity among butterflies has led to many interesting studies. Butterfly wings are intensively studied by many researchers because of their charismatic colour, hydrophobic behaviour, wettability, fluorescence, self-cleaning property, and also for their flexibility and lightness (Devi *et al*., 2021, Krishna *et al*., 2020, Kaspar *et al*., 2019).

Pieridae is one of the largest families under Lepidioptera which consist of more than 1100 species. Pierid butterflies exhibit sexual dichroism (Giraldo and Stavenga, 2006).Incident light on the scale creates a thin film interference, which changes the colour of the reflected light. Based on the information gained from the Atomic Force Microscopic images of scales, it is known that colour of the reflected light depends upon the periodic arrangements of ridges (Kaspar *et al*., 2019).

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Scanning Electron Microscopic analysis of yellow and white wing scales of Pierid butterflies has shown the presence of densely decorated ovoid beads in their cross ribs (Giraldo, 2008). The presence of beads on the cross ribs is one of the general characteristics of Pierid butterflies.

Fourier transform infrared (FTIR) spectroscopic technique can be used to study the spectrochemical analysis of diverse materials ranging from small molecules to supra molecular structures (Xu and Gowen, 2021).Structural features can be analyzed by using FTIR which is based on the inter and intra molecular interactions of functional groups present in that molecule in all aggregation states (Kannan *et al.*, 2020; Kamnev et *al.*, 2021). Many studies using FTIR have been done on biological samples such as live cells, tissue samples, body fluids (Baker *et al*., 2014), and also in tissues of certain disorders like myopathies and brain tumours (Petibois *et al*., 2009), body fluid traces (Mistek and Lenev, 2018). The biochemical information provided by FTIR will be used for diagnosis and forensic purposes. Only few studies of FTIR have been done on arthropods like honeybees, dragonflies (Machovic *et al*., 2017). Very few reports are available on FTIR analysis in butterflies (Tian *et al.*, 2015, Krishna *et al*., 2020). An attempt has been made in the spectrochemical analysis of various functional groups present in the wings of different Pierid butterflies by using the FTIR technique.

MATERIALS AND METHODS

Butterflies of the family Pieridae were collected from four regions around Madurai, Tamilnadu, India namely Alagar Hills Reserve Forest (10°04’39.0"N; 78°12’60.0"E), Sirumalai Hills Reserve Forest (10°11’01.1"N; 77°59’49.9"E), Kiluvamalai Reserve Forest (10°04’18.1"N; 78°10’11.0"E) and The American College campus (9°55’46.1"N; 78°08’01.3"E). The specimens of Pierid butterflies were collected with the help of insect net and hand picking during the month of December 2018 to September 2019. The collected specimens were stretched on stretching board, and then transferred to the insect boxes. The collected butterflies were preserved by mixing phenol and camphor (3:1 ratio). The cotton was dipped in the phenol- camphor mixture and kept in four corners of the insect box. Identification was done on the basis of literature and keys. Identified species of Pierid butterflies were labelled properly. Nine species of Pieridae butterflies were selected for the study which included *Eurema brigitta*, *Catopsilia pyranthe, Colotis danae*, *Pareronia valeria, Appias lyncida, Pareronia ceylanica, Colotis etrida, Eurema hecabe* and *Ixias pyrene.*

The study focused on spectrochemical analysis of Pierid butterflies by using FTIR technique. The forewings of nine butterflies were chosen for this study. The functional groups in the wings were identified by Fourier Transform Infrared spectroscopy (FT-IR Model- Thermo fisher 380). The biomolecules in butterfly wings are characterized at room temperature. The FTIR spectrum was obtained in the mid IR Region of 400 – 4000 cm^{-1}. With potassium bromide crystals, the wings of the butterflies were directly placed and the spectrum was recorded (Sackey *et al*., 2018). The recorded readings were plotted in the graph using Origin 2021b software (version - 9.85).

RESULTS AND DISCUSSION

The spectrochemical analyses of different regions of nine Pierid butterflies revealed the cuticular hydrocarbons. The selected wing regions of nine Pierid butterflies for FTIR analysis includes apical forewing region of *C. nerissa,* basal forewing region of *C. pyranthe*, discal forewing region of *P. valeria,* basal forewing region of *P. ceylanica,* apical forewing region of *C. danae*, discal forewing region of *E. hecabe*, discal forewing region of *A. lyncida*, basal forewing region of *C. etrida* and discal forewing region of *I. pyrene*. Different regions of wings were selected based upon their different colors. (Table 1; Fig. 1, 2, 3, 4 and 5). FTIR measurement of nine different butterflies of the Pieridae family was carried out to find the chemical composition spectra.

Alkanes are saturated hydrocarbons, characterized by absorption due to C-H Stretching and bending. The structure of n-alkane with a longer hydrocarbon chain allows the close packing of molecules that

acts as a barrier to water and well suited for hydrophobic property (Drijfhout *et al*., 2009). Alkanes are the most predominant hydrocarbon present in all the selected butterflies. In the present study, in *C. nerissa*, the observed absorbance spectra bands at 1339, 2967, 2915, 2836, 1374 and 1339 cm^{-1} were assigned to be alkanes. The absorbance spectral values of alkanes in P. valeria correspond with 2959, 2847, 1464 and 1377 cm^{-1}. In *P. ceylanica*, the absorbance spectra band at 2966, 2901, 1436, and 1351 cm^{-1} were assigned to be alkanes. In *C. danae*, the spectra bands at 2953 and 2919 cm^{-1} correspond with alkanes. In *E. hecabe*, the absorbance spectra bands at 2949, 2924, 2861 and 1365 cm^{-1} represent alkanes. Spectral peak value of alkanes in *A. lyncida* is 2919 and 1370 cm^{-1}. In *C. etrida*, the observed bands at 1382 cm-1 belong to alkanes. In *I. pyrene*, the spectral peak value for alkanes was observed at 2970, 2919, 2842 and 1374 cm^{-1}.

The outer surface layer of cuticle is composed of a matrix of alkenes into which n-alkanes are embedded. Alkenes have three structural features which include length of the long hydrocarbon chain, either sides of the double bond and the angle formed by the double bond. This structural complexity renders the molecule more specific (Rundel *et al*., 2005). In *C. nerissa*, the absorbance band at 1645 cm^{-1} was assigned to be alkenes. The alkenes group has C=C stretch. In *C. pyranthe*, the observed absorbance spectra bands at 1800 and 1647cm^{-1} belong to alkenes. These are characterized as C=O and C=C stretch. In *C. danae*, the observed band is at 1655 cm^{-1} represent alkenes. In all other selected butterflies alkenes were absent.

In *P. valeria*, the absorbance spectra band at 3133cm^{-1} represent alkynes. In *C. etrida*, the observed absorbance spectra bands at 3363 and 3235cm^{-1}correspond to alkynes (terminal). In *I. pyrene*, the band at 3227cm^{-1} represent alkynes (terminal). These are characterized as Ca”C-H, C-H stretch. These are terminal alkynes with strong, narrow bands. This can be an important diagnostic tool because very few organic compounds show absorption in this region. The mobility of these compounds (alkanes, alkenes and alkynes) is important for its functions, for example in wasps, the state of outer layer (solid or liquid) is important to the function of ant repellents. In honeybees and wasps, the role of n-alkanes (a dominated hydrocarbon) was well studied in the field of nest mate and egg recognition (Martin and Drijifhout, 2009).

A study based on the proboscis extension reflex (PER), in honeybees showed that honeybees were unable to discriminate between n-alkanes, whereas they could learn and discriminate between alkenes. When the n-alkanes composition in hydrocarbon profile was altered, it had little effect on protection from ‘guard’ bees. In contrast, when the n- alkenes (the minor peaks of hydrocarbon profile) composition was altered, honeybees were attacked more intensively by ‘guard’ bees. Thus, n-alkenes even though present in the small quantity, gives protection against ‘guard’ bees. It is reported that different colonies of honeybees significantly differ in their hydrocarbon profiles (Machovic *et al*., 2017). There are three major types of hydrocarbons present in all insects including alkanes, alkenes and methyl hydrocarbons. According to previous FTIR studies of insect wings, the proportion of chemical components of wing was found to be similar among different species (Gibbs and Pomonis, 1995; Ibitoye *et al.,* 2018).

The second predominant compounds present in the butterfly wing region include alkyl halides, alcohols and phenols. These compounds act as a signalling molecule which is useful for insect communication. Understanding the concept of chemical signalling and communication is of vital importance because it is responsible for fundamental processes like species and gender recognition (Schlick- Steiner *et al*., 2006). In *C. nerissa*, the spectra band at 630 cm^{-1} belong to alkyl halides. Alkyl halides are compounds that carbon group banded with halogen. In *C. pyranthe*, the spectra band at 597 cm^{-1} represent alkyl halides. In *P. ceylanica*, the band at 1539cm^{-1} was assigned to be carbonyl group. These are characterized as C=O stretch. The spectral bands at 649, 560 and 830 cm^{-1} correspond with alkyl halides. In *A. lyncida*, the spectra band

Table 1. FTIR Spectra (Hydrocarbons and Functional groups) of Pierid Butterflies

Hydrocarbons and Functional groups	Butterfly species with its spectral peak values (cm^{-1})								
	Cepora nerissa	*Catopsilia pyranthe*	*Pareronia valeria*	*Pareronia ceylanica*	*Colotis danae*	*Eurema hecabe*	*Appiasly ncida*	*Colotis etrida*	*Ixias pyrene*
Alcohols and Phenols	3667	3687	3703, 3435	3287	3286	3256		3704	
Aldehydes							1647	2936	
Aliphatic amine	1234,1164, 1112	1160,1092	1153,1075, 1013	1257,1073, 1017	1049	1077	1237, 1066	1187	1272, 1083
Alkanes	1339,2967, 2915,2836, 1374,1339	1442,1365	2959,2847, 1464,1377	2966,2901, 1436,1351	2953, 2919	2949, 2924, 2861, 1365	2919, 1370	1382	2970, 2919, 2842, 1374
Alkenes	1645	1800, 1647			1655				
Alkyl halides	630	597	626	649,560,830	630	893, 587	850, 623	674, 597	614
Alkynes			3133					3363, 3235	3227
Aromatic amines		1279, 1160				1269		1272	
Aromatic groups		3064, 3004, 2962	893				1433		1433, 896
Bromine	690	690	690	649, 560	690	690	623	674, 597	690
Carbonyl Groups				1539					
Carboxylic acid	3273			1625		1654,1532, 1444	1134	1741, 1678	
Chlorine				830		850	850	830	
Ketones							1647		
Nitrocompound	1444	1545	1326		1533		1527		1527
Primary, Secondary amines		3286	3314						

at 630 cm^{-1} represent alkyl halides. This methyl branched hydrocarbons among insects functions as a sex pheromones and also acts as a kairomones i.e. a signal molecule that is advantageous to the receiver but not to the donor. These physiologically important hydrocarbon molecules also function as an anti-aphrodisiac agent (Gibbs and Pomonis, 1995; Chung and Carroll, 2015). The bands belonging to the asymmetric and symmetric stretching vibrations of aliphatic C-H bonds of methyl, methylene and methane groups can be found in the wave number region between 3000-2700 cm^{-1}. The bands of bending vibrations of CH_2 and CH_3 can be found at about 1450 and 1370 cm^{-1}.

The absorbance spectral peak value of alcohols and phenols are characterized as O-H stretch. In *C. nerissa*, the absorbance peak at 3667 cm^{-1} represent alcohols and phenols. These are characterized as O-H stretch. In *C. pyranthe*, the observed band at 3687 cm^{-1} was assigned to be alcohol and phenols. In *P. valeria*, the peaks at 3703 and 3435cm^{-1} correspond with alcohols and phenols. In *P. ceylanica*, absorbance band at 3287cm^{-1} represent alcohols and phenols. In *C. danae*, the peak at 3286 cm^{-1} correspond with alcohols and phenols. In *E. hecabe*, the spectra band at 3256 cm^{-1} was assigned to be alcohols and phenols. In *C. etrida*, the absorbance band at 3704 cm^{-1} represent alcohols and phenols. Alcohols and phenol were absent in *I. pyrene*. In addition to communication and signalling, these components are involved in nest-mate recognition, task-specific cues, dominance and fertility cues, chemical mimicry,

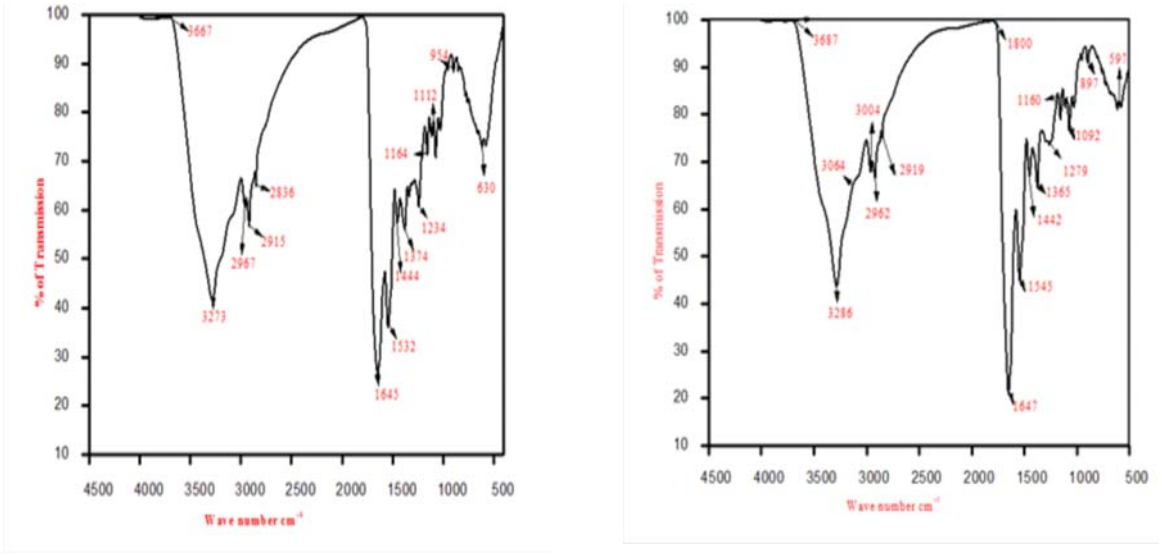

Fig. 1 FTIR analysis of *Cepora nerissa* and*Catopsillia pyranthe*

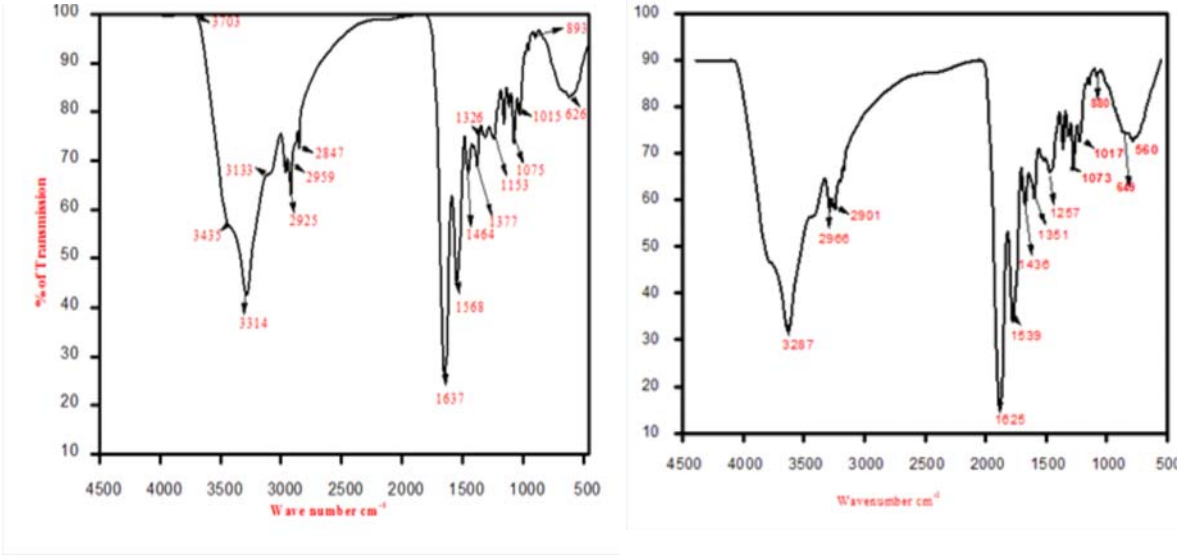

Fig. 2 FTIR analysis of *Pareronia valeria* and *Pareronia ceylanica*

mate selection and kin recognition. All these help to drive the evolutionary force in terms of speciation in butterflies (Rundel *et al.*, 2005).

Insects are smaller in size and have large surface area to the volume ratio, hydrocarbons in the cuticle gives protection against desiccation, by controlling the transcuticular water flux (Martin *et al*., 2004). Hydrocarbon molecules also prevent the wetting of insect's body. Hydrophobic property of hydrocarbons acts against desiccation. Carboxylic acids in the cuticle mainly include amino acids and fatty acids. In *C. nerissa*, the band at 3273 cm^{-1} was assigned to carboxylic acid. These have O-H stretch and carboxylic acids show a strong wideband for the O-H stretch. In *E. hecabe*, the bands at 1654, 1532 and 1444 cm^{-1} represent carboxylic acid. These have C-O stretch of

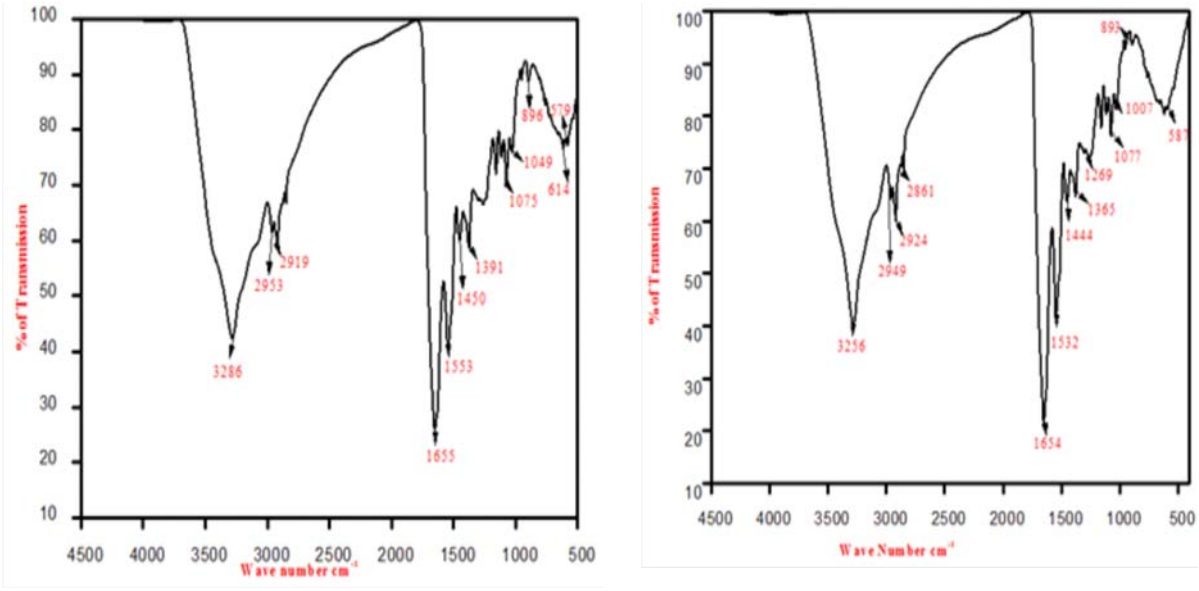

Fig. 3 FTIR analysis of *Colotis danae* and *Eurema brigitta*

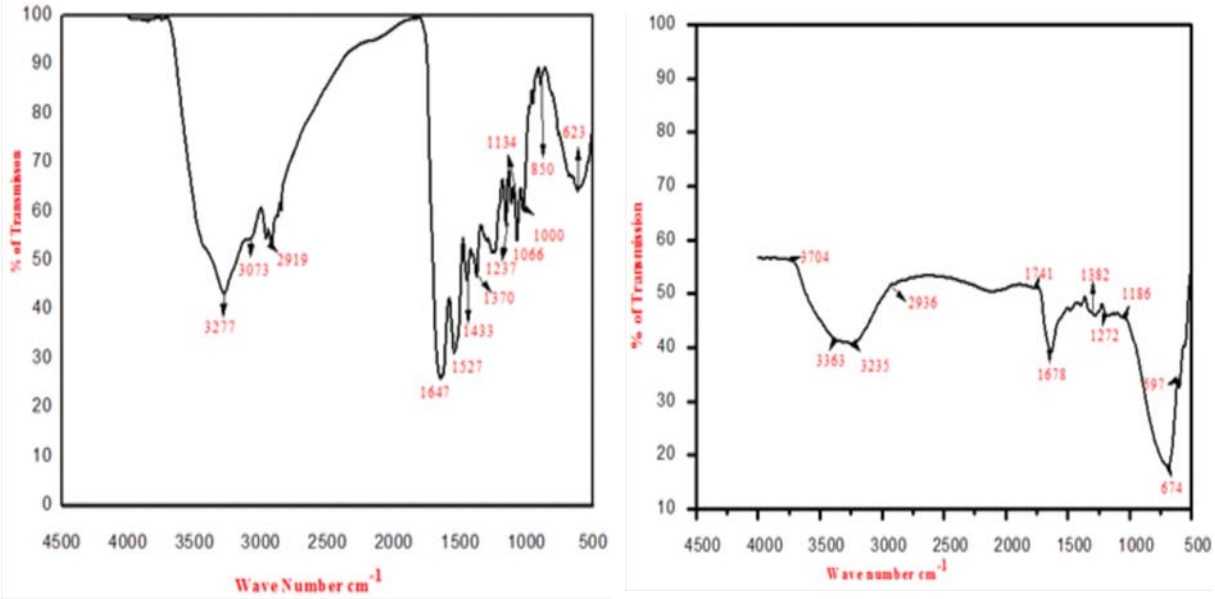

Fig. 4 FTIR analysis of *Appias lyncida* and *Colotis etrida*

carboxylic acids shows a strong wide band. In *A. lyncida*, the spectra bands at 1134 cm^{-1} belong to carboxylic acids. In *C. etrida*, the bands at 1741and 1678 cm^{-1} were assigned as carboxylic Acids. Carboxylic Acids show C=O stretch. The Carboxylic acids present in the insect's cuticle plays an important role in saving the life of insects by acting as an anti-desiccation agent and water proofing agent (Chung and Carroll, 2015). Pieridae butterflies have a specific pigment called pterin (Shamim *et al*., 2014). All pterin pigments have the same basic structure; they differ based on the functional radicals attached to the nucleus. Not all pterin pigments are coloured, colouration depends upon their chemical structure. Majority of butterflies with brown and black scales have melanin, a

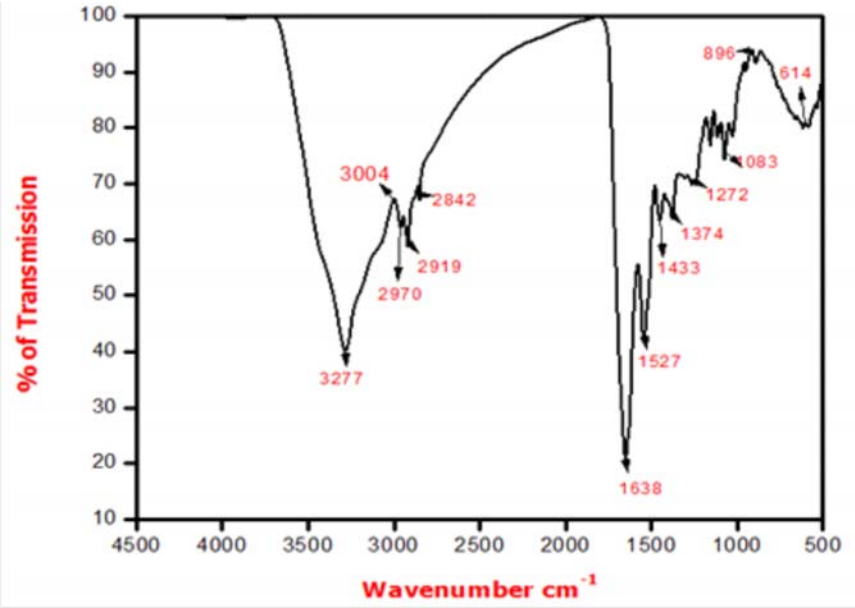

Fig. 5 FTIR analysis of *Ixias pyrene*

universal pigment (Ghiradella, 1998). Pigment melanin contains an essential amino acid phenylalanine, a key compound responsible for wing pigmentation. Insect's wings predominantly have eumelanin rather than pheomelanin. In addition to pigmentation, phenylalanine also has physiological importance, it helps the insects to cope up with parasitic infections (Stuhr *et al.*, 2018). The band at 3087cm^{-1} was assigned to be NH stretching modes with some contributions of the aromatic CH stretching vibrations. The protein repeated units give rise to nine characteristic infrared absorption bands namely Amide A, B and I-VII (Tian *et al.*, 2015).

In *C. nerissa*, the absorbance band at 1444 cm^{-1} correspond with N-O asymmetric stretch and the observed absorbance spectra band at 3286 cm^{-1} represent primary and secondary amines and amides. These are characterized as N-H stretching frequency in primary amines and amides are derived from ammonia. Secondary amines and amides show only one peak in the infrared. The observed band at 1545 cm^{-1} was nitro compounds with N-O asymmetric stretch. In *P. valeria*, absorbance peak at 1326 cm^{-1} represent nitro compounds with N-O symmetric stretch and 1568 cm^{-1} was characterized as N-O asymmetric stretch. Band at 3314 cm^{-1} correspond primary and secondary amines and amides. In *C. danae***,** the observed peak at 1533 cm^{-1} belong to nitro compounds with N-O asymmetric stretch. In *A. lyncida*, the peak at 1527 cm^{-1} correspond to nitro compounds with N-O asymmetric stretch. In *I. pyrene*, the band at 1527 cm^{-1} belong to nitro compounds with N-O asymmetric stretch.

In *C. nerissa*, the bending of aliphatic amines with C-N stretch and O-H bend was produced at 1234, 1164 and 1112 cm^{-1}. In *C. pyranthe*, the absorbance peak at 1160 and 1092 cm^{-1} represent aliphatic amines. In *P. valeria*, the bands at 1153, 1075 and 1013 cm^{-1} belong to aliphatic amines with C-N stretch and O-H bend. In *P. ceylanica*, the observed bands at 1257, 1073 and 1017 cm^{-1} correspond with aliphatic amines with C-N stretch. In *C. danae*, the absorbance spectra band at 1049 cm^{-1} was assigned as aliphatic amines with C-N stretch and O-H bend. In *A.lyncida*, the observed absorbance bands at 1237 and 1066 cm^{-1} represent aliphatic amines with C-N stretch.

Catecholamine is a precursor of melanin pigment derived from aromatic amino acids such as tyrosine and phenylalanine incorporated into cuticle (Stuhr *et al.*, 2018). In *C. pyranthe***,** the absorbance peaks

at 3064, 3004 and 2962 cm^{-1} belong to the aromatic group. The C-H stretch in aromatic group was observed. The observed absorbance bands at 1279, 1160 cm^{-1} represent aromatic amines, characterized by C-N stretch. In *P. valeria*, the observed absorbance peak at 893 cm^{-1} belong to the aromatic group. In *E. hecabe*, the peak at 1269 cm^{-1} assigned to aromatic amines. The C-N stretch in aromatic amines was observed. *In A. lyncida*, the absorbance peak at 1433 cm^{-1} represent the aromatic group. The C-H stretch in aromatic group was observed. In *C. etrida*, the peak at 1272 cm^{-1} assigned to aromatic amines. This is characterized as a C-N stretch. In *I. pyrene*, the absorbance peaks at 1433 and 896 cm^{-1} were assigned to the aromatic groups. The C-H stretch and C-C stretch in aromatic group were observed.

In all the selected nine butterflies, C-Br stretch (690 - 515 cm^{-1}), was confirmed as bromine. In *P. ceylanica*, a peak at 830 cm^{-1} was characterized as C-Cl stretch. The C-Cl (850-550 cm^{-1}) stretch was confirmed as chlorine. In *E. hecabe*, a peak at 587 cm^{-1} was characterized as chlorine. The presence of bromine and chlorine may be influenced by the colour of the wings. In *A. lyncida* the observed absorbance spectra band at 1647 cm^{-1} correspond with unsaturated aldehydes and ketones. These are characterized as C=O stretch. As in ketones, if the carbons adjacent to aldehyde groups are unsaturated and this vibration is shifted in lower wave number. Spectral peak at 850 cm^{-1} was characterized as C-Cl stretch. In *C. etrida*, the observed absorbance spectra band at 2936 cm^{-1} represent aldehyde. This is characterized as H-C=O; C-H stretch. The spectral peak values at 674 and 597 cm^{-1} were characterized as C-Br. The peak at 830cm^{-1} was characterized as a C-Cl stretch. The C-Cl stretch was confirmed as chlorine. Elements like bromine, sulphur and silica were predominant in wings X-ray fluorescence spectrometry (XRF) analysis of wings revealed the presence of high concentration of Cl, Br and Ca in the supporting structures of wings (Stuhr *et al*., 2018; Kaspar *et al*., 2019).

Cuticular hydrocarbons are also used as a taxonomic tool. These hydrocarbons among insects, they exhibit natural variation and that too under direct selection. During allopartic speciation, these hydrocarbons rapidly diverge due to differences in diet, environmental conditions and this could lead to reproductive isolation (Buckley *et al.,* 2012). Thus cuticular hydrocarbons are better indicator for recent speciation and reproductive isolation (Simmons and Thomas, 2004). Identification of cryptic species is a common problem in taxonomy. Even morphologically indistinguishable species can be discriminated by their hydrocarbon profiles (Schlick-Steiner *et al*., 2006). Long term stability of hydrocarbons allows analyzing the museum specimens and even an extinct species (Martin *et al.,* 2009).

FTIR analysis of nine different Pierid butterfly wings showed many relative sizes of peaks. There are no significant differences in the chemical composition of wings but the colour differences observed among the nine different Pierid butterflies and it was inferred that not majorly due to the differences in the cuticular hydrocarbons. It may be due to the presence of different microstructures like scales, ridges and grooves.

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Design and testing of a novel cost-effective lethal ovitrap for the control of *Aedes aegypti* (Linnaeus 1762)

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ABSTRACT: An indigenous, novel, cost-effective, non-electric lethal ovitrap was designed for the dengue vector. The bacteria *Bacillus cereus* VCRC 641 was grown in a cost-effective culture medium of chicken feather waste. The culture supernatant was used as ovitrap attraction of the dengue vector of *Aedes aegypti* (Linnaeus 1762). Twenty experiments with 80 ovitraps, three experiments with 15,000 ovitraps, and three experiments with 3,000 ovitraps in the laboratory, outdoor and households respectively conducted, to assess the efficacy, showed that the ovitrap with *B. cereus* culture supernatant (10%) significantly attracted the gravid *A. aegypti* to lay eggs in all experimental traps. More importantly, the first instar larvae from the eggs on the traps had the mortality immediately. The novelty of this trap is proved to exhibit a dual function for attracting gravid mosquitoes and killing the newly emerged larvae. This is the first report asserting the dual role of a cost-effective, non-electric ovitrap to control dengue vectors. The ovitrap contains only simple components of a plastic bowl, a cylinder, and a plastic plate. The total cost of these materials per trap was Rs. 15 only. The laboratory study showed that the experimental ovitrap attracted >10 times the control traps (experimental: 1465.90±251.48 and control: 140.00±23.95). This is the first report about the efficacy of an indigenously designed lethal ovitrap for attracting dengue vector.

KEY WORDS: Dengue vector, first report, dual application, non-electric ovitrap, *Bacillus cereus*, chicken feather waste, oviposition active index

INTRODUCTION

Dengue is an arbovirus infection transmitted by female *Aedes aegypti* (Linnaeus 1762) and *A. albopictus* (Skuse, 1894). Globally 128 countries reported with dengue infection, and 50-100 million cases per annum were reported (WHO, 2017). Dengue and dengue hemorrhagic fever are endemic in nearly 100 countries, including India. Dengue is prevalent in every state, and the National Vector Borne Disease Control Program (NVBDCP) reported 123106 laboratory confirmed cases with 90 deaths till October in India (Ganeshkumar *et al.*, 2018; NVBDCP, 2021). Climate change plays an important part in the dengue epidemiology. Increase in temperature and

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rainfall could lead to increase in transmission of virus and eventually leads to geographical expansion of vector species. Some studies in India stated that the upsurge in dengue vector density during monsoon and post-monsoon period was owing to the abundant of breeding habitat of *Aedes* mosquito (Bhadauriya and Ramteke, 2020). *A. aegypti* has a specific characteristic of effectively adapting to a new environment and being colonized in many countries. *A. aegypti* used to take blood meal than sugar source for egg development, and that aid in the transmission of dengue viral infection (Scott and Takken, 2012; Shih-Che Weng *et al*., 2021). It feeds several times in a single gonographic cycle to increase the chance of acquiring and transmitting pathogens (Scott *et al.*, 1993; Brackney *et al*., 2021). *Aedes* mosquitoes breed in various household containers, including different coloured flower pots. *Aedes* mosquitoes are generally active during the daytime and mostly prefer dark container openings for resting and oviposition. Visual and chemical cues induce the oviposition to lay eggs to maintain successful offspring (Mcgregor and Connelly, 2021). Mosquito control, in general, targeted the larval source reduction such as identifying breeding sites, removing or applying larvicide in the breeding habitat, and adult mosquito control measures by chemical insecticide fogging.

Source reduction of breeding sites of the *Aedes* is a mandatory need for mass trapping interventions to target egg laying females and killing the larval progeny. *Aedes* can survive in the small pocket of water positioned in inaccessible areas and seems to be a significant challenge for public health. For dengue prevention and control programs, ovitrap design and its application is an alternate strategy for long-standing vector surveillance to provide new information on the dynamics of dengue vector population and the spatiotemporal distribution of the mosquitoes. Ovitrap is a user-friendly and effective tool for monitoring dengue vectors, and earlier was applied in various countries like Singapore, Australia, Indonesia (Sasmita *et al.,* 2021). The lethal oviposition trap was used to attract gravid female mosquitoes, subsequently laying eggs eventually, the mosquitoes twig on the substratum. Many such ovitraps were reported earlier, such as autocidal gravid ovitrap (AGO), sticky trap (ST), double sticky trap (DST) and gravid *Aedes* trap (GAT), Attractive baited lethal ovitrap (ALOT) (Chadee *et al.,* 2010; Eiras *et al.*, 2014; Paz-Soldan *et al*., 2016). Despite all these ovitrap designs, so far, no report on the dual application of ovitrap for attracting and killing immature larvae was found, except a preliminary attempt using *Pseudomonas fluorescence* (Pushpanathan, 2017). With these contextual studies, the present work was undertaken on the dual application of a novel, cost-effective, non-electric lethal ovitrap to control dengue vectors of *A. aegypti* using *Bacillus cereus* VCRC 641 culture supernatant as an attractant.

MATERIALS AND METHODS

Designing cost-effective, non-electric lethal ovitrap for mosquito control

The ovitrap in the present study has a simple scientific design and is convenient to attract adult mosquitoes in the field condition. The ovitrap consists of four components made of black-colored recyclable plastic: a bowl (250 ml), a container (500 ml), a lid, and a plastic plate to cover the ovitrap setup to prevent dust. The container's lid was fixed inside the bottom of the bowl using adhesive paste. Ten percent diluted bacterial culture supernatant solution (attractant) was filled in the container, and the lid was screwed-up to the cylinder intact and inverted into the bowl. The attractant was flown into bowl up to desired level via a small hole made above the mouth of the cylinder at a distance of 3.5 cm, fixed the plastic plate on the bottom of the cylinder to prevent the dust and other materials from falling inside the bowl. The working principle of this trap design is to maintain the attractant level constant. Whenever the attractant level decreases due to evaporation, the attractant will flow from the container into the bowl, keeping the level consistent. Therefore, the ovitrap is prolonged for longer days (> 20 days) in the field environment. The simple features of this ovitrap are suitable to carry out experiments in all field conditions (Fig. 1 and 2).

Bacterial strain

Bacillus cereus VCRC 641, which was isolated earlier from the midgut of freshwater fish, *Clarias*

A. Components of Lethal ovitrap.

B. Final outlook of the assembled ovitrap

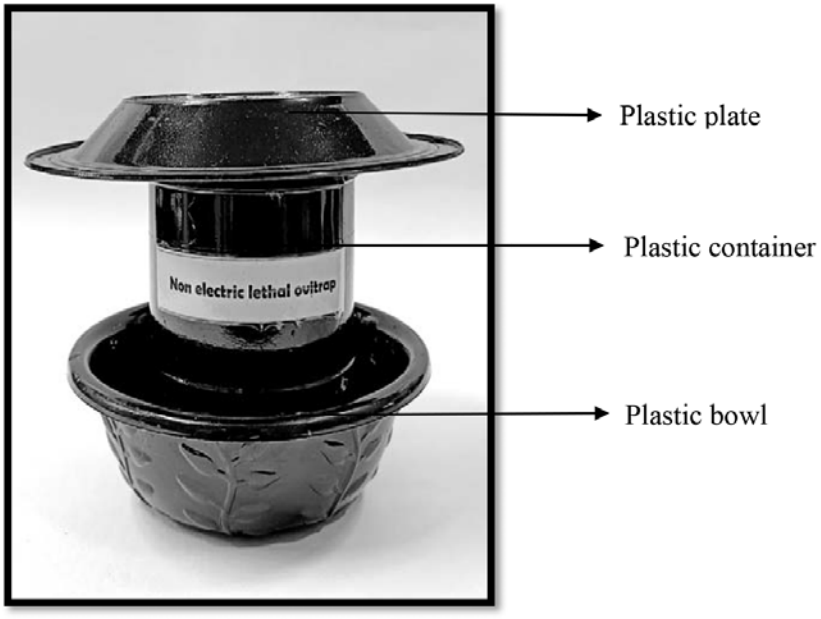

Fig. 1 Components of non-electric lethal ovitrap for mosquito attraction.

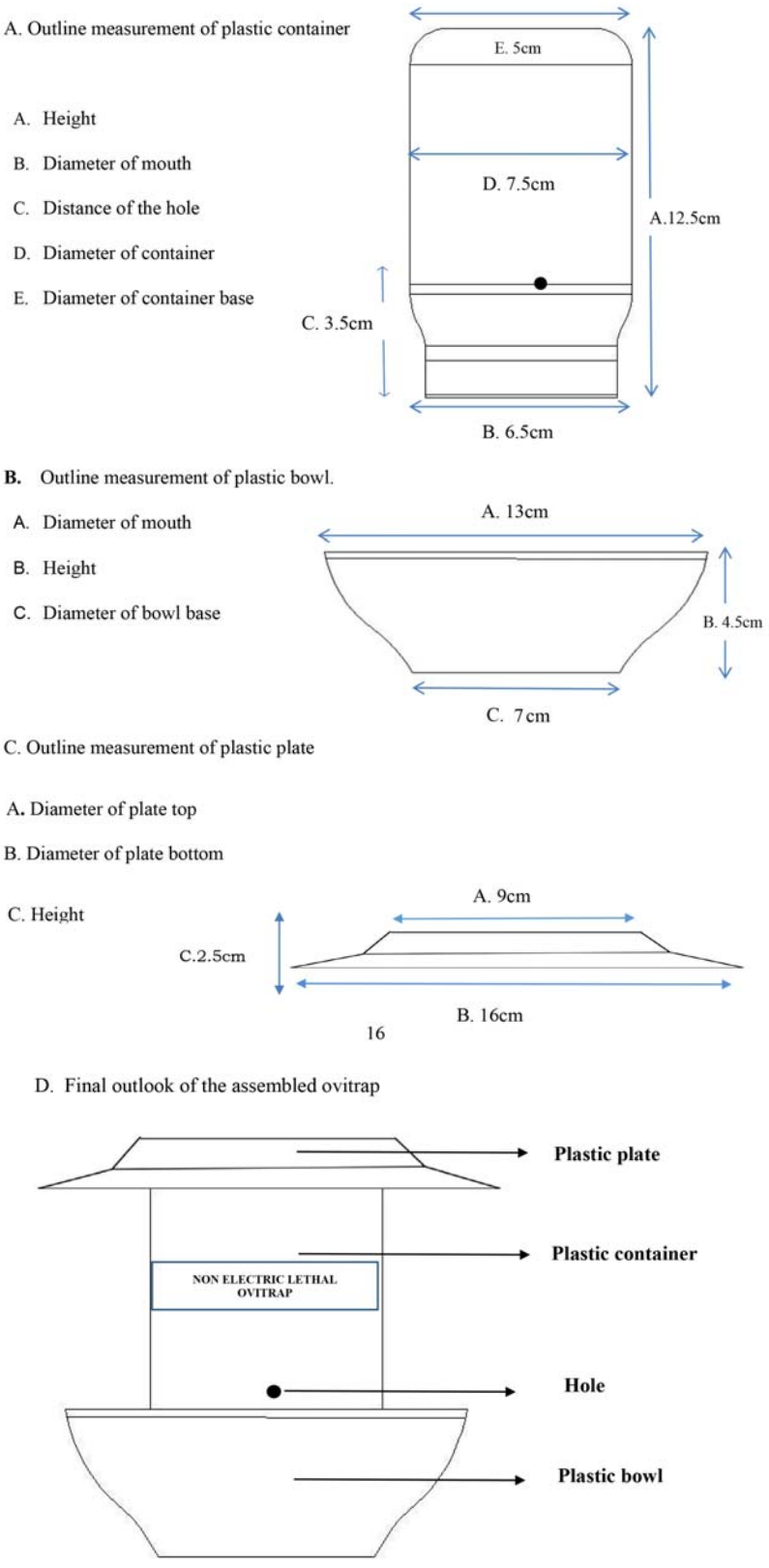

Fig. 2 Presentation of outline dimension image of lethal ovitrap design.

Fig. 3 Preparation of chicken feather waste (CFW) culture medium

Fig. 4 Lethal ovitrap setup in the field outdoor for the attraction of *Aedes aegypti* mosquitoes

Fig. 5 Lethal ovitrap setup in the indoor households for attraction of *Aedes aegypti* mosquitoes

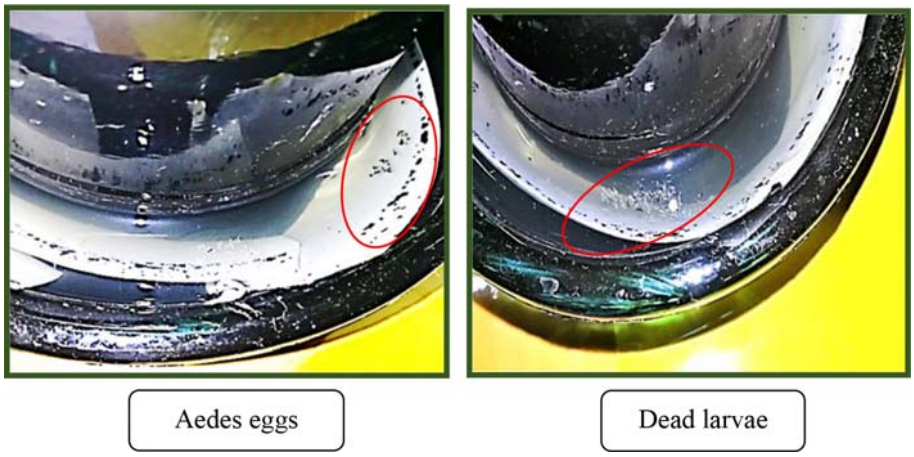

Fig. 6 Eggs laid by the *Aedes aegypti* and dead larvae on the lethal ovitrap

batrachus, was used in the present study. This strain was proved to possess potential mosquitocidal activity against major vector species *viz*: *Culex quinquefasciatus*, *Anopheles stephensi*, and *A. aegypti* (Manikandan, 2022 personal communication). A loopful of *B. cereus* was sub-cultured, and 50 µl of the sub-culture was inoculated into a bacterial culture medium prepared from chicken feather waste (CFW) (0.5%). After 72 hours, the bacterial culture was centrifuged using Hitachi high-speed refrigerated centrifuge, and the bacterial cell pellet was separated by centrifugation at 12,000 rpm for 15 min. The present study used the cell-free culture supernatant, which was generally discarded as a waste, as a substrate for oviposition attraction. The supernatant was freeze dried at - 80^0 and preserved for bioassays against larvae of mosquito species.

Chicken feather waste (CFW) culture medium

Chicken feather waste (CFW) culture medium as described earlier by Poopathi *et al.* (2011) was used in the present study to culture the potent isolate *B. cereus* VCRC 641. CFW was collected from poultry industry and washed thoroughly with normal water and air-dried in the shadow places. The completely dried feathers then finely crushed and powdered from the flour mill and later on preserved in room temperature (28^0C) until further use. The CFW culture medium was prepared (0.5%) in water (5g/l) and pH adjusted to 7.5. The culture medium was dispensed in 2 litre conical flasks (volume 500 ml) and autoclaved. A loopful of *B. cereus* stock was sub-cultured in 10 ml test tube and incubated overnight. 50 µl of the pre-culture/ overnight culture was inoculated into 2 litre flasks containing 500ml of CFW medium and incubated in orbitek shaker in room temperature at 200 rpm for 3 days (72 hours). After homogeneous sporulation, the bacterial culture was centrifuged at 10,000 rpm for 30 minutes and the bacterial cell mass were separated and the culture supernatant was collected and stored at 4^0C. The supernatant was diluted with water (10%) and used as gravid mosquito attractant in the newly invented ovitrap (Fig. 3).

Lethal ovitrap – Laboratory evaluation

Gravid female *A. aegypti* mosquitoes (100 numbers) received from mosquito colony section of this research institute were released inside the wooden mosquito cage (2' x 2' x 2'). The ovitraps containing the *B. cereus* culture supernatant (10%) and control traps comprising normal water were positioned in the opposite direction of each experimental cage and three replicates were maintained. The cages were maintained at room temperature (28±2^0C, 70-75% RH) and the entire experiments were arranged at 16 hours and the eggs laid by the mosquitoes were recorded on the next day at 10 hours. The total number of eggs laid on the paper strip surface was carefully counted using the hand lens, and experiments were repeated 20 times in different days and in total 80 traps were tested (2 traps each for experimental and control) to ascertain the mosquito oviposition attractancy in the laboratory.

Lethal ovitrap – field evaluation

The ovitraps were experimented in five different arid-urban study sites where dengue incidences were reported more often. The ovitraps were placed in selected outdoor locations such as garden, near corporation dust bin, places where tyres were dumped, barren land, garbage dumping places, and backyard of houses, which preferred breeding places of *Aedes* mosquitoes (Fig.4). For experimental and control 500 traps each were placed in each study site and experiments were repeated three times. Totally 15,000 ovitraps were systematically tested.

Similarly, for indoor experiments, 20 houses per study site were selected and five each for experimental and control ovitraps were placed in different locations inside the houses like kitchen, hall, bedroom, store room, verandah and pooja rooms (Fig. 5). Consequently, 100 ovitraps each for experimental and control were placed in indoor households (5 study sites) repeated three times. In total, 3,000 ovitraps were comprehensively tested. The total number of eggs per trap laid by *A. aegypti* was recorded.

Ovitrap indices –application

The data generated from experimental and control ovitraps were subjected to statistical analysis to ascertain four major parameters, *viz*,

Oviposition Active Index (OAI): $OAI = \frac{Nt - Ns}{Nt + Ns}$

Where the Nt denotes number of eggs in the test medium, Ns denotes number of eggs in water as control. If OAI is more than 0.3, it is an attractant; if OAI is less than 0.3 means, it is repellent (Hwang *et al.,* 1982).

PHI (Positive House Index):

$$PHI = \frac{Number\ of\ house\ with\ positives}{Total\ number\ of\ houses\ inspected}$$

OI (Ovitrap Index):

$$OI = \frac{Number\ of\ ovitrap\ containing\ eggs}{Total\ number\ of\ ovitrap\ observed}$$

ODI (Ovitrap Density Index) = Average egg density per positive ovitrap

A mixed effect model was used to build the model for eggs density with linear, Poisson and square root separately, with place and replicates kept as random effects and group (experimental and control) observed as fixed effects. Lower values of Akaike information criterion (AIC) and Bayesian information criterion (BIC) were considered as a good model. McNemar's test was used to compare experimental and control ovitrap data. The analyses were carried out applying STATA 14.2 (USA, Texas).

RESULTS AND DISCUSSION

Mosquitocidal efficacy of *Bacillus cereus* VCRC 641

Bacillus cereus VCRC 641 culture supernatant lyophilized powder was highly toxic (Table 1) to late third instar mosquito larvae of *A. aegypti*, *Cx. quinquefasciatus* and *A. stephensi*. Extra cellular toxins were highly toxic to all the three mosquito species. The LC_{50} values of these mosquito larvae were 0.23, 0.17, and 1.38 mg/l respectively. Considering the mosquitocidal property of *B. cereus*, the culture supernatant was selected for oviposition attractant through newly designed lethal ovitrap.

Lethal ovitrap –design

The ovitrap consists of four recyclable components, a plastic bowl (diameter at top 13cm, at base 7cm and volume 250 ml), a recyclable plastic container (diameter at top 6.5cm, at base 5cm, height 12.5cm and volume 500 ml) with a plastic lid (diameter 6.5cm and height 1cm) and a plastic plate (diameter at top and base 16 and 9cm and height 2.5cm respectively). The plastic container can hold 500ml of the attractant (culture supernatant of *B. cereus*). The lid of the container was permanently fixed at the bottom of bowl using adhesive. Two small holes were made in the container at a distance of 3.5 centimeters from its mouth. This will facilitate the release of attractant into the plastic bowl and maintain the level constant. *B. cereus* culture supernatant (10%) was used as an attractant, which was filled in the container and closed with the lid fixed to the bowl and inverted. A plastic plate was used to cover the ovitrap to avoid dust and other

Table 1. Toxicity of extracellular metabolites from culture supernatant of *B cereus* VCRC 641

Species	Slope	Intercept	LC_{50} *	LC_{90} **	χ^2
Culex quinquefasciatus	6.75	1.001	0.17(0.22-0.14)	0.62(0.91-0.42)	2.96
Anopheles stephensi	4.70	0.89	1.38(1.8-1.07)	5.79(8.86-3.78)	2.42
Aedes aegypti	5.93	0.65	0.23(0.33-0.16)	1.69(3.07-0.92)	2.94

*LC_{50} (mg/L)- (90% UCL-LCL); **LC_{90} (mg/L) (90% UCL-LCL)

Table 2. Oviposition Active Index (OAI) of outdoor study sites and indoor households

Area	**Outdoor**	**Indoor**
Gorimedu	0.801	0.758
Lawspet	0.739	0.756
Mettupalayam	0.829	0.817
Reddiyarpalayam	0.741	0.734
Villianur	0.725	0.750
Average	0.767±0.045	0.763±0.031

Table 3. Vector Indices (PHI, OI and ODI) and OAI of outdoor study sites and indoor households of the *Aedes sp* in five different study sites

Area	Ovitrap	PHI	OI	ODI		OAI	
		In	Out	In	Out	In	Out
Mettupalayam	Exp.	1	0.99	159	300	0.817	0.829
	Cont.	0.97	0.96	16	8		
Gorimedu	Exp.	1	0.99	80	172	0.758	0.801
	Cont.	0.97	0.96	11	19		
Lawspet	Exp.	1	0.99	65	80	0.756	0.739
	Cont.	0.90	0.94	9	12		
Villianur	Exp.	1	0.99	78	88	0.75	0.725
	Cont.	0.97	0.94	11	14		
Reddiyarpalayam	Exp.	1	0.99	72	81	0.734	0.741
	Cont.	0.96	0.90	11	12		

In - indoor households; Out - outdoor

Table 4. Comparative analysis between indoor household and outdoor study sites

Area	Ovitraps	Eggs	
		Positive	Negative
Indoor	Experimental	1500 (100.0%)	0
	Control	1435 (95.7%)	65 (4.3%)
Outdoor	Experimental	7488 (99.8%)	12 (0.2%)
	Control	7111 (94.8%)	389 (5.2%)

Table 5. Goodness of fit statistics

Model	Indoor		Outdoor	
	AIC	*BIC*	*AIC*	*BIC*
Linear	29152	29182	165235	165273
Poisson	36390	36414	278185	278215
Square root linear model	10872	10902	66718	66756

AIC- Akaike information criterion, *BIC*- Bayesian information criterion

things from falling into the trap. This plate was fixed permanently using adhesive at the bottom of the inverted container. This ovitrap setup was kept in the experimental sites. The attractant in the container flows continuously until it reaches the level of outlets (holes) already made on the two opposite sides of the container. In the laboratory study, ovitraps were placed in opposite sides of the mosquito cages along with control traps. Similarly, the ovitraps were kept in suitable places in the five experimental field areas (outdoor and indoor).

Lethal ovitrap –Laboratory evaluation

Ovitrap attraction of *A. aegypti* mosquitoes in the laboratory showed that the density of eggs laid was significantly more in the experimental ovitraps containing the *B. cereus* culture (Fig. 6). OAI between the experimental and the control ovitraps showed significantly a high level of attraction. The average OAI was 0.8145±0.052. The average number of eggs laid by gravid female mosquitoes was 1465.90 ±251.48 (95% CI: 1348 – 1584) in the experimental traps while in the control it was 140.00±23.95 (95% CI: 129 – 151) (P>0.001), indicating a >10-fold effective attraction.

Lethal ovitrap –outdoor field evaluation

In Gorimedu outdoor study area, the total mean eggs laid by *A. aegypti* was 166.5 in the experimental ovitraps whereas, only 18.2 eggs in the control ovitraps. Similarly, in Lawspet study site, 78.2 eggs were collected in the experimental traps and only 10.8 eggs were collected in the control ovitraps. In Mettupalayam area, 282.9 and 26.7 eggs were recorded in experimental and control ovitraps respectively. In Reddiyarpalayam study site, 77.9 and 10.73 eggs and in Villianur study site, 81.6 and 12.4 eggs were collected in experimental and control respectively. If the OAI is above 0.30, then it is taken as effective attractant or if it is less than 0.3 then it is repellent (Hwang *et al.*, 1982). From all five outdoor study sites, observed mean OAI was only 0.767±0.045 (Table 2), indicating the effectiveness of the trap in the attractive.

Statistical analysis between experimental and control ovitraps from five different study site were

further analysed. Linear mixed effects model with square root transformation of eggs density associated with group (experimental and control ovitraps) was built (eggs = 3.91+7.37 X Group). The mean egg density for experimental ovitrap was 127 (95% CI: 29 – 226), whereas, for control trap, it was 17 (95% CI: 0 – 50). The results depicted that the experimental ovitrap had significant higher egg density than the control (P<0.001).

Lethal ovitrap –indoor household evaluation:

In indoor households of Gorimedu area, totally, 79.3 eggs were collected in experimental ovitraps whereas only 10.5 eggs were collected in control. Similarly, in Lawspet area, 63.3 and 7.7 eggs were collected in the experimental and control ovitraps. In the houses of Mettupalayam, Reddiyarpalayam and Villianur areas, 144.9 and 14.8, 70.59 and 9.72, 76.81 and 10.1 eggs were collected from experimental and control ovitraps respectively. The average OAI from the households was 0.763±0.031 (Table 2).

Statistical analysis between experimental and control ovitraps placed in the indoor houses were further analysed. Linear mixed effects model with square root transformation of eggs density associated with group (experimental and control ovitrap) was built (eggs = 3.91+7.37 X Group). Mean egg density for experimental ovitrap was 85 (95% CI: 62 – 138), whereas, for control trap it was only 11 (95% CI: 0 – 30). Statistical analysis from the results depicted that the experimental ovitrap had significant number of egg density than the control. From this study, it was observed that the experimental lethal ovitrap was found to be very effective in the field for 10 to 15days. It is very important to mention that, the newly emerged first instar larvae died immediately in all experimental ovitrap from laboratory and field experiments.

Lethal ovitrap –Indices

Supplementary indices for measuring the dynamics on the presence of *A. aegypti* in all field sites including the indoor households were also investigated in the present study. The results showed that the target dengue vector of *A. aegypti* population was significant in existence in all outdoor study sites and indoor households during the study period of August to December, 2018 (Table 3).

Comparative analysis

In indoor households, all 1,500 traps were positive for eggs (100.0%) in experimental and 1435 were positive in control (95.7%). Likewise, in outdoor study sites, 7,488 traps were positive (99.8%) in experimental and 7,111 were positive in control (94.8%) out of 15,000 traps. Experimental ovitraps had significantly greater than control in terms of egg positivity in indoor households (P<0.001, using chi-square test) as well as outdoor (P<0.001, using chi-square test) (Table 4). Three statistical models for assessing egg density were used, AIC and BIC values were well-fitted models. Among the three models, the linear mixed model with the square root of egg density had lower values of AIC and BIC for the indoor and outdoor models (Table 5).

Mosquito vectors are renowned to recognize their definite hosts and oviposit the eggs through chemical stimulations, physical provocations that are perceived by sensory receptor sites present on their antennae (Davis and Bowen, 1994). With the aid of oviposition attractants, mosquito vectors can identify the preferred sites for egg laying. Several synthetic chemicals were identified as oviposition attractants for mosquito vectors even when used in comparatively lesser quantity (Beehler and Mulla 1993; Boullis *et al*., 2021). Microbial organisms can also produce the oviposition attractants (Hazard *et al.*, 1967; Rockett 1987; Hasselschwert and Rockett 1988; Beehler *et al*., 1994; Ponnusamy *et al*., 2008, 2015). Protein hydrolysate from bacterial contaminants attracts the gravid female mosquitoes of *Cx.quinquefasciatus* (Beehler *et al*., 1994). *Trichoderma*, a known *Deuteromycetes* fungi synthesize perfumed, unstable metabolites (Kikuchi *et al*., 1974; Keszler *et al*., 2000; Sarhy-Bagnon *et al*., 2000; Kalyani *et al*., 2000; Geetha *et al*., 2003). In the present study, the extracellular metabolites from *B. cereus* culture supernatant (cell-free extract) was used as attractant for gravid *A. aegypti* mosquitoes in laboratory and field sites, using a newly designed cost-effective lethal oviposition traps and the results were presented. Ovitrap surveillance was applied in different

countries such as Malaysia (Lau *et al*., 2017; Afizah *et al.,* 2018), Taiwan (Wu *et al*., 2013), Trinidad (Chadee *et al*., 1987) and Philippines and dengue vector management was improved considerably.

Presently, many types of ovitraps are reported to be in use such as standard lethal trap, NEAS-sticky ovitrap, Mosquito TRAP sticky ovitrap, Bioagents gravid *Aedes* trap and CDC-autocidal gravid ovitrap (Brian *et al.,* 2017). Ovitrap used in the present work is a simple and economically cheap lethal ovitrap with no need of electricity. The attractancy of ovitrap can be maintained continuously for more than a month. Medium sized ovitrap was used in the present study and large sized traps were found to be ideal for egg laying of female gravid *A.* species (Reiskind *et al.,* 2012; Panigraphi *et al.,* 2014). Non-electric lethal Ovitrap used in this present study provided virtuous oviposition attraction especially for *A. aegypti* as compared with the conventional ovitrap. The attraction efficiency by the ovitrap is more with *A. aegypti* than the other mosquito species (*Cx. quinquefasciatus* and *A. stephensi*) which may be due to the black colour painted on the traps and odour of the attractant. This result was in corroboration with earlier findings that black colour attracts more *Aedes* mosquitoes than the other species (Williams 1962; McDaniel *et al*., 1976; Colton *et al*., 2003; Panigrahi *et al*., 2014). It is very important to emphasis in the present study that *B. cereus* extracellular metabolites from the culture supernatant acted not only as attractant but also as larvicide for newly emerging first instar larvae. This is a first report stating the above perception in vector control. Therefore, this ovitrap is entirely different from the other lethal ovitraps such as In2Care ovitrap (Snetstellar *et al*., 2016) and the trap design of the present study originated from the design of bird feeder in the poultry industry.

The lethal ovitrap investigated in the present study is entirely different from already existing trap designs reported from various countries. The entire trap design is functioning as an automatic device to enable slow release of attractant into the plastic bowl. The working principle behind the trap is to control attractant level constantly in the plastic bowl due to evaporation of attractant by sun light exposure. Lethal ovitrap used in this study revealed more number *Aedes* eggs found in experimental ovitraps comparatively at outdoor than indoor and the similar observation was documented by Martin *et al.* (2019). Ovitrap indices like PHI, OI and ODI showed that the existence of *A. aegypti* was seen in all study sites during the study period and this result was in corroboration with earlier workers (Sasmita *et al.,* 2021). Similarly, positive results from OAI in the experimental traps in the study sites were also in agreement with previous report (Poonam *et al*., 2002). Therefore it is concluded in the present study, that the newly designed lethal ovitrap will immensely helpful to vector control researchers on the control of dengue vectors in the field.

The use of cell free supernatant of *B. cereus* cultured in chicken feather waste media in order to attract gravid *Aedes* to lay their eggs in the ovitrap is a novel strategy. Pushpanathan *et al.* (2017) described the *P. fluorescence* culture supernatant for attraction of *Ae. aegypti* and *Ae. albopictus* mosquitoes in the breeding sites and thereafter the egg laying capacity of gravids and known down effects by the exotoxins. This lethal ovitrap design is environment-friendly and easily refillable by the end users. The cost of the lethal ovitrap in Indian rupees is 15 per trap only (US 0.20$). It is very economic and can be used in sufficient numbers to control *Aedes* mosquitoes. It is an indigenous, innovative cost effective device for controlling *Aedes* mosquito population.

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Persistence of cyantraniliprole in sandy loam soil and effect of organic manure amendment

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ABSTRACT: The persistence of cyantraniliprole, a new systemic insecticide of the class anthracitic diamide, was studied under the laboratory conditions in sandy loam soil and the soil amended with farmyard manure (0.5%), after spiking at 1, 2 and 4 µg kg^{-1} levels in three different soil moisture regimes *viz.* air dry, field capacity level and also in saturated conditions. Degradation was comparatively faster in saturated than at field capacity and air dry condition. There was an increase in the persistence with increase in the spiking concentration. The persistence of cyantraniliprole was dependent on soil moisture condition, organic matter content and concentration of cyantraniliprole used.

KEYWORDS: Degradation, farmyard manure, soil moisture regimes, concentration

INTRODUCTION

Pesticides are chemicals intended for preventing, destroying, repelling, or mitigating any pest during the production, shipment, transit, storage or distribution of any food or agricultural product. Only 10 per cent of the applied pesticide reaches the targeted pest and remaining 90 per cent will get transported through air, soil and water (Moses *et al*., 1993). The pH of soil, type of clay, extent of organic matter, amount of pesticide reaching the environment and moisture regime are the most important parameters influencing the transfer and transformation pathway of pesticide in soil (Worrall *et al*., 2001). The length of time a chemical remains in soil without losing the molecular integrity, physical, chemical and biological characteristics through which it is transported and distributed is termed as "soil persistence" (Navarro *et al*., 2007) of that chemical. Half-life is used to evaluate the persistence. Some pesticides those are resistant to transformation last longer in the soil, posing a greater risk to the environment and subsequent crop. Pesticides and their toxic metabolites persisting in soil is a major global concern since it leads to an increase in hazardous load in the ecosystem (Luo *et al*., 2008; Das and Mukherjee, 2012).

Cyantraniliprole (Benevia) is a novel broad spectrum systemic insecticide of anthranilic diamide category. Its mode of action is ryanodine receptor activation; hence it is coming under Insecticide Resistance Action Committee (IRAC) group 28. Documented half-life of cyantraniliprole in soil under aerobic condition is 16.20-89.4 days (PPDB, 2019). In India it is registered for utilisation in six crops including grapes, pomegranate, chilli, tomato, cabbage and gherkins for the control of both

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lepidopteran and dipteran pests (CIBRC, 2021). Even though highly effective against agricultural pests, cyantraniliprole is highly toxic to aquatic and benthic vertebrates and honey bees. The major metabolites are IN- J9Z38 and IN-JCZ38. The mobility of the compound was found to be moderate with a water solubility of 14.2 mg l^{-1}, the dissipation kinetics of cyantraniliprole in soil given main emphasis and was done in sandy loam soils characterized by low clay content, high porosity and faster leaching. Hence the present study aims to investigate the effect of various moisture regimes on persistence of cyantraniliprole.

MATERIALS AND METHODS

Soil required for the study was sampled (0-15 cm top soil) using tube auger and spade from Kazhakkuttam, Thiruvananthapuram, Kerala, India with no recent history of pesticide application. The soil was ground, shade dried and sieved through a 2 mm mesh screen. The physico-chemical properties of the soil *viz*. pH, organic carbon, and percentage of sand, silt and clay fractions (textural analysis) in the soil, the major nutrients were estimated adopting standard analytical procedures.

Analytical standard of cyantraniliprole (99.2% purity) was procured from Bayer Crop Science (Frankfurt, Germany). Cyantraniliprole (Benevia 10.26 % OD), was obtained from M/S Bayer CropScience. The reagents such as methanol, acetonitrile used were of analytical and HPLC grade. The chemicals PSA, NaCl, Na_2SO_4 and $MgSO_4$ were activated at 450° C for 4h before use. The organic manure for the soil amendment was dry powdered cow dung which was procured locally.

The Dionex Ultimate 3000 UHPLC system (Thermo Scientific) was used for chromatographic separation using column Accucore a Q column (100 x 2.1, 2.6µ) maintained at a temperature of 30 °C. Elution was done using two elutents (solvent mixtures). The mobile phase consisted of [A] 0.1 per cent formic acid + 5 mM ammonium formate in water; [B] 0.1 per cent formic acid + 5 mM ammonium formate in methanol. The flow rate was maintained at 0.30 ml min^{-1} and 6 minutes run time. Then the effluent from LC was introduced into Thermo Scientific TSQ Quantiva mass spectrometer. The source parameters were, ion source type is H-ESI (Heated electrospray ionization), Sheath gas, 60.00 (Arbitrary units), Auxillary gas, 5.00 (Arbitrary units) and Sweep gas, 1.00 (Arbitrary units) with ion transfer tube temperature, 320 °C and ion spray voltage source of 3800 V (positive ion). The vaporization temperature is 200 °C. The residues were quantified in MS/MS system (Rao and Davidson, 1980).

Studies on the persistence and dissipation of cyantraniliprole in normal sandy loam soils and 0.5 per cent dry farm yard manure (FYM) amended soil were performed under different conditions *viz*., air dry, field capacity and saturation at three levels (1, 2 and 4 mg kg^{-1}) in the laboratory incubation condition. The commercial formulation of cyantraniliprole (Benevia) was used for the study. For conducting the laboratory study, one kg each of the normal soil and FYM amended soil maintained at air dry, brought to field capacity (90 ml kg^{-1} of sandy loam) and in saturated condition (2-3 cm layer of water) were taken in conical flask and spiked separately at 1, 2 and 4 mg kg^{-1} levels of cyantraniliprole, homogenized and kept aside for 2 hours (0^{th} day). Whole apparatus is kept under incubation chamber. Ten gram soil was taken from the conical flask in triplicate and analyzed for residue estimation of cyantraniliprole. Likewise, samples were drawn on 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 10^{th}, 15^{th}, 20^{th} and 30^{th} day after application for estimation of residues and to identify the metabolites formed if any. Residues of cyantraniliprole persisting at different time intervals were estimated, from which the half-life was calculated.

The general procedure for pesticide residue analysis is sample collection, extraction, clean-up and estimation. The extraction and clean-up of residues of cyantraniliprole from soil was performed by QuEChERS method, wherein soil samples were extracted using acetonitrile and the efficiency of extraction was assessed. For this a 10 g of soil samples were weighed in 50 ml centrifuge tube, to which, 4 g magnesium sulphate, 1 g sodium chloride and 20 ml acetonitrile were added. The mixture

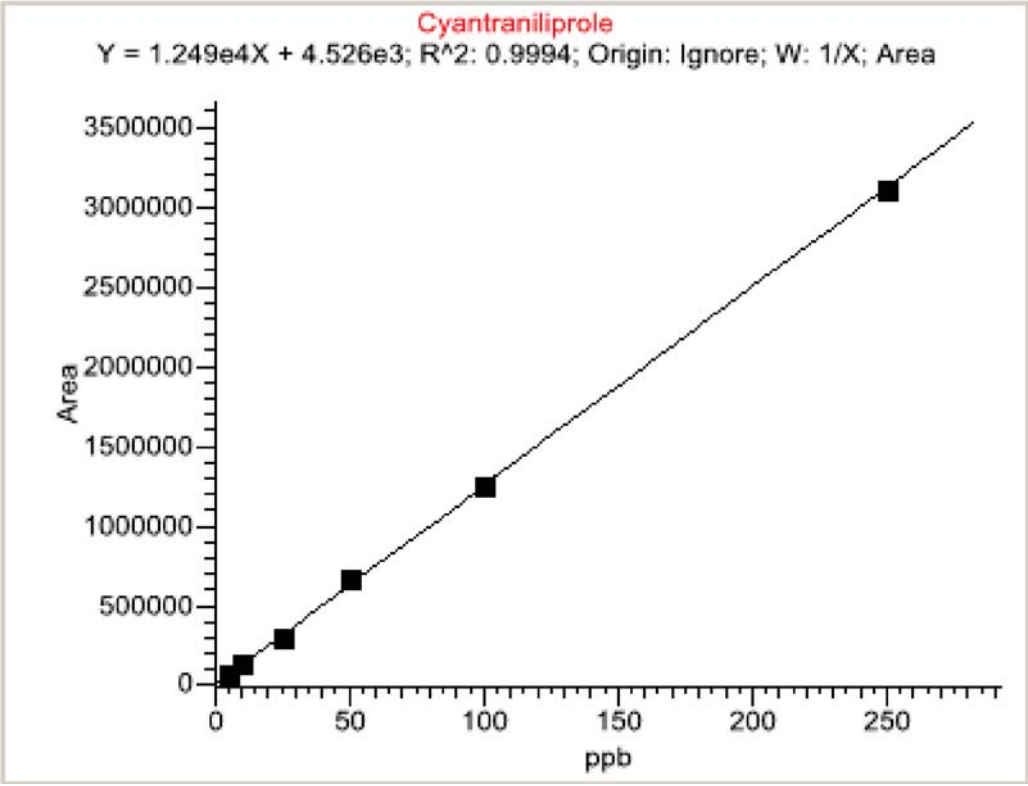

Fig. 1 Calibration curve (Linearity Curve) showing $R^2 = 0.9994$

shaken for 2 minutes in a vortex shaker and was centrifuged for 4 minutes at 3300 rpm. Ten ml supernatant was transferred to a 15 ml centrifuge tube using a micropipette and 0.25 g primary secondary amine and 1.5 g magnesium sulphate were added and was shaken for 30 seconds in a vortex followed by centrifugation at 4400 rpm for 10 minutes. After the centrifugation, 4 mL of the cleaned supernatant extract was transferred to a turbo tube and evaporated to dryness at 40°C using turboVap. The dry residue was redissolved in methanol and the volume was made up to 1ml, filtered through 0.22µm poly vinylidene fluoride (PVDF) syringe filter and collected in a glass vial and injected to UHPLC for estimation and confirmation of residues.

The single laboratory analytical method was validated in terms of linearity, specificity, limit of quantitation, limit of detection, accuracy and precision as per European Union guidelines (Sante, 2021) prior to real samples analysis. For recovery studies, untreated soil samples were fortified at 0.01, 0.05 and 0.1 mg l^{-1}. Linearity was assessed with the coefficient of correlation (R^2) derived from 5-point calibration. Absence of any peak at or near propinquity of retention time of the target molecule indicates the specificity of the method. The lowest concentration with signal to noise (S/N) of 3:1 was considered as the limit of detection (LOD) and the S/N of 10:1 was considered as the limit of quantification (LOQ) reliable linearity within a range of 0.01 to 1 µg ml^{-1}. The accuracy was expressed in terms of recovery (%) and the relative standard deviation of repeatability (RSDr %) was accounted as the precision of the method. The recovery of cyantraniliprole from soil was determined by analyzing the fortified samples in triplicate. The residue dissipation and half-life of cyantraniliprole were subjected to statistical analysis outlined by Hoskins (1961). $C_t = C_o e^{-kt}$, where C_t indicates concentration of cyantraniliprole at time t, C_o shows the initial deposits after application and k indicates rate constant of degradation. The half-life ($t_{1/2}$) is expressed as the time required to reach the half of

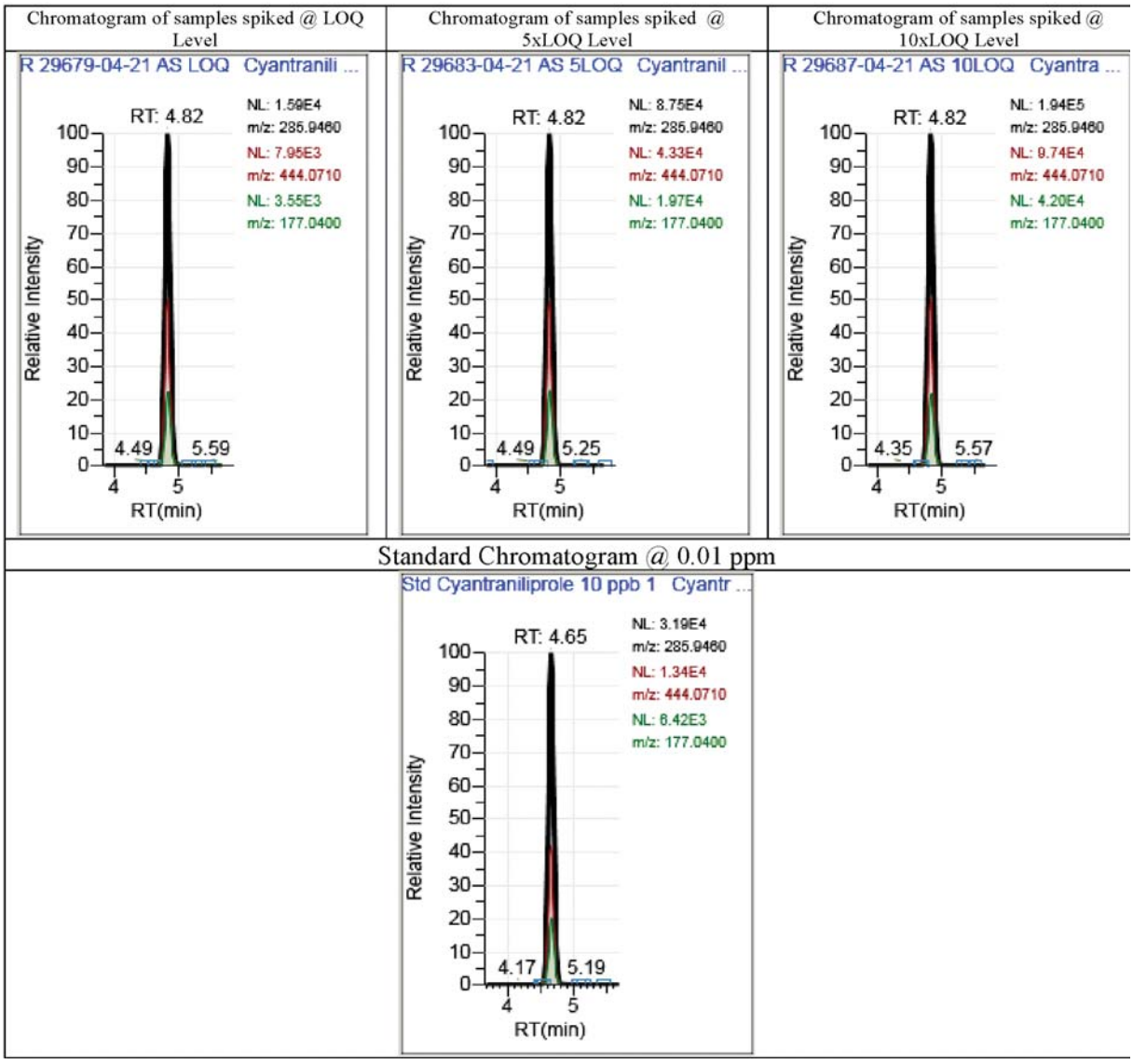

Fig.2 Chromatograms of spiked samples at three different levels

Table 1. Mass depending parameters in LC-MS/MS

Retention time (min)	Precursor ion *Q1*	Product ion *Q3*	Collision energy	Quantitative/ qualitative
4.82	475.04	177.04	42.815	Quantitative
		285.946	13.539	Qualitative
		444.071	19.455	Qualitative

initial concentration and was calculated from the equation, $t_{1/2} = \ln (2) / k$.

RESULTS AND DISCUSSION

The residues were quantified in MS/MS system. The compound dependent parameters of cyantraniliprole are retention time (min), precursor ion Q1, product ion Q3 and collision energy (Table 1). To validate the suitability of the analytical method for the experimental samples, various analytical parameters were tested, including recovery, precision, linearity, quantitation limit (LOQ) and detection limit (LOD). The values of recovery experiment obtained in the validation indicated the efficiency of the method adopted for extraction and clean-up of residues. The method showed a good linearity from 0.001–0.25 mg kg^{-1} with coefficient of correlation, R^2 value greater than 0.9994 (Fig.1).

The soil used for the study was sandy loam in texture with a proportion of 65.9 per cent sand, 22.3 per cent silt and 11.8 per cent clay content. The bulk density and particle density of the soil were 1.59 and 2.63 Mg m^{-3}, respectively. The water holding capacity was found to be 18.21 per cent. The soil was found to be moderately acidic with a pH of 5.97. The organic matter content of the soil was 0.84 per cent. The availability of primary nutrients in the soil was 177.89 kg ha^{-1} of available nitrogen, 61.04 kg ha^{-1} of available phosphorous, 148.6 kg ha^{-1} of available potassium. The secondary nutrients such as calcium, magnesium and sulphur were found to be 182, 55.7 and 3.0mg kg^{-1} respectively. Thus, the soil was found to be low in available nitrogen, medium in available potassium and high in available phosphorus. The secondary nutrients were found to be deficient in soil (Table 2). The recovery of cyantraniliprole from soil samples fortified at 0.01, 0.05 and 0.1 mg kg^{-1} (Fig. 2) varied from 81.35, 86.56 and 90.26 per cent with RSD of 1.52, 3.25 and 5.11 per cent. Since recoveries were more than 80 per cent, and the RSD values below 20, the method was selected for extraction and estimation.

At 1, 2 and 4 mg kg^{-1} the half - lives were 27.60, 28.25, 30.45 days at air dry condition, respectively whereas it was 24.83, 25.44, 25.94 days at field capacity soil moisture conditions and 18.50, 20.33, 22.31 days at saturated conditions, respectively in normal soil. The data on the metabolism of cyantraniliprole under laboratory condition revealed that no major metabolites were detected in the soil in the experiment conducted for assessing the persistence of cyantraniliprole. In all the three soil conditions, slower dissipation of cyantraniliprole was observed in soils amended with 0.5per cent FYM as the half-life values increased to 28.21, 29.29, 32.44 days in air dry condition, 25.66, 25.97, 26.24 days in field capacity condition and 19.07, 21.15 and 23.73 days in saturated soil condition at 1, 2, 4 mg kg^{-1} levels of fortification. Soil moisture regimes do have an influence on the persistence in the soil and also for the soil amended with FYM. Maximum persistence of cyantraniliprole was observed in 4

Table 2. Physical and chemical properties of the soil

Parameters	Properties
Texture	Sandy loam
Sand (%)	65.90
Silt (%)	22.30
Clay (%)	11.80
Bulk density (Mg m^{-3})	1.59
Particle density (Mg m^{-3})	2.63
Porosity (%)	39.54
Field moisture (%)	8.20
Water holding capacity (%)	18.21
Hydraulic conductivity (mL min^{-1})	0.4
pH	5.97
Electrical conductivity (dS m^{-1})	0.30
Cation exchange capacity (cmol (+) kg^{-1})	4.30
Anion exchange capacity (cmol (+) kg^{-1})	1.21
Organic matter (%)	0.81
Available nitrogen(kg ha^{-1})	177.89
Available phosphorus (kg ha^{-1})	61.04
Available potassium (kg ha^{-1})	148.60
Exchangeable calcium (mg kg^{-1})	182.00
Exchangeable magnesium (mg kg^{-1})	55.70
Exchangeable sulphur (mg kg^{-1})	3.00

Table 3. Persistence of cyantraniliprole in soil

DAA	Residues (mg kg^{-1})					
	Sandy loam soil			0.5% FYM amended soil		
	T1 (1 mg kg^{-1})	T2 (2 mg kg^{-1})	T3 (4 mg kg^{-1})	T4 (1 mg kg^{-1})	T5 (2 mg kg^{-1})	T36(4 mg kg^{-1})
Air dry soil						
0	0.68±0.05	1.29±0.2	2.96±0.7	0.96±0.09	1.71±0.09	3.25±1.1
1	0.67±0.04	1.28±0.02	2.93±0.3	0.92±0.1	1.69±0.2	3.23±0.6
3	0.65±0.02	1.26±0.06	2.91±0.08	0.89±0.1	1.65±0.01	3.21±0.3
5	0.62±0.05	1.19±0.10	2.63±0.2	0.81±0.08	1.58±0.1	3.15±0.2
7	0.59±0.04	1.08±0.04	2.41±0.03	0.75±0.04	1.49±0.05	3.09±0.4
10	0.50±0.06	0.96±0.2	2.06±0.3	0.67±0.06	1.27±0.07	2.98±0.2
15	0.47±0.02	0.87±0.20	1.87±0.2	0.60±0.1	1.18±0.1	2.54±0.1
20	0.41±0.10	0.71±0.05	1.75±0.08	0.53±0.03	0.98±0.04	2.27±0.1
30	0.33± 0.02	0.68±0.01	1.62±0.01	0.48±0.01	0.91±0.01	1.73±0.09
$t_{1/2}$(days)	27.60	28.25	30.45	28.21	29.29	32.44
Field capacity soil						
0	0.61±0.01	1.13±0.6	2.43±0.1	0.65±0.01	1.17±0.1	2.41±0.2
1	0.57±0.02	1..09±0.6	2.41±0.3	0.63±0.04	1.10±0.1	2.35±0.1
3	0.51±0.01	1.01±0.6	2.37±0.3	0.57±0.05	1.03±0.02	2.21±0.5
5	0.48±0.01	0.94±0.7	2.31±0.07	0.51±0.01	0.96±0.01	2.13±0.5
7	0.41±0.02	0.85±0.4	2.24±0.05	0.49±0.02	0.87±0.03	1.76±0.4
10	0.38±0.01	0.79±0.5	1.98±0.1	0.41±0.01	0.81±0.07	1.53±0.1
15	0.31±0.02	0.62±0.4	1.69±0.4	0.37±0.02	0.68±0.05	1.37±0.2
20	0.29±0.01	0.58±0.3	1.51±0.1	0.34±0.04	0.61±0.01	1.29±0.06
30	0.27±0.03	0.53±0.1	1.12±0.4	0.29±0.03	0.54±0.01	1.17±0.1
$t_{1/2}$(days)	24.83	25.44	25.94	25.66	25.97	26.24
Saturated soil						
0	0.93±0.03	1.26±0.03	2.52±0.3	0.95±0.01	1.30±0.07	2.60±0.1
1	0.91±0.05	1.21±0.02	2.49±0.2	0.93±0.02	1.28±0.01	2.57±0.4
3	0.87±0.08	1.12±0.07	2.35±0.1	0.89±0.06	1.16±0.07	2.51±0.1
5	0.75±1.1	1.02±0.01	2.19±0.3	0.75±0.03	1.09±0.05	2.35±0.1
7	0.61±0.07	0.93±0.01	2.02±0.1	0.69±0.03	0.96±0.03	2.14±0.02
10	0.58±0.01	0.86±0.04	1.84±0.2	0.60±0.02	0.87±0.02	1.89±0.04
15	0.51±0.07	0.71±0.01	1.58±1.1	0.53±0.01	0.79±0.02	1.61±0.06
20	0.48±0.07	0.63±0.04	1.34±0.02	0.49±0.01	0.71±0.04	1.45±0.02
30	0.29±0.02	0.45±0.01	1.02±0.2	0.31±0.01	0.47±0.01	1.14±0.03
$t_{1/2}$(days)	18.50	20.33	22.31	19.07	21.15	25.73

DAA - Days after application

mg kg^{-1} fortified amended soil under air dry condition (Table 3).

The results are in accordance with the earlier findings of study of persistence of chlorpyriphos by George *et al*. (2007) where higher organic matter application increased the persistence and also persistence was found increasing with the increase in concentration of applied chemical. The increased retention of pesticide by the organic matter reduced its amount in soil solution and thus making it available for microbial degradation. The effect of moisture was prominent and longer persistence was observed under dry conditions followed by field capacity moisture and submerged condition (Gupta *et al*., 2008).The study on persistence and degradation of cyantraniliprole under various moisture regimes also showing similar results with higher persistence in air dry condition and the half-life obtained were 33, 28.9 and 18.2 days for air dry, field capacity and saturated condition respectively (Kumar and Gupta., 2020).

A faster dissipation of cyantraniliprole occurred in saturated soil conditions, could probably‘ be due to the anaerobic condition. Kulshrestha and Singh (1992) also reported a faster dissipation of pendimethalin under submerged soil conditions. Similarly, Smith *et al*. (1995) also reported faster dissipation of cyfluthrin under anaerobic soil conditions and George *et al*. (2007) reported faster degradation in flooded condition. The amended soil with 0.5 per cent organic matter showed an increase in the half-life at various concentration levels. Similarly, a higher persistence of fipronil was obtained in organic matter rich soils than in sandy loam soils (Mohapatra *et al*., 2010).

The moisture status of the soil affected the persistence of cyantraniliprole. From first day of application to 30 th day of application the residue concentration was found reducing. The half- life of the compound was found reducing when the moisture content was increased and the highest half-life was obtained for the soil under air- dry condition and the lowest under the saturated soil condition. As the concentration of fortification increased, the half-life also increased. The persistence data also revealed that the half-life was found slightly increased in the soil amended with farmyard manure in all the three soil conditions. None of the major metabolites of cyantraniliprole were detected in the persistence study.

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Microstructure of wing scales in butterfly species from Alagar Hills, Tamil Nadu, India

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ABSTRACT: Study undertaken to find the structural arrangement of scales in similar coloured regions within butterfly wings of different species revealed their scale structure was architecturally different. The scales under scanning electron microscope showed difference among butterflies. Scales of *Papilio polymnestor* (Cramer) had high concentration of windows, *Pareronica ceylanica* (Felder) with beads and other species such as *Talicada nyseus* (Guerin Meneville), *Atraphaneura aristolochiae* (Fabricius), *Junonia heirta* (Fabricius), *Neptis hordonia* (Moore), *Acrae violae* (Fabricius) and *Danus chrysippus* (Linnaeus) had network and lamina. Tonality of colours showed differences in the arrangement of scales.

KEYWORDS: Butterfly Scales, SEM, Nanostructure, Beads, Window, Lamina and Pillar

INTRODUCTION

Butterflies come under the order Lepidoptera that exhibit brilliant iridescence (Stavenga *et al*., 2008). Just like a pointillist painting the surface of the wing is a collection of coloured dots called scales. The scales of butterflies are detached easily when their wings are touched. Colours that appear in butterflies are diverse and serve different functions (Nobre *et al*., 2021). Even by using computer generated images, these colours are highly complex to reproduce. Colours are created in two different ways via pigments and nanostructures (Wu *et al*., 2012; Yoshidha *et al*., 2001; Zhu *et al*., 2009). The patterns on the wings enable butterflies to recognize their own species at a distance and differentiate between males and females. Many reports have shown two mechanisms for colour generation in butterfly wings (Tilley and Eliot, 2002). Pigmentary colours of organisms are produced by differential absorption of visible wavelength by pigment molecules (Fox, 1976). Majority of colour patterns in Lepidoptera correspond to pigments incorporated in scales or cuticle (Ghiradella, 1998). But the most beautiful tonalities of iridescent colour exhibited by some butterflies are generated by light interference in specialized scales (Vukusic *et al*., 2000). The structural colours of organisms are produced by physical interaction of light waves with biological nanostructures that vary in refractive index (Prum and Torres, 2003). Bright animal colours are some of the most intriguing and poorly understood natural phenomena that continue to preoccupy scientists working at all levels of scientific inquiry. Ghiradella (1998) carried out studies on butterfly scales patterning in insect cuticle and morphology of

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Andean butterfly population. It was found that vivid colours are caused by pepper pot nanostructures. Vertesy *et al.* (2006) observed the wing scale microstructure and nanostructure in butterflies and structural coloration of blue colour of three Lycaenids. Fiber optic spectrophotometry and 2D-fourier analysis have been used to investigate the physical mechanism of colour production in twelve Lepidoptera species of four families by Prum *et al.* (2006). Novel photo anode structures have been templated by using butterflies wing scale (Zhang *et al.*, 2009; Zhu *et al.*, 2009). Studies have been carried out in butterflies on their diversity (Alturi *et al.*, 2011; Devi *et al.*, 2021), and lifecycle (Appalanaidu and Venkataramana, 2010), but very limited studies have been carried out on the scales of butterflies which will have applications in nano material sector. Thus, the present work was carried out to investigate the structural pattern of scales pertaining to different colours on the wings of butterflies found in Alagar Hills, Tamil Nadu.

MATERIALS AND METHODS

Specimen collection and preparation

Samples of coloured butterfly wings were taken from the specimens collected from Alagar Hills (10°04' N; 78°12'E), Madurai, Tamil Nadu, India. Alagar hills is one of the reserve forest in Tamil Nadu which houses the richest diversity of butterflies (Sharmila *et al.*, 2020). The scales studied were blue, red, yellow and orange belong to the Pieridae, Papilionidae, Lycaenidae and Nymphalidae families. The eight species are *Pareronica ceylanica* (Felder), *Papilio polymnestor* (Cramer), *Talicada nyseus* (Guerin Meneville), *Atraphaneura aristolochiae* (Fabricius), *Junonia heirta* (Fabricius), *Neptis hordonia* (Moore), *Acrae violae* (Fabricius) and *Danus chrysippus* (Linnaeus).

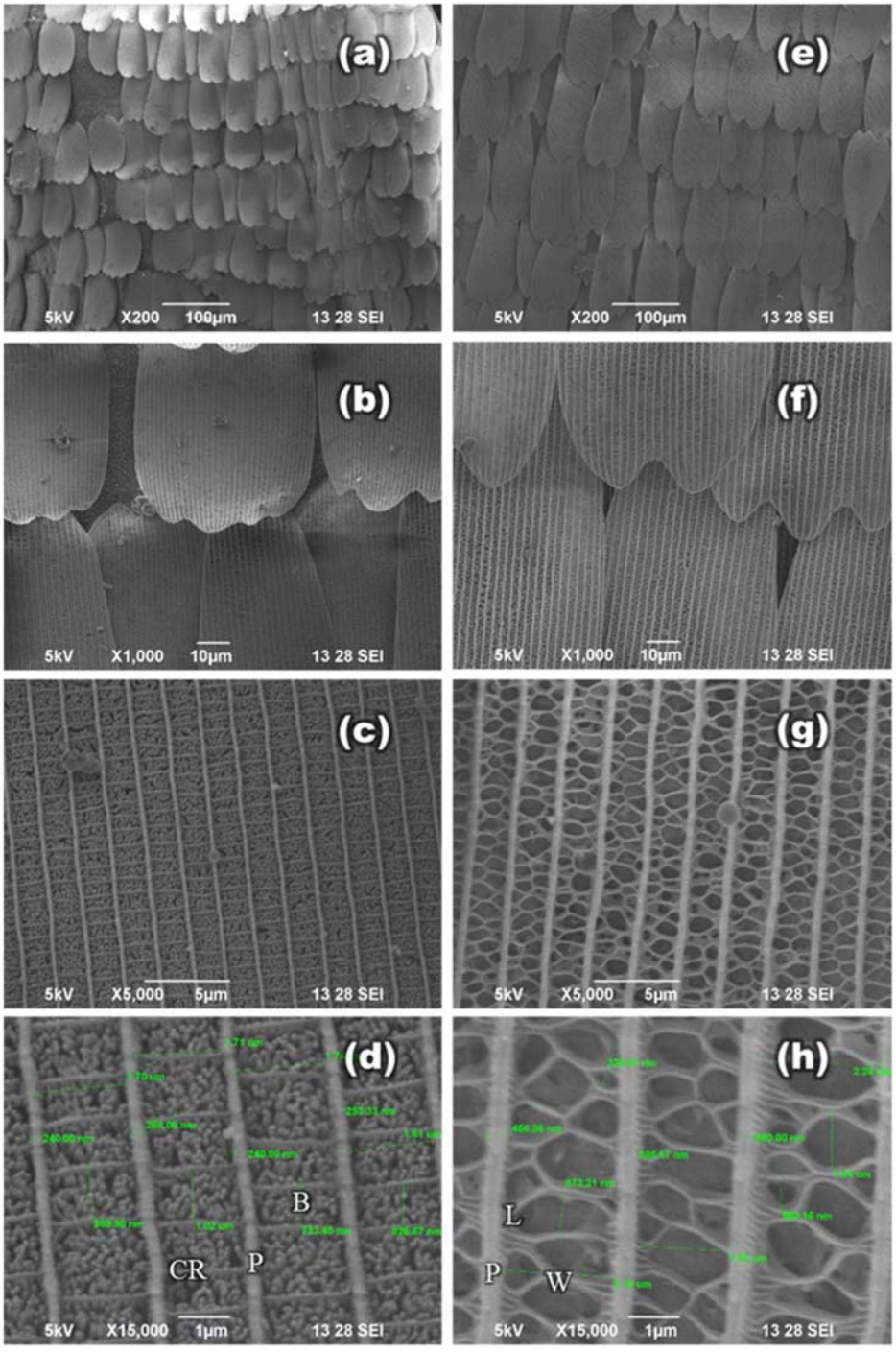
Scanning electron microscopy (SEM)

Dried specimens of butterflies were used to find out the qualitative approximation for the trends in different wing scales related to colour. The wing related to the specific colour was cut, stuck to microscope stubs and coated with iridium. The micro-configuration of scales was observed under the scanning electron microscope JSM – IT 300 L V (Prum *et al.*, 2006).The butterfly scales examined include four colours from eight species of four Lepidoptera families. The microstructures were categorized into pillar, window, lamina and cross rib for determining the wings architecture. Scales were observed in different magnifications such as 1µm, 5µm, 10µm and 100µm.

RESULTS AND DISCUSSION

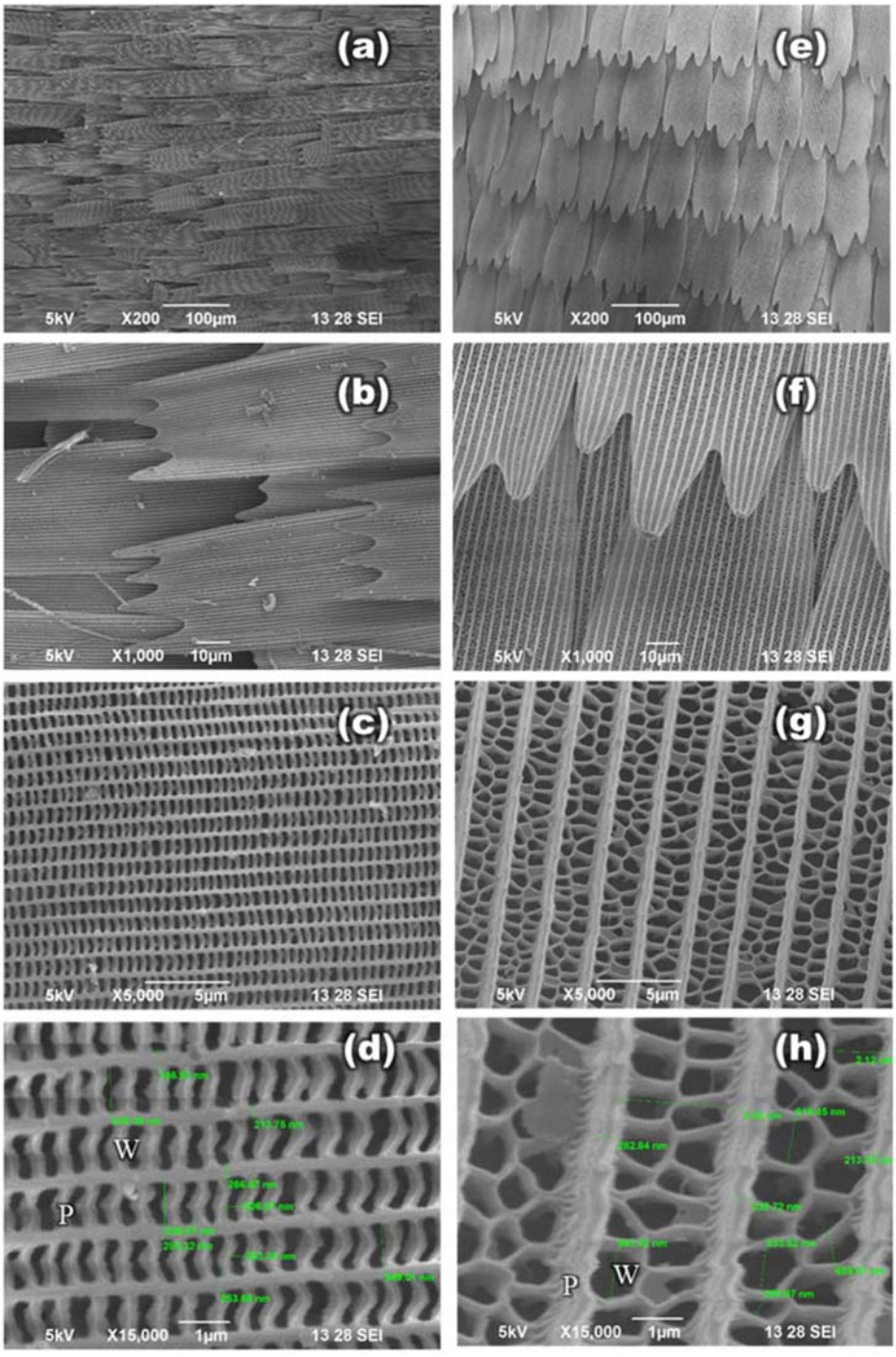
The present study highlights the diversity in the arrangement of scales for different colours in different families of butterflies. The SEM has helped in the investigation of butterfly wings not only in the scale shape but has helped to observe micro and nano-sized structures. *Pareronica ceylanica* and *Papilio polymnestor* with blue colour have scales that have grooves, but at higher magnification the pattern within the scales was different. The pillar of *P. ceylanica* were of the size 240-268nm and the distance of cross ribs ranged from 1.70 to1.74µm, and the cross ribs in between were filled with beads. The scales of *P. polymnestor* had pillars of the size 360-446nm and the distance of cross ribs ranged between 1.82 and 2.24µm, few open windows were found while some windows were filled with lamina. The structure between the cross ribs had network like arrangement (Fig. 1). *Talicada nyseus* and *A. aristolochiae* with red colour had teethed scales and the number of grooves vary between them. *T. nyseus* had pillars of the size 826-949nm and the distance between cross ribs ranged from 1.34 to 1.69µm, and showed open windows. In *A. aristolochiae* the pillar size ranged from 213-282nm and distance between pillars ranged between 2.12 and 2.34µm, and the open windows in a network like arrangement (Fig. 2).

In *J. heirta* and *N. hordonia* with yellow colour the scales look alike with grooves, but at higher magnification they showed slight difference. The pillars of *J. hierta* ranged from 248 to 333nm and the distance of cross ribs ranged from 824nm to1.07µm. *N. hordonia* pillars were in the range between 200 and 223nm and cross ribs ranged from 162 to 223nm. In *J. hierta*, the cross ribs were



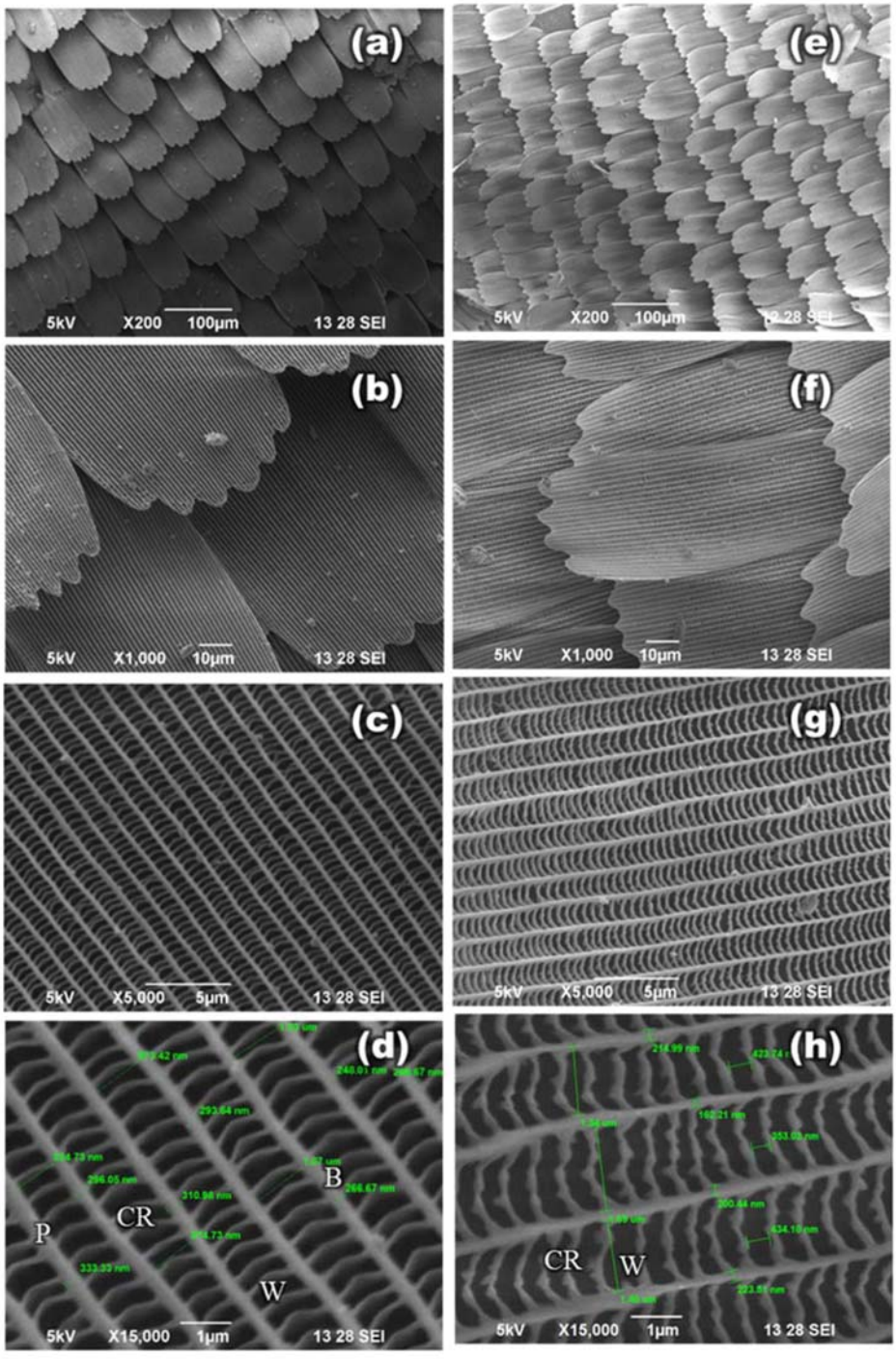
(a-d) Scales of *Pareronia ceylanica* ; (e-h) Scales of *Papilio polymenestor* P-Pillar; CR-Cross Rib; B-Beads; L-Lamina; W-Window

Fig. 1 Scanning electron micrograph of blue colour scales of *Pareronia ceylanica* and *Papilio polymenestor* butterflies



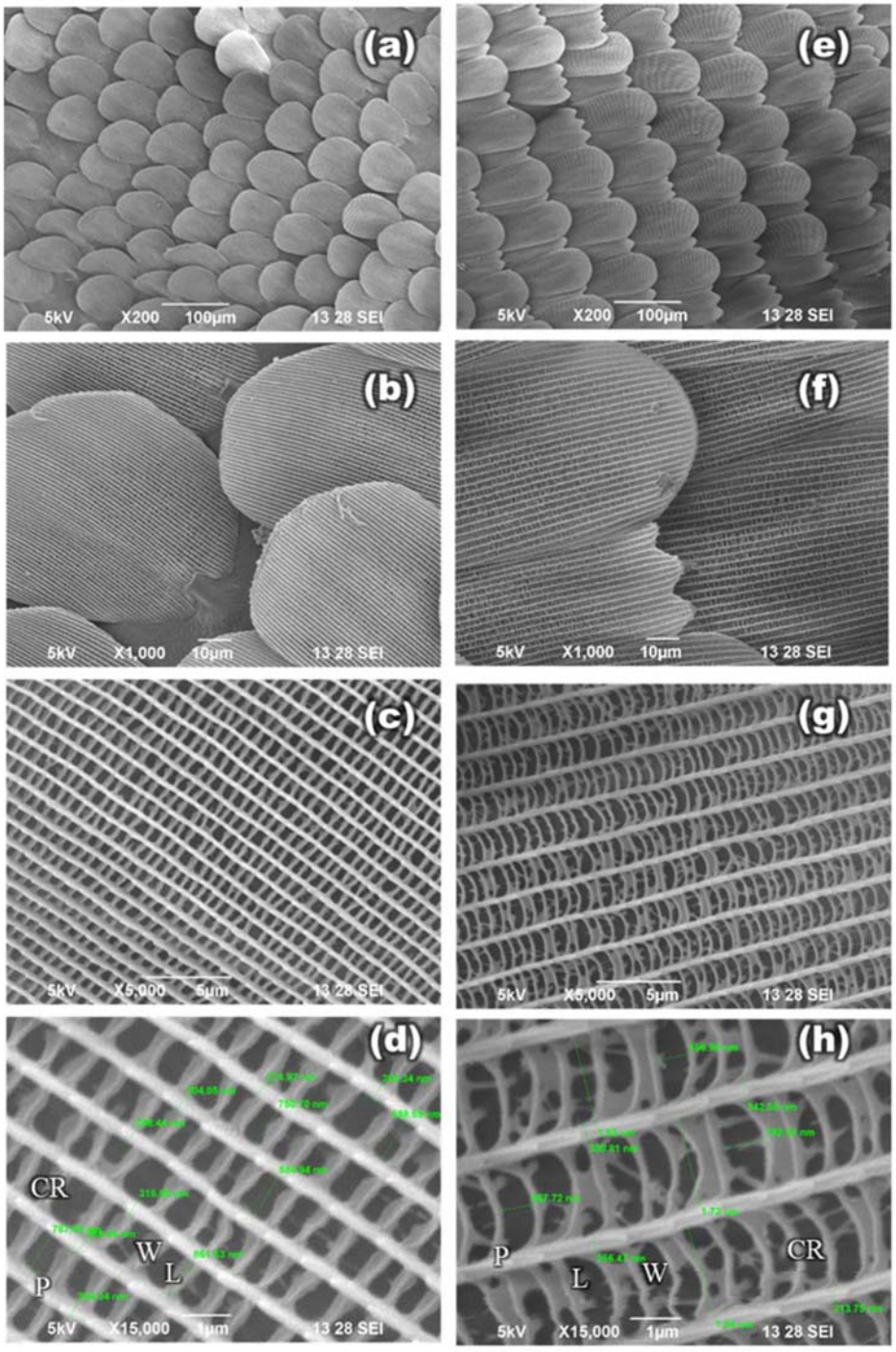
(a-d) Scales of *Talicada nyseus* ; (e-h) Scales of *Atraphaneura aristolochiae.* P- pillar; CR-Cross Rib; B-Beads; L-Lamina; W-Window

Fig. 2 Scanning electron micrograph of red colour scales of *Talicada nyseus* and *Atraphaneura aristolochiae* butterflies



(a-d) Scales of *Junonia hierta*; (e-h) Scales of *Neptis hordonia* P- Pillar; CR-Cross Rib; L-Lamina; W-Window

Fig. 3 Scanning electron micrograph of light yellow colour scales of *Junonia hierta* and *Neptis hordonia* butterflies



(a-d) Scales of *Acraea violae* ; (e-h) Scales of *Danaus chrysippus ;* P- Pillar; CR-Cross Rib; L-Lamina; W-Window

Fig. 4 Scanning electron micrograph of dark orange coloured scales of *Acraea violae* and *Danaus chrysippus* butterflies

smooth and in *N. hordonia* they had slight projection and beaded appearance (Fig. 3). *A. violae* with orange colour at lower magnifications exhibit the scales with smooth round edge, while the pillars size ranged from 274 to 304nm and the cross ribs ranged from 750 to 898 nm with the scales of open windows. *D. chrysippus* showed two types of scales which have smooth edge and with grooves. The pillar size ranged from 214 to 232nm and cross ribs of the size from 1.72 to1.84µm. Most of the windows had lamina and cross veins between them (Fig. 4).

Scales in the under surface are smooth and the upper surface has parallel ridges. The spatial structure of a scale depends on the type of butterfly. Distribution of beads had been seen in Pierid scales. The beads effectively absorb short wavelength light and at the same time scatter long wavelength light (Moorehouse *et al*., 2007; Parnell *et al.,* 2018). There was a slight similarity in the structure of scales of *J. heirta* and *N. hordonia* and for others there was a vast difference. Coloration in certain species of butterflies of subfamily Nymphalidae is due to species specific patterning of different coloured scales on their wings (Stavenga *et al*., 2014). The scales in *Morpho agea* showed pine shaped structures (Yoshioka and Kinoshita 2003). In *Uranea fulgens* they were characterized by laminar nanostructures of air cavities within the body of scales (Vukusic *et al*., 2000). In Marcedus Blue the upper surface of the scale is formed of high longitudinal ridges with open microcells (Balint *et al*., 2004). The present study also highlights that even though the colours in species of butterflies look similar, the architectural plan of the scales was different. The morphology of the scales had thrown light on their structure and the structure was unique to each species and colour. The construction of scale structure is malleable that allowing extravagantly varying shapes (Thayer *et al*., 2020). This study has limitations on explorations of different dimension of the nano-architecture however this investigation will add the biomimetic values from butterfly scales and promising application for nano-scale photonics in future.

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Note on *Thereuopoda longicornis* (Fabricius, 1793) (Scutigeromorpha: Scutigeridae) from Kerala, India

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ABSTRACT: Description and variations of *Thereuopoda longicornis* (Fabricius, 1793) from Kerala, India, with a key to two widespread species of the genus is provided. It is the first attempt to describe a scutigeromorph centipede from Kerala, and one of very few, from India.

KEYWORDS: Centipede, Chilopoda, Scutigeromorpha, *Thereuopoda*, first description

Being a tropical country with four biodiversity hotspots, India is home to all kinds of centipedes, except the order Craterostigmomorpha (Palita, 2016). But there have been no comprehensive contributions ever made to the taxonomy of order Scutigeromorpha in India. The genus *Thereuopoda* Verhoeff, 1904 comprises two geographically widespread species, i.e., *T. clunifera* and *T. longicornis*, each showing significant morphological variations across regions (Würmli, 1979). Scutigeromorphs are unique among centipedes in terms of their distinctive features such as compound eyes, multisegmented tarsi, dorsal tergal spiracles, domed head capsule, and the mode of deposition of the spermatophore. They are the only living representatives of the subclass Notostigmomorpha. Comprehensive molecular data, along with their unique morphological characteristics have placed them as the sister group of all other centipedes (Murienne *et al.*, 2010). Despite having a worldwide distribution, the Scutigeromorpha is the least studied and most poorly documented centipede order under the class Chilopoda, phylum Arthropoda (Negrea, 2003; Stoev and Geoffroy, 2004; Bonato *et al.*, 2010; Bonato and Zapparoli, 2011).

Globally, scutigeromorph diversity stands at around 200 described species. However, further re-examinations of the type specimens have synonymized many of them and reduced the current valid species to about 100 (Würmli, 1979; Stoev and Geoffroy, 2004; Minelli, 2006). The scutigeromorph classification was pioneered by Karl Wilhelm Verhoeff in the early 20th century. Currently, this order comprises three families, Scutigeridae, Pselliodidae, and Scutigerinidae. The latter two families are comparatively less abundant and their distribution is limited to the Neotropics and tropical Africa (Pselliodidae) and Africa and Madagascar (Scutigerinidae) (Koch and Edgecombe, 2006; Edgecombe, 2011). On the other hand, the family Scutigeridae enjoys an extensive distribution across the continents and is further divided into two subfamilies, Scutigerinae and Thereuoneminae (Acosta, 2003; Edgecombe, 2011).

The scutigeromorph diversity in India is never been recorded before and the country has recently gained

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momentum in centipede taxonomy, but it is limited to the order Scolopendromorpha (Khanna, 1998; Joshi and Edgecombe, 2018; Joshi *et al.*, 2020). Published data on other chilopod orders like Scutigeromorpha, Lithobiomorpha and Geophilomorpha from India are scanty. Order Lithobiomorpha and Geophilomorpha are comparatively better documented globally than Scutigeromorpha.

The present paper describes *Thereuopoda longicornis* (Fabricius, 1793), a scutigeromorph centipede that belongs to the family Scutigeridae and subfamily Thereuoneminae. The regional description of this species is useful because, Würmli (1979) observed this animal shows extensive morphological transitions at various levels (especially colouration, distribution of pigments, maximum length, shape of the head sutures, and female gonopod). He also argued that the widespread *T. longicornis* is not a homogenous species, as it is the most variable chilopod known until recently. In the same study, he synonymized many specimens from different regions, indicating the regional variation of this species. A distribution map (Würmli, 1979, fig. 26) indicates several widely distributed records of *T. longicornis* in India, including Kerala.

The specimen was obtained from Mayiladummugal, Thekkada (8°37’57.1"N; 76°57’19.8"E, elevation 128m above MSL), a village area located 23 km away from Ponmudi (the nearest stretch of the Western Ghats, 8°46’00.2"N; 77°06’41.0"E). The sampling site is a rocky area surrounded by rubber monoculture. Active searching in the possible hideouts within the selected quadrant (10x10 m) exposed the centipede under a rock (Fig. 1). The animal was captured alive and brought to the lab without losing any appendages. After microscopic examination (Labomed – Luxeo 6z), the specimen was preserved in alcohol (70%) and deposited in the museum of the Department of Zoology, University of Kerala. The identification was done by using the taxonomic key published by Würmli (1979).

Description

Length: 25 mm, total body segments - 15 (mature), sex- female (Fig. 2A, D)

Head: Globular slightly longer than wide. Large laterally placed eyes composed of many facetted ommatidia. Flagelliform antennae composed of a scape and three sections (flagella) composed of a large number of annuli, separated from each other by a node. The antennal annuli wider than long. The cephalic sutures have a characteristic “dog-leg” kink with their posterior part deflected outwards, and then the anterior part kinked inwards (Fig. 2B). The cephalic plate has a pale-yellow colour with dark greenish-brown patches at the center, on the anterolateral sides of the cephalic sutures, and behind the eyes. On the ventral side, the anterior margins of the coxites provided with four long spine bristles (Fig. 2G). A similar spine bristle also found at the trochanteroprefemurs of the forcipules.

Tergites: A brownish median longitudinal stripe passes along the tergites flanked by dark greenish-brown colouration on the lateral sides. The spiracles on the posterior ends of tergites long, with two swollen orange-brown stoma saddles on either side (Fig. 2F). The stigmatotergites elongated and have “shouldered” posterolateral margins rather than evenly rounded (two projecting swellings behind the stoma saddles) (Fig. 2E). The spines on the stigmatotergites strong and abundant (medially and on the stoma saddles) except the first, which has low spine count.

Legs: Length of legs increases from the anterior to the posterior (Fig. 2A). Each leg comprises six segments, including coxa, trochanter, prefemur, femur, tibia, and tarsi (I&II). On the ventral side, the coxa of each leg is provided with a long spine bristle. The distal ends of the prefemur, femur, and tibia of legs 1 to 14 are provided with spine bristles. The tarsi of walking legs are extensively divided into many annuli and form a long flagellum. The flagella of these legs (1 to 14) terminate with an apical claw. The ultimate legs are inserted parallel to the body axis and do not have apical claws.

Gonopods: Maximum length 1.3 times maximum width (ratio A/B of Würmli 1973, fig. 1). Proarthron 1.4 times length of mesarthron (ratio C/D of Würmli 1973). The sinus between mesarthron broadly parabolic. Width of mesarthron 0.5 maximum width

Fig. 1 Location and habitat type of *Thereuopoda longicornis*

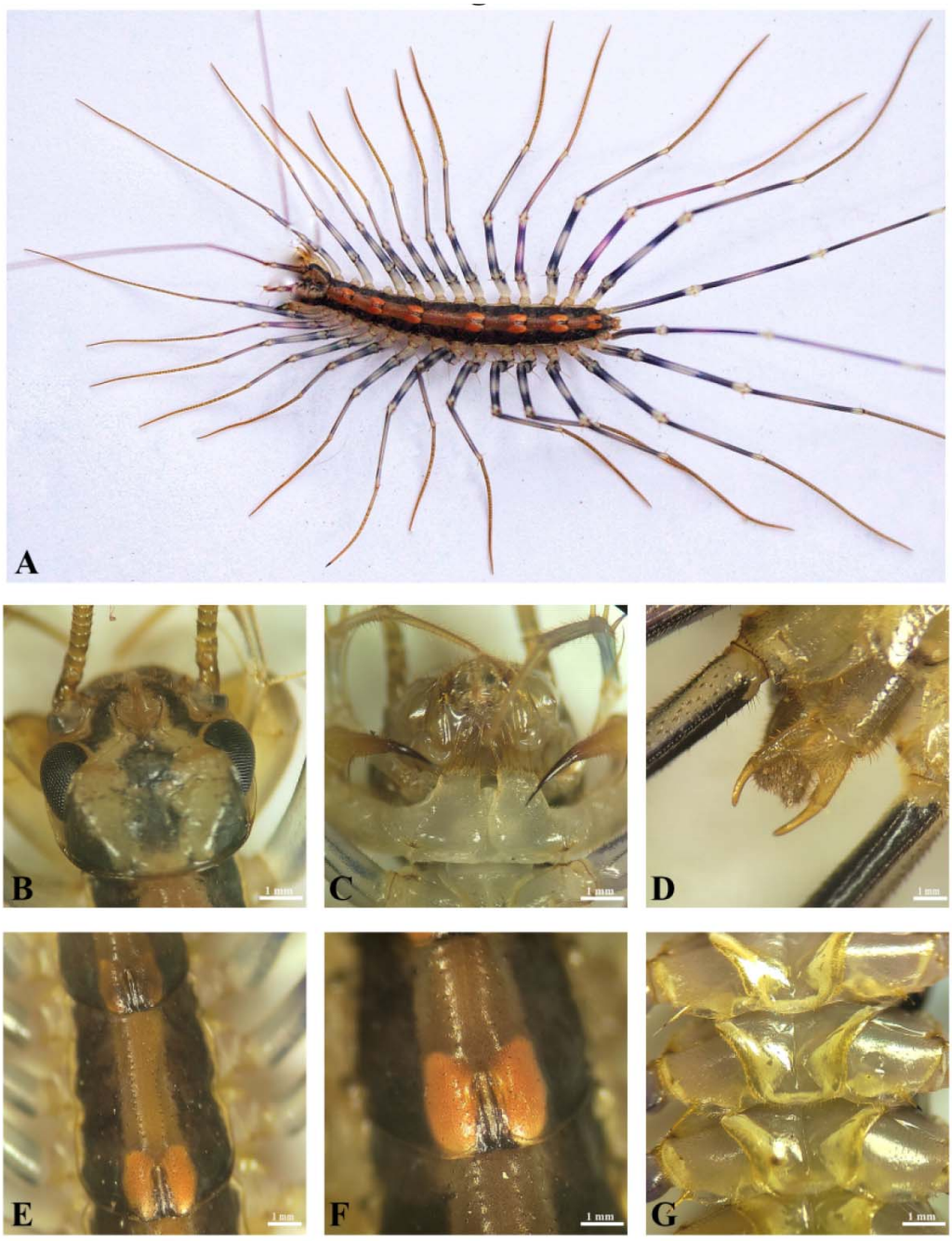

Fig. 2 *Thereuopoda longicornis* and parts

A - Thereuopoda longicornis
B - Cephalic plate and proximal part of antennae, dorsal view
C - Head ventral view showing the long spine bristles on the coxites
D - The female gonopods
E - Tergite (4th), dorsal view
F - Stoma saddles
G - Sternites, ventral view

of sinus (ratio F/G of Würmli 1973). Proarthron + Mesarthron 1.25 times length of metarthron (ratio C+D/E, Würmli 1973).

Assignment of the specimen to *Thereuopoda* is based on the kinked cephalic sutures, spines on anterior stigmatotergites, vaulted stoma saddles, straight ventral margin of the female subanal plates, and divergent metarthron of the female gonopods. Würmli (1979) delimited two geographically widespread species of Thereuopoda, i.e., *T. longicornis* and *T. clunifera* and considered the posterior border of tergites, the degree of divergence of the gonopods, colouration, the longitudinal stripe on the tergites, and the shape of female subanal plates as delimiting criteria. The present specimen has a pale-yellow cephalic plate with dispersed greenish-brown patches. The anterior ends of the head sutures show slight inward kink. The longitudinal stripe on tergites has apparent brown colouration rather than being colourless. Stoma saddles have a strong orange colouration that makes them prominent. These features not static as they vary in different regions, i.e., the strong inward kink of head sutures and colourless longitudinal median stripe in other variants (Würmli, 1979).

Key for the identification of two wide spread species of *Thereuopoda* (Würmli, 1979)

1. The posterior border of the tergites is evenly rounded; the gonopods often diverge less, more slender; in general blue-green with less brown pigments; there are no unpigmented longitudinal stripes on the tergites; the pigment is cloudy on the lateral thirds of the tergites, and they are lacking in greater rounded places; female subanal plates do not have appendixes…… *Thereuopoda clunifera* (Wood, 1862).

2. The posterior border of the tergites is unevenly rounded; the gonopods diverge more and are larger; colouration is brown to or dark brown, sometimes with blue-green pigment, especially in juveniles; a longitudinal stripe on the tergites is without pigmentation; the female subanal plates often have an appendix …… …… …… …… …… …… …… *Thereuopoda longicornis* (Fabricius, 1793)

Indian terrain supports different types of centipedes. Scutigeromorph taxonomy is still a puzzle and demands more robust contributions.

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First report of *Amegilla dizona* Engel and *Ceratina dentipes* Friese (Hymenoptera: Apidae) from Kerala, India

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ABSTRACT: Two bees from family Apidae, *Amegilla (Dizonamegilla) dizona* Engel, 2009 and *Ceratina (Neoceratina) dentipes* Friese, 1914 are reported for the first time from Kerala along with their current geographical distribution.

KEY WORDS: *Amegilla dizona*, *Ceratina dentipes*, geographical distribution

Apidae is one of the most diverse bee families in the world with three subfamilies, Apinae, Nomadinae and Xylocopinae (Michener, 2007). According to Ascher and Pickering (2022), Apidae contains 5950 described species around the world. In Kerala, 37 species under eight genera were reported so far (Prakash *et al.*, 2020). Apidae comprises social bees, solitary bees and also cleptoparasitic bees. *Amegilla (Dizonamegilla) dizona* and *Ceratina (Neoceratina) dentipes* are solitary bees. They belong to subfamily Apinae and Xylocopinae respectively. Both the bee species were collected from Kole wetland ecosystems of Kerala, which is globally acknowledged as Ramsar sites (Islam and Rahmani, 2008).

The genus *Amegilla* Friese is a diverse group with 19 species reported from India (Ascher and Pickering, 2022). Bees of the subgenera *Zonamegilla* Popov and *Notomegilla* Brooks of the genus *Amegilla* are commonly known as blue-banded bees, even though some of them lack blue bands (Leijs *et al.*, 2017). The subgenus *Dizonamegilla* Brooks got its name from the presence of two white apical hair bands in the abdomen (T3 and T4) (Brooks, 1988). *A. dizona* possesses white bands instead of blue. It is an Indian species and the replacement name for this species is given by Engel (2009). *A. dizona* specimens were identified with the help of key and description provided by Bingham (1897).

The genus *Ceratina* Latreille of India consists of 21 reported species (Ascher and Pickering, 2022) and are commonly known as small carpenter bees. The subgenus *Neoceratina* Perkins consists of small, black or feebly metallic bees, widely distributed in Australian, oriental and Palearctic regions (Michener, 2007). Detailed description and key provided by Prashantha (2017) was helpful in confirming the identity of *C. dentipes*.

***Amegilla (Dizonamegilla) dizona* Engel, 2009**

Specimens examined: 2 ♀♀, 04.ix.2021, Srayilkadavu, Malappuram district (10°42' 4.32'' N; 76° 1' 38.64'' E), Coll. Anju Sara Prakash, sweep net, host plant: *Leucas aspera* (Willd.) Link, family Lamiaceae.

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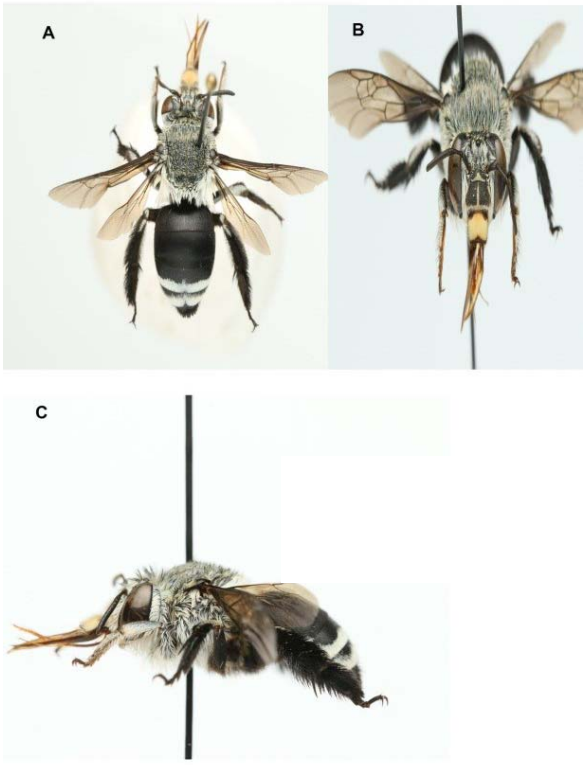

Fig. 1 *Amegilla (Dizonamegilla) dizona:*
(A) Habitus (dorsal view), (B) Head (frontal view), (c) Habitus (lateral view)

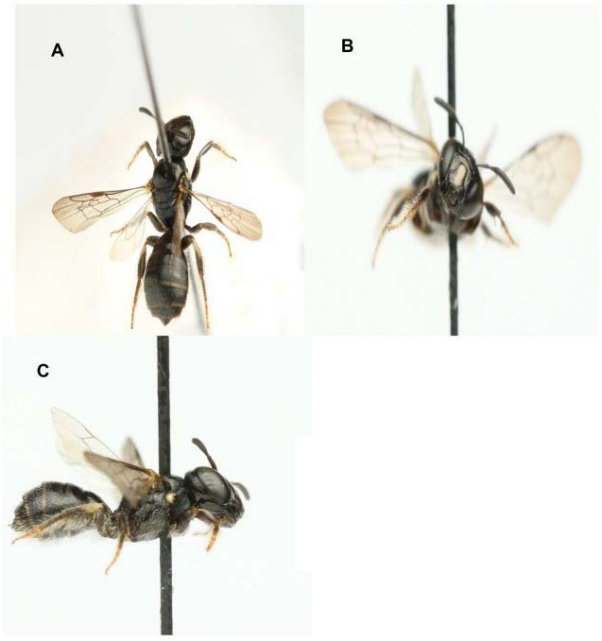

Fig. 2 *Ceratina (Neoceratina) dentipes:*
(A) Habitus (dorsal view), (B) Head (frontal view), (c) Habitus (lateral view)

Diagnosis (Fig. 1 A-C): Body length 13 mm; integument black; labrum and base of mandibles pale white; medial line, triangular mark above medial line and transverse sub-marginal line of clypeus pale white; vertex and dorsal part of thorax mixed with pale white and black pubescence; lateral and posterior margin of thorax with long white hairs; T3 and T4 with white apical hair bands; T2 with lateral white pubescent spot.

Remarks: According to Bingham (1897), the abdominal segment 4 and 5 has white apical hair bands. But by observing the specimens collected and following the terminology of Michener (2007), the pubescent bands are found in the 3rd and 4th abdominal segments.

Distribution: India: Kerala (new report), Puducherry, Odisha, Jammu and Kashmir (Ascher and Pickering, 2022), Tamil Nadu, Karnataka, Gujarat (Saini *et al.*, 2020), Rajasthan (Saini *et al.*, 2016), Allahabad (Uttar Pradesh), Kolkata (Bingham, 1897).

***Ceratina (Neoceratina) dentipes* Friese, 1914**

Specimens examined: 2 ♀♀, 04.ix.2021 and 18.ii.2022, Srayilkadavu, Malappuram district (10°42' 4.32'' N; 76° 1' 38.64'' E), Coll. Anju Sara Prakash, sweep net, host plant: *Aniseia martinicensis* (Jacq.) Choisy, 2 ♀♀, 16.ii.2022, Thommana, Thrissur district (10° 20' 46.68''; N 76° 15' 14.76'' E), pan trap collection.

Diagnosis (Fig. 2 A-C): length 4.63mm; Integument black, shiny; pale white or yellowish white markings on clypeus, pronotal tubercles, fore tibia and hind tibia; scape of antenna rectangular and flat; anterior and posterior margins of scutum finely punctate, middle portion impunctate; scutellum finely punctate; propodeal dorsum finely granulate; T1 without punctures; T2-T3 finely punctate; T4-T6 strongly punctate.

Distribution: India: Kerala (new report), Karnataka (Prashantha, 2017, Saini *et al.*, 2020), Nepal, Thailand, Vietnam, China, Singapore, Philippines, Indonesia, Papua New Guinea, Solomon Islands, Vanuatu, Fiji, Samoa, Cook Islands, French Polynesia, USA, Mauritania (Ascher and Pickering, 2022).

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Mulberry varieties for chawki rearing of *Bombyx mori* L. (Lepidoptera: Bombycidae) in subtropical conditions in India

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ABSTRACT: To identify best mulberry variety for chawkie rearing and its impact on economic characters of silkworm (*Bombyx mori* L.), experiments were conducted in three seasons viz., autumn -2019, spring -2020 and autumn- 2020, with six mulberry varieties (C-2038, Tr-23, PPR-1(S-140), S-1635, S-146 and G-2). Based on the weight of 10 mature larvae (g), cocoon yield by weight (kg), single cocoon weight (g), single shell weight (g) and shell ratio (%), S-1635 and S-140 (PPR-1) were found suitable for chawki rearing in sub-tropical conditions.

KEY WORDS: Silkworm, chawkie rearing, bioassay, biochemical analysis, moisture retention, cocoon characters

The silkworm *Bombyx mori* L. is a typical monophagous insect and mulberry is its sole food plant. The quality of mulberry leaf is one of the most important factors for the production of good cocoon crop (Ravikumar, 1988). The growth and development of silkworm larvae and the economic characters of cocoon are known to be influenced by the nutritional content of mulberry leaves (Krishnaswamy *et al.*, 1971; Machi and Katagiri, 1991; Singhal *et al.*, 2005; Rahmathulla, 2012). All through the first four instars and former half of fifth instar, the mulberry leaf consumed is invariably utilized for only its growth. The leaf consumed during latter half of the fifth instar, on the other hand, is utilized for building the cocoon (Nair and Kumar, 2004). About 92.20 per cent of the silk produced in the world is obtained from *B. mori* reared solely on mulberry leaves (*Morus* spp.). It is well-established fact that in sericulture, more than 60 per cent of the total cost of cocoon production goes towards mulberry production alone.

Mulberry varieties like S36, S41, S46, S54, S1, S146, S1635, AR12, AR14, TR10, BR2 were evaluated for nutritional potential and silkworm rearing. In recent years more new mulberry varieties like S1708, MS5, C6, C10, were evolved and variety S1708 turns out to be superior in bioassay tests compared to other varieties (Yoganandamurthy *et al.*, 2013 a, b, c). To identify best mulberry genotypes for chawkie rearing and its impact on economic characters of silkworm, experiments were conducted in three seasons viz., autumn -2019, spring -2020 and autumn- 2020, with six varieties.

Nursery of six mulberry genotypes viz; C-2038, Tr-23, S140, S1635, S 146 (as control) and G 2 was raised in January'2018. Bush (irrigated) type mulberry varieties with a spacing 3'X3' were planted in complete randomized block design replicated thrice. There were 24 plants in each variety under four treatment combinations (96 plants in each replication in three replications. Gap filling was taken

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up during the month of January, 2019. Maintenance was done as per recommended package of practices of RSRS, Sahaspur, Dehradun. Data on leaf yield, moisture per cent, moisture retention capacity after six and twelve hours, analysis of leaf quality for different biochemical parameters viz; concentration of total soluble carbohydrates (TSC), total soluble protein (TSP) and total chlorophyll content were recorded.

Moulting test of mulberry silkworm: During autumn 2019, in spring 2020 and in autumn 2020, moulting was more than 90 per cent in all six varieties, in all the three instars. Moulting per cent was recorded maximum in S-140 (PPR-1).

Bioassay studies: During autumn-2019, the cocoon yield/10000 larvae by weight (kg) was found maximum in S-1635 mulberry variety (18.440 kg). Single cocoon weight, single shell weight and S/R ratio, were 1.751g, 0.374g and 21.36 per cent, respectively, in S-1635 and were found statistically significant (Table 1). However, data recorded on other parameters such as fecundity, hatching per cent, larval period and weight of 10 matured larvae was found statistically non-significant in all the three rearing seasons.

During spring the yield/10000 larvae by weight (kg) was maximum in S-1635 (17.267 kg). The variety also recorded maximum single cocoon weight, single shell weight and S/R per cent with 1.751(g), 0.370(g) and 21.10 per cent, respectively. It was followed by S-140 and was statistically significant on all the parameters to control. Bioassay studies

Table 1. Bioassay studies during autumn-2019, spring and autumn 2020

Variety	Autumn 2019				Spring 2020				Autumn 2020			
	Yield* (kg)	SCW (g)	SSW (g)	SR %	Yield (kg)	SCW (g)	SSW (g)	SR %	Yield (kg)	SCW (g)	SSW (g)	SR %
C-2036	16.000	1.551	0.301	19.41	15.530	1.611	0.311	19.31	13.267	1.524	0.306	20.09
Tr-23	16.110	1.590	0.330	20.75	15.620	1.600	0.310	19.38	12.577	1.615	0.322	19.94
S-140	16.660	1.490	0.290	19.46	16.770	1.721	0.350	20.38	14.400	1.653	0.350	21.17
S-1635	18.440	1.751	0.374	21.36	17.267	1.751	0.370	21.10	14.550	1.637	0.350	21.38
S-146	18.220	1.720	0.340	19.77	15.557	1.620	0.321	19.82	12.967	1.613	0.333	20.65
G-2	17.220	1.601	0.320	19.99	15.533	1.630	0.331	20.31	13.130	1.630	0.340	20.86
CD	0.190	0.015	0.017	0.992	0.190	0.015	0.017	0.460	0.498	0.041	0.015	0.717

*Cocoon Yield/ 10000 larvae by weight (kg); SCW - Single cocoon weight; SSW - single shell weight; SR - shell ratio percentage

Table 2. Moisture content of leaves during 2020

Variety	Spring		Autumn	
	*Leaf (g)	Moisture (%)	* Leaf (g)	Moisture (%)
C-2036	143	74.470	187	75.45
TR-23	136	72.694	138	74.74
S-140	212	76.343	255	77.17
S-1635	126	75.165	159	74.24
S-146	138	74.413	140	73.53
G-2	133	73.056	168	75.16
CD	29.8	1.522	8.3	1.68

*Fresh wt. of 25 leaves

Table 3. TSC, TSP and total chlorophyll of the varieties (mg/ g dwt)

Variety	TSC	TSP	Chlorophyll
S-1635	301.55	48.01	3.46
G-2	288.51	40.44	2.83
S-140	294.05	48.16	4.33
C-2038	244.50	30.40	2.84
Tr-23	255.58	48.06	3.63
S-146	251.99	37.73	3.73
CD	17.26	4.18	0.56

140 followed by Tr-23 and S-1635. Total chlorophyll content was maximum in S-140 followed by S-146, Tr-23 and S-1635. Concentration of TSC, TSP and total chlorophyll were found significant to control (Table 3).

Assessment of post cocoon parameters: During spring 2020, S-1635 and S-140 were found at par with others; however filament length was maximum in S-140. During autumn 2020, S-1635 and S-140 were found at par with others, however filament length was found maximum in S-1635. There were no differences in denier, renditta and Reelability among the genotypes (Table 4).

Table 4. Assessment of post cocoon parameters among varieties in spring and autumn

Variety	Filament length (m)		Denier		Renditta		Reelability (%)	
	spring	autumn	spring	autumn	spring	autumn	spring	autumn
C-2038	1070	821	2.6	2.5	7.95	7.50	80.04	78.67
Tr-23	926	924	3.0	2.5	7.08	6.50	82.13	81.35
S-140	1096	903	2.7	2.6	7.54	7.59	80.10	78.16
S-1635	901	937	2.4	2.4	7.50	7.07	79.79	81.21
S-146	798	859	2.6	2.4	6.54	7.21	81.96	80.20
G-2	958	936	2.8	2.8	9.03	6.77	79.06	80.22

during autumn revealed that yield/10000 larvae were higher in S-1635 variety (14.550 kg). The single cocoon weight, single shell weight and shell ratio percentage, 1.637(g), 0.350(g) and 21.38 per cent, respectively, were also higher in S-1635 followed by S-140 and it was found significant on all the parameters (Table 1).

Moisture and moisture retention capacity: During spring-2020 mean moisture (%) and mean moisture retention capacity (%), after 6 hours and 12 hours was found maximum in S-140 (PPR-1) followed by S-1635 and was significant on all the parameters to control. During autumn 2020 mean moisture per cent, after twelve hours was found maximum in S-140 and more than 60 per cent after twelve hours in S-1635 and was found significant on all these parameters to control (Table 2).

Bio-chemical analysis: TSC was higher in S-1635 followed by S-146 and G-2. TSP was higher in S-

As per the objective to identify best mulberry variety for chawkie rearing and its impact on economic characters of cocoon, varieties S-1635 and S-140 were found suitable for sub-tropical conditions.

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Wing scale patterns of *Hypolimnas bolina* (Linnaeus, 1758) (Lepidoptera: Nymphalidae)

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ABSTRACT: *Hypolimnas bolina* (L), exhibit a stunningly bright and lustrous colour wing patches. The present study is focused on the variety of pigmented scales that cover the wings of *H. bolina*. A total of 128 different types of scales (white, brown, blue as well as black scales) were investigated, which includes 63 morphologically different types of scales on the dorsal part and 65 scales from ventral side. For the analysis the scales are taken from the black and violet portion of dorsal part and brown and white colored patches in ventral region. Micrometry of scales on the dorsal side showed a length range of about 86.6 to 102.4µ and width range of 63 to 78.8µ. Dimension analysis of ventral region range from 86.6 to 106.3µ in length and width of 66.8 to 86.6µ. The shape and distribution of scale depends on their exact location on the wing which are responsible for boggling pattern and brilliant visual appearance.

KEYWORDS: Blue moon butterfly, micrometry, pigmented wings, scale dimension

Many butterflies exhibit specific spatial colour patches on wings that are stunningly bright and iridescent in their appearance generating a spectacular vision. The pigmented scales on the dorsal and ventral surface of the wing are responsible for the colour pattern (Smetacek, 2000). This form of coloration is phenotypically variable (Brunton and Majerus, 1995; Kemp, 2006). Structurally colour wing patches are blown up in males showing sexual dimorphic ornamentation (Kemp and Macedonia, 2006). *Hypolimnas bolina* (Linnaeus, 1758) also called the Blue moon or Great eggfly butterfly is a sexually dimorphic belonging to the family Nymphalidae under the order Lepidoptera. It is distributed from west to east, from Madagascar to Easter Island, and a north to south one from Japan to Australasia (Marsh *et al.*, 1977). The female is both monomorphic and a mimic of *Euploea* in the west region. Also polymorphic and most of the forms are non-mimetic in the east part. Polymorphism is a sex-limited character in the *H. bolina*. It is a black-bodied butterfly with a wingspan of about 7-8cm. The upper side of the wings is jet black, offset with three pairs of white spots, two on the forewing and one on the hindwing. These spots are surrounded by purple iridescence. In addition, the upper side of the hindwing bears a series of small white dots with brown surroundings.

The butterflies of *H. bolina* were collected from the premises of Sree Narayana College, Kollam (8°52 55 N; 76°36 4 E) by using handheld insect net. The scales were dislodged from the wing surface as per the standard method of Grodnitsky and Kozlov (1991). Scale samples were shredded from each pigmented region of wing separately on a slide. A drop of xylene was used for fixation of scales and the samples were studied under the light

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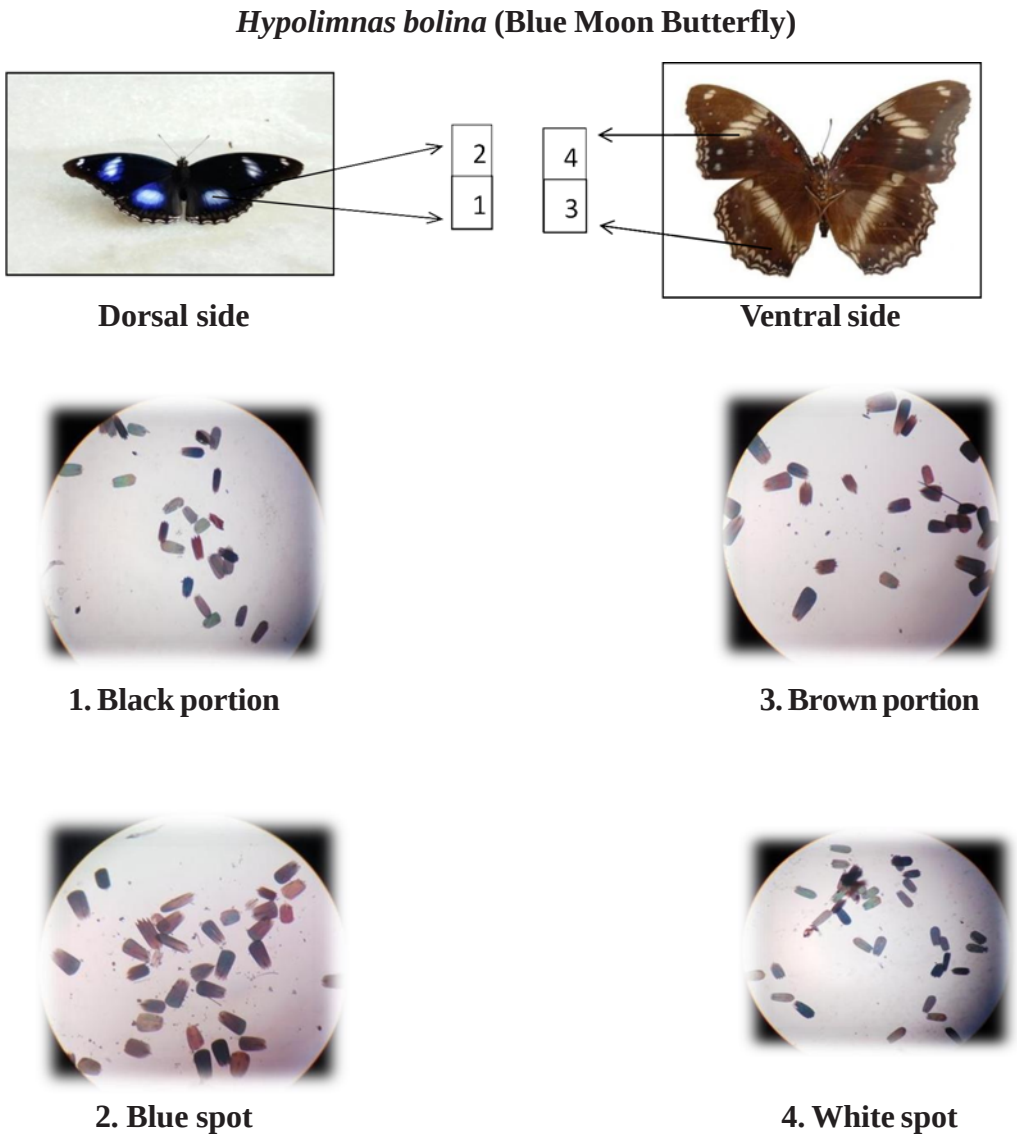


Fig. 1 Different types of scales - white, brown, blue as well as black scales

microscope. The measurements of the scales were made using micrometry. Photomicrographs of the sample scales were prepared for comparison.

In the scales extracted from distinct pigmented regions of the wing, a total of 128 morphologically different types of scales (white, brown, blue as well as black scales) were identified (Fig.1). There were 63 types in the dorsal side and 65 in the ventral side (Plate 1- 4). An examination of scales from the dorsal side revealed presence of white, blue as well as black scales (Plate 1). Majority of the scales were short and broad. The black region consisted of 32 different scales and the blue region contained 31 types. The apical part of certain scales seen with two or more pointed edges and through this structure difference between the scales are observed.

Plate 1

Dorsal side

1. Black region

Plate 2

2.Blue spot

Plate 3

Ventral side

3. Brown portion

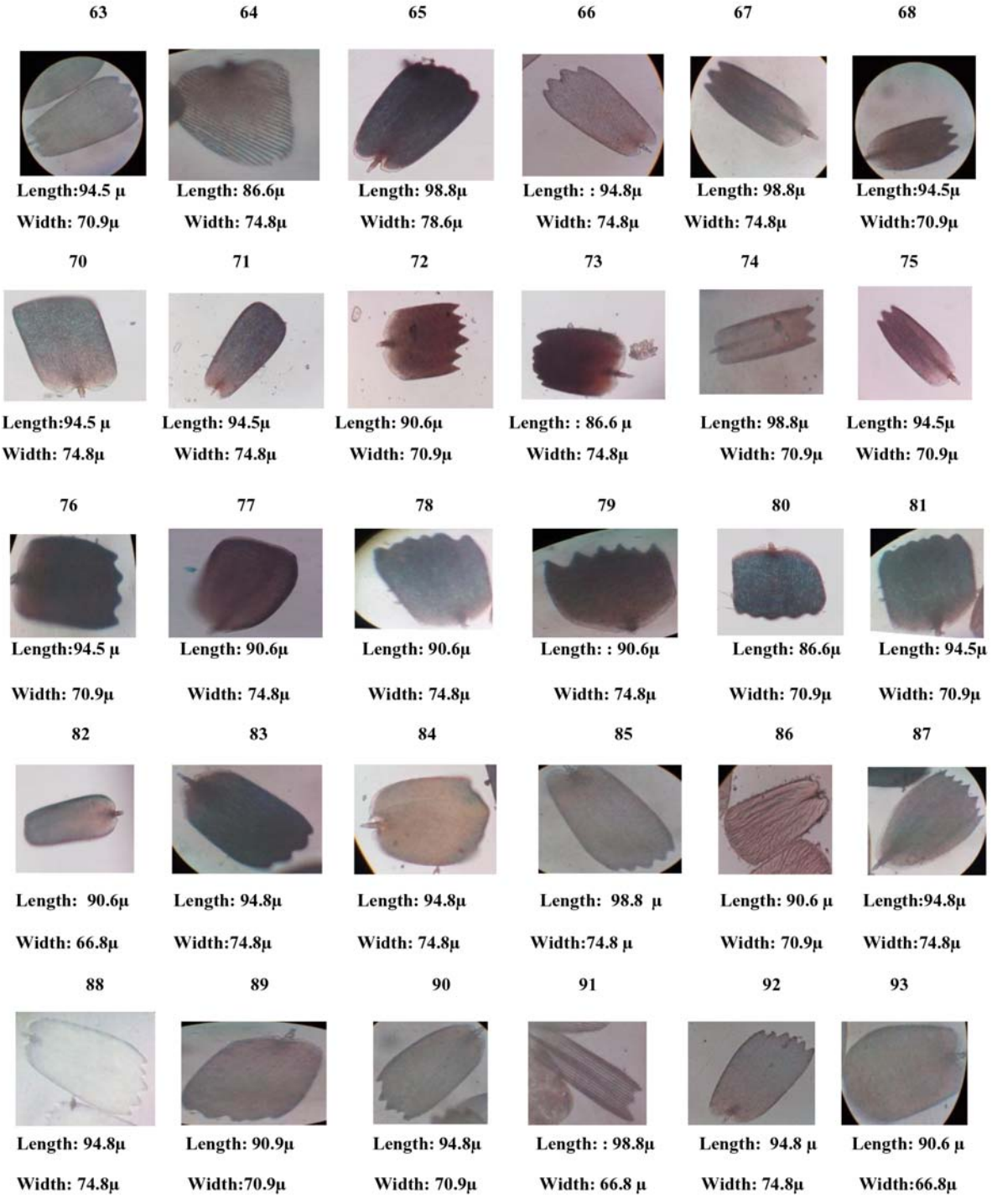
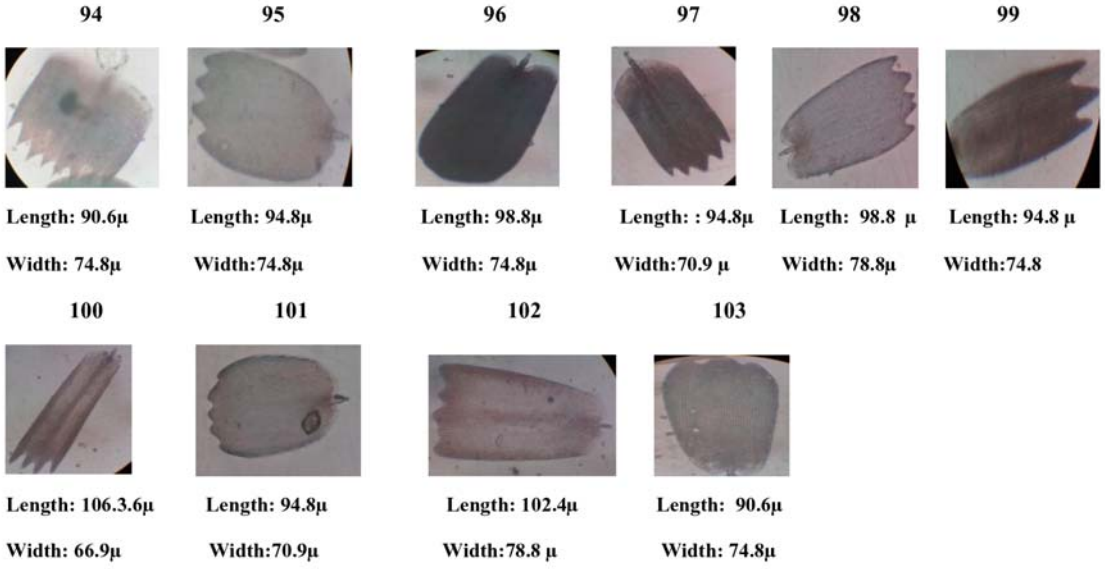
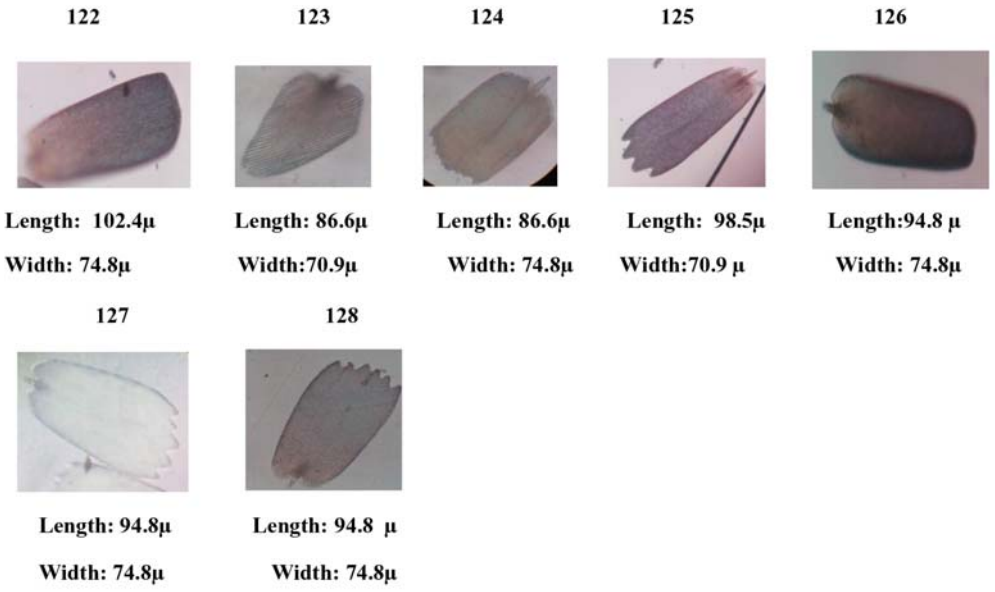


Plate 4



4. White spot

PLATE 5



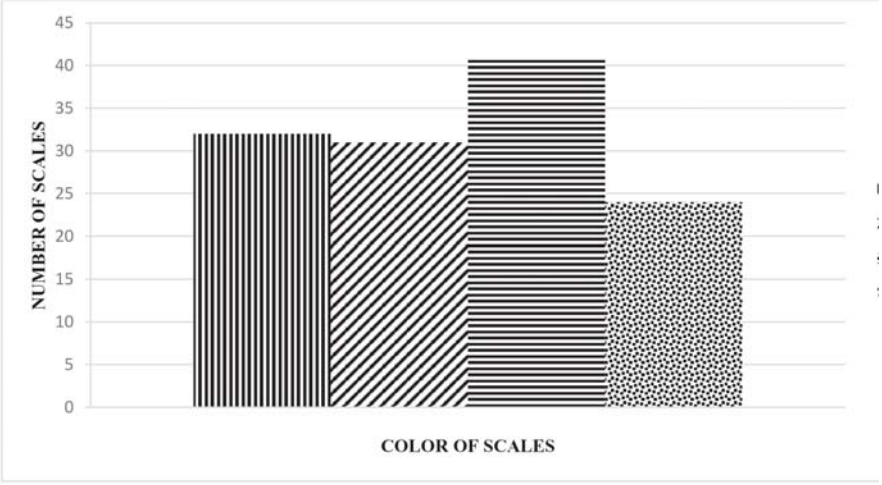


Fig. 1 Dorsal and ventral side pigmented wing scales in *Hypolimnas bolina*

The colour pattern and shape of the scales on the ventral side was similar to that of the dorsal wing. In the ventral side brown colour scales were relatively more. There were 41 types of brown scales and 24 white scales. The coloration of scales is based on the light absorbed by them. The scales on the ventral side were flat and wide. The dorsal scale ranged from 86.6 to 102.4µ in length and 63 to 78.8µ in width (Plate 1, 2) and in the ventral side scales, the length and width ranged from 86.6 to 106.3µ and 66.8 to 86.6µ respectively (Plate 3, 4).

Wing scale coloration in *H. bolina* exhibit high phenotypic variation, as a sexual ornament, and is a male-limited trait (Kemp and Macedonia, 2006). Total diffusive reflection spectra measured in different regions of the *H. salmacis* wing scales

was in agreement with *H. bolina* indicating similar kind of structural colouration wing scale pattern (Siddique *et al.*, 2016). The colour formation on the scale in *H. bolina* serves as sexual signals and exhibit sexual dimorphism (Kemp and Jones, 2001). The comparison of dorsal side and ventral side scales indicate that the shape of all scale types depends on the location of the scale on the wing. The knowledge on colour iridescence in scales of *H. bolina* is useful for studying mating preferences and signal variation with other *Hypolimnas* butterflies. Besides these several functions of the wing colour, a better understanding on the optical and radiative properties of wing scales provide a better vision on the biological behavior of butterflies. The mechanism of color interaction in wing scales of butterfly might be useful in the field of nano-optics and photonics.

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Cross sectional studies on the ectoparasites among rodents in scrub typhus cases in Karnal and Kaithal Districts of Haryana, India

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ABSTRACT: *Orientia tsutsugamushi* is a mite-borne bacterium belonging to the family Rickettsiaceae and is responsible for a disease called scrub typhus in humans, which is transmitted by the vector mite *Leptotrombidium deliense* (common ectoparasite on rodents) in most of Asian countries including India. The study conducted in selected villages of Karnal and Kaithal districts of Haryana state, India revealed four species of rodents - *Rattus rattus, R. norveigicus, Bandicota indica* and *Suncus murinus.* Dust mite *Dermatophagoides farina*; chigger mite *L. deliense and* fleas *Xenopsylla astia* and *X. cheopis* were prevalent on the rodents.

KEY WORDS: Vector mite, chigger mite, dust mite, flea, rickettsial disease

Scrub typhus is a rickettsial disease caused by *Orientia tsutsugamushi* a mite-borne bacterium belonging to the family Rickettsiaceae. It is transmitted to humans by the bite of infected vector chigger mites. The trombiculid chigger mites are common ectoparasites on rodents and belong to the genus *Leptotrombidium* (Acariformes: Trombiculidae). Of these, the most common are *L. pallidum* (Nagayo), *L. deliense* (Walsh), *L. scutellare* (Nagayo) and *L. akamushi* (Brumpt) (Acosta-Jamett *et al.,* 2020). Historically, scrub typhus had been endemic in Asia, Australia, and islands in the Indian and Pacific Oceans, known as the "tsutsugamushi triangle" (Bonell *et al.,* 2017). However, there have been recent reports of scrub typhus from Africa, the Middle East, and South America suggesting that the disease is no longer restricted to this triangle (Jiang and Richards, 2018). But no indigenous cases have been reported from North America and Europe. Scrub typhus is frequently reported from many Asian countries and is endemic in Nepal and its neighboring countries including India (Sub-Himalayan belt) and Bhutan, where it is considered an emerging infectious disease (Jeromie Wesley Vivian, 2017; Ranjan and Prakash, 2018; Tshokey *et al.,* 2018). Now it is re-emerging in almost in all states of India (Tilak and Kunte, 2019). The other vector mite spe-cies

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detected based on molecular techniques are *Ascoschoengastia indica* (Hirst), *Guntheria cassiope* (Womersley), *Odontacarus* sp. (Ewing), *Eutrombicula wichmanni* (Oudemans), and *Microtrombicula chamlongi* (Nadchatram and Kethley) (Elliott *et al.*, 2019). These mites contribute for the transmission in different regions of India (Ranjan and Prakash, 2018). Knowledge of the vector, including species, distribution, density, and habitats, is important to understand the epidemiology of scrub typhus in a given area or region (Park *et al.*, 2016). Clinical scrub typhus cases were reported in the districts of Karnal and Kaithal of Haryana state and most of them were confirmed in the laboratory of the PGIMER, Chandigarh. This study was undertaken with the main objective in finding out the vector mites among rodents that takes part in transmission of scrub typhus in the villages.

Study site: Haryana State has been contributing more number of scrub typhus cases from Karnal, Kaithal, and Panchkula districts with reference to the monthly periodical reports of National Centre for disease control (NCDC), Delhi, Based on that, a cross sectional entomological investigation was made in Sagger ($29^0$41'8.2644''N; $76^0$59'25''E) and Dadapur (28^0N; 76.6629^{0}E) villages of Karnal District; Pundri (29.7621^{0}N; 76.5546^{0}E) and Harsola ($29^0$48'5.51"N; $76^0$23'58.52"E) villages of Kaithal District of Haryana State. The GPS locations of areas from where rodents were trapped. Sherman and Wonder's traps were used. Single trap was placed in the scrub typhus positive house and two traps in the neighboring houses in each village. The sources of rat dwelling were identified by the presence of burrows, their paws, and excreta. Traps were laid at 6 PM and were collected in the next day morning by 6 AM (12 hours). A piece of Chapatti and roasted coconut were used as rodent baits. Rodents were identified using morphometric characteristics (Agrawal, 2000). Chigger mites were gathered from the rodents after they were euthanized using chloroform (Sigma–Aldrich, Bangalore, India) as described previously (Park *et al*., 2016). The chiggers were identified using the standard key for iden-tification of Indian *Trombiculidae* (Fernandes and Kulkarni, 2003). The identification features included the shape of the scutum, specialized leg setae, palpal chaetotaxy and chelicerae (Kuo *et al.*, 2015, Kumlert *et al.*, 2018; Philip *et al.*, 2021). The confirmation of the genus and species were based on pub-lished keys (Fernandes and Kulkarni 2003; Philip *et al.*, 2021). The dust mites were identified using the pictorial keys (Calloff and Stewart, 1997).

About 500g of soil and litter (humus samples) in and around rat burrows was collected and packed in zipped sachets and brought to the laboratory for detection of chigger mites employing Berlese's Funnel method. Berlese funnels are used for extracting the arthropods from soil and litter samples (Philip *et al.*, 2021). The organisms were identified using a binocular microscope (Dewinter) at 100X magnification. The Indices have been computed adopting the following standard formulae (Philip *et al*., 2021; Basker *et al*., 2022).

Chigger index: It is exclusively for Mite Borne Disease. It is measured by number of chiggers infested by a single host.

Prevalence rate of mites: Number of Hosts with ectoparasites / Total number of hosts examined

Mean intensity of ectoparasites from host animals: Total number of ectoparasites collected / Number of hosts infected with ectoparasites

Total flea index = Total number of fleas collected (regardless of species), divided by the total number of hosts examined.

Among the 12 traps laid at villages, viz., Sagger, Dadapur of Karnal District, and Pundri and Harsola villages of Kaithal District, six traps successfully trapped rodents. Four species of rats were encountered viz., *Rattus rattus, R. norveigicus, Bandicota indica and Suncus murinus*. Four species of ectoparasites isolated from the rats were - *Dermatophagaoides farinae, Leptotrombidium deliense* and fleas, *Xenopsylla astia* and *X. cheopis.* A total of 39 ectoparasites of all species could be collected. Among the mites, *D. farinae* was more prevalent (22 nos. and 56.4%) in rodents

Table 1 Prevalence of ectoparasites among the rodent species

Village	Rodents/ source	Ectoparasites - no./ species/(%)
Sagger	*Rattus rattus*	1 *Dermatophagoides* spp (4.55%)
Dadapur	*Bandicota indica*	17 *Dermatophagoides* spp (77.27%)
		5 *Trombiculid* spp (33.3%)
Dadapur	*Suncus murinus*	6 *Trombiculid* spp (40%)
Pundri	*R. rattus*	1 female *Xenopsylla cheopis*
		1 male *X. astia*
		3 *Trombiculidae* spp (33.3%)
		2 *Dermatophagiodes* spp (9.09%)
Harsola	*R. norveigicus*	1 *Trombiculidae* spp (6.7%)
		2 *Dermatophagoides* spp (9.09%)
Dadapur	soil and litter	2 *Dermatophagiodes* spp.

Table 2. Ectoparasites and their indices in scrub typhus reported villages in Karnal and Kaithal

Village (District)	Rodent	Dust mites – no./ (%)/ Index	Chiggers spp no./ (%) / Index	Fleas no./ Index	Prevale-nce rate	Mean intensity
Sagger (Karnal)	*Rattus rattus*	1 - (4.54%) - 0.5	0 - 0 - 0	0 - 0	0	0
Sagger (Karnal)	*Suncus murinus*	0	0 - 0 - 0	0 -0	0	0
Dadapur (Karnal)	*Bandicota indica*	17 - (77.27%) - 17	5 - (33.3%) - 5	0 -0	1	14
Dadapur (Karnal)	*S. murinus*	0	6 - (40%) - 6	0 - 0	1	14
Pundri (Kaithal)	*R. rattus*	2 - (9.09%) - 2	3 - (20%) - 3	2* - 1	1	7
Harsola (Kaithal)	*Rattus norveigicus*	2 - (9.09%) - 2	1 - (6.7%) -1	0	1	3

*1 female *Xenopsylla cheopis* and1 male *X. astia* flea

followed by chigger mite *L. deliense* (15 nos. and 38.4%) and species of fleas (2 and 5.1%). All the species were found on the rodents. From the soil and litter sample extraction *D. farinae* (2 no.) could be recorded (Table 1).

Chigger trombiculid mites, *L. deliense* were infested maximum on *S. murinus* (40%) followed by in *B. indica (*33.3%), *R. rattus* (20%) and *R. norveigicus* (6.7%*)*. Dadapur of Karnal, was identified as more vulnerable for scrub typhus. *D.*

farinae was more detected in Dadapur and most of them were infesting *B. indica*. The soil sample collected in and around the rodent burrows from Dadapur village also showed the presence of *D. farinae*. The chigger index of Dadapur was 5.5; in Pundri, chigger index was 3 and in Horsala of Kaithal district, it is 2. Among these villages, Dadapur showed higher chigger index. Since these indices are greater than the critical chigger index of 0.69 (Olson *et al.,* 1979; Basker *et al*., 2022), the probability of scrub typhus transmission in these villages is more. The prevalence rate of mite is one for all the rodent species. Mean intensity of ectoparasites from host animals is 14 each for *B. indica* and *S. murinus*. Of the fleas detected there were both the primary and secondary vectors of plague and were isolated from *R. rattus* which was captured in the semi-urban of Kaithal. Mean ectoparasites present per animal is dust mites 3.66, chigger mites 2.50 and fleas 0.33 (Table 2).

The bacteria *O. tsutsugamushi* cause scrub typhus and is transmitted by *Leptotrombidium* mites. It is responsible for a potentially fatal tropical infection which is a grossly under-recognized public health problem in India (Bonell *et al.,* 2017; Behera *et al.,* 2019). This disease is known to occur in diverse ecological settings in India with large numbers of cases being reported from Tamil Nadu, Andhra Pradesh, Karnataka and Kerala in the South, Himachal Pradesh, Uttaranchal, Jammu and Kashmir in the North, Meghalaya, Assam and Nagaland in the North-East, West Bengal and Bihar in the East and Maharashtra and Rajasthan in the West (Xu *et al*., 2017; Philip *et al.,* 2021).

Dust mites feed mostly on dead skin and hairs shed from humans. In Haryana state, dust mites *Dermatophagoides pteronyssinus* and *Chyletus malaccenus* were previously reported (Voorhorst *et al.,* 1969). House dust mites, especially certain species of pyroglyphids, are the cause of allergic reactions of the respiratory tract (asthma and rhinitis). In recent years, house dust mite allergy has been identified as a frequent cause of asthma, especially among children (Traub and Wisseman Jr, 1968).

Rodents are not only the reservoir of *O. tsutsugamushi* the causative organism for scrub typhus but also known to transmit human and animal diseases such as *Leptospira* spp, *Borrelia* spp., *Yersinia pestis,* and *Bartonella* spp. (Raharivolona and Ganzhone, 2009). In the present study, the potential primary and secondary plague vectors in India *X. cheopis* and *X. astica* have been isolated from the *R. rattus* which was captured from Pundri semi-urban of Kaithal district, Haryana and these species are potential enough to enhance the spread of plague within the communities in future if an outbreak occurs (Eisen *et al.,* 2014). It is imperative that high priority be given to the research and development of effective integrated rodent management programs against domestic, peri-domestic, and sylvatic rodent species to reduce the chances of parasite transmission. Since dust mite and chigger mite infestation found in rodents and soil, the surveillance on morbidity related to scrub typhus, allergy and other complications in the human community is highly essential.

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Evaluation of oviposition substrates and mating duration on fecundity and egg hatchability of *Samia ricini* Donovan (Lepidoptera: Saturniidae)

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ABSTRACT: Assessment of oviposition substrates and mating duration on the fecundity and egg hatchability of eri silkworm *Samia ricini* Donovan, revealed that maximum disease free layings were recorded in the control (353 eggs with 98 % hatchability) followed by the treatment, mating duration of six hours (332 eggs with 96 % hatchability). Poor egg laying and egg hatchability was noticed in one hour (151 eggs with 41 % hatchability). Correlation coefficients between mating duration, fecundity and egg hatchability showed strong positive correlation. Oviposition substrate's impact on fecundity and egg hatchability revealed that gada cloth gave better results (344 eggs with 96 % hatchability) whereas polythene cover recorded poor fecundity (203 eggs) and jack fruit kharika recorded poor hatchability (90 %). A minimum of six hours of mating duration is needed for the eri moth to attain its full fecundity potential and better egg hatchability.

KEY WORDS: Eri silkworm, substrates, gada cloth, correlation, regression

India is the second largest producer of silk in the world with a total raw silk production of 36,152 MT. Its uniqueness lies in having all the four types of commercial silkworm species. Among them, mulberry silk accounts for about 70.21% (25,384 MT) followed by eri silk 19.80% (7,157 MT), tasar silk 9.3% (3,370 MT) and muga silk 0.66% (240 MT) (CSB, 2020). Eri silkworm *Samia ricini* Donovan, is a polyphagous and multivoltine species, and 95 per cent of its total silk production in the world is contributed by India. Its primary hosts are castor (*Ricinus communis* Linn.) and kesseru (*Heteropanax fragrans* Roxb.). Secondary host plants include cassava (*Manihot esculenta* Crantz.) and many other plants belonging to the family Euphorbiaceae. Eri silk has excellent thermal properties and offers tremendous blending possibilities with other natural silks such as wool, cotton, jute and synthetic fibers (Gautam and Goel, 2006). Eri silkworm has its own advantage of being more disease resistant and requires fewer resources. Hence, it is known as poor man's silk. However, its potential remains unexploited because of poor quality eggs. In silkworm rearing, quality of eggs is the major factor affecting the cocoon yield. Production of good quality eggs (disease free layings - DFLs) and high fecundity rate are influenced by oviposition substrate and mating duration. A study was undertaken to assess different oviposition substrates and mating durations on fecundity and

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egg hatchability, in the Department of Entomology, Faculty of Agriculture, Annamalai University during 2021.

Eggs of eri silkworm were procured from eri silkworm seed production centre, Central Sericulture Germplasm Resource Centre, Hosur, Tamil Nadu. Eggs were surface sterilized with two per cent formalin and thoroughly washed in water and incubated at 26°C and 85 per cent relative humidity. Rearing room was disinfected seven days before rearing using five per cent bleaching powder solution and kept closed for 24 hours. Rearing room was well ventilated and fly proofing was done for avoiding uzi fly attack. Neonates were brushed immediately after hatching on young leaves in a plastic tray and covered with paraffin paper to maintain temperature and humidity. The worms were reared using leaves of castor. First and second instar larvae were fed two times a day and third, fourth and fifth instar larvae were fed thrice. Bed cleaning was done daily. Ripened fifth instar larvae were identified by gently rubbing, on which they produced sound of hollowness. They were transferred to plastic collapsible mountages (Netrikas) for cocoon spinning. Cocoon harvest was done five days after spinning. After harvesting the cocoons were cut opened and healthy pupae were collected for experiments. Collected healthy pupae were placed in cardboard box for adult emergence. After emergence, moths were allowed to mate for various mating durations *viz.,* one, two, three, four, five and six hours on gada cloth. Naturally decoupling pairs were treated as control. Each treatment was replicated thrice and each replication consisted of three pairs of eri moth (Subramanian *et al.,* 2012). After the respective mating duration was over, the male moths were separated gently without damaging their genital organ. Each mated female eri moth from respective replications was kept separately in cardboard boxes for egg laying. Fecundity was measured and the DFLs were maintained till fifteen days in the same cardboard boxes for calculating hatchability.

$$\text{Hatchability (\%)} = \frac{\text{No. of normal eggs} - \text{No. of unhatched eggs}}{\text{No. of normal eggs}} \times 100$$

Seven treatments *viz.,* Kharikas (sticks) of castor, mulberry, jack fruit tied vertically along a rope, plastic tray, cardboard box, polythene bags and gada cloth lined container were evaluated for their suitability as oviposition substrates. All the treatments were replicated thrice, and each replication consisted of three mated females. Naturally decoupled female moths were collected after mating and allowed to lay eggs in respective oviposition substrates. Fecundity and hatchability were calculated.

The experiments revealed that eri moth required a minimum mating duration of six hours to produce fertile DFLs with a fecundity of 300 – 350 eggs/ moth (Table 1). It was also found that when moths allowed for decoupling naturally the fecundity increased. Egg laying and hatchability was poor in one hour (151 eggs, 41 %), two hours (194 eggs, 48 %) and three hours (241 eggs, 66 %). Maximum fecundity was obtained in the control treatment (353 eggs, 98 %). This was followed by six hours mating duration (332 eggs, 96 %). Subramanian *et al.* (2012) who indicated poor egg laying when moths were allowed to mate for only an hour. However, their finding of reduction in fecundity below five hours mating duration was in contradiction to the present finding, wherein it was found that mating duration below 6 hours recorded poor egg laying and hatchability. Similarly, the findings of Behura and Panda (1978) who recorded that four hours of mating duration as minimum was also contradictory with present results.

Correlation between mating duration and fecundity indicated a strong positive correlation ($r = 0.992$),. Regression analysis revealed 98.41 per cent variation ($\beta = 0.9841$) in fecundity as influenced by mating duration. The regression equation indicated that for every one-hour increase in mating duration fecundity increased by 35 numbers of eggs. Correlation between mating duration and egg hatchability ($r = 0.971$) indicated a strong positive correlation. Regression analysis showed 97.1 per cent variation ($\beta = 0.971$) in egg hatchability as influenced by mating duration. The regression equation indicated that for every one-hour increase in mating duration, hatchability increased by 11 per cent.

Table 1. Effect of different mating durations on fecundity and egg hatchability

Mating duration	Fecundity*	Hatchability#
1 hr	151 (12.30)^{g}	41 (38.93)^{g}
2 hrs	194 (13.93)^{f}	48 (42.60)^{f}
3 hrs	241 (15.52)^{e}	66 (53.13)^{e}
4 hrs	253 (15.91)^{d}	71 (58.50)^{d}
5 hrs	292 (17.09)^{c}	92 (71.57)^{c}
6 hrs	332 (18.23)^{b}	96 (76.31)^{b}
Control	353 (18.80)^{a}	98 (80.12)^{a}
CD 0.05	1.920	5.556
SE(d)	0.887	2.545

Mean of three replications
*Values within parenthesis are square root transformed
#Values within parenthesis are arc sine transformed
Values with different alphabets with in a column differ significantly

Table 2. Effect of different substrates on fecundity and egg hatchability

Substrates	Fecundity*	Hatchability#
Castor kharika	325 (18.04)^{a}	96 (76.00)^{ab}
Mulberry kharika	298 (17.27)^{d}	92 (73.78)^{b}
Jack fruit kharika	308 (17.56)^{c}	90 (70.79)^{c}
Cardboard box	319 (17.86)^{b}	96 (78.25)^{a}
Gada cloth	344 (18.55)^{a}	96 (78.74)^{a}
Polythene cover	203 (14.27)^{e}	93 (74.34)^{b}
Plastic tray	317 (17.80)^{bc}	95 (78.30)^{a}
CD 0.05	0.456	2.938
SE(d)	0.223	1.170

Mean of three replications
*Values within parenthesis are square root transformed
#Values within parenthesis are arc sine transformed
Values with different alphabets with in a column differ significantly

The influence of oviposition substrates on fecundity and hatchability, revealed that gada cloth recorded maximum egg laying (344 eggs) and was followed by castor kharika (325 eggs) and both were found on par with each other. Cardboard box treatment recorded a maximum fecundity of 319 eggs and plastic tray treatment recorded 317 eggs. Poor egg laying was noticed in polythene cover treatment (203 eggs). Egg hatchability was maximum in gada cloth (96 %), cardboard box (96 %) and plastic tray (95 %). All the three treatments were on par with each other. Jack fruit kharika recorded poor hatchability (90 %) among the other treatments (Table 2). The present finding was in contradiction to the findings of Subramanian *et al.* (2012) who reported that egg laying pattern of eri moths were not influenced by the substratum. From the results of the present study, it may be concluded that eri moths need six hours minimum mating duration to obtain better fecundity and hatchability. Further as gada cloth performed as the best oviposition substrate, it can be utilized in commercial grainages.

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Preliminary study on the wing scales of moth *Creatonotus transiens* Walker, 1855 (Lepidoptera: Erebidae)

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ABSTRACT: Dimensions of different types of scales on the wings of the moth *Creatonotos transiens* Walker were analyzed using micrometry. A total of 65 morphologically distinct types of scales were investigated, including 35 from dorsal side and 30 from ventral portion. Dorsal side scales have a length range of 82.7 to 141.3µ and width range of 55.1 to 78.8µ. The length of scale on the ventral region ranged from 82.7 to 133.9µ and width 63 to78.8µ. On comparison with ventral side of wing most of the dorsal side scale forms are long narrow and dentate.

KEYWORDS: Morphometric, colour patches, structural diversity, tiger moth

Tiger moth, *Creatonotos transiens* Walker, 1855 (Lepidoptera: Erebidae), is distributed from south-east Asia from India to China, South Japan, Borneo and Lombok. The pigments for various colour patterns are contained in scales of wings. These wing scales can have different shapes and pigments, enabling the formation of various colour patterns on both upper- and lower sides of the wing membranes (Eliasson *et al.,* 2005). The colours in certain scales are due to chemical pigments, while others contain ridges and prominences that cause light to reflect resulting in bright and iridescent appearance (Vukusic and Sambles, 2003). Tiger moths have been a challenging group for systematic study because their wing patterns and colours, traditionally used for species identification, are highly variable (Weller *et al.*, 1999; Schmidt, 2007) but lack reliable synapomorphies. The scale forms of moth provide an acoustic advantage by decreasing predation risk specifically to bats. A preliminary study was undertaken on morphological differences in the wing scales of moth, *C. transiens*.

Moths of *C. transiens* were collected from the gardens of Sree Narayana College, Kollam (8°52'55''N; 76°36'4''E). Scale samples from white and light brown colour portion of wings were dislodged as per the standard method of Grodnitsky and Kozlov (1991). Scales were shredded from each region of wing into a glass slide. A drop of xylene was used for fixation and the samples were studied under a light microscope. The measurements of the scales were carried out using micrometry and photomicrographs were prepared for analysis.

The fore wings are dark chestnut with the costa and cell of fore wing suffused with white in *C. transiens.* Three orbicular and reniform spots outlined in grey on discocellulars are present. It has narrowed fore wing with very pale fuscous. The costa and base of inner margin are white and black spots in and just beyond each angle of cell. Hind wings pale fuscous with broad irregular black margin bearing a black spot on discocellulars. The dorsal side and ventral side are same, so the scales are

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Plate I

Dorsal wing

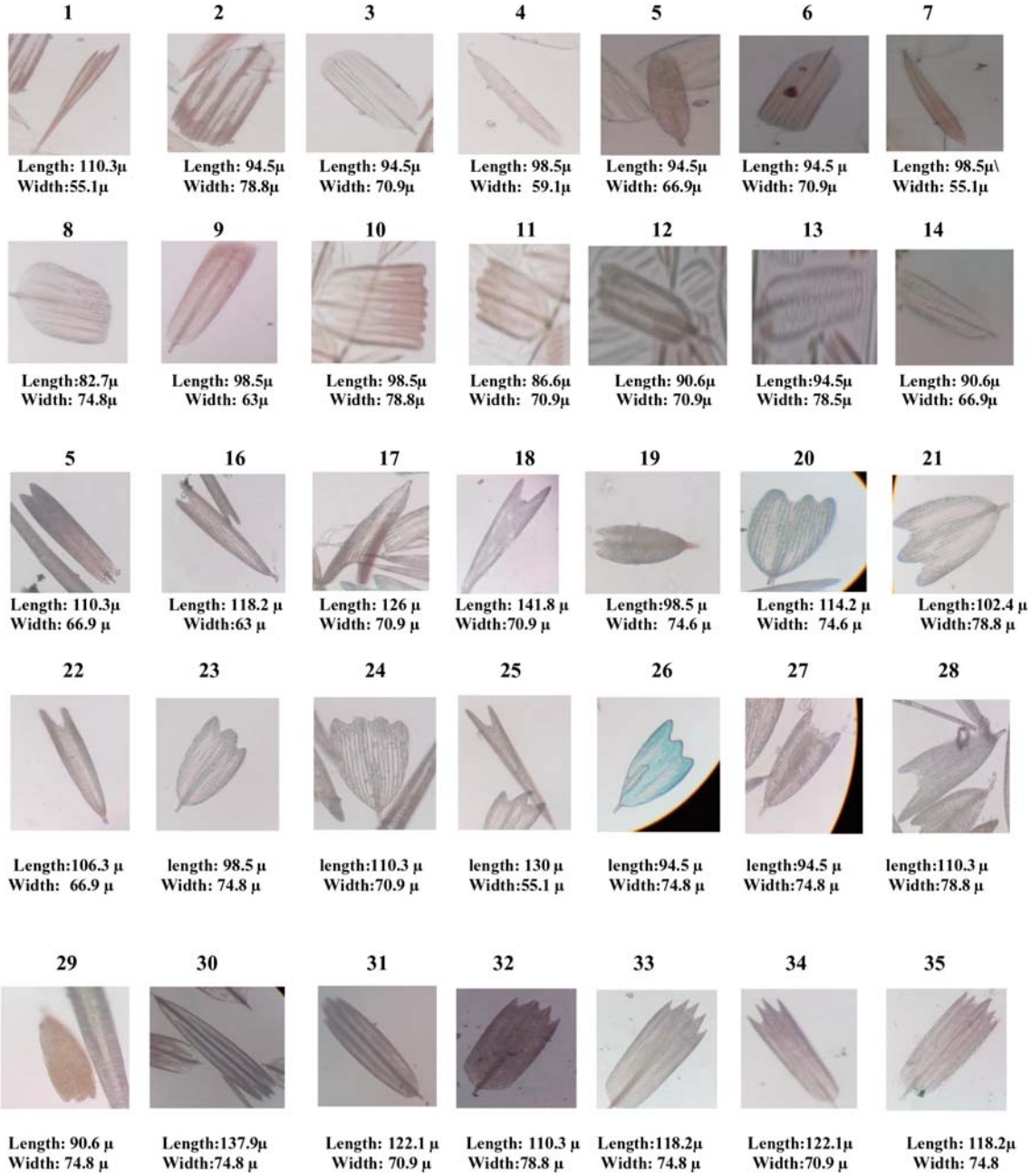
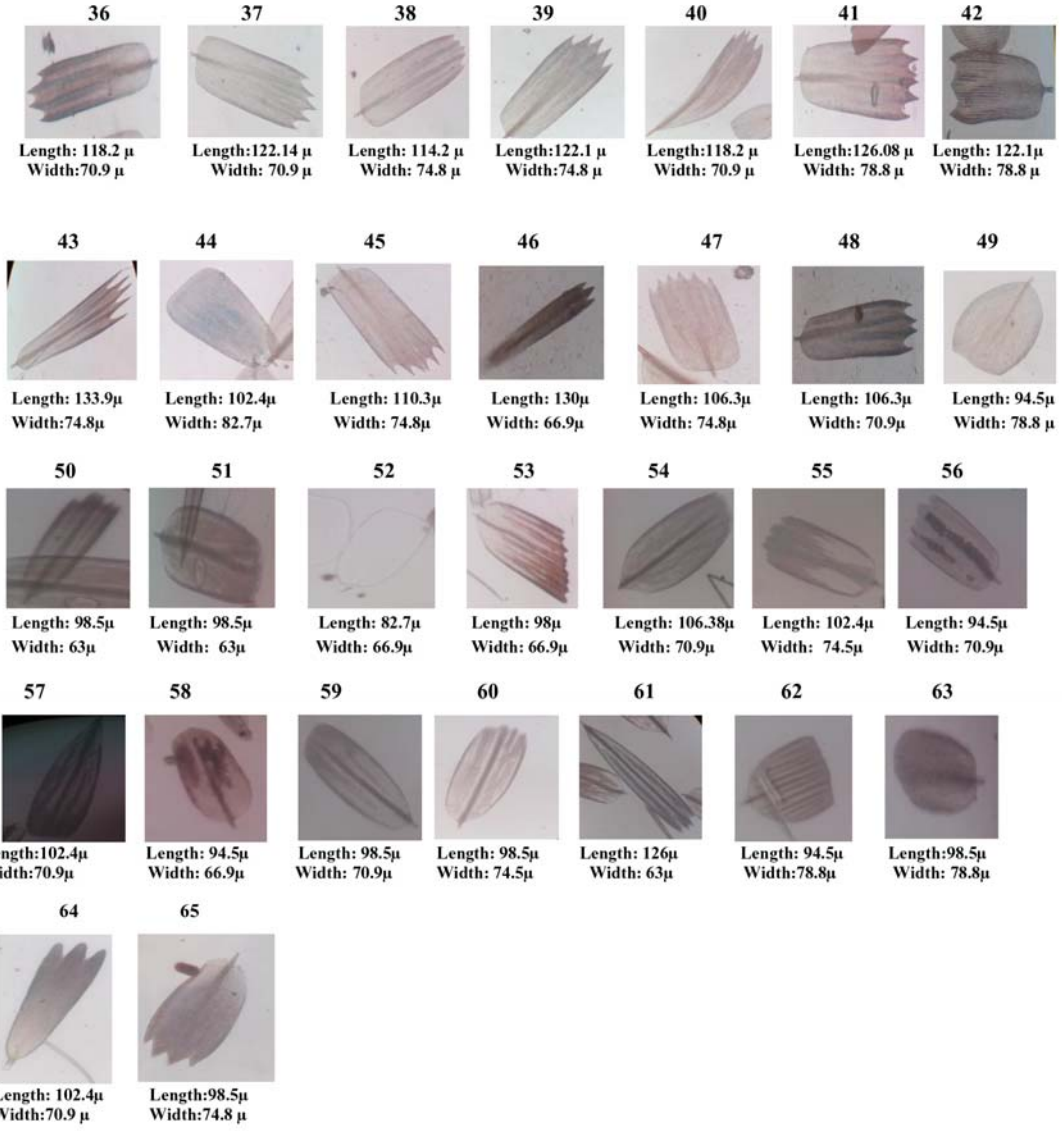
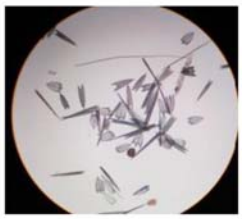


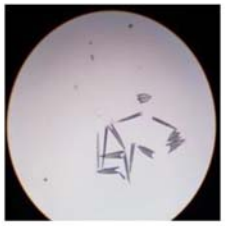
Plate II

Ventral wing





Dorsal wing scales



Ventral wing scales

Creatonotus transiens

extracted commonly. The cream colour and black spot are there in the dorsal side wing but due the too small size the individual scales cannot be taken separately. About 65 scales are observed from dorsal and ventral side. Among them 35 scales are from dorsal and 30 scales from ventral side are taken. The white colour and light brown colour scales were identified. The sharp edged end was seen in majority of scales. The dimension of the dorsal wing range from 82.7 to 141.3µ length and 55.1 to 78.8µ in width (Plate I – 1 to 35) and the dimension of the ventral wing range from 82.7 to 133.9µ in length and 63 to 78.8µ in width (Plate II - 36 to 65). On comparison with ventral side of wing most of the dorsal side scale forms are long narrow and dentate.

The colour of scales function as thermoregulatory and the scale structure also protect moths from trapped in spider webs (Zeng *et al.*, 2011). Moth scales are composed of honeycomb-like hollows analogous to sound-absorbing material which is an advantage for decreasing the predation by echolocating bats and decreases perception of moth by predators (Zeng *et al.*, 2011).

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Record of *Coranus siva* Kirkaldy (Hemiptera: Reduviidae) on coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae) in India

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ABSTRACT: The coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae) is a serious pest affecting coffee worldwide. The present study provides the first report of the natural predation of *Coranus siva* Kirkaldy on *H. hampei*. As of now, there is no report on the genus *Coranus* or other reduviid predation on coffee pests from India or form other coffee growing countries. The predator captured the adult and sucked the body fluid. The predator found to paralyze many adults of *H. hampei* whenever more prey adults were provided. Predation of *C. siva* and its sequence of behavioural events are reported.

KEYWORDS: Predation, first report, behaviour, biological control agent

Efforts towards the elimination of chemical based pest control methods have led to the identification of important ecosystem service provided by natural enemies (Naylor and Ehrlich, 1997). Members of Reduviidae (Hemiptera) have been recorded as natural enemies of various groups of agricultural pests worldwide (Ambrose, 2003; Sahayaraj, 2014). About 7,000 known species and sub-species from 913 genera belonging to 25 sub-families make them the largest group of predators among terrestrial bugs (Froeschner and Kormilev, 1989; Cassis and Gross, 1995). Reduviid predatory species from the genus *Coranus* are known for their effective role as biological control agents (Wallace, 1953; Ambrose, 1988; Kumar *et al.*, 2011).

Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae) is one of the major insect pests that attacks arabica coffee, *Coffea arabica* L. and robusta coffee, *C. canephora* Pierre ex A. Froehner (Rubiaceae) and causes a loss of about US$ 500 million annually (Vega *et al.*, 2002). An excessive reliance on insecticide endosulfan 35EC coupled with labour intensive cultural practices have long been a main hurdle in *H. hampei* management. Although several parasitoids, entomopathogens and nematode parasites have been used in the biological control of *H. hampei*, its life cycle within coffee berries has resulted in a limited success (Damon, 2000; Jaramillo *et al.*, 2006). No native predators or parasitoids are recorded on *H. hampei* until now in India**.** Thus, the study aimed to search for the new natural enemies from the field and document their impact on *H. hampei*. *Coranus siva* (Kirkarldy) (Hemiptera: Reduviidae) has long been recognized as a generalist predator regulating pests affecting different crops.

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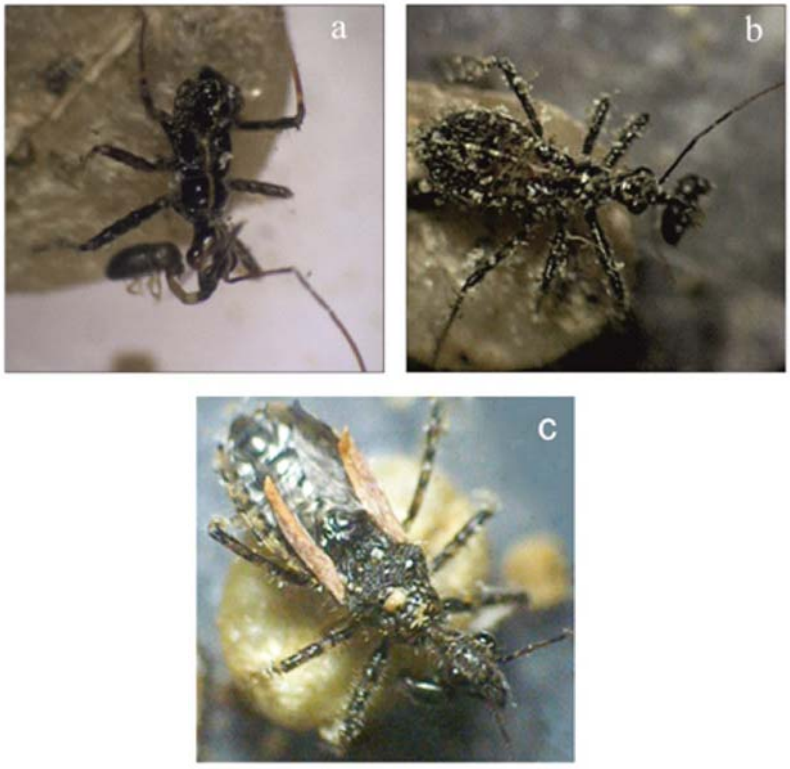

Fig. 1 Predation of *Coronus siva* on *Hypothenemus hampei* --
a) Predator nymph attacking at anterior region of *C. siva* adults
b) Predator nymph attacking at middle region of *C. siva* adults
c) Adult predator attacking C. siva adult

Field surveys were conducted for assessing the *H. hampei* population in an arabica coffee plantation in the Chikkamagaluru region (13°26'41.4"N: 075°48'29.5"E, 1067m elevation), Karnataka, India. A few nymphs of reduvid bug feeding on *H. hampei* adults in the field were brought to the laboratory along with *H. hampei* infested coffee berries. The predator and *H. hampei* infested coffee berries were initially kept in a nylon mesh covered plastic tray (21 x 15 x 10cm). The tray was maintained in a growth chamber (GC-300TLH, Jeiotech, Korea) at 25±1ºC and 70±5% RH. The nymphs were fed on *H. hampei* adult females emerging from infested coffee berries in the tray.

The reduvid bug was identified as *C. siva*. For making observations on predation by *C. siva*, five fresh females of *H. hampei* emerging from infested coffee berries and the nymph of *C. siva* were released into a vial (3cm diameter x 5cm height). Predation behavioural events of *C. siva* on *H. hampei* adults were observed under a stereo zoom microscope (Lawrence and Mayo, Trinocular Research Microscope).

In the presence of *H. hampei* adults in the vial**,** the antennae of *C. siva* pointed forward, and it remained stationary for a while until the potential prey displayed any motion. Once the prey made a slight body movement, the predator captured the prey with its first two legs, rendering it immobile. Then the predator scanned the prey by rolling the body to an appropriate site to insert its stylet. The coffee berry borer adults triggered a response by continuous leg movements in defence to the predator attack. However, the defence appeared futile as the prey was lifted off the substratum with the predator's support of a long rostrum. The predator inserted the stylet either at the anterior

(base of the antennae), median (junction of the head and thorax) or posterior (anal segments) region (Fig. 1) and the activity of the prey ceased after it was paralysed. Subsequently, the predator sucked the body fluid off the prey. After the exhaustion of the body fluid of the prey at a particular region, the predator changed its original site and selected a new site for feeding on body fluid. The predator survived on *H. hampei* for over 70 days.

Coranus siva, a generalist predator recorded to feed on pests i.e., *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae), *Helicoverpa armigera* Hubner, *Earias insulana* Boisduval (both Lepidoptera; Noctuidae), *Oxycarenus hyalinipennis* Costa (Heteroptera: Oxycarenidae) and *Odontotermes obesus* Rambur (Isoptera: Termitidae) (Kumaraswami, 1991; Kumar, 1993).

In this study, observations were also made to quantify the attack rate on different prey adults when there was an increase in the prey density to simulate the outbreak of *H. hampei*. It was observed that even after making a successful attack on *H. hampei* adult, on the sight of another actively moving adult, the predator made a shift by attacking a new prey while abandoning the previously attacked one. The same behaviour of paralyzing many individuals of *H. hampei* would eventually suppress the infestation rate of coffee at a higher prey density in the field. Attacking many individual preys can induce increased prey mortality. The visual stimulus of an active prey was one of the major factors for the initiation of a quick orientation towards a prey. The response of the predator was dependent on the activity of the prey and its abundance. This predatory behaviour of *C. siva* can be considered as the most desirable feature in biological pest suppression.

This study provides the first record of natural predation of *C. siva* on *H. hampei*. It is worth noting that there is no report on the genus *Coranus* or other reduviid predation on coffee pests from India or other coffee-growing countries. In addition, Reduviids from sub-family Harpactorinae are known to prey on soft-bodied caterpillars, grubs and termites (Resh and Cardé, 2009). However, in the present study, the first evidence of Reduviid *C. siva* was found feeding on hard coleopteran adult *H. hampei*.

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Nesting structure of stingless bees, *Lophotrigona canifrons* Smith and *Tetragonula iridipennis* Smith (Hymenoptera: Apidae) in natural forests of Nagaland, India

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ABSTRACT: The nesting sites of two species of stingless bees were located in Medziphema and Punglwa village which fall under the Dimapur and Peren districts of Nagaland. The nest of *Lophotrigona canifrons* Smith is subterranean in nature constructed inside cavities below ground and that of *Tetragonula iridipennis* Smith is arboreal in nature constructed on tree trunks inside cavities. The length and width of the entrance tube of *T. irridipennis* measured 26mm in length, 22mm in width and the total length of the tube including the accessed tube that connected with the nest measured 70mm. The area of the honey in *T. irridipennis* was 80x60x50mm, with 112g stored honey and 0.72g individual honey pot. In the case of *L. canifrons* length of the entrance tube was 20 mm and width 15 mm for the underground nest-1 (UG-1); and for the UG-II, the length and width measured 10mm and 14mm respectively. The honey pots of *L. canifrons* were dark brown to black in colour just like that of pollen pots. For UG-I, the honey area was 100x65x59mm; with 127g honey and 0.78g single honey pot. Similarly, in UG-II the honey area was 70x62x55mm with 114g honey and 0.80g of a single honey pot.

KEYWORDS: Nest architecture, entrance tube, brood cell, pollen pots, honey pots,

Stingless bees belong to the family Apidae and are close relatives of true honey bees, carpenter bees, orchid bees and bumble bees. Stingless bee is the smallest (4.0 to 5.0 mm long) of the honey bees living in social colonies which are are perennial, shows polymorphism (queen, worker and males) with division of labour. They make their nests in dark places like empty logs, cavities in tree trunks, cracks and crevices in old walls etc., where the nest entrance mostly projects as an external tube (Roopa, 2000; Gajanan; 2005). They prefer closed structure for nesting rather than open space. One major component of the stingless bee's nests is the excellent insulation made by propolis-structure called batumen, especially with the exposed nests. Nests in large trunks or in soils are particularly well insulated. Stingless bees also produce hive products like honey, pollen, bee wax and resins; they have been reported to be important pollinators of many crops in tropical and subtropical regions (Roubik, 1995). Stingless bees have been reported to be efficient pollinators of many crops and in almost sixty crops were efficiently pollinated by stingless bees (Heard, 1988). Studies on the nesting habit

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and nest structure of stingless bees is well documented in southern parts of India (Roopa, 2000; Gajanan *et al*., 2005 and Danaraddi *et al*. 2009), however, it is still in infant phase in North eastern part of India. Hence, the present study has been formulated to know the details of nesting habits and nesting structure of *Lophotrigona canifrons* Smith a subterranean in nature constructed inside cavities below ground and that of *Tetragonula iridipennis* Smith in Nagaland.

The study was carried out at the Experimental farm AICRP Honey Bees and Pollinators, Department of Entomology, School of Agricultural Sciences and Rural Development (25°.75'961N, 93.853698°E), Medziphema under Dimapur district and at Punglwa (25°372 20"N; 93°502 14"E), under Peren district of Nagaland, India. The surveys were conducted to locate the arboreal nest of the stingless bee, *T. iridipennis* and underground nest of stingless bee, *L. canifrons* in all the natural vegetation in and around the University campus, Medzihema, Dimapur district and in Punglwa, Peren District during 2020-2021. The observations on natural nest architecture in two species of stingless bees were observed. Initially the activity of bees was recorded consecutively for three days at the nesting site and then the nest was extracted and brought to the laboratory for further studies. Nesting habit was studied based on shape, size and location of nest. Dimensions of the cavities, entrance length and width were recorded. The internal structure of the nest was studied by dissecting the colonies. The nest characteristics *viz*., area of pollen, honey and brood, their location in the nest, no. of brood/food cells per linear, weight of single pollen, honey and brood cells, were recorded.

Total of four colonies were located in the survey, two colonies each of *L. canifrons* in Punglwa, Peren district and *T. iridipennis* in Medziphema, Dimapur district. The first nest of *L. canifrons* was located nearby a stream in a forest area at the end of Punglwa village (N 25°39'22.05621''; E 93°51'31.83443''). The area was sloppy in nature with big trees. The underground nest I (UG-I) was located in between small shrubs at about 45cm below the ground level and was found in a hollow area with many stones. The size of the cavity was around 40x45x60cm. The nest was built inside the cavities, attached to the side walls through slight evaginations of the outer involucrum. The second underground nest (UG-II) was located in a grassy area near to the Punglwa village (N 25ï 39'31.61152''; E 93ï 51'12.34298''). Similar to the previous nest, the nesting area was sloppy in nature and nested among stones but compactly within the cavity (Table 1, Fig. 1). As of *Tetragonula iridipennis*, two arborreal nest was found nested on a tree. However, due to the presence of one nest in a large tree, the insides of the nest could be studied only for one.

The shape of the two underground nests were somewhat elliptical to oval in shape and the colour of the nest was totally black (Fig. 2). It was covered

Table 1. Nesting habits of stingless bees- *Tetragonula iridipennis* Smith and *Lophotrigona canifrons* Smith

Nesting place	Height above/below ground (cm)	Cavity dimension (cm)	Nest dimension (cm)	Tube (mm)		Colour of entrance
				Width	Length	
Tetragonula iridipennis						
Tree trunk	274.6	……...	…..	22	26, 70	Creamy white
Lophotrigona canifrons (Subterranean)						
Nest—I	45	40x45x60	35 x24x17	15	20	Black
Nest—II	38	43x38x45	31 x27x19	14	10	Black

Fig. 1 Nesting site of *L. canifrons*

Fig. 2 Nest of *L. canifrons*

Fig. 3 Entrance tube of *L. canifrons*

Fig. 4 Entrance tube of *T. iridipennis*

Fig. 5 Internal structure of nest of *T. iridipennis*

Fig. 6 Brood cells of *L canifrons*

Fig. 7 Pollen pots of *T. iridipennis*

Fig. 8 Honey pot of *L. canifrons*

Fig. 9 Honey pots of *T. iridipennis*

by a thick and multiple layer of involucrum or batumen which provided thick insulation to the colony. The colour of the entrance was black, circular in shape and the structure of the entrance was made up of sand and soil particles mixed with cerumen in the case of *L. canifrons* (Fig. 3). The entrance tubes exposed to the outside was very short for both the underground nest. The length and width of the entrance tube were 20 mm and 15 mm for the UG-I and 10mm and 14mm for UG-II, respectively. The entrance tube of *T. iridipennis* that was exposed to the outside measured 26mm in length, 22mm in width and the total length of the tube including the accessed tube that connected with the nest measured 70mm. The colour was creamy white in color with small brown specks and was very soft in texture (Fig. 4).

The internal colony structure consisted of brood area and food area in both nests (Table 2, Fig. 5). The brood cells of *L. canifrons* were oval in shape, light brown in colour and were smaller in size when compared to honey and pollen pots (Fig. 6). The brood cells were arranged in a loose manner and present in the middle, surrounded by pollen and honey pots on the sides and below it. The dimension of the brood cell of the UG-I measured (110x90x50mm) mm and the weight of the total brood was 159g with 0.0065g (mean of 10) weighed single brood cell. Similarly, in UG-II, area of brood cell was 170x118x70mm, total brood weight was 198g and single brood cell was 0.0066g respectively. The number of cells per linear cm (4) and per cubic cm (12) was recorded. As for the brood cell of *T. iridipennis*, the brood cell dimension was

Table 2. Internal characters of stingless bee nest

Parameters/ Type of nests	Underground nest of *Lophotrigona canifrons*		Arborreal nest of *Tetragonula irridipennis*
	Nest—I	Nest—II	
Brood cell area (l X b X h) mm	110 x 90 x 50	170 x 118x 70	250 x 130 x 60
Wt. of brood area (g)	150	198	233
Wt. of single brood cell (g)	0.0065	0.0066	0.0061
No. of cells/linear cm	4	4	4
Pollen area (l x b x h) mm	100 x 80 x50	85 x 60 x 75	162 X 125 X 50
Wt. of pollen pots (g)	147	153	188
Wt. of single pollen pots (g)	0.65	0.54	0.49
No. of pots/linear cm	1 (0.8)	1(0.8)	1 (0.9)
Honey area (l x b x h) mm	100 x 65 x 59	70 x 62x 55	80 x 60 x 50
Weight of honey pots (g)	127	114	112
Weight of single honey pot (g)	0.78	0.80	0.728
No. of pots/linear cm	1 (0.9)	1 (0.9)	1 (0.8)

250x130x60 mm. The brood cells were oval in shape, brown in colour with a light tinge on the top surface. The newly constructed brood cells were lighter in colour as compared to the old ones. The cells were attached to each other on the sides and arranged in a horizontal comb with one layer after another forming a kind of terrace. The brood cells were surrounded by several layers of waxy sheets and vertical pillars which provided strength and insulation. The dimensions of the nest vary according to the species and age of the stingless bee colony. The weight of the total brood and single brood cell were 233g and 0.0061g, respectively.

The food area was divided into pollen area and honey area. In *L. canifrons*, the pollen pots were deep dark brown to black in colour. They were made up of soft cerumen and the pollen pots were located on the sides surrounding the brood. For UG-I, the dimension of the pollen area was 100x80x50 mm; the total weight of the stored pollen was 147g and the weight of a single pollen pot was 0.65g. The dimension of the pollen area was 85x60x75 mm, total weight of the stored pollen and single pollen pot were 153g and 0.54g, respectively in UG-II. As for *T. iridipennis*, the honey and pollens were stored in separate pots. The pollen pots were found to be located at the sides or the periphery of the colony surrounding the brood cells. The pollen pots varied in shape from circular to oval and were light brown in colour (Fig. 7). The pollen area measured 162x125x50 mm; the weight of the stored pollen mass was 188g and weight of a single sealed pollen pot was 0.49g.

The honey pots of *L. canifrons* were dark brown to black in colour just like that of pollen pots (Fig. 8). They were located on the periphery and here in this case it was also found below the brood cells. For UG-I, the dimension of the honey area was 100x65x59mm; the total weight of the honey was 127g and single honey pot was 0.78g. Similarly, in UG-II the dimension of honey area was 70x62x55mm, the total weight of the honey recorded as114g and the weight of a single honey pot as 0.80g. In the nest of *T. iridipennis*, the honey pots were dark brown in colour (Fig. 9) and in all the cases, once filled, the honey pots were found sealed and

stored. The dimension of the honey area was 80x60x50mm; the weight of the stored honey was 112g and the weight of a single honey pot was 0.72g, respectively. The honey was slightly sour to taste with a distinct odour.

Dannaradi *et al*. (2009) reported nesting site of *T. iridipennis* on wall crevices and tree trunks at Dharwad, Karnataka. Barbosa *et al*. (2013) reported subterranean nest of *Geotrigona subterranea* in a simple cavity between the ground and a masonry structure. The present findings of entrance tube are in conformity with the findings of Roopa (2002) and Gajanan *et al,* (2005) who observed variations in the length of *T. iridipennis* in and around Bangaluru and in Dharwad area, Karnataka (Dannaradi *et al*., 2009). The size of the nest varies with age and number of individuals in a colony. The length of the entrance tube appears to be a species-specific character and also depended on the type of nesting site as the entrance tube of *T. gribodei* reported by Pooley and Michener, (1969) was very short. Barbosa *et al*. (2013) observed entrance holes to be circular with a diameter that ranges from 0.85 cm to 1.20 cm and an average value of 1.0 cm. Dannaradi *et al*. (2009) reported the brood cells as oval, brownish in colour that looked like jowar grains and arranged in a network of narrow vertical pillars with horizontal connectives. Similar findings were observed by Roopa (2002) and Gajanan *et al*, (2005) reported oval shaped pollen pots made up of soft cerumen which were dark brown in colour that were usually observed at the periphery of the colony. Barbosa *et al*. (2013) observed combs were supported by many pillars, with varying thickness. The investigations provided better understanding of the nest characteristics of stingless bees which paved the way in carrying out research to rearing them under domesticated conditions.

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Parapoynx diminutalis Snellen, 1880 (Lepidoptera: Crambidae): A pest of submerged aquatic weed *Hydrilla verticillata* (L.f.) Royle

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ABSTRACT: *Parapoynx diminutalis* was observed in *Hydrilla verticillata* (a weed) in artificial tanks at Kerala Agricultural University. The incidence was so severe that the entire biomass of hydrilla was eaten away by the larvae which pupated in water. Pupal case was made of stem pieces of hydrilla, thus curtailing the growth and multiplication of the weed was suppressed in a period of one month indicating that this is a potential biocontrol agent for the weed.

KEY WORDS: Weed, water thyme, biocontrol agent, caterpillars, severe feeders

Water thyme, *Hydrilla verticillata* (L.f.) Royle (Family: Hydrocharitaceae), is a submerged obligate aquatic plant with cosmopolitan distribution (Cook and Luond, 1982). *Hydrilla* is a profusely branched, herbaceous perennial with very slender stems with pointed leaves arranged in a whorl fashion (CAIP, 2021). Generally, when hydrilla takes over an aquatic habitat, it gradually eliminates all other aquatic plants, leaving just hydrilla. However, submerged weeds like najas, utricularia are also found to co-exist in many freshwater ecosystems in Kerala. During mid- September 2020, *Hydrilla* was collected from a pond at Ollukara, Thrissur, Kerala (10.53" N; 76.25" E) and was grown in cylindrical concrete tanks with 40 cm height and 38 cm inner diameter, maintained in the AICRP centre. By late September, 2020, egg mass of a moth was first observed, under the leaves of hydrilla. It took 4-6 days to hatch. The hatched out larvae were very active and started feeding voraciously. The caterpillar constructed case out of hydrilla plant part and moved around by remaining within the case (Fig. 1). The caterpillar is creamy white with yellowish brown head, prothorasic shield and thorasic legs; with brown spots on head, and thorasic segments around the base of setae; branched gills present on meso and metathorax and abdominal segments 1-9. Larval length was about 10 mm and was light yellow in colour with brown patch on head and tip of thorax. The larvae caused damage to hydrilla by feeding on the plant parts and preparing cocoon case from hydrilla stem cuttings and were found to retreat in between feeding cycles inside the case. It caused

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Fig 1. Larva of *Parapoynx diminutalis*

Fig 2. Pupal case of *Parapoynx diminutalis*

Fig 3. Hydrilla stem with pupal cases of the moth

Fig 4. Adult moth immediately after emergence

extensive damage by cutting the stems and defoliation (Fig. 3). The larval period was 20-30 days. The hydrilla samples with pupal cases (Fig. 2) were kept in glass tumblers containing water and covered with fine mesh net, so as to observe the emergence of adult moth. Adults were emerged in 6-8 days. The adult moth was small, straw-colored with white and brown stripes, and resembled a rice leaf roller moth in size and colour. Male moths were smaller than female moths (Fig. 4).

The insect species was identified as *Parapoynx diminutalis* Snellen, 1880 based on the larval description provided by Habeck and Balcuinas (2005). According to Habeck (1996), the presence of branched gills and brown patches on the head and tip of the thorax, distinguish larvae of this moth from those of other aquatic species. *P. diminutalis* is an adventive moth, found in a variety of water bodies, including rivers, backwaters, lakes, and ponds. It was first reported in Florida in 1976 by Del Fosse, Perkins and Steward, but gradually appeared in wider areas (Habeck, 1996). Larval stage of the moth causes damage to *Hydrilla*. *Hydrilla* and other aquatic plants are regularly attacked by aquatic larvae (Buckingham and Bennett, 1989). This moth has been reported earlier in 1971 in India and Pakistan during an attempt to determine potential biological control agents in *Hydrilla* (Baloch *et al*., 1980). Purcell *et al*. (2019) have reported its incidence in hydrilla from China, Indonesia, Malaysia, Singapore, Thailand and Vietnam. The search for a potential biocontrol agent for *Hydrilla* began in early 1980s and though some snails and pathogens were detected, the results were not promising (CAIP, 2021). A detailed study on life cycle and host range of this moth is required to establish its potential as a biocontrol agent for aquatic weed management.

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The identity of the insect was also confirmed by Dr. N. Chitra, Professor (Entomology), Tamil Nadu Agricultural University, Coimbatore, as *Parapoynx diminutalis* and her expert service is greatly acknowledged.

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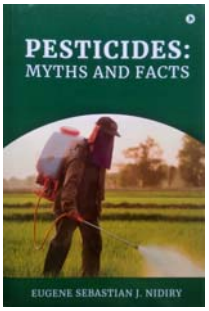

Pesticides : Myths and Facts, Notion Press, 2021. 330 pages

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Pesticides are one among the most misunderstood entities in the world. Being anathema for most people, administrators and even many scientists find it difficult to support and justify use of pesticides. History shows that the human kind has been experimenting with a variety of toxins to protect his crops and finally ended up with an array of synthetic chemicals. These are toxic chemicals, however, when diluted and used as prescribed, they are safe, like a wild elephant which is tamed. Many protocols, developed over time, are in place to study the acute and chronic poisoning effects in laboratory animals, which can be extrapolated to humans. Around 80 different types of tests with well-defined protocol are conducted. The acute toxicity tests include estimation of LD_{50} values allergenicity, skin and eye irritation etc. The chronic toxicity studies investigate carcinogenicity, mutagenicity, developmental toxicity, teratogenicity and so on.

Those molecules which are proven to be carcinogenic or mutagenic during the preliminary screening are summarily rejected. Thus usually out of only one in 15000 promising molecules will be taken forward for further studies. To protect the users and consumers, different indices like Acceptable Daily Intake (ADI), Maximum Residue Limit (MRL), Acute Reference Dose and Waiting period are worked out. To overcome the limitations of laboratory studies, reviews are conducted periodically which also examines the adverse impact of the chemical in field situations.

However, even with all these precautions and safety measures, pesticides are still misunderstood mostly because of the mass chemophobia among the people. The chemohobia mainly have its roots in the much publicised 'Silent Spring' by Racheal Carson, who wrote "Chemicals are the sinister and little recognized partners of radiation… Entering into living organisms, passing from one to another in a chain of poisoning and death". Her heart moving but exaggerated description of a 'Silent Spring' due to use of DDT shocked many generations and the awe is still continuing. Many other events added strength to this feeling and presently all are looking for pesticide-free food produced through organic agriculture.

There are very few voices that explain the facts about pesticides. Dr. Eugene Sebastian Nidiry, former Principal Scientist, ICAR– IIHR has taken up the role of devil's advocate. In his book "Pesticides: Myths and Facts" the basic science of pesticides are explained. From second chapter onwards, different myths about pesticides and their rebuttal are explained. What is outstanding about this rebuttal is the extensive use of statistics and data to nail the arguments so beautifully. For example in chapter 9, on the alleged relationship between pesticides use and cancer, 18 tables and 10 graphs which clearly explain the topic and drives home the rebuttal: "the correlation existing between pesticide use and higher incidence of cancer is spurious. Higher percentage of cancer deaths is due to lower death rate and higher life expectancy, achieved mainly through modern medicine and modern agriculture". Thus the book refutes 12 common myths about pesticides. The reader is likely to have differences of opinion, however, in case he wants to prove a point, he also has to present credible data, which is the way of science and logic.

This book will be an asset to any library and agricultural scientist and extension worker. Data gives information and information gives knowledge and knowledge gives wisdom. The author did not look into the much hyped Endosufan 'tragedy' of Kasaragod, probably due to unavailability of data. May be in the next edition, the real side of the Endosulfan 'Tragedy' can be incorporated. Scientific temper is an important quality required for any citizen. Continuous learning is inevitable to check one's preconceived notions. I recommend this book to the academia and intelligentsia of the country and liberate themselves from the detention of Silent Spring.

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