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Description of two new species of *Lisotrigona* (Hymenoptera, Apidae, Meliponini) from Central India and their nests

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ABSTRACT: *Lisotrigona darbhaensis* **sp. nov**. and *L. kosumtaraensis* **sp. nov** from Chhattisgarh and Maharashtra states, respectively, are described along with the additional description of the male of *L. chandrai*. *Lisotrigona darbhaensis* nested in the tree trunk of teak (*Tectona grandis*) while *L. kosumtaraensis* in the Indian frank incense (*Boswellia serrata*) and Indian boxwood (*Gardenia latifolia*). Brood cells of *L. kosumtaraensis* were arranged in clusters. The colony of *L. kosumtaraensis* consisted of 921 female and 40 male bees. The detailed studies on male genitalia, metasomal sterna, and morphometry with associated female bees collected from Maharashtra and Chhattisgarh provided conclusive evidence as these bees were found different from the known species of *Lisotrigona* and new to science. Both the new species are distinct and different from other known species of *Lisotrigona*. The diversity of *Lisotrigona* bees in India is rich with six valid species and the action of synonymizing all Indian species of *Lisotrigona* with *L. cacciae* is arbitrary. © 2023 Association for Advancement of Entomology

KEY WORDS: *Lisotrigona darbhaensis*, *L. kosumtaraensi*s, stingless bees

INTRODUCTION

Stingless bees belonging to the tribe Meliponini of the family Apidae are doing yeoman service to the humans along with the honey bees by yielding honey of high medicinal value and pollinating several plant species including cultivated crops (Crane, 1999; Heard 1999; Cortopassi-Laurino *et al.,* 2006). Honey of stingless bees fetches a premium price in India ranging from rupees 1,500 to 10,000 per kilogram (Viraktamath *et al*., 2021a). The pollination services of honey bees, stingless bees, and other pollinators are worth US $ 577 billion per year (Lautenbach *et al*., 2012).

Among the three genera (*Tetragonula* Moure, 1961*, Lepidotrigona* Schwarz, 1939 and *Lisotrigona* Moure,1961) that occur in India, the genus *Lisotrigona* is characterized by smaller size (usually measuring 3.00 mm or less in body length), short linear malar space, converging inner eye margins, and reduced wing venation (Michener 2000). The first species of *Lisotrigona* from India (type locality: Hoshangabad: Madhya Pradesh) was described as *Melipona cacciae* by Nurse (1907), which was later transferred to the new genus *Lisotrigona* described by Moure (1961). After a gap of 97 years, Jobiraj and Narendran (2004) described *L. mohandasi,* and two species

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(*L. revanai* and *L. chandrai*) were added by Viraktamath and Sajan Jose (2017).

Females of most of the species of stingless bees are remarkably similar with very weak diagnostic characters resulting in difficulty in the identification of the species. Contrarily, the males have strong diagnostic characters that are species-specific and very useful in identifying the species (Schwarz, 1939; Sakagami, 1978; Rasmussen, 2013; Attasopa *et al*., 2018). Recently Engel *et al.* (2022) transferred *L. carpenteri* Engel to a new genus *Ebaiotrigona* when the males of the species were discovered after 22 years, and male genitalia were found unique and different from the typical *Lisotrigona* genus.

Bees from Kerala and Maharashtra were described as *L. chandrai* and *L. revanai* by Viraktamath and Sajan Jose (2017), respectively. However, Rasmussen *et al*. (2017) synonymized *L. mohandasi, L. revanai,* and *L. chandrai* with *L. cacciae* without giving justification. Recent studies by Viraktamath *et al*. (2021b) on morphometry of female *Lisotrigona* bees collected from seven places of India from Maharashtra, Chhattisgarh, and Mizoram in comparison with *L. mohandasi, L. chandrai, L. revanai* and *L. cacciae* (Primary type specimens) revealed that the dataset formed more than one cluster in Principal Component Analysis and Canonical Discriminant analysis indicating the occurrence of more than one species in India.

In this paper, *Lisotrigona darbhaensis* from Chhattisgarh and *L. kosumtaraensis* from Maharashtra are described as new species along with the additional descriptions of metasomal sterna of male *L. chandrai*. Brief notes on the nests of these two new species are also provided.

MATERIALS AND METHODS

In the endeavour to collect male with associated female stingless bees from various parts of India, four colonies of *Lisotrigona* in Kerala, six in Maharashtra, two in Chhattisgarh were found besides many foraging female bees in Maharashtra and Mizoram; and the authors were successful in collecting both males and females from three states (Kerala, Chhattisgarh, and Maharashtra). Male and female bees were collected from one colony of *Lisotrigona* nested in a teak tree (*Tectona grandis*) at Darbha, Chhattisgarh (18.85° N; 81.8689° E) (Figs. 6 A-1, A-2) by inserting one end of a narrow glass tube (15 cm long) in the colony and aspirating from the other end where a rubber tube (45 cm long) was connected. Glass and rubber tubes were separated by a thin muslin cloth to prevent the entry of bees in the mouth while aspirating. However, in Maharashtra adopted two methods for collecting male and female bees. All the bees of one *Lisotrigona* colony nested in an Indian frankincense tree (*Boswellia serrata*) at Kosumtara (21° 16’ 6’’N; 80° 32’ 37’’ E). were captured by using chloroform (Fig. 6 B-1). At Navatolla (21° 16’ 52’’N; 80° 33’ 36’’ E) a water trap (Viraktamath *et al.,* 2020) was used to collect male and female bees from one colony nested in an Indian boxwood tree (*Gardenia latifoila*). All the collected bees were transferred to vials containing ethyl alcohol (95%), labeled, and later sexed at the Systematic laboratory of the Department of Entomology, University of Agricultural Sciences Bengaluru (UASB) under a stereo binocular microscope. All the male bees and representative female bees were mounted on a triangular point and each bee was labeled with collection data (place, date, and collector’s name).

Followed the methods described by Viraktamath and Rojeet (2021) for the description of the species by studying the morphometry of 36 morphological traits, structure of male metasomal sterna and genitalia. Besides, subjected the morphometry dataset to the Principal Component Analysis (PCA) and mapped the species on a PCA plot to understand the relationships among the species. The terminology used in the description was that of Sakagami (1978) and Rasmussen (2013). The procedure to study the male genitalia, metasomal terga, and sterna were similar to that described by Viraktamath and Rojeet (2021). Images of the bees, genitalia, and metasomal sterna were taken under Leica microscopes (M205C and DM2000) fitted with a digital camera (DFC425) having a range of magnification from 7.8 to 160x and 40 to 400 x,

respectively. The images were later processed using Photoshop. Measured the length and width of the penis valve, gonostylus, and gonocoxite, under the microscope using Leica measurement software.

Holotypes and paratypes were deposited at the Department of Entomology, UASB. One paratype of each species will be deposited at the Zoological Survey of India, Kolkata, ZSIK.

The nests of both the species besides the internal structure of *L. kosumtaraensis* by breaking open the colony in Kosumtara were also studied. Since the entire colony was captured, total female and male bees were counted to estimate the strength of the colony excluding the brood. A sample of brood cells, honey, and pollen pots was measured (20 cells each) by using an ocular micrometer fitted in a stereo binocular microscope. Photographs were taken to depict the internal structure of the colony.

RESULTS

***Lisotrigona darbhaensis* Viraktamath sp. nov.**

LSIDurn:lsid:zoobank.org:act:E5D496A5-2B97-4EEF-B06B-444953E6297C

Diagnosis. Male bees measure a mean of 3.20 mm in body length and 1.14 mm in head width with forewings 2.65 mm long and 1.00 mm wide. Female bees measure a mean of 3.10 mm in body length, 1.18 mm head width with 2.59 mm long, and 0.97 mm wide forewings. This species differs from *L. kosumtaraensis* and males of other known species (*L. chandrai* and *L. furva*) in the following features. In the metasomal sternum 4, the gradulus briefly touches the antecosta medially as found in *L. kosumtaraensis* but does not touch in *L. chandrai;* the setae arising from the basal area of gradulus do not extend beyond the apical margin but in *L. kosumtaraensis* and *L. chandrai,* they extend beyond the apical margin (Figs. 3 A, B, C S4). The gradulus in sternum 5 touches the antecosta medially as in *L. kosumtaraensis* but in *L. chandrai* the gradulus runs very close to the antecosta without touching it; the apical margin is weakly bisinuate in *L. darbhaensis* but nearly straight in *L. kosumtaraensis,* and distinctly bisinuate in *L. chandrai* (Figs. 3A, B, C S5), distinctly inverted U-shaped in *L. furva* (see fig. 5 of Michener 2007). Antecosta nearly straight medially in sternum 6 in *L. darbhaensis* whereas it is distinctly convex in *L. kosumtaraensis*, distinctly concave in *L. chandrai,* and weakly concave in *L. furva* (see fig. 4 of Michener 2007)*;* apicomedian lobe of sternum 6 wider than long (as long as broad in *L. kosumtaraensis,* longer than wide in *L. chandrai* and *L. furva* (Figs. 3A, B, C S6). Gonocoxae elongate (0.81 mm), flipped J-shaped with a maximum width of 0.35 mm in *L. darbhaensis* and *L. kosumtaraensis* whereas they are shorter (0.79 mm), characteristically C-shaped, distinctly wider (0.41 mm) in *L. chandrai* (Figs. 4 A-1, B-1, C-1). The terminal part of penis valves distinctly curved and pointed in *L. darbhaensis, L. kosumtaraensis, L. chandrai* (Figs. 4 A-2, B-2, C-2) but straight and bluntly pointed in *L. furva* (See the fig. 2 of Michener, 2007). Gonostylus long (1.06 mm) arising from the basal part of gonocoxa in *L. darbhaensis* and *L. kosumtaraensis* but shorter, arising from basal half of gonocoxa in *L. chandrai* (0.87 mm) and *L. furva;* gonostylus broader with the tapered bluntly rounded, curved apex in *L. darbhaensis, L. kosumtaraensis, L. chandrai* (Figs. 4 A-3, B-3, C-3) but very slender in *L. furva* (see description and fig. 2 of Michener 2007).

Female bees are distinctly punctate on mesoscutum as also in *L. kosumtaraensis, L. revanai,* and *L. chandrai.* However, *L. cacciae* has exceedingly minute faint punctures while *L. furva* has strong punctures. The ratio of inter-ocellar to ocello-ocular distance is higher in *L. darbhaensis* (1.93) and *L. kosumtaraensis* (1.92) while lower in *L. chandrai* (1.44), *L. cacciae* (1.50), and *L. revanai* (1.61) (Table 2).

Description Males:

Coloration. Head, mesosoma and metasoma shiny, black (Fig. 1a, b). Labrum yellowish-brown; clypeus blackish brown; scape dark reddish-brown except the basal bulb and the socket yellowish-brown; pedicel, flagellar segments blackish-brown on the upper side but lighter on the lower side; ocelli transparent, shiny, light brown; compound eyes blackish brown (Fig. 1c). Wings hyaline; tegula,

Table 1. Morphometry* of two new species of *Lisotrigona* in comparison with type specimens of *L. chandrai* and *L. cacciae*

↓Parameter / Species→	*L. darbhaensis sp. nov.*		*L. kosumtaraensis sp. nov.*		*L. chandrai***		*L. cacciae****
	Male	Female	Male	Female	Male	Female	Holotype
	n-2	n-10	n-10	n-10	--	--	--
Length of body	3.20 ± 0.01	3.10 ± 0.14	3.32 ± 0.20	3.12 ± 0.25	3.01	2.78	2.95
Width of head including eyes	1.14 ± 0.02	1.18 ± 0.02	1.14 ± 0.03	1.19 ± 0.04	1.18	1.19	1.19
Length of head	0.88 ± 0.01	0.94 ± 0.01	0.89 ± 0.04	0,94 ± 0.04	0.88	0.86	1.01
Length of eye	0.84 ± 0.01	0.84 ± 0.02	0.88 ± 0.02	0,87 ± 0.04	0.82	0.83	0.83
Width of eye	0.38 ± 0.01	0.34 ± .01	0.36 ± 0.02	0.33 ± 0.02	0.35	0.35	0.33
Upper interocular distance	0.67 ± 0.03	0.75 ± 0.02	0.71 ± 0.02	0.79 ± 0.02	0.68	0.76	0.75
Diameter of median ocellus	0.13 ± 0.00	0.11 ± 0.01	0.14 ± 0.01	0.12 ± 0.00	0.13	0.09	0.11
Inter ocellar distance	0.29 ± 0.01	0.28 ± 0.00	0.29 ± 0.01	0.28 ± 0.00	0.26	0.26	0.27
Ocello-ocular distance	0.09 ± 0.00	0.14 ± 0,01	0.10 ± 0.01	0.15 ± 0.00	0.13	0.18	0.18
Length of clypeus	0.25 ± 0.00	0.26 ± 0,01	0.27 ± 0.02	0.26 ± 0.01	0.33	0.25	0.24
Maximum width of clypeus	0.45 ± 0.00	0.55 ± 0.01	0.49 ± 0.01	0.58 ± 0.05	0.49	0.38	0.42
Malar space length	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.01	0.03	0.02
Length of scape	0.33 ± 0.00	0.42 ± 0.01	0.33 ± 0.02	0.43 ± 0.02	0.38	0.49	0.37
Width of scape	0.09 ± 0.01	0.08 ± 0.00	0.10 ± 0.00	0.08 ± 0.00	0.09	0.08	0.07
Length of pedicel + flagellum	1.22 ± 0.00	0.89 ± 0.04	1.27 ± 0.06	0.93 ± 0.05	1.26	0.92	--
Length of flagellomere 1	0.05 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.06 ± 0.02	0.04	0.08	0.06
Length of flagellomere 2	0.11 ± 0.00	0.07 ± 0.00	0.11 ± 0.01	0.09 ± 0.01	0.11	0.07	0.06
Length of flagellomere 3	0.10 ± 0.00	0.08 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.11	0.07	0.07
Width of flagellomere 3	0.13 ± 0.01	0.10 ± 0.00	0.12 ± 0.00	0.10 ± 0.01	0.13	0.10	0.11
Length of mandible	0.30 ± 0.02	0.43 ± 0.02	0.31 ± 0.01	0.55 ± 0.00	0.30	0.48	0.45
Width of mandible	0.15 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.22 ± 0.00	0.14	0.16	0.12
Length of forewing + tegula	2.65 ± 0.00	2.59 ± 0.05	2.82 ± 0.13	2.64 ± 0.09	2.55	2.71	2.65
Width of forewing	1.00 ± 0.00	0.97 ± 0.03	0.99 ± 0.06	0.96 ± 0.03	0.95	1.00	0.95
Length of pterostigma	0.45 ± 0.00	0.45 ± 0.01	0.43 ± 0.02	0.45 ± 0.01	0.41	0.41	0.36
Length of marginal cell	0.88 ± 0.02	0.77 ± 0.02	0.85 ± 0.01	0.78 ± 0.03	0.90	0.78	0.89
Width of marginal cell	0.20 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.21 ± 0.01	0.20	0.18	0.18
Wing diagonal length	0.71 ± 0.01	0.74 ± 0.01	0.79 ± 0.01	0.74 ± 0.01	0.70	0.66	0.73
Number of Hamuli	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00	5.00	6.00
Length of mesoscutum	0.75 ± 0.02	0.77 ± 0.02	0.79 ± 0.02	0.77 ± 0.02	0.77	0.60	0.71
Width of mesoscutum	0.88 ± 0.02	0.92 ± 0.02	0.94 ± 0.03	0.97 ± 0.05	0.91	0.90	0.93
Length of scutellum	0.27 ± 0.01	0.25 ± 0.01	0.26 ± 0.02	0.23 ± 0.02	0.25	0.23	0.23
Width of scutellum	0.77 ± 0.02	0.75 ± 0.01	0.84 ± 0.02	0.78 ± 0.03	0.76	0.45	0.48
Length of hind tibia	0.80 ± 0.00	0.89 ± 0.02	0.80 ± 0.01	0.91 ± 0.03	0.81	0.81	0.86
Width of hind tibia	0.26 ± 0.01	0.35 ± 0.01	0.26 ± 0.01	0.37 ± 0.02	0.23	0.33	0.31
Length of hind basitarsus	0.39 ± 0.00	0.44 ± 0.01	0.41 ± 0.01	0.48 ± 0.02	0.39	0.42	0.35
Width of hind basitarsus	0.14 ± 0.01	0.21 ± 0.01	0.15 ± 0.00	0.23 ± 0.02	0.13	0.23	0.18

* All measurements in mm ± SD, **: Data based on Viraktamath & Sajan Jose (2017); ***: Data based on Rasmussen (2013)

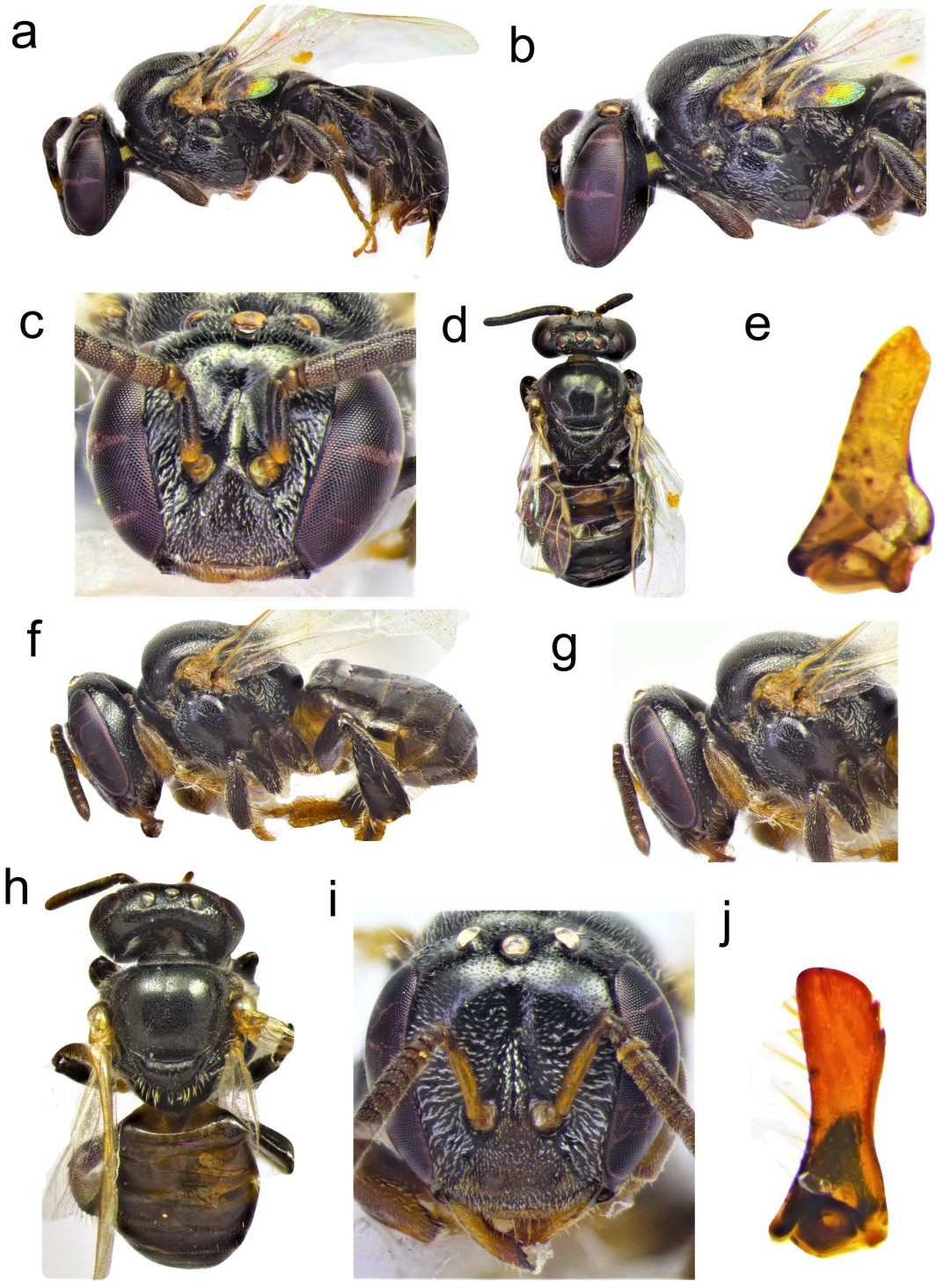

Fig. 1 *Lisotrigona darbhaensis* **sp. nov.** (a). Male lateral, (b). Head and thorax lateral (c). Head frontal, (d). Head, mesosoma and metasoma dorsal, (e). mandible (f). Female lateral, (g). Head and mesosoma lateral (h). Head, mesosoma, metasoma dorsal, (i). Head frontal (j). Mandible

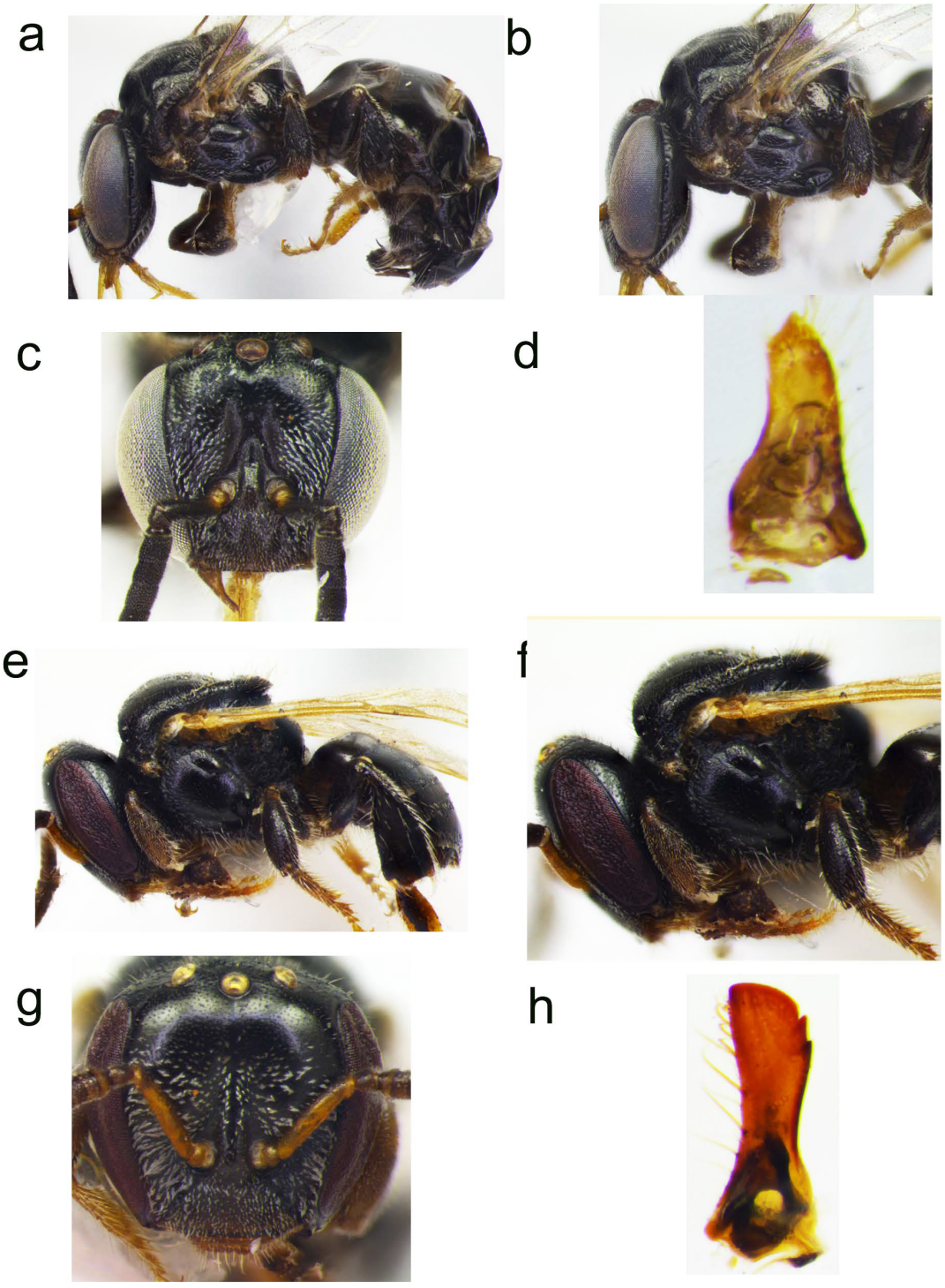

Fig. 2 *Lisotrigona kosumtaraensis* **sp. nov.** (a). Male lateral, (b). Head and thorax lateral (c). Head frontal (d). Mandible (e). Female. Lateral (f). Head and thorax lateral (g). Head frontal (h). Mandible

pterostigma, veins light brown. Mandibles ochraceous on the upper half with dark brown mottling on the basal half (Fig. 1e) All legs dark reddish-brown except the coxae, trochanters, and tarsi light brown. Metasomal terga black with light brown mottling on T2 to T4 (Fig. 1d); metasomal sterna dark reddish-brown.

Pilosity. Labrum fringed with long ochraceous hairs. Clypeus, the lower part of face covered with yellowish plumose tiny hairs without obscuring underlying integument; the upper and middle part of the face with fine yellowish hairs (Fig. 1c); vertex with brownish erect hairs. Mesoscutum, scutellum clothed with whitish fine hairs; mesoscutellar margin fringed with long whitish hairs (Fig. 1b, d). Mesepisternum clothed with white plumose hairs while metepisternum largely bare with a few long white hairs on the lower part (Fig. 1b). Propodeum with white, plumose short hairs on both lateral areas. Coxae, trochanters heavily fringed with yellowish hairs while femora, tibiae covered with short white hairs. The upper surface of the hind tibia with sparse white hairs while anterior and posterior margins fringed with white hairs. Metasomal terga T1 to T4 with sparse simple hairs along apical margins; apical terga with a transverse row of fine hairs near the apical margin; lateral and apical margins of terminal terga with a few long, stiff hairs.

Integument. Clypeus, entire face, vertex distinctly punctate. Integument surface in between antennal sockets distinctly depressed. Mesoscutum and pleural area punctate densely; mesoscutellum shiny, finely punctate. The middle part of propodeum imbricate; metasomal terga sparsely punctate otherwise glabrous.

Morphometry. Males measure a mean of 3.20 ± 0.01 mm in body length and 1.14 ± 0.02 mm in head width including eyes (Table 1). Head length 0.88 ± 0.01 mm; upper interocular distance 0.67 ± 0.03 mm; interocellar distance 0.29 ± 0.01 mm; diameter of median ocellus 0.13 mm; malar space length 0.02 mm; wing length 2.65 mm; wing width 1.00 mm; wing diagonal length 0.71 ± 0.01 mm; hind tibial length 0.80 mm and hind basitarsus length 0.39 mm. The ratio of head length to head width 0.77 (Table 2); eye length to upper interocular distance 1.25; interocellar distance to cello-ocular distance 3.22; scape length to eye length 0.39; forewing diagonal length to head width 0.63; hind tibial width to hind tibial length 0.33; hind basitarsus width to hind tibial width 0.54.

Metasomal sterna and genitalia. The following description is based on dissection of two male bees. Metasomal sternum 4, 0.25 mm long and 0.95 mm wide (Table 3); gradulus briefly touches the antecosta in the middle; lower 1/3rd area with setae that do not extend beyond the apical margin (Fig. 3 A-S4). Sternum 5 measures 0.13 mm long and 0.82 mm wide; gradulus touches the antecosta in the middle; apical margin weakly bisinuate with long setae arising from the margin (Fig. 3 A-S5). Antecosta in sternum 6 nearly straight in the middle region; the apicomedian lobe 0.20 mm long and 0.23 mm wide, broadly rounded at the apex; apodemal lobes moderately separated by a distance of 0.55 mm (Fig. 3 A-S6). Genitalia yellowish brown except the apical half of penis valves dark reddish-brown; if not exerted, lie within the abdomen cavity occupying from sternum 3 to 6. Each gonocoxa with elongate, slightly curved basal extension the apex of which curved inwards and then upwards resembling a flipped J-shape (Fig. 4 A-1); both gonocoxae with the widest region at the upper 1/3rd where both nearly touch each other, then separated and again coming close at the basal curved region. gonocoxae measure 0.81 mm long with a maximum width of 0.35 mm. Penis valves arise from the anterior margin of gonocoxae, 0.18 mm wide at the base, 0.50 mm long, gently curved with pointed curved apices (Fig. 4 A-1, A-2). Each gonostylus arises from basal 1/3rd length of gonocoxa, elongate (1.06 mm), tapering towards the terminal end with bluntly rounded, curved apex from where two long curved setae arise and short setae on the lateral side of terminal 1/3rd region (Fig. 4 A-3).

Females:

Coloration. Head and mesosoma black while metasoma dark reddish to black (Fig. 1f, g). Labrum, clypeus, compound eyes reddish brown to blackish brown. Antennal socket, scape, and its basal bulb yellowish-brown to dark reddish-brown; flagellar

Table 2. Ratios* of body parts of two new species of *Lepidotrigona* and holotypes of *L. chandrai, L. cacciae* and *L. revanai*

↓Ratio / Species→	*L. darbhaensis* **sp.nov.**		*L. kosumtaraensis* **sp.nov.**		*L. chandrai***		*L. cacciae****	*L. revanai***
	Male	Female	Male	Female	Male	Female	Holotype (Female)	Holotype (Female)
	n-2	n-05	n-05	n-05	--	--	--	--
Head length / Head width	0.77 ± 0.01	0.79 ± 0.01	0.78 ± 0.02	0.77 ± 0.06	0.75	0.72	0.85	0.82
Eye length / Upper interocular distance	1.25 ± 0.05	1.10 ± 0.05	1.24 ± 0.01	1.12 ± 0.02	1.21	1.09	1.11	1.13
Interocellar / Ocello-ocular distance	3.22 ± 0.11	1.93 ± 0.06	2.90 ± 0.19	1.92 ± 0.05	2.00	1.44	1.50	1.61
Scape length / Eye length	0.39 ± 0.01	0.50 ± 0.02	0.38 ± 0.02	0.48 ± 0.04	0.46	0.59	0.45	0.53
Forewing length / Forewing width	2.65 ± 0.00	2.72 ± 0.05	2.85 ± 0.24	2.75 ± 0.13	2.68	2.71	2.79	2.59
Forewing diagonal length / Head width	0.63 ± 0.01	0.62 ± 0.01	0.69 ± 0.01	0.61 ± 0.03	0.83	0.55	0.61	0.61
Hind tibial length / Head width	0.70 ± 0.01	0.77 ± 0.01	0.70 ± 0.01	0.76 ± 0.06	0.69	0.68	0.72	0.75
Hind tibial length / Forewing diagonal length	1.13 ± 0.01	1.24 ± 0.02	1.01 ± 0.02	1.21 ± 0.06	1.12	1.23	1.18	1.23
Hind tibial width / Hindi tibial length	0.33 ± 0.01	0.39 ± 0.01	0.33 ± 0.01	0.41 ± 0.02	0.28	0.41	0.36	0.34
Hind basitarsus width / Hind tibial width	0.54 ± 0.01	0.57 ± 0.00	0.58 ± 0.01	0.56 ± 0.05	0.57	0.69	0.58	0.72

* All measurements in mm ± SD, **: Data based on Viraktamath & Sajan Jose (2017); ***: Data based on Rasmussen (2013)

segments blackish-brown on the upper side but lighter on the lower side. Ocelli shiny, yellowish-brown (Fig. 1i). Mandibles reddish-brown except for the lower 1/3rd mottled with dark reddish-brown (Fig. 1j). Tegula yellowish-brown; wings hyaline with pale brown pterostigma and veins. Legs dark reddish-brown except for the coxae, trochanters, and tarsi pale yellowish-brown; hind basitarsus with a reddish-brown broad longitudinal stripe in the middle. Metasomal T1 to T4 light brown anteriorly, dark brown posteriorly while the T5-T6 darker (Fig. 1h). In some all metasomal terga dark brown to black. Metasomal sterna yellowish-brown anteriorly and dark brown posteriorly.

Pilosity. Labrum fringed with long yellowish hairs. Clypeus, lateral areas of face clothed with white plumose short hairs without obscuring the underlying integument. Middle and upper part of the face with fine white hairs (Fig. 1i); vertex with brownish erect hairs. Mesoscutum, mesoscutellum with fine white hairs while the mesoscutellum fringed with long yellowish hairs. Anterior half of mesepisternum with short, plumose white hairs density and length of which increases on the lower quarter; metepisternum largely bare on the upper half while the lower half with fine short white plumose hairs (Fig. 1g). Coxae, trochanters heavily fringed with yellow hairs. The upper part of the hind tibia with sparse white hairs; anterior and posterior margins fringed with plumose white hairs. Metasomal T1-T4 with few short white hairs arranged in the transverse band, the density and length increasing on the terminal terga; sterna with a dense transverse band of short hairs on each with density increasing on the terminal segments.

Integument. Similar to the males.

Morphometry. Females measure 3.10 ± 0.14 mm in body length and 1.18 ± 0.02 mm in head width (Table 1); head 0.94 ± 0.01 mm long; upper interocular distance 0.75 ± 0.02 mm while interocellar distance 0.28 ± 0.00 mm; malar space length 0.01 mm; forewing 2.59 ± 0.05 mm long, 0.97 ± 0.03 mm wide; forewing diagonal length 0.74 mm ± 0.01 mm; hind tibial and hind basitarsus 0.89 ± 0.02 and 0.44 ± 0.01 mm long and 0.35 ± 0.01

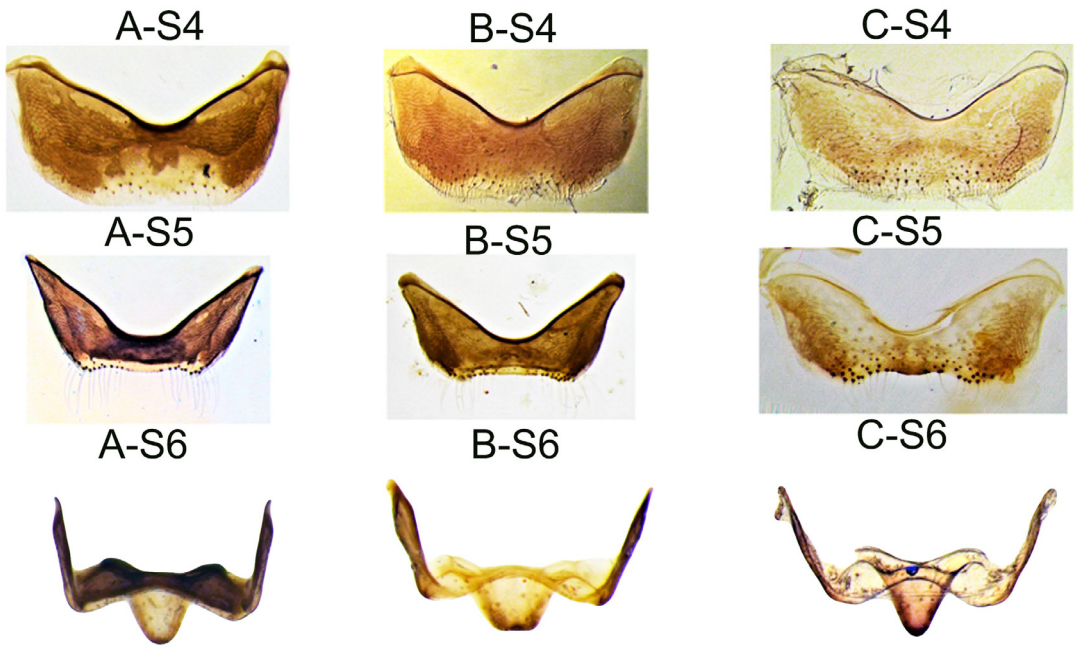

Fig. 3 Metasomal sterna: (A). *Lisotrigona darbhaensis* **sp. nov.** (B). *L. kosumtaraensis* **sp. nov.** and (C). *L. chandrai* (S4). Sternum 4, (S5). Sternum 5, (S6). Sternum 6

Table 3. Morphometry of male metasomal sterna and genitalia structures of two new species of *Lisotrigona* in comparison with male paratypes of *L. chandrai*

↓Parameter / Species→	*L. darbhaensis* **sp. nov.**	*L. kosumtaraensis* **sp. nov.**	*L. chandrai*
	n-05	n-05	n-03
Length of sternum 4	0.25	0.28 ± 0.02	0.27 ± 0.04
Width of sternum 4	0.95	1.03 ± 0.05	1.04 ± 0.02
Length of sternum 5	0.13	0.15 ± 0.00	13.5 ± 0.01
Width of sternum 5	0.82	0.93 ± 0.09	0.89 ± 0.04
Width of sternum 6	0.55	0.60 ± 0.02	0.58 ± 0.00
Length of the median lobe of sternum 6	0.20	0.19 ± 0.01	0.18 ± 0.00
Width of the median lobe of sternum 6	0.23	0.19 ± 0.01	0.15 ± 0.00
Length of gonocoxa	0.81	0.83 ± 0.04	0.79 ± 0.06
Width of gonocoxa at the mid-region	0.35	0.35 ± 0.03	0.41 ± 0.02
Length of penis valve	0.50	0.57 ± 0.04	0.54 ± 0.04
Width of penis valve at the base	0.18	0.18 ± 0.02	0.17 ± 0.02
Length of gonostylus	1.06	1.06 ± 0.22	0.87 ± 0.04

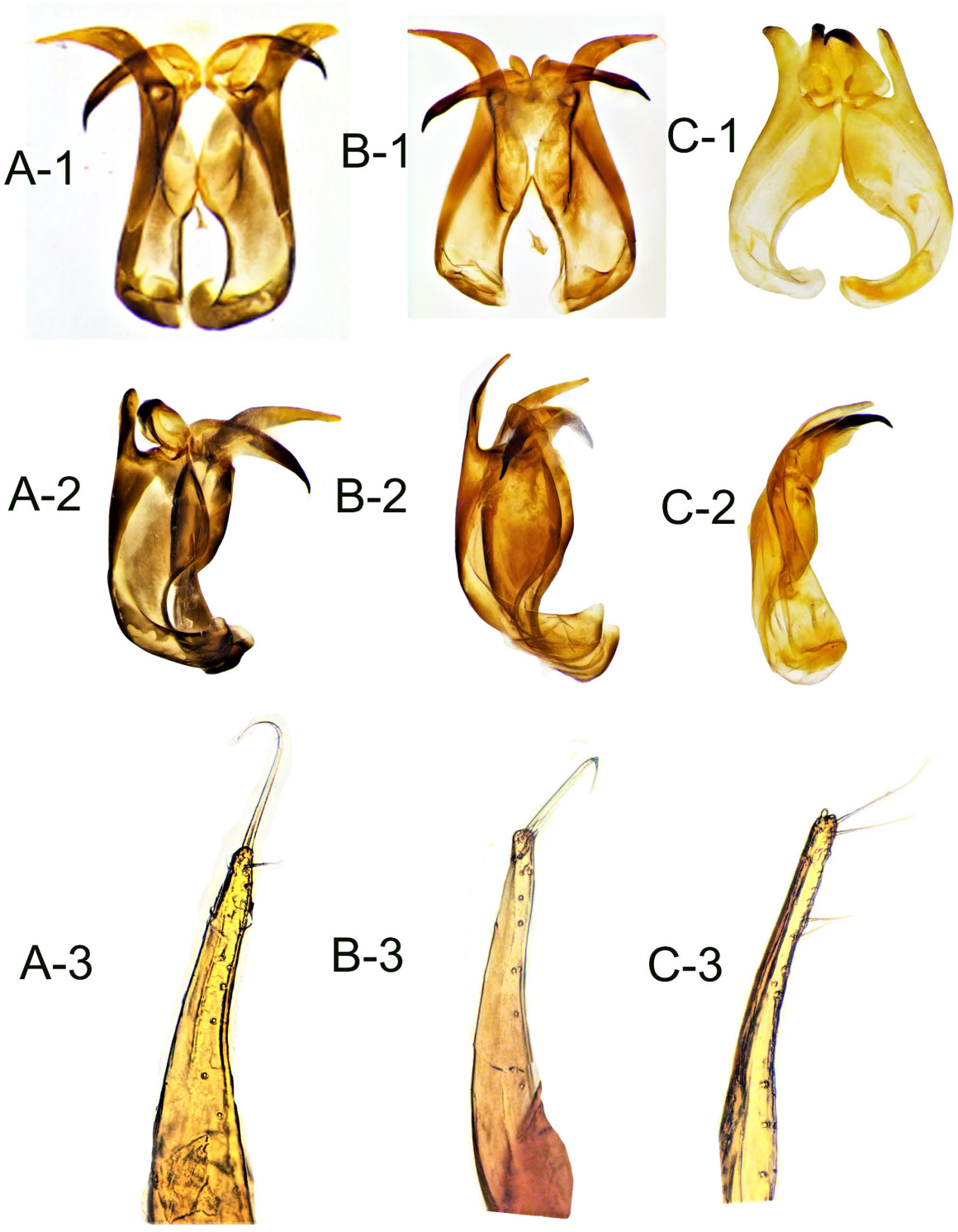

Fig. 4 Male genitalia: (A). *Lisotrigona darbhaensis* **sp. nov.** (B). *L. kosumtaraensis* **sp. nov.** (C). *L. chandrai*, (1). Genitalia ventral view, (2). Lateral view (3). Terminal part of gonostylus

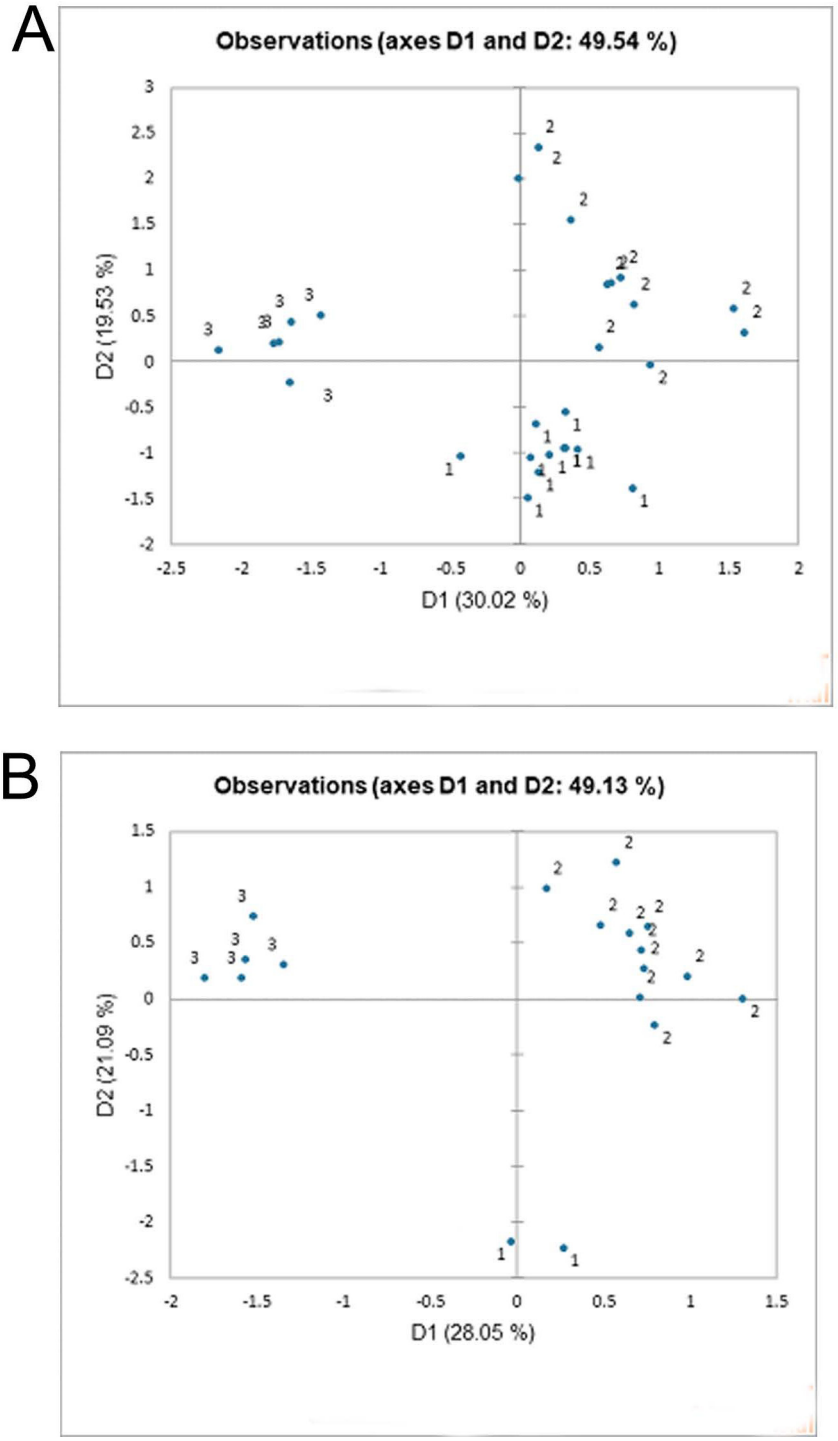

Fig. 5 Clusters of (1) *Lisotrigona darbhaensis* **sp. nov.** (2) *L. kosumtaraensis* **sp. nov.** and (3) *L. chandrai* on PCA plots formed by using 36 morphological traits. (A) Females, (B) Males

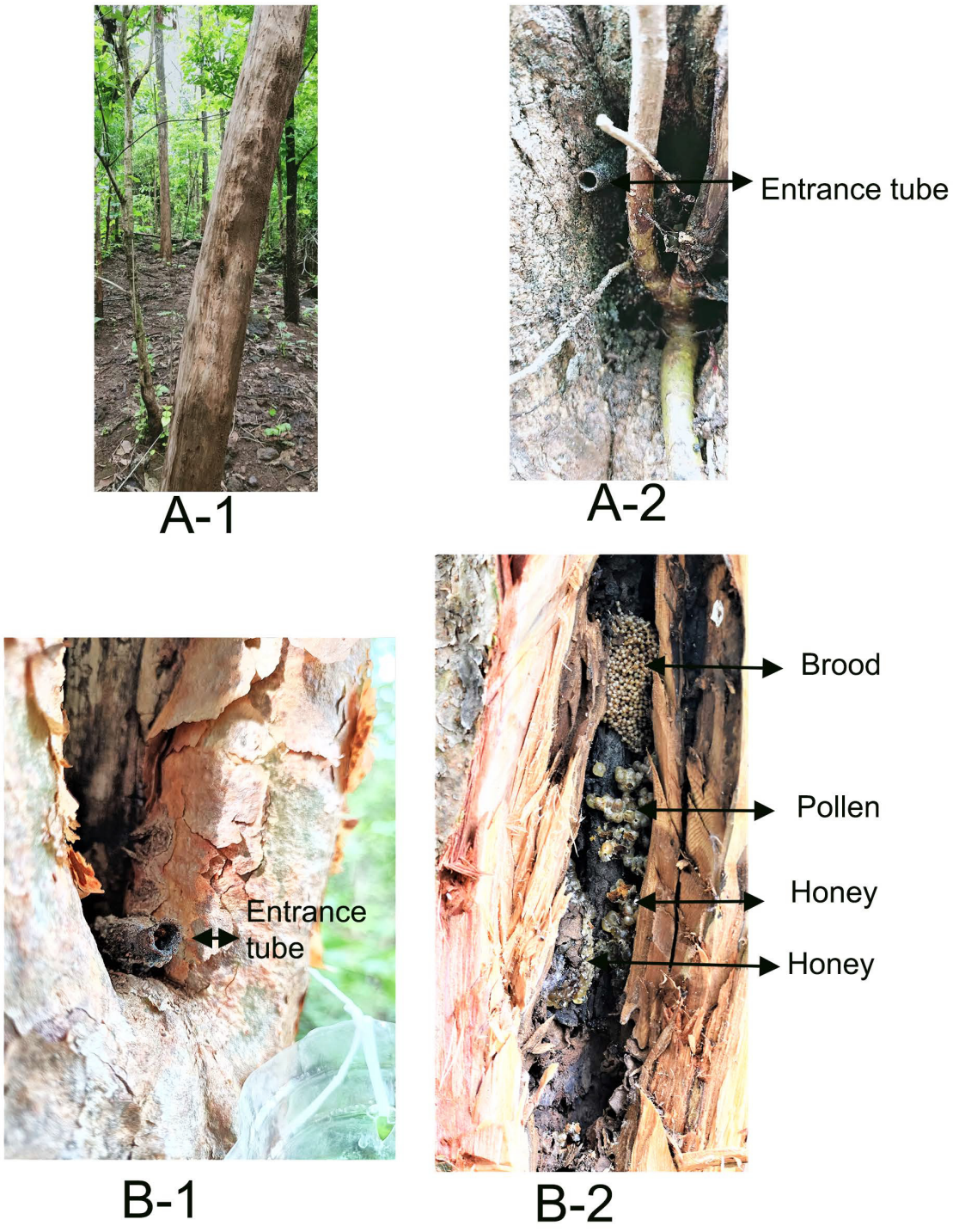

Fig. 6 Nests of (A) *Lisotrigona darbhaensis* **sp. nov.** and (B) *L. kosumtaraensis* **sp. nov.** (A-1) Nest in teakwood tree (*Tectona grandis*), (A-2) Close view of the colony showing entrance tube, (B-1) Nest in Indian frankincense tree (*Boswellia serrata*), (B-2) Inner structure of the nest showing brood, pollen and honey areas

and 0.21 ± 0.01 mm wide, respectively. Ratio of head length to width 0.79 ± 0.01 (Table 2); interocellar to ocello-ocular distance 1.93 ± 0.06; forewing diagonal to head width 0.62 ± 0.01; hind tibial length to forewing diagonal length 1.24 ± 0.02; hind basitarsus width to hind tibial width 0.57.

Nest. A single colony of *L. darbhaensis* was found in a trunk of a young teak wood tree (*Tectona grandis*) at Darbha, Chhattisgarh (Fig. 6 A-1). The colony had an entrance tube of 2 cm in length with a round opening of 1 cm in diameter (Fig. 6 A-2). The bees were very shy and stopped foraging with a slight disturbance.

Material examined. Holotype 1♂ adult. Chhattisgarh: Darbha ((18.85° N; 81.8689° E, Altitude 557 m a.s.l), 17. xi. 2020, leg. Shubham Rao with genitalia stored in genitalia vial pinned to the same pin, deposited at UASB. *Paratypes*: 1♂, 13♀ with the same collection data deposited at UASB; 1♀ paratype to be deposited at ZSIK.

Etymology. This species is named after the type locality Darbha.

***Lisotrigona kosumtaraensis* Viraktamath and Jagruti sp. nov.**

LSIDurn:lsid:zoobank.org:act:1A5D7F3B-048C-49E1-B588-67433B0B7682

Diagnosis. This species is larger than *L. darbhaensis* with males measuring a mean of 3.32 mm in body length and 1.14 mm in head width; forewings 2.82 mm long and 0.99 mm wide. The species is distinct in the following aspects: setae arising from basal area of gradulus of the metasomal sternum 4 extend beyond the apical margin (Fig. 3 B-S4); apical margin of metasomal sternum 5 nearly straight (Fig. 3 B-S5); antecosta in metasomal sternum 6 distinctly convex medially (Fig. 3 B-S6); apicomedian lobe of the metasomal sternum 6 as long as broad with broadly rounded apex; gonocoxae elongate and narrower, flipped J-shaped as in *L. darbhaensis* (Figs. 4. B-1, B-2); penis valves with distinctly curved pointed apices as in *L. darbhaensis,* and *L. chandrai* (Figs. 4 B-2); gonostyli long and wider. Female bees with distinct punctures on mesoscutum as in *L. darbhaensis, L. chandrai* and *L. revanai*.

Description. Males:

Coloration. Head, mesosoma and metasoma black (Fig. 2 a,b). Labrum yellowish-brown to dark reddish-brown; clypeus dark reddish-brown; scape, pedicel, flagellar segments dark reddish-brown approaching to black except for the basal bulb of scape and antennal sockets light reddish-brown. Pedicel and flagellar segments brownish-black. Ocelli shiny, light reddish-brown; compound eyes brown to dark reddish-brown approaching black, but in some appearing greyish black in frontal view (Fig. 2c). Mandibles ochraceous with light brown mottling in the basal and middle region (Fig. 2d). Pronotal lobe, tegulae light reddish-brown. Wings hyaline with brownish pterostigma and veins. Mesoscutellum light reddish-brown to black. Legs dark reddish-brown except for coxae, trochanter, and tarsi ochraceous. Metasomal terga with light brownish irregular mottling; sterna ochraceous medially

Pilosity. Labrum with sparse white hairs. Clypeus clothed with fine white plumose hairs without obscuring underlying integument. The lower part of the face with white plumose prominent hairs while the upper part with fine white hairs (Fig. 2c). Vertex with white erect hairs. Mesoscutum covered with fine white hairs. Mesoscutellum fringed with long white hairs. Coxae, trochanters, tarsi heavily fringed with long yellowish hairs while femora, tibiae are clothed with short white hairs; Upper surface of the hind tibia with sparse white hairs while anterior margin fringed with white hairs and posterior margin with dense plumose white hairs. The anterior and lower part of mesepisternum, the lower part of metepisternum with white, plumose long hairs; the lateral surface of propodeum with dense short white plumose hairs (Fig. 2b). Posterior margin of metasomal terga and sterna with a transverse band of very fine white hairs the density and length of which increasing towards terminal metasoma.

Integument: Clypeus, entire face, vertex distinctly punctate. Integument surface in between antennal sockets distinctly depressed. Mesoscutum, mesoscutellum, pleural area punctate densely;

mesoscutellum shiny, finely punctate. The middle part of propodeum imbricate; metasomal terga sparsely punctate otherwise glabrous.

Morphometry. Male bees measure a mean of 3.32 ± 0.20 mm long with 1.14 ± 0.03 mm wide head; head length 0.89 ± 0.04 mm; upper interocular distance 0.71 ± 0.02 mm; interocellar distance 0.29 ± 0.01 mm; diameter of median ocellus 0.14 ± 0.01 mm; forewings 2.82 ± 0.13 mm long, 0.99 ± 0.06 mm wide; forewing diagonal length 0.79 ± 0.01 mm; hind tibiae 0.80 ± 0.01 mm long, 0.26 ± 0.01 mm wide while the hind basitarsus 0.41 ± 0.01 mm long, 0.15 mm wide (Table 1). Mean ratio of head length to width 0.78; eye length to upper interocular distance 1.24; interocellar to ocello-ocular distance 2.90; scape length to eye length 0.38; forewing diagonal length to head width 0.69; hind tibial width to length 0.33 and hind basitarsus width to hind tibial width 0.58 (Table 2).

Metasomal sterna and genitalia. The following description is based on the dissection of five male paratypes. Sternum 4 measures 0.28 mm long, 1.03 mm wide (Table 3); gradulus touching the antecosta briefly at the medial region; lower 1/3rd area of the sternum with long setae extending beyond the apical margin (Fig. 3 B-S4); Sternum 5 densely pigmented, 0.13 mm long with widely separated apodemal lobes (0.93 mm); gradulus in touch with antecosta medially; apical margin nearly straight with long setae arising submarginally (Fig. 3 S-5); in sternum 6, the antecostal margin distinctly convex medially; apicomedian lobe as long as broad (0.19 mm) with broadly rounded apex (Fig. 3 S-6). Genitalia yellowish brown except the terminal half of penis valves dark reddish-brown; each gonocoxa with an incurved basal extension (0.83 mm long) the terminal part of which curved inwards and then upwards resembling a flipped J-shape (Figs. 4 B1, B2); broadest (0.35 mm) at the apical half where both gonocoxae come in close contact; penis valves arise from the anterior margin of gonocoxae, 0.18 mm wide at the base, tapering towards apical region terminating in curved pointed apex (Figs. 4 B-2); gonostylus arises from the lateral side at basal 1/3rd of each gonocoxa, wider at the midlength and tapering terminally with broad rounded, curved apex; two long and curved setae arise from the apical margin; a circlet of short spines submarginally and a longitudinal row of short spines along the terminal half (Fig. 4 B3); each gonostylus 1.06 mm in length.

Females:

Coloration. Head, mesosoma, metasoma black (Fig. 2 e,f.) Labrum reddish brown; clypeus dark reddish-brown; compound eyes blackish brown; ocelli shiny with brownish tinge; scape reddish-brown to black except for basal bulb and antennal socket light reddish-brown; flagellar segments blackish-brown on the upper side but light brown on the lower side (Fig. 2g); mandibles reddish-brown with dark reddish-brown mottling on basal 1/3rd (Fig. 2h) Pronotal lobe, tegula yellowish-brown; wings hyaline with reddish-brown tinge; pterostigma, veins light brown. All legs black except coxae, trochanters, and tarsal segments reddish-brown; hind basitarsus with the broad reddish-brown longitudinal band.

Pilosity. Labrum fringed with reddish-brown long hairs. Clypeus with fine white hairs; lower part of the face with distinct plumose white hairs; (Fig. 2g) vertex with short brownish erect hairs; gena, post gena fringed with sparse long white hairs; occipital area with fine white hairs. Mesoscutum and mesoscutellum with fine white hairs; mesoscutellar margin fringed with long ochraceous hairs. Anterior half of mesepisternum with distinct, long plumose white hairs; metepisternum with sparse white hairs; the lateral surface of propodeum with thick short plumose white hairs (Fig. 2f). Metasomal tergum with a transverse band of white hairs near basal margin the density and length of which increases towards the caudal end; sterna with a very dense transverse band of yellowish hairs with density increasing towards caudal end.

Integument. Head, mesosoma and metasoma shiny. Clypeus with dense fine punctures; entire face, vertex with distinct punctures; gena glabrous. Mesoscutum, pleural area, mesoscutellum with dense minute punctures. Middle area of propodeum imbricate. Metasomal terga with very sparse punctures otherwise glabrous.

Table 4. Differentiating characters among the males of known species of *Lisotrigona* from the world

↓Parameter / Species→	*Lisotrigona darbhaensis* **sp. nov.**	*Lisotrigona kosumtaraensis* **sp. nov.**	*Lisotrigona chandrai*	*Lisotrigona furva* ----
Metasomal sternum 4	Gradulus briefly touches antecosta medially	Gradulus briefly touches antecosta Medially	Gradulus does not touch antecosta	
	Setae from gradulus do not extend beyond the apical margin	Setae from gradulus extend beyond the apical margin	Setae from gradulus extend beyond the apical margin	
Metasomal sternum 5	Gradus briefly touches antecosta medially	Gradus briefly touches antecosta medially	Gradulus does not touch but is very close to the antecosta.	Apical margin distinctly inverted U-shaped
	Apical margin weakly bisinuate	Apical margin weakly bisinuate	Apical margin distinctly bisinuate	
Metasomal sternum 6	Antecosta straight in the middle	Antecosta distinctly convex in the middle	Antecosta distinctly concave in the middle	Antecosta weakly concave
	Apicomedian lobe wider than long	Apicomedian lobe as long as broad	Apicomedian lobe longer than wide	Apicomedian lobe longer than wide
Gonocoxa	Elongate, narrower, flipped J-shaped	Elongate, narrower, flipped J- shaped	Shorter, wider, C-shaped	Shorter, wider, C-shaped
Penis valve	Elongate with curved pointed terminally	Elongate with curved pointed terminally	Elongate with curved pointed terminally	Broader with straight, slender apically
Gonostylus	Long, broader with bluntly rounded apex	Long, broader with bluntly rounded apex	Long, broader with bluntly rounded apex	Shorter, very slender

Morphometry. Female paratypes measure 3.12 ± 0.25 mm, 1.19 ± 0.04 and 0.94 ± 0.04 mm in body length, head width and head length, respectively (Table 1); upper interocular distance 0.79 ± 0.02 mm; interocellar distance 0.28 ± 0.00 mm; diameter of median ocellus 0.12 ± 0.00 mm; forewings 2.64 ± 0.09 mm long, 0.96 ± 0.03 mm wide; wing diagonal length 0.74 ± 0.01 mm; hind tibia and hind basitarsus 0.91 ± 0.03 and 0.48 ± 0.02 mm long, respectively. Ratio of head length to width 0.77; interocellar to cello-ocular distance 1.92; forewing diagonal length to head width 0.61; hind tibial length to forewing diagonal length 1.21; hind basitarsus width to hind tibial width 0.56 (Table 2).

Nest. One colony of *L. kosumtaraensis* was found in a vertical cavity (54.40 x 5.6 cm) of an Indian frankincense tree at a height of 124 cm from the ground (Fig. 6 B-1). The colony had a long, dark brown entrance tube of 6.9 cm in length with an oval opening of 0.6 x 0.8 cm. The inner surface of the entrance tube was thin, but the exterior surface was rough made of propolis. Brood was a mix of spheroid and ellipsoid cells, arranged in a cluster in the middle of the cavity (Fig. 6 B-2). The brood cell was 2.24 ± 0.09 mm wide and 2.72 ± 0.36 mm in height. All the brood cells were connected by thin cerumen connectives and to the cavity by the pillars. Honey cells were located above the brood area as well as at the bottom of the cavity. Pollen cells were found just below the brood (Fig. 6 B-2). Each honey and pollen cell measured 0.84 ± 0.25 and 0.87 ± 0.23 mm in width and 1.10 ± 0.18 and 1.11 ± 0.16 mm in length, respectively. The colony consisted of 621 female and 40 male bees which formed 4.16 percent of the total population of the bees.

Material examined. Holotype: 1♂ adult. Maharashtra: Kosumtara, (21°16' 6"N; 80° 32' 37" E, Altitude 355 m.s.l), 25. vii. 2021, leg. Jagruti Roy deposited at UASB. *Paratypes*: 13♂, 13♀ with same collection data; 7♂, 2♀ with same data but collected on 16. vii. 2021, 10♂, 9♀ collected at Maharashtra: Navatolla (21° 16' 52"N; 80° 33' 36" E), 16. vii. 2021, leg. Jagruti Roy, deposited at UASB; 1♀ paratype to be deposited at ZSIK.

Etymology. This species is named after the type locality Kosumtara.

***Lisotrigona chandrai* Viraktamath and Sajan Jose, 2017**

Lisotrigona chandrai was described and illustrated by Viraktamath and Sajan Jose (2017) from the type locality Kanhangad, Kerala. Detailed morphometry of male and female type specimens is presented in table 1 (Viraktamath and Sajan Jose 2017) and the ratios of body parts in table 2. Male metasomal sterna provide important diagnostic characters along with genitalia structures to identify the species (Attasopa *et al*., 2018). However, Viraktamath and Sajan Jose (2017) did not describe the male metasomal sterna of *L. chandrai* in their publication. Hence, we describe these sterna to compare with all the known species of *Lisotrigona* after re-examining the type specimens deposited at the UASB.

Description of metasomal sterna. Sternum 4 is lightly pigmented with gradulus not touching the antecosta; setae arising from gradulus extend beyond the apical margin (Fig. 3 C-S4); sternum 5 lightly pigmented, 0.15 mm in length, 0.93 mm in width; gradulus very close to antecosta without touching it medially; short setae arise from most of gradulus (Fig. 3 C-S5); the apical margin distinctly bisinuate; sternum 6 lightly pigmented except lateral apodemal lobes and apicomedian lobe; antecosta distinctly concave medially; the apicomedian lobe longer (0.18 mm) than wide (0.15 mm) with broadly pointed apex (Fig. 3 C-S6).

DISCUSSION

Stingless bees that belong to the genus *Lisotrigona* are rare (Engel 2000; Viraktamath *et al*., 2021b) and occur in India and Southeast Asia. *Lisotrigona cacciae* the type species of the genus described from India is also reported from Thailand, Borneo, and Sri Lanka (Michener 2007; Karunaratne *et al*. 2017). Engel (2000) described *L. carpenteri* from Vietnam, Cambodia, and *L. furva* from Thailand. However after 22 years males of *L. carpenteri* were discovered and the male genitalia were found unique and different from those of other known species of *Lisotrigona.* Hence, Engel et al (2022) transferred *L. carpenteri* to a new genus *Ebaiotrigona* with *L. carpenteri* as type species of the new genus. So far five species of *Lisotrigona* are known in the world. Michener (2007) compared the populations of *L. cacciae* and *L. furva* from Thailand and reported that both species are extremely similar and all the characters (except the head width) which seemed to differentiate both species failed. Engel (2000) reviewed the genus *Lisotrigona* from Indo-Malayan region and compared *L. cacciae* and *L. scintillans* (Cockerell). Since he did not find differences in size, integument sculpturing and major differences in coloration, he synonymized *L. scintillans* with *L. cacciae.* Interestingly no efforts have been made to collect and describe males, queens, and nest structure of *L. cacciae* which is reported to be very common in Thailand and Southeast Asia.

In India, besides *L. cacciae,* three species of *Lisotrigona* namely, *L. mohandasi, L. revanai,* and *L. chandrai* were described (Jobiraj and Narendran 2004; Viraktamath and Sajan Jose 2017). However, Rasmussen *et al.* (2017) synonymized these species with *L. cacciae* though Viraktamath and Sajan Jose (2017) provided critical differences in morphometry, male genitalia structures among known species of *Lisotrigona* besides describing queen and nest structure for *L. chandrai*.

Recent efforts in the collection of large samples of *Lisotrigona* female bees from feral colonies and analysis of morphometric data of these samples indicated the occurrence of more than one species in India (Viraktamath *et al.* 2021b). The authors success in collecting male bees in association with females from two states (Chhattisgarh and

Maharashtra) and the study of morphometry of 36 parameters, structure of male metasomal sterna, male genitalia along with mapping of the species on a PCA plot, revealed the distinctiveness of these bees which led to the description of *L. darbhaensis* and *L. kosumtaraensis* as new species. These two new species are different from each other as well as other known species of *Lisotrigona* in morphometry, the structure of male metasomal sterna and genitalia as enumerated in (Tables 1 to 4 and Figs. 3, 4; also see the diagnostic characters under *L. darbhaensis*).

Female bees are distinctly punctate on mesoscutum of *L. darbhaensis, L. kosumtaraensis, L. revanai,* and *L. chandrai.* However, *L. cacciae* has exceedingly minute faint punctures while *L. furva* has strong punctures. Both the new species differ from *L. scintillans* in having the face, vertex, mesoscutum, abdomen clothed with short white hairs while in *L. scintillans,* these body parts are not hairy except little pale hairs at the sides of face (Cockerell, 1920). The ratio of interocellar to ocello-ocular distance is higher in *L. darbhaensis* (1.93) and *L. kosumtaraensis* (1.92) while lower in *L. chandrai* (1.44), *L. cacciae* (1.50), and *L. revanai* (1.61). Analysis of morphometry data of both male and female bees of *L. darbhaensis, L. kosumtaraensis* and *L. chandrai* also confirmed the distinctiveness of these new species as all the three species formed distinct clusters on the PCA plots (Fig. 5A, B).

The colony strength of 961 bees (921 females + 40 males) in *L. kosumtaraensis* is an approximate estimation as it was not sure whether all the foraging bees had returned at the time of capturing the colony and hence not considered the brood cells. Interestingly male bees formed 4.16 percent of the total bee population. However, Viraktamath (unpublished) obtained only 6 males (2.68%) and 224 females when an entire colony of *L. chandrai* was captured.

The discovery of these two new species increases the number of *Lisotrigona* species to six from India. However, a critical study of male metasomal sterna, genitalia with associated female bees may reveal the underlying rich diversity of India which remains largely unexplored. The results strongly indicate the necessity of revision of the genus from the Indian subcontinent.

Based on the evidence of the present study, it is proposed that all the three species namely, *L. mohandasi, L. chandrai,* and *L. revanai* synonymized with *L. cacciae* by Rasmussen *et al.* (2017) need to be considered as valid and distinct from *L. cacciae*. The authors are also of the opinion that the occurrence of *L. cacciae* outside India needs to be verified by collecting males with correctly associated females and a critical study of male genitalia along with metasomal sternal structures.

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A new species of *Purana* Distant, 1905 (Hemiptera, Cicadidae), from the Western Ghats, with comments on the erroneous records of *Purana tigrina* (Walker, 1850) in south India

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ABSTRACT: A new species of cicada is described from south India. The new taxon, *Purana cheeveeda* Sadasivan **sp. nov.** is easily differentiated from all the other known species of the *Purana tigrina* species group based on its operculum apex not reaching beyond the anterior margin of sternite 3; tubercles on sternite 4 black and almost as large as those on sternite 3; relatively short rostral length reaching unction of abdominal sternite II and sternite III; the absence of dark fasciae on the transverse grooves of postclypeus, forewing venation, basal lobes of pygofer with large diverging triangular spines and the characteristic pentagonal uncus of the male. The status of *Purana tigrina* (Walker, 1850) from South India is discussed. The topotypes of the taxon commonly identified as *P. tigrina* from south India did not match the morphology of the holotype of *P. tigrina*. In addition, the study of the type specimen of *P. tigrina* demonstrated that the external morphology and male genital characters of the holotype of *P. tigrina* match that of *P. tigrina* from the Malayan region. Hence the type locality is mislabelled. This common taxon from southern India which has been traditionally misidentified is described here as a new species of *Purana*. As per Articles 76A.1.4 and 76A.2, of the *International Code of Zoological Nomenclature* (1999), the type locality of *P. tigrina* is hereby corrected as Malaysia. Based on the findings, *P. tigrina* is removed from the south Indian cicada fauna. The new species has some features common to both the *Purana carmente* group and *Purana tigrina* group, but most characters agree to *Purana tigrina* group, hence is tentatively placed in this group. The characters based on coloration may not be useful in species group classification in *Purana*, hence structural features like male genitalia and venation are taken to revise the existing species group keys. A modified key to the *Purana* species groups and members of the *P. tigrina* species group is also provided.
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INTRODUCTION

Purana Distant (1905) is a speciose Oriental genus of cicadas distributed from Sri Lanka and south India to Malaysia and the Philippine Islands (Distant, 1906; Sanborn, 2013). This genus comprises many cryptic species with high endemism and is distinguishable based only on the morphology of male genitalia and their singing calls, as exemplified in the works of Boulard (2006, 2007, 2013). Price *et al.* (2016) on the distribution of cicadas in the Indian subcontinent, listed four known species from the genus namely *P. tigrina* (Walker, 1850) ranging from south India to Sumatra, *P. morrisi* (Distant, 1892) limited to south India, *P. guttularis* (Walker, 1858) from north-eastern India to Sumatra, and *P. campanula* Pringle, 1955 from Sri Lanka. *Purana tigrina* was described as *Dundubia tigrina* "From Mr. Walker's collection" in the then British Museum of Natural History (NHM) and the type locality was given as 'Malabar' (Walker, 1850: 70). Later, it was treated as *Leptopsaltria tigrina* by Distant, (1889), and the distribution was mentioned as Continental India (Malabar and Trivandrum), and Malay Peninsula (Province Wellesley) as per Distant (1889–1892). Later in 1905, Distant placed the species in the newly erected genus *Purana* and designated it as the type species (Distant 1905). Distant (1906), mentioned the localities of specimens of *P. tigrina* as Malabar, Trivandrum, Tibet, and the Malay Peninsula. Another species from south India, *P. morrisi* (Distant, 1892) was described as *L. morrisi* Distant, 1892. The type specimen of *P. morrisi* BMNH(E) 1009417 was collected from Shivarai Hills, Salem district, Tamil Nadu (Distant, 1906; Price *et al.,* 2016). This species is known only from Madras Province (Tamil Nadu), as per Distant (1906) and Price *et al.* (2016). The Western Ghats and south India thus far have only two known species *viz. P. tigrina* and *P. morrisi*.

Walker's (1850) original description of *P. tigrina* provides only a basic account of its coloration and morphology with no illustrations or any mention of the male genitalia. Similarly, Distant (1889–1892) in his *Monograph of Oriental Cicadidae*, and later in Rhynchotal Notes (Distant 1905), and the Fauna of British India series (Distant 1906) did not examine or describe the male genitalia. It is possible that species lumping was committed by Distant (1905), wherein he used the same name for all similar-looking species from Malabar, Travancore, and Malaya, thus expanding the distribution of that taxon from south India to the Indo-Malayan sub region. Price *et al.* (2016) provided a provisional catalogue, regional checklist, and bibliography of cicadas of the Indian Subcontinent. Price *et al.* (2016), and their online catalogue of images of species and types from the NHM London accessible at https://www.indiancicadas.org (Marathe *et al.,* 2022), does not illustrate the male genitalia of any of the *Purana* species, nor did they discuss the taxonomic validity of any of these taxa.

A similar taxonomic issue in another cicada species from south India was resolved recently by Sadasivan (2021), where, taxonomic lumping had occurred with *Pomponia linearis* (Walker, 1850). Although *P. linearis* was said to occur in the Western Ghats as per Distant (1906) and Price *et al.* (2016), taxonomic confirmation was lacking. It was observed that none of the early descriptions of *Pomponia* from the Western Ghats were complete, and details of male genitalia were absent in Walker (1850) and Price *et al.* (2016). It was finally established that the records of *Pomponia linearis* from the Western Ghats, were erroneous (Sadasivan 2021), and this taxon, which was a hitherto undescribed species, was described as *P. pseudolinearis* Sadasivan, 2021.

During the documentation of cicadas of Kerala State in south India, noted a very common morphotype of cicada similar to *P. tigrina* in external appearance, but differed considerably from the holotype in the structure of the male genitalia and operculum. This species commonly referred to as *P. tigrina* in the published literature on cicadas from the region (Marathe *et al.,* 2022) is very common and abundant throughout Kerala. Of the specimens examined from Kerala, none matched

the holotypes of *P. tigrina* or *P. morrisi* previously said to inhabit the area as per Distant (1905) and Price *et al.* (2016). Moreover, Duffels *et al.* (2007) had established that *P. tigrina* is a taxon inhabiting the Malayan region. On comparing the morphotype with the holotype and specimens of *P. tigrina* from Malaya in NHM London, along with the illustrations of the genitalia of Malayan specimens of *P. tigrina* from Duffels *et al.* (2007) with those of the holotype and that of our taxon, it was observed that specimens from Malabar, Cochin, and Travancore regions of Kerala State differed considerably from the type of *P. tigrina* and Malaysian material of this species in numerous morphological characteristics, especially the male genitalia. Hence it is described here as a new species. Correcting the nomenclature of this extremely common species is important for other taxa awaiting description from the region.

MATERIALS AND METHODS

During the faunal exploration of the state of Kerala in the Western Ghats of south India, numerous cicadas were documented, including a few morphotypes of the *Purana tigrina* species group. Of them, the commonest and most abundant morphotype matching the descriptions of *P. tigrina* was found to be extensively distributed throughout the lowlands of the state and was the only species found to inhabit the previous collection localities of *P. tigrina* in Kerala, as per published data from Distant (1906) and Price *et al.* (2016). Specimen sampling was done from the locations representing the erstwhile state of British Malabar (the apparent type locality of *P. tigrina*), and the old kingdoms of Cochin and Travancore. The study locations were Nilambur in Malappuram District in northern Kerala representing Malabar; Mukundapuram, Trichur district, and Pala in Kottayam district from central Kerala representing Cochin region; and Palode and Kulathupuzha in Trivandrum District representing the erstwhile Travancore (Fig. 1). Images of the male genitalia of the type specimen of *P. tigrina* were obtained from NHM London with the following labels: "Malabar" NHMUK 013585498 (formally BMNH(E) # 1009413). Reference was also made to the type of *P. morrisi* (Distant, 1906) (NHMUK 013585498, formally BMNH(E) # 1009413), also from south India. Field photographs were taken with a Canon 70D Digital SLR camera, Canon 180 mm macro lens, and MPE 65 f 2.8 1–5x Lens. The morphology was studied and measurements were taken with a HEADZ Model HD81 stereomicroscope. Terminology for morphological description and venation follows Moulds (2005). The basic cicada taxonomy follows Dmitriev *et al.* (2021). Measurements follow a modification of morphometrics from Sarkar (2019) and Sadasivan (2021). Orientation of spines as per their mid-axis and their attachment to the body is referred to as 'erect', 'semi-erect', 'semi-decumbent', 'decumbent' and 'adpressed' (Sadasivan, 2021). The structure of the male genitalia was studied in situ for the type specimens, and for detailed illustrations, they were dissected and treated with 10 per cent KOH overnight and later preserved in glycerol. Illustrations were hand-drawn by the first author and then digitalized. The original descriptions, type specimens, and field photographs were analysed.

Measurements (in mm taken in the dorsal view, unless specified) and indices used in descriptions as per Sadasivan (2021) are as follows (arranged in cephalocaudal order) –

HL—Head length; length of the head in the midline from the anterior-most point of the postclypeus to the mid-posterior margin of the head, measured dorsally.

HW—Head width; width of the head including the compound eye, measured between the lateral-most points of convexity of the compound eye in dorsal view in the transverse plane.

EL—Eye length in the dorsal view.

PL—Pronotum length at the mid-dorsal line.

PW—Pronotum width; maximum width, measured in dorsal view.

ML—Mesonotum mid-dorsal length to the cruciform elevation, in dorsal view.

MW—Mesonotal width.

FWL—Forewing length; the maximum expanse of the forewing from its medial most attachment to the mesonotum to the most convex part of its apex.

FWW—Forewing width; distance between the node and the tornus across the forewing.

HWL—Hindwing length; the maximum expanse of the hindwing from its medial most attachment to the mesonotum to the most convex part of its apex.

AL—Abdomen length; mid-dorsal length of the abdomen measured from the posterior-most point on the cruciform elevation to the tip of the pygofer or anal style, whichever is the farthest, in the freshly killed insect.

AW—Abdomen width; the maximum width measured in the transverse plane in dorsal view, in the freshly killed insect.

OPL—Operculum length, in lateral view.

RL—Rostrum length.

ABL—Anterior body length; length of the specimen from the anterior tip of postclypeus to the posterior of scutellum in the midline, HL + PL + ML.

TL—Total Length; HL + PL + ML + AL.

CI—Cephalic Index; (HW/HL) × 100.

OI—Ocular Index; (EL/HW) x 100.

PI—Pronotal Index; (PW/PL) × 100.

MI—Mesonotal Index; (MW/ML) × 100.

OPI—Opercular Index; (OPL/ABL) × 100.

API—Anteroposterior Index; ABL/AL × 100.

RI—Rostral Index; (RL/ABL) × 100.

FAR—Forewing Aspect Ratio; high aspect ratio indicates long, narrow wings, and a low aspect ratio indicates short, wide wings (FWL/FWW) × 100.

FI—Forewing Index; (FWL/ABL) × 100.

IWR—Inter-Wing Ratio; (FWL/HWL) × 100.

GI—Gastral Index; AW/AL x 100, high index value indicates a relatively wider abdomen.

RESULTS

On the misidentification of *Purana tigrina* in south India

Duffels *et al.* (2007), in their revision of the *Purana tigrina* species group, stated that they had examined the holotype of *P. tigrina* but the collections studied by them revealed no further specimens of *P. tigrina* from India or adjacent areas and they concluded that the type specimen may have been mislabelled. While the holotype may be mislabelled, Price *et al.* (2016) recorded the type locality as Malabar itself. None of the early works mentioned male genitalia morphology in species descriptions and hence we think that species lumping (taxonomic lumping) occurred in Distant (1905), where he used the same taxon name for similarly looking taxa from Malabar, Travancore, and Malaya, thus expanding the distribution to southeast Asia. This was reiterated later in Distant (1889–1892, 1905, 1906) and Price *et al.* (2016) due to the lack of examination of male genitalia. Many morphological features, including the male genitalia, of any of the known south Indian species from the *P. tigrina* species group, did not match that of the type specimen of *P. tigrina* which matches specimens of *P. tigrina* from the Malayan region (see Fig. 2 in Duffels *et al.*, 2007). Similarities include, elongated triangular uncus (compared to trapezoidal uncus in south Indian specimens) and slightly converging, ridge-like basal pygofer lobes, compared to spine-shaped basal pygofer lobes in south Indian specimens. This implies that the original type locality as "Malabar" is erroneous, and the *P. tigrina* holotype is mislabelled. Despite extensive efforts in Trivandrum over a decade, we were able to collect only a single morphotype described as a new species below, the male genitalia of which significantly differed from that of the type specimen of *P. tigrina*.

A similar error was recently corrected on the identity of *Pomponia linearis* (Walker, 1850) from south India (Sadasivan, 2021) where taxonomic lumping had occurred with species from northeast India to Vietnam and southeast Asia, and the misidentified one turned out to be a new species *Pomponia pseudolinearis* Sadasivan, 2021. Similar-looking species from a large geographical area were taxonomically lumped into a single taxon due to a lack of study of the male genitalia.

According to Duffels *et al.* (2007), *P. tigrina* is a common species in the Malayan Peninsula, Bunguran Island, South Borneo (Kalimantan Timur),

Sumatra, and Nias. Distant (1905) used the same species name for similar-looking taxa from Malabar, Travancore, and Malaya, thus expanding the distribution to South India. This was followed later by Distant (1906) and Price *et al.* (2016) who did not examine the male genitalia. In agreement with Duffels *et al.* (2007), the older records of occurrence of *P. tigrina* from south India must be treated as misidentifications of the very common and widespread new species of the region described below. As per Articles 76A.1.4 and 76A.2, of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999), the type locality of *P. tigrina* is corrected to be Malaysia. Based on these findings *P. tigrina* is removed from south Indian cicada fauna, while the exact limits of distribution in north-east India need to be delineated with extensive fieldwork and study of male genitalia.

Systematic Part

Family Cicadidae Latreille, 1802

Subfamily Cicadinae Latreille, 1802

Tribe Leptopsaltriini Moulton, 1923

Subtribe Puranina Lee, 2013

Genus *Purana* Distant, 1905

Type species: *Purana tigrina* (Walker, 1850), as per Distant (1905).

Type locality: Malaysia (fixed in this paper as per Articles 76A.1.4 and 76A.2, of the *International Code of Zoological Nomenclature*, 1999).

Diagnosis. Head including eyes about as wide as mesonotum; vertex long, its anterior end situated far beyond the level of anterior margin of eyes; lateral margin of pronotum anteriorly dentate; forewing with marginal areas narrow; anterior longitudinal vein (alv) of apical cell 5 as long as that of apical cell 7; bases of apical cell 2 & 3 strongly infuscated; timbal cover well-developed, without black patch; male operculum small, scale-like, short, reaching or just passing posterior margin of sternite II; male abdomen about as long as the distance from head to cruciform elevation; a pair of tubercle-like projections on abdominal sternites III and IV with projection on each posterolateral surface nearly longitudinally protruding posteriorly; uncus lobes fused and undivided; basal lobe of pygofer (blp) distinct with apex spine-shaped; aedeagus thin (Walker 1850; Distant 1905; Lee 2009; Lee and Emery 2013).

Purana cheeveeda Sadasivan **sp. nov.** (Fig. 2–5)

LSIDurn:lsid:zoobank.org:act:43B6F099-DC3F-45C1-AF60-3E787470D7B1

Holotype—Male, Pala, Kottayam District, Kerala State, India. Col. Jebine Jose 15. vi.2021, 100m A.S.L., from a private estate, male located by its call. Dry pinned specimen. Holotype number THRG 0039. Will be deposited in the National Center for Biological Sciences (NCBS), Bengaluru, India.

Paratypes (4 males and 3 females)—Female THRG 0040; bearing the same data as the holotype; dry pinned specimen; will be deposited in the National Center for Biological Sciences (NCBS), Bengaluru, India. Male THRG 0042, THRG 0049, and female THRG 0050; Mukundapuram, Trichur District, Kerala State, India; Col. Muralimohan; 26.v.2021; 20m A.S.L., dry pinned specimens; will be deposited in National Center for Biological Sciences (NCBS), Bengaluru, India. Male THRG 0041 & female THRG 0048; Palode, Trivandrum District, Kerala State, India; Col. Kalesh Sadasivan; 21. vii.2019; 50m A.S.L.; Dry pinned specimens; will be deposited in the Western Ghats Regional Station, Zoological Survey of India (ZSI), Calicut. Male THRG 0043 (wet specimen in ethanol) and THRG 0047 (dry pinned specimen); Nilambur, Malappuram District, Kerala State, India; Col. Bernard M Thampan; 10. vi.2021; 400m A.S.L.; will be retained as voucher specimens in TNHS collections.

Description of the Holotype (male, THRG 0039). (Fig. 2–5)

The description of the holotype is given in the live state. Upon preservation in alcohol, the yellows and greens lose saturation and become yellow-brown, while blacks and greys become dark brown.

Head. In dorsal view, head small, postclypeus with anterior margin rounded, tip angular; head much wider than long (CI–400); general color of head dull green, with black markings; ocelli pale pink; ocular tubercles dark greenish-black; distance between lateral ocelli and medial margin of eyes twice distance between lateral ocelli; cephalic spots small, black (inconspicuous in dry pinned specimen), epicranial suture with its anterior arms black; vertex on each side bears an L-shaped black patch; ocular socket bordered with black; eyes anteriorly brown, posterolaterally dark green; scape and pedicel black, and flagella brown; frons green, marked with a V-shaped black mark extending anterolaterally from frontal ocelli bordering anterior arms of frontoclypeal suture; supra-antennal plate black; frontoclypeal suture unmarked; dorsum of postclypeus brownish-green with dark greenish-black lines in transverse grooves (Figs. 2A, D).

In anteroventral view, eyes prominent, brown anteriorly, superolaterally greenish-blue, inferolaterally yellowish-white; postclypeus unmarked (Fig. 2C), vertically oval, swollen, inferior aspect triangular, tapering towards anteclypeus on its inferior third, transverse grooves on frons unmarked; lorum green, with sparse greyish pruinescence, its junction with postclypeus and anteclypeus has a thin black streak; anteclypeus green, tipped with black on its region near labrum and with paramedian black streak on superior half, whole surface covered with greyish pruinescence; genae yellowish-green, marked with a thin black transverse black line extending from postclypeus towards eyes; labrum and mentum pale brownish and centrally streaked with dark brown; mentum tipped with brown; labium pale brown with median groove dark brown, with black apex; rostrum reaches junction of abdominal sternite II and sternite III (Figs. 2B, E). In lateral view, posteroinferior border of eyes bluish yellow, superior two-thirds brown.

Pronotum. Pronotal width almost thrice its length (PI–266.67) and its general color green; lateral margin of pronotum dentate; lateral angle of pronotal collar broad and rounded and its posterolateral margin well-developed, rest of collar thinner (Fig. 2D). Median hourglass-shaped black mark prominent, its arms run anteriorly and expand bilaterally along suture with head; a short black streak runs parallel to paramedian fissure; paramedian fissures not prominently marked, lateral fissures marked with an irregular black band; medial and lateral lobes of pronotum brownish-green; lateral lobe bounded with irregular black band; ambient fissure marked with thin brown line; pronotal collar green, a squarish patch of black near lateral angle and a small black spot just dorsal it; posterior margin of collar thinly lined with black (Figs. 2A, D).

Mesonotum. In dorsal view, mesonotum marginally wider than long (MI–116.67), greenish-brown; a mid-dorsal black band with prominent expansions at its middle and on its termination on anterior border of cruciform elevation; submedian sigillum (ssig) brown, bounded by a black vertical lateral streak reaching almost half length of mesonotum and expands at its tip; lateral sigillum (lsig) brown, marked by a central black band which is broken into a distal black spot anterolaterally near pronotal collar and a distal J-shaped streak with a bulbous distal tip; J-shaped marks on lsig never touch black spot on scutal depression; black of scutal depression encroaches posteriorly into anterior arms of cruciform elevation; cruciform elevation greenish-brown and lateral depressions green, rest of it green. Edges of the mesonotum and sides of cruciform elevation covered with sparse silvery pruinescence, which may be lost in preservation. In ventral view, basisterum-2 bears a large black spot (Figs. 2A, D).

Operculum. Triangular, longer than broad, short reaching distal margin of sternite II (OPI–44.12); its lateral margin almost straight with small lateral convexity at its middle, thick and edged with black at its middle; lateral angle is rounded and almost square; posterior margin straight and runs parallel to sternite II; medial angle rounded and acute and medial margin oblique; medial angles of operculum separated from each other by a distance almost equal to width of one operculum; diaphanous light green, with sparse whitish pruinescence (Fig. 2C).

Fig. 1 Map showing the known distribution and type locality of *Purana cheeveeda* Sadasivan **sp. nov.**, and type locality of *P. morrisi* from south India

Wings. Wings hyaline, FW long, apex rounded; transparent with black infuscations only on basal veins of apical segments 2 and 3; very faint spot like marginal smoky infuscations on transverse veins of apical cells 1 to 5, near their junction with ambient vein, which is less discernible towards lower cells; FW apices faintly tinted with amber; HW with 6 apical cells; 9 minor transparent veinlets/folds in anal lobe space between veins 3A and 2A on magnification. Veins of FW reddish-brown and nearing the joints and distally black; anterior wing margin till node brown bordered thinly in the proximal half with black, later part brown; those of HW black. Nodes of both wings pale yellowish-white (Figs. 2A, 4A).

Legs. Brownish-green, more brownish in anteroventral aspect of coxa and femur; tibiae greenish-brown proximally with distal half brown; tarsus brown, claws black. Femur with three black spines; a primary spine on proximal aspect of femur (long, sharp-tipped, oblique, semi-erect); a secondary spine (long, sharp-tipped, semi-erect, thinner than primary spine); small tertiary spine (very short, sharp-tipped, tooth-like) present just distal to secondary spine (Fig. 2E, 4A). Meracanthus (mc) flat, elongated triangular with short base, translucent greenish-white, passes slightly distal to distal border of hind trochanter.

Abdomen. Longer than head and thorax together (AL–14.00, API–78.57); widest at distal aspect of tergite 3; sides gently tapering from 3rd to 7th tergites and thereafter truncated, including VII and VIII. General color bluish olive green marked with black lines and amber-brown to orange-brown suffusion on paradorsal regions of tergites; dorsal aspect of abdomen covered sparingly with silvery pubescence, more so on caudal end. Each tergite bordered black along distal margin, this black band thicker and ingresses into tergite at paradorsal and more so on lateral aspect, tergites 7 and 8 almost fully black. Timbal covers almost enclosing the timbal cavity, incomplete laterally, exposing timbals through a rectangular window between it and superior border of operculum; color pale pinkish-brown with basal black suffusion, lateral margins with thin black border slightly broader posteriorly;

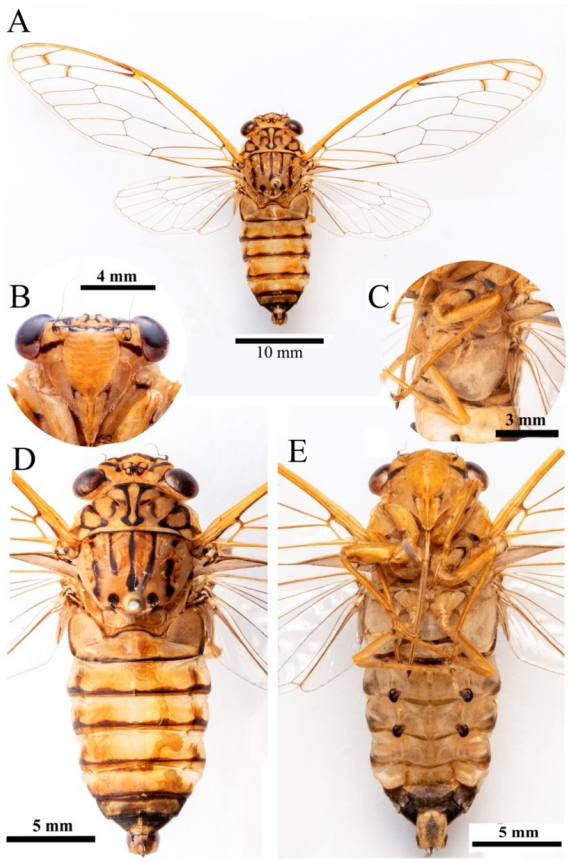

Fig. 2 *Purana cheeveeda* Sadasivan **sp. nov.**, Holotype (THRG 0039): A, dorsal view of the whole insect; B, close-up of head and postclypeus; C, lateral view of the operculum; D, close-up of the dorsum; E, closeup of ventral view. © Kalesh Sadasivan

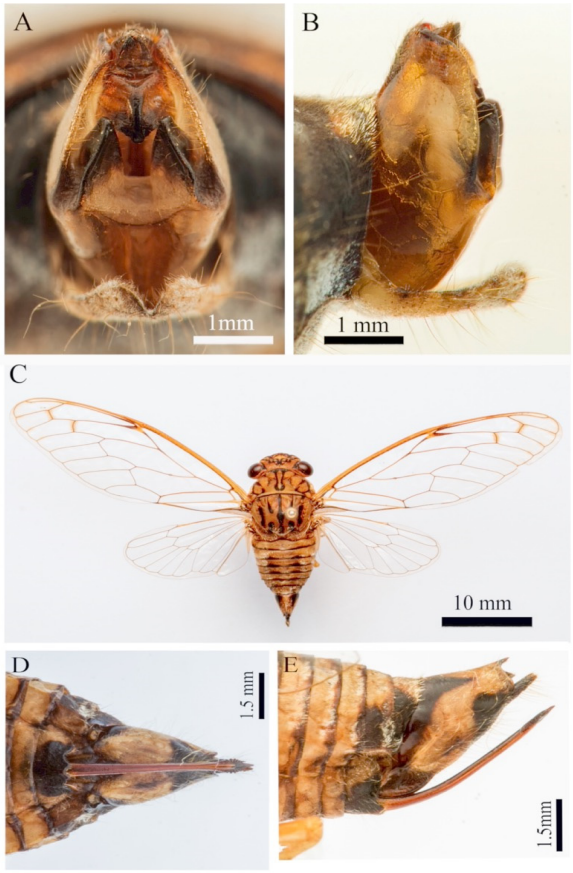

Fig. 3 *Purana cheeveeda* Sadasivan **sp. nov.**: A, male genitalia ventro-posterior view; B, male genitalia lateral view; C, dorsal view of a paratype female (THRG 0040); D, ventral view of the female terminalia (THRG 0040); E, lateral view of the female terminalia (THRG 0040). © Kalesh Sadasivan

medial margin uniformly curved, apex curved and directed towards wing base and its lateral margin straighter. In ventral view, abdominal sternites diaphanous bluish-green, covered with sparse white pubescence; sternite I is exposed in its middle between the operculum on each side; tubercles on distal end of sternite III & IV equal in size and shiny black; 7th sternite and its tergite matt black; tergite 8 similarly black; sternite VIII rounded triangular with a central V-shaped indentation presenting a bifid appearance, greyish white with a central blackish suffusion (Figs. 2A, D, E, 4A, C).

Genitalia. Male genitalia sclerotized at tip of uncus and basal lobes of pygofers. In ventro-posterior view, distal shoulder of pygofer acute, with a short sharp apex directed straight posteriorly; laterally with smooth curve; pygofer bears sparse setae; medial lobe of fused uncus pentagonal (Figs. 5A, 6F) with median incision suggesting a fusion of lobes, its tip slightly notched at exit of aedeagus; distinctly protruding well sclerotized triangular lower basal pygofer lobes directed posteriorly with their blunt apices slightly directed posterolaterally (Figs. 3A, B). In lateral view, distal shoulder with a short,

upturned tip directed dorsally with slight recurvation; basal lobes of pygofer prominent. Aedeagus fish hook-shaped, with its proximal three-fifths thicker and irregular, rest curved, tapers finely to its inwardly directed bevelled tip (Fig. 5D). In dorsal view, apex of distal shoulder of pygofer reaches short of anal style, and dorsal beak of pygofer small (Figs. 5A, C).

Description of females. General color and appearance as in males, major differences are mentioned below. The wing measurements and those of head and thorax are equal, or only marginally smaller than that in males. Length of abdomen, however, is much shorter (AL–11.50±0.71; API–110.52±13.57); operculum small (OPL–1.00±0.05; OPI–9.95±2.82) but more triangular than in males, widely separated and medial border ends at level of meracanthus (mc), posterior margin just reaches level of distal end of sternite I; tympanal covers absent; in ventral view, sternite VII shiny black with its midventral part notched; tergite IX basally orange-yellow and distally black; genitalia with an acute dorsal beak of sternite IX is much longer and prominent than in males; it reaches level of protruding ovipositor sheath. Ovipositor is marginally longer than sheath and dorsal beak together. In lateral view, tergite IX has a black triangular mark on its base and another small black triangular spot on its posterosuperior free end (Figs. 3C–E, 4B, D).

Measurements (mm).

Holotype.

Male. FWL–29.00; FWW–9.00; HWL–14.00; HL–2.00; HW–8; EL–2.00; PL–3.00; PW–8.00; ML–6.00; MW–7.00; AL–14.00; AW–8.00; OPL–3; RL–9.00; ABL–11; TL–25.00; CI–400; OI–250; PI–266.67; MI–116.67; OPI–44.12; API–78.57; RI–81.82; FAR–322.22; FI–263.63 IWR–207.14; GI–57.14.

Paratypes.

Males (n=5): FWL–29±2.00; FWW–8.67±0.58; HWL–13.67±1.53; HL–1.92±1.44; HW–7.67±0.58; EL–1.93±0.12; PL–2.83±0.29; PW–7.50±0.50; ML–5.67±0.58; MW–6.83±0.29; AL–13.67±0.58; AW–7.67±0.58; OPL–3.00±0.00; RL–9.00±1.00; ABL–10.42±1.01; TL–24.08±1.59; CI–400±.0.00; OI–25.23±0.41; PI–265.56±15.03; MI–121.11±7.79; OPI–28.99±2.98; API–76.10±4.28; RI–86.40±4.55; FAR–334.72±11.36; FI–279.11±14.32; IWR–212.93±10.45; GI–56.04±1.09.

Females (n=3): FWL–28.00±1.41; FWW–8.50±0.71; HWL–13.50±0.71; HL–1.86±0.18; HW–7.50±0.71; EL–2.00±0.00; PL–3.00±0.00; PW–7.00±0.71; ML–5.25± 1.06; MW–6.75±0.35; AL–11.50±0.71; AW–7.50±0.71; OPL–1.00±0.05; RL–9.00±0.00; ABL–10.13±1.24; TL–21.63±1.94; CI–400.00±0.00; OI–26.79±2.52; PI–233.33±23.57; MI–130.56±19.64; OPI–9.95±2.82; API–110.52±13.57; RI–76.38±8.16; FAR–321.08±11.25; FI–289.52±14.30; IWR–194.52±1.21; GI–65.15±2.14.

Variation. Individuals vary to some extent in their markings and size. Streaks and marks on the head, pronotum, and mesonotum may be heavy and smudged, and lsig and ssig may have darker brown markings obscuring the black streaks in some individuals. However, on careful observation, the lsig black marks are discontinuous. The J-shaped marks on middle aspect of lsig never touch the black spot on the scutal depression. As far as we could see, this had no particular relation to the population or time of emergence of the year and the variants are often caught together. The pronotum between central fasciae and lateral fissures was generally marked but markings were less discernible in less heavily marked specimens (1 out of 10 specimens studied). There was not much variation in black lines on transverse grooves of the postclypeus, though occasional individuals had them represented by a black medial spot on the first two transverse grooves. In venation, the black infuscations and smoky tint to FW change to amber-brown in preservation. The marginal infuscations may be less obvious in some specimens, though visible at a slanting angle. Wing lengths vary marginally, males have a FL of 29±2.00 mm and females 28.00±1.41mm. In males (n=5), the length of the abdomen (10.42±1.01) was marginally variable. The operculum length (OPL–3.00±0.00) was constant in males. The rostral length was slightly

variable with RL–9.00±1.00 in males and relatively constant in females RL–9.00±0.00. In females (n=4), the length and width of the abdomen were slightly variable (AL–11.50±0.71; AW–7.50±0.7). Variation in the anterior body length was minimal (ABL males–10.42±1.01; females–10.13±1.24), compared to the total body length (TL males–24.08±1.59; females–21.63±1.94). Regarding the male genitalia, the lateral sharp ends of the sclerotized thin black lamina on the free lower margin of the male uncus were sometimes blunted and, in some specimens, the tip of the basal lobe of pygofer was less pointed than normal. The relative position of uncus is variable probably according to the mated status of the males, the tenerals having the uncus flush and lying within the pygofer (Figs. 3A, B), and in mated males, they are conspicuously extruded (Fig. 5B).

Distribution. South-western Peninsular India. As per our observations, the species is very common in the lowlands and midlands of the western slopes of the Western Ghats of South India from Kanyakumari District in Tamilnadu State, extending northwards through all districts of Kerala State, reaching south-western Karnataka State till about Mangalore.

Ecological notes. The species is very abundant in homesteads and jungles up to 300m elevation (Fig.1). The calls start in the fall of twilight, continue into the late evenings, and end at dusk. They are heard calling from the bases of trees and stems of shrubs up to 6 meters. The call is very loud and unmistakable. A group of males will be calling from a patch of the woodland and another group of males will start calling from a different patch as the first batch of calls dies down. They will stop calling as one approach and do not fly away unless disturbed. They sit well camouflaged against the bark of trees. Both sexes are occasionally attracted to light. The main activity is centered around the southwest monsoon from May to July. They are common in cocoa and nutmeg plantations. The songs are continuously heard lasting for hours. The song consists of individual stretches (echemes), each of which lasts for about 20–25 seconds. The initial 5–88 seconds are formed by a series of echemes that increase in tempo and volume as the time progresses, becoming continuous for about 7–10 seconds, and during the last 5–7 seconds, the call decreases in volume and tempo. Each echeme then continues into the next echeme without a pause.

Etymology. The species name *'cheeveeda'* is derived from the Malayalam word *cheevedu* meaning cicada in vernacular and the name *cheevida* means *'it's a cicada!'*.

DISCUSSION

The new species differs from *P. tigrina* of the Malayan subregion as well as the holotype by the following characteristics–the opercula are short and do not extend beyond the distal margin of sternite II, the apex is not acute and is almost straight and lies parallel to the distal margin of sternite II (Figs. 7F, 8C), while the tip is acute and crosses into the proximal aspect of sternite II in Malayan specimens and holotype (Figs. 7D, E, 8C). The lateral margin is straight in the new species while it is oblique in Malayan specimens and the holotype of *P. tigrina* (Figs. 7D, E, 8C). The rostrum is longer and reaches the distal margin of sternite II in *P. cheeveeda* **sp. nov.** (while much shorter in the type specimen of *P. tigrina* only reaching just beyond the distal coxa); tubercles in sternite III and sternite IV are equal sizes (Fig. 7F), while the sternite IV tubercles much smaller than sternite III in Malayan specimens and the holotype of *P. tigrina* (Figs. 7D, E); and male genitalia with strongly sclerotized, tra-pezoidal uncus and spine-shaped basal pygofer lobes (Figs. 6A, B, 7E) in contrast to the elongated triangular uncus and slightly converging, ridge-like basal pygofer lobes with a narrow triangular apex in the type specimen of *P. tigrina* and Malayan specimens (Duffels *et al.,* 2007) (Figs. 6C, D). The length of FW apical cell 2 is half the length of apical cell 3 in *P. tigrina,* while in new species apical cell 2 is almost three-fourths of the length of apical cell 3. With respect to colouration, the new species is less prominently marked on the thoracic segments than in *P. tigrina*; has unmarked post-clypeus and hence no transverse fasciae or hourglass marking of ground color (Fig. 8B) while the transverse fasciae enclosing the hourglass pale area are seen in both Malayan specimens and the holotype of *P. tigrina* (Fig. 8A).

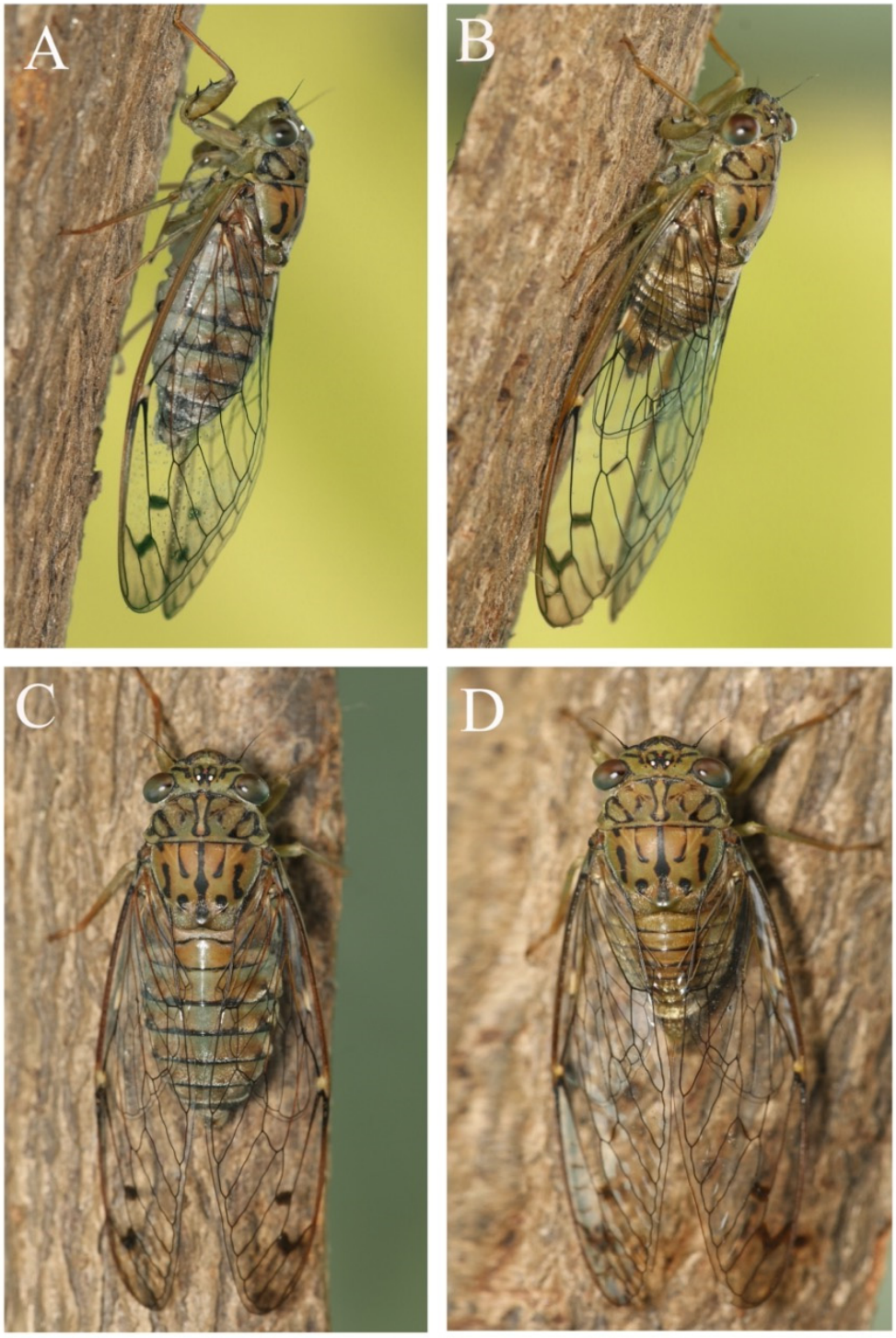

Fig. 4 *Purana cheeveeda* Sadasivan **sp. nov.**, live insect in nature: A, lateral view of the male; B, lateral view of the female; C, dorsal view of the male; D, lateral view of the female. © Kalesh Sadasivan

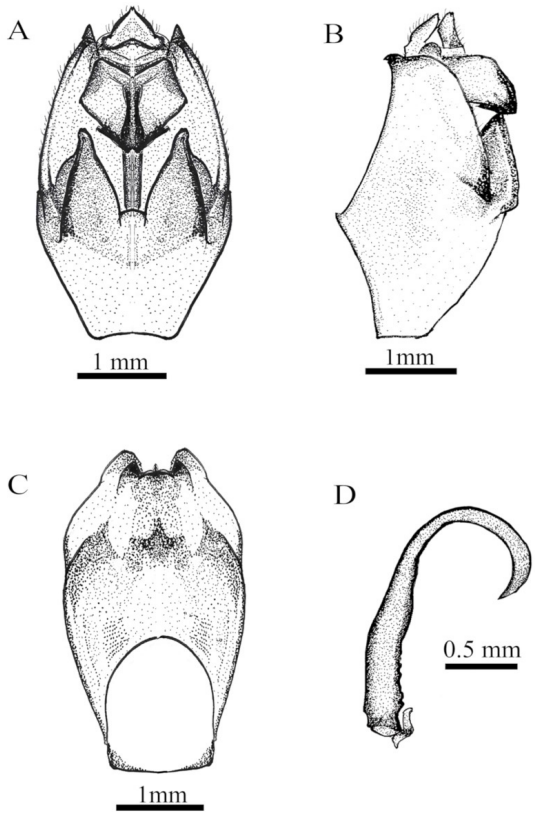

Fig. 5 *Purana cheeveeda* Sadasivan **sp. nov.**, male genitalia of paratype, KOH treated wet specimen preserved in glycerol: A, ventro-posterior view; B, lateral view; C, dorsal view; D, aedeagus left lateral view

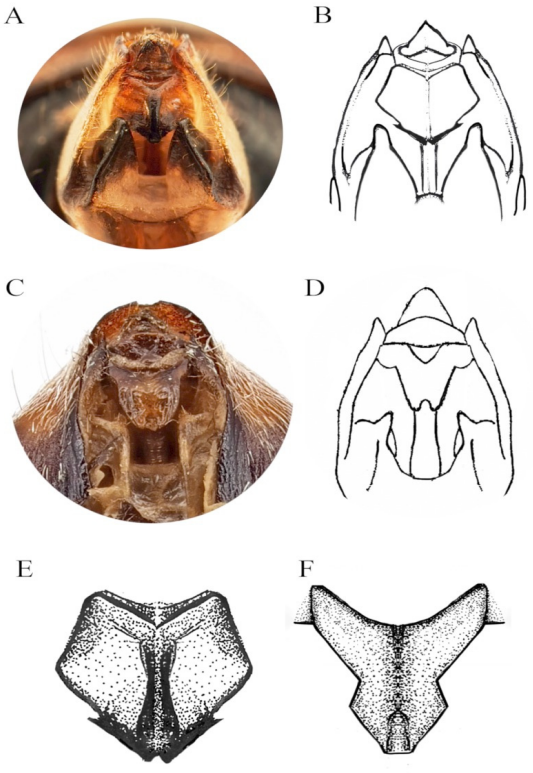

Fig. 6 Male genitalia of *Purana* species. A–B, male genitalia of *P. cheeveeda* Sadasivan **sp. nov.** holotype © Kalesh Sadasivan; C, male genitalia of *P. tigrina* (Walker,1850) holotype Image © Mick Webb; D, Illustration of the male genitalia of *P. tigrina* (Walker,1850) Malayan specimen, redrawn based on Duffels et al. (2007); E, Trapezoid uncus of *P. cheeveeda* Sadasivan **sp. nov.**; F, elongated triangular uncal lobes of *P. morrisi* © Kalesh Sadasivan

Purana morrisi another species known from south India, is easily distinguished from the new species by its long rostrum reaching just beyond the sternite III tubercles (short in the new species never reaching beyond the sternite III tubercles); the operculum in males just reaches the proximal aspect of sternite III in *P. morrisi*, while it ends just short of the distal margin of sternite II in the new species; the presence of transverse fascia on postclypeus (absent in *P. cheeveeda* **sp. nov.**); and the venation with the basal (transverse vein) of a2 (apical segment of FW) when extrapolated meets the junction of basal and middle thirds of RA1 i.e., more directed to the anterior wing margin, in *P. tigrina,* this vein, when extrapolated bisects RA1 i.e., more directed towards the wing apex. With respect to coloration, *P. morrisi* is heavily marked, with the cephalic spots joined by dark brown marks (unmarked in *P. cheeveeda*), the medial lobe of the pronotum having heavy marks (less heavily marked in *P. cheeveeda*), the mesonotum also is heavily marked with the submedian sigillae (ssig) converging to meet the median line (not meeting in *P. cheeveeda* **sp. nov.**), lateral sigillae (lsig) sinuous

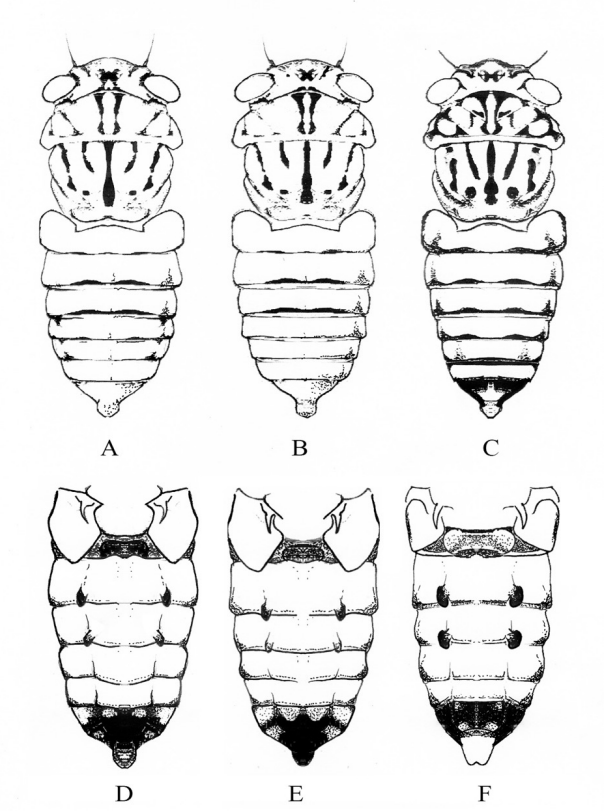

Fig. 7 Body of *Purana* species. A, *P. tigrina* (Walker, 1850) dorsal view of holotype; B, *P. tigrina* (Walker, 1850) dorsal view of the Malayan specimen, redrawn based on Duffels *et al.* (2007); C, *P. cheeveeda* Sadasivan **sp. nov.**, dorsal view; D, *P. tigrina* (Walker, 1850) ventral view of holotype; E, *P. tigrina* (Walker, 1850) ventral view of the Malayan specimen, redrawn based on Duffels *et al.* (2007); F, *P. cheeveeda* Sadasivan **sp. nov.**, ventral view. © Kalesh Sadasivan

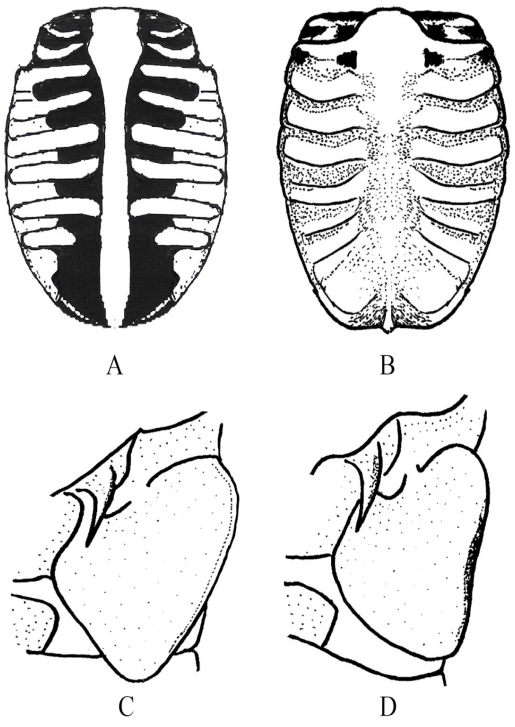

Fig. 8 Post-clypeal markings and operculum of *Purana* species. A, *P. tigrina* (Walker, 1850) Malayan specimen, postclypeus in ventral view, redrawn based on Duffels *et al.* (2007); B, *P. cheeveeda* Sadasivan **sp. nov**., postclypeus in ventral view; C, *P. tigrina* (Walker, 1850) Malayan specimen, male operculum in lateroventral view, redrawn based on Duffels *et al.* (2007); D, *P. cheeveeda* Sadasivan **sp. nov**., male operculum in lateroventral view. © Kalesh Sadasivan

and broad with its distal end bulbous and contiguous with the spot in the scutal depression (lsig is thin, the distal end is less bulbous and far away from the scutal spots in *P. cheeveeda* **sp. nov.**). Male genitalia reveal the most useful character to distinguish it from the new species as the uncus is pentagonal in *P. cheeveeda* **sp. nov.** while it is elongated triangular in *P. morrisi* (Figs. 6E, F). A redescription of *P. morrisi* is under preparation) with morphometric data based on freshly collected topotypes.

All other known species of *Purana* are extralimital to south India and are restricted to various other areas of Southeast Asia. *Purana guttularis* (Walker, 1858) from Eastern India, Myanmar, and the rest of Southeast Asia to Philippine Islands, is distinguished by the overall paucity of markings on the head and thorax; length of FW a2 is half the length of a3 (a2 is almost three-fourths of the length of a3 in *P. cheeveeda* **sp. nov.**); the three-segmented lsig of mesonotum (single thin continuous mark in *P. cheeveeda* **sp. nov.**) and the male opercula being broad, transverse, nearly together than in *P. tigrina*, *P. morrisi,* and

P. cheeveeda **sp. nov.** with inner margins oblique, apices sub-truncate and rounded. *Purana campanula* Pringle, 1955 from Srilanka is distinguished by its extremely long fused uncal lobes (Pringle, 1955), while the new species has short, fused uncus. *Purana metallica* Duffels and Schouten, 2007 and *P. kpaworensis* Boulard 2006 from Southeast Asia have simple, bilobate uncus and slightly converging, ridge-like basal pygofer lobes with a narrow triangular apex, while the new species has strongly sclerotized, triangular uncus and spine-shaped basal pygofer lobes (Duffels *et al.*, 2007; Boulard, 2006). Tubercles on sternite III and IV are equal in size and that on IV reaches the posterior margin of the segment in the new species and hence differs from *P. mulu* Duffels and Schouten, 2007 in which the tubercles on sternite IV do not reach the posterior margin of the segment and is distinctly smaller than in sternite III. Male operculum reaches beyond the anterior margin of sternite III in *P. latifascia* Duffels and Schouten, 2007, and *P. karimunjawa* Duffels and Schouten, 2007; while in the new species, the male operculum is short and does not reaching beyond the anterior margin of the sternite III. The absence of a black-brown margin of the medial aspect of the male operculum differentiates the new species from *P. usnani* Duffels and Schouten, 2007. Other species of the *P. tigrina* group such as *P. ptorti* Boulard, 2007, *P. tigrinaformis* Boulard, 2007, and *P. khaosokensis* Boulard, 2007 are locally distributed in Southeast Asia and are hence extralimital to South India (Boulard, 2007). The new taxon is easily distinguished from other similar taxa in Srilanka and Southeast Asia from the *P. tigrina* group (See Key given).

***Purana* species groups**

The key to *Purana* species groups was proposed by Lee (2009). A minor modification of the key has been attempted with the retention of stable structural characters of male genitalia, while characters of coloration, which may be relatively less reliable, are modified or deleted. We observed that the coloration character mentioned in Lee (2009) 'pronotum between central fasciae and lateral fissures unmarked' was variable in *P. cheeveda* **sp. nov**., (markings less discernible in less heavily marked specimens 1 out of 10 specimens studied, but present in all heavily marked specimens) while the structure of male genitalia was consistent. This mark was however present on *P. morrisi.* Hence, we think that utility of coloration as characters for identifying species groups may be questionable. Similarly, the coloration character 'male timbal cover with black marking' mentioned for *P. carmente* was also found to be present in a milder intensity in both *P. cheeveda* **sp. nov.** and *P. morrisi* by the lateral margin of the male timbal covers being margined in black. With these minor modifications of characters in coloration in the key, the two known south Indian taxa are comfortably placed in the *P. tigrina* group.

Revised key to species groups of *Purana* based on male morphology modified from Lee (2009)

1. Uncal lobes fused, with tip occasionally bifid (Fig. 6E); anterior longitudinal vein of FW apical cell 5 about as long as anterior longitudinal vein of apical cell 7 (Fig. 2A).....2

– Uncal lobes widely separated till its base; anterior longitudinal vein of FW apical cell 5 distinctly shorter than anterior longitudinal vein of apical cell 7…. *Purana nebulilinea* group

2. Basal lobe of pygofer either ridge-like with a small medial triangular spine (Fig. 6D) or is large triangular tooth-like with a pointed apex (Fig. 6A)...3

– Basal lobe of pygofer not ridge-like nor triangular, but instead with a curved apex, or angulate medially.....4

3. Male operculum relatively long and slender, always passing anterior margin of sternite III to reach more than 1/3rd of its length, or nearly reaching or passing posterior margin of sternite III.....*P. carmente* group

– Male operculum short, not reaching anterior margin of sternite III (Fig. 8D) or if crosses it, never more than 1/3rd of the length of sternite III (Fig. 8C).....*P. tigrina* group

4. Basal lobe of pygofer medially angulate.....5

– Basal lobe of pygofer not angulate medially, its apex curved.....6

5. Tergite 3 of male abdomen much wider than mesonotum; abdominal tubercles very long and thick; basal vein of apical cell 1 shorter than a half as long as longitudinal vein of apical cell 1.....Genus *Maua*

– Tergite 3 of male abdomen about as wide as base of mesonotum; abdominal tubercles tiny and slender; basal vein of apical cell 1 more than three-quarters as long as longitudinal vein of apical cell 1.....*P. ubina* group

6. Male pygofer spherical; uncus with a comparatively narrow apex.....Genus *Formosemia*

– Male pygofer obovate; uncus with a wide apex in ventral view.....*P. abdominalis* group

There are several overlapping characters between the species groups. Some groups seem to be heterogenous in constitution. For instance, the male genitalia with bifid uncus, ridge-like basal pygofer lobes are seen in some species of *P. carmente* group as well as the *Purana tigrina* group (Schouten and Duffels, 2002; Duffels *et al.,* 2007; Lee, 2009). This means that the current species group concepts may be provisional and a phylogenetic analysis is warranted to delineate the relations between the various species groups and decide the species nestled within them. Until such a large comprehensive phylogenetic analysis and revision of *Purana* and related genera is available, this modified and artificial species group classification may be followed.

Purana tigrina species group can be diagnosed with the members having anterior longitudinal vein of FW apical cell 5 about as long as the anterior longitudinal vein of apical cell 7; uncus undivided; basal lobe of pygofer spine-shaped with a narrow apex; male timbal cover without black marking, but may be margined with black on its lateral edge; bases of apical cells 2 and 3 of forewing prominently infuscated; male operculum short, not reaching or slightly passing posterior margin of sternite II as per Lee (2009). Amongst *Purana tigrina* species group there were two distinct sub-groups, one sub-group with ridge-like basal lobe of pygofer with a small medial triangular spine and simple (not strongly sclerotized) terminally bifid uncus, and the second subgroup with large triangular tooth-like basal lobe of pygofer with a pointed apex and strongly sclerotized triangular uncus (Schouten and Duffels, 2002). *P. morrisi* is a member of the former sub-group, while *P. cheeveda* sp. nov. falls in the latter sub-group. A phylogenetic study of this species group is warranted to decide the morphological characters useful for taxonomy. The key to members of *P. tigrina* species group is given below.

Key to the species of the *P. tigrina* species group based on males with their distribution ranges (modified from Boulard, 2007; Duffels *et al.,* 2007; Lee, 2009)

1. The apex of operculum not reaching beyond the anterior margin of sternite III (Fig. 8D).....2

– The apex of operculum reaching or extending beyond anterior margin of sternite III.....5

2. Tubercles on sternite 4 black, as almost large as those on sternite III (Fig. 2E).....3

– Tubercles on sternite 4 brownish, conspicuously smaller than those on sternite III (Sarawak and Brunei).....*P. mulu*

3. Basal lobes of pygofer with large diverging triangular spine (Fig. 3A) (South India) .....***P. cheveeda* sp. nov.**

– Basal pygofer lobes ridge-like, apical spines small 4

4. Basal pygofer lobes with apical spine divergent (Khun Pawor in North Thailand)..... .....*P. kpaworensis*

– Basal pygofer lobes with apical spine convergent (Langkawi Island and Tarutao Island west coast of the Malayan Peninsula) ..... *P. metallica*

5. Basal lobes of male pygofer consisting of large triangular projections.....7

– Basal pygofer lobes ridge-like, apical spines small and converging.....6

6. The apex of operculum rounded triangular extending beyond anterior margin of sternite III; tubercles on sternite IV brownish, much smaller than those on sternite III; rostrum short never reaching anterior margin of sternite III (Nias Island, Sumatra, Malayan Peninsula, Greater Natuna, and Borneo).....*P. tigrina*

– The apex of operculum rounded reaching anterior margin of sternite 3; tubercles on sternite 4 black, as almost large as those on sternite III; rostrum long reaching the tubercles on sternite 3 (Tamil Nadu, South India).....*P. morrisi*

7. Medial margin of male operculum black-brown. Lateral fasciae on mesonotum broken up or continuous, widest part of lateral fascia narrower than the distance between paramedian and lateral fasciae.....8

– The medial margin of the male operculum of its ground color, not marked in black-brown; lateral fasciae on mesonotum continuous, widest part of lateral fascia as wide as the distance between paramedian and lateral fasciae (Sabah, north Borneo).....*P. latifascia*

8. Lateral fasciae on mesonotum broken up into a linear part and a black dot, linear part 2–3 times as wide as the anterior part of median fascia (Karimun Archipelago north of central Java) .....*P. karimunjawa*

– Lateral fasciae on mesonotum continuous, rarely broken up into a linear part and a black dot, linear part as wide as or slightly broader than the anterior part of median fascia (Singapore, Sumatra, Bunguran, North Borneo).....*P. usnani*

A new taxon of the *P. tigrina* group is described from Kerala in southern India. The new species falls in the *P. tigrina* group following Duffels *et al.* (2007) and Lee (2009), by the following characteristics–the apex of operculum not reaching beyond the anterior margin of sternite 3; tubercles on sternite 4 black, as almost large as those on sternite 3; basal lobes of pygofer with large diverging triangular spines, along with its short rostral length, the extent of dark fascia on the transverse grooves of postclypeus, forewing venation and the characteristic pentagonal uncus of the male. The species was earlier confused with *P. tigrina* (Walker, 1850). As per our findings, *P. tigrina* must refer to the taxon distributed in the Malayan bioregion. Identification of the commonest cicada of the region, which was traditionally misidentified as *P. tigrina,* as a new species highlights the need to study male genitalia in species determination. It must be noted that in contrast to what was previously thought, cicadas may be geographically and altitudinally restricted in distribution, implying a high degree of endemism. Wider species distribution probably implies the existence of a species complex or taxonomic lumping arising out of suboptimal studies.

Abbreviations:

NHM	Natural History Museum, London
FW	Forewing
NCBS	National Center for Biological Sciences, Bengaluru
TNHS	Travancore Nature History Society, Thiruvananthapuram
UAS	University of Agricultural Sciences, Bengaluru
ZSI	Zoological Survey of India

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Efficacy of compounds used in mosquito repellents (DEET, picaridin, prallethrin and IR3535) against odorant binding protein (OBP20) of *Anopheles gambiae*: A molecular docking study

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ABSTRACT: The study is to use AutoDock software to determine the binding affinity or binding energy of DEET, picaridin, prallethrin, and IR3535 components with the odorant receptor of the *Anopheles gambiae* say (Diptera, Culicidae) mosquito species. The binding energy (ÄG) of prallethrin was determined to be highest at -10.55 kcal/mol followed by picaridin at -7.1 kcal/mol, DEET at -6.57 kcal/mol and IR3535 at -5.6 kcal/mol being the lowest among all. By comparing their binding energy levels after AutoDocking, it is to decide which mosquito repellent is the most effective. © 2023 Association for Advancement of Entomology

KEY WORDS: AutoDocking, olfactory receptor, binding energy, efficacy

INTRODUCTION

The most significant carrier of *Plasmodium falciparum* malaria in the world, female *Anopheles* mosquitoes, largely uses olfactory cues to locate their human hosts. A component of human sweat triggers a response in the female-specific protein AgOr1 of the *Anopheles gambiae,*which belongs to a family of putative odorant receptors. Odorant-binding proteins (OBPs) serve as a bridge between odorant receptors, which are found in olfactory structures of the mosquito's peripheral sensory system (primarily the antennae and maxillary palps), and the air medium that broadcasts chemical signals, serving as the first relay in semiochemicals reception in mosquitoes. OBPs are hypothesised to be involved in the transfer of odorants to odorant receptors (ORs) for the particular signal transduction of behaviorally active odorants (Venthur and Zhou, 2018). These proteins might be used as molecular targets for the creation of mosquito attractants. Dipteran OBPs lack the extended C-terminus needed to occupy the binding pocket at low pH because they are shorter (125 amino acid residues). *An. gambiae* is the only mosquito whose OBP structure has been documented (Leite *et al*., 2009). AgamOBP1 is a member of the medium subclass and is 125 residues long with six cysteines and three disulfide linkages. It also features an extended C-terminal section that is buried inside the protein core and forms a wall with the internal cavity (Cali and Persaud, 2020).The odors emanating from human skin and sweat serve as the *An. gambiae's* primary means of locating its hosts. These odours cause the insect to react in a certain way to OBP. *Anopheles gambiae* OBP

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20 or AgamOBP20, a particular form of OBP, has recently been defined. During the height of its host-seeking behaviour, the female mosquito's antennae exhibit high levels of this OBP, which suggests that it may be involved in olfactory sensing. Hydrophobic residues make up the majority of the AgamOBP20's binding site.

It is believed that the amino acids Leu106, Leu107, and Met53, which have been identified as possible critical residues, are crucial for the interaction between the protein and the ligand. Important considerations for the way the ligand interacts to AgamOBP20 are the steric restriction and hydrophobic interaction. The molecular characteristics and parameters discovered may be used to create novel insecticides and repellents that can interfere with AgamOBP20's function and cause *An. gambiae* to behave differently when looking for a host (Janeiro *et al*., 2016).The major goal of this study is to use AutoDock software to determine the binding affinity or binding energy of DEET, picaridin, prallethrin, and IR3535 components with the ORs of the *An. gambiae*.

MATERIALS AND METHODS

Softwares required: The following softwares were downloaded and installed from online sources which were used to carry out our molecular docking procedure:

- Open Babel GUI (http://openbabel.org)
- UCSF Chimera (http://www.cgl.ucsf.edu/chimera/)
- BIOVIA Discovery Studio
- MGL Tools
- AutoDock 4.2.6

Ligand Retrieval: Ligands were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). The three-dimensional (3D) structures of the chemical compounds were obtained from PubChem database in the form of SDF files (structure-data files). For molecular docking, the following five ligands were retrieved:

- DEET- PubChem CID 4284); IUPAC name: N,N-diethyl-3-methylbenzamide
- Picaridin (PubChem ID 125098); IUPAC name: butan-2-yl 2-(2-hydroxyethyl) piperidine-1-carboxylate
- Prallethrin (PubChem ID 9839306); IUPAC name: (2-methyl-4-oxo-3-prop-2-ynylcyclopent-2-en-1-yl)2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropane-1-carboxylate
- IR3535 (PubChem ID 104150); IUPAC name: ethyl 3-[acetyl (butyl) amino] propanoate
- PG4 (Substance SID 7889818; Compound CID 8200); IUPAC name: Tetraethylene glycol

DEET, Picaridin, Prallethrin, and IR3535 were the test ligands among the aforementioned ligands, whilst PG4 was the co-crystal ligand of the template protein OBP20.

File Conversion from SDF to PDB: Using the software Open Babel (http://openbabel.org), the ligands that were retrieved from the PubChem database in the form of SDF files were then translated to PDB (Protein data bank) file format. SDF was chosen as the output format, and PDB was chosen as the input format. The input name of the file contained the ligands in their SDF form. The file was named ligand.pdb in the output file and saved to the desktop. The "Convert" button was then clicked. The ligands were eventually prepared for docking after being converted to PDB format.

Retrieval and Preparation of Protein: In the RCSB database (http://www.rcsb.org/pdb/), the 3D structure of the odorant binding protein was looked up. The search results included odorant binding proteins from a number of different organisms, including *Drosophila melanogaster, Aedes aegypti, Anopheles gambiae* and *Bombyx mori*. The odorant binding protein from *An. gambiae* was chosen to perform the molecular docking process. The protein macromolecule known as AGAP005208-PA and an already bound specific

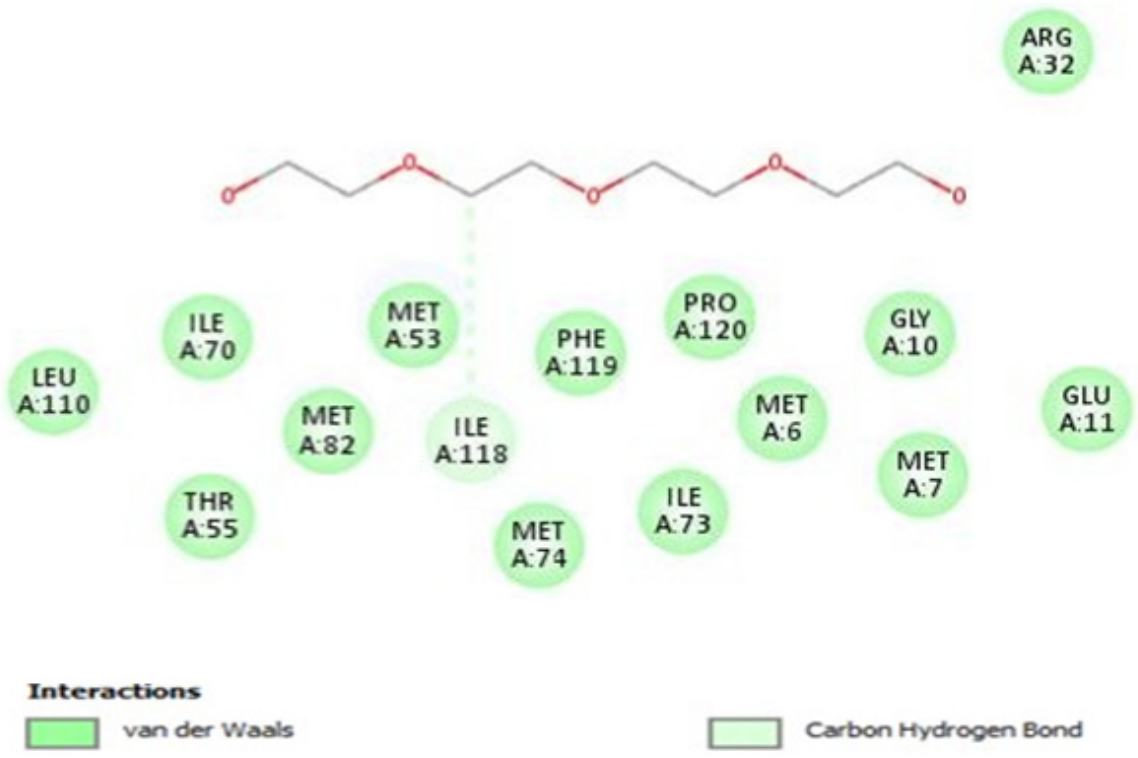

Fig.1 BIOVIA Discovery Studio image showing 2D interactions of co-crystal ligand (PG4) with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20

ligand PG4 or polyethylene glycol were both present in the chosen 3D structure of *Anopheles gambiae's* odorant binding protein (PDB ID 3V2L).

The 2D interactions of bounded co-crystal ligand (PG4) with OBP20 of *Anopheles gambiae* were visualized in BIOVIA Discovery Studio (Fig.1). The amino acid residues which showed Vander Waals interaction were Met6A, Met7A, Gly10A, Glu11A, Arg32A, Met53A, Thr55A, Ile70A, Ile73A, Met74A, Met82A, Leu110A, Phe119A and Pro120A. On the other hand, the amino acid residue which showed Carbon Hydrogen Bonds was Ile118A.

Following retrieval, the protein structure was opened in the UCSF Chimera programme (http://www.cgl.ucsf.edu/chimera/) to get rid of its co-crystal ligand (PG4) before docking. It was discovered that Chain A was the binding chain for the co-crystal ligand, hence Chain A was chosen. Once the co-crystal ligand had been chosen from the Residue dropdown list, it was deleted from the protein structure. The newly modelled protein structure without the co-crystal ligand was saved which was then ready for docking.

Docking: The ligands and the protein were prepared for docking by using Auto dock Tools (ADT). Using the Lamarckian Genetic Algorithm, the docking software Auto Dock 4.2.6 was utilised for the investigation of protein-ligand complexes (LGA). The template protein (*An. gambiae* OBP20) and its co-crystal ligand PG4 were initially docked. Then DEET, Picaridin, Prallethrin and IR3535 were molecularly docked with the *A. gambiae* OBP20 odorant binding protein receptor. The software Auto Dock 4.2.6 makes advantage of free binding energy to score the ligand-protein complexes (10 numbers). The 2D interactions between the ligand and the amino acids in the protein's LBD (Ligand Binding Domain) were visualised using BIOVIA Discovery Studio (Fig.1). Docking was carried out individually, and

Table 1. Grid box measurements of the LBD of receptor protein used for docking

Protein	Ligand	Gridboxdetails				
		Dimensions	Spacing	Coordinates		
				X	Y	Z
Anopheles gambiae OBP20	DEET	60×60×72	0.375Å	2.083	1.056	-7.222
	Picaridin	56×50×68	0.375Å	3.222	2.083	-4.861
	Prallethrin	58×64×70	0.375Å	1.806	0.167	-5.667
	IR3535	50×52×72	0.375Å	3.139	2.833	-5.306

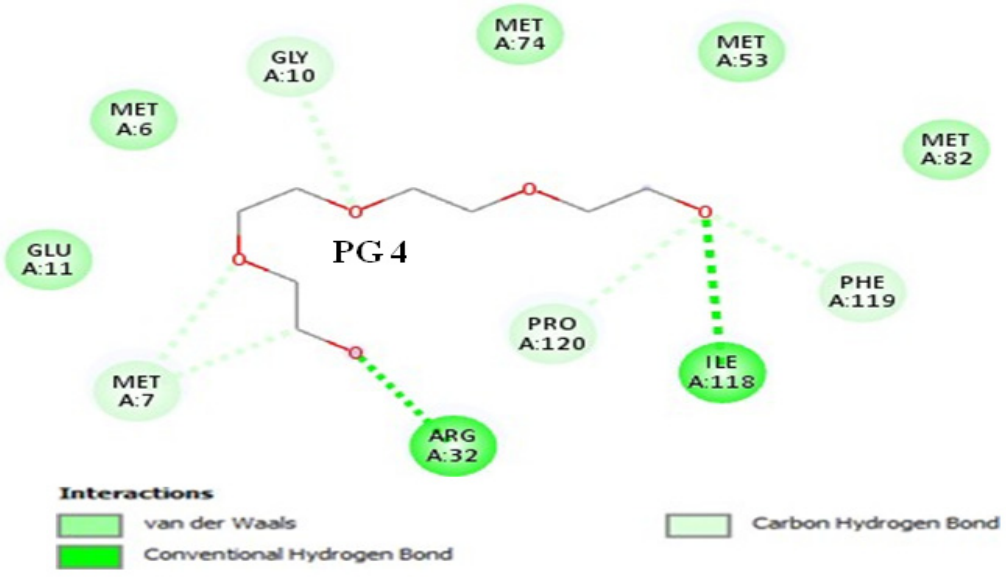

Fig. 2 BIOVIA Discovery Studio image showing 2D interactions of co-crystal ligand (PG4) with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

measurements of every grid box used for each protein-ligand docking (Table 1).

RESULTS AND DISCUSSION

Docking of co-crystal ligand (PG4) with *Anopheles gambiae* OBP20: PG4 exhibited ligand binding interactions with OBP20 of *An.gambiae*. After docking, the most favourable protein-ligand complex which was obtained had a binding energy (ÄG) of -3.89 kcal/mol. Two amino acid residues demonstrated conventional hydrogen bonding with LBD of OBP20 of *An. gambiae*, four amino acid residues demonstrated carbon-hydrogen bonding, and five amino acid residues shown Van der Waals interaction (Fig. 2, Table 2).

Table 2. 2D interactions between the co-crystal ligand (PG4) and the ligand binding domain (LBD) of *Anopheles gambiae* OBP20

VanderWaalsInteraction	CarbonHydrogenBonds	Conventional HydrogenBonds
Met6A	Met7A	Arg32A
Glu11A	Gly10A	Ile118A
Met53A	Phe119A	
Met74A	Pro120A	
Met82A		

Docking of DEET with *Anopheles gambiae* OBP20: DEET demonstrated ligand binding interactions with OBP20 of *An. gambiae* (Fig. 3).

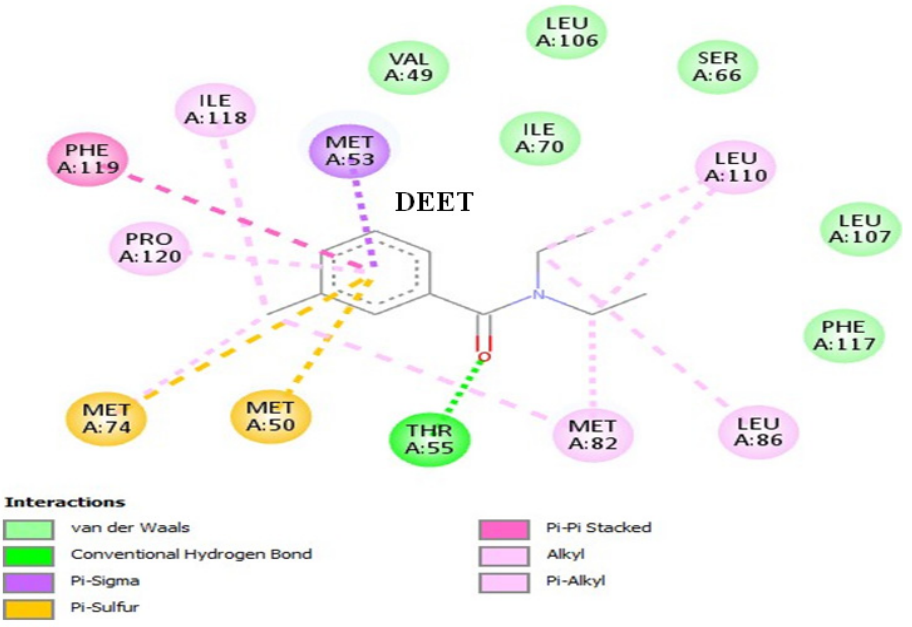

Fig. 3 BIOVIA Discovery Studio image showing 2D interactions of DEET with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

Table 3. 2D interactions between DEET and *Anopheles gambiae* OBP20

Vander Waals Interaction	Conventional Hydrogen Bonds	Pi-Sulfur Interaction	Pi-Sigma Interaction	Pi-PiStacked Interaction	Alkyl Interaction	Pi-Alkyl Interaction
Ile70A	Thr55A	Met74A	Met53A	Phe119A	Leu110A	Met82A
					Ile118A	
					Pro120A	

After docking, the most favourable protein-ligand complex which was obtained had a binding energy (ÄG) of -6.57 kcal/mol. As listed in Table 4, one amino acid residue demonstrated Van der Waals interaction, one demonstrated conventional hydrogen bonding, one demonstrated Pi-Sulfur interaction, one demonstrated Pi-Sigma interaction, one demonstrated Pi-Pi stacked interaction, three demonstrated alkyl interaction, and one demonstrated Pi-Alkyl interaction with LBD of OBP20 of *An. gambiae* (Table 3).

Docking of Picaridin with *Anopheles gambiae* OBP20: Picaridin demonstrated ligand binding interactions with OBP20 of *An. gambiae* (Fig.4). The protein-ligand combination with the best docking results has a binding energy (ÄG) of -7.1 kcal/mol. One amino acid residue exhibited Van der Waals interaction, two exhibited conventional hydrogen bonding, three exhibited alkyl interaction, and three exhibited Pi-Alkyl interaction with LBD of OBP20 of *An. gambiae* (Table 4).

Docking of Prallethrin with *Anopheles gambiae* OBP20: Prallethrin demonstrated ligand binding interactions with OBP20 of *An. gambiae* (Fig. 5). The protein-ligand complex with the best docking results had a binding energy (ÄG) of -10.55 kcal/

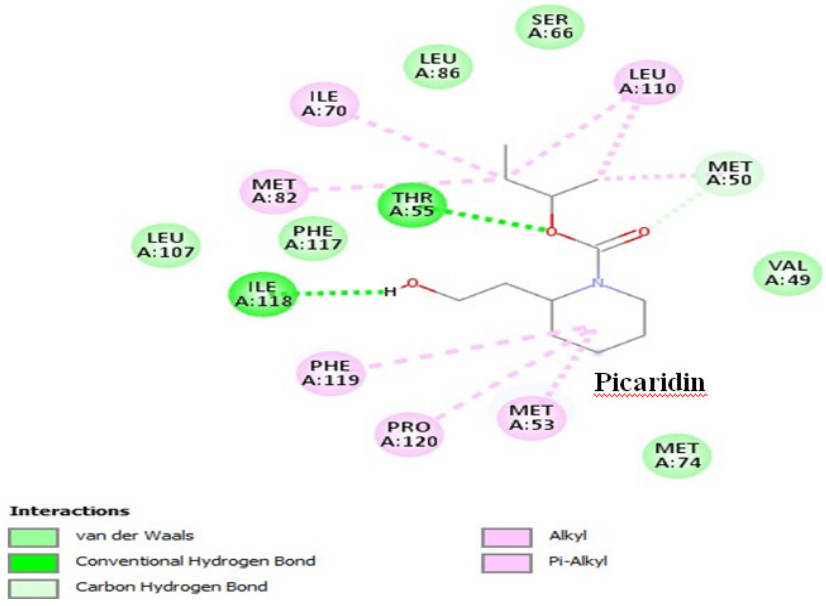

Fig. 4 BIOVIA Discovery Studio image showing 2D interactions of Picaridin with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking.

Table 4. 2D interactions between Picaridin and *Anopheles gambiae* OBP20

Vander Waals Interaction	Conventional Hydrogen Bonds	Alkyl Interaction	Pi-Alkyl Interaction
Met74A	Thr55A	Ile70A	Met53A
	Ile118A	Met82A	Phe119A
		Leu110A	Pro120A

mol. Seven amino acid residues showed alkyl interaction with LBD of OBP20 of *An. gambiae*, six amino acid residues demonstrated Van der Waals interaction, and one residue demonstrated carbon hydrogen bonding (Table 5).

Docking of IR3535 with *Anopheles gambiae* OBP20: OBP20 from *An. gambiae* had ligand binding interactions with IR3535 (Fig. 6). The most favourable protein-ligand combination that could be formed after docking had a binding energy (ÄG) of -5.8 kcal/mol. Five amino acid residues showed alkyl contact with LBD of OBP20 of *An. gambiae,* three amino acid residues demonstrated Van der Waals interaction, one amino acid residue demonstrated typical hydrogen bonding (Table 6).

The obtained results have been interpreted statistically (Fig. 7).

Utilizing Auto Dock 4.2.6, molecular docking was performed for the current study. Finding the strongest component (among those chosen for the study) used in the manufacturing of insect repellents was the main goal of this investigation. This study's findings will help determine which compound is most effective at keeping mosquitoes away, one of the most dangerous insect vectors on the planet. In this study, *An. gambiae*, a mosquito which plays a major role in the transmission of malaria, is the organism against which the efficacies of the chemicals were evaluated.

Following molecular docking, it was discovered that the binding energy (ÄG) of DEET bound to the odorant binding protein of *An. gambiae* was -6.57 kcal/mol. This demonstrates that the DEET chemical is a key component of insect repellents. The efficiency of several commercial mosquito repellent sprays and items containing DEET was investigated in a study conducted by Rodriguez and

Table 5. 2D interactions between Prallethrin and *Anopheles gambiae* OBP20

Vander WaalsInteraction	Alkyl Interactions	Carbon Hydrogen Bonds
Gly10A	Met6A	Pro120A
Arg32A	Met7A	
Met53A	Ile70A	
Thr55A	Ile73A	
Ile118A	Met74A	
Phe119A	Met82A	
	Leu110A	

Table 6. 2D interactions between LBD of IR3535 and *Anopheles gambiae* OBP20

Vander Waals Interaction	Alkyl Interaction	Conventional Hydrogen Bond
Met74A	Met53A	Thr55A
Ile118A	Ile70A	
	Met82A	
	Leu110A	
	Pro120A	

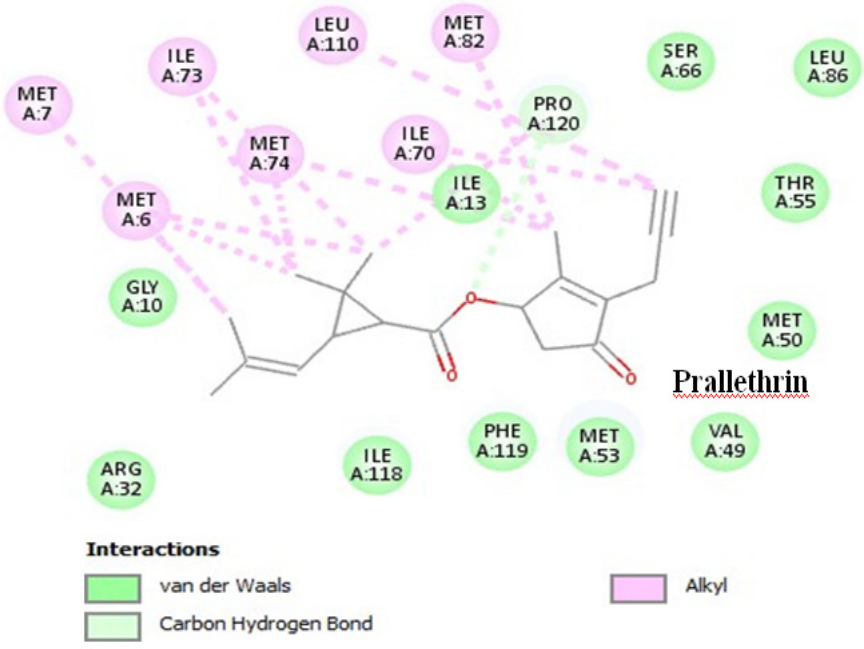

Fig. 5 BIOVIA Discovery Studio image showing 2D interactions of Prallethrin with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

Hansen (2015) which showed that they were effective and lasted for a fair amount of time. DEET-containing products have been proven to be both safe and efficient. The chemical N,N-diethyl-meta-toluamide is known by the acronym DEET.

However, after conducting more docking experiments with several other compounds that are also included in repellents, it could be understood that Prallethrin is the most efficient substance out of the four that were chosen in this study. The binding energy (ÄG) of Prallethrin was found to be -10.55 kcal/mol. It is a synthetic pyrethroid with quick knock-down action against domestic insect pests and vectors. It is utilised in household insecticides to combat cockroaches, houseflies, and mosquitoes (Matsunga *et al.,* 1987). It is most frequently utilised in liquid insect repellents and it is thick yellow to amber liquid. These chemicals, in the form of vapor obstruct mosquitoes' respiratory tracts and chemo receptor. The second most effective compound is Picaridin, which has a binding energy (ÄG) of -7.1 kcal/mol. With a binding energy (ÄG) of -5.8 kcal/mol, IR3535 exhibits the lowest effectiveness.

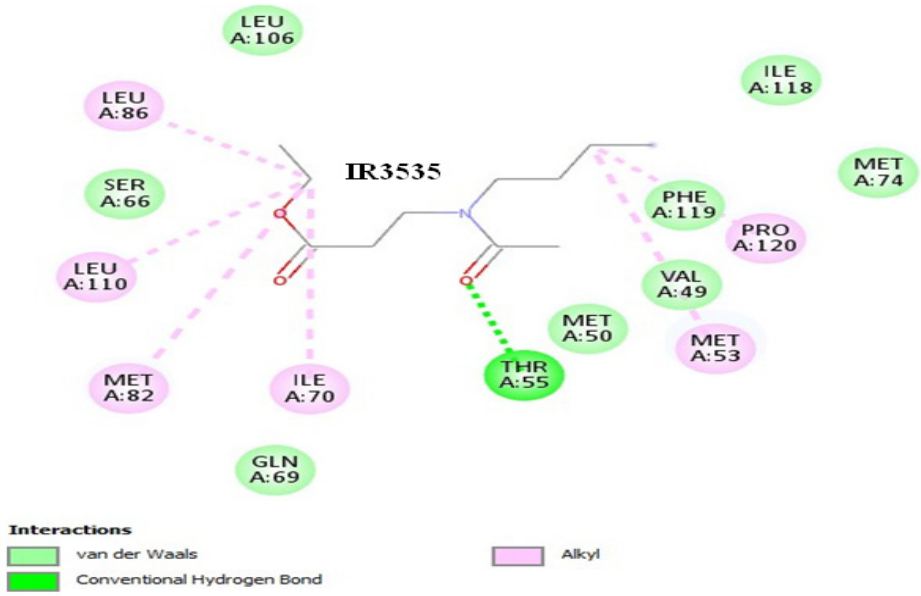

Fig. 6 BIOVIA Discovery Studio image showing 2D interactions of IR3535 with the amino acid residues of ligand binding domain (LBD) of *An. gambiae* OBP20 after docking

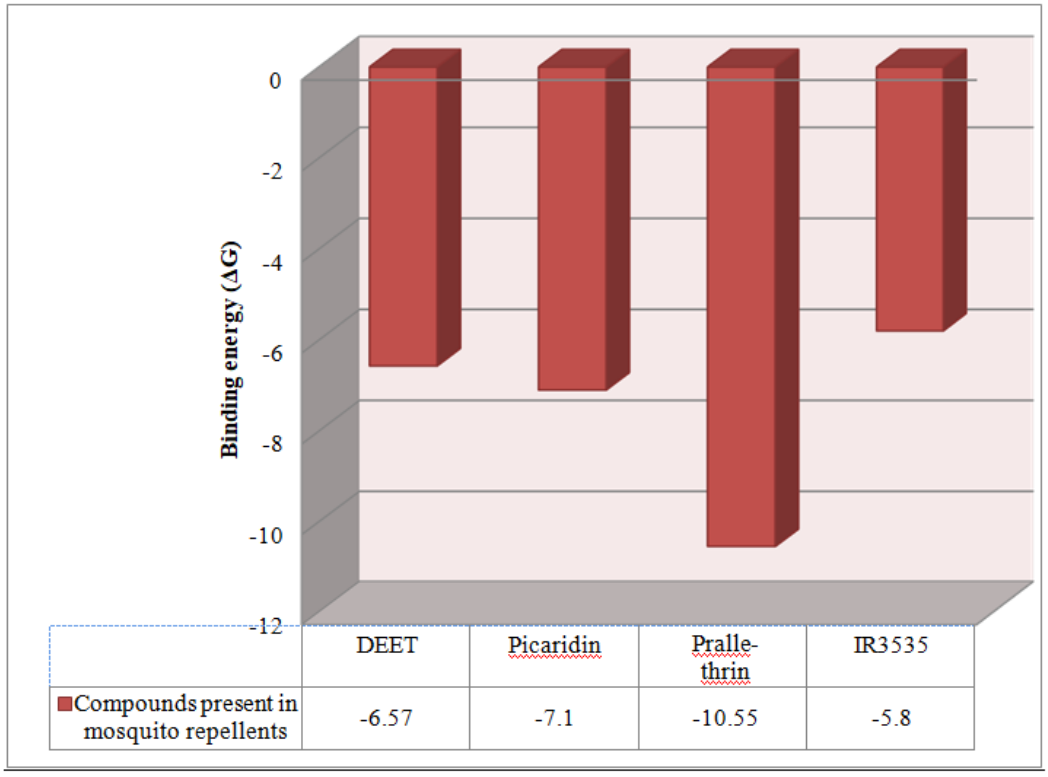

Fig. 7 Statistical data interpretation of binding energy (ÄG) of various chemical compounds present in mosquito repellents. Here, x-axis represents the compounds present in mosquito repellents and y-axis represents of binding energy of each of them

Therefore, DEET is not the only weapon. Dr. Dan Strickman, who oversees the Global Health Program at the Bill and Melinda Gates Foundation and is the author of "Prevention of Bug Bites, Stings, and Disease", claims that products containing the active components Picaridin and IR3535 are equally effective. Picardin has won the advantage, according to Strickman. Mosquitoes may land on individuals using DEET but refrain from biting them. When they use a picaridin-containing product, mosquitoes are less likely to even settle on them. However, Strickman notes that IR3535 repellents don't have the overpowering aroma of other products and are just marginally less effective. The Centre for Disease Control and Prevention recommends repellents containing any of those active components as being secure and reliable. They are easily accessible everywhere in the world.

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Species list and pictorial key to the dung beetles (Coleoptera, Scarabaeidae, Scarabaeinae) in the Chinnar Wildlife Sanctuary in the south Western Ghats, India

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ABSTRACT: Thirty five species comprising 13 genera were recorded from the thorny forests in Chinnar Wildlife Sanctuary, Kerala, India. Genera *Onthophagus* (14 species) and *Caccobius* (six species) were the most specious in the region. Pictorial key to the dung beetles in the thorny forest of Chinnar Wildlife Sanctuary collected during 2010-2015 is given. © 2023 Association for Advancement of Entomology

KEY WORDS: Coprinae, Onthophagus, Caccobius, dry forests, distribution records

INTRODUCTION

Scarabaeinae dung beetles are a globally distributed group of insects that are scavengers, which are predominantly coprophagous (faeces-eating), but may also feed on dung from other animals and decomposing animals, fungi and rotten fruits (Halffter and Mathews, 1966). Their feeding behaviour is important for the ecosystem services such as nutrient recycling, biological pest control and secondary seed dispersal (Hanski and Cambefort,1991; Nichols *et al*., 2008). Based on their feeding and nesting strategies dung beetles are classified into three functional guilds namely; rollers (telecoprid nesters), tunnelers (paracoprid nesters) and dwellers (endocoprid nesters) (Cambefort and Hanski, 1991). Aside from their functional importance in ecosystems, dung beetles have been proposed as a useful indicator group of habitat disturbance due to their fast response to environment modifications (Halffter and Favila, 1993, 1997; Davis *et al*., 2001).

The Western Ghats in India is one of the biodiversity hotspots of the world. Species composition and community structure of dung beetle in the moist western slope of the south Western Ghats have been studied in detail (Sabu and Vinod, 2005; Anu, 2006; Sabu *et al*., 2006, 2007; Vinod and Sabu, 2007; Vinod, 2009; Latha *et al*., 2011; Sabu *et al*., 2011; Mathews, 2013; Sathiandran *et al*., 2015). However, limited data is available about the dung beetles in the dry eastern slopes of the south Western Ghats. In the present study an analysis of the taxonomic composition of dung beetles in the thorny forest belts in Chinnar Wildlife Sanctuary (WLS) in the Western Ghats, along with a species list and pictorial key is compiled.

MATERIALS AND METHODS

The study was carried out at a southern tropical thorny forest at Chinnar WLS in the eastern dry slope of south Western Ghats in Kerala, south India (Fig. 1). Chinnar WLS with an area of 90.422 km^2

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is located 54 km south to Munnar and Marayoor, in Idukki district, Kerala.

Dung beetles were collected with dung-baited pitfall traps on a seasonal basis in the dry season and northeast monsoon seasons during the 2009–2012 period. The collected beetles were preserved in alcohol (70%) over night and later identified to species level using taxonomic keys available in Arrow (1931) and Balthasar (1963 a, b) and by comparing with the identified specimens available in the Zoological Survey of India, Western Ghats Regional Station, Kozhikode, Kerala. Verified specimens were deposited in the Zoological Survey of India museum, Western Ghats regional station, Kozhikode, Kerala, India.

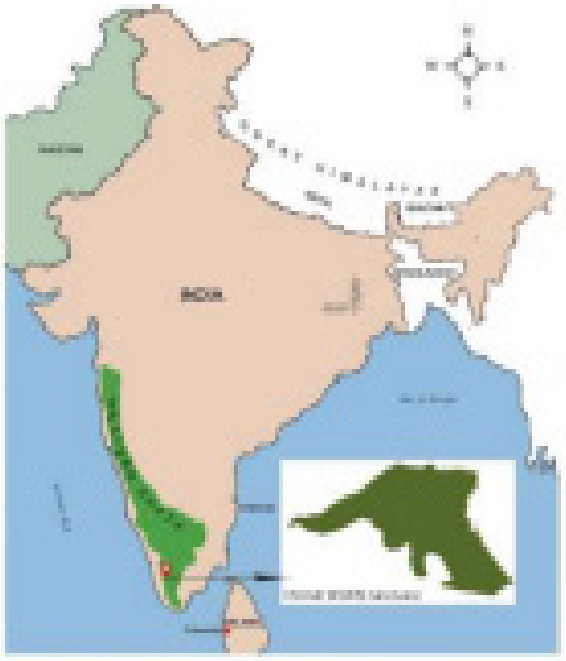


Fig. 1 Map showing the study region

Key to the tribes of subfamily Scarabaeinae in the Chinnar Wildlife Sanctuary with images:

1. Middle coxae widely separated, parallel or only little converging (Fig. 4 A).....3

—Middle coxae not widely separated (Fig. 4 B)…….. 2

RESULTS AND DISCUSSION

Thirty-five species comprising 13 genera (*Caccobius, Catharsius, Cleptocaccobius, Garreta, Gymnopleurus, Heliocopris, Onthophagus, Paracopris, Paragymnopleurus, Scarabaeus, Sisyphus, Tibiodrepanus* and *Tiniocellus*) and six tribes (Coprini, Gymnopleurini, Oniticellini, Onthophagini, Scarabaeini and Sisyphini) were recorded from the thorny forests in Chinnar Wildlife Sanctuary. *Onthophagus,* with 14 species and *Caccobius,* with six species, were the most specious genera in the study region. Two rare species, *Garreta smaragdifer* (earlier known from Sri Lanka and north eastern India) (Arrow, 1931; Sobhana *et al.,* 2017) and *Caccobius rufipennis* (earlier known from Sri Lanka and Eastern Ghats in India) (Arrow, 1931; Priyadarsanan, 2006), in addition to the female of the latter species is reported (Table 1).

Three species, *Caccobius rufipennis* (Motschulski, 1858), *Garreta smaragdifer* Walker, 1858 and *Scarabaeus sanctus* Fabricius, 1798 were rare species from the dry forest region of Chinnar (Fig. 3). Comparison of dung beetles collected in the present study with the earlier reports from the south western Ghats (Arrow,1931; Balthasar, 1963, 1974; Paulian, 1980, 1983) and the checklist of dung beetles of the moist western slope of the South Western Ghats (Sabu *et al*., 2011) revealed the presence of four species (*Gymnopleurus cyaneus*, *Onthophagus spinifex*, *Onthophagus ephippioderus* and *Onthophagus pardalis*) in Chinnar Wildlife Sanctuary which was mentioned as lost species from the south Western Ghats. These findings indicate that extensive studies in the eastern slope of the Western Ghat may lead to the unearthing of more rare species.

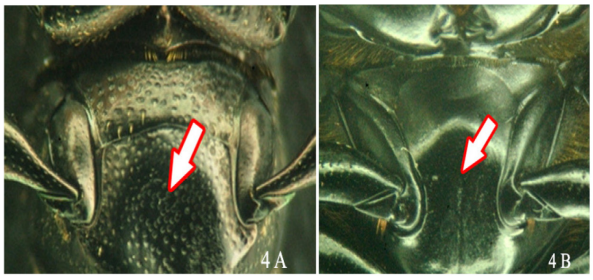


Fig. 2 (A) *Garreta smaragdife* - Male, (B) *Garreta smaragdifer* Female, (C) *Caccobius rufipennis*

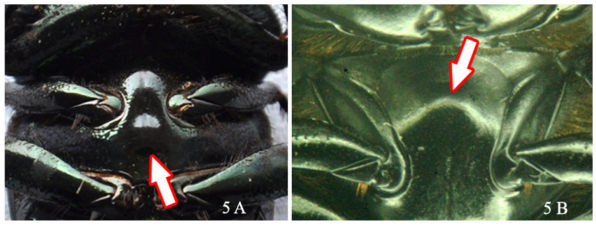
Fig. 3 Rare dung beetle species recorded from Chinnar WLS - D) *Caccobius gallinus*, (E) *Paracopris davisoni* (F) *Scarabaeus sanctus*

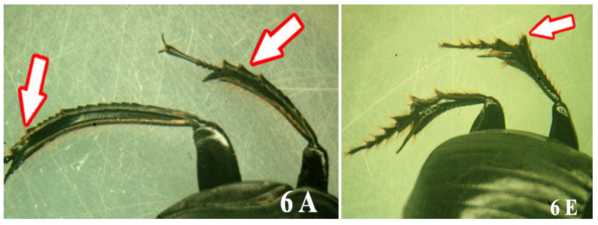
2. Middle coxa converges strongly behind (Fig. 5 A)……..... 3

—Middle coxa diagonally placed (Fig. 5 B..... Gymnopleurini

3. Middle and hind tibiae are slender and not dilated at end (Fig.6 A). ……..... 4

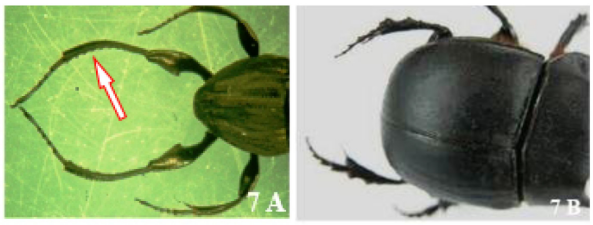
—Middle and hind tibiae moderately broad at the extremity (Fig. 6 B). 5





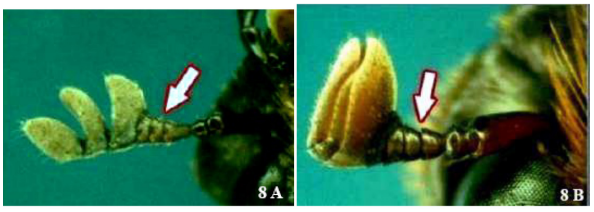
4. Posterior legs extremely long, tarsi filiform (Fig.7 A). ..... Sisyphini

—Posterior legs not extremely long, tarsi more or less flat and tapering (Fig. 7 B).....Coprini



5. Antenna 8 segmented (Fig. 8 A). ..... Oniticellini

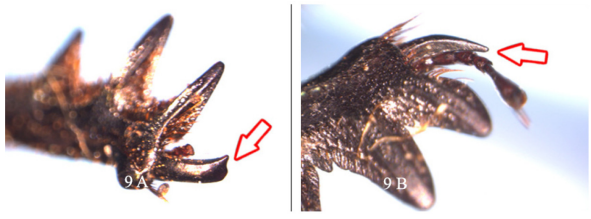
—Antenna 9 segmented (Fig. 8 B). ..... Onthophagini



Key to the genera of the tribe Gymnopleurini

1. Terminal spur of front tibia bidentate (Fig. 9 A). ..... *Paragymnopleurus* Shipp

— Terminal spur of front tibia not bidentate (Fig. 9 B). 2



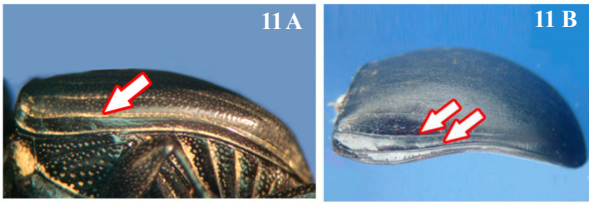
2. Clypeus bidentate(Fig. 10 A)..... .....*Gymnopleurus* Illiger

—Clypeus with 4-6 anterior teeth (Fig. 10 B). ..... *Garreta* Janssens

Key to genera of the tribe Coprini

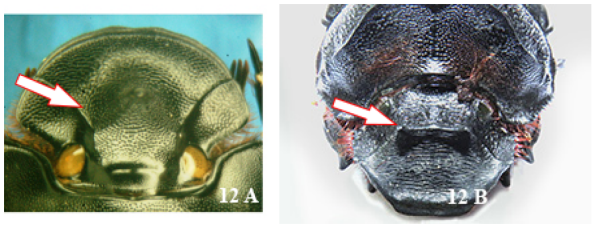
1. Elytra with one lateral carina (Fig. 11 A).....3

—-Elytra with two lateral carina (Fig. 11 B).....2



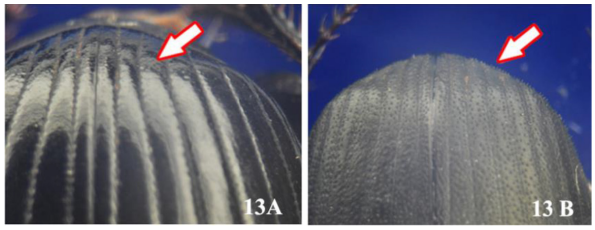
2. Ocular lobes separated by carinate sutures from clypeus (Fig. 12 A).....*Catharsius* Hope

—— Ocular lobes united by carinate suture with clypeus (Fig. 12 B).....*Heliocopris* Hope



3. Punctures at apex and sides of elytra without hairs (Fig.13 A).....*Copris* Geoffroy

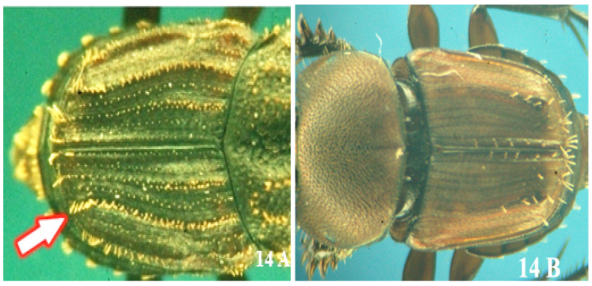
——Punctures at apex and sides of elytra bearing short stiff hairs (Fig. 13 B)..*Paracopris* Balthasar



Key to the genera of the tribe Oniticellini

1. Elytra fringed before hind margin (Fig. 14 A). ..... *Tibiodrepanus* Krikken

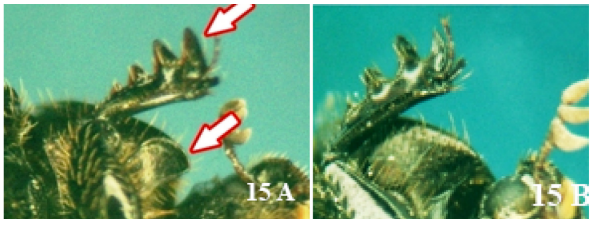
—Elytra fringed only at hind margin (Fig. 14 B). .....*Tiniocellus* Peringuey



Key to the genera of the tribe Onthophagini

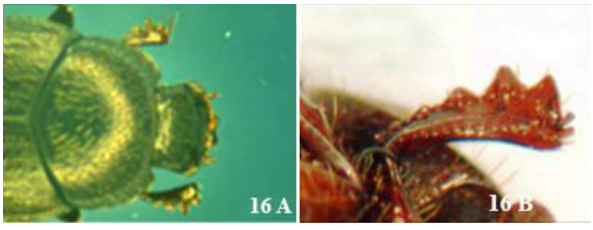
1. Apical margin of fore tibia at right angles to inner margin; anterior angles of prothorax hollowed beneath (Fig. 15 A).....2

—Either one or none of the above character presents (Fig. 15 B).....*Onthophagus* Latreille



2. Apical tooth of tibia thin and translucent; apical tooth in obtuse angle with tibia (Fig. 16 A).....*Cleptocaccobius* Cambefort

— Apical tooth of tibia not thin and translucent; apical tooth in right angle with tibia (Fig. 16 B).....*Caccobius* Thomson



Key to the species of genus *Scarabaeus*

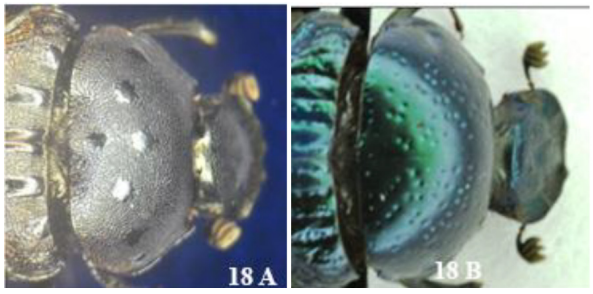
1. Clypeal teeth separated by sharp notches (Fig. 17 A). ..... *sanctus* Fabricius

—Clypeal teeth separated by acute notches (Fig. 17 B). ..... *erichsoni* Harold

Key to the species of genus *Gymnopleurus*

1. Pronotum with about six shining spots (Fig. 18 A). ..... *parvus* Macleay

— Pronotum without spots (Fig. 18 B) ..... *cyaneus* Fabricius



Key to the species of genus *Sisyphus*

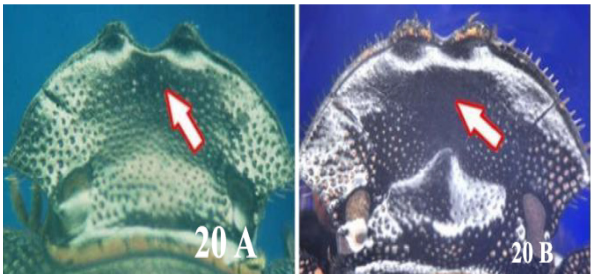
1. Hind femur gradually dilated (Fig. 19 A). .....*longipes* Olivier

—Hind femur abruptly dilated (Fig. 19 B). .....*neglectus* Gory

Key to the species of genus *Paracopris*

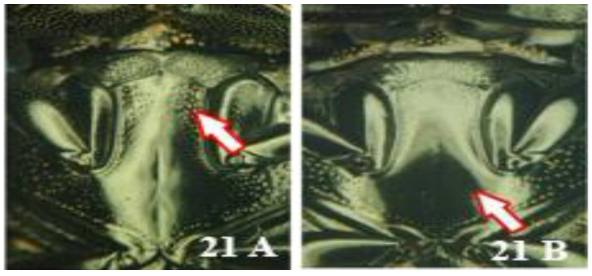
1. Clypeus strongly punctured (Fig.20 A). .....*cribratus* Gillet

—Clypeus rather smooth (Fig. 20 B).....2



2. Metasternal shield punctured in front (Fig. 21 A). ..... *davisoni* Waterhouse

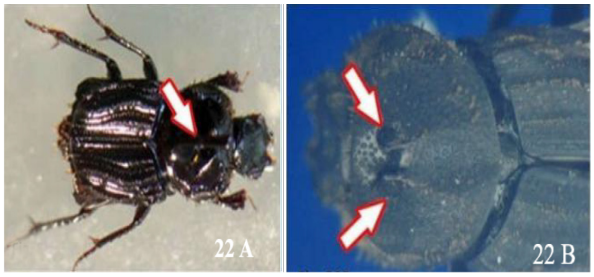
— Metasternal shield not punctured in front (Fig. 21 B). .....……..…… *signatus* Walker



Key to the species of genus *Tibiodrepanus*

1. Male with single pronotal horn (Fig. 22 A). ..... *setosus* Wiedemann

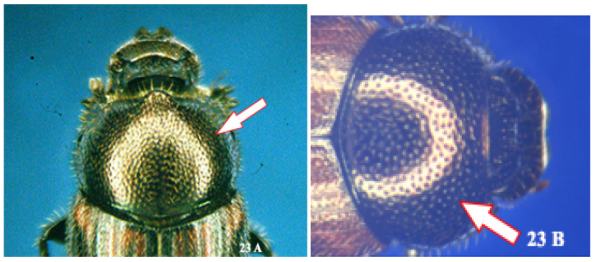
— Male with two pronotal horn (Fig. 22 B). ..... *sinicus* Harold



Key to the species of genus *Caccobius*

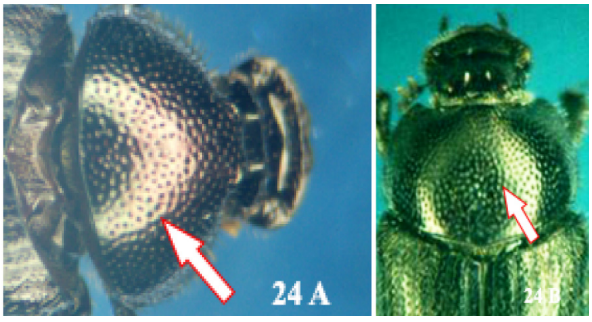
1. Pronotum granulate at sides (Fig. 23 A).....2

—Pronotum punctured at sides (Fig. 23 B).....5



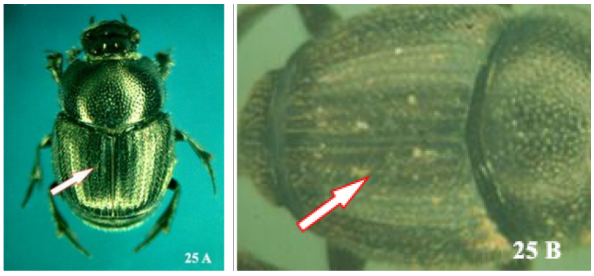
2. Pronotum not closely punctured (Fig. 24 A). ..... *vulcanus* Fabricius

—Pronotum closely punctured (Fig. 24 B).....3



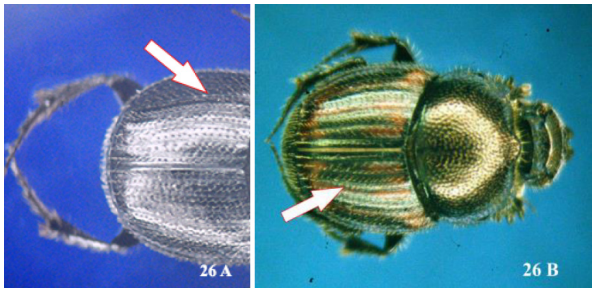
3. Elytra very shining (Fig. 25 A)..... *gallinus* Arrow

—Elytra not shining (Fig. 25 B).....4



4. Elytra entirely black (Fig. 26 A).....*ultor* Sharp

—Elytra brown, variegated (Fig. 26 B). ..... *meridionalis* Boucomont



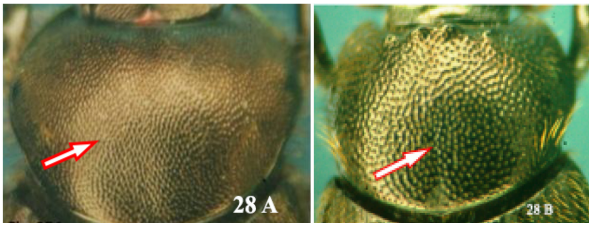
5 Upper surface clothed with minute setae (Fig. 27 A). .....*unicornis* Fabricius

— Upper surface devoid of setae (Fig. 27 B). ..... *rufipennis* Motschulsky

Key to the species of genus *Onthophagus*

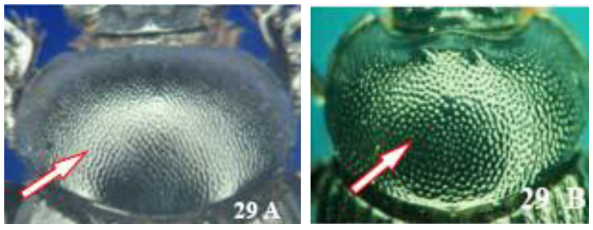
1. Pronotum wholly or partly or granular or rugose (Fig. 28 A) 2

—Pronotum punctured without granules (Fig. 28 B). 7



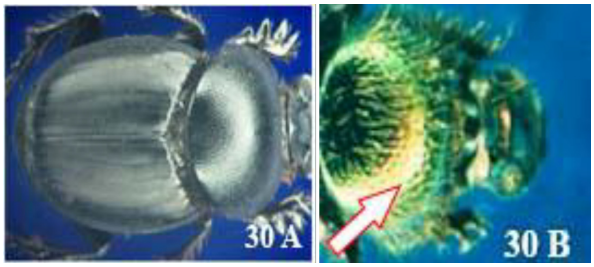
2. Pronotum entirely granular or rugose, without distinct punctures (Fig. 29 A).....3

—Pronotum partly granular or rugose, with some punctures (Fig. 29 B).....4



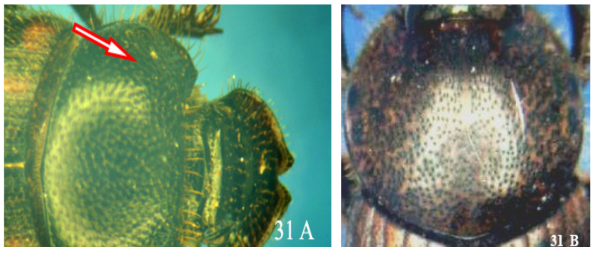
3. Upper surface not clothed with dense pile (Fig. 30 A).....*amphinasis* Arrow

—Upper surface clothed with very dense pile (Fig. 30 B).....*tarandus* Fabricius



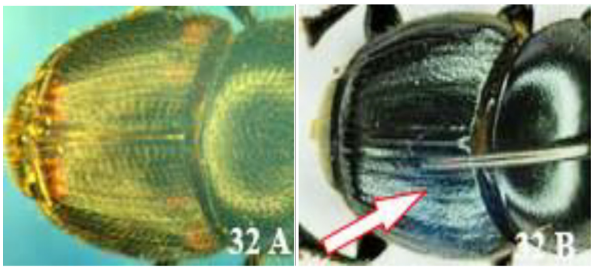
4. Pronotum with evenly distributed granules (Fig. 31 A).....7

—Pronotum without evenly distributed granules (Fig. 31 B).....6



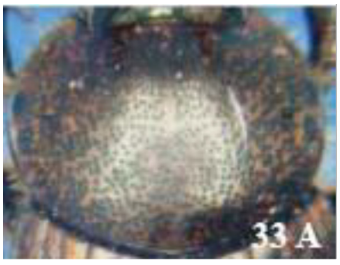
5. Elytra not very shining (Fig. 32 A) .....*furculus* Fabricius

— Elytra very shining (Fig. 32 B) .....*spinifex* Fabricius



6. Pronotum not shining (Fig. 33 A) .....*retecornutus* Lansberg

—Pronotum shining (Fig. 33 B). .....*vividus* Arrow

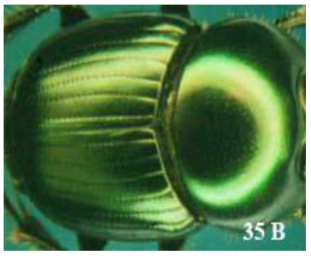


7. Upper surface without hairs (Fig. 34 A). 8

— Upper surface with distinct hairs or setose (Fig. 34 B). 11

8. Elytral suture with a minute elevation (Fig. 35 A).....9

— Elytral suture without minute elevation (Fig. 35 B).....10

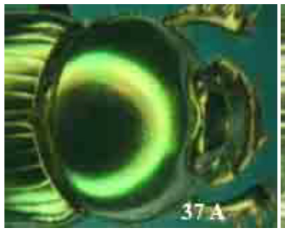


9. Head produced in front (Fig. 36 A). ..... *ephippioderus* Arrow

—Head not produced in front (Fig. 36 B).....*pardalis* Fabricius

10. Pronotum metallic green (Fig. 37 A). ..... *dama* Fabricius

—Pronotum black (Fig. 37 B) .....*quadridentatus* Fabricius

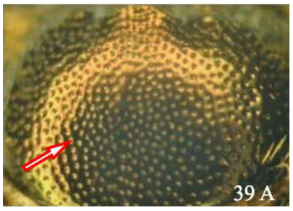


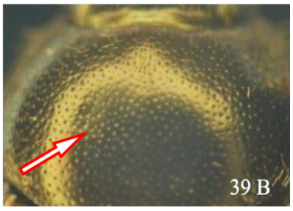
11. Pronotum pale at sides (Fig. 38 A).....12

—Pronotum uniformly coloured (Fig. 38 B).....14

12. Punctures of pronotum large, close and umbliicate (Fig. 39 A).....*Furcillifer* Bates

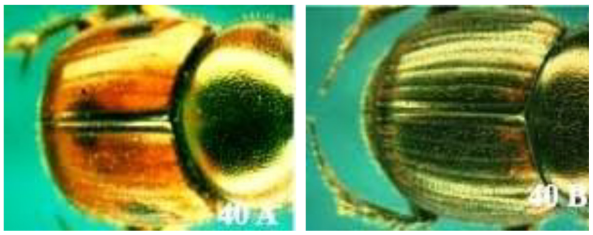
— Punctures of pronotum not large, close and umbliicate (Fig. 39 B).....13





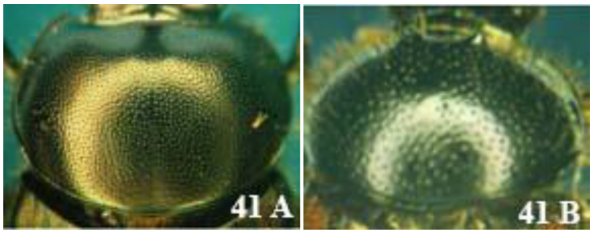
13. Base, apex and sides of the elytra pale (Fig. 40 A).....*fasciatus* Boucomont

— Base, apex and sides of the elytra not entirely pale (Fig. 40 B).....*favrei* Boucomont



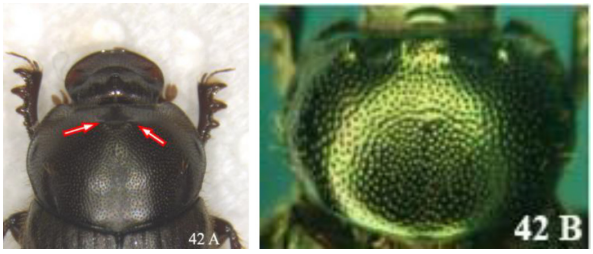
14. Pronotum finely and closely punctured (Fig. 41A).....15

—Pronotum strongly punctured (Fig. 41 B). 16



15. Pronotum with two thoracic prominences (Fig. 42 A).....*turbatus* Walker

— Pronotum with four thoracic prominence (Fig. 42 B)..... *ensifer* Boucomont



16. Body short and broad (Fig. 43 A).....17

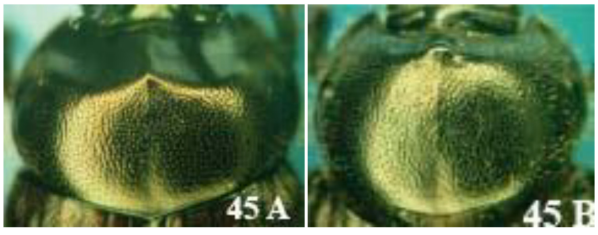
—Body rather elongate (Fig. 43 B).....18

17. Pronotum with blunt tubercles anteriorly (Fig. 44 A)..... *ludio* Boucomont

— Pronotum without blunt tubercles (Fig. 44 B). 19

18.Front angels of the pronotum pointed (Fig.45 A). ..... *cervus* Fabricius

— Front angels of the pronotum not pointed (Fig. 45 B). ..... *falsus* Gillet



19. Pronotum with median longitudinal groove (Fig. 46 A).....*bifasciatus* Fabricius

— Pronotum without median longitudinal groove (Fig. 46 B)..... *unifasciatus* Schaller

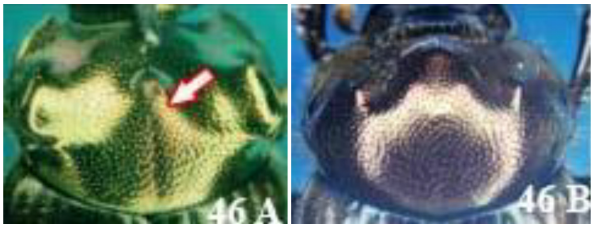


Table 1. Dung beetle species collected from Thorny forest of Chinnar in south Western Ghats during 2010-2015 with distribution records

Caccobius (Caccophilus) gallinus Arrow, 1907	India (Kerala: Nelliampathi, Chinnar, Wayanad; Tamil Nadu: Nilgiri Hills) (Arrow, 1931; Sobhana *et al.,* 2013).
Caccobius (Caccophilus) meridionalis Boucomont, 1914	India (Karnataka; Kerala: Erumaiyoor, Mahe, Nelliampathi, Chinnar, Ranipuram, Shendurney, Silent valley, Thekkady, Wayanad; Gujarat; Maharashtra; Tamil Nadu: Anaimalai Hills, Nilgiri Hills), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Caccobius (Caccobius) rufipennis (Motschulski, 1858)	India (Kerala: Chinnar), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Caccobius (Caccophilus) ultor Sharp, 1875	India (Haryana: Kanneri; Karnataka, Budipadaga; Kerala:Nelliampathi, Chinnar, Ranipuram; Maharashtra: Bombay, Khandesh; Punjab; Rajasthan; Uttar Pradesh) (Arrow, 1931, Sobhana *et al.,* 2013).
Caccobius (Caccophilus) unicornis (Fabricius, 1798)	China, India (Assam; Kerala: Silent valley, Chinnar, Wayanad; Madhya Pradesh; Tripura; W. Bengal), Indonesia (Borneo, Java, Sumatra), Malay Peninsula, Myanmar, Philippines, Sri Lanka, Taiwan (Arrow, 1931; Sobhana *et al.,* 2013).
Caccobius (Caccophilus) vulcanus (Fabricius, 1801)	India (Bihar; Karnataka, Bangalore; Kerala: Erumaiyoor, Chinnar, Ranipuram), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Catharsius (s. str.) molossus (Linnaéus, 1758)	Afghanistan, Cambodia, China, India (Andaman; Arunachal Pradesh; Assam; Bihar; Gujarath; Hariyana; Karnataka; Kerala: Kinavellore, Nelliampathi, Chinnar, Wayanad; Maharashtra: Mumbai; Meghalaya; Orissa; Rajasthan; Sikkim; Tamil Nadu; Uttaranchal; West Bengal), Laos, Malay (Sunda Island), Malaysia, Nepal, Sri Lanka,Taiwan, Thailand, Vietnam (Annam) (Arrow, 1931; Sobhana *et al.,* 2013).
Cleptocaccobius arrowi Cambefort, 1985	India (Karnataka, Bangalore; Kerala: Malabar, Ranipuram, Chinnar, Shendurney; Maharashtra: Mumbai; Nagpur) (Arrow, 1931; Sobhana *et al.,* 2013).
Garreta smaragdifer Walker, 1858	India (Kerala: Chinnar), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Gymnopleurus (s. str.) cyaneus (Fabricius, 1798)	Bangladesh, India (Andhra Pradesh; Gujarat; Haryana; Karnataka: Anaimalaihills; Kerala: Chinnar, Malabar, N. Malabar; Maharashtra: Mumbai; Tamil Nadu: Coimbatore; W. Bengal: Dhoni forest, Kannirode), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Heliocopris bucephalus (Fabricius, 1775)	Bangladesh, India (Bihar; East and Peninsular India;Kerala: Chinnar, Wayanad; Madhya Pradesh; Maharashtra; Tamil Nadu: Hassanur; Tripura; Uttar Pradesh; W. Bengal), Laos, Malay Peninsula, Myanmar, Thailand (Siam), Vietnam (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (Paraphanaeomorphus) bifasciatus (Fabricius, 1781)	India (Assam; Bihar; Kerala: Nilgiri hills, Ranipuram, Chinnar, Thekkady, Wayanad; Sikkim; W. Bengal), Myanmar (Arrow, 1931; Sobhana *et al*., 2013).

Onthophagus (s. str.) cervus (Fabricius, 1798)	India (Karnataka; Kerala: Calicut, Nilgiri hills, Ranipuram, Chinnar, Thekkady, Wayanad; Madhya Pradesh; Maharashtra; Tamil Nadu: Coimbatore, Puducherry; Uttaranchal; W. Bengal), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (s. str.) dama (Fabricius, 1798)	Bhutan, India (Bihar; Karnataka; Kerala: Nilambur, Nilgiri hills, Ranipuram, Chinnar, Thekkady, Wayanad; Maharashtra; Sikkim; Tamil Nadu: Anaimalai hills; Uttaranchal; W. Bengal), Nepal, Sri Lanka (Arrow, 1931, Sobhana *et al.,* 2013).
Onthophagus (Colobonthophagus) ephippioderus, Arrow, 1907	India (Kerala: Nilgiri hills; Karnataka: Belgaum (Arrow, 1931; Sobhana *et al.,* 2013).
*Onthophagus (s. str.) falsus,*Gillet, 1925	Afghanistan, Bangladesh, India (Assam; Kashmir; Kerala: Ranipuram, Chinnar, Thekkady, Wayanad; W. Bengal) (Arrow, 1931; Sobhana *et al*., 2013).
Onthophagus (s. str.) fasciatus Boucomont, 1914	India (Karnataka; Kerala: Nilgiri hills, Ranipuram,Thekkady, Chinnar, Wayanad; Madhya Pradesh; Maharashtra: Mumbai; Uttaranchal; W. Bengal; Tamil Nadu: Anaimalai hills, Madhura) (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (s. str.) furcillifer Bates, 1891	India (Assam; Kashmir; Kerala: Ranipuram, Chinnar, Thekkady, Wayanad; Punjab; Uttaranchal) (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (s. str.) furculus (Fabricius, 1798)	India (Tamil Nadu: Puthuchery) (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (s. str.) ludio Boucomont, 1914	India (Kerala: Nilgiri hills; Maharashtra: Belgaum, Bombay, Nagpur), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (Colobonthophagus) pardalis (Fabricius, 1798)	India (Kerala: Chinnar, Nilgiri hills; Maharashtra: Bombay;Kanara) (Arrow, 1931; Sobhana *et al.,* 2013).
*Onthophagus (s. str.) quadridentatus (*Fabricius, 1798)	India (Assam; Karnataka: Bangalore, Belgaum; Kerala:Chinnar, Mahe, Malabar, Chinnar, Nilgiri hills, Palakkad; Maharashtra: Bombay,Pune; Tamil Nadu: Coimbatore; W. Bengal: Calcutta), Sri Lanka (Colombo) (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (s. str.) spinifex (Fabricius, 1781)	India (Bengal; Bihar; Kerala: Chinnar, NilgiriHills; Maharashtra: Bombay; Tamil Nadu: Madurai), Sri Lanka (Colombo) (Arrow, 1931; Sobhana *et al*., 2013)
Onthophagus (s. str.) turbatus Walker, 1858	India (Karnataka; Kerala: Chinnar, Mahe, Malabar, Nelliampathi, Chinnar, Nilgiri hills; Maharashtra; Tamil Nadu: Puducherry), Sri Lanka (Arrow, 1931; Sobhana *et al.,* 2013).
Onthophagus (s. str.) unifasciatus Schaller, 1783	India (Bengal; Bihar; Kerala: Nilgiri hills; Chinnar, Maharashtra: Bombay; Tamil Nadu: Coimbatore, Madras), Sri Lanka (Colombo, Kandy) (Arrow, 1931; Sobhana *et al.,* 2013).
Paracoprisdavisoni (Waterhouse, 1891*)*	India (Karnataka; Kerala: Nelliyampathy, Chinnar, Nilgiri hills, Peerumade, Ranipuram, Thekkady, Travancore, Wayanad; Maharashtra: Mumbai; Tamil Nadu: Palni hills) (Arrow, 1931; Sobhana *et al.,* 2013).

Paracoprissignatus (Walker, 1858)	India (Karnataka; Kerala: Mahe, Malabar, Thekkady, Chinnar, Travancore, Sendurney, Wayanad; Maharashtra; Tamil Nadu: Coimbatore), Laos, Sri Lanka, Vietnam (Annam) (Arrow, 1931; Sobhana *et al.,* 2013).
Paragymnopleurussinuatus Olivier, 1789	India (Arunachal Pradesh; Karnataka; Kerala:Nelliampathi, Chinnar, Nilambur, Palghat, Ranipuram, Shendurney; Maharashtra: Kanara, S. Bombay; Sikkim; W. Bengal), Myanmar, Nepal (Arrow, 1931; Sobhana *et al.,* 2013)
Scarabaeus (Kheper) erichsoni Harold, 1867	India (Karnataka: Bangalore; Tamil Nadu: Madras; Kodaikanal; Podanur), Kerala: Chinnar, Sri Lanka (Colombo, Kandy) (Arrow, 1931; Sobhana *et al.,* 2013).
Scarabaeus (Kheper) sanctus Fabricius, 1798	India (Bihar; Karnataka: Bangalore, Belgaum; Kerala: Chinnar, Nilgiri hills; Maharashtra: Mumbai; Orissa: Sholapur), Sri Lanka (Kinavallore) (Arrow, 1931; Sobhana *et al.,* 2013).
Sisyphus (s. str.) longipes (Olivier, 1789)	India (Karnataka; Kerala: Nilgiri hills; Maharashtra; Orissa; Tamil Nadu: Ooty; W. Bengal), Myanmar, Sri Lanka, Thailand (Arrow, 1931; Sobhana *et al.,* 2013).3
Sisyphus (s. str.) neglectus Gory, 1833	China, India (Karnataka; Kerala: Nelliampathi, Chinnar, Wayanad; Uttaranchal), Myanmar, Thailand (Arrow, 1931; Sobhana *et al.,* 2013).
Tibiodrepanus setosus (Wiedemann, 1823)	India (Kerala: Nelliampathi, Nilgiri hills, Chinnar, Wayanad; TamilNadu: Anamalai hills) (Arrow, 1931; Sobhana *et al.,* 2013)
Tibiodrepanus sinicus (Harold, 1868)	India (Central and Northern India; Kerala: Nelliampathi), Laos, Myanmar, North Vietnam, Southern China (Arrow, 1931; Sobhana *et al.,* 2013).
Tiniocellus spinipes (Roth, 1851)	Angola, Brazil (Natal), Congo, Ethiopia, Guinea, India (Karnataka; Kerala: Calicut, Nilambur, Wayanad; Chinnar, Madhya Pradesh; Maharashtra; Punjab: Chari; Uttaranchal), Malawi, Somalia, SouthAfrica (Transvaal), Tanzania, Uganda, Zimbabwe (Arrow, 1931; Sobhana *et al.,* 2013).

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Compatibility of carbosulfan 25 EC with certain agrochemicals in brinjal ecosystem

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ABSTRACT: The combined use of chemical insecticides with fungicides and fertilizers in a single application is a promising pest-control option to maximise productivity and minimize labour efficiency. Studies were conducted to assess the compatibility of systemic insecticide, carbosulfan 25 EC with a fungicide, micronutrient and insecticide in brinjal ecosystem. The optimum and effective dose of carbosulfan (250 g a.i.ha^{-1}) was compatible with other agro chemicals used in combination *viz.,* copper oxy chloride 50 WP @500 g a.i.ha^{-1}, zinc sulphate 0.5 per cent and dimethoate 30 EC @ 300 g a.i.ha^{-1}, without any creaming matter and/or sediment formation in any of the combinations. The tank mix foliar application of the combination chemicals *viz.,* carbosulfan @ 250 g a.i.ha^{-1} + copper oxy chloride 50 WP @ 500 g a.i.ha^{-1}, carbosulfan @ 250 g a.i.ha^{-1} + zinc sulphate 0.5 per cent and carbosulfan @ 250 g a.i.ha^{-1} + dimethoate @ 300 g a.i.ha^{-1} did not inflict any phytotoxic effect on the treatment imposed plants and a mean grade of '1' (0-10% injury) was awarded to all the treated plants in the brinjal ecosystem. The bioefficacy trials after two rounds of spraying with carbosulfan and combination with fertilizer and fungicides revealed that maximum per cent reduction is noticed in insecticide combination (carbosulfan @ 250 g a.i.ha^{-1} + dimethoate @ 300 g a.i.ha$^{-1)}$ as well as recommended and four times the dose of carbosulfan (250 and 1000 g a.i.ha^{-1}), which similarly effects in managing the shoot and fruit damage caused by the shoot and fruit borer, *Leucinodes orbonalis.* The treatments with fertilizer and fungicides alone marked the least reduction in fruit and shoot damage.The yield of brinjal ranged from 24.1to 28.7 t ha^{-1} in different treatments. Plots treated with carbosulfan @ 250 g a.i.ha^{-1} + dimethoate @ 300 g a.i.ha^{-1} recorded the highest fruit yield (28.7 t ha^{-1}) followed by carbosulfan @ 250 g a.i.ha^{-1} + copper oxy chloride @ 500 g a.i.ha^{-1} (28.3 t ha^{-1}) and carbosulfan @ 250 g a.i.ha^{-1}+ zinc sulphate (27.8 t ha^{-1}). The current findings states that carbosulfan in combination with other agrochemicals have given better results in terms of phytotoxicity, bioefficacy and yield. © 2023 Association for Advancement of Entomology

KEY WORDS: Combination, copper oxy chloride, zinc sulphate, dimethoate

INTRODUCTION

Brinjal, *Solanum melongena* Linnaeus is highly cosmopolitan and popular vegetable grown as known as "King of vegetables" globally (Lalia *et al.,* 2021). It is grown throughout the year under tropical and subtropical conditions and usually finds its place in common men's kitchen (Ajit *et al.,* 2017). It is also popularly known as poor man's crop; serves a meal for poor people's diet. Being a major vegetable crop in India, brinjal is cultivated in about 7.27 lakh hectares with an annual

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production of 123.23 Lakh tonnes during 2016-17 (Borah and Saikia, 2017).

Brinjal is attacked by more than 70 insect pests (Borah and Saikia, 2017) and the pest problems in brinjal are becoming more serious because of favourable conditions which are provided by present methods of cultivation. The monoculture, overlapping of crops, dense cropping, excess use of fertilizers and pesticides, continuous availability of preferred host plants etc., are some of the major reasons for pest outbreak (Misra, 2008; Rao, 2003). Insecticides provide an acceptable solution to overcome these pests, as they are highly effective, rapid in curative action and adoptable to most situations, flexible in meeting changing agronomic conditions and relatively economical. Various insecticides belonging to, organophosphates, carbamates and synthetic pyrethroids are recommended to control these pests (Jagginavar *et al.,* 2009; Anil Kumar *et al.,* 2000). Carbosulfan, (2,3-dihydro-2,2-dimethyl-7-benzo-furnanyl (din-butyl amino) thio methyl carbamate) a relatively new methyl carbamate insecticide is reported to be effective against insect pests of this crop. Carbosulfan is found to be very efficient in reducing the population of the most notorious pest of brinjal, the fruit and shoot borer as well as sucking pests (Sheeba Jasmine, 2002) and hence the chemical is recommended for brinjal pests.

In plant protection schedule very often, it becomes necessary to combine the application of different agrochemicals. At times, the insect pests and diseases occur simultaneously on a crop requiring foliar application of different insecticides as well as fungicides. In addition, foliar spray of fertilizers may also be required at the same time to meet the fertility of the crop (Chandrakumar *et al.,* 2008). Considering the need for application of insecticides, fungicides and fertilizers repeatedly, the combined application *i.e.* tank mix is preferred by the farmers since it could save time, money, energy and wear and tear of equipment. However, the major problem associated with this practice is the possibility of reduction in bioefficacy owing to physical and chemical incompatibility. Pesticides and fertilizers when mixed together tend to react with each other in some cases and in this process compounds may be formed which may produce harmful effects rather benefits to the crop plants. Sometimes toxicity may be increased or decreased (Patial and Mehta, 2008). Carbosulfan was tried in combination with other chemicals in few crops and found compatible. Carbosulfan (5%) was compatible with fungicides like captan, captafol, thiram and do not have any adverse effect on germination of sorghum seeds. The shootfly, *Atherigona soccata* (Rond.) was effectively controlled by five per cent carbosulfan followed by carbosulfan + carbendazim, carbosulfan + captan, carbosulfan + captafol and carbosulfan + thiram. A similar trend was observed in grain yield also (Morale *et al.,* 1991). The information on the above aspect is very much limited with respect to carbosulfan combinations in brinjal ecosystem. In view of above facts, a study of carbosulfan with different combination of agrochemicals was attempted in brinjal crop to evaluate its phytotoxic effects, bioefficacy against the shoot and fruit borer, *Leucinodes orbonalis* and its yield impacts.

MATERIALS AND METHODS

Laboratory studies: Emulsion stability test was conducted to study the compatibility of carbosulfan emulsion alone and in combination as detailed below.

Preparation of standard hard water: Standard hard water was prepared by dissolving 0.302g $CaCl_2$ and 0.139g $MgCl_2$ in one litre of distilled water.

Emulsion stability test: The test was carried out as prescribed by Indian Standard Specification (Anonymous, 1973). 0.2 ml of formulated chemical (carbosulfan 25 EC) was added into 30 ml of standard hard water taken in a beaker at the rate of 25 to 30 ml per minute with the material pouring directly into the beaker and not along the sides. The contents of the beaker was stirred with a glass rod at a rate of 4 revolutions per second during the addition. The diluted emulsion was made up to 100 ml with hard water and it was transferred immediately to a clean dry graduated cylinder. The graduated cylinder with its contents was kept in a thermostat at $30 \pm 1^{o}C$ for 1 hour. After the

specified time, the volume of the creamed matter at the top and/or the sediment if any at the bottom was observed. For stable emulsion, the creaming matter and/or the sediment if any should not exceed 2.0 ml. To 30 ml of standard hard water taken in a beaker, 0.2 ml of formulated chemical (carbosulfan 25 EC) was added. Similarly, diluted solutions of copper oxy chloride (0.2g), zinc sulphate (0.5 g), dimethoate (0.2 ml) were prepared separately using standard hard water. To 30 ml of the formulated chemical suspension (carbosulfan) prepared, 30 ml of the combination chemical (copper oxy chloride, zinc sulphate and dimethoate) was added and transferred to a clean dry graduated cylinder and the volume was made upto 100 ml with standard hard water, shaken well and was kept in a thermostat at 30 ± 1°C for 1 hour without any disturbance. The volume of the creamed matter at the top or the sediment if any at the bottom was observed. The creaming matter and/or the sediment not exceeding 2.0 ml was considered as criteria for the compatibility.

Field experiment: A replicated and randomized field experiment was conducted to study the compatibility of carbosulfan with other agrochemicals at Urumandampalayam village, Vellakinaru, Coimbatore, with the variety CO-2, during the period of Jan - May, 2013, with ten treatments replicated four times and the plot size was 20m^2.

The treatment details are mentioned in table 3. Three rounds of insecticidal spraying were given in the field trials at the vegetative stage as soon as the pest infestation starts at 14 days interval commencing from 10th day after transplanting with pneumatic knapsack sprayer using 500 litres of spray fluid per hectare. The control plots were water sprayed with pneumatic knapsack sprayer using 500 litres of spray fluid per hectare. All treatments were replicated thrice with a plot size of 20 m^2.

Phytotoxicity assessment: In the field, symptoms like leaf injury, wilting, vein clearing, necrosis, epinasty and hyponasty were observed in each plot from ten randomly tagged plants at 1, 3, 7 and 14 days after spraying as per Central Insecticide Board Registration Committee (CIBRC) protocol.

Leaf injury was assessed on visual rating from 1 - 10 scale such as

Rating	Phytotoxicity (%)
1	0 - 10
2	11 - 20
3	21 - 30
4	31 - 40
5	41 - 50
6	51 - 60
7	61 - 70
8	71 - 80
9	81 - 90
10	91 - 100

The per cent leaf injury was calculated using the formula ,

$$\text{Per cent leaf injury} = \frac{\text{Total grade points}}{\text{Max. grade x no. of leaves observed}} \times 100$$

Bioefficacy: Observation on shoot damage was recorded on five randomly selected and tagged plants from each plot on 7th and 14th day after the spray and expressed as shoot damage per cent. Observation on fruit damage was made by counting total number of fruits and damaged fruits with bore holes from 10 randomly selected plants per plot at each picking and converted into fruit damage per cent.

Yield Assessment: Brinjal fruits were harvested as replicated plot wise at an interval of 3 days and pooled to arrive the total yield from the first harvest which commenced 15 days after third spraying.

Statistical analysis

Laboratory studies: The data related to safety

tests were transformed to $\sqrt{x+0.5}$ and analysed by completely randomized design. The treatment mean values of the experiment were compared using Duncan's Multiple Range Test (DMRT). The corrected per cent mortality for lab studies was worked out by using Abbott's (1925) correction.

$$\text{Corrected percent mortality} = \frac{P_o - P_c}{(100 - P_c)} \times 100$$

Where,

Po - Observed mortality in treatment

Pc - Observed mortality in untreated check

The values of the corrected per cent mortality were transformed using arc sine transformation for normalisation of data (Snedecor and cochran, 1967)

Field studies: The yield data in the field experiment were transformed to $\sqrt{x+0.5}$ and analysis of variance was carried out by randomized block design (Panse and Sukhatme,1958) and means were separated by Duncan's Multiple Range Test. The corrected per cent reduction in field population was worked out by using the formula of Henderson and Tilton (1955).

$$\text{Corrected per cent reduction} = 1 - \frac{(T_a X C_b)}{(T_b X C_a)} \times 100$$

Where,

Ta - Number of insects in the treatment after spraying

Tb - Number of insects in the treatment before spraying

Ca - Number of insects in the untreated check after spraying

Cb - Number of insects in the untreated check before spraying

RESULTS

Evaluation of compatibility of carbosulfan 25 EC by emulsion stability test: The emulsion stability test conducted to assess the compatibility of carbosulfan 25 EC with copper oxy chloride 50 WP, zinc sulphate (0.5%) and dimethoate 30 EC revealed that no creaming matter and/or sediment formation was observed in any of the combinations *viz*., carbosulfan 25 EC @ 250 g a.i.ha^{-1} + copper oxy chloride 50 WP @ 500 g a.i.ha^{-1}, carbosulfan 25 EC @ 250 g a.i.ha^{-1} + zinc sulphate 0.5 per cent, carbosulfan 25 EC @ 250 g a.i.ha^{-1} + dimethoate 30 EC @ 300 g a.i.ha^{-1}. The results indicated that carbosulfan at the optimum and effective dose was compatible with other agrochemicals used in the present study.

Evaluation of carbosulfan 25 EC for phytotoxicity on brinjal in the compatibility study: The results of the investigation on the compatibility of carbosulfan 25 EC with copper oxy chloride 50 WP, zinc sulphate (0.5%) and dimethoate 30 EC as tank mix foliar application on brinjal were furnished in the table 2. The observations showed that none of the combination treatments *ie.* carbosulfan 25 EC @ 250 g a.i.ha^{-1} + copper oxy chloride 50 WP @ 500 g a.i.ha^{-1}, carbosulfan 25 EC @ 250 g a.i.ha^{-1} + zinc sulphate (0.5 %), carbosulfan 25 EC @ 250 g a.i.ha^{-1} + dimethoate 30 EC @ 300 g a.i.ha^{-1} caused any phytotoxic effect and were not differed symptomatically from control. During the entire period of observations the mean grade of '1' (0-10% injury) was awarded for all the treatment imposed plants. Hence it was concluded that the combination of carbosulfan with other fungicide, micronutrient and insecticide did not inflict any phytotoxic effect on brinjal.

Evaluation of carbosulfan 25 EC in combination with other chemicals on the bioefficacy of brinjal against shoot and fruit borer, on shoot damage: Two sprays of carbosulfan with its recommended, double and triple the dose (250, 500 and 1000 g a.i.ha^{-1}) and the combinations were given and the shoot and shoot damage percent was worked out. The results of the evaluation of carbosulfan 25 EC in combination with other chemicals on the bioefficacy of brinjal against shoot and fruit borer, *L. orbonalis* on shoot damage after first spray revealed that a maximum of 81.58 per cent reduction in shoot damage was noticed in

Table 1. Effect of carbosulfan 25 EC in combination with other chemicals on shoot damage by shoot and fruit borer, *Leucinodes orbonalis* in brinjal ecosystem (Mean of three replications)

Treatments	Per cent Shoot Damage														
	Days after first application								Days after second application						
	PTC	1	3	7	10	14	Mean	% Redn	1	3	7	10	14	Mean	% Redn
Carbosulfan 25 EC @ 250 g a.i.ha^{-1}	6.33	0.83^{a} (1.15)	0.57^{a} (1.03)	1.27^{ab} (1.31)	1.87a^{b} (1.52)	2.37^{ab} (1.68)	1.38	81.47	0.60^{a} (1.05)	0.47^{a} (0.98)	0.50^{a} (1.00)	0.93^{a} (1.19)	1.23^{a} (1.31)	2.53	91.46
Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + Copper oxy chloride @ 500 g a.i ha^{-1}	6.13	1.47^{b} (1.40)	1.43^{c} (1.39)	1.57^{bc} (1.44)	1.90^{d} (1.55)	2.17^{a} (1.63)	1.71	77.10	1.30^{b} (1.34)	1.23^{b} (1.31)	1.50^{ab} (1.39)	1.97^{c} (1.51)	2.20^{c} (1.59)	0.79	81.24
Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + Zinc sulphate - 0.5%	5.80	1.83^{c} (1.49)	1.60^{c} (1.37)	2.23^{c} (1.57)	2.77^{c} (1.80)	2.93^{b} (1.81)	2.05	72.49	1.97^{c} (1.56)	1.83^{b} (1.51)	2.13^{c} (1.61)	2.73^{d} (1.79)	3.03^{d} (1.87)	2.43	73.25
Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + Dimethoate 30 EC @ 300 g a.i.ha^{-1}	7.27	0.80^{a} (1.14)	0.47^{a} (0.97)	1.23^{a}b (1.28)	1.90^{ab} (1.55)	2.47^{b} (1.68)	1.37	81.58	0.70^{a} (1.07)	0.47^{a} (0.98)	0.79^{a} (1.09)	0.93^{a} (1.18)	1.10^{a} (1.25)	0.80	90.87
Copper oxy chloride @ 500 g a.i ha^{-1}	6.60	5.03^{e} (2.35)	4.77^{e} (2.29)	5.23^{d} (2.39)	5.40^{d} (2.43)	6.27^{d} (2.60)	2.78	62.72	1.90^{d} (1.55)	2.10^{d} (1.61)	2.60^{e} (1.76)	2.90^{e} (1.84)	3.13^{d} (1.91)	2.34	71.10
Zinc sulphate- 0.5%	7.40	2.20^{d} (1.64)	2.00^{d} (1.58)	2.50^{c} (1.73)	2.90^{c} (1.84)	3.37^{c} (1.97)	2.59	65.28	1.97^{d} (1.49)	1.90^{c} (1.46)	2.27^{d} (1.60)	2.70^{e} (1.74)	3.33^{d} (1.92)	2.43	72.15
Dimethoate EC @ 300 g a.i.ha^{-1}	7.07	1.43^{b} (1.39)	1.33^{c} (1.35)	1.53^{b} (1.43)	1.90^{ab} (1.55)	2.07^{a} (1.60)	1.65	77.86	1.57^{c} (1.44)	1.23^{b} (1.30)	1.33^{b} (1.34)	1.37^{b} (1.35)	1.63^{b} (1.43)	1.43	83.68
Carbosulfan 25 EC @ 500 g a.i.ha^{-1}	7.38	1.10^{ab} (1.25)	1.03^{b} (1.22)	1.27^{ab} (1.30)	1.90^{ab} (1.55)	1.93^{a} (1.50)	1.43	81.15	1.03^{ab} (1.24)	0.87^{b} (1.17)	1.27^{b} (1.32)	1.53^{b} (1.42)	1.87^{b} (1.53)	1.314	84.97
Carbosulfan 25 EC @ 1000 g a.i.ha^{-1}	7.53	0.97^{ab} (1.21)	0.87^{b} (1.15)	1.10^{a} (1.25)	1.70^{a} (1.41)	2.10^{a} (1.61)	1.52	81.39	0.77^{a} (1.12)	0.53^{a} (1.01)	0.63^{a} (1.06)	0.93^{a} (1.19)	1.10^{a} (1.26)	0.79	90.94
Untreated control	6.80	6.97^{f} (2.72)	7.20^{f} (2.77)	7.63^{e} (2.84)	7.43^{e} (2.81)	8.07^{e} (2.92)	7.46		8.20^{e} (2.94)	8.43^{e} (2.98)	8.80^{e} (3.04)	9.17^{f} (3.10)	9.10^{e} (3.09)	8.74	-

PTC – Pre - treatment count; Figures in parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by a common letter(s) are not significantly different by DMRT (p=0.05)

carbosulfan 25 EC @ 250 g a.i.ha^{-1} and dimethoate 30 EC @ 300 g a.i.ha^{-1} combination, followed by recommended, four times and double the dosage of carbosulfan recording 81.47, 81.39 and 81.15 per cent damage, which are on par. The treatments without insecticides *ie.* fertilizers and fungicides alone marked the lowest percent damage of 65.28 and 62.72 per cent damage. After the second spray, the highest per cent reduction was noticed for the recommended dose of carbosulfan 25 EC @ 250 g a.i.ha^{-1} displaying 91.46 per cent followed by four times the dose of carbosulfan (1000 g a.i.ha$^{-1)}$ and the insecticides combination (carbosulfan 25 EC @ 250 g a.i.ha^{-1} and dimethoate 30 EC @ 300 g a.i.ha^{-1}) portraying 90.94 and 90.87 per cent reduction (Table 1). Two times the dose of carbosulfan (500 g a.i.ha^{-1}), the insecticide, dimethoate EC @ 300 g a.i.ha^{-1} and the combination of carbosulfan and fungicide (carbosulfan 25 EC @ 250 g a.i.ha^{-1} + copper oxy chloride @ 500 g a.i ha^{-1}) offered 84.97, 83.68 and 81.24 per cent reduction in shoot damage and were

Table 2. Effect of carbosulfan 25 EC in combination with other chemicals on fruit damage by shoot and fruit borer *Leucinodes orbonalis* in brinjal ecosystem (Mean of three replications)

Treatments	Per cent Fruit Damage															
	Days after first application								Days after second application							
	PTC	1	3	7	10	14	Mean	% Redn	PTC	1	3	7	10	14	Mean	% Redn
Carbosulfan 25 EC @ 250 g a.i.ha-1	16.33	1.37^{a} (1.37)	1.30^{a} (1.34)	1.50^{a} (1.41)	1.73^{ab} (1.49)	1.97^{a} (1.57)	1.57	77.18	4.70	1.30^{b} (1.34)	1.17^{b} (1.29)	1.40^{b} (1.38)	1.60^{a} (1.45)	1.97^{ab} (1.57)	1.49	90.96
Carbosulfan 25 EC @ 250 g a.i.ha-1 + Copper oxy chloride @ 500 g a.i ha-1	17.27	2.10^{c} (1.56)	2.03^{c} (1.54)	2.47^{b} (1.68)	2.93^{c} (1.82)	3.20^{c} (1.89)	2.55	63.09	6.07	2.73^{c} (1.80)	2.57^{c} (1.75)	2.97^{c} (1.86)	3.73^{c} (2.06)	3.97^{d} (2.11)	3.19	64.06
Carbosulfan 25 EC @ 250 g a.i.ha-1 + Zinc sulphate - 0.5%	16.83	2.03^{c} (1.59)	1.97^{c} (1.57)	2.23^{c} (1.65)	2.90^{c} (1.84)	3.03^{c} (1.88)	2.43	64.74	4.67	2.67^{c} (1.78)	2.37^{c} (1.67)	2.43^{c} (1.71)	2.77^{bc} (1.79)	2.90^{c} (1.84)	2.63	61.87
Carbosulfan 25 EC @ 250 g a.i.ha-1 + Dimethoate 30 EC @ 300 g a.i.ha-1	17.07	1.43^{ab} (1.39)	1.33^{a} (1.35)	1.53^{a} (1.43)	1.90^{b} (1.55)	2.07^{b} (1.60)	1.65	76.05	7.73	0.70^{a} (1.09)	0.57^{a} (1.03)	0.63^{a} (1.06)	1.60^{a} (1.44)	1.83^{ab} (1.53)	1.07	88.00
Copper oxy chloride @ 500 g a.i ha-1	16.07	2.73^{c} (1.80)	2.57^{d} (1.75)	2.97^{c} (1.86)	3.73^{d} (2.06)	3.97^{c} (2.11)	3.19	53.75	8.07	4.07^{d} (2.13)	3.87^{d} (2.09)	3.93^{c} (2.10)	4.60^{d} (2.26)	6.60^{f} (2.66)	4.61	48.08
Zinc sulphate- 0.5%	17.97	2.20^{d} (1.64)	2.23^{d} (1.65)	3.63^{d} (2.03)	4.87^{d} (2.32)	5.40^{d} (2.43)	3.67	46.85	6.37	4.27^{d} (2.18)	4.17^{d} (2.16)	5.10^{d} (1.37)	6.80^{e} (2.70)	9.23^{e} (3.12)	5.91	33.45
Dimethoate EC @ 300 g a.i.ha-1	16.78	1.73^{b} (1.48)	1.57^{bc} (1.43)	2.03^{ab} (1.51)	2.57^{c} (1.70)	2.93^{b} (1.81)	2.17	68.60	8.33	2.03^{b} (1.58)	1.90^{b} (1.54)	1.97^{b} (1.57)	2.27^{b} (1.65)	2.83^{c} (1.82)	2.20	75.24
Carbosulfan 25 EC @ 500 g a.i.ha-1	16.73	1.47^{ab} (1.40)	1.43^{bc} (1.39)	1.57^{b} (1.44)	1.90^{b} (1.55)	2.17^{b} (1.63)	1.71	75.24	4.93	1.30^{b} (1.34)	1.23^{b} (1.32)	1.47^{b} (1.40)	1.73^{ab} (1.49)	2.03^{b} (1.59)	1.55	82.53
Carbosulfan 25 EC @ 1000 g a.i.ha-1	15.42	1.17^{a} (1.28)	1.26^{a} (1.32)	1.37^{a} (1.34)	1.60^{a} (1.43)	1.87^{a} (1.53)	1.45	78.92	3.57	0.80^{a} (1.14)	0.67^{a} (1.08)	0.93^{a} (1.20)	1.03^{a} (1.24)	1.37^{a} (1.36)	0.96	89.20
Untreated control	16.43^{d} (2.63)	6.70^{e} (2.68)	6.87^{e} (2.71)	7.07^{e} (2.75)	7.03^{e} (2.74)	6.82^{e}	6.90		7.87	8.43^{e} (2.99)	8.67^{e} (3.03)	8.73^{e} (3.04)	9.10^{f} (3.10)	9.50^{g} (3.16)	8.89	

PTC – Pre treatment count; Figures in parentheses are $\sqrt{x+0.5}$ transformed values; In a column, means followed by a common letter(s) are not significantly different by DMRT (p=0.05)

on par. The lowest per cent reduction in shoot damage was noticed in fungicide and fertilizer treatments (71.10 and 72.15%).

Evaluation of carbosulfan 25 EC in combination with other chemicals on the bioefficacy of brinjal against shoot and fruit borer, on fruit damage: Two rounds of sprays of carbosulfan with its recommended, double and triple the dose (250, 500 and 1000 g a.i.ha^{-1}) and the combinations were given and the shoot and fruit damage percent was worked out. The results of the evaluation of carbosulfan 25 EC in combination with other chemicals on the bioefficacy of brinjal against shoot and fruit borer on fruit damage after first spray revealed that a maximum of 78.92 per cent reduction is noticed in four times the dose of carbosulfan (1000 g a.i.ha^{-1}), followed by the recommended dose of carbosulfan (250 g a.i.ha^{-1}) portraying 77.18 per cent, insecticides combination

Table 3. Effect of carbosulfan 25 EC in combination with other chemicals on the yield of brinjal

No	Treatments	kg plot^{-1}			Mean kg plot^{-1}	Mean t ha^{-1}
		I harvest	II harvest	III harvest		
T_1	Carbosulfan 25 EC @ 250 g a.i.ha^{-1}	52.2^{c}(7.26)	56.6^{a}(7.54)	54.0^{bc}(7.38)	54.2^{c}(7.39)	27.1
T_2	Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + Copper oxy chloride @ 500 g a.i ha^{-1}	57.2^{ab}(7.59)	56.0^{a}(7.51)	56.6^{ab}(7.57)	56.6^{ab}(7.55)	28.3
T_3	Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + Zinc sulphate - 0.5%	54.5^{abc}(7.41)	57.0^{a}(7.58)	55.2^{ab}(7.46)	55.6^{abc}(7.49)	27.8
T_4	Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + Dimethoate 30 EC @ 300 g a.i.ha^{-1}	57.5^{a}(7.61)	56.8^{a}(7.57)	58.0^{a}(7.64)	57.4^{a}(7.61)	28.7
T_5	Copper oxy chloride @ 500 g a.i ha^{-1}	47.3^{d}(6.91)	48.3^{b}(6.98)	49.0^{d}(7.03)	48.2^{e}(6.98)	24.1
T_6	Zinc sulphate- 0.5%	48.5^{d}(6.99)	49.0^{b}(7.03)	48.0^{d}(6.96)	48.5^{e}(6.99)	24.2
T_7	Dimethoate 30 EC @ 300 g a.i.ha^{-1}	51.5^{c}(7.21)	50.0^{b}(7.10)	52.0^{c}(7.24)	51.2^{d}(7.18)	25.6
T_8	Carbosulfan 25 EC @ 500 g a.i.ha^{1}	54.3^{bc}(7.41)	55.5^{a}(7.48)	56.0^{ab}(7.51)	55.2^{bc}(7.46)	27.6
T_9	Carbosulfan 25 EC @ 1000 g a.i.ha^{-1}	53.6^{c}(7.40)	55.2^{a}(7.46)	54.8^{abc}(7.43)	54.5^{c}(7.47)	27.3
T_{10}	Untreated control	32.0^{e}(5.70)	31.5^{c}(5.65)	33.0^{e}(5.78)	32.1^{f}(5.71)	16.05

Values are mean of four observations; In a column means followed by a common letter are not significantly different by DMRT (p = 0.05); Values in the parentheses are transformed values

(carbosulfan 25 EC @ 250 g a.i.ha^{-1} + dimethoate 30 EC @ 300 g a.i.ha^{-1}) displayimg 76.05 per cent and double the dose of carbosulfan (500 g a.i.ha^{-1}) with 75.24 per cent reduction in fruit damage. The treatments with fertilizer and fungicides alone represented the highest damage with 46.85 and 53.75 per cent reduction. After the second spray the highest per cent reduction was noticed for the recommended dose of carbosulfan 25 EC @ 250 g a.i.ha^{-1} displaying 90.96 per cent followed by four times the dose of carbosulfan (1000 g a.i.ha^{-1}) with 89.2 per cent reduction and the insecticides combination (carbosulfan 25 EC @ 250 g a.i.ha^{-1} and dimethoate 30 EC @ 300 g a.i.ha^{-1}) portraying 88 per cent reduction in fruit damage. Two times the dose of carbosulfan (500 g a.i.ha^{-1}), the insecticide and dimethoate EC @ 300 g a.i.ha^{-1} offered 82.53 and 75.24 per cent reduction in shoot damage and were on par. The combination of carbosulfan and fungicide (carbosulfan 25 EC @ 250 g a.i.ha^{-1} + copper oxy chloride @ 500 g a.i ha$^{-1)}$ and combination of carbosulfan and fertilizer posed (carbosulfan 25 EC @ 250 g a.i.ha^{-1} + zinc sulphate - 0.5%) 64.06 and 61.87 per cent reduction in fruit damage respectively (Table 2). The lowest per cent reduction in fruit damage was noticed in zn sulphate and copper oxy chloride treatments (33.45 and 48.08% respectively) (Table 2).

Evaluation of carbosulfan 25 EC in combination with other chemicals on the yield of brinjal: The field experiment conducted to assess the yield of brinjal by tank mix application of carbosulfan 25 EC with copper oxy chloride 50 WP, zinc sulphate (0.5%) and endosulfan 35 EC showed that all the combination treatments registered higher yields than these chemicals when used alone. The yield of brinjal ranged from 24.1 to 28.7 t ha^{-1} in different treatments (Table 3). Carbosulfan 25 EC @ 250 g a.i.ha^{-1} + dimethoate 30 EC @ 300 g a.i.ha^{-1} recorded maximum yield of 28.7 t ha^{-1} followed by carbosulfan 25 EC @ 250 g a.i.ha^{-1} + copper oxy chloride 50 WP @ 500 g a.i.ha^{-1} (28.3 t ha^{-1}) followed by carbosulfan 25 EC @ 250 g a.i.ha^{-1} + zinc sulphate 0.5 per cent (27.8 t ha^{-1}) which were on par with each other. The different doses of

carbosulfan 25 EC alone *ie*. 250, 500, 1000 g a.i.ha^{-1} registered 27.1, 27.6 and 27.3 t ha^{-1} which were on par with each other, of which carbosulfan 25 EC @ 500 g a.i.ha^{-1} was on par with the combination treatments (Table 3).

DISCUSSION

The results of the physical compatibility test showed that the carbosulfan in combination with other agro chemicals like copper oxy chloride, zinc sulphate, dimethoate in standard hard water did not produce any sediment and/or creamy matter which showed that the chemical is compatible with other agrochemicals tested. Concordant results were obtained by Judge and Natti (1972) that carbofuran + captan were compatible and not showed any adverse effect on each other, which was again supported by Padmanaban (1980) who obtained concordant results that no creaming matter at the top when carbaryl, endosulfan or monocrotphos was mixed with urea. Similar findings were also reported by Morale *et al.* (1991) that five per cent carbosulfan was compatible with fungicides like captan, captafol and thiram. This was again in agreement with the findings of Paul Mohan Roy (1988) who stated that the addition of mancozeb and/or urea did not produce any creaming or sedimentation with fenvalerate, cypermethrin, deltamethrin and methyl-o-demeton. In the field, the combination treatments did not cause any phytotoxic effects on brinjal and awarded with grade '1' (0-10% injury). This result was in accordance with Morale *et al.* (1991) who reported that five per cent carbosulfan with fungicides like captan, captafal and thiram as seed treatment did not have any adverse effect on the germination of sorghum seeds. Similarly, Poe and Jones (1972) also arrived the same results that carbaryl, methomyl, parathion and dimethoate in combination was compatible and did not produce any adverse effect on tomato crop. Similar reports were also reported by Judge and Natti (1972) in carbofuran + captan combination. In another study by Maduri *et al.* (2021) the physical compatibility of 18 combinations involving 6 insecticides (clothianidin 50 WDG @ 0.1g/l, acetamiprid 20 SP@ 0.2 g/l, flonicamid 50WG@0.2 g/l, buprofezin 25SC @1.5 ml/l, novaluron 10EC @1ml/l and dimethoate 30EC@1.7 ml/l) and 3 fungicides (propiconazole 25EC @ 1ml/l, carbendizm 50 WP @1g/l and carbendizm 12% + mancozeb 63% WP @2G/l) were evaluated. All 18 combinations of insecticides and fungicides tested were physically compatible. The combinations (*viz.,* novaluron + carbendazim, acetamiprid + carbendazim, acetamiprid + carbendazim 12% + mancozeb 63% andbuprofezin + carbendazim 12% + mancozeb 63%) showed phytotoxic symptoms like vein clearing and scorching onleaves, remaining all other treatment combinations were compatible.

The bioefficacy studies revealed that carbosulfan treatments alone and in combinations significantly reduced the shoot and fruit damage caused by shoot and fruit borer, *L. orbonalis*. The per cent reduction in shoot and fruit damage was maximum for the recommended and four times the dose of carbosulfan (250 and 1000 g a.i.ha^{-1})and combination treatment of insecticides (carbosulfan 25 EC at 250 g a.i.ha^{-1} + dimethoate 30 EC at 300 g a.i.ha^{-1}). Two times the dose of carbosulfan (carbosulfan at 500 g a.i.ha^{-1}) and the insecticide (dimethoate 30 EC at 300 g a.i.ha^{-1}) trails its efficacy in managing the shoot and fruit damage by shoot and fruit borer of brinjal. The results are in conformity with Mahla *et al.* (2017) who stated that carbosulfan 25 EC @1500 ml/ha, followed by 1250 ml/ha, reduced the damage of shoot and fruit borer considerably with high marketable yield, without any phytotoxic effects on brinjal crop. The results are in agreement with Misra (2008) that carbosulfan 25 EC @ 500 g a.i.ha^{-1} led to 84.73 and 71.93 per cent reduction in shoot damage and 76.94 and 77.31 per cent fruit damage. The present results fall in line with Roy *et al.* (2016) who observed a reduced infestation of shoot and fruit borer after application of carbosulfan 25 EC@375g. ai/ha yielding 9.23 q/ha. Much studies are not conducted in combination of chemicals, especially with carbosulfan to compare the current results.

The harvest of healthy brinjal fruits was found to be maximum from the combination treatments than the treatment with individual chemicals, which ranged from 27.8 to 28.7 t ha^{-1}. Such an observation

has been reported by Morale *et al*. (1991) that the combination of carbosulfan with fungicides like captan, captafal and thiram recorded maximum grain yield of sorghum. Concordant results were also reported by Srinivasan *et al.* (1986) that the carbamate insecticide BPMC (0.025%) or carbaryl (0.075%) applied with chitin inhibitor SIR 8514 (0.0325%) recorded a higher yield of brinjal fruit of 19.97 t ha^{-1} and 18.5 t ha^{-1} respectively which coincides with the findings of Sathyanarayana Moorthy *et al*. (1988) when insecticides like monocrotophos, quinalphos, chlorpyriphos and carbaryl were combined with neem oil in rice crop. Similar results were also obtained by Peter *et al.* (1989), Rao *et al.* (1995), Singh and Tripathi (1996) and Pawar and Mali (1997).

Carbosulfan at 250 g a.i.ha^{-1} was found to be compatible with the recommended doses of copper oxy chloride 50 WP, zinc sulphate (0.5%) and dimethoate 30 EC in the emulsion stability test, as there was no creaming up at the top layer and/or sediments at the bottom. Carbosulfan at 250 g a.i.ha^{-1} when sprayed as tank mix to brinjal crop, in combination with copper oxy chloride 50 WP, zinc sulphate (0.5%) and dimethoate 30 EC did not exhibit any phytotoxic symptoms in the field. The bioefficacy of carbosulfan with its combinations against the shoot and fruit damage of brinjal shoot and fruit borer revealed a positive effect with carbosulfan at recommended, double and four times the dosage performing the best efficacy. The bioefficacy of combinations revealed that, the insecticides combination (carbosulfan + dimethoate) portrayed a similar better performance, but combination of carbosulfan with fungicide and fertilizer, showed a neutral effect. The treatments with fungicides and fertilizer alone, displayed a very minimal effect in managing the damage due to shoot and fruit borer. The combination of carbosulfan 25 EC at 250 g a.i.ha^{-1} + dimethoate 30 EC at 300 g a.i.ha^{-1} recorded maximum yield of 28.7 t ha^{-1} followed by carbosulfan 25 EC at 250 g a.i.ha^{-1} + copper oxy chloride 50 WP at 500 g a.i.ha^{-1} (28.3 t ha^{-1}) and carbosulfan 25 EC at 250 g a.i.ha^{-1} + zinc sulphate (27.8 t ha^{-1}). All the combinations (carbosulfan + dimethoate, carbosulfan + copper oxy chloride, carbosulfan + zinc sulphate) at the recommended doses exhibited additive effect.

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Studies on influence of various stages of mulberry leaf in the growth and cocoonic parameters of silkworm *Bombyx mori* (L.)

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ABSTRACT: The objective of this work was to determine the effects of different stages of mulberry leaves on growth performance of treated larvae of *Bombyx mori* (L.) (Lepidoptera, Bombycidae) and characters of the resulted cocoons were evaluated. Results showed that the food consumption rate and assimilation rate were greatest for larvae fed on tender and over matured mulberry leaves as compared to larvae fed on mature leaves. Assimilation and conversion efficiencies were very high in the larvae grown on matured mulberry leaves (0.189 and 1.99% respectively). Moreover, the larvae fed on matured mulberry leaves showed higher growth rate, cocoon weight and shell ratio. Among the three different growth stages of leaves, the matured leaves were found to be the best food for final instar larvae of *Bombyx mori* L. which promotes maximum larval growth and it gains to quality cocoon and raw silk production.
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KEY WORDS: Larval growth, assimilation, shell ratio, cocoon characters

INTRODUCTION

Sericulture is an industrial trade that involves the cultivation of mulberry plant species, the raising of silkworms, and the manufacture of silk. It is a sustainable, environment friendly and ago-forestry focused trade, because it is integration of mulberry plants and silkworm farming system. It begins with the cultivation of mulberry trees and ends with the raising of silkworms on mulberry leaves to generate cocoons. It is difficult to transport mulberry leaves across vast distances or keep them for lengthy periods of time since they must be fresh. As a result, silkworm raising and mulberry tree farming is now essentially one and the same business (Rama Kant *et al*., 2004).The mulberry silkworm *Bombyx mori* (L.) produces the majority of commercial silk in the world (Yogananda Murthy *et al*., 2013). Apart from environmental issues, the amount and quality of mulberry leaf also have a major impact on silk yield. The nutritional content of the mulberry host leaves, which seems to be a decisive factor of silk quality, has a significant impact on silkworm growth (Chauhan and Tayal, 2017). Silkworm, *B. mori* is a poikilothermic insect that is the primary source of silk manufacturing. Nutritional parameters have a strong influence on silkworm food consumption and growth. Silkworm feeding efficiency iscrucial in the

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conversion of mulberry leaves to silk (Anantha Raman *et al*., 1994).

Several researchers have analysed the quality of mulberry varieties through feeding experiments and reported that MR2 is the best variety in which cocoon weight and silk weight were maximum (Etebari and Matindoost, 2005). The monophagous mulberry silkworm exclusively consumes mulberry leaves, and its growth and development are solely dependent on the nutrients found in those leaves (Shilpi and Praban, 2020).The major goal of silkworm breeding is to enhance the profit for silk producers and other sericultural industries by improving commercially valued economic features including cocoon weight (CW), cocoon shell weight (CSW), cocoon shell percentage (CSP) and shell ratio (SR) (Pouya Zamani *et al*., 2019). Mulberry trees are cultivated in a variety of climates;therefore, leaf quality has a big impact on their growth and development (Rukmangada *et al*., 2019). Mulberry leaves provide silkworms with all of the nutrients and water they require, as a result of massive co-evolution and selective breeding between silkworms and mulberry trees. Proteins, carbohydrates, lipids, inorganic matter, moisture, and vitamin A are the key nutrients in mulberry leaves that silkworms may consume (Fanchi Li *et al*., 2016). Supplemental nutrient enrichment of mulberry leaves is one of the alternate methods of improving larval development and cocoon formation (Thangapandiyan and Dharanipriya, 2019). Masoud *et al*. (2020) found that the leaves of the Kenmochi tree promoted good silkworm growth and development, as evidenced by superior performance qualities. *B. mori* strains reared on Jorhat and TR10 mulberry plant types showed the higher levels of fibrous protein, calcium, potassium, magnesium, and phosphorus than those grown on other mulberry types, resulting in higher silk output and the consumption rate (Lalfelpuii *et al*., 2019). Food conversion efficiency influences the cost effectiveness of silkworm rearing directly or indirectly, and it is regarded as a significant physiological parameter for comparing silkworm breeds (Rahmathulla *et al*., 2005). Slow progress and a longer larval lifespan occurred from restricted feeding period. With the rise in feeding length, absorption, assimilation, conversion, and metabolism, as well as their levels, exhibited a gradual rise (Mathavan *et al*., 1987). In this investigation, an attempt has been made to find out the effect of food quality silk production of the final instar larvae of *B. mori* on the basis of feed, which is focused mainly on different growth stages of mulberry leaves as nutritional intake of growth.

MATERIALS AND METHODS

The study region was located in Nagercoil, southern region of the state of Tamilnadu, India at 8º 10' N; 77º 25' E, with an average altitude of 157 ft. The summer season is from March to May, followed by the monsoon season from June to mid of September, and the winter season from mid of September to February. The highest temperature recorded in Nagercoil was 34.5ºC, and winter temperatures as low as 23ºC have been recorded. The average annual rainfall received is 985 mm. CSR2 × TN hybrid variety of the silkworm was used in the present investigation, which is a hybridwell-known for its excellent survival, yield, silk ratio, and capacity to produce high-quality bivoltine silk that meets international standards which suits best to the climatic conditions of the study area (Nihal Nila and Stevens Jones, 2021).

The final instar larvae of *B. mori* were obtained from the industrial sericulture training centre at Konam, Nagercoil, India, reared under standard environmental conditions of 28°C, 85 per cent RH (Krishnaswami, 1986) and three types of MR2 variety mulberry leaves were given as food. MR2 is identified as a one of the best mulberry varieties in this area mentioned in the literatures. Therefore, in this analysis, different mulberry leaf stages of MR2 are identified based on the agro-climatic conditions of the study region, to evaluate the cocoon production efficiency and the influence of food, nutritional qualities and silk production of silkworm *B. mori*.

Freshly moulted final instar larvae of *B. mori* were divided into three separate groups containing 50 larvae each. The volume of the terraria (surrounding area of the larvae of caterpillar) as 6.5 × 2.1cm was kept constant for all the groups.

The worms in group I were fed with freshly matured mulberry leaves.

The worms in group II were fed with tender leaves and the

The worms in group III were fed with over matured leaves respectively.

Silkworms were given known quantities of fresh, high-quality mulberry leaves five times, as per the bivoltine rearing package's standard recommendation (Chinnaswamy Ramesha *et al*., 2012). The food given each day was weighed and recorded. Faeces and the unfed leaves were collected every day and oven-dried at 90°C to get the constant weight.

Determination of food utilization and nutritional indices

Healthy larvae were measured daily in each replica of each intervention, and uneven, unwell, or dead larvae were removed and replaced from different batches as discovered. After oven drying at a constant temperature of 80°C, the dry weight of residual leaf, excreta, and larvae weight were measured daily. Mounting is the final stage of the rearing process. Mountage is the most significant tool that assists silkworm larvae in spinning their cocoon comfortably. Larvae were replicated and treated individually in plastic, foldable cocoon–making frames .It determines both the quality and quantity of the cocoons. The rectangular- shaped mountage is made from plastic supported by plastic reapers on all sides and a corrugated form with eleven peaks and 0.9m width of plastic mountage is used for this analysis. After the 6th day of mounting, the cocoons were collected and assessed. The weights of larva, cocoon, shell, and filament were measured using electronic balance. The following are the formulas for calculating various nutritional metrics (Rahmathulla and Suresh, 2012; Ayandokun and Alamu, 2020).

Food Utilization: The scheme of energy budget followed in this present study is the slightly modified IBP formula (Petrusewicz and Macfadyen, 1970) usually represented as -

C = P+R+F+U

Where, "C" is refereed as consumption, "P" is refereed as production, "R" is referred as energy loss via respiration and "F" is refereed as faeces excretion products and the "U" is referred as nitrogenous excretion products.

The quantity of uric acid in the faeces is negligible in insects (Roy and Kvenberg, 1981). In the present work, the faeces (F) therefore represent the undigested food as well as nitrogenous excretory wastes. "P" was estimated by subtracting the initial dry weight of the larva from the terminal weight at the end of the experiment. Food assimilated was calculated by subtracting the faeces weight from the food consumed. The metabolic loss of energy was found out by subtracting the food converted from the food assimilated. Rate of feeding, assimilation, conversion and metabolism were calculated at the experimentation.

Feeding rate: Amount of food consumed per unit body weight of larvae per unit time (g live body wt/ day).

Feeding rate = [Total food consumed (Joules/ Larvae) / Mid body weight (g) × Instar duration (days)] × 1000

Assimilation rate: Amount of food assimilated per unit weight of larvae per unit time (g live body wt/ day).

Assimilation rate = [Total food assimilated (Joules/ Larvae) / Mid body weight (g) × Instar duration (days)] × 1000

Conversion rate: The food converted per unit weight of larvae per unit time (g live body wt/day).

Conversion rate = [Total food converted (Joules/ Larvae) / Mid body weight (g) × Instar duration (days)] × 1000

Metabolic rate: It is difference between the assimilation rate and conversion rate.

Metabolic rate = Assimilation rate × Conversion rate

Assimilation efficiency: The percentage of food energy assimilated in relation to the food energy consumed.

Assimilation efficiency = [Total food assimilated (Joules/ Larvae) / Total food consumed (Joules/ Larvae)] × 100

Conversion efficiency: Percentage of food converted in relation to food consumed is known as gross conversion efficiency. Net conversion efficiency represents the percentage of food converted in relation to assimilated food.

Gross conversion efficiency = [Total food converted (Joules/ Larvae) / Total food consumed (Joules/ Larvae)] × 100

Net conversion efficiency = Total food converted (Joules/ Larvae) / Total food assimilated (Joules/ Larvae)] × 100

Shell ratio: The amount of silk that may be generated from each cocoon is determined by the weight of the shell. As a result, calculating the shell ratio is essential.The cocoon weight includes the weight of shell and weight of pupa inside (Anantha Raman and Magadum, 1994).

Shell ratio = [Weight of cocoon shell / Weight of cocoon] × 100

Using a commercially available statistical software tool (SPSS® for Windows, V. 16.0, Chicago, USA), data were analysed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The results were given as means ± SD, with $P < 0.05$ considered statistically significant (Masoud *et al*., 2020).

RESULTS AND DISCUSSION

The focus of this research was to determine the effects of various stages of mulberry leaf feed on the growth and characteristics of the final instar larvae of *B. mori*. Several studies have shown that silkworms seem to be more receptive to nutrition supplements during the 4th and 5th stages, which is important for identifying and selecting nutritionally effective silkworm varieties for commercial reasons. As a result, the nutrient usage research was limited to the 5th instar larvae, as this period of silkworm growth saw 80-85 per cent of total leaf consumption (Chinnaswamy *et al*., 2012), which is used in this investigation.

The conversion is greater (0.055± 0.03221g) in mature mulberry fed larvae when compared to the worms fed on tender (0.038± 0.202221 g) and very matured (0.045± 0.03671 g) leaves. Growth is higher in larvae fed with matured leaves of mulberry when compared to other. This shows that the matured leaves are rich in food constituents that promote the growth of the larvae (Table 1). According to Chinnaswamy *et al*. (2012), mulberry leaves are high in protein, carbohydrates, vitamins, sterols, phago-stimulants and minerals all of which aid silkworm larvae in their growth and development. The much-reduced larval growth seen in worms fed solely tender and over matured leaves might be owing to all of the abovementioned nutrients being lower than that in matured leaves, affecting development. When silkworms were fed on mulberry variety on matured leaves or tender or over matured leaves, and when feeding was switched from one mulberry variety to the other, the single pupal weight did not change considerably. This is likely due to the fact that the growth-development effects of protein and vitamins obtained from mulberry leaves, which might impact economic parameters such as pupal weight and cocoon weight, were likely similar in the three mulberry plant kinds studied. There was a favourable association among silkworm larval growth and cocoon production, showing that increasing larval growth will boost silk gland production, which will result in improved cocoon production (Pouya Zamani *et al*., 2019). Mathavan *et al*. (1987) and Hideshi *et al*. (1982) found that leaf ingestion had a direct impact on silkworm body weight and, as a result, silk-producing capacity. Similar findings were observed in the current investigation – silkworm fed on matured leaves had a fast growth rate owing to the influence of high nutritional content.

Insects' growth may be slowed not only by a lack of nutrients in their food, but also by the impact of

Table 1. Growth of fifth Instar *B. mori* fed on various types of mulberry leaves

Type	Initial dry weight (g)	Final dry weight (g)	Growth (g)
Mature	0.062± 0.03235^{a}	0.117± 0.04221^{b}	0.055± 0.03221^{ab}
Tender	0.063± 0.01046^{b}	0.101± 0.032231^{c}	0.038± 0.202221^{b}
Over Mature	0.0625± 0.04215^{a}	0.106± 0.02321^{c}	0.045± 0.03671^{a}

Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)

Table 2. Food consumption, assimilation and conversion of fifth Instar *B.mori* fed on various types of mulberry leaves

Types	Consumption (g)	Assimilation (g)	Conversion (g)
Mature	2.7586± 0.13425^{b}	0.5214± 0.12235^{a}	0.055± 0.13109^{ab}
Tender	6.6462± 0.21235^{a}	4.0162± 0.16235^{c}	0.038± 0.19687^{a}
Over Mature	5.6092± 0.16285^{b}	1.9492± 0.21025^{a}	0.0435± 0.18615^{a}

Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)

Table 3. Feeding rate, assimilation rate, conversion rate and metabolic rate of fifth Instar *B.mori* fed on different types of mulberry leaves

Types	Feeding*	Assimilation*	Conversion*	Metabolic*
Mature	3092.6± 0.19316^{a}	584.52± 0.18460^{b}	6.16± 0.19225^{a}	0.4664± 0.18925^{a}
Tender	8144.85± 0.21435^{c}	4921.81± 0.19421^{a}	4.65± 0.18315^{a}	3.978± 0.19425^{a}
Over Mature	6693.55± 0.17425^{b}	2326± 0.16215^{b}	5.19± 0.18405^{c}	1.906± 0.17365^{b}

**In g live body weight/day; Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)*

Table 4. Assimilation (%) and conversion efficiency (%) of fifth Instar *B. mori* fed on different types of mulberry leaves

Type	Type	Conversion	
		Gross	Net
Mature	Mature	1.99± 0.13478^{ab}	10.54± 0.13969^{ab}
Tender	Tender	0.57± 0.16823^{b}	0.94± 0.16215^{b}
Over Mature	Over Mature	0.78± 0.14622^{a}	2.25± 0.15102^{a}

Values are mean ± SD of ten observations. Values in the same column with different super script letters (a, b, and c) differs significantly at p < 0.05 (DMRT)

other environmental factors. Growth is also influenced by food consumption and assimilation, which differs across species. Furthermore, in the bivoltine race, a reduction in feeding time at the most active feeding stage (5th instar) resulted in a shorter larval duration, which negatively influenced larval weight and other connected features (Rahmathulla and Suresh, 2012). Consumption and assimilation are separate characteristics that are affected by the type of diet used and the silkworm breeds used. The amount of food digested, is influenced by a number of variables. Higher enzyme production, as seen by increased digestibility, protease, and lipase activity, corresponds to increased food consumption (Muniraju *et al*., 2004).

Consumption and assimilation of larvae fed with different types of mulberry leaves and the experimental values during the calculation of consumption, assimilation and conversion are shown in table 2. Food consumed and food assimilated are higher in larvae fed on over matured leaves (1.9492± 0.21025 g; 5.6092± 0.16285 g) and tender leaves (6.6462± 0.21235 g, 4.0162± 0.16235 g) than that on matured leaves (2.7586± 0.13425 g, 0.5214± 0.12235 g). Thus, food consumption and assimilation are greater when the larvae are fed on tender and over matured leaves. But growth is found to be greater when the larvae are fed with matured leaves. Thus, the type of food which is consumed in small amounts results in high growth. Conversion is greatest in larvae fed on matured leaves and recorded (0.055± 0.03221g) compared to (0.038± 0.202221 g and 0.045± 0.03671 g) for larvae fed on tender and over matured leaves. The food consumption, assimilation effective responses of mulberry silkworm, *B. mori* and its cocoon characteristics enhanced by feeding the last larval instar with mature mulberry leaves. This may be due to the nutritional supplements (Thangapandiyan and Dharanipriya, 2019). Since the matured mulberry leaves were fed to silkworm at the subsequent stage of growth and development, the food consumption and assimilation were found to be lower in the current study. In general, the current findings are consistent with those of previous researchers (Ahmad Nawaz *et al*., 2020; YangYang Li *et al*., 2016; Marilucia Santorum *et al*., 2020; Ming Lei *et al*., 2019).

Protein metabolism is a crucial biochemical process that aids in defining distinct phases of development, and the conversion of host plant nutrients into silk protein occurs mostly during the larval stages. Poor nutrition diets will have a direct impact on insects' basic biochemical and physiological metabolism, altering the detoxification system and increasing illness resistance (Lalfelpuii *et al*., 2019). Similarity rate of feeding and assimilation are high in larvae fed on mature and tender mulberry leaves but rate of conversion is high in mature leaves fed larvae. This corresponds to the high metabolic rate in larvae fed with over matured leaves and tender mulberry leaves (Table 3). Thus, it is strong evident that though the food consumption, assimilation and rate of feeding are high in larvae feed on over matured leaves and tender mulberry leaves, because of high of metabolic rate the conversion and the conversion rate are low in these larvae.

Assimilation and conversion efficiencies are very high in the larvae grown matured mulberry leaves (0.189± 0.12565%, 1.99± 0.13478%) when compared to that of larvae fed on tender mulberry leaves (0.005± 0.14231%, 0.57± 0.16823%) and over matured leaves (0.007± 0.14952%, 0.78± 0.14622%). The findings indicate the food which is assimilated and converted with high efficiency by organisms is proved to be the best food for the larvae (Table 4).

The nutritional efficiency of an insect may be considered a significant factor in a basic entomological situation. Nutritional efficiency, on the other hand, becomes a major concern in sericulture. The potential of various silkworm races and variants to digest, assimilate, and convert mulberry leaves to body substance, and then to the major commercial product, the cocoon, varies significantly. The conversion efficiency is influenced by numerous factors such as rearing procedures, rearing conditions, leaf quality, feeding proportion, and numerous compounds like as food additives, vitamins, antibiotics, and hormones (Rahmathulla and Suresh, 2012).

Silkworm growth, development, and production are all influenced by temperature. Evaluating the effect of temperature variations rather than constant-temperature rearing has been proven to be more effective in commercial silkworm rearing. Food consumption, production, and larval duration are all affected by the temperature combination (Rahmathulla, 2012). Variations in the amount of food consumed in a range of temperature combinations might explain variations in the amount of poor matter generated. During early instars, raising rearing temperature reduced food absorption and increased assimilation efficiency. Increased consumption may increase the pace at which food passes through the multivoltine silkworm larva's stomach, leaving less time for digestion and thereby lowering food assimilation efficiency (Stuart and Stephen, 1985). Despite modest food consumption, the conversion rate was higher at higher rearing temperatures. This finding suggests that larvae reared with little input can collect more nutrients by improving efficiency and feeding durations, allowing them to retain crucial levels of growth-limiting nutrients like nitrogen and water (Muniraju *et al.,* 2004).

Cocoon and shell weights are crucial production indicators, while shell ratio indicates the quantity of raw silk spun from cocoons, which varies depending on silkworm age and strain. It is identified that the shell ratio is high in the larvae fed on matured leaves (16.22± 0.21114%) and low in larvae fed on over matured leaves (11.29± 0.19112%) shown in Table 5. A higher value of Shell ratio was evident on the *B. mori* strains fed with the matured mulberry leaves. Feeding mulberry leaves enriched in potassium, magnesium, and calcium greatly enhanced the shell ratio,which has been revealed in larvae raised on matured mulberry leaves, which could be due to the high levels of amino acids, potassium, magnesium, and calcium in the leaves (Lalfelpuii *et al*., 2019). It is showed that the larvae fed on matured mulberry leaves have spun cocoons with highest shell ratios, which might be attributed

Table 5. Cocoon weight (g), shell weight (g)and shell ratio of fifth Instar *B. mori* fed on different types of mulberry leaves

Types	Cocoon	Shell without pupa	Shell Ratio
Mature	11.4± 0.21015^{c}	1.85± 0.21116^{c}	16.22± 0.21114^{c}
Tender	8.62± 0.20315^{c}	1.32± 0.19273^{c}	15.31± 0.19547^{c}
Over Mature	10.8± 0.19208^{c}	1.22± 0.18748^{c}	11.29± 0.19112^{c}

Values are mean ± SD of ten observations. Values in the same column with different super script letter (c) differs significantly at $p < 0.05$ (DMRT)

to high amino acid and carbohydrate levels.

Food utilization studies on *Riccinus communis* (Sujatha *et al*., 2014) and *B. mori* (Muniraju *et al*., 2004) support the facts from this present study: it is evident that the matured mulberry leaves is the best food for the final instar larvae of *B. mori* because of its high assimilation and conversion efficiencies. Moreover, the conversion and the rate of conversion are also high. The larvae fed on matured leaves shows high growth rate. Shell ratio and cocoon weight are high in the worms fed in matured mulberry leaves. This might be due to the presence of more amount of nutrients in their leaves. It is also found that amino acid presents in the food will plays an important role in growth of the larvae, cocoon weight and shell ratio.

In this study it is identified that the significant growth was observed on larvae fed on matured mulberry leaves as 0.055± 0.03221 g. Amount of food consumed and assimilated were high in larvae fed on tender (6.6462± 0.21235 g; 4.02± 0.16235 g) and over matured leaves (5.6± 0.16285 g; 1.95± 0.21025 g) of mulberry compared with matured mulberry leaves (2.75± 0.13425 g; 0.52± 0.12235 g), because of the nutritional supplements in proper availability aspect. The same results were reflected in rate of feeding and metabolism, which is higher in tender and over matured leaves of mulberry. It results that the poor nutrition diets will have a direct impact on the insect's basic biochemical and physiological metabolism. Since the matured mulberry leaves are high in nutritional qualities, the shell ratio, assimilation and conversion efficiencies are high in larvae fed on matured mulberry leaves. Thus the results reveal that the matured leaves are best food for final instar larvae of *B. mori* which promotes maximum possible growth and silk production.This information is vital for suitable management of healthy silkworms, improvement of silkworm strains and production of high quality cocoons with good commercial value.

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Age-specific ecological life table of *Spodoptera litura* (F.) (Lepidoptera, Noctuidae) on groundnut

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ABSTRACT: Experiments were conducted to study the life history traits of *Spodoptera litura* under controlled environmental conditions on groundnut host. To construct the age-specific fecundity life tables, adults emerged on the same day were caged for oviposition and the number of eggs laid on each day was recorded. The key mortality factors involved in each life stages were also accounted. Females contributed highest number of progeny (m_x = 346.12) on 39th day of pivotal age. The net reproductive potential (R_o) was 858.52 females/female/generation with the mean generation period (T_c) of 38.86 days. The life table analysis revealed that the late instar larvae were more vulnerable to natural mortality factors (64.95%) and total mortality per cent recorded was 83.02. The various key mortality factors *viz*., parasitoids (*Cotesia* sp., *Chelonus* sp. and Tachinids), virus (NPV), malformed pupa and adults were recorded from the field population of *S. litura* from groundnut ecosystem.
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KEY WORDS: Vital statistics, net reproductive rate, intrinsic rate of increase, mortality factors

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual, herbaceous legume and important oil seed crop. Tobacco caterpillar, *Spodoptera litura* (Fab.) is one of the important pests, infesting more than 290 species of plants belonging to 99 families (Wu *et al.,* 2004). Among the various insect pests attacking groundnut, leaf eating caterpillar, *S. litura* commonly known as tobacco caterpillar, causes extensive damage and it is found to be serious pest on groundnut (War *et al*., 2011). The frequent outbreaks of *S. litura* occurs mainly due to insecticide resistance and favourable environmental conditions (Rao *et al*., 2020). Hence, it is important to study the vital statistics and ecological parameters that influence *S. litura* population in groundnut ecosystem.

Understanding the most vulnerable stage in the biology of an insect is the key to its management, most such weaknesses in the insect life cycle can be best understood by studying its life-tables (Deevey, 1947). To know the comprehensive description of the key mortality factors, survivorship, development and expectation of life, life table is an important analytical tool, which provides detailed information of population dynamics (Southwood

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1978). Life table studies provide an opportunity to assess and evaluate the impact of specific mortality factors acting on insect population (Carey 1993). Through life table studies, age-specific mortality factors and survival rates of a pest species can be determined (Birch, 1948). Life-tables constructed using laboratory data collected under controlled conditions are useful in revealing the maximal growth potential of a population (Ashok *et al*., 2020). The construction of several life tables might be used to prepare a predictive model which can be tested against natural population fluctuations. In this view, the present study was conducted to determine age specific survivals, fecundity and different population growth statistics and ecological mortality factors of *S. litura* on groundnut.

MATERIALS AND METHODS

Age-specific life table analysis of *S. litura*

To construct age-specific life and fertility tables, 10 pairs of newly emerged adults of laboratory reared population were enclosed for oviposition in wooden cages of size 45×45×60 cm. Potted groundnut plants were provided for oviposition along with cotton swab dipped in 10 per cent honey solution to serve as food for the adults. After oviposition, batch of 100 eggs were collected and placed in ten plastic containers in ten batches of each. Immediately after hatching, the larvae were transferred individually to plastic containers with fresh leaves of groundnut (replaced daily until pupation) and reared to record age-specific mortality in different developmental stages. To construct age-specific fecundity life tables, adults emerged on the same day were caged for oviposition and the number of eggs laid on each day was recorded. The observation on fecundity was continued until all the females died. As the sex ratio will be 1:1, the number of eggs obtained per female was divided by two to get the number of female birth. The following parameters of age-specific life tables were worked out (Howe, 1953):

X = pivotal age in days
L_x = survival of female at age 'x'
m_x = age schedule for female births at age 'x'
R_o = net reproductive rate
r_m = innate capacity for increase in number
T_c = mean duration of generation

Net reproductive rate (Ro)

The 'Ro' is the rate of multiplication of population in generation measured in terms of females produced per generation. The sum total of the products 'lx.mx' is the net reproductive rate (Ro).

$$R_o = \sum l_x.m_x$$

Mean duration of generation (Tc)

The mean age of the mothers in a cohort at the birth of female offspring.

$$T_c = \frac{\sum x.lx.mx}{Ro}$$

Innate capacity of increase in number (r_m)

Total number of individuals survived and mean number of female offspring births were recorded at each age interval. From these data, the arbitrarily value of 'rm' was derived by the following formula:

$$r_m = \frac{\log_e Ro}{Tc}$$

Where, e = 2.71828, Tc = Mean generation time

The intrinsic rate of increase (rm) was subsequently calculated from the arbitory 'rm' by taking two trial values selected on either side of it differing in the second decimal places by establishing the relationship (Atwal and Bains, 1974).

$$e^{7-rmx.lxmx} = e^{7} = 1097$$

The values of obtained from the two trials were plotted against their respective arbitory 'rm' which give a straight line. The straight line was intersected by a vertical line drawn from the described values of =1097. The points of intersection gave the value of true 'rm' accurate to four decimal points.

The finite rate of natural increase (λ)

The number of females per female per day *i.e.,* finite rate of increase was determined as:

λ = antilog e^{rm}

From this data, the weekly multiplication of the population was calculated. The hypothetical F_2 females were also worked out with the formula $(Ro)^2$.

Age-specific distribution

Age-specific distribution (per cent distribution of various age groups) of *S. litura* on groundnut was worked out with the knowledge of 'rm'. The stable age distribution was constructed by following the method of Andrewartha and Birch (1954). The 'Lx' (Life table age distribution) was calculated from the 'lx' table by using the following formula:

$$Lx = \frac{lx + (lx + 1)}{2}$$

Per cent distribution of each age group (x) was calculated by multiplying the Lx with . By putting together, the percentage under each stage *viz*., egg, larval, pupal and adult stages, the expected per cent distribution was worked out.

Life expectancy of *S. litura*

Life expectancy of the pest was worked out by using columns x, l_x, d_x, $100q_x$, L_x, T_x and e_x.

Where, x = Pivotal age (days), lx = Number of surviving at the beginning of age interval out of 100, dx = Number dying during 'x', T_x = Number of individual's life days beyond 'x', Mortality rate per hundred alive at the beginning of age interval, Alive between x and x + 1; X_2= Expectation of further life

Ecological life table analysis of *S. litura*

To study the various key mortality factors of *S. litura* in the groundnut ecosystem, sampling of different life stages *viz*., eggs, larvae, pupae was done. The egg masses were collected and recorded the mortality of eggs either due to infertility, parasitization or unknown causes. Similarly, different larval stages were collected at weekly interval and reared in the laboratory in plastic boxes to record the mortality in each instar either due to parasitization, disease or due to unknown causes. The absolute population of egg, larvae per ten quadrates (each quadrate measuring 4x4.2m) was recorded in the field throughout the cropping season. To study the mortality factors in the pupal stage, ten random spots were dug in the cropped area to calculate the pupal population. The number of malformed pupae, infected pupae and incompletely pupated ones were counted and the percentage of the same was calculated, besides per cent adult emergence was also computed. As different developmental stages of *S. litura* were collected at weekly interval, the developmental stages were reared till the adult emergence and the different mortality factors at each stage was recorded. The natural enemies collected during the study were identified at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, Karnataka, India.

Survivorship curve and mortality of *S. litura*

The survivorship curves were drawn by plotting the number living at a given age (lx) against the age (x). The shape of the curve will describe the distribution of mortality with age (Slobodkin, 1962). Different mortality factors were identified and corresponding K-values were assigned for each mortality factors at different developmental stages and the relationship between mortality of *S. litura* and K-values was worked out. the following parameters proposed by Morris and Miller (1954) were accounted.

x = Stage or age interval at which the sample was taken

l_x = The number surviving at the beginning of the stage stated in the x-column

d_x = The number dying within the age interval stated in the x-column

$d_x f$ = The mortality factors responsible for d_x

$100q_x$ = Percentage mortality (dx as percentage of l_x)

S_x = Survival rate within the stage mentioned in the x column

RESULTS AND DISCUSSION

Survival of different life stages of *S. litura*

The maximum duration of different developmental stages of *S. litura* on groundnut *i.e.,* eggs, larvae and pupae were 3, 17 and 10 days, respectively.

day of pivotal age (l_x=0.76) and mortality increased slowly, indicated by a gradual decrease in the l_x values (Table 2). Females contributed maximum mean progeny production per day (m_x=346.12) on the 39th day of pivotal age which declined (m_x=62.00) on 42nd day (Fig. 1). Age-specific fecundity indicated slow raise in the fecundity at initial stages and it gradually raised to reach the peak followed by gradual decrease in the fecundity. Pre-oviposition period of *S. litura* on tobacco ranged from 36th to 37th days of pivotal age and females contributed highest number of progeny (m_x=508.92) on the 41st day of pivotal age (Patil *et al*., 2014).

Life history parameters of *S. litura*

The net reproductive rate (R_o) was 858.52. The data on mean length of generation time (T_c) was 38.86 days. The intrinsic rate of natural increase in number (r_m) was 0.1738 females per female per day with a daily finite rate of increase in number (ë) 1.19 females per female per day and population of *S. litura* would be able to multiply 3.38 times per week under the given set of conditions. The hypothetical female's population in F_2 generation was found to be 733056.60 and the potential fecundity was 1215.49 eggs per female (Table 3). The intrinsic rate of natural increase (r_m) of the population on different host plants ranged from 0.153 to 0.195 females /female/day (Sooravan *et al.,* 2005). Intrinsic rate of laboratory reared *S. litura* on peanuts was 0.1828 females /female/day (Tuan *et al.,* 2013). According to Sundaram *et al.* (2006) the population doubling time of *S. litura* on cauliflower leaves was 3.85 days.

Age-specific distribution of *S. litura* on groundnut

The investigation on the contribution of each developmental stage of *S. litura* on groundnut towards the stable age distribution was calculated by observing the age schedule of birth rate and death rate (m_x and l_x). Adults contributed only 0.20 per cent to the population of stable age, whereas eggs, larvae and pupae contributed 52.46, 45.71 and 1.60 per cent, respectively (Table 4). This indicates that immature stages contributed highest to the stable age distribution of the population. The contribution of eggs, larvae, pupae and adults of *S. litura* were 52.0, 46.4, 1.3 and 0.3 per cent, respectively on groundnut (Gedia *et al*., 2008).

Life expectancy of *S. litura*

The life expectancy (e_x) of *S. litura* declined gradually with the advancement of age. Life expectancy of newly deposited eggs was 13.30 days. The mortality rate (d_x) increased gradually which is indicated by a decrease in the l_x values

Table 1. Survival of different developmental stages of *S. litura* on groundnut

Replications	No. of eggs	Egg hatched (0 to 3 days)	Larval survival (4 to 21days)	Pupal survival (22 to 32 days)
1	10	8	7	6
2	10	10	10	9
3	10	10	8	8
4	10	10	10	9
5	10	7	6	6
6	10	10	8	8
7	10	10	10	9
8	10	8	7	7
9	10	10	9	8
10	10	9	8	8
Cumulative mortality (%)	-	8	17	22

Table 2. Age-specific fecundity of *S. litura* on groundnut

x	l_x	m_x	$l_x m_x$	$xl_x m_x$
0-32	0.78	-	-	Immature
33	0.78	-	0.78	25.74
34	0.78	-	0.78	26.52
35	0.78	-	0.78	27.30
36	0.78	48.75	38.02	1368.72
37	0.78	131.75	102.76	3802.12
38	0.78	231.37	180.46	6854.82
39	0.76	346.12	263.05	10258.95
40	0.67	256.75	172.02	6880.80
41	0.55	138.75	76.31	3128.71
42	0.38	62.00	23.56	989.52
43	0.23	0.00	0.00	0.00
			Ro=$\sum l_x m_x$=858.52	$\sum xl_x m_x$=33363.20

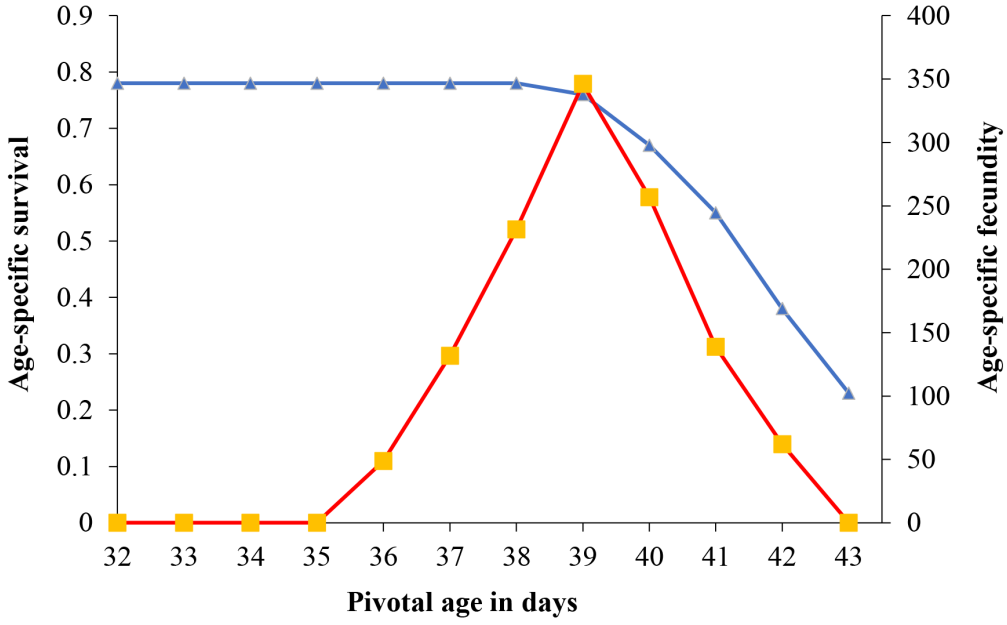

Fig. 1 Age-specific survival and fecundity of *S. litura* on groundnut

Out of 100 eggs 92 eggs hatched successfully into larva, 83 larvae successfully pupated out of 92 larvae and total of 78 adults successfully emerged (Table 1). The cumulative mortality during egg, larval and pupal stages was 8, 17 and 22 per cent respectively. Egg, larva and pupa of *S. litura* on tobacco was having duration of 4, 18 and 13 days, respectively (Patil *et al.,* 2014).

Age-specific fecundity of *S. litura*

Pre-oviposition period of *S. litura* ranged from 33rd to 35th day of pivotal age. Females deposited the first batch of eggs on the 36th day (m_x=48.75) and continued up to 42nd day (m_x=62.00) with l_x values being 0.78 and 0.38 respectively. The first female mortality was observed on the 7th day *i.e.,* on 39th

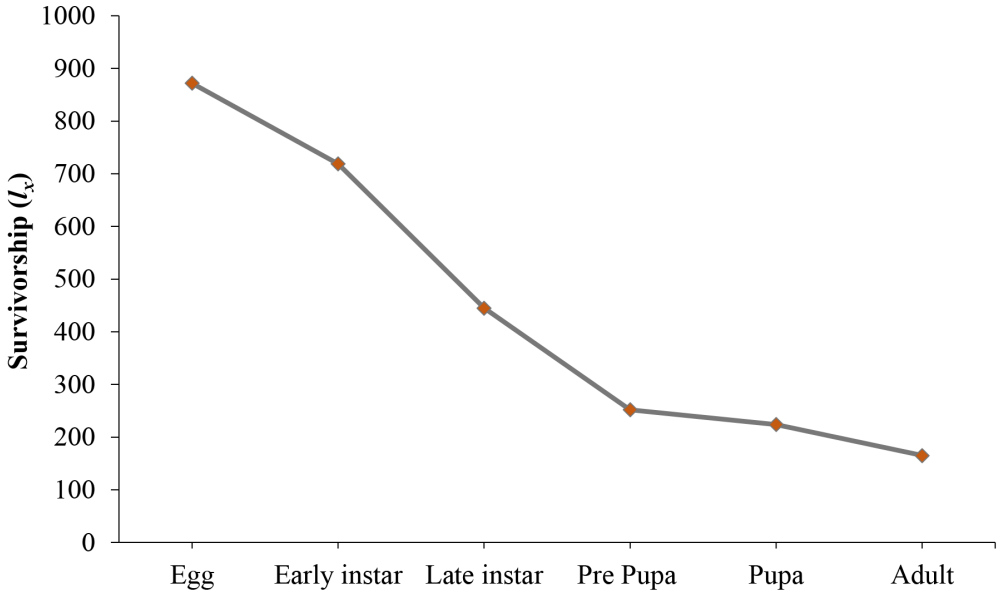

Fig. 2 Survivorship curve of *S. litura* on groundnut

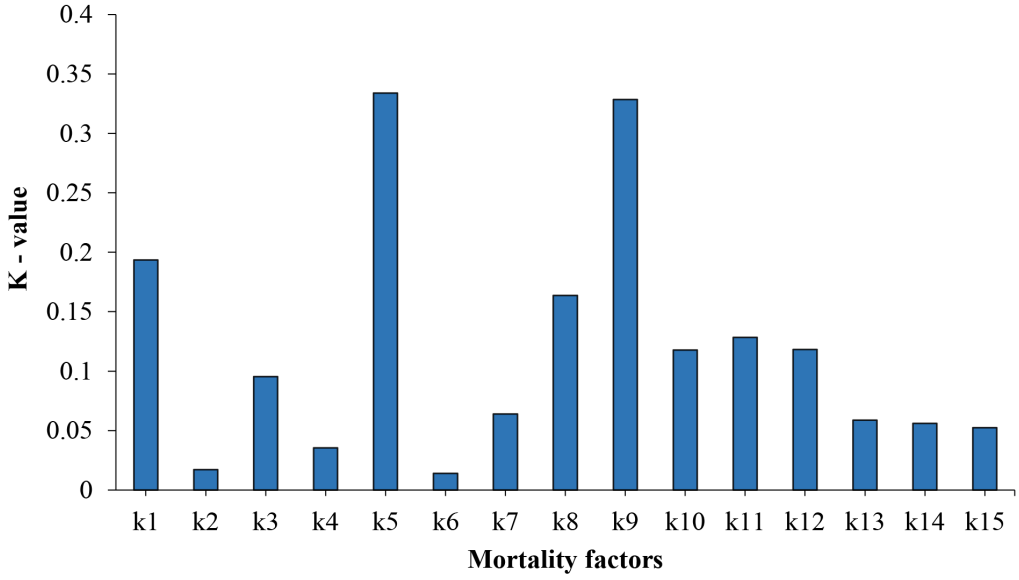

Fig. 3 Key mortality factors of *S. litura* in groundnut ecosystem

and mortality was comparatively high at 35th to 40th day of pivotal age (Table 5), which is indicated by the reduction in the life expectancy to 1.00 day from 13.30 days in the beginning. Life expectancy of *S. litura* eggs was 17.34, 17.44, 16.39, 17.45 and 17.98 on castor, tobacco, groundnut, cotton and cabbage, respectively (Maghodia and Koshiya, 2008). The expected further life of *S. litura* at the age of 15 to 20 days was reduced to 7.08 from 12.82 days (Dhabi *et al*., 2009).

Ecological life table analysis of *S. litura*

Ecological life table was constructed for the field population to understand the role of various key mortality factors that influence the population of *S. litura* in groundnut ecosystem during Kharif, 2017. The total mortality of *S. litura* was 83.02 per cent. The highest mortality was observed in the larval stage (64.95%); followed by pupal stage (26.33%), egg stage (17.54%) and adult stage (10.30%). The egg mortality in *S. litura* was mainly due to unknown causes (17.54%). In the early instar larvae, the total mortality was 42.47 per cent, of which unknown causes alone contributed to 28.34 per cent mortality, followed by tachinids parasitization upto 9.05 per cent. NPV accounted for mortality of 3.42 per cent and *Cotesia* sp. accounted for low mortality *i.e.,* 1.66 per cent. The number of early instar larvae that survived at the end were 445 (Table 6).

In case of late instar larvae, the total mortality recorded was 50.53 per cent, unknown causes alone contributed to 28.00 per cent mortality, whereas, tachinid parasitoids, *Peribaea orbata*, *Carcelia* sp., *Chelonus* sp. and NPV accounted for 6.15, 1.34 and 15.04 per cent mortality, respectively (Table 6). The number of late instar larvae that survived at the end was 252. Larval parasitoids, *Perbaea orbata* (Wiedemann) and *Apanteles ruficrus* (Haliday) caused 13.7 and 8.2 per cent mortality, respectively in *S. litura* on groundnut (Sridhar and Prasad, 1996). In the first generation the early instars larval mortality was 5.7 per cent and the late instar larval mortality was due to unidentified parasitoid and unknown reasons, 6.05 and 3.24 per cent mortality, respectively. In the second generation, mortality of early instar larvae due to unknown reason was 9.1 per cent and mortality in late instar larvae due to unknown reason was 25.0 per cent (Jadhav *et al.*, 2006). Key mortality factors like *Apanteles* spp., green muscardine fungus and an unidentified tachinid fly were the major mortality factors of *S. litura* infesting cabbage during the rainy season and *C. chloridae* during the winter season (Patait *et al.*, 2009).

Table 3. Life history traits of *S. litura* on groundnut

Population growth statistics	Formula	Calculated value
Net reproductive rate (R_o)	$\sum l_x m_x$	858.52
Mean length of generation (T_c)	$\sum x l_x m_x / R_o$	38.86 days
Innate capacity for increase in number (r_m)	$\text{Log}_e R_o / T_c$	0.1738 females/female/day
Finite rate of increase in Number (λ)	antilog e^{rm}	1.19 females/female/day
Arbitary 'r_m' (r_c)	-	0.18
Weekly multiplication of population	(λ) w	3.38
Doubling time (DT)	$\log 2 / r_m$	3.98 days
Potential fecundity (Pf)	$\sum m_x$	1215.49
Hypothetical F_2 females	$(R_0)^2$	737056.59

Table 4 Age-specific distribution of *S. litura* on groundnut

x	l_x	X+1	r_m*(x+1)	exp(r_m*x+1)	Lx(exp(r_m*x+1)	% contribution	
0	1	1	-0.1738	0.8404	0.8404	16.7068	Egg 52.46
1	1	2	-0.3476	0.7063	0.7063	14.0415	
2	1	3	-0.5214	0.5936	0.5936	11.8013	
3	1	4	-0.6952	0.4989	0.4989	9.9186	
4	1	5	-0.8690	0.4193	0.4193	8.3362	Larvae 45.71
5	0.92	6	-1.0428	0.3524	0.3242	6.4458	
6	0.92	7	-1.2166	0.2962	0.2725	5.4175	
7	0.91	8	-1.3904	0.2489	0.2265	4.5037	
8	0.91	9	-1.5642	0.2092	0.1904	3.7852	
9	0.91	10	-1.7380	0.1758	0.1600	3.1813	
10	0.90	11	-1.9118	0.1478	0.1330	2.6444	
11	0.90	12	-2.0856	0.1242	0.1118	2.2225	
12	0.87	13	-2.2594	0.1044	0.0908	1.8057	
13	0.87	14	-2.4332	0.0877	0.0763	1.5176	
14	0.86	15	-2.6070	0.0737	0.0634	1.2608	
15	0.85	16	-2.7808	0.0619	0.0526	1.0473	
16	0.85	17	-2.9546	0.0520	0.0442	0.8802	
17	0.85	18	-3.1284	0.0437	0.0372	0.7398	
18	0.84	19	-3.3022	0.0368	0.0309	0.6145	
19	0.84	20	-3.4760	0.0309	0.0259	0.5164	
20	0.84	21	-3.6498	0.0259	0.0218	0.4340	
21	0.84	22	-3.8236	0.0218	0.0183	0.3648	
22	0.83	23	-3.9974	0.0183	0.0152	0.3029	Pupae 1.60
23	0.83	24	-4.1712	0.0154	0.0128	0.2546	
24	0.83	25	-4.3450	0.0129	0.0107	0.2140	
25	0.83	26	-4.5188	0.0109	0.0090	0.1798	
26	0.83	27	-4.6926	0.0091	0.0076	0.1511	
27	0.83	28	-4.8664	0.0077	0.0063	0.1270	
28	0.81	29	-5.0402	0.0064	0.0052	0.1042	
29	0.81	30	-5.2140	0.0054	0.0044	0.0875	
30	0.80	31	-5.3878	0.0045	0.0036	0.0727	
31	0.80	32	-5.5616	0.0038	0.0030	0.0611	
32	0.78	33	-5.7354	0.0032	0.0025	0.0500	
33	0.78	34	-5.9092	0.0027	0.0021	0.0420	Adult 0.20
34	0.78	35	-6.0830	0.0022	0.0017	0.0353	
35	0.78	36	-6.2568	0.0019	0.0014	0.0297	
36	0.78	37	-6.4306	0.0016	0.0012	0.0249	
37	0.78	38	-6.6044	0.0013	0.0010	0.0210	
38	0.78	39	-6.7782	0.0011	0.0008	0.0176	
39	0.76	40	-6.9520	0.0009	0.0007	0.0144	
40	0.67	41	-7.1258	0.0008	0.0005	0.0107	
41	0.55	42	-7.2996	0.0006	0.0003	0.0073	
42	0.38	43	-7.4734	0.0005	0.0002	0.0042	
				Total	5.0306	100.00	100.00

In the pre-pupal stage, the unknown causes were noted to be the major mortality factor (11.11 %). The population at the beginning of pupal stage was 224. Pupal mortality due to malformation, non emergence of adult from pupae and unknown causes during the pupal stage were 12.05, 11.16 and 5.71 per cent, respectively. In the adult stage, 5.45 and 5.12 per cent mortality due to adult malformation and unknown causes in moths was observed. The malformed moths with twisted wing were incapable of reproduction and the final population that survived was 148 (Table 6). Pupal mortality due to unknown reasons was 20.0 per cent (Jadhav *et al.*, 2006). The key mortality factors of *S. litura* on groundnut were NPV, parasites like *Cotesia* spp. and tachinid maggot (Kumar *et al.*, 2015).

The generation survival of 0.1697 indicated that only 16.97 per cent of the initial population could survive and successfully complete the generation. The number of larvae dead due to unknown causes contributed maximum to high 'K' value of 0.6625, followed by NPV infection, tachinids and *Cotesia* sp. with 'K' values of 0.1992, 0.1594 and 0.0171, respectively (Table 6). The remaining minor mortality factors like *Chelonus* sp. and malformed stages contributed less to 'K' value in the age-specific life table of *S. litura.*

Survivorship curves

The survivorship curve pattern of *S. litura* during *Kharif* 2017 plotted on a semi-logarithmic scale. It was observed that the curve obtained in the present study was almost similar to type III curves indicating that the mortality rate was constant in all the stages. Further, the drop in survivorship was glaringly high in the late instar larvae but the end result of all the curves suggested a steady drop in the survivorship of *S. litura* by the adult stage. This survivorship curve indicates that there was comparatively high mortality in early stages of development and the population *S. litura* stabilizes over the period of time as there is constant mortality and it showes distinct steps at each developmental stages (Fig. 2). So this indicates that intervention in the earlier stages of development can manage population of *S. litura*. Survivorship curves of 22 species of herbivores, out of these seven species (40%) belonged to convex type of survivorship curves. The rest, however, followed the intermediate forms between Type II and Type III curves for lepidopteran insects (Price, 1980). On five soya bean cultivars the survivorship curves of *S. exigua* was type III (Farhani *et al.,* 2011).

Table 5. Life expectancy of *S. litura* on groundnut

Pivotal age (Days) 'x'	Number Surviving to the beginning of the age interval	Number dying during 'x'	Mortality rate per hundred alive at beginning of the age interval $\frac{(dx.100)}{lx}$	Alive between age 'x' and 'x+1' $\frac{lx + (1x + 1)}{2}$	No. of the individuals life days beyond 'x'	Expectation of further life $\frac{Tx}{lx} \times 2$
(x)	(l_x)	(d_x)	(100 q_x)	(L_x)	(T_x)	(e_x)
0-5	100	8	8.00	96	665	13.30
5-10	92	2	2.17	91	569	12.36
10-15	90	5	5.55	87.50	478	10.62
15-20	85	1	1.17	84.50	390.5	9.18
20-25	84	1	1.19	83.50	306	7.28
25-30	83	3	3.61	81.50	222.5	5.36
30-35	80	2	2.50	79.00	141	3.52
35-40	78	55	70.51	50.50	62	1.58
40-45	23	16	69.56	11.50	11.50	1.00

Table 6. Ecological life-table of *S. litura* in groundnut ecosystem

Stages	No. alive at the beginning of x (l_x)	Factors responsible for dx (d_xF)	No.dead (a)	Mortality per cent $100q_x$	Mortality $d = a/l_x$	Survival S = 1-d	'K' value (-ln(s))
Egg (N1)	872	Unknown causes	153	17.54	0.1754	0.824	0.1935
Early instars	719	*Cotesia* sp.	12	1.66	0.0166	0.983	0.0171
	707	Tachinids	64	9.05	0.0905	0.909	0.0954
	643	NPV	22	3.42	0.0342	0.965	0.0356
	621	Unknown causes	176	28.34	0.2834	0.716	0.3340
Late instars	445	*Chelonus* sp.	6	1.34	0.0134	0.986	0.0140
	439	Tachinids	27	6.15	0.0615	0.938	0.0640
	412	NPV	62	15.04	0.1504	0.849	0.1636
	350	Unknown causes	98	28.00	0.2800	0.720	0.3285
Pre pupa	252	Unknown causes	28	11.11	0.1111	0.8889	0.1177
Pupa	224	Malformed pupae	27	12.05	0.1205	0.8795	0.1284
	197	Not emerged pupae	22	11.16	0.1116	0.8884	0.1183
	175	Unknown causes	10	5.71	0.0571	0.9429	0.0587
Adults	165	Malformed adult	9	5.45	0.0545	0.9455	0.0560
	156	Unknown causes	8	5.12	0.0512	0.9488	0.0525
Total			724	83.02			1.7773
Normal females x 2 (N2)			148				
Reproducing females x 2			74				
Generation survival (N2/N1)			0.1697				

Key mortality factors (K-factors)

A total of 15 mortality factors (K1 to K15) were identified in *S. litura* on groundnut during the study period. Larval parasitoids (*Cotesia* sp., *Chelonus* sp. and tachinids), virus (NPV), malformed pupa, adult malformation and unknown causes (Fig. 3). This indicates that there are good number of mortality factors operating in groundnut ecosystem in this area that can be utilised for management of *S. litura* on groundnut but the unknown causes dominated the K-values and the K-values of biotic factors *i.e.*, natural enemies contributed very less in the population reduction, this indicates that there is no sufficient population of natural enemies and efforts should be made to increase natural enemy population. In sunflower total of 14 mortality factors of *S. litura* were identified (Geetha and Jagadish, 2014).

Life-table provides an insight to assess and evaluate the impact of specific mortality factors acting on insect population. Various age-specific population growth statistics data like reproductive potential, age-specific fecundity, generation time and life expectancy of *S. litura* are useful in assessing population dynamics of the pest. Significant levels of mortality were found in different developmental stages of the pest and there are good number of key mortality factors especially tachinid parasitoids which are responsible for population reduction, these factors can be successfully employed in IPM to tackle population of *S. litura* in groundnut ecosystem.

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Trap crop selection and economic threshold based ecological management of *Spilarctia obliqua* (Walker) (Lepidoptera, Erebidae) for sesame

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ABSTRACT: The stage-specific life table and nutritional ecology of a *Spilarctia obliqua* (Walker) along with respective economic thresholds (ETs) on four different crops such as sesame (*Sesamum indicum*), castor (*Ricinus communis*), jute (*Corchorus capsularis*) and sunflower (*Helianthus annuus*) were studied during 2020-2022. The feeding and population dynamics of *S. obliqua* were significantly affected by the hosts in terms of host suitability or susceptibility (sunflower>jute>castor>sesame). The ET of *S. obliqua* on sesame (40.59±2.12 pests m^{-2} area) was significantly ($F_{3,8}$=4.72, P=0.031) higher than the other crops (sunflower<jute<castor). Subsequently, the three most suitable hosts (sunflower> jute>castor) were tested in a multi trap cropping system for sesame as main crop in a specific pattern depending on respective susceptibility. Data from the model trap cropping (without pesticide) supported minimum infestation of *S. obliqua* and other pests along with more predators on sesame with higher benefit cost ratio (BCR) and more (11.82%) carbon sequestration (CS) in same area relative to monoculture (with pesticide) of sesame. It supports pesticide free high production and better CS than sole culture of sesame for climate smart agriculture. © 2023 Association for Advancement of Entomology

KEY WORDS: Host suitability, sesame, castor, jute, sunflower, carbon sequestration, phytoconstituents

INTRODUCTION

Spilarctia obliqua (Walker) (Syn. *Diacrisia obliqua*) (Lepidoptera, Erebidae) is a major polyphagous (generalist) pest of different economic crops including sesame, castor, jute and sunflower throughout the South East Asian countries (Gotyal *et al*., 2015; Mobarak *et al.,* 2020). Management of *S. obliqua* is throgh broad-spectrum synthetic pesticides (triazophos, lambda cyahlothrin, indoxacarb, cypermethrin, deltamethrin**,** etc.) and plant extracts (nimbicidine, ultineem, neemoil, etc.) (Bhardwaj and Kumari, 2016; Mohapatra and Gupta, 2018). The basic information on bio-ecology of an insect pest is necessary before deciding any strategy to combat with the pest (Slansky and Scriber, 1985; Chen *et al*., 2017). Trap crops can attract and divert pests from the main crop by exploiting their different sensory modalities toward most preferred (trap crops) hosts (Rhino *et al*., 2016; Srinivasan *et al*., 2008). But, till date none of the studies has been performed with *S. obliqua* on different crop cultivars using age-stage life table

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and nutritional ecology-based ET calculation along with carbon sequestration efficiencies (CSEs) or trap crop designing.

The objectives of the present study were to (i) find out the biochemical basis of host preference of *S. obliqua* by their food utilization efficiency measures, (ii) unfold the impact of different host plants on their population growth, (iii) find the appropriate ETs of *S. obliqua* for time based application of any control measures for the crops, (iv) selection of trap crops based on host choice and their cropping pattern for optimum management of the pest other than any pesticides, and (iv) suggest new strategy for climate smart agriculture (CSA) in terms of economic profit and sustainable management of *S. obliqua* by using multilayer trap cropping.

MATERIALS AND METHODS

Series of laboratory experiments were conducted during 2020-2022 to study the feeding dynamics and population ecology of *S. obliqua* on four economic crops like, sesame, *Sesamum indicum* (Pedaliaceae) cv. Rama; castor, *Ricinus communis* (Euphorbiaceae) cv. DPC-9; jute, *Corchorus capsularis* (Malvaceae) cv. Sonali, JRC-321 and sunflower, *Helianthus annuus* (Asteraceae) cv. PAC-36. Fieldworks were also conducted to determine ETs for *S. obliqua* and CSEs of respective crop cultivars along with trap crop selection for designing a suitable trap cropping system.

Sesame, castor, jute and sunûower crops were cultivated in a field situated near Chinsurah Rice Research Center (CRRC), Chinsurah, 22°53' N; 88°23' E, 13m above sea level, Hooghly, West Bengal, India, in their growing season during late summer to monsoon (June-September) in 2020-2022. Total twenty four [4 crop×(3 treated+3 control)=24] plots [each plot 10m× 10m; soil organic matter 5.3 ± 0.2%, pH 7.7, average photoperiod of about 13:11 (L:D) at 30–32°C] were prepared for cultivation of sesame, castor, jute and sunûower with average plant density of 30±2, 08±2, 30±2 and 12±2 plants m^{-2}, respectively. Fieldworks were conducted by growing the crop cultivars in randomized block design (RBD) with a gap of 1 m between two plots. The yield potential of the selected crop cultivars was observed over a traditional synthetic pesticide, triazophos 40 EC (@ 40g ai. ha^{-1}) for two times at pre-flowering and flowering stage along with control (without pesticide) side-by-side. The crops were naturally infested by *S. obliqua* in the field and the pests were collected separately for their mass culture. Intact mature leaves from 4-5 weeks old crop plants from respective control plots were collected separately for phytochemical analysis as well as food for *S. obliqua* neonates.

Phytochemical analysis: Freshly collected intact leaves of sesame, castor, jute and sunflower were initially rinsed with distilled water and dried separately for different phytochemical analysis such as total carbohydrates (Dubois *et al*., 1956), total proteins (Miller, 1959), total lipids (Folch *et al*., 1957), total amino acids (Moore and Stein, 1948), total nitrogen (Humphries, 1956), moisture (Nayek and Banerjee, 1987), ash content (Banerjee and Haque, 1984), total phenols (Bray and Thorpe, 1954), total flavonoids (Zhishen *et al*., 1999), Tanins, saponins (Trease and Evans, 1983), alkaloids (Harborne, 1973), phytates (Reddy and Love, 1999) and oxalates (Day and Undrwood, 1986). Determination of each biochemical analysis was repeated three times and expressed in µg mg^{-1} dry weight basis.

Insect mass culture: The initial population of *S. obliqua* larvae were collected from each crop (sesame, castor, jute and sunflower) cultivar separately from the cultivated. The larvae were incubated separately in the laboratory at 26±1°C, 60±5% RH and a photoperiod of 12:12 (L:D) on intact mature leaves of the selected crops in glass jars (20 cm dia. × 30 cm ht.) until their pupation. After emergence of adults from the reared pupae, six pairs of newly emerged male (bipectinate antennae) and female (filiform antennae) were placed in an oviposition cage of fine nylon net (25×25×25 cm) containing a small cotton ball soaked with 10% honey solution for their feeding. The paired moths (male and female) were kept with respective fresh foliage separately for their

oviposition. The stock culture of *S. obliqua* was initiated with the F_1 eggs on the selected crop cultivars with three replications at same conditions in a growth chamber [ten eggs in a glass jar (20 cm dia. × 30 cm ht.)] up to three generations. On each crop cultivar, newly laid eggs by the F_3 females were collected in order to obtain the same aged eggs of defined cohort (n=100) for the crops to study population and nutritional ecology with three replications. The petiole of each fresh mature leaf was inserted into a moist piece of cotton, which was wrapped with aluminium foil to prevent moisture loss and replaced daily with fresh ones. Ten larvae were placed in a glass jar containing a particular mature fresh leaves of selected crop cultivars in same condition up to their last instar (6^{th} instar) for pupation. The pupae obtained from each glass jar were placed in separate glass jar (6 cm dia. × 10 cm ht.) covered with fine mesh nylon net in same condition up to their adult emergence on respective crop cultivars. Similarly, newly emerged six pairs of moths (male and female) derived from the respective crop cultivars were kept with respective fresh foliage separately in the same type of oviposition cage. Mortality and developmental durations from egg to adult along with newly emerged female's fecundity were recorded. The feeding dynamics of the neonates and population data throughout their life cycle were recorded separately on the selected crop cultivars.

Feeding ecology: Feeding ecology was determined by taking the F_4 newly emerged first instar larvae that had been reared in the laboratory condition on the selected crops (sesame, castor, jute and sunflower) separately as described in previous experiments. Food utilization indices were calculated by the formulae of Waldbauer (1968). All the feeding indices like, growth rate (GR), consumption rate (CR), relative growth rate (RGR), consumption index (CI), egestion rate (ER), host consumption rate (HCR), approximate digestibility (AD%), efficiency of conversion of ingested food (ECI%), efficiency of conversion of digested food (ECD%) and host utilization efficiency (HUE%) were estimated on the selected host plants with three replications.

Life table study: The data on survival, developmental duration and oviposition of all individuals on the selected four crop (sesame, castor, jute and sunflower) cultivars were analyzed separately based on age-stage life table. It includes parameters, probability of survival from birth to age x (l_x), proportion of dying (d_x), mortality rate (q_x) and survival rate (s_x) per day per age class from egg to adult stages, which were calculated based on the formulae of Southwood (1978), Carey (1993), Krebs (1994) and Price (1998). Using these parameters, the statistics like total individuals at age x and beyond k (T_x), average population alive in each stage (L_x), life expectancy (e_x), gross reproductive rate (GRR or m_x), net reproductive rate (NRR or R_0), mean generation time (T_c), doubling time (DT), intrinsic rate of population increase (r_m), and finite rate of population increase (ë) were computed, using Carey's formulae (1993).

Yield loss, ET and CSE calculation: The occurrences of *S. obliqua* were recorded by random quadrat sampling (RQS) from each treated and control plots of the selected crop (sesame, castor, jute and sunûower) cultivars. Each plot was considered with 24 quadrats for 3 times (pre-flowering, flowering and post flowering stage) i.e., total 72 quadrats/plot. Economic injury (EI) of *S. obliqua* was determined according to the methodology proposed by Pedigo *et al.* (1986) that expressed as numbers or injury equivalents which governed by four primary variables viz., cost of the management tactic per production unit (*C*), market value per production unit (*V*), percent yield loss per pest *(DÊ)* and the proportional reduction in pest attack (*K*). If the relationship of these variables is linear or roughly so, the EI can be given as *EI = C/VDÊK* (Pedigo *et al.,* 1986; Pedigo and Buntin, 1994). The economic threshold (ET) is the population density at which control action should be determined (initiated) to prevent an increasing pest population (injury) from reaching the EI (Pedigo and Higley, 1992). The cost of control (C) includes cost of the insecticide plus application, although others could be added (Higley and Wintersteen, 1992). On the basis of BHCs infestation and the efficacy of the traditional synthetic pesticide were determined in terms of yield damage reduction

(Yr%), proportion of insect controlled (PC%) and percent yield loss per pest per plant (D%) along with the management costs (C) for calculation of EI, ET, time to reach the EI (Ti) and ET (Tt) when a plant was infested by a single pest in the field (Roy, 2019). The average management cost was calculated using the cost of the insecticide (triazophos 40 EC) and its application accounted @ Rs. 3500 ha^{-1}. The market value of the produced crops was considered accordingly prevailed in West Bengal, India (Roy, 2020). The benefit cost ratio (BCR) was also determined to find the production efficiency of the selected crop (sesame, castor, jute and sunflower) cultivars over *S. obliqua* as sole pest infestation. The organic biomass production and CSE of the selected crop cultivars were also determined as in Roy (2020) to find the ability of the crops to mitigate the GHGs emission and climate change for CSA (Albrecht and Kandji, 2003; Lal, 2008, 2011; Heeb *et al*., 2019; Roy, 2021).

Trap crop selection and cultivation pattern: Another fieldwork was conducted similarly during 2020-2022 in the same field for designing suitable trap crop and their cultivation pattern. Six plots [each plot 10 m× 10 m; soil organic matter 5.3 ± 0.2%, pH 7.7, average photoperiod of about 13:11 (L:D) at 30–35°C] were prepared similarly in the same field near CRRC for cultivation of sesame as main crop followed by sunflower, jute and castor as trap crop according to preference of *S. obliqua*. The sesame cultivar was grown in the six plots side by side and there was a gap of 0.125 m between two plots which was kept for cultivation of single row (3.184% land area) of sunflower. Surrounding the six plots a border of 0.25m diameter was used for jute (3.815% land area) followed by same diameter of 0.25m for castor (3.872% land area). The land used for main crop cultivation was 8.184 times than the trap crops which occupy only 10.889% of land area. The sesame cultivar (cv. Rama) was also cultivated in the same condition as sole crop (monoculture) in same land area (673.33 m^2) with keeping only 3.889 per cent land area as ecotone. All the six plots of sesame and inter-plot sunflower along with the borders of jute and castor in composite culture as well as only sesame as sole crop (monoculture) with three replications were maintained without pesticide. Different crop parameters were determined in the manipulated crop ecosystem to find the production efficiency of trap crop system (polyculture) in comparison with the monoculture of sesame over BHC of *S. obliqua* along with other pest infestation. Biomass production and CSEs were also determined for both agro ecosystems of sesame.

Statistical Analysis: Experimental data of different phytoconstituents of the selected crop cultivars (sesame, castor, jute and sunflower) and the pest (*S. obliqua*) population parameters feeding indices including ETs related values were homogeneous among treatments as confirmed by Levene's homogeneity test. All the data were normally distributed as determined by Shapiro–Wilk tests and so data were analysed with one-way ANOVA. Means associated with all the data were separated using Tukey's (HSD) test when significant values were obtained. The RBD data of the selected cultivars, respective CSEs and the RQS data from the field with ET values of the pest were analyzed using one-way ANOVA (Zar, 1999). All the statistical analysis was performed by using SPSS, version 16.0.

RESULTS AND DISCUSSION

Host Phytochemicals: All the PMs and SMS were varied significantly ($F_{3,8} \geq 6.30$, $P \leq 0.017$) in the crop cultivars and they were present in reverse order with each other. Among the PMs, total carbohydrates and proteins were in the order of sesame<castor< jute<sunflower. Similarly, total lipids and amino acids were the lowest in sesame followed by castor, jute and sunûower, respectively. All the SMs were in the order of sesame> castor> jute> sunflower. Ultimately, the ratio of PMs to SMs was significantly ($F_{3,8} \geq 5.76$, $P < 0.021$) varied in the selected crop (sesame<castor< jute<sunflower) cultivars (Table 1).

Feeding dynamics: All the food utilisation indices of *S. obliqua* on the selected crops were displayed significant ($F_{3,8} \geq 10.67$, $P \leq 0.004$) variations within the selected crops. The average GR and CR of *S. obliqua* larvae on sesame, castor, jute and sunûower were 6.72±0.09, 6.73±0.09, 6.83±0.09,

7.09±0.10 and 33.48±0.09, 33.91±0.10, 34.36±0.10, 37.13±0.13 mg per day, respectively. The GR, CR, AD and HUE values were in the order of sesame<castor<jute<sunflower while, ECD and ECI were exactly in reverse order (Table 2).

Population dynamics: The stage-specific life tables of *S. obliqua* were investigated in the laboratory on intact mature leaves of sesame, castor, jute and sunflower cultivars and showed four distinct stages (i.e., egg, larva, pupa and adult) with six larval instars. The l_x, T_x and e_x of *S. obliqua* gradually decreased throughout the developmental stages on the selected crop cultivars and they also produce type-III survivorship curve like most of the insects. Whereas, q_x was varied in different developmental stages and comparatively higher in egg and pupae stage with a rapid surge in adult stage on the selected crop cultivars. The average Pf significantly varied on the crops (sesame< castor<jute<sunflower) ($F_{3,8}$=36.75; P<0.001). The F_x, GRR or m_x and NRR or R_0 of *S. obliqua* also differed significantly ($F_{3,8}$=13.99-23.23; P≤0.002). Average T_c for the crop cultivars were without any significant ($F_{3,8}$=2.96; P=0.098). Whereas, the average DT for the crop (sesame>castor>jute> sunflower) varied significantly ($F_{3,8}$=3.87, P=0.031). The r_m and λ also varied significantly ($F_{3,8}$=6.81-7.06; P≤0.012) like their respective Pf (Table 3).

Yield losses, ETs and CSEs: The efficacy of the synthetic pesticide over the control indicated EI and ET of 25.20 - 42.65 and 20.31- 40.59 pests

Table 1. Phytochemical variations (Mean ± SE of 3 observations /cultivar) of sesame (*S. indicum*; cv. Rama), castor (*R. communis*; cv. DPC-9), jute (*C. capsularis*; cv. Sonali; JRC-321) and sunflower (*H. annuus*; cv. PAC-36)] determined during 2020-2022

Phytochemicals (µg mg^{-1} dry weight)	Sesame	Castor	Jute	Sunflower
Total carbohydrates	49.42±1.43[a]	50.12±1.31[b]	51.21±1.31[c]	54.33±1.49[d]
Total proteins	5.62±0.43[a]	6.32±0.29[b]	6.89±0.37[b]	11.53±0.39[c]
Total lipids	4.22±0.41[a]	4.69±0.23[b]	4.92±0.21[b]	9.13±0.32[c]
Total amino acids	1.38±0.22[a]	1.85±0.20[b]	2.03±0.15[c]	2.18±0.16[d]
Total nitrogen (%)	0.69±0.10[a]	1.41±0.13[b]	1.54±0.12[b]	2.24±0.11[c]
Moisture (%)	76.13±1.43[a]	77.85±1.45[b]	77.98±1.38[b]	78.68±1.44[b]
Ash content (%)	9.05±0.25[a]	11.37±0.45[b]	11.50±0.47[b]	12.20±0.41[c]
Total phenols	14.84±0.29[a]	13.01±0.49[b]	12.14±0.41[c]	11.69±0.39[d]
Total flavonoids	13.80±0.29[a]	12.97±0.42[b]	11.10±0.38[c]	10.65±0.39[d]
Tanins	10.98±0.27[a]	8.85±0.40[b]	9.28±0.31[c]	6.53±0.28[d]
Saponins	14.08±0.24[a]	11.95±0.44[b]	10.38±0.32[c]	9.63±0.41[d]
Alkaloids	12.48±0.28[a]	10.35±0.44[b]	9.78±0.36[c]	8.03±0.26[d]
Phytates	8.78±0.21[a]	6.65±0.38[b]	5.08±0.28[c]	4.33±0.20[d]
Oxalates	7.52±0.29[a]	5.39±0.24[b]	4.82±0.25[c]	3.07±0.19[d]

Within the rows means followed by same letter(s) are not significantly different at P<0.05 by Tukey (HSD) test along with *F* values (ANOVA)

Table 2. Average feeding indices of *S. obliqua* Walkar (Mean ± SE of 3 observations) on selected host plants [sesame (*S. indicum*; cv. Rama), castor (*R. communis*; cv. DPC-9), jute (*C. capsularis*; cv. Sonali; JRC-321) and sunflower (*H. annuus*; cv. PAC-36)] determined during 2020-2022

Parameter	Sesame	Castor	Jute	Sunflower
GR (mg day^{-1})	6.72±0.09^{a}	6.73±0.09^{a}	6.83±0.09^{b}	7.09±0.10^{c}
CR (mg day^{-1})	33.48±0.09^{a}	33.91±0.10^{b}	34.36±0.10^{a}	37.13±0.13^{c}
RGR (mg day^{-1})	3.41±0.02^{a}	3.43±0.02^{a}	3.37±0.02^{b}	3.21±0.02^{c}
CI (mg day^{-1})	55.57±0.08^{a}	57.84±0.08^{b}	59.95±0.08	57.33±0.08
AD (%)	69.32±0.06^{a}	69.69±0.06^{b}	69.73±0.06^{b}	71.17±0.05^{c}
ECI (%)	12.14±0.08^{a}	11.95±0.07^{b}	11.88±0.08^{b}	11.47±0.07^{c}
ECD (%)	20.07±0.16^{a}	19.60±0.15^{b}	19.56±0.16^{b}	18.29±0.14^{c}
HUE (%)	77.63±0.03^{a}	77.84±0.03^{b}	77.85±0.03^{b}	78.67±0.03^{c}
ER (mg day^{-1})	17.46±0.02^{a}	17.81±0.02^{b}	17.37±0.02^{a}	16.07±0.02^{c}
HCR (mg day^{-1})	73.03±0.09^{a}	75.64±0.09^{b}	77.33±0.09^{c}	73.39±0.09^{d}

Within the row means followed by same letter(s) are not significantly different at P<0.05 by Tukey (HSD) test along with *F* values (ANOVA). Here, GR: growth rate, CR: consumption rate, RGR: relative growth rate, CI: consumption index, AD: approximate digestibility, ECI: efficiency of conversion of ingested food, ECD: efficiency of conversion of digested food, HUE: host utilization efficiency, ER: egestion rate, HCR: host consumption rate

m^{-2}, respectively on the crop cultivars (castor< sunflower<jute<sesame) and were significantly ($\boldsymbol{F}_{3,8}$=4.89; P=0.032 and $F_{3,8}$=4.72; P=0.031, respectively) differed from each other. For a single pest observation per plant the possible time that can be taken to reach EI (Ti) and ET (Tt) were calculated as 36.72-75.38 and 35.72-74.38 days, respectively on the cultivars (sunflower<jute <castor<sesame) and were also significantly ($F_{3,8}$=44.53; P<0.001) varied. The benefit cost ratio (BCR per ha) were 0.23-0.58 for the crop cultivars (sesame<castor<jute<sunflower) as in EY and NP with significant ($F_{3,8}$=8824.47; P<0.001) variation. The CS were with significant ($F_{3,8}$=34.00; P<0.001) variations due to different biomass production ($F_{3,8}$=205.06; P<0.001) by the cultivars (sesame<sunflower<jute<castor). The crop parameters including production values and CSEs were changed according to specific pest infestation depending on their host preference, population growth and even on host itself (Table 4).

Efficacy of trap crops over sole crop: The cultivation of sesame as main crop with the most preferred crop cultivars (sunûower> jute> castor) as trap crop (polyculture) in a specific pattern had shown more production efficacy than monoculture of sesame. Different attributes related with the crop production were significantly ($F_{1,4}$= 9.34; Pd=.037) higher in the said manipulated (designed) agro ecosystem than monoculture system of sesame. The infestation of *S. obliqua* and other pests were 16.23±2.41, 7.44±2.12 and 11.62±2.32, 5.74±2.15 individuals m^{-2}, respectively in mono and poly culture of sesame. Whereas, the occurrence of different predators, mainly Hymenopterans, were significantly ($F_{1,4}$=84.00; P<0.05) higher in the defined multi-trap (sunflower>jute>castor) cropping system (8.98±2.06 individuals m^{-2}) of sesame than its monoculture (6.53±2.14 individuals m^{-2}) system.

Table 3. Population dynamics and reproductive table (Mean ± SE of 3 observations) of the 12 cohorts (n=100) of *S. obliqua* Walker on selected four host plants [Sesame (*S. indicum*; cv. Rama), castor (*R. communis*; cv. DPC-9), jute (*C. capsularis*; cv. Sonali; JRC-321) and sunflower (*H. annuus*; cv. PAC-36)] cultivated during 2020-2022.

Population parameters	Sesame	Castor	Jute	Sunflower
Potential fecundity (Pf)(eggs/female)	172.67±9.53[a]	210.15±8.42[b]	270.64±8.12[c]	300.57±6.41[d]
Total fertility rate (F_x) (offspring/total mature females)	1914.40±758.64[a]	1982.67±888.09[b]	9022.67±1345.17[c]	13622.67±1573.77[d]
Gross reproductive rate (GRR or mx) (offspring/ individual)	26.98±9.68[a]	62.89±5.89[b]	70.43±5.35[c]	86.37±4.81[d]
Net reproductive rate (NRR or R_0) (offspring/ individual)	9.07±3.81[a]	10.67±3.81[b]	33.07±3.81[c]	45.07±3.81[d]
Generation time (T_c) (days)	44.49±0.29[a]	44.73±0.31[b]	45.39±0.37[c]	44.13±0.26[d]
Doubling time (DT) (days)	20.03±1.45[a]	15.41±1.49[b]	9.05±0.38[c]	8.05±0.14[d]
Intrinsic rate of increase (r_m) (per day)	0.04±0.01[a]	0.05±0.01[b]	0.08±0.00[c]	0.09±0.00[d]
Finite rate of increase (ë) (per day)	1.05±0.01[a]	1.05±0.01[a]	1.08±0.00[b]	1.09±0.00[c]

Within the row means followed by same letter(s) are not significantly different at P<0.05 by Tukey (HSD) test along with *F* values (ANOVA)

The BCR ha^{-1} was 0.23±0.00 and 0.34±0.00, respectively for sesame as sole crop (monoculture) and main crop (polyculture) depending on respective production cost and profit. Habitat manipulation by the trap crops (sunflower>jute>castor) leads more carbon sequestration (11.82%) due to significantly ($F_{1,4}$=40.22, *P*=0.003) higher biomass production than sesame as sole crop.

Sunflower (cv. PAC-36) leaves were noted with good nutritional (PMs) quality compared to other three crops (jute> castor> sesame) and anti-nutritional factors (SMs) were in reverse order. The PMs (carbohydrates, proteins, lipids, amino acids) including moisture content was used for their general growth and reproduction like other animals (Turunen, 1990; Genc and Nation, 2004). Such variations in host plants were directly affected the potential and achieved development and growth of *S. obliqua* as in other insects (Awmack and Leather, 2002; Roy and Barik, 2012). The GR, CR, AD and HUE of *S. obliqua* were higher on sunflower leaves followed by jute, castor and sesame, while ECI and ECD were in reverse order (sesame>castor>jute>sunflower) due to respective host chemical regime. Trap cropping by habitat manipulation is an attractive option to reduce dependency on conventional pest management practices through insecticides (Srinivasan *et al*., 2008; Rhino *et al.,* 2016). In this finding, all these parameters (GRR or m_x, NRR or R_0, r_m, T_c, DT and ë) were higher in sunflower followed by jute, castor and sesame like most of the insects (Roy and Barik, 2013; Mobarak *et al*., 2020; Roy, 2020).

Table 4. Different crop parameters and ETs (Mean ± SE of 3 observations) of *S. obliqua* Walkar on selected four host plants [sesame (*S. indicum*; cv. Rama), castor (*R. communis*; cv. DPC-9), jute (*C. capsularis*; cv. Sonali; JRC-321) and sunflower (*H. annuus*; cv. PAC-36)] including their CSEs observed over a traditional synthetic pesticide (Triazophos 40 EC) along with control (without pesticide) side by side during 2020-2022

Crop Parameter	Sesame	Castor	Jute	Sunflower
Yield damage without treatment (Yd %)	21.77±2.89[a]	31.71±0.83[b]	23.93±2.15[c]	25.39±3.81[d]
Proportion of insect controlled (PC %)	80.24±3.09[a]	83.61±2.17[b]	83.02±1.66[c]	80.24±3.09[a]
Yield damage reduction after treatment (Yr %)	17.63±2.92[a]	26.52±1.12[b]	19.93±2.13[c]	20.57±3.62[d]
Damage per pest per plant (D%)	4.13±0.32[a]	5.19±0.68[b]	3.99±0.15[c]	4.83±0.56[d]
Economic injury (EI) (pests/m^2)	42.65±2.54[a]	21.22±1.13[b]	32.16±1.07[c]	25.20±1.47[d]
Economic threshold (ET) (pests/ m^2)	40.59±2.13[a]	20.31±1.04[b]	29.78±1.77[c]	23.12±1.94[d]
Time to reach EI/pest/ m^2 (Ti days)	75.38±3.83[a]	69.55±2.41[b]	44.98±1.57[c]	36.72±2.92[d]
Time to reach ET/pest/ m^2 (Tt days)	74.38±3.83[a]	68.54±2.41[b]	43.98±1.57[c]	35.72±2.92[d]
Production value				
Total production cost [TPC] (Rs/ha)	21900.00±57.74[a]	21900.00±57.74[a]	21900.00±57.74[a]	21900.00±57.74[a]
Economic yield [EY](Rs/ha)	26783.95±57.74[a]	27287.42±52.35[b]	31063.45±50.66[c]	34587.75±55.45[d]
Net Profit [NP] (Rs/ha)	4983.95±57.74[a]	5487.42±52.35[b]	9263.45±50.66[c]	12787.75±55.45[d]
Benefit cost ratio (BCR/ha)	0.23±0.00[a]	0.25±0.00[b]	0.42±0.00[c]	0.58±0.00[d]
Carbon sequestration efficiency (CSE)				
Biomass produced (lbs dry wt/ m^2)	2.26±0.04[a]	3.48±0.05[b]	3.052±0.04[c]	2.381±0.05[a]
Carbon sequestration (lbs/ m^2)	0.97±0.04[a]	1.47±0.05[b]	1.298±0.04[c]	1.024±0.05[a]
Equivalent CO_2 sequestration (lbs/ m^2)	3.36±0.04[a]	5.19±0.05[b]	4.547±0.04[c]	3.540±0.05[d]
Carbon sequestration (Kg/ha)	4136.87±40.17[a]	6406.96±40.29[b]	5611.504±40.02[c]	4364.001±40.68[d]
Equivalent CO_2 sequestration (kg/ ha)	14954.31±40.17[a]	23277.98±40.29[b]	20361.291±40.02[c]	15787.115±40.68[d]

Within the row means followed by same letter(s) are not significantly different at $P<0.05$ by Tukey (HSD) test

Table 5. Different attributes of the selected trap crops [sunflower (*H. annuus*; cv. PAC-36), jute (*C. capsularis*; cv. Sonali; JRC-321) and castor (*R. communis*; cv. DPC-9)] for sesame (*S. indicum*; cv. Rama) cultivation as main crop for ecosystem service-based management of *S. obliqua* Walker and or other such pest species

Parameters	Sole crop Sesame	Main crop Sesame
Plant density (number/m^2)	30±2[a]	30±2[a]
Trap crop density (number/m^2)	-	Sunflower:12±2; Jute:30±2; Castor:8±2
Land area used for sesame (%)	96.11±0.00[a]	89.11±0.00[b]
Land area used for others (%)	3.89 (Boundary gap)[a]	10.89 (Trap crop)[b]
S. obliqua infestation (number/m^2)	16.23±2.41[a]	7.44±2.12[b]
Other pests infestation (number/m^2)	11.62±2.32[a]	5.74±2.15[b]
Occurrence of predators (number/m^2)	6.53±2.14[a]	8.98±2.06[b]
Production cost (Rs/ha)	21900.00±57.74[a]	22500.00±52.44[b]
Seed produced (kg/ha)	635.33±6.43[a]	712.23±7.22[b]
Net profit (Rs/ha)	4983.95±57.74[a]	7613.45±52.44[b]
Benefit cost ratio (BCR/ha)	0.23±0.00[a]	0.34±0.00[b]
Biomass produced (lbs dry wt/ m^2)	2.26±0.04[a]	2.62±0.06[b]
Carbon sequestration (lbs/ m^2)	0.97±0.04[a]	1.12±0.06[b]
Equivalent CO_2 sequestration (lbs/ m^2)	3.36±0.04[a]	3.89±0.06[b]
Carbon sequestration (kg/ha)	4136.87±40.17[a]	4807.32±42.03[b]
Equivalent CO_2 sequestration (kg/ ha)	14954.31±40.17[a]	17412.60±42.03[b]

Within the row means followed by same letter(s) are not significantly different at $P<0.05$ by Tukey (HSD) test

Thus, relatively low food quality of sesame was made it less preferred host to *S. obliqua* than other crops and which was ultimately supported trap crops (sunflower> jute>castor) selection for sustainable production of sesame. In the present investigation, the mean EI and ET values of *S. obliqua* were in the order of sesame> jute>sunflower>castor with significant variations due to their respective damage potential. Even, trap cropping had also supported CSA of sesame because of lower pest infestation without any pesticide use as well as higher BCR value and CSEs than its monoculture. This study will also inform about the susceptibility and or severity of host cultivars towards *S. obliqua* for their judicious management by using ETs as well as defined trap cropping system of sesame or other such crops.

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A review of *Macromia* Rambur, 1842 (Odonata, Macromiidae) of Western Ghats, with taxonomic notes on *Macromia miniata* Fraser, 1924 and *M. irata* Fraser, 1924

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ABSTRACT: A review of the genus *Macromia* Rambur, 1842 (Odonata, Macromiidae) of Western Ghats of Peninsular India is presented with its updated distribution. An attempt is made to collate the scattered data in peer-reviewed literature published to date and is supplemented with field data gathered by the authors over two decades. Although *Macromia* is represented by nine species including six endemics in the Western Ghats, not much has been published on them from the region. *Macromia irata* Fraser, 1924 was described from Coorg but was rarely reported in peer-reviewed literature since its very brief original description by Fraser in 1924. The detailed morphology including that of the genitalia of *M. irata* is discussed. A revised classification based on the species groups and a key to the species of *Macromia* of the Western Ghats of Peninsular India is provided. To quantify the ratios of the number of the prenodal and postnodal veins in Odonata, a new nodal range expression called Standardised Species Nodal Range (SSNR) and a new index termed Standardised Species Nodal Index (SSNI) is also proposed.
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KEY WORDS: Dragonflies, standardised species nodal index, morphometric index, revised classification

INTRODUCTION

Macromia Rambur, 1842 (Macromiidae Needham, 1903), are large to medium-sized dragonflies with large globular eyes and metallic blue or green pterothorax with yellow stripes (Fraser, 1936). The genus presently has about 80 species that range from Afrotropical (Madagascar), Oriental, Australian (Australia, Papua New Guinea), Nearctic, and Palearctic regions (Davies and Tobin, 1985; Paulson *et al.*, 2021). Even though they are primarily found in the Indo-Australian region, a few species are also found in Europe (Boudot, 2010) and North America (Paulson *et al.*, 2021). In India, the genus *Macromia* is represented by 14 species (Subramanian and Babu, 2017). Nine species have been recorded in the Western Ghats (WG) (Fig. 1) as per Fraser (1936), since then, no new species

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have been added (Subramanian *et al.,* 2018). *Macromia annaimallaiensis* Fraser, 1931, *M. bellicosa* Fraser, 1924, *M. cingulata* Rambur, 1842, *M. ellisoni* Fraser, 1924, *M. flavicincta* Selys, 1874, *M. flavocolorata* Fraser, 1922, *M. ida* Fraser, 1924, *M. indica* Fraser, 1924, and *M. irata* Fraser, 1924 are the known species from the WG of Peninsular India. Fraser (1924) described *M. miniata* from the WG but later synonymised it with *M. flavocolorata* (Fraser, 1936). These are rare insects, as evidenced by the paucity of records in published literature and compilations (Tiple *et al.,* 2013; Emiliyamma, 2014; Tiple and Koparde, 2015; Subramanian and Babu, 2017; Subramanian *et al.,* 2018; Tiple *et al.,* 2022). Recently, Nair *et al.* (2021) listed nine species in the WG, of which six are endemic to the area, and they updated the status and distribution of the species for Kerala. Odonates of the genus *Macromia* generally inhabit sub-montane streams and are seen above 600 m ASL, though some are found at the foothills of the WG, especially in the post-monsoon period. They all exhibit strong sustained flight manoeuvres and patrol territories along forest streams and paths (Fraser, 1936).

The *Macromia* species of the Oriental Region had been segregated into species-groups by Laidlaw (1922), based on the colour of the postclypeus, antehumeral stripe, and presence or absence of dorsal spine on S10. Fraser (1924) adapted this concept in his work on the Indian species and referred to the *cincta*, *cingulata,* and *calliope* groups. In the WG, the *flavicincta-bellicosa-irata* species belongs to the *cincta* group; the *indica-annaimallaiensis* cluster is of the *cingulata* group; and *ida-flavocolorata* species constitute the *calliope* group. According to Fraser (1924), the presence or absence of the following characteristics are useful for the species-level identification of *Macromia*– the yellow stripe across the postclypeus, the antehumeral stripe, the spine on the dorsum of segment 10, the spine on the outer side of the superior anal appendage and the shape of genital hamule and lobe of the males. The most useful diagnostic characteristic among them is the structure of the male genitalia (Fraser, 1936).

In this article, the scattered literature on the genus *Macromia* from the WG were presented together, updated the current distribution, provided additional taxonomic notes on species and species groups, illustrations of marking on abdominal segments 2, 7 and 8, and a revised key to the males of *Macromia* of WG. To quantify the ratios of the number of the prenodal and postnodal veins in Odonata, a new nodal range expression called Standardised Species Nodal Range (SSNR) and a new index termed Standardised Species Nodal Index (SSNI) is proposed.

MATERIALS AND METHODS

All published information on *Macromia* from Peninsular India has been reviewed, with additional data from the personal records of the authors. The current distribution is based on Emiliyamma (2014), Subramanian and Babu (2017), Subramanian *et al.* (2018), and Nair *et al.* (2021). Unless specified, all locality records are based on observations by the authors. The general taxonomy of *Macromia* follows Fraser (1936). The species group concepts and classifications from Laidlaw (1922) and Fraser (1924) are also used, and the taxonomic keys have been revised. The current checklist is based on Subramanian and Babu (2017), Nair *et al.* (2021), and Paulson *et al.* (2021). The morphological terminology follows Fraser (1936) and Garrison *et al.* (2006). Wing terminology is based on Riek and Kukalová-Peck (1984). Morphometric data are based on both field-collected specimens and Fraser (1924, 1936). High-resolution images of all *Macromia* holotypes, allotypes, and lectotypes were obtained from the Natural History Museum London (BMNH) online portal https://data.nhm.ac.uk/ as well as the Naturalis Biodiversity Centre, https://bioportal.naturalis.nl., of Leiden Museum.

The classical Nodal Index has been modified into a new concept termed – SSNR, to account for individual variability and thus include the highest and lowest values ever recorded for specimens of a species.

$$SSNR = \frac{\text{Postnodal Range}^{LFW}\text{: Prenodal Range}^{LFW}}{\text{Postnodal Range}^{LHW}\text{: Prenodal Range}^{LHW}} :: \frac{\text{Prenodal Range}^{RFW}\text{: Postnodal Range}^{RFW}}{\text{Prenodal Range}^{RHW}\text{: Postnodal Range}^{RHW}}$$

SSNR is further simplified into the concept of SSNI with the average value of range i.e. (Max +Min)/2, of pre/postnodals, rounded to the nearest integer, expressed as an index for the number of specimens of a species studied.

SSNI is thus the nodal ratios between the forewing and hindwing, expressed as follows:

$$SSNI = \frac{\text{Forewing (Prenodal Average : Postnodal Average)}}{\text{Hindwing (Prenodal Average : Postnodal Average)}}$$

Abbreviations:

BMNH –Natural History Museum, London; OD–Original Description; TNHS–Travancore Nature History Society; TORG–TNHS Odonate Research Group; WLS–Wildlife Sanctuary

RESULTS

Macromia Rambur, 1842

Type species: *Macromia cingulata* Rambur, 1842
OD: Rambur (1842). *Histoire Naturelle des Insectes. Névroptères*. Paris: Librairie Encyclopédique de Roret xvii 534 pp. 12 pls. [137].

Diagnosis: "Tibia of ♂ with a long membranous keel on the flexor surface; the base of hindwing angulated and eyes with a small sinuous projection towards the middle of the posterior border. The discoidal cell of the hindwing distal to the level of arculus, hindwing strongly angulated in ♂s. Discoidal cells on forewing and hindwing always entire and never traversed by veins and discoid field of cells in forewing begin with two rows of cells" *Macromia* can be easily differentiated from the closely resembling *Epophthalmia* Burmeister, 1839 in the WG, by the cell in forewing and hindwing being entire and not traversed by veins, while in *Epophthalmia* the cells in forewing are always traversed (Fraser, 1936).

Macromia annaimallaiensis Fraser, 1931

(Figs.1, 3:1A–C, 10A, 11A, 12C)

OD: Fraser (1931). *Macromia annaimallaiensis* Fraser, *Rec. Ind. Mus.* Vol. xxxiii pp.447, 452, 453 (1931)

Material studied: Images of Holotype, ♂; 013384048 NHMUK London, Mudis Hills, 12.v.29, S. India, F.C. Fraser; Allotype, ♀ 013384047 NHMUK London, Mudis Hills, 12.v.29, S. India, F. C. Fraser.

Measurements: ♂ abdomen 56–59 mm, hindwing 45–48 mm. ♀ abdomen 53–58 mm, hindwing 48–51mm.

Nodal Ratio and index: SSNR– 9:15/12:10:: 15:9/10:11; SSNI–15:9/10:12

Historical Distribution: Mudis Hills in *Anamalais*, May, Tamilnadu (Fraser 1931); Kallar and Shaliyar Rivers Kerala (Fraser, 1936). Confined to hills south of Palghat Gap (Fraser, 1936).

Recent records and Current Distribution: Ponmudi-Kallar near Rajakumari, Munnar, June 2013, 700m (Nair *et al.*, 2021). Thus, the current distribution is confined to the Anamalai Landscape, only in the southern WG.

Taxonomic group: cincta group.

Field Identification: Eyes bottle green; antehumeral stripe absent; S2 with a crown-shaped yellow spot on dorsum (Fig. 11A); S3–6 with paired mid-dorsal spots, S8 is unmarked (Fig. 12C), segment 10 with a dorsal robust obtuse spine.

Anal appendages: similar to that of *M. ellisoni* as per Fraser (1936).

Genitalia: with long hamules (Fraser, 1936).

Ecological notes: This is said to be the most dominant *Macromia* south of the Palghat Gap (Fraser, 1936). A high elevation species as per Subramanian *et al.* (2018). While patrolling, they

Table 1. Distribution and endemic status of *Macromia* species of Western Ghats

No.	Species	Distribution	Endemicity
1	*Macromia annaimallaiensis* Fraser, 1931	Anamalai hills south of Palghat Gap	Endemic to southern WG
2	*Macromia bellicosa* Fraser, 1924	Western Ghats south of Coorg	Endemic to southern WG
3	*Macromia cingulata* Rambur, 1842	Throughout the Western Ghats, Central India, Bengal, and parts of Eastern Ghats	Endemic to Peninsular India
4	*Macromia ellisoni* Fraser, 1924	Western Ghats south of Coorg	Endemic to southern WG
5	*Macromia flavicincta* Selys, 1874	Whole Western Ghats and parts of Eastern Ghats, Central India	Endemic to Peninsular India
6	*Macromia flavocolorata* Fraser, 1922	Peninsular India, Bengal, and Northeast India	None
7	*Macromia ida* Fraser, 1924	Western Ghats south of South Kanara	Endemic to WG
8	*Macromia indica* Fraser, 1924	Western Ghats north of Palghat Gap south of Coorg, Mumbai	Endemic to WG
9	*Macromia irata* Fraser, 1924	Whole Western Ghats from Maharashtra to Agasthyamalais	Endemic to WG

keep to the scrub side and are difficult to follow (Fraser, 1936). The species is a large one like *M. ellisoni*. It flies in the Anamalai hills in May (Fraser, 1931). The species is not strictly a jungle insect.

***Macromia bellicosa* Fraser, 1924**

(Figs. 1, 3: 2A–C, 8H, 10C, 11E, 12E, 15B)

OD: Fraser (1924). A survey of the Odonata (Dragonfly) fauna of Western India with special remarks on the genera *Macromia* and *Idionyx* and description of thirty new species, with Appendix I and II. *Rec. Indian Mus.,* 26: 453–454. (PI. XXV, fig. 9).

Material studied: 1) Images of Lectotype, ♂; 013322948 NHMUK London, India, Coorg, Cannanore Ghat, 28. v. 1923, F. C. Fraser. 2) Field specimens examined (not collected): ♂, Thirunelli, Wayanad Kerala, Broad-leaved Evergreen Forest, 2016 June, 600 m (KS).

Measurements: ♂ abdomen, 45–47 mm, hindwing 40–43 mm. ♀: Unknown.

Nodal Ratio and index: SSNR– 7:16/9:11::15:7/10:9, 7:14/9:10::14:7/10:10; SSNI–15:7/11:10

Historical Distribution: Kudremukh in South Kanara, Cannannore Ghats; Madapur, and Hatti River in Coorg (Fraser, 1924).

Recent records and Current Distribution: Thattaekkad, Kerala, <200 m, Secondary Forests-Tropical Evergreen Forest, 2014 (Aby P Varghese); ♂, Neriamangalam in Lower Periyar Valley, Kerala State, <200 m, Tropical Wet-Evergreen Forest in Varghese *et al.* (2014) as 'Image 10. Macromia annaimalaiensis'; ♂, Thirunelli, Wayanad Kerala, Broad-leaved Evergreen Forest, 2016 June, 600 m (KS); ♂, Coorg, Karnataka, 600 m, March 2018, (Daniel V Raju); Aryanad, Thiruvananthapuram (Chandran and Chandran, 2021); Aaralam Wildlife Sanctuary, Kannur (Nair *et al.,* 2021). Thus, the current range of its distribution includes Coorg, Wayanad, Annamalai, and Agasthyamalai Landscapes in the WG south of Coorg.

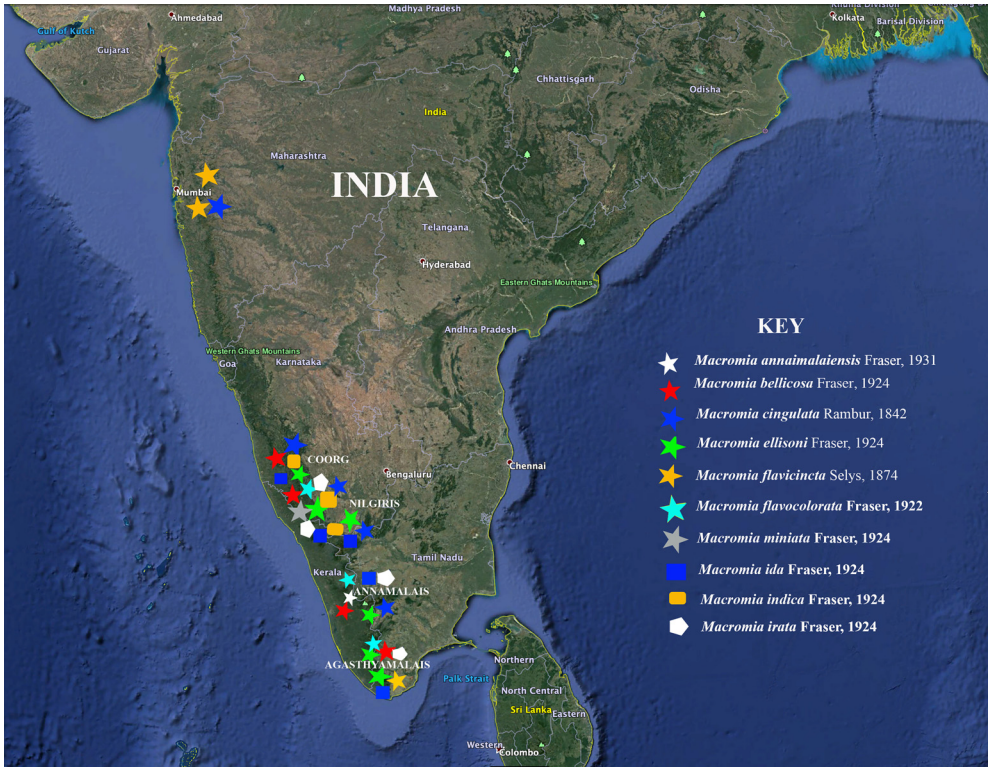

Fig. 1 Map of Western Ghats with current distribution of *Macromia* species

Taxonomic group: cingulata group.

Field Identification: A small species with wings less than 45 mm; face black marked by citron-yellow and black; antehumeral stripe present; S2 with whole of dorsum yellow, distal border bow-shaped, convex (Fig. 11E); S7 basal region up to jugum yellow, this yellow extends broadly across jugum, expands laterally forming a bilobed transverse patch; S8 with a bilobed transverse patch of yellow occupying basal third (Fig. 12E). Appendages ochreous. Restricted yellow markings and the shape of genitalia will distinguish it from *M. flavicincta.* Fraser (1936) mentioned that in specimens from Kanara, the basal annulus in S7 covers half, and S8 may be interrupted widely with a dorsal black line, separating the spots.

Anal appendages: Dark ochreous to reddish-yellow, of equal length. Superior, flattened, tapering to a fine point with a medial robust spine on outer side. A few small teeth beneath apex. Inferior curved gently up, narrowly triangular, faintly bifid apically (Fraser, 1924). Generally structured like that of *M. flavicincta* but more curved and more robust than lateral spine (Fraser, 1936).

Genitalia: Hamules broad at base, thinning to a robust long hook lying parallel to lobe, its end curved like a button hook; lobe tiny, lying in same sinuous line as ventral border of segment, not angulated out at all to latter, produced backward and well angulated with an apical border of segment (Fraser, 1924). Resembles that of *M. flavicincta*, however, ventral border of segment 2 in same straight line as lobe, hamules as long as lobe.

Ecological notes: Jungle insect of submontane streams (Subramanian *et al.,* 2018). The flight period is May.

***Macromia cingulata* Rambur, 1842**
(Figs.1, 8A, 9C,D, 10B, 11D, 12G, 15F)

OD: Rambur (1842). Ins.Névrop.P.137;

Material studied: Field specimens examined (not

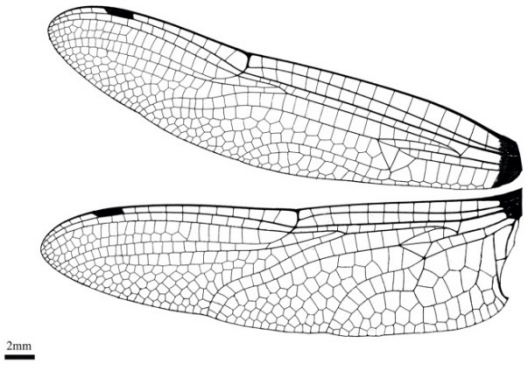

Fig. 2 *Macromia* wing venation. Drawn based on *Macromia irata* Fraser, 1924 **(**TORG 1006). © Kalesh Sadasivan

collected): ♂, Ponmudi, Munnar, Kerala, 800 m, June 2013 (KS).

Measurements: ♂ abdomen 39–45 mm, hindwing 32–36 mm. ♀ abdomen 42–43 mm, hindwing 38 mm.

Nodal Ratio and index: SSNR– 5:12/6:7::12:6/7:6, 7:14/9:8::14:6/8:8; SSNI–13:6/8:7

Historical Distribution: Mullah canal and Byrobah nullah in Poona. Distributed all along the WG from Khandala to Coorg. Mahableshwar, Cauvery River, Fraserpet, Coorg (Fraser, 1924). Hasanur on Mysore border, Coimbatore, Totapalle in Agency Tracts (Eastern Ghats?) (Fraser, 1936).

Recent records and Current Distribution: ♂, Ponmudi, Munnar, Kerala, 800 m, June 2013; ♂ (Teneral), Palghat, Kerala, 50 m March 2020 (Sharan Venkatesh); ♂, Kovilakathumuri in Nilambur, Kerala, 40 m, Teak Plantation, October 2015 (Divin Murukesh), Aryanad, Thiruvananthapuram (Chandran and Chandran, 2021) and November 2020 at Attingal, Thiruvananthapuram (KS). Thus, it is seen in the northern WG, Coorg, Wayanad, Nilgiris, Anamalais, and Agasthyamalais landscapes in the WG. It has been recorded from Bhor Wildlife Sanctuary, Wardha, Maharashtra (Tiple, 2020), Jabalpur, Madhya Pradesh (Tiple *et al.*, 2022), Amboli-Chaukul-Parpoli region of Maharashtra (Sawant *et al.*, 2022), Purulia, West Bengal (Dawn, 2021) and Bankura district, West Bengal (Roy *et al.,* 2022). Hence, the current distribution is Peninsular India, narrowly reaching up to the north-eastern plains.

Taxonomic group: cingulata group.

Field Identification: A small species with wings less than 45 mm; face black marked by citron-yellow and black; antehumeral stripe present; S2 with dorsum yellow, proximal margin bow-shaped with a central concavity, paradorsally yellow extends as small triangular extension distally (Fig. 11D); S7 with basal yellow annulus that extends distally on carina as a diamond-shaped yellow spot, S8 basal annulus with convex distal border, centrally trilobed, annulus grossly resembling a crown (Fig. 12G). Anal appendages black.

Anal appendages: "Black, as long as segment 9, superiors tapering to a point, directed straight back, inner border straight, outer border slightly convex with a robust spine nearer to apex than to base, followed by a row of teeth below. Inferior appendage paler, narrowly triangular, apex slightly upturned, overlapping apex of superiors" (Fraser, 1936).

Genitalia: Basal three-fourths wider and flat, with distal fourth hooked. Genital lobes long, triangular, and sharp-tipped (Fig. 8A).

Ecological notes: This is a species of low-mid-elevation jungle streams, seen on territorial patrols along streams and river edges. Males may be seen hanging on shrubs and trees, sunning themselves at the edges of forests. Flight is usually long and sustained, going back and forth along the same region of the river every 5 minutes or so, usually on bright sunny mornings and afternoons. This is probably the commonest of all *Macromia* as per our field data. They prefer slow-flowing, shallow, pebble streams. The flight period is April-November.

***Macromia ellisoni* Fraser, 1924**

(Figs.1, 3: 3A,B, 8C, 9A,B, 10D, 11F, 12B, 15E)

OD: Fraser (1924). A survey of the Odonata (Dragonfly) fauna of Western India with special remarks on the genera *Macromia* and *Idionyx* and

description of thirty new species, with Appendix I and II. *Rec. Indian Mus.,* 26: 457–458. (PI. XXV, fig. 3).

Material studied: 1) Images of Holotype, ♂, 013384050 NHMUK London, 4,000 ft., Sigur, Nilgiris, Mysore Ditch, S. India, 7. x. 21, F.C. Fraser. 2) Field specimens examined (not collected): ♂, Pandipathu in Peppara Agasthyamalais, Kerala, 700 m, Broad-leaved Evergreen Forest, April 2013 (KS); ♂, Kalakkad, Tamilnadu, Broad-leaved Evergreen Forest May 2013 (KS).

Measurements: ♂ abdomen 49-52 mm, hindwing 47-49 mm. ♀abdomen 54-56 mm, hindwing 53-59 mm.

Nodal Ratio and index: SSNR– 12:17/12:11::18:11/12:12, 9:16/13:11::15:10/11:11: SSNI– 17:11/12:12

Historical Distribution: Coorg (Sampaji River) Karnataka and Nilgiris (Sigur, Devalashola), Nilgiri-Wayanad (Fraser, 1924). It is recorded that *M. ellisoni* is rare in the Nilgiris and more common in Coorg (Fraser, 1936).

Recent records and Current Distribution: ♂, Pandipathu in Peppara, Agasthyamalais, Kerala, 700 m, Broad-leaved Evergreen Forest, April 2013 (KS); Edamalakudi, Mangulam, May 2022 (Nair *et al., in press*); ♂, Kalakkad, Tamilnadu, Broad-leaved Evergreen Forest May 2013 (KS); Kurichi in Konni Forest Division, Kerala, November 2014 (Pradeepkumar *et al.,* 2014); ♂, Pampadum Shola National Park, Kerala, May 2015, Montane Temperate Forest, 2000 m (KS); and Aaralam, Kannur, Kerala, October 2016 (Palot and Kiran, 2016). Thus, from Coorg, Wayanad, and Nilgiris Landscape, the range is extended further southward to include Anamalais and Agasthyamalais. Hence the current landscape distribution is from Agasthyamalais to Coorg.

Taxonomic group: cingulata group.

Field Identification: Eyes brilliant bluish emerald green; well-defined yellow humeral stripe, antehumeral stripe present; abdomen in dorsal view–S2, with a central diamond-shaped black patch that interrupts yellow on its dorsum (Fig. 11F); S3–S6 paired middorsal spots, S7 basal annulus dorsally not extending beyond jugum. S8 unmarked, anal appendages black.

Anal appendages: "Cerci and inferiors of equal length, superior tapering, pointed, without external spine at middle, a few minute spines beneath middle third. Inferior narrowly triangular, almost straight, curving a little up at apex" (Fraser, 1924). "Cerci longer than segment 10, straighter than in *M. indica*, apex not curled up as in *M. indica*, small spine on outer border almost vestigial, a row of small teeth below, which extends to base of appendage. Inferiors shorter than superiors resemble that of *M. indica"* (Fraser, 1936). *Macromia ellisoni,* according to Fraser (1924), does not have a spine on lateral aspect of cerci, however, in all specimens studied as well as in figure 53 (page 170) of Fraser (1936), the rudimentary lateral tooth on cerci is discernible.

Genitalia: Hamules very stout and tumid, not tapering but with a tiny spine springing abruptly from the apex, lobe short and rounded, directed ventrad (Fraser, 1924), (Fig. 8C).

Ecological notes: Adult males were seen far away from water, patrolling forest paths at mid-elevations in the sunny and humid forenoons. They also hang on bushes and shrubs in the late afternoons and overcast weather. Fraser (1936) conversely comments that they are seldom seen away from water. They prefer fast-flowing forest streams.

Macromia flavicincta **Selys, 1874**
(Figs. 1, 3:4A–C, 8B, 11C, 12J, 15D)

OD: Selys (1874). Bull. Acad. Belg. (2) Vol. xxxvii p. 25.

Material studied: Images of Lectotype: ♂ 013322958 NHMUK, London, Allotype: ♀ 013322941 NHMUK, London.

Measurements: ♂ abdomen 47"50 mm, hindwing 41"43 mm. ♀abdomen 50"53 mm, hindwing 43"44 mm.

Nodal Ratio and index: SSNR– 6:14/10:10:: 16:7/10:9; SSNI–15:7/10:10

Historical Distribution: The type locality is Bengal,

it was recorded from Mahableshwar and Poona in Maharashtra (Fraser, 1924), Padera in Agency Tracts (Eastern Ghats?), Andhra Pradesh.

Recent records and Current Distribution: The species is restricted to suitable localities in Peninsular India and adjoining areas of Bengal. It has been reported from Ponmudi Hills, in Agasthyamalais (KS); Nagpur city (Tiple *et al.*, 2013); Bhankura District, West Bengal (Roy *et al.*, 2022) and Bhor Wildlife Sanctuary, Wardha, Maharashtra (Tiple, 2020). The current distribution is thus northern WG, Bengal, Eastern Ghats, and Agasthyamalais.

Taxonomic group: cingulata group.

Field Identification: A small species with wings less than 45 mm; face ferruginous/reddish brown/ amber-brown, and yellow, with a 'T' shaped mark on crest of frons; Antehumeral stripe present; S2 broadly yellow with distal mid-dorsal end bifid (Fig. 11C); S8 with basal yellow annulus/spot; anal appendage dull ochreous. Genital lobe much more angulated with caudal margin of S2 than its ventral margin. As per Fraser (1936), S7 has a broad annulus covering more than half of basal part, S8 with a similar ring occupying less than basal half, and S9 with a small basolateral transverse spot; this is evident in the Lectotype ♂ 013322958 NHMUK, London (Fig. 12J). Field specimens vary in the pattern of colour. S7 has a basal yellow annulus with a broad extension beyond jugum, this extension tends to spread transversely in some specimens forming a short transverse band. Annulus on S8 usually replaced by a pair of small yellow paradorsal basal streaks separated by a wide black area along carina in southern specimens; while samples from drier eastern slopes much yellower with boldly marked annuli. S9 unspotted dorsally with basolateral transverse spot on each side (Fig. 15D).

Anal appendages: "Dull ochreous; cerci as long as segment 9, inner surface slightly concave, outer border nearly straight with a robust spine at its middle tipped with black, apex of appendage curved out but directed straight back as seen in profile, a row of small teeth below following lateral spine" (Fraser 1936).

Genitalia: Genitalia similar to that of *M. bellicosa,* however, with posterior border of genital lobe more angulated. Hamule similar to that of *M. bellicosa,* however, distal two-thirds are rather straight (Fig. 8B).

Ecological notes: After *M. cingulata,* this seems to be a common species, though not much is known about its habits and breeding ecology. It may be seen resting at noon on shrubs, sometimes in small, loose groups.

Macromia flavocolorata **Fraser, 1922**

(Figs. 1, 4:1A,B, 5A,B, 5C,D, 5E,F, 6B,D, 11J, 12I)

OD: Fraser (1922b). JBNHS. Vol. XXVIII, p.702, fig.2 (♀, as *Macromia flavocolorata* Fraser, 1922). Fraser (1922a). New and rare Indian Odonata in the Pusa collection. Mem. Dept. Agric. India (Ent.), 7: 67–68. (♂, as *Macromia atuberculata*)

Material studied: 1) Images: Holotype, ♂ NHMUK 013384052; NHM London, Hasimara, Duars, Bengal, 20. x.21, H. V. O'Donel as per type label; Allotype: ♀NHMUK 013384051, Hasimara T. E., Duars, Bengal, 7. viii.31, H. V. O'Donel, as per type label and Kimmins (1966); Allotype: ♂ NHMUK013322959 as *M. atuberculata* Fraser, 1932, collected by C.M.Inglis without a date as per original description in Fraser, F.C. 1922a. (C.M. Inglis coll. 1920 from Hasimara, Duars, [Bengal], as per Kimmins 1966); Images of Lectotype, ♂, RMNH.INS.JVT.3858 Leiden Museum, Coorg, Somwarpet, 1.vii.23.

2) Field Specimens examined (not collected): ♂, Thenmalai, Kerala, 200 m (Fig. 15G), November 2013 (KS); Thirunelli in Wayanad, Kerala, Broad-Leaved Evergreen Forest, May 2016 (KS).

Measurements: ♂Abdomen 44–47 mm. Hindwing 37 mm. ♀Abdomen 43 mm. Hindwing 38 mm.

Nodal Ratio and index: SSNR– Holotype ♂ NHMUK 013384051– 6:15/7:9::15:6/9:9; Allotype ♀NHMUK 013384052– 9:18/10:11::16:8/10:11, ♂ NHMUK 013322959– 8:15/9:10::15:6/11:8; SSNI– 16:8/11:10; Lectotype, ♂, RMNH.INS.JVT. 3858 Leiden Museum– 7:16/11:9: :14:6/10:11; SSNI– 15:7/ 10:11

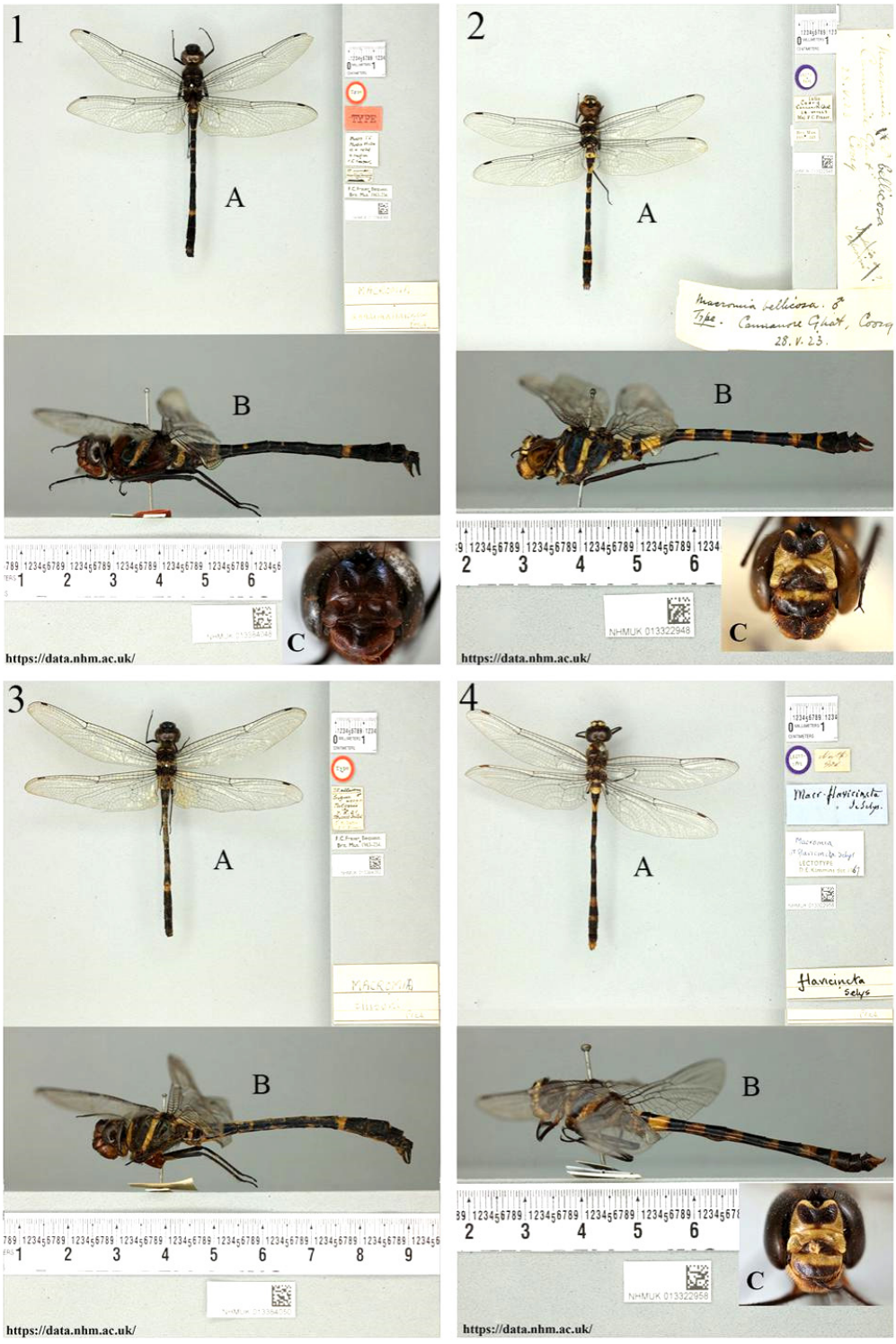

Fig. 3 NHMUK Holotype and Lectotype Images**:** 1–*Macromia annaimallaiensis* Fraser, 1931 Holotype: A–dorsal view, B–lateral view, C–Head front view; 2–*Macromia bellicosa* Fraser, 1924 Lectotype: A: dorsal view, B–lateral view, C–Head front view; 3–*Macromia ellisoni* Fraser, 1924 Holotype: A–dorsal view, B–lateral view; 4–*Macromia flavicincta* Selys, 1874 Lectotype: A–dorsal view, B–lateral view, and C–Head front view. All images © Natural History Museum London

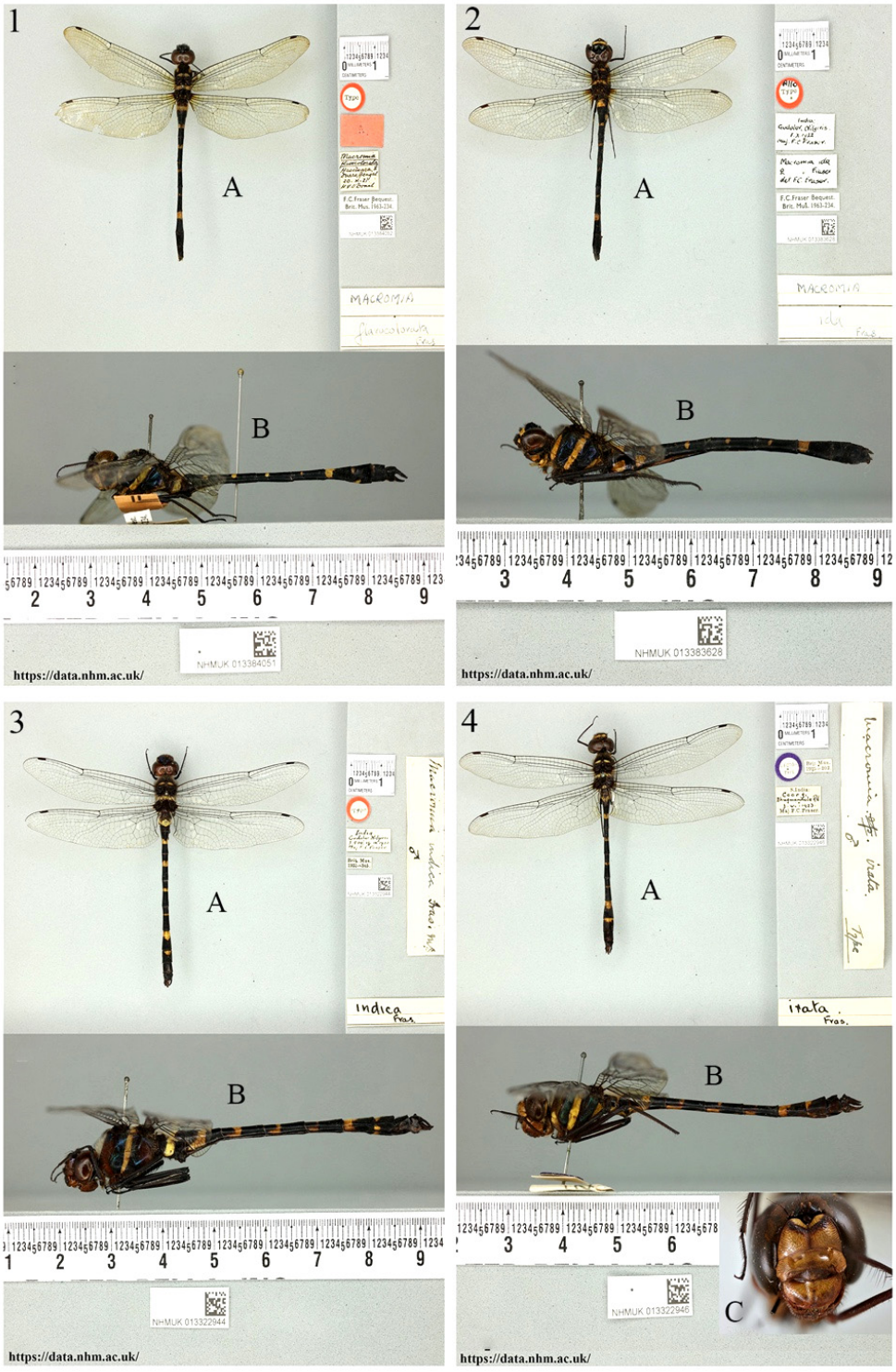

Fig. 4 NHMUK Holotype, Allotype, and Lectotypes: 1–*Macromia flavocolorata* Fraser, 1922, Holotype: A–dorsal view, B–lateral view; 2–*Macromia ida* Fraser, 1924 Allotype: A–dorsal view, B–lateral view; 3–*Macromia indica* Fraser, 1924 Holotype: A–dorsal view, B–lateral view; 4–*Macromia irata* Fraser, 1924 Lectotype: A–dorsal view, B–lateral view, C–Head front view. All images © Natural History Museum London

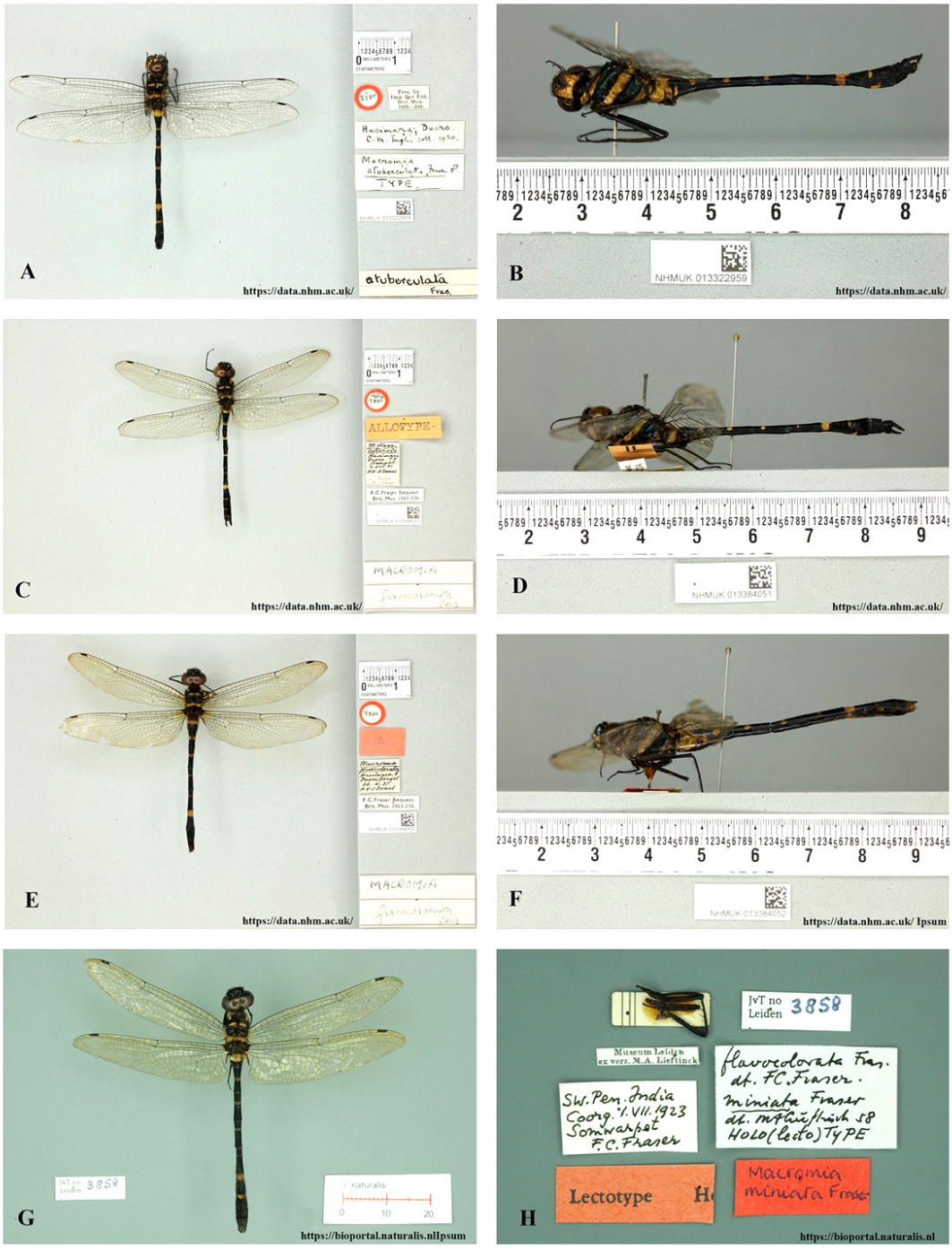

Fig. 5 A and B–Holotype ♂ *M. atuberculata* Fraser, 1932 (*Macromia flavocolorata* Fraser, 1922) NHMUK 013322959; C, D–♂ *Macromia flavocolorata* Fraser, 1922 NHMUK 013384051; E, F–Holotype ♂ *Macromia flavocolorata* Fraser, 1922, NHMUK 013384052; G, H–*Lectotype*: ♂ *Macromia miniata* Fraser, 1924 (*M. flavocolorata* Fraser, 1922) RMNH.INS.JVT.3858 ©A–F images © Natural History Museum London and G, H - RMNH Leiden Museum

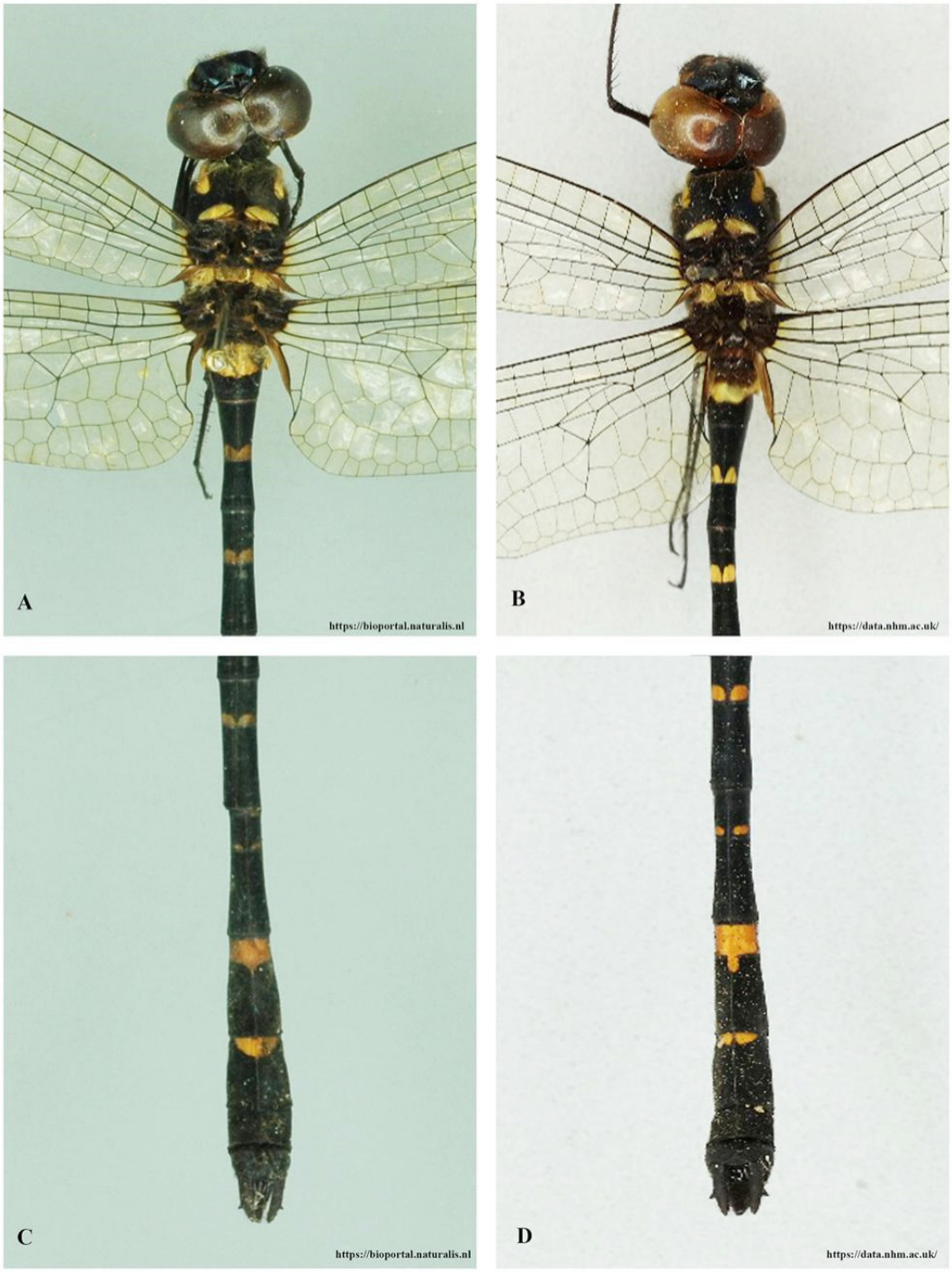

Fig. 6 A–Dorsum of proximal abdomen of *Macromia miniata* Fraser, 1924 (*M. flavocolorata* Fraser, 1922) RMNH.INS.JVT.3858; B–Dorsum of proximal abdomen of *Macromia flavocolorata* Fraser, 1922 ♂ NHMUK 013384051; C–Dorsum of terminal abdominal segments of *Macromia miniata* Fraser,1924 RMNH.INS.JVT.3858; D–Dorsum of terminal abdominal segments of *M. atuberculata* Fraser, 1932 (*M. flavocolorata* Fraser, 1922), holotype ♂ NHMUK 013322959. © Natural History Museum London and RMNH Leiden Museum

Historical Distribution: Somwarpet in Coorg Karnataka; Cannannore Ghat, Kerala (Fraser, 1924); the whole of the west coast of Peninsular India north of the Palghat Gap and rarely southwards. Anamalai and Malabar "in synonymy with *M. miniata* Fraser, 1924 (Fraser, 1936). Somwarpet in Coorg, Karnataka and Cannannore Ghat, Kerala (Fraser, 1924)

Recent records and Current Distribution: ♂, Thenmalai, Kerala, 200 m (Fig.15G), November 2013 (KS); Kanichar, Kannur June 2021 (Nair *et al.*, in *press*); Thirunelli in Wayanad, Kerala, Broad-leaved Evergreen Forest, May 2016 (KS); ♂ Mukkali, Silent Valley, secondary forest. Kerala (Fig. 15C), June 2012 (Biju PB), and Aryanad, Thiruvananthapuram (Chandran and Chandran, 2021). The current distribution is the WG from Agasthyamalais to Coorg, Nilgiris, Bengal, Maharashtra, further eastwards to Laos and Vietnam as per Fraser (1936) and Sawant *et al.* (2023).

Taxonomic group: calliope group (see below under *M. miniata* Fraser, 1924 for taxonomic comments).

Field Identification: Small size and restricted markings will separate it from all other *Macromia*, except *M. ida* which is similar (Fraser, 1924). Eyes emerald green; base of labium ochreous, borders diffusely dark brown, two colours gradually blending; antehumeral stripe well-developed extending beyond halfway to dorsum of pterothorax and superior edge well-defined; Abdomen dorsal view-S2 dorsum generally yellow, this may be reduced in some specimens, as in NHMUK 01338405; this yellow interrupted with black from sides at base, one-third of distal marking always bilobed (Fig. 11J); S3–S6 with paired mid-dorsal spot; S7 dorsal basal spot extends into carina as a tongue-shaped yellow carinal band; S8 with paired small basal paradorsal yellow triangular spot (Fig. 12 I); S10 without dorsal spine; ♂ genitalia posterior hamule with apical two-thirds narrow; anal appendages black. The specimens from the WG have some differences as follows: abdomen in dorsal view-S2 with single large dorsal yellow shield-shaped patch having mid-border produced distally into a small triangular extension (Fig. 11I); S3–S6 with paired mid-dorsal spots; S8 with single basal dorsal yellow triangular spot (Fig. 12 H).

Anal appendages: Described as *M. atuberculata* in Fraser (1922b). Superior anal appendages black, armed with a robust spine at outer side of apical third. Inferior black, subtriangular, curling up at apex. S10 smooth, without spine. No illustrations were given by Fraser (1922b), (Fig. 10E). "Anal appendages black, equal in length, superior tapering but slightly, ending in a fine point turned slightly outward. Outer border a little distal to middle of the appendage, with a very robust tooth. Inferior appendage triangular, concave above as seen in profile, its apex turning up between superiors" (Fraser, 1924).

Genitalia: Posterior hamule of ♂ genitalia with apical two-thirds narrow. Posterior hamules tumid at base but rapidly thinning and drawn out into a very long attenuated spine running parallel with genital lobe, reaching its apex. Genital lobe small, triangular, directed straight back (as in *M. miniata* in Fraser, 1924). It was not mentioned or illustrated in the original description of *M. atuberculata* Fraser, 1932.

Ecological notes: May–July is the flight period otherwise not much is known about its ecology.

Taxonomic comments:

Macromia miniata was described by Fraser from ♂ specimens in 1924, from Coorg and Cannannore. Later in 1936, he synonymised it with *M. flavocolorata*. *Macromia flavocolorata* was originally described from Bengal based on a ♀ specimen (Fraser, 1922a), the ♂ was later described in the same year from Bengal as *M. atuberculata* in Fraser (1922b). The description of the ♂ did neither include description or illustration of the genitalia nor illustration of the anal appendage. Under *M. atuberculata,* Fraser mentioned that the superior anal appendage was black, armed with a robust spine at the outer side of the apical third, inferior black, sub-triangular, and curling up at its apex.

Fraser (1936), mentions some morphological differences between the Bengal and South Indian specimens of *M. flavocolorata* that the latter are generally darker with fewer yellow marks and the former have more yellow markings. In the males - 1) segment 2 basal half is citron-yellow in South Indian specimens (Fig. 6A) while in Bengal and Burmese specimens, basal three-fourth is citron-yellow (Fig. 6B); 2) a large quadrate black spot that interrupts the annulet just above the oreillet in segment 2 is present in South Indian specimens and absent in Bengal and Burmese samples; 3) S8 with large triangular dorsal basal spot (Fig. 6C), and a quadrate spot at the base on each side in South Indian specimens, while the mid-dorsal basal spot is replaced by paired spots in specimens from Bengal and Burma (Fig. 6D). In the case of the ♂ specimen as per Fraser (1936), the S2 yellow markings, though variable in females, was restricted in South Indian form and was very broad in Bengal specimens with only a small apical black margin; and S3 with very large confluent spots in Burmese specimen, while south Indian specimens have distinct paired dorsal spots adjoining the jugum and basolateral triangular spots.

The images of *Lectotype* ♂; RMNH.INS. JVT.3858 Leiden Museum were compared with ♂ NHMUK 013384052, ♂NHMUK 013384051, and ♂ NHMUK 013322959 at NHM London. The original descriptions of Fraser (1922a, b) and Fraser (1924) were also compared. The male abdomen was slightly longer in *M. miniata* (47mm) compared to 44 mm in *M. flavocolorata.* Hindwing length was equal in both specimens at 37mm. Labium was brownish yellow at the base, broad black at the borders in *M. miniata,* and brown in *M. flavocolorata.* There are some differences in the venation. The hypertrigones are traversed thrice in forewing and twice in hindwing in *M. miniata*, while 3–4 times in forewing and two times in hindwing in *M. flavocolorata.* The anal loop in hindwing is 6–7 celled in *M. miniata* (007519114-RMNH), while it is 13–14 celled in ♀ NHMUK 013384052, 6 celled in NHMUK 013384051, and 6–7 celled in NHMUK 013322959 of *M. flavocolorata*. The nodal index in *M. miniata* (007519114-RMNH) is 7:14/9:10::15:6/11:9 and 7:16/11:9:: 14:6/10:11 in the original description (could be the data for the lost Cannannore Ghat specimen). The nodal index for ♀ NHMUK 013384052 is 9:18/10:11::16:8/10:11, NHMUK 013384051 is 6:15/7:9::15:6/9:9 and NHMUK 013322959 is 8:15/9:10::15:6/11:8. The SSNR for *M. miniata* male is thus 7:15/10:9.5::14.5:6/10.5:10 (7:15/10:10::15:6/11:10) and for *M. flavocolorata* males are 7:15/8:8::15:6/10:8.5 (7:15/8:8::15:6/10:9), thus, lower nodal counts for the hindwings in the latter.

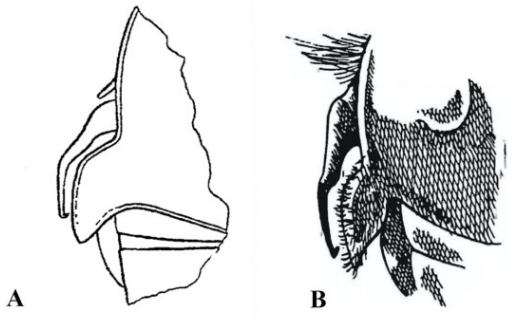

Fig. 7 Comparison of original illustrations of *M. flavocolorata* Fraser, 1922 (A) from Fraser (1936) and *M. miniata* Fraser, 1924 (B), adapted from Fraser (1924)

The colouration of the abdomen in *M. miniata* is also different from that of *M. flavocolorata.* S2 basal half is citron-yellow with its distal border trilobed, while it is bilobed in *M. flavocolorata* males. S3–S5 has a pair of fused sub-dorsal spots in *M. miniata,* while subdorsal triangular spots are almost separate in *M. flavocolorata.* S6 spots in both taxa are separately represented by yellow spots. S7 basal third has a yellow annule with a short tongue-shaped extension to the jugum in *M. flavocolorata,* while its basal third with a yellow annulus with a short linear extension to the dorsal carina in *M. miniata.* S8 with a pair of small baso-dorsal triangular spots in *M. flavocolorata,* while these spots are fused across the midline to form a large triangular base-dorsal yellow spot *in M. miniata.* The cerci are black in both taxa. The lateral tooth is robust, placed on the outer border a little distal to the middle in *M. flavocolorata* while it is less robust and placed on the junction of middle

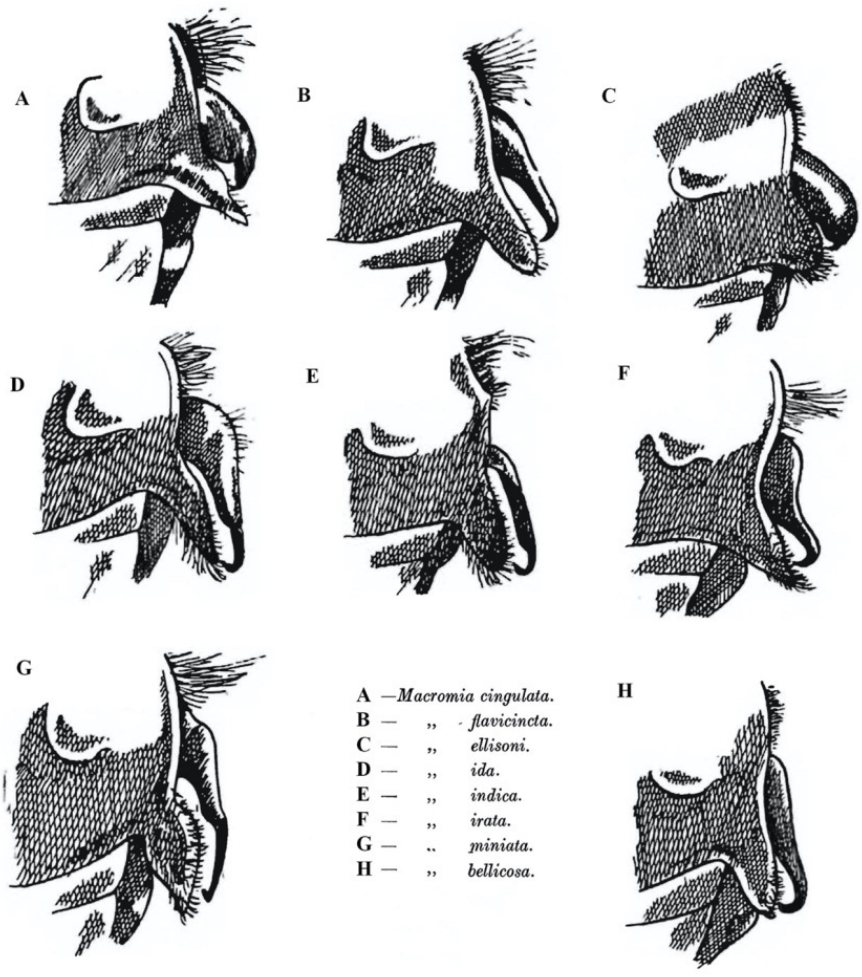

Fig. 8 Genitalia of *Macromia*, adapted from Fraser (1924)

and distal third in *M. miniata.*

The yellow dorsal spot in S2 is shield-shaped with distal end convex or pointed, in *M. miniata* (Fig. 11I); while it occupies the whole segment in well-marked individuals of *M. flavocolorata* and the distal margin is bilobed (Fig. 11J). The markings on S7–8 are very different between the two insects. The S7 in *M. miniata is* having a basal annulus with a sharp dorsal extension along the carina, while it is a broader tongue-shaped extension in *M. flavocolorata.* The S8 has a single yellow basal triangle in *M. miniata*, while it is broken into smaller triangles in *M. flavocolorata.*

Fraser (1936), did not mention any significant difference in male genitalia or appendages. The drawing of the male genitalia of *M. flavocolorata* in Fraser (1936) has differences from that of *M. miniata*, illustrated in Fraser (1924). The hamule is much longer in *M. miniata* while shorter in *M. flavocolorata.* The genital lobe posterior border is leaf-shaped, sharp-tipped, and clefted in *M. miniata,* while it is shallowly angulated in *M. flavocolorata* (Fig. 7).

Fraser had given one male syntype of *M. miniata* Fraser, 1924 to M. A. Lieftinck synonymising it with *M. flavocolorata*. Dr. Lieftinck who examined this specimen considered *M. miniata* Fraser, 1924, to be distinct from *M. flavocolorata* according to Kimmins (1966). The whereabouts of the second syntype (taken from Cannanore) are unknown (Kimmins, 1966). Lieftinck (1971) considered the

species valid and the Somwarpet male specimen was designated as the Lectotype, with southern Peninsular India, Coorg, Somwarpet, 1. VIII. 1923, F.C. Fraser as the specimen data. Since there were no types in the Fraser collection, the description was enclosed in square brackets and it was designated as the Lectotype of *M. miniata* Fraser, 1924 (Kimmins, 1966). Van Tol (1992) referred to this taxon as *M. flavocolorata* Fraser, 1922, which was followed by later authors.

The taxon *M. flavocolorata* Fraser, 1922, is widely distributed from south India to Laos, and this might indicate that it is a species complex. Examination of the original and subsequent descriptions (Fraser 1922 a, b, 1924, 1936), and the analysis of type specimen images, shows that there are some morphological differences between the type specimens from NHMUK and Leiden Museum. This, together with the biogeographical aspects of the collection localities (Coorg in the WG vs Bengal) and substantiated by the taxonomic comments by Lieftinck in Kimmins (1966), might indicate that the WG taxon might be a good species. It is possible that the WG congener of this species complex was possibly described as *M. miniata* Fraser, 1924. The morphological similarities between the specimens later led to Fraser (1936), synonymising *M. miniata* Fraser, 1924 with *M. flavocolorata* Fraser, 1922**.** Integrated taxonomic studies with fresh specimens are needed to confirm the status of *M. flavocolorata* and *M. miniata* in the WG of Peninsular India.

Macromia ida **Fraser, 1924**
(Figs.1, 4: 2A,B, 8D, 11H, 12A)

OD: Fraser (1924). A survey of the Odonata (Dragonfly) fauna of Western India with special remarks on the genera *Macromia* and *Idionyx* and description of thirty new species, with Appendix I and II. *Rec. Indian Mus.,* 26: 449–450. (PI. XXV, fig. 4).

Material studied: Images of Lectotype, ♂ 013322952 NHMUK London, India, Gudalur, Nilgiris, 3,500 ft., 20. ix. 1922, F. C. Fraser; Allotype: ♀013383628 NHMUK London, Gudalur, Nilgiris, 1. x. 1922, F.C. Fraser.

Measurements: ♂ abdomen 42 mm, hindwing 38 mm. ♀abdomen 41 mm, hindwing 35 mm.

Nodal Ratio and index: SSNR– 8:16/10:11::17:7/10:11; SSNI–17:8/11:11

Historical Distribution: Gudalur, Nilgiri-Wayanad, Tamil Nadu, and Bhagmandala in Coorg and S. Kanara in Karnataka (Fraser, 1924).

Recent records and Current Distribution: The WG both north and south of the Palghat Gap, up to south Kanara (Subramanian *et al.*, 2018). Kanichar, Kannur June 2021 (Nair *et al.*, in *press*); Peppara WLS of Agasthyamalais in June 2020 at 200m elevation (KS); Aryanad, Thiruvananthapuram (Chandran and Chandran, 2021). Thus, distributed in Coorg, Nilgiris, Anamalai, and Agasthyamalai Landscapes.

Taxonomic group: calliope group.

Field Identification: Eyes emerald green; base of labium bright chrome-yellow with borders jet-black, two colours being sharply defined; antehumeral stripe well-developed with superior edge well-defined; abdomen in dorsal view-S2 with a butterfly-shaped patch on dorsum, which may be reduced to fine spots in some specimens (Fig. 11H); S3–S6 with paired middorsal spots; S7 dorsal basal spot with convex distal border, not extending beyond jugum (Fig. 12A); S10 without dorsal spine; appendages black; genitalia with posterior hamule broad at base up to distal 3/4th, abruptly narrow distally.

Anal appendages: "Anal appendages black, of equal length. Superiors sloping strongly down and back, tapering to a fine point, parallel, and with a robust external spine situated slightly apicad to the middle of the segment. Inferior triangular, convex dorsally its apex curling gently up between apices of superiors" (Fraser, 1924).

Genitalia: Lobe rather long, truncate, sinuous, pointed; hamules foliate, tumid in basal two-thirds, then abruptly narrowed into a long fine spine with an imbricated apex extending slightly beyond apex of lobe (Fraser, 1924). Hamules basal two-thirds

very broad, lobe is sinuous and attenuated (Fraser, 1936).

Ecological notes: Nothing is known about the ecology of the species except that it is a species of mid-high elevation jungles. They fly over shallow submontane streams with gravel bottoms. The habits resemble that of *M. flavocolorata* and the flight period is October-December.

Macromia indica **Fraser, 1924**

(Figs.1, 4: 3A,B, 5E, 8E, 10A, 11B, 12D)

OD: Fraser (1924). A survey of the Odonata (Dragonfly) fauna of Western India with special remarks on the genera *Macromia* and *Idionyx* and description of thirty new species, with Appendix I and II. *Rec. Indian Mus.,* 26: 448–449. (PI. XXV, fig. 5).

Material studied: Images of Holotype, ♂ 013322944 NHMUK London; India, Gudalur, Nilgiris, 3,500', 14. ix. 1922, F. C. Fraser.

Measurements: ♂ abdomen 57–58 mm, hindwing 45–46 mm. ♀ abdomen 52–56 mm, hindwing 46–50 mm (Fraser, 1924).

Nodal Ratio and index: SSNR– 8:15/11:10 ::15:10/9:10; SSNI–15:9/10:11

Recent records and Historical Distribution: Sigur, Gudalur, Pandy River and Burliyar, Nilgiris Tamilnadu; Cauvery River, Fraserpet in Coorg-Mysore Frontier in Karnataka (Fraser, 1924). Thus, confined to the WG North of the Palghat Gap (Fraser 1936).

Current Distribution: ♂, Karaekattai, Kudremukh National Park, Karnataka, Broad-leaved Evergreen Forest, (Emiliyamma and Radhakrishnan, 2007); the WG north of Palghat Gap according to Subramanian *et al.* (2018). Thus, the current distribution is Coorg, Wayanad, and Nilgiris Landscapes of the WG north of the Palghat Gap.

Taxonomic group: cincta group.

Field Identification: Segment 10 with a dorsal tooth; antehumeral stripe absent; S2 with dorsal distal border convex; abdomen in dorsal view " S2 with a minaret-shaped mark on distal end with lateral extensions along distal margin (Fig. 11B); S3–6 with mid-dorsal yellow annuli; S7 with basal yellow annulus and a large triangular extension through carina across jugum; S8 with dorsal basal yellow triangular spot (Fig. 12D), base of which may extend laterally as a thin annulus (Fig. 15A). Broad annuli on the abdomen and the dark black-brown rays at the base of the wing diagnostic amongst the congeners (Fraser, 1936).

Anal appendages: "Similar to that of *M. moorei* Selys 1874 (Fraser 1936), black, equal in length. Superior a little compressed, sloping and tapering to a fine point turned up and a little out, inner border slightly concave, outer bearing a minute spine at its middle, some fine teeth beneath the apex. The superiors are upturned abruptly and the lateral spines are situated slightly nearer to the apex. Inferior triangular, concave above as seen in profile, its apex turning slightly up between the superiors" (Fraser, 1924).

Genitalia: Similar to that of *M. annaimalaiensis* as per Fraser (1936). Hamules long, fine, tapering, a little tumid at base, apex with a fine imbricated point that extends to extreme apex of lobe, latter directed almost straight back, very narrow, tongue-like (Fraser, 1924) (Fig. 8E).

Ecological notes: The males were seen patrolling stream edges and were always seen along similar water bodies. The flight period is November–December.

Macromia irata **Fraser, 1924**

(Figs.1, 4: 4A–C, 8F, 9E,F, 10F, 11G, 12F, 13, 14)

OD: Fraser (1924). A survey of the Odonata (Dragonfly) fauna of Western India with special remarks on the genera *Macromia* and *Idionyx* and description of thirty new species, with Appendix I and II. *Rec. Indian Mus.,* 26: 454–455. (PI. XXV, fig. 6).

Material studied: 1) Images of *Lectotype,* ♂; 013322946 NHMUK London; S. India, Coorg, Bhagmandala Road, 3. vi.1923, F.C. Fraser; Allotype: ♀013322940 NHMUK London, Napoklu Road, Coorg, 18.v.1924, F.C. Fraser. 2) Field

specimens examined: 4 males, Thenmalai, Kollam District, Rubber Estate, 200 m May 2018 (KS) (TORG 1006, 1007, and 1008, voucher specimens in TNHS); Chatancode in Peppara, Kerala, Semi-Evergreen Forest, 200 m (KS) (not collected).

Measurements: ♂ abdomen 47 mm, hindwing 43 mm. ♀abdomen 46 mm, hindwing 46 mm.

Nodal Ratio and index: SSNR– 9:17/10:12::18:8/12:13, 7:17/10:12::17:8/13:1; SSNI–18:9/13:12

Historical Distribution: Confined to Coorg, Bhagmandala (Fraser, 1924), South Kanara, Bettaferi (Bhasin, 1953) all in Karnataka, Vythiri, Malabar Wayanad, Kerala, May (Fraser, 1931and1936); the WG above the Palghat Gap till Coorg (Subramanian *et al.*, 2018). Reported from Central India by Tiple *et al.* (2013) and Tiple and Koparde (2015), and Amboli, Maharashtra (Swant *et al.,* 2023). Thus, all records are north of the Palghat Gap.

Recent records and Current Distribution: 4 males, Thenmalai, Kollam District, Rubber Estate, 200 m May 2018 (KS); Chatancode in Peppara, Kerala, Semi-Evergreen Forest, 200 m (KS); Vallakadavu, in Periyar, Kerala, May 2016, Tropical Wet-Evergreen Forest, 600 m (Abraham Samuel); Thenmalai, Kollam District, May 2018 (KS); Kanichar, Kannur June 2021 (Nair *et al.*, in *press*); Aryanad, Thiruvananthapuram (Chandran and Chandran, 2021). Thus, the range extends to the south of Palghat Gap, the current distribution is the southern WG south of the Coorg landscape including Nilgiris and Agasthyamalais.

Taxonomic group: cingulata group.

Field Identification: A smaller species with wings less than 45 mm; face ferruginous/reddish brown/amber-brown, and yellow; antehumeral stripe present; genital lobe angulated well with ventral margin of S2 than its caudal margin; characteristic twin diamond-shaped saddle markings on S2 (Fig. 11G) narrowly transected by dorsal carina (Fraser, 1936); S7 with a fan-shaped expansion of dorsal basal yellow spot; S8 with basal yellow annulus/spot; a butterfly spot in S8 finely bisected by a black line along carina (Fig. 12F). The antehumeral stripe is sometimes very rudimentary and represented by only a dirty brown spot, in which case the abdominal markings– the fan-shaped expansion on S7 and butterfly spot in S8 are diagnostic.

Anal appendages: "Anal appendages are generally black but in some the inferior dark reddish-brown. Superior tapering to a fine point turned slightly out and upwards and bearing a sharp, robust spine at the middle of its outer border. The sub-apical fine teeth are not at all evident. Inferior narrowly triangular, curved up as seen in profile and extending slightly but distinctly beyond the superiors" (Fraser, 1924). Superiors as long as segment 10, with a robust spine on its outer border, apex very acute and turned slightly out, similar to *M. flavicincta* Selys, 1874. Inferiors markedly longer than superiors, narrowly triangular, apex gently curved up (Fraser, 1936).

Genitalia: Genitalia is very similar to that of *M. bellicosa*, differing only in the shape of the loop which is of the same small size but is strongly angulated out from the ventral border of the segment and in nearly the same straight line as the apical border, i. e. exactly the opposite condition to that found in *M. bellicosa* (Fraser, 1924). Genitalia is also very similar to *M. flavicincta* Selys, 1874 (Fraser, 1936) (Fig. 8F).

Ecological notes: A considerable number of these insects were seen by Fraser towards the end of April 1923, all flying high and often resting on the uppermost branches of the tallest forest giants. Several males were subsequently seen hawking over a neighbouring stream (Fraser, 1924). It was described as the commonest *Macromia* on the West Coast (of Peninsular India) by Fraser (1936) but had escaped scientific documentation until 1953 (Bhasin, 1953), since its original description in 1924. These are exceptions to the general rule of distribution of the genus as being commoner on lower elevations (<200 m) compared to others of the genus. They prefer stagnant shallow water around Myristica Swamps in shade in the Agasthyamalais. It flies in May (Fraser, 1931). The flight period is from April to June.

Endemicity of *Macromia* in the WG and Kerala

The known distribution of *Macromia* in the WG is given in Table 1, based on all available sources mentioned above.

Species Groups in *Macromia* of Western Ghats

The *Macromia* of the WG is classified according to the principles laid down by Laidlaw (1922), which was followed by Ris (1916). And later, Lieftnick (1929) followed them both in his treatment of the Malayan *Macromia*. The grouping was based on– 1) The colour of the postclypeus, which may be yellow, or may agree with that of the rest of the front of the head which may be reddish-brown or dark brown, 2) The presence or absence of a humeral stripe on the pterothorax; note that a lateral oblique stripe of yellow is present in all Oriental species of the genus, and 3) the presence or absence of a flattened, pointed, triangular process on the dorsum of S10 in ♂.

Laidlaw (1922) classified Oriental *Macromia* into three groups.

I. Group of *M. westwoodi* Selys: Segments 2–6 of abdomen unicoloured, all with a more or less metallic lustre. The front of the head is uniformly dark brown, but the pyramidal processes of the frons metallic green or violet. Males with a pointed triangular process on the dorsum of the tenth segment of the abdomen. Pterostigma small (2 mm or less).

II. Group of *M. cincta* Rambur: Segments 2–6 of abdomen black or brownish-black without metallic lustre. Segments 2–3 at least with yellow markings on the dorsum. Front of head very dark brown, pyramids of frons black, slightly metallic. No definite humeral band on the dorsum of pterothorax. Costal nerve black. Males with the pointed triangular process on the dorsum of segment 10 of the abdomen. Pterostigma about 3 mm.

III. Group of *M. calliope* Ris: Pterothorax black, with a rich metallic lustre, with a humeral band incomplete above. Frons black with metallic lustre, anteclypeus- black or dark brown, postclypeus yellow. The costal nerve is usually entirely black. Segments 2–6 of abdomen black (or in one or two species metallic), the second segment at least with yellow markings on the dorsum. Segment 10 without dorsal process. In most species, the upper anal appendages are straight or incurved apically.

Fraser (1924), classified Indian *Macromia* into four groups–

Group 1–*westwoodi*: Segments 2 to 5 metallic coloured, the humeral band present or absent, no well-defined yellow stripe on face, spine on the dorsum of 10th abdominal segment present. No Indian representatives.

Group 2–*cincta*: Segments 2 to 5 matt black, marked with yellow, humeral band absent, no well-defined yellow stripe on face, spine on the dorsum of 10th abdominal segment present (*M. indica*).

Group 3– *calliope:* Segments 2 to 5 matt black, humeral band present, a well-defined yellow stripe on face, spine on 10th abdominal segment absent (*M. ida; M. flavocolorata*).

Group 4–*cingulata:* Segments 2 to 5 matt black, humeral band present, no well-defined yellow stripe on face, spine on 10th abdominal segment present (*M. cingulata, M. flavicincta, M. irata, M. bellicosa, M. ellisoni*).

Fraser (1936) comments that S2 and S3 in *M. ellisoni* have metallic reflux. This is also clearly evident in live field specimens where segments 2 and 3 and the proximal aspect of S4 and S5 also have a metallic green sheen. Going by Laidlaw (1922), it is seen that S2–S6 must be unicoloured all with more or less metallic lustre for the *westwoodi* group. In *M. ellisoni,* S2–S6 is not unicoloured and is marked in yellow. Group of *M. cincta* according to Laidlaw (1922) must have S2–S6 black or brownish-black without metallic lustre and no definite humeral band on the dorsum of pterothorax, which is not true in the case of *M. ellisoni* which, has a metallic lustre and a humeral band. It does not fall in the group of *M. calliope*

because S10 has a dorsal process in *M. ellisoni,* which must be absent in that group. The front of the head is uniformly dark brown, but the pyramidal processes of the frons metallic green or violet, according to Laidlaw (1922), is a character of the *westwoodi* group. All the species in the cingulata group of Fraser (1924), namely *M. cingulata*, *M. irata, M. flavicincta,* and *M. bellicosa* have non-metallic colours on the pyramid of frons in strict contrast to metallic greenish of *M. ellisoni.* The length of pterostigma is more than 2 mm in *M. ellisoni.* Thus, in summary, *M. ellisoni* has more features of the *westwoodi* group than that of the *cingulata* group. Group 4 of Fraser (1924) had no well-defined yellow stripe on the face according to Fraser (1924) but, Fraser (1936) contradicts it under the description of *M. cingulata* on page 180, *M. flavicincta* on page 172 which states that the postclypeus is citron-yellow in both the species. Thus, the relations among the species in the cingulata group are still unresolved. The Group of *M. moorei* has no representatives in the WG.

Considering the above, a revised classification for *Macromia* species of the WG and Peninsular India is proposed as follows–

Group 1–*ellisoni group*: Segments 2 to 5 with metallic lustre and yellow markings; well-defined humeral band; Front of head uniformly dark brown, but the pyramidal processes of the frons metallic green or violet, postclypeus not yellow; males with S10 dorsal spine *(M. ellisoni).*

Group 2–*cincta group*: Segments 2 to 5 matt black, marked with yellow, humeral band absent; postclypeus not yellow, front of head very dark brown, pyramids of frons black, slightly metallic; males with S10 dorsal spine (*M. indica, M. annaimalaiensis).*

Group 3–*cingulata group*: Segments 2 to 5 matt black, humeral band present; postclypeus citron/ chrome-yellow, the pyramid of frons non-metallic; males with S10 dorsal spine. The anal appendages are closer to the calliope group in *M. cingulata* and anal appendages structurally similar to cincta group *in M. flavicincta, M. irata,* and *M. bellicosa.*

Group 4–*calliope Group*: Segments 2 to 5 matt black; antehumeral band present; Frons black with a metallic lustre, postclypeus yellow; Spine on S10th absent (*M. ida; M. flavocolorata). M. flavicincta, M. irata* and *M. bellicosa* have a similar scheme of genitalia and anal appendage. *M. cingulata* has a scheme of the anal appendages and male genitalia slightly different from others of the same group.

Based on anal appendages, *Macromia* of the WG falls into four groups. *M. ida-flavocolorata* group has a lateral spine at the junction of the distal and middle third, distal third rapidly tapers and angulated inwards but the tip directed outwards. *M. bellicosa-flavicincta-irata* cerci with the lateral robust spine at the middle third, the distal half of cerci knife-shaped, tapering gradually and the distal half of the cerci directed inwards but angulated outwards. *M. indica-annaimallaiensis* is similar to the *M. bellicosa-flavicincta-irata* group. *M. ellisoni* has a spine at the junction of the distal and middle third and the distal third is directed outwards. *M. cingulata* is interesting with the spine in the junction of the distal and middle third, distal third rapidly tapering and angulating inwards.

Based on the anal appendage structure, considering the morphology of the genital lobe and the hamulus, the following five groups are evident. *M. bellicosa-flavicincta-irata* group with the triangular genital lobe and the elephant trunk hamulus. *M. indica-annaimallaiensis* has a triangular genital lobe with a convex anterior border and a hamulus with a constriction at the junction of the proximal and middle third. *M. ida-flavocolorata* group has the tongue-shaped genital lobe with a convex anterior margin and a swollen base of the hamulus with a sinuous end. *Macromia ellisoni* has a rounded genital lobe with a very broad hamulus with a short-curved tip. *Macromia cingulata* has a triangular genital lobe with a sharp extension and a broad hamulus with an angulated distal third and hook at the end.

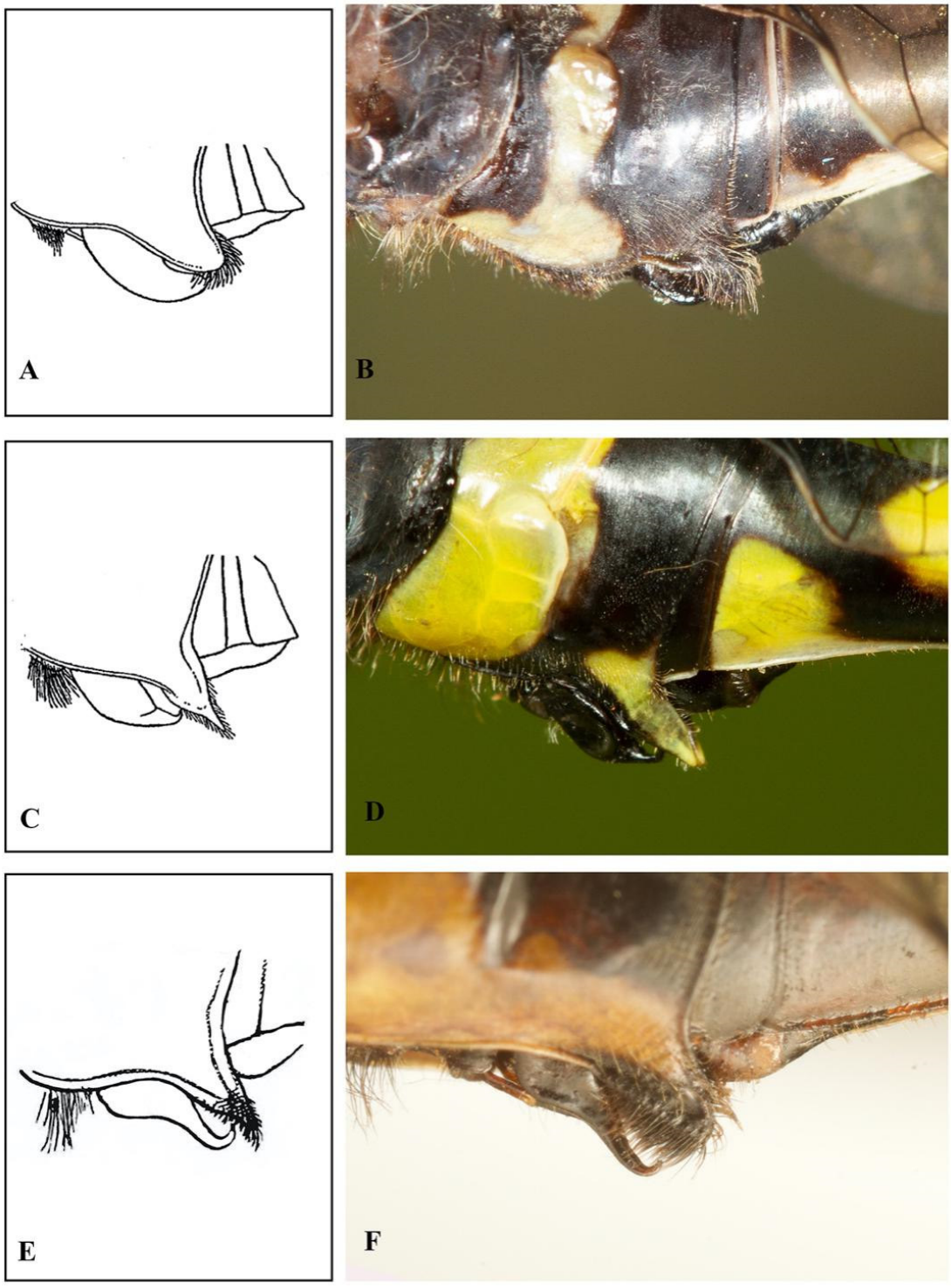

Fig. 9 Genitalia of *Macromia: M. ellisoni* Fraser, 1924 (A, B); *M. cingulata* Rambur, 1842 (C, D); *M. irata* Fraser, 1924 (E,F)

The exact status, composition of these species groups, and their interrelations need to be investigated and ascertained by molecular techniques and integrated taxonomy.

***Notes on Macromia irata* Fraser, 1924**
(Figs.1, 8F, 9E,F, 11G, 12F, 13,14)

Material examined:

1. TORG 1006–♂, dry pinned specimen, India, Kerala, Thenmalai, Kollam District, 50 m ASL., 5th June 2018, Private Rubber estate, collected by the authors, deposited in the collection facility at the TNHS, Trivandrum, India.

2. TORG 1007–♂, dry pinned specimen from the same locality as TORG 1006, collected by the authors on 5th June 2018 deposited in the collection facility at the TNHS, Trivandrum, India.

3. TORG 1008–♂, Wet specimen in 100% Alcohol, from the same locality as TORG 1006, collected by the authors on 5th June 2018 with the same data as the Holotype.

Measurements (in mm)

TORG 1006: Total length 62; length of abdomen 45; length of cerci 2; Fw 40; Hw 39.

TORG 1007: Total length 63; length of abdomen 46; length of cerci 2; Fw 42; Hw 42.

TORG 1008: Total length 63; length of abdomen 46; length of cerci 3; Fw 42; Hw 41.

Description of male

Head. Labium brown, bases of mandible amber yellow; labrum inferior half dark amber-brown, superior half yellow without a sharp demarcation. Anteclypeus brown, postclypeus orange-yellow. Frons yellow, with its medial margin of apices broadly black-bordered. Interface between yellow and black a narrow amber-brown band. Lateral aspect of frons yellow. Dorsal aspect of frons bordering vertex with a yellow triangular patch, one on each side. Vertex dark metallic black with a dark metallic blue reflex. Epicranium, antennae, and occiput black. Eyes bright emerald green anteriorly, bordered with brilliant blue and turquoise laterally. Orbits are black superiorly, yellowish-brown inferiorly, bordered with black laterally near sinuous projection at middle of posterior border.

Prothorax: Simple, as in the genus, without spines or other structure.

Pterothorax. General colour dark metallic green, with two lateral stripes on Epm2 and Epm3, tiny rudiment of antehumeral stripe pale citron-yellow. Underside brown. In dorsal view, dorsal carinae black, crest lined by a thin coppery line that expands into a coppery brown triangle with base caudally. Ante-alar ridge black, thicker medially, ante-alar sinus white. Episternum (Eps2) metallic green on medial half; on coppery brown in lateral half, in the region of the antehumeral stripe. Notum N2, N3 black, post-notum PN2 pale yellowish-white. In lateral view, antehumeral stripe reduced to a small pale citron-yellow rounded triangular spot on either side of suture between episternum (Eps2) and infra-episternum (Ips2); rest of Ips2 brown. Epimerum (Epm 2) metallic green in its superior third, inferior third yellow. This metallic green band on Epm2 wider inferiorly. Superior part of yellow stripe finely spotted in reddish-brown. Eps3 metallic green. Ips3 dark brown. Superior third of Epm3 metallic green, inferior third pale citron-yellow. Metallic green band on Epm3 wider superiorly.

Legs. Coxae brown, whole of trochanter, initial part of femora on extensor aspect brownish-black, rest of legs black.

Wings. Hyaline, edges effumed, pterostigma small covering two cells (2 mm in both wings); proximal border, parallel to preceding cross vein, its distal margin being oblique. Membrane white, eight cells in anal loop, hypertrigones traversed 4 times in forewings, twice in hindwing; six cubital nervures in forewing, four in hindwing. Nodal Index: forewing with 16 prenodal and hindwing with 11, forewing 7 postnodal and hindwing 10 postnodal. Discoidal field two-celled in forewing up to four cells before the node level and extends to eight cells distally and in hindwing it is one cell, to begin with, extends to three cells. Base of hindwing deeply excavated, tornal angle prolonged inwards

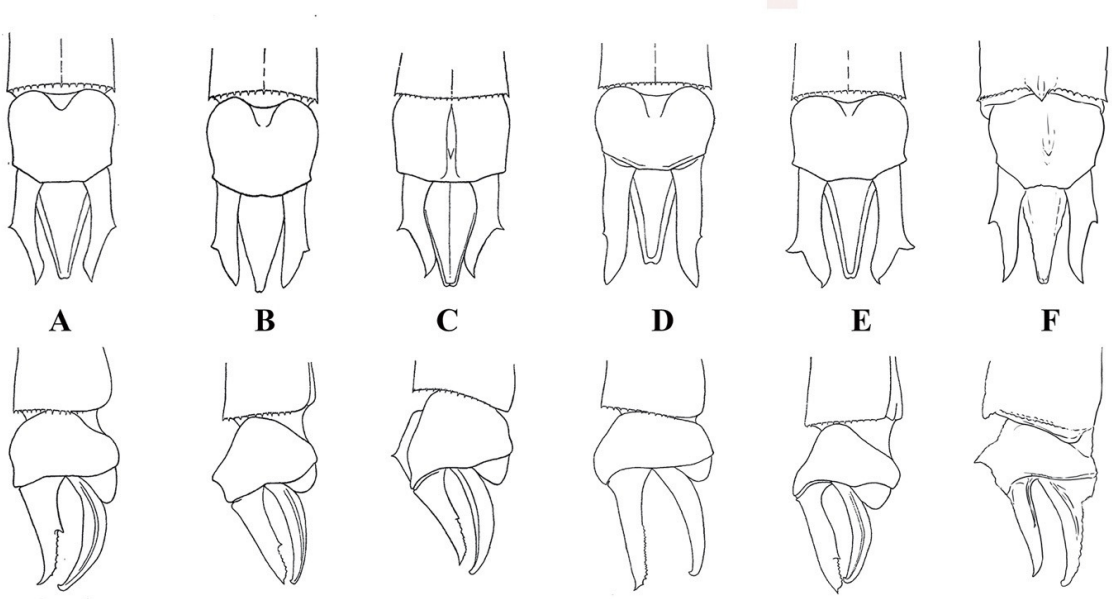

Fig. 10 Anal appendage schemes of *Macromia* of Western Ghats from Fraser (1936). A–*M. annaimallaiensis* Fraser, 1931 & *M. indica* Fraser, 1924; B–*M. cingulata* Rambur, 1842; C–*M. bellicosa* Fraser, 1924 & *M. flavicincta* Selys, 1874; D–*M. ellisoni* Fraser, 1924; E–*M. flavocolorata* Fraser, 1922, *M. miniata* Fraser, 1924 & *M. ida* Fraser, 1924; F–*M. irata* Fraser, 1924. Illustrations © Kalesh Sadasivan

markedly. Anal triangle two-celled.

Abdomen. Black marked with yellow. Segment 1 black; dorsal aspect of segment 2 predominantly black except for pair of pale yellowish-green spots paradorsally. In lateral view, its anteroinferior half, region immediately adjoining genitalia pale citron-yellow. Segments 3–6 black with a pair of mid-segmental paradorsal spots, decreasing in size caudally from segments 2–6. Mid-ventrally S3 bears a large triangular yellow spot at its junction with segment 2. In S4–S6 this triangular spot is reduced to a yellowish-brown streak. Segment 7 has a thick yellow almost annular ring at its base and mid-dorsally this ring forms a small contiguous yellow spot. Width of yellow basal ring almost one-fourth dorsal length of S7. Abdomen widest at segment 8, which is black, dorsally with a butterfly-shaped yellow spot and ventrally with a pair of rudimentary yellowish spots. Segment 9 tergite wholly black, sternite dark brownish-black except near junction with S2 sternite, which has a small ill-defined quadrangular yellow spot. Mid-dorsally caudal end of carina on segment 9 produced into a small tooth. Segment 10 on dorsum, at its middle, with a robust spine; whose cranial border is convex and caudal border with keel concave.

Genitalia. Posterior hamulus resembles an elephant's head and trunk. Basal third spatulate tapers off into an inwardly directed hook at its apex. Genital lobe tooth-like. Posterior hamule slightly longer than genital lobe (Fig. 8F, 9E, F).

Anal appendages. Cerci black, epiproct dark brownish-black, with a robust lateral spine at its middle, directed posterolaterally. Cranial junction of spine with appendage convex, caudal junction concave. Tip of cerci directed posterolaterally. Epiproct dark brown dorsally and its tip blunt, minimally bifid in dorsal view. In lateral view, lateral margined portion of cerci extends till its middle, till lateral spine. Epiproct slightly longer than cerci. Both cerci and epiproct gently curved dorsally.

Variation: Segment 6 sometimes lacks yellow mid-segment dorsal spots otherwise not much variability is observed. Segment 7 with the small contiguous spot on basal annulus can be oval or diamond-shaped. Compared to the types and specimens from

north of the Palghat Gap, the southern specimens are much more extensively marked in black. The frons was marked with black in the upper fourth in northern specimens, while it was more extensive and covered almost the upper third in the southern examples. The sulcus of the frons was marked with a thin black line and the adjacent slopes were fully yellow in Coorg specimens; while the black line in the sulcus was thicker, slopes black enclosing a small yellow triangular spot with the apex directed to the floor of sulcus on each side, on the slope nearer the border with the vertex. In the case of venation, the hypertrigones are traversed 3–4 times in forewings and 2–3 times in hindwings in Coorg specimens while in Agasthyamalai specimens it is 4 in forewings and 2 in hindwings. The SSNR for Coorg specimens 8:17/10:12::18.5:8/12.5:12 and SSNR for Agasthyamalai specimens 7:17.5/10.5:11.5::17.5:7/11.5:10. The abdominal markings have some variations. The ventrobasal streaks in Coorg specimens are well-defined and prominent in contrast to the Agasthyamalai specimens with a narrow annulus which is laterally touching the ventrobasal streak in S8 in Coorg while it is disjunct in Agasthyamalai specimens. S10 spine location is at the mid-dorsum on Coorg specimens and slightly distal to it in Agasthyamalai specimens. The insects are otherwise similar including the genitalia of the ♂. The variations may be a clinal one and do not qualify for a separate subspecies taxon. However, this needs to be investigated in molecular terms.

Habitat and Ecology: This species was observed flying at the bases of the foothills of Agasthyamalais, below 200 m above ASL, in the southern WG. The flight period is May to June after the onset of southwest monsoon rains in Kerala. The males were seen patrolling along 2 m wide streams throughout the day in a largely shaded rubber plantation at the edge of a Myristica swamp forest, in sunny mornings and afternoons. The streams have gravel and rubble in the bottom and the depth was less than 50 cm. Each male was seen defending an area of 8–10 m of the stream in its patrol flight at about

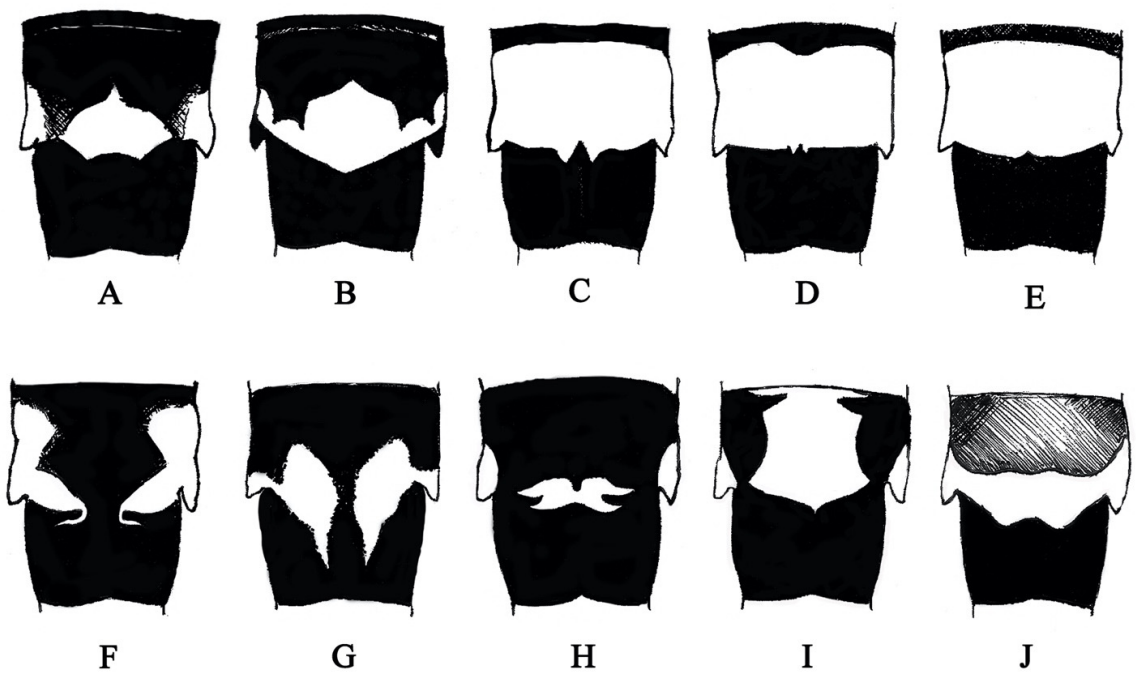

Fig. 11 Dorsal view of abdominal segment 2 of *Macromia* based on museum types: A–*M. annaimallaiensis* Fraser, 1931; B–*M. indica* Fraser, 1924; C–*M. flavicincta* Selys, 1874; D–*M. cingulata* Rambur, 1842; E–*M. bellicosa* Fraser, 1924; F–*M. ellisoni* Fraser, 1924; G–*M. irata* Fraser, 1924; H–*M. ida* Fraser, 1924; I–*M. miniata* Fraser, 1924 (*M. flavocolorata* Fraser, 1922); J–*M. atuberculata* Fraser, 1932 (*M. flavocolorata* Fraser, 1922). Illustrations © Kalesh Sadasivan

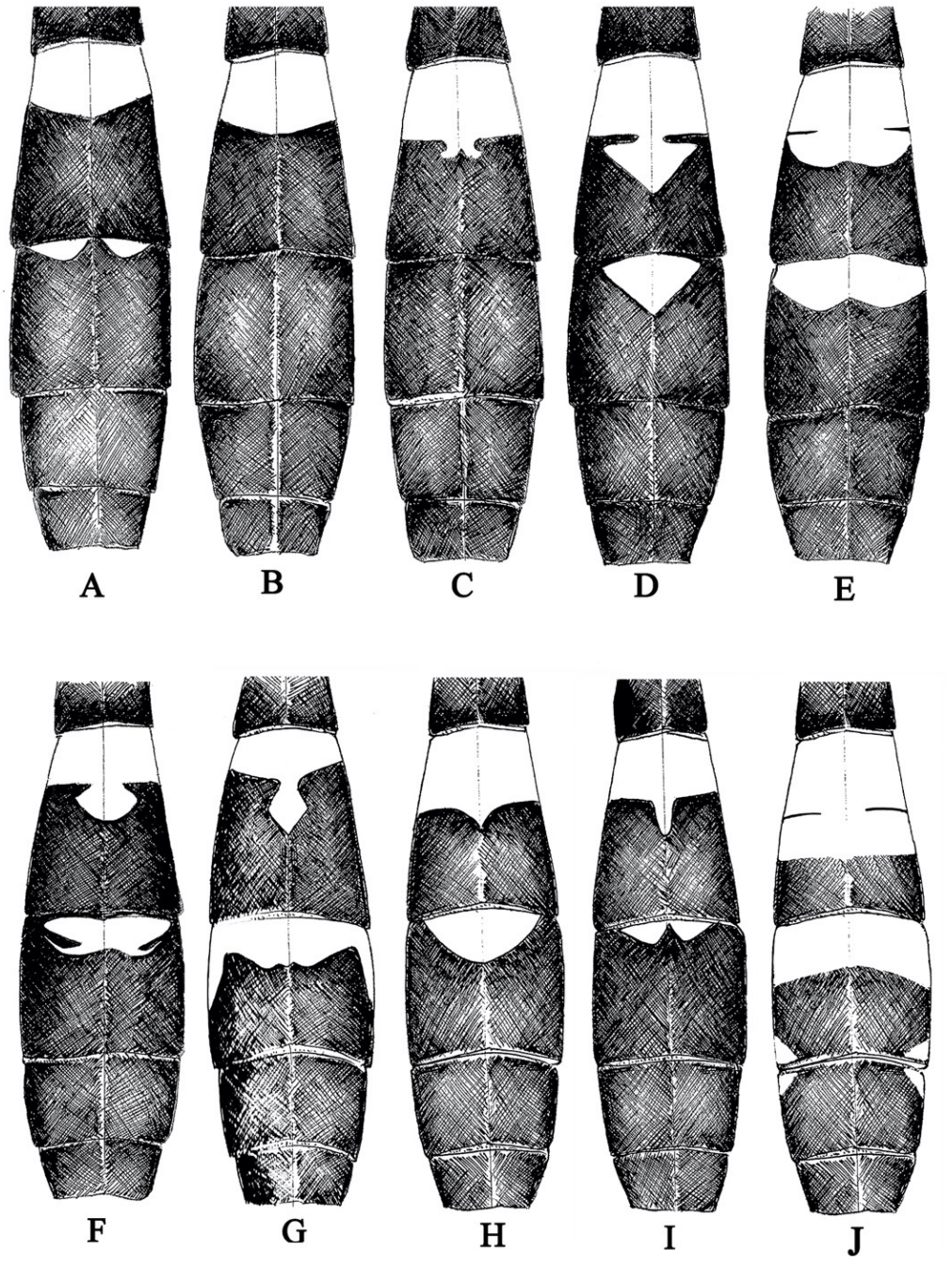

Fig. 12 Dorsal view of abdominal segments 7 and 8 of *Macromia* based on museum types: A–*M. ida* Fraser, 1924; B–*M. ellisoni* Fraser, 1924; C–*M. annaimallaiensis* Fraser, 1931; D–*M. indica* Fraser, 1924; E–*M. bellicosa* Fraser, 1924; F–*M. irata* Fraser, 1924; G–*M. cingulata* Rambur, 1842; H–*M. miniata* Fraser, 1924 (*M. flavocolorata* Fraser, 1922); I–*M. atuberculata* Fraser, 1932 (*M. flavocolorata* Fraser, 1922); J–*M. flavicincta* Selys, 1874. Illustrations © Kalesh Sadasivan

3 feet height from the water surface. Four individuals were engaged in patrolling a 250 sqm of water surface.

Differential Diagnosis: *M. irata* shows an affinity to two possibly monophyletic groups of *Macromia* in the WG considering the similarities in anal appendage and genitalia. These are the *cingulata-flavicincta-bellicosa* cluster forming the *cingulata* group and the *indica-annaimallaiensis* cluster forming the *cincta* group of *Macromia.*

The cingulata group: The three species, *M. flavicincta, M. bellicosa,* and *M. irata,* have well-developed but short or reduced antehumeral stripes and also consider the scheme of anal appendage and genitalia to form a natural group. The general structure of the anal appendage and the colour is dull ochreous (*M. flavicincta)*, reddish-brown (*M. bellicosa*), and black with epiproct dark reddish-brown (*M. irata*). The S10 dorsal spine is more prominent and acute in *M. irata* compared to the other two species. *M. flavicincta* has a well-defined broad antehumeral stripe changing to red-brown, labrum black-bordered, ante-alar sinus bright citron-yellow, tibial keels conspicuously yellow, segment 8 base with a complete annular yellow ring, segment 7 lacks the contiguous yellow spot while in *M. irata* the anal appendage is dark brownish-black, segment 8 has a butterfly-shaped yellow spot and the yellow annuli at the base of segment 7 has a mid-dorsal extension as a contiguous yellow spot. S10 bears a spine near its mid-dorsum slightly towards the apex while the spine is exactly at the middle or a little distal in *M. irata*. The male genitalia is similar to *M. irata* but the hamule is much shorter and the apex of the genital lobe is more rounded, posterior margin of the genital lobe is much concave in *M. flavicincta.* The tip of the lobe is rounded in *M. flavicincta* while it is acute in *M. irata. Macromia bellicosa* has a short but well-defined antehumeral stripe, labium bright yellow, labrum, anteclypeus, lower postclypeus black and rest of face is bright citron-yellow; while labium is brown, labrum inferior half dark amber-brown and superior half yellow in *M. irata*, anteclypeus is brown and postclypeus is orange-yellow. The underside of pterothorax black in *M. bellicosa* while it is brown in *M. irata*. Segment 7 with the basal half yellow while in *M. irata* the basal third is yellow with the dorsal contiguous yellow spot. S8 has a narrow annule in this species while in *M. irata* it is reduced to a butterfly-shaped basal spot. S10 bears a spine near its mid-dorsum slightly towards the apex while the spine is exactly in middle in *M. irata*. The anal appendage is similar to *M. flavicincta* (but more curved, and S10 spine is more robust). The colour of the anal appendage is reddish-yellow in contrast to blackish-brown on *M. irata*. The genitalia are slightly different from the similar *M. bellicosa* in that the genital lobe is strongly angulated from the ventral border and it is nearly in a straight line as the caudal border, and vice versa in *M. irata*. The labrum in *M. irata* is superiorly yellow and inferiorly brown and distinguishes it clearly from the black-bordered labrum of *M. flavicincta* and the fully black labrum of *M. bellicosa* and *M. flavicincta. Macromia bellicosa* has ochreous to reddish-brown appendages with more inwardly angular cerci with a mid-lateral spine while *M. irata* has dark brownish-black appendages with less angled cerci with a tooth-like mid-lateral spine. The lateral spine is almost perpendicularly directed with respect to the long axis of the cerci in *M. flavicincta* and *M. bellicosa,* while it is at a postero-lateral acute angle to it in *M. irata.* The cranial junction of the mid-lateral spine with the cerci is concave in *M. flavicincta* and *M. bellicosa,* while it is convex in *M. irata.* The curvature of cerci and the lateral angulation of the tip of the cerci are smooth and gradual in *M. irata*, in contrast to *M. flavicincta,* while in *M. bellicosa* the curve is more angular and the tip of the cerci abruptly deviates outwards in relation to the inner margin. The shape of the anterior and posterior hamulus and its relative length with respect to the lobe distinguishes this species from the closely similar *M. flavicincta.* and *M. bellicosa* (Figs. 8 B,H)

Other similar species groups are the *ellisoni* group and the *cingulata* subgroup of *cingulata* group. *Macromia ellisoni,* a large species, has a well-developed citron-yellow antehumeral stripe, a less robust spine on S10, and the genitalia is much different with a broader hamulus. Anal appendage with shorter epiproct compared to the cerci, and

the rudimentary lateral spines on the cerci, located more towards the apex and the ends of cerci are diverging. The *cingulata* subgroup has a different anal appendage with a rudimentary spine on the distal third of the lateral aspect of the cerci, the distal third of which is directed inwards; while in *M. irata* the spine is in the middle of the lateral aspect of the cerci and tips of the tapering cerci are directed outwards. *M. cingulata* also has a broad hamulus in comparison with *M. irata.*

The callipoe group: M. ida and *M. flavocolorata* are easily diagnosed from *M. irata* by the absence of a dorsal spine on S10 and the structure of the hamule (Figs. 10 E,F).

The cincta group: The general scheme of genitalia and the anal appendage has some affinity to the *indica-annaimallaiensis* group of *Macromia*. These species have a bluish metallic reflex on the anterior pterothorax instead of the dark green in *M. irata. M. indica* has a labrum edged with black; mid-dorsal yellow annules on S3–6, the lateral spines on cerci are slightly nearer to the apex and are more angular with the apex abruptly upturned. The genitalia of *M. indica* is similar to *M. annaimallaiensis* with a short hamulus in comparison with the genital lobe (Fig. 8E). *M. annaimallaiensis* lacks a butterfly-shaped yellow spot at the base of segment 8, has anal appendages similar to *M. indica*, and has a shorter, less angulated hamulus with a middle constriction, on genitalia compared to *M. irata.*

From other Indian Macromia species: M. moorei and *M. cupricincta* are two species similar to *M. irata* with respect to the general morphology of anal appendage and genitalia but are extralimital in distribution. In *M. moorei,* the labrum and clypeal region are reddish-brown, without antehumeral stripe, one lateral yellow stripe, a yellow ante-alar stripe, an anal loop with 7 cells, the absence of a robust spine on the dorsum of S10, and the short and much more angulated hamulus in comparison with the genital lobe. The lateral border cerci are more angulated inwards on the dorsal view, and the cerci and epiproct are more up-curved in *M. moorei*. The genital lobe has a rounded tip in contrast to the acute one in *M. irata.* This species is distributed from the Northeast Himalayas to the rest of Southeast Asia. *M. cupricincta* has a general cupreous-brown colour, bright citron-yellow ante-alar sinus, single citron-yellow stripe on lateral pterothorax, tibial keels conspicuously yellow, S7 basal half yellow, apical half S7–10 is coppery brown, and anal appendages dark ochreous. The genitalia, especially the genital lobe, and the shape and position of anterior hamules are very similar to *M. irata,* however, this species lacks the inferior concavity at the middle third, seen in the posterior hamule of *M. irata.* This species is distributed from Assam to Myanmar.

DISCUSSION

The species groups of *Macromia* of the WG of Peninsular India have been revisited and a revised scheme is suggested. The dependable characters for species identification of *Macromia* are the details of colouration of the face, especially the postclypeus; presence or absence of antehumeral stripes; structure of genital lobe and hamulus; markings on segment 2, 7, and 8; presence or absence of S10 dorsal spine, and the scheme of anal appendages. The variable characters are the length of the pterostigma and that of antehumeral stripes. Additional characters that may be useful are markings on the dorsum of S2, S7, and S8; the shape of the anal angle of the hindwing in males; tip structure of hamule and scales in male genitalia; and prothorax structure in females. A revised key to *Macromia* of the WG and Peninsular India is provided. The endemicity and distribution of all peninsular Indian taxa are updated.

Macromia irata, probably forms a monophyletic lineage with *M. flavicincta* and *M. bellicosa*, inside the *cingulata* group. The species is very distinct from *cincta* group which is possibly another closely related monophyletic group. The taxa *M. irata* has been re-described with details of variation and current distribution. *Macromia irata* can be easily differentiated from all the known taxa by the sulcus of frons black enclosing two triangular yellow spots, rudimentary antehumeral stripe, metallic green ground colour of the pterothorax, paler yellow colour instead of bright citron-yellow markings, S3–S6 with paired mid-dorsal spots, S7 with annulus and

a contiguous yellow dorsal spot, S8 with a butterfly-shaped dorsal spot, S9 with a dorsal carinal tooth at its caudal end, S10 with a robust dorsal spine, black anal appendages, relatively less angular cerci with a prominent spine at the middle of the lateral border, epiproct slightly longer than the cerci, and genitalia with hamulus as long as the genital lobe and caudal margin of the genital lobe being more angular than its ventral border with S2. As far as known, its present range is from Agasthyamalais in southern Kerala to the northern WG in Maharashtra.

Fraser (1931) remarked that not a single species of *Macromia* was found in Travancore, and the genus appears to become increasingly scarce south of the Palghat Gap, although extremely rich in species to the north of that barrier. But here *M. ellisoni*, *M. irata,* and *M. cingulata* are now reported from the Agasthyamalai region of old Travancore.

A revised key to males of *Macromia* of the Western Ghats

The key given by Fraser (1936) has some errors, for example, the third couplet second part says "Ground colour of pterothorax dark metallic blue at sides and upper part of the dorsum, dark reddish-brown at the lower part of dorsum; segment 10 without a dorsal spine". This is mentioned for *M. indica* and *M. annaimallaiensis*, which is misleading as both species have a strong keel that is developed to almost a tooth or spine on the dorsum of S10. Hence, the keys have been revised for males of *Macromia* of WG as given below. The location of the S10 dorsal spine may vary from the mid-dorsum to the distal end. The extension of the pterostigma varies even in a wing of a single specimen. The extent of the dorsal projection of the antehumeral streaks is variable. The colour of the eyes is reddish-grey and paler in the very short teneral phase. The robustness and position of the S10 spine were found to be variable even between individuals of a species. So, its presence or absence is a more useful character than its attributes. Other characteristics that may be useful are the shape of the tip of the hamulus and the anal angle of the hindwing in males.

1. Segment 10 with a dorsal tooth/spine 2

- S10 without dorsal spine……3
(Calliope Group: Eyes emerald green; Antehumeral stripe well-developed and superior edge well-defined, S3–S6 paired middorsal spots; Appendages black)

2. Antehumeral stripe present ……..4
(Cingulata Group: Antehumeral stripe may extend beyond halfway to the dorsum of pterothorax or may be reduced to less than halfway to the dorsum in which case it is usually continued thereof dorsally as a reddish band or vestigial yellow spots)

- Antehumeral stripe absent..…….....8
(*Cincta* Group)

3. Labium bright chrome-yellow basally, borders jet-black, the two colours sharply defined; in dorsal view, S2 with a yellow butterfly spot, S7 dorsal basal spot with convex distal border and does not extend beyond the jugum; S8 with a pair of small paradorsal basal triangular spots; posterior hamule broad at the base up to the distal 3/4th and abruptly narrow distally (Figs. 10E,11H, 12A)……
.....*M. ida* Fraser, 1924

- Labium ochreous basally borders diffusely dark brown, the two colours gradually merging; S2 with yellow annulus with trilobed distal dorsal border, S7 with dorsal spot extending into the dorsal carina as a small triangle; S8 single basal dorsal yellow triangular spot; posterior hamule with its apical two-thirds narrow (Figs. 10E, 11I, 12 H)…….....…….
.....*M. flavocolorata* Fraser, 1922

4. S8 unmarked, a large species with wings more than 45mm; Eyes brilliant bluish emerald green; in dorsal view, S3–S6 with paired middorsal spots, S7 yellow basal annulus dorsally not extending beyond the jugum (Figs. 10D, 12B)….....*M. ellisoni* Fraser, 1924

- S8 with basal yellow annulus/spot, small species with wings less than 45mm ….....…….5

5. Face black, marked by citron-yellow and black.....6

Fig. 13 *Macromia irata* Fraser, 1924 (TORG 1006): A–Lateral view; B–Close up of anterior pterothorax; C–Close-up of Head; D–Antero-lateral view of head and pterothorax, E–Lateral View of pterothorax.
Photographs © Kalesh Sadasivan

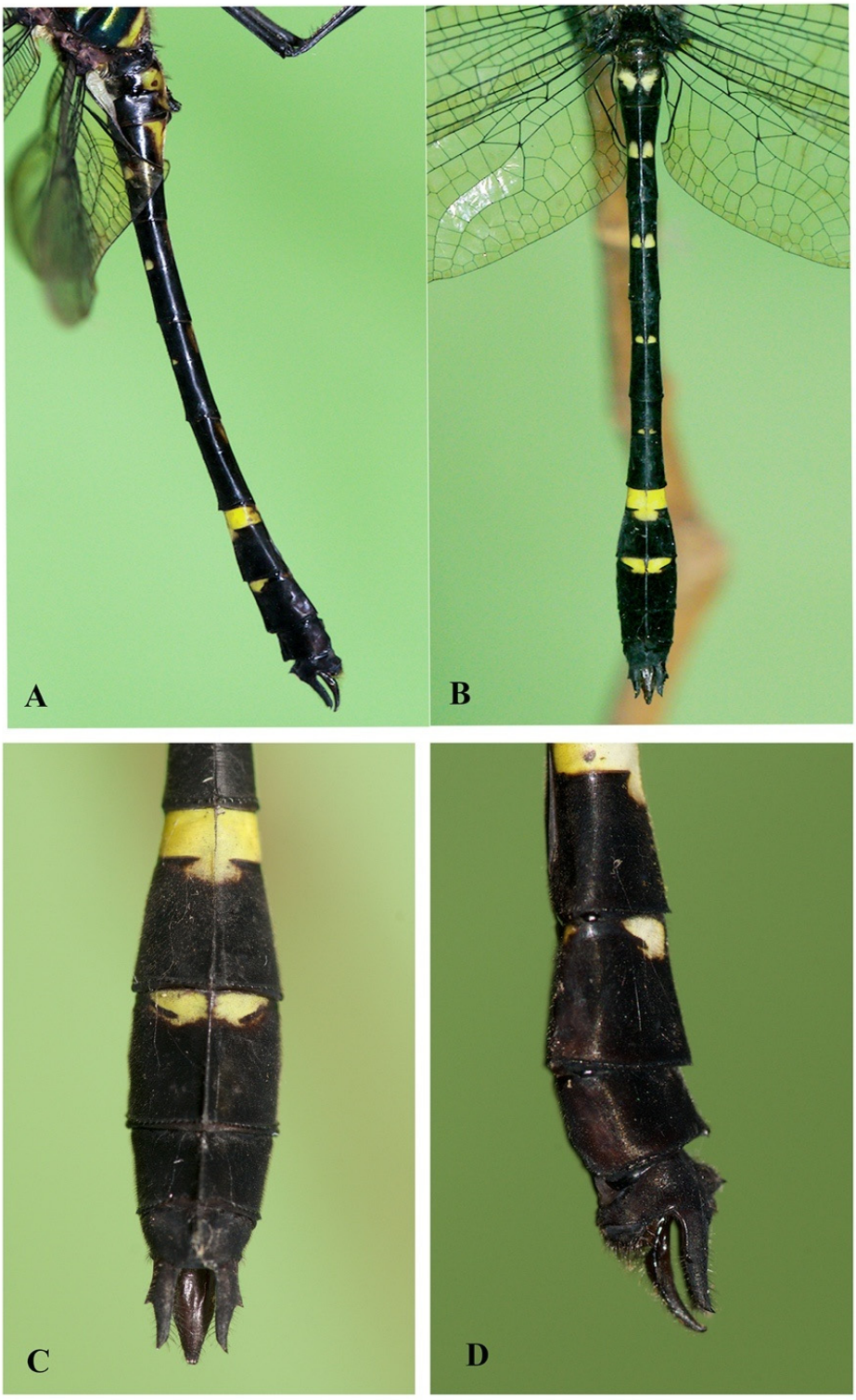

Fig. 14 *Macromia irata* Fraser, 1924(TORG 1006): A–Lateral view of abdomen; B–Dorsal view of abdomen; C–Dorsal view of anal appendage; D–Antero-lateral view of the anal appendage.
Photographs © Kalesh Sadasivan

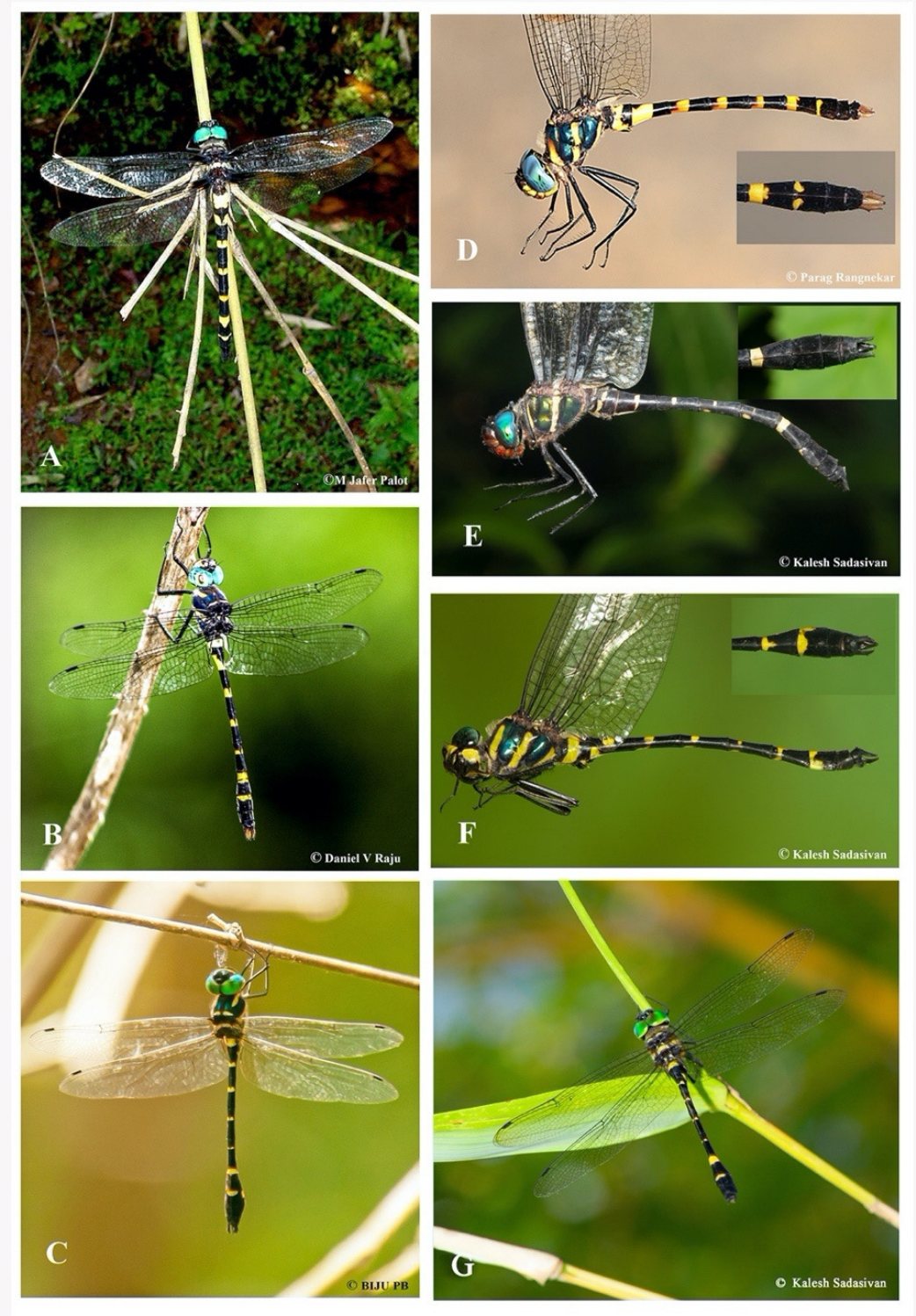

Fig. 15 Field images of *Macromia* from the Western Ghats**.** A–*M. indica* Fraser, 1924 © M. Jafer Palot from Kudremukh, Karnataka; B–*M. bellicosa* Fraser, 1924 by Daniel V Raju, from Coorg, Karnataka; C–*M. flavocolorata,* Fraser, 1922 (♂) © Biju PB, from Silent Valley, Kerala; D–*M. flavicincta* Selys, 1874 (♂) © Parag Rangnekar, from Maharashtra; E–*M. ellisoni* Fraser, 1924 (♂) © Kalesh Sadasivan, from Agasthyamalais, Kerala; F–*Macromia cingulata* Rambur, 1842 (♂) © Kalesh Sadasivan, from Munnar, Kerala, and G–*M. flavocolorata,* Fraser, 1922 (♂) © Kalesh Sadasivan, from Thenmalai, Kerala

- Face ferruginous/reddish brown/amber-brown and yellow.....7

6. Anal Appendages ochreous; Eyes green; Abdomen- S3–S6 with basal annuli, S7 dorsal basal spot expands to a transverse bilobed band beyond the jugum, thus yellow occupies a little less than half of the S7, S8 dorsal basal annulus distal border bilobed dorsal laterally and thus with a concave middorsal margin (Figs. 10C, 12E)…….....…....... .....*M. bellicosa* Fraser, 1924

- Anal Appendages black; Eyes pale blue; An inverted 'T' shaped mark on face formed by black spots on frons and the black stripe on the sulcus of frons; in dorsal view, S3 with paired middorsal spots, S4–S6 spots almost conjoint, S7 basal dorsal yellow spot with a short tongue/ elongated diamond-shaped extension beyond the jugum, S8 basal annulus crown-shaped with a convex distal border; Small species with wings less than 45mm (Figs. 10B, 12G)…….....*M. cingulata* Rambur, 1842

7. Anal appendages dull ochreous; Eyes bluish, 'T' shaped mark on crest of frons; S2 bilobed with dorsal distal border of yellow annulus, S3–S6 annuli, S7 with dorsal basal ring expanding into a transverse band beyond the jugum thus yellow occupies almost half of the S7, S8 with the butterfly wing spots separated with a broad black streak of the dorsal carina, or when well-marked joins across midline forming basal yellow annuli with dorsal concave distal margin, but always occupying less than half of S8, Genital lobe much evidently angulated with the caudal margin of S2 than its ventral margin (Figs. 10C, 11C, 12J)….....*M. flavicincta* Selys, 1874

- Anal appendages dark brownish-black; eyes emerald green; in dorsal view of abdomen, S2 with twin diamond-shaped spots; S3–S6 with paired middorsal spots, a butterfly spot in S8 finely bisected by a black line along the carina, S7 with the fan-shaped expansion of the dorsal basal yellow spot; Genital lobe angulated well with the ventral margin of S2 than its caudal margin (Figs. 10 F, 11G, 12F)…...……..…*M. irata* Fraser, 1924

8. S3–6 with mid-dorsal yellow annuli and S8 with dorsal basal yellow triangular spot; S7 with a triangular extension beyond the jugum (Figs. 10A, 12D).....……..*M. indica* Fraser, 1924

- S3–6 with paired mid-dorsal spots, S7 with a yellow basal annulus with a very short, broad but bifid mid-dorsal extension beyond the jugum, and S8 is unmarked (Figs. 10A,12C)..... .....*M. annaimallaiensis* Fraser, 1931

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Courtship and mating behaviour of pupal parasitoid, *Xanthopimpla flavolineata* Cameron (Hymenoptera, Ichneumonidae, Pimplinae)

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ABSTRACT: The present study investigated the courtship and mating behaviour of pupal parasitoid, *Xanthopimpla flavolineata* in the laboratory. The results revealed that the premating period of day-old virgin females of *X. flavolineata* with newly emerged males was 23.21 ± 0.41 h, and day-old virgin males with newly emerged females was observed as 3.59 ± 0.07 h. The mating and preoviposition periods were 63.57 ± 3.39 seconds and 4.05 ± 0.16 days, respectively. The male approached the female with its antennal movement and body language. The females were mated on the day of emergence and mated many times during their life span. Almost immediately after emergence, the males became sexually active. When freshly emerging females were allowed to mate with males after their first mating, 53.33 per cent of females remated. The premating duration was shorter (2.73±0.62 h) and the mating period was longer (79.53±2.43 sec) when male rivalry occurred during mating. Mating success was more likely when two males were paired with a single virgin female. Males of greater and medium-size had a much higher chance of mating than males of lesser size. The courting and mating behaviour of *X. flavolineata* will be useful in improving laboratory mass rearing techniques. © 2023 Association for Advancement of Entomology

KEY WORDS: Biological control, courtship sequence, male size, mating success

Xanthopimpla flavolineata Cameron (1907) is a solitary endoparasitoid of agriculturally important lepidopteran pests like *Cnaphalocrocis medinalis* Guenee and *Scirpophaga incertulas* Walker in rice. It belongs to the subfamily, Pimplinae, family, Ichneumonidae of Hymenoptera order, is the most biologically diverse group (Gauld, 1991). The subfamily, Pimplinae group of insects are big, strikingly coloured parasitoids and best represented subfamily under the family Ichneumonidae (Fitton *et al.,* 1988). Classification of this group is changed several times by taxonomists, the subfamily is divided into four tribes *viz.,* Ephialtini, Pimplini, Delomeristini and Perithoini (Gauld *et al.,* 2002). All the species of tribe, Pimplini are generalized parasitoids that feeds on more than one species of host. The parasitic wasps, pimplines are idiobionts of the endopterygote insects which concealed within plant tissues (Fitton *et al.,* 1988). A few species of ichneumonid wasps have been utilized for biological control of pests, but a great majority of them are yet to be exploited for such control methods. Their non-utilization is apparently due to our inadequate knowledge of their ethology (Gupta and Tikar, 1976; Gitau *et al.,* 2007; Dung *et al.,* 2011). The behavioural responses of parasitoids to various host

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stages and their adaptability to the particular temperature in the tropical climate are less understood. The present study aimed to investigate and retrieve information about the courtship behaviour of *X. flavolineata* and the effect of male competition and body size of male on mating success. This knowledge can now enhance the mass rearing techniques and employ these parasitoids in biological control programs to manage economically important agricultural pests.

The experiment on courtship and mating behaviour of *X. flavolineata* was carried out at the Insectary, Department of Entomology, Annamalai University, Tamil Nadu, India and the following methods were followed.

***X. flavolineata* and their host rearing:** The larvae and pupae of rice pink stem borer, *Sesamia inferens* Walker, one of the host insects of *X. flavolineata* and adults of *X. flavolineata* were collected from rice fields of Experimental Farm, Annamalai University, Tamil Nadu. The host insect was reared as described by Lingappa (1978) and *X. flavolineata* parasitoids were reared using the obtained pupae of *S. inferens* under laboratory conditions [27±2°C, 13:11 h (light: dark) and 65±5% RH]. Freshly emerged adults were kept in vials (Diameter: 30 mm; Height: 15 cm) individually closed with nylon cloth at the top and fed with 50 % honey solution. All *X. flavolineata* parasitoids were used just once in the experiments.

Courting and mating sequence: Fifteen individual pairs (one male and female) were released in the rearing cages (15 x 15 x 15 cm) to mate in the early morning at 0700 - 0900 h with mesh-sided enclosure and were provided with honey, streaked on plastic strips and placed inside the cage. Each pair was observed for the successful courtship until they ended up with the male and female physically separating after copulation was observed and photographed using Cannon 24 MP DSLR camera. The mating duration was recorded using a stopwatch. Immediately after mating, the female parasitoid was provided with one day old *S. inferens* pupa and continuously observed for oviposition to know the preoviposition period, if any. Fifteen such mated females were observed in sequence.

Influence of male competition on mating success: To assess the effect of male competition on male mating success when paired with more than one male with a single female in the rearing cages (15 x 15 x 15 cm) with mesh sided enclosure. The observations of the premating, mating, and preoviposition periods were recorded. The mating success was also recorded for comparison between a single male or more than one male.

Impact of male size on successful mating: To determine the effect of male body size on the mating success, the experiment was conducted. Three different sized males with a single medium-sized virgin female were introduced in the same experimental setup as described above and mating observations were recorded. A total of 15 individual observations (replicates) of mating were conducted. However, the unmated pair was replaced with the new pair.

Table 1. Reproductive behaviour of *X. flavolineata*

No.	Treatments	Premating period (h)*	Mating period (Seconds)*	Preoviposition period (Days)**
1.	Day-old virgin females X newly emerged males	23.21 ± 0.41	42.63 ± 2.33	5.23 ± 0.17
2.	Newly emerged females X day-old virgin males	3.59 ± 0.07	63.57 ± 3.39	4.05 ± 0.16

* Mean of 15 observations; Mean ± SE; Single pair used per observation; ** Single female used per observation

Table 2. Effect of male competition on the mating (with medium-sized adults: newly emerged females with a day-old virgin males) in *X. flavolineata*

No.	Treatments	Premating period (h)*	Mating period (Seconds)*	Preoviposition period (Days)**
1.	One male X One female	4.02 ± 0.07	62.51 ± 1.93	4.13 ± 0.14
2.	Two males X One females	2.73 ± 0.62	79.53 ± 2.43	4.08 ± 0.11

* Mean of 15 observations; Mean ± SE; Single pair used per observation; ** Single female used per observation

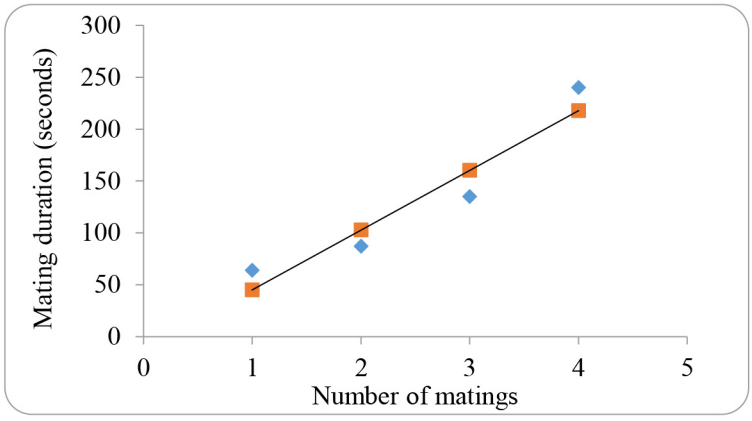

Fig. 1 Relationship between the number of mattings and the duration of the mattings in *X. flavolineata*

Statistical analysis: Wherever a single observation was made on behaviour of the parasitoid, the standard error of the mean (SE ±) was calculated. The relationship between the mating numbers and duration was analyzed using regression analysis (Panse and Sukhatme, 1961).

Premating: The premating period of day-old virgin females of *X. flavolineata* with newly emerged males was 23.21 ± 0.41 h, and day-old virgin males with newly emerged females was observed as 3.59 ± 0.07 h. (Table 1). The premating period was varied based on the adult males, it showed that males became sexually active almost a day after emergence. The females mated on the day of emergence itself and mated many times during their life span. Pillai and Nair (1983), reported that females mated on the day of emergence or on the subsequent day, with earlier emerged males. If multiple mattings occurred in this period, the mating time was also recorded. When newly emerged females were allowed to mate with the males after the first mating, 53.33 per cent of females were remated. On average the females mated 3.6±0.28 times when left with the males. The mating duration increased significantly with the number of mating (Linear regression: $Y = 57.6x - 12.5$, $F = 19.15$, $R^2 = 0.90$*) (Fig. 1). Khatri *et al.* (2009) reported similar results in *Diadegma semiclausum* Hellen on Diamond back moth, *Plutella xylostella* (L.).

Courtship and mating behaviour: The male approached the female with their antennal movement and body language (Fig. 2a). Vinson (1998) observed the buzzing action of males to locate the females 3 to 5 cm away. After acceptance of the female, the males mounted on the females by placing the prothoracic legs just below the bases of the wings and held the wings and abdomen of the female firmly with his meso and metathoracic

Table 3. Influence of male size on the mating (newly emerged females with day-old virgin males in different sizes) in *X. flavolineata*

No.	Treatments	Premating period (h)*	Mating period (Seconds)*	Preoviposition period (Days)**
1.	Small sized males X Medium sized females	5.81 ± 0.13	72.57 ± 3.39	4.57 ± 0.13
2.	Medium sized males X Medium sized females	3.51 ± 0.41	63.57 ± 3.39	4.23 ± 0.18
3.	Large sized males X Medium sized females	3.03 ± 0.37	58.61 ± 2.13	4.07 ± 0.13

* Mean of 15 observations; Mean ± SE; Single pair used per observation; ** Single female used per observation

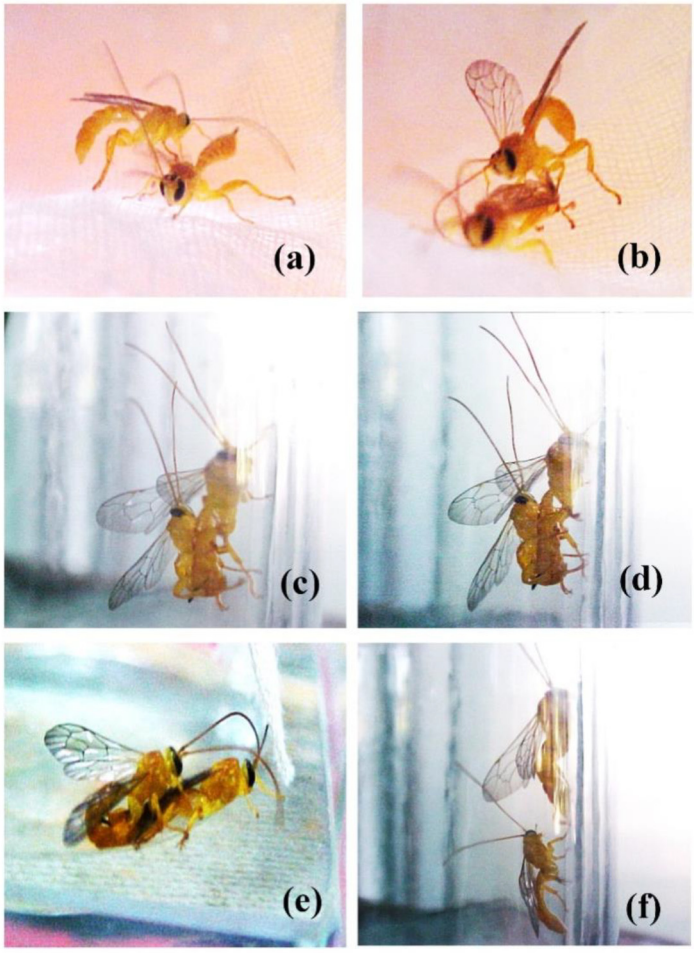

Fig. 2 Courtship and mating sequence of *X. flavolineata* (a) male approaching female with his antennal movement and body language; (b) male placing his prothoracic legs on the female; (c) male bends its abdomen and hold the female; (d) male searching the genital pore of female after proper mounting; (e) actual mating with antennal vibrations; (f) pair moving away and relaxing

legs (Fig. 2b). The male parasitoid bent his abdomen (Fig. 2c), sought out the genital pore, and mated (Fig. 2d). During the process of mating the male slightly vibrated his antennae which were anteriorly oriented, fanned his wings in high speed for one/ two seconds, then stopped for two to three seconds and repeated the whole process during the entire period of mating (Fig. 2e), which normally lasted for 63.57 ± 3.39 seconds in *X. flavolineata* (Table 1). The male terminated the mating by releasing the female from its hind legs. After mating, both males and females moved away from each other and rested for 7 to 9 minutes, then dispersed to other places (Fig. 2f). Similar results were reported by Shannon (1977) in *X. stemmator* and Kainoh (1999) in *Cardiochiles nigriceps* Viereck.

Pre-oviposition: After mating, the adult female *X. flavolineata* took 4.05 ± 0.16 days to oviposit on the host insect. Only after this pre-oviposition period, adult females search for a suitable host for oviposition (Table 1). The results are in accordance with Moutia and Courtois (1952), Moore and Kfir (1996) and Henry (2008).

Influence of male competition on mating: When two males were allowed to mate simultaneously, it was found that the premating period was significantly shorter (2.73 ± 0.62 h). The mating period was significantly more extended (79.53 ± 2.43 sec), and the preoviposition period (4.08 ± 0.11 days) was similar or significantly on par when compared with the single pair (Table 2). The females preferred to mate with the more vigorous males, as reported by Joyce *et al.* (2009). This study found that pairing at least two males with a single virgin female will increase the probability of mating success that happened with the stronger one often.

Influence of male size on mating: When virgin females were directly introduced to large, medium and petite males had the same mating success. Comparatively, the medium-sized male showed a shorter duration of premating period (3.51 ± 0.41 hours), mating period (63.57 ± 3.39 sec), and preoviposition period (4.23 ± 0.18 days) followed by larger sized males (3.03 ± 0.37, 58.61 ± 2.13, 4.07 ± 0.13) (Table 3). In this experiment, larger and medium-sized males had higher probability of mating significantly than smaller ones. Similar findings where male size had impact on mating success found in other parasitic insect species *viz., Cotesia marginiventris*, *C. flavipes, Mastrus ridens, Trichogramma euproctidis, C. urabae* reported by Joyce *et al.* (2009), Granger *et al.* (2011), Sandanayaka *et al.* (2011) and Avila *et al.* (2016).

The experiment concluded that the premating period was shorter and the mating period was longer when male competition occurs on mating in *X. flavolineata*. This indicates that pairing at least two males with a single virgin female will increase mating success. Further, larger and medium-sized males had significantly higher probability of mating than smaller ones. The mating sequence was also explored step by step, which provides baseline information on the behaviour of *X. flavolineata.* Hence, the pupal parasitoid, *X. flavolineata,* may have opted for mass production in the laboratory under careful handling. Avoiding disruptive techniques and understanding parasitoid courting behaviour will expand the range of naturally occurring biocontrol agents used in the agricultural ecosystem. The focus is on biocontrol to meet the twin objectives of pest management and environmental safety. Its success will be exclusively dependent on the demonstration of parasitoid based technology and the assured provision of high-quality parasitoid to farmers. Efforts to conserve current natural enemies should also be prioritized.

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Termite sampling methods: A comparative study in four habitats of north Kerala

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ABSTRACT: Initially, three termite sampling methods were compared in a coastal area of North Kerala to check the efficiency of the widely followed standardized belt transect protocol (Jones and Eggleton, 2000) with a simplified belt transect protocol and random search method. Of the total 11 species that belong to 4 genera collected during the study, the standardized belt transect protocol recorded only two genera and 5 species in 20 hours of sampling effort, while the simplified protocol with half the effort (10 hours) recorded two genera and 4 species. Random search method with least effort (6h) recorded 4 genera and 11 species that included all the species collected in the earlier methods. There was a marked difference in sampling efficiency; the random sampling method yielded 1.87 species per hour while standardized belt transect protocol and simplified belt transect protocol yielded only 0.25 and 0.4 species per hour respectively. The result of the study was further verified in three more habitats viz. natural forest, coffee plantation and tea plantation which gave similar results. The study indicates that the random search method which covers more area in less time yield more representative termite fauna in all the four habitats tested, than the standardized belt transect protocol which spends more time covering less area.
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KEY WORDS: Random search, standardized transect protocol, sampling efficiency

Appropriate sampling methods are important for studying the diversity of any organism. There are different sampling protocols for different organisms and different habitats. Absence of adequate sampling strategies is an impediment to our understanding of many groups of organisms. Soil organisms in general and termites in particular are among such organisms. In spite of their high diversity and importance, an efficient and foolproof sampling strategy is still lacking.

Davies *et al*. (2012) reported that a particular sampling method may not be the most appropriate or effective for all habitats and active searching (modified version of standardized belt transect of Jones and Eggletton, 2000) was most effective method of termite sampling in mesic savannas and baiting in arid savannas. Zeidler *et al.* (2004) also suggests baiting experiments to arid environments. The major limitation with bait experiment is that it only document cellulose feeding termite species, thereby excluding the soil feeding termites and it is less effective in wet season (Davies *et al*., 2021).

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The standardized belt transect (Jones and Eggleton, 2000) protocol is considered as the most appropriate method for sampling of soil termites in tropical areas (Eggleton *et al.,* 2002; Donovan *et al.*, 2002; Varma and Swaran, 2007; Shanbhag and Sundararaj, 2013). Other suggested methods include different forms of baiting (Abensperg-Traun, 1993; Taylor *et al.,* 1998; Gromadzki, 2003), mound density counts (Traore and Lepage, 2008) and litter bags (Yamashita and Hiroshi, 1998). The standardized belt transect protocol is designed to cover only a small area (100m x 4m transect), within which, much time (20 h) is spent for thorough searching. There is a possibility that if more area is sampled, more species could be obtained. The present study was designed to check this hypothesis by comparing the efficiency of the standardized belt transect protocol with simplified methods of termite sampling in different habitats with lesser effort covering more area.

The study was completed in two steps - Initial comparison of three methods in the coastal area and then verification of the results in three other habitats in Wayanad district.

Study area: The initial field trials were conducted in the coastal area at Mavilakadappuram, a small village near Cheruvathur in Kasaragod district, (12.191733 N; 75.124300 E). The area was selected after a pilot survey that indicated high termite diversity. The plot selected for study had dimensions of 150m x 30m and located about 60m away from the coastline. Further verification of the results was done in three habitats in Wayanad district viz., a forest ecosystem at Thrissileri (11.8325 N; 76.0392 E), a coffee plantation at Thonichal (11.4550 N; 75.5910 E) and a tea plantation at Thalappuzha (11.8332 N; 75.9677 E).

Sampling Methods:

A. Testing three sampling methods in coastal area: Three sampling methods compared in the study were: standardized belt transect protocol (Jones and Eggleton, 2000), a simplified belt transect protocol and random search method.

1) Standardized belt transect protocol (Jones and Eggleton, 2000) – 20h. sampling: Standardized sampling protocol is based on a belt transect of 100 m length and 4 m width. The transect was divided into 20 contiguous sections of 5m x 2m and numbered sequentially (Figure 1). A total of one hour (20 minutes each by 3 investigators) time spent searching for termites in every alternate section. So a total of 20 man hours spent for sampling. Microhabitat like surface soil, accumulations of litter and humus at the base of trees; all subterranean nests, inside of dead logs, mounds and runways on vegetation were observed in each section up to a height of 2m above the ground level. Belowground searches were also made in 12 small plots per section each about 12×12cm, to 10cm depth.

2) Simplified belt transect protocol – 10h. sampling: This is a modified form of the standardized belt-transect protocol by reducing the sampling time from one hour to half an hour (10 minutes by 3 investigators) per each section. Only 6 plots of 12×12cm, to 10cm depth were sampled per each section, instead of 12. All other things remain the same.

3) Random search method – 6h sampling: The whole area of the plot (150m x 30m) was divided into six parts and randomly searched for termites. One hour was spent searching for termites in each part. Searching was done over the soil surface, dead

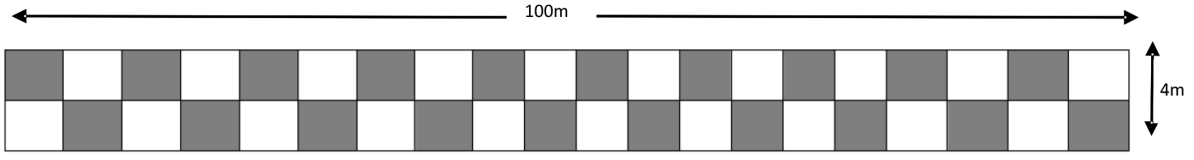

Fig. 1 Lay out of the sampling methods (Shaded area-Standardized belt transect protocol, unshaded areas - Simplified belt transect protocol)

wood, subterranean nests and other microhabitats up to a height of 2m above the ground. Search was not done in soil below ground level.

B. Testing sampling methods in three other habitats: The result of the study in coastal area was further verified in three ecologically different areas: a natural forest (unaltered natural ecosystem), a coffee plantation (least managed monoculture land), and a tea plantation (intensively managed monoculture land). Plots selected with 150m x 30m area in each habitat. Only the standardized belt-transect protocol (with reduced time of 6hrs.; 18 minutes per section and 3 plots of 12×12cm, to 10cm depth per each section) and random search method (6h) were compared. The collection methods followed were the same as mentioned before.

Collection and preservation of samples: Specimens were sampled from every encountered termite population. Preferably workers and soldiers were collected by using wet brush or forceps; placed in a vial containing (70%) ethanol and labeled with specimen number, habitat, collection locality, date and time. The preserved specimens were examined under a stereo zoom microscope at 45x magnification and identified up to species level using key published by Roonwal and Chhotani (1989) and Chhotani (1997). All collected specimens were deposited in the Zoology museum, Payyanur College, Edat, Kerala, India.

Statistical analysis: The efficiency of sampling methods is calculated as the number of samples and species obtained for unit effort as follows.

Sampling efficiency = total number of samples and species / total hours spent.

Efficiency of different methods were compared through analysis of variance (ANOVA) using SPSS and Shannon index of biodiversity (H) using the PAST software (Hammer *et al.,* 2001).

Shannon index $$H = -\sum_{i=1}^{s} (p_i * \ln p_i)$$

where:

H = the Shannon diversity index

Pi = proportion of the population made up of species i

s= numbers of species in sample

A. Sampling in coastal area

The number of termite samples and species recorded from the three termite sampling methods in coastal area are presented.

1) Standardized belt transect protocol: Twenty two samples were collected during the 20 hours of observation, 18 samples from surface soil and 4 samples from belowground soil (upto 10 cm depth). All the samples collected from deeper soil were devoid of soldiers, which could be identified only upto genus level. They belonged to two genera which were already recorded - *Odontotermes* and *Microcerotermes*. Thus a total of 5 species under 2 genera were documented (Table 1).

2) Simplified belt transect protocol: A total of 7 samples were collected during the 10 hours of observation that belonged to 4 species under 2 genera (Table 1). Two samples could be identified only up to generic level and they both belonged to *Microcerotermes*.

3) Random search method: A total of 19 samples were collected during the 6 hours of observation and they belonged to 11 species under 4 genera (Table 1). Three samples were identified only up to generic level and they belonged to the three genera - *Odontotermes, Heterotermes* and *Microcerotermes*.

The random search method is distinctly efficient when compared to the other two methods. The highest number of termite species per hour (1.83) and samples per hour (3.17) were recorded by the random search method. Standardized belt transect protocol recorded only 0.25 species and 1.1 samples per hour and simplified belt transect protocol recorded 0.4 species and 0.7 samples per hour sampling effort. The Shannon index of diversity was also found much higher in the random search method (2.253) compared to standardized belt transect protocol (1.413) and simplified belt transect protocol (1.332).

B. Sampling in three other ecosystems

Results of the two termite sampling methods in three

Table 1. Species and number of samples collected in three sampling methods in the coastal area

Species	No. of samples per protocol/ method			Total
	Standardized belt transect (20h)	Simplified belt transect (10h)	Random search (6h)	
Heterotermes balwanti	-	-	1	1
Heterotermes indicola	-	-	1	1
Odontotermes feae	-	-	1	1
Odontotermes giriensis	-	-	2	2
Odontotermes obesus	4	-	4	8
Odontotermes redemanni	5	1	2	8
Odontotermes vaishno	1	1	1	3
Odontotermes yadevi	-	-	1	1
Microcerotermes fletcheri	2	2	1	5
Microcerotermes pakistanicus	1	1	1	3
Synhamitermes quadriceps	-	-	1	1
Unidentified samples	9	2	3	14
Total samples	22	7	19	48
Total species	5	4	11	11
Samples per hour	1.1	0.7	3.17	
Species per hour	0.25	0.4	1.83	

different ecosystems are given in Table 2**.** A total of 14 species from 96 samples were recorded in this study. Thirteen species were collected in random search method and standardized belt transect protocol could collect only eight species. The highest number of termite species per hour was recorded in the forest ecosystem (1.33) by random search method. In the standardized belt transect protocol it was only 0.67. The lowest number of termite species per hour was recorded in coffee plantation and tea plantation by standardized belt transect protocol (0.5 each). In the random search method, it was 1.17 and 0.67 respectively.

Number of samples per hour recorded by random search method in coffee plantation was 3.5 and in forest ecosystem and tea plantation it was 3.33. In standardized belt transect protocol it was 1.83 for both forest ecosystem and coffee plantation and 2.17 for tea plantation).

The highest number of termite samples was found in random search method. Out of 96 samples, 61 samples were collected in random search method. Standardized belt transect protocol recorded 35 samples only. During the study, the maximum efficiency was obtained in the random search method.

Comparing the effectiveness of the two sampling methods, there was a significant difference (ANOVA, $P<0.05$). The diversity indices calculated, also depicts the efficiency of random search over standardized belt transect protocol. By random search method, Shannon diversity index value for forest ecosystem was 2.056, coffee plantation was 1.493 and tea plantation was 1.277 and it was 1.121, 0.759 and 0.687 by standardized belt transects protocol. The values show more termite diversity obtained via random search method in all the three different ecosystems when compared with standardized belt transect protocol. The result of the current study reveals that the random search method with least effort yield the maximum number of samples and species.

Table 2. List of species recorded from three ecosystems by different sampling protocol/method

Species	Forest ecosystem		Coffee plantation		Tea plantation		Total
	Standardized belt transect	Random search	Standardized belt transect	Random search	Standardized belt transect	Random search	
Odontotermes anamallensis	-	-	1	1	-	-	2
O. assmuthi	-	-	-	-	1	5	6
O. boveni	-	-	-	-	2	-	2
O. ceylonicus	-	-	2	1	-	-	3
O. feae	1	3	-	1	-	1	6
O. obesus	-	-	-	-	-	3	3
O. redemanni	6	1	8	12	10	10	47
O. vaishno	1	1	-	-	-	-	2
O. sps	3	3	-	-	-	-	6
O. yadevi	-	2	-	2	-	-	4
Narulitermrs indicola	-	5	-	-	-	-	5
N. sp.	-	1	-	2	-	-	3
Ampoulitermes wynaadensis	-	2	-	-	-	-	2
Ceylonitermellus peryarensis	-	-	-	1	-	-	1
Unidentified sp.	-	2	-	1	-	1	4
Total samples	11	20	11	21	13	20	96
Total species	4	8	3	7	3	4	14
Samples per hour	1.83	3.33	1.83	3.5	2.17	3.33	
Species per hour	0.67	1.33	0.5	1.17	0.5	0.67	

There were some attempts to modify the standardized belt transect protocol by reducing sampling size and/or sampling time (Davies *et al*., 2013; Schyra and Korb, 2019; Effowe *et al*., 2021). In the present study, the modified version with reduced time did not yield promising results. The random search method with least effort yielded the maximum number of samples and species for all the four habitats tested. It is also important to note that except for a single species (*O. boveni;* in the tea plantation), all the species recorded from the standardized belt transect protocol were recorded from the random search method at all the four sampling sites. On the other hand, the random sampling recorded 6, 4, 4 and 2 species each, in addition to that recorded in standardized protocol, from the four sites.

The random search method, covering more surface area, yielded more samples and species and the time and effort spent for standardized belt transect protocol did not yield comparable results. The probable reason for low efficiency being the high effort for sampling below ground (12 sections of 10 cm depth as mentioned before) which yielded only few samples; that too mostly devoid of the soldier caste, that made identification difficult. Majority of the termite samples and species were collected from the soil surface within mudplasters. There is every chance to get majority of the termites from the surface soil as the deeper termites too come up to the soil surface for foraging. Thus covering maximum surface area and microhabitats seems more important, at least for preliminary termite sampling of a tropical habitat. Searching

belowground can be still relevant for extensive sampling as it may help to collect any missed out species.

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New distributional records of egg parasitoids (Hymenoptera, Chalcidoidea, Trichogrammatidae) from Chhattisgarh, India

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ABSTRACT: Egg parasitoids from the Trichogrammatidae family, including *Aphelinoidea, Paracentrobia,* and *Ufens*, parasitize the hemipteran eggs on crops, orchards, and forest tree species. In agro-forestry areas, trichogrammatids are widely recognised as effective biocontrol agents for lepidopterous insect pests of different crops. They play a significant role and are widely accepted in the biological management of insect pests. Many trichogrammatid egg parasitoids were explored in sweep net collecting samples during surveys carried out in the agro-forestry regions of Chhattisgarh, India, during February–March and October–November 2017. Ten species of trichogrammatids, namely *Aphelinoidea gwaliorensis* Yousuf & Shafee; *Lathromeroidea ajmerensis* Yousuf & Shafee*, Oligosita debaiensis* Yousuf & Shafee*, Oligosita gilvus* Yousuf & Shafee*, Pseudoligosita nephotetticum* (Mani)*, Oligosita novisanguinea* Girault*, Oligosita sanguinea* Yousuf & Shafee*, Paracentrobia magniclavata* Yousuf & Shafee*, Ufens gurgaonensis* Yousuf & Shafee and *Ufens jaipurensis* Yousuf & Shafee were recorded as indigenous species, with new distribution in Chhattisgarh. © 2023 Association for Advancement of Entomology

KEY WORDS: Biocontrol agents, new records, forest, agro-forestry

Biodiversity of parasitic fauna explored in India represents only 5 per cent of the world parasitic species (Rathod and Karnataka, 2009). Egg parasitoids constitute an economically important group of parasitic insects in terrestrial ecosystem as they regularly exercise a natural check on the populations of various insect pests. The minute egg parasitoids, generally about less than a 0.5 mm, parasitize various groups of insect pests but mostly recorded attacking members of Lepidoptera and Hemiptera. These egg parasitoids are universally accepted bio-control agents of lepidopteran pests of agro-forestry (Doutt and Viggiani, 1968; Debach and Rosen, 1991; Smith, 1996) and *Trichogramma* spp. have world-wide popularity as biocontrol agents.

Some species of the genus Trichogramma: *Trichogramma kankerensis* Yousuf & Hassan; *Trichogramma plasseyensis* Nagaraja; *Trichogramma raoi* Nagaraja; *Trichogramma thalense* Pinto & Oatman (Yousuf *et al.*, 2015)

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were reported from Chhattisgarh. So, due to lack of research in exploration of egg parasitoids this study was carried out in several locations in Chhattisgarh state to assess the diversity of egg parasitoids belonging to the trichogrammatidae family. Thus, Chhattisgarh has flora including mango, litchi, jackfruit, bamboo, shisam, karanj, ber and sal dominant forest area. So, insect pests attacking on aforementioned vegetation and natural enemies associated with them Trichogrammatid egg parasitoids were collected from a various locations in agro-forestry zones in Chhattisgarh, India.

All the Collected and examined specimens have been submitted in National Forest Insect collection (NFIC), Dehradun, Uttarakhand, India.

Surveys were conducted in agro-forestry areas of different districts, namely Bilaspur, Dhamatari, Durg, Korba, Jangir-Champa, Raipur, Rajnandgaon in Chhattisgarh for the collection of trichogrammatid egg parasitoids. During monsoon and post monsoon periods, collection of trichogrammatids was carried out by sweeping method. Sweep net sampling was adopted in greenland areas of forest and agro-forests and the insect fauna collected were preserved in alcohol (70%). From these samples, trichogrammatid egg parasitoids were sorted out for the present study. After going through the normal course of dehydration, minute egg parasitoids were dissected in clove oil and mounted in euparal under glass cover slips. For identification of the collected material, important literature on trichogrammatid taxonomy (Doutt and Viggiani, 1968; Yousuf and Shafee, 1988; Lin, 1994) was consulted.

A total of 10 species of trichogrammatid egg parasitoids, namely *Aphelinoidea gwaliorensis* Yousuf & Shafee; *Lathromeroidea ajmerensis* Yousuf & Shafee, *Oligosita debaiensis* Yousuf & Shafee, *Oligosita gilvus* Yousuf & Shafee, *Pseudoligosita nephotetticum* (Mani), *Oligosita novisanguinea* Girault, *Oligosita sanguinea* Yousuf & Shafee, *Paracentrobia magniclavata* Yousuf & Shafee, *Ufens gurgaonensis* Yousuf & Shafee and *Ufens jaipurensis* Yousuf & Shafee were identified up-to species level. Brief account of these trichogrammatids collected from Chhattisgarh is presented.

1. *Aphelinoidea gwaliorensis* Yousuf and Shafee

Aphelinoidea gwaliorensis Yousuf and Shafee, 1985b: 303.

Hosts: Unknown

Distribution: INDIA: Andhra Pradesh, Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir Madhya Pradesh, Odisha, Punjab, Uttar Pradesh, West Bengal.

Specimens examined: INDIA: Chhattisgarh: Bilaspur, Khootaghat, 1♀(on slide), 1.iii.2017; Koni, 1♀ (on slide), 30.x.2017. coll. Manendra Kaneria (sweeping).

2. *Lathromeroidea ajmerensis* Yousuf and Shafee

Lathromeroidea ajmerensis Yousuf and Shafee, 1988: 160.

Hosts: Unknown

Distribution: INDIA: Madhya Pradesh, Punjab, Rajasthan.

Specimens examined: INDIA: **Chhattisgarh:** Bilaspur, Ratanpur, Khootaghat; 1♀, 1.iii.2017; coll. Manendra Kaneria (Sweeping); 1♀; Bilaspur, Koni; 30.x.2017; coll. Manendra Kaneria (sweeping).

3. *Oligosita debaiensis* Yousuf and Shafee

Oligosita debaiensis Yousuf and Shafee, 1988: 152-153.

Hosts: Unknown

Distribution: INDIA: Punjab, Uttar Pradesh.

Specimens examined: INDIA: Chhattisgarh, Korba, Kechuna; 1♀, 5.iii.2017; coll. Manish Kaneria (sweeping); 1♀; Dhamtari, Jamtara; 2.xi.2017; coll. Manish Kaneria (sweeping).

4. *Oligosita gilvus* Yousuf and Shafee

Oligosita gilvus Yousuf and Shafee, 1984: 17.

Hosts: Unknown

Distribution : INDIA: Madhya Pradesh, Punjab and Uttar Pradesh).

Specimens examined: INDIA: Chhattisgarh: Bilaspur, Ratanpur, Khootaghat, 1♀, 1.iii.2017; Rajnandgaon, Dongargarh, Pragyagiri; 2♀, 10.iii.2017; coll. Manish Kaneria (sweeping);

Bilaspur, Koni; 1♀, 30.x.2017; coll. Manendra Kaneria (sweeping).

5. *Pseudoligosita nephotetticum* (Mani)

Oligosita nephotetticum (Mani): Pinto & Viggiani, 2004: 289.

Hosts: *Nephotettix bipunctatus* (Uhler) on rice (Hemiptera: Cicadellidae).

Distribution: INDIA : Uttar Pradesh, Punjab, Haryana and Uttarakhand.

Specimens examined: INDIA: Chhattisgarh: Raipur, Barauda; 1♀, 9.iii.2017; coll. Manendra Kaneria (sweeping); Bilaspur, Koni; 1♀, 30.x.2017; coll. Manendra Kaneria (sweeping).

6. *Oligosita novisanguinea* Girault

Oligosita novisanguinea Girault, 1912:79-80.

Oligosita ruficorpa Yousuf and Shafee, 1988:146.

Oligosita novisanguinea Girault: Hayat 2008: 7.1.

Hosts: *Asphondylia miki*

Distribution: INDIA: Uttar Pradesh, Himachal Pradesh, Jammu and Kashmir, Haryana, Punjab); USA and Italy.

Specimens examined: INDIA: Chhattisgarh: Bilaspur, Ratanpur, Khootaghat; 1♀, 1.iii.2017; coll. Manendra Kaneria (Sweeping); Korba, Kechuna; 2♀, 5.iii.2017; Manish Kaneria (sweeping); 1♀; Bilaspur, Koni; 30.x.2017; coll. Manendra Kaneria (sweeping); 1♀, Rajnandgaon, Dongargarh, Pragyagiri; 10.iii.2017; coll. Manish Kaneria (sweeping);

7. *Oligosita sanguinea* Yousuf and Shafee

Oligosita sanguinea (Girault): Doutt & Viggiani, 1968: 540.

Hosts: Unknown

Distribution: INDIA: **Chhattisgarh,** Madhya Pradesh, Punjab.

Specimen examined: INDIA: Chhattisgarh: Jangir-Champa, Kotmi Sonar; 1♀, 15.vii.2016; coll. Manendra Kaneria (sweeping).

8. *Paracentrobia magniclavata* Yousuf and Shafee

Paracentrobia magniclavata Yousuf and Shafee, 1985a:301.

Hosts: Egg of coleopteran insect.

Distribution: INDIA: Uttar Pradesh, Madhya Pradesh and Punjab.

Specimen examined: INDIA: Chhattisgarh: Bilaspur, Ratanpur, Khootaghat; 1♀, 1.iii.2017; coll. Manendra Kaneria (Sweeping).

***9.* Ufens gurgaonensis** *Yousuf and Shafee*

Ufens gurgaonensis Yousuf and Shafee, 1988: 75.

Hosts: Unknown

Distribution: INDIA**:** Haryana, Gurugram, Madhya Pradesh, Punjab.

Specimens examined: INDIA: Chhattisgarh: Raipur, Kacheri; 1♀, 9.iii.2017; coll. Manendra Kaneria (sweeping); 2♀, 11.iii.2017; Durg, Pulgaon road; coll. Manendra Kaneria (sweeping)

10. *Ufens jaipurensis* Yousuf and Shafee

Ufens jaipurensis Yousuf & Shafee, 1988: 80.

Hosts: Unknown

Distribution: INDIA: Rajasthan, Punjab.

Specimen examined: INDIA: Chhattisgarh, Raipur, Kacheri; 1♀, 9.i0ii.2017; coll. Manendra Kaneria (sweeping).

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Report of *Nonartha birmanicum* (Jacoby) (Coleoptera, Chrysomelidae) on mango inflorescence

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ABSTRACT: *Nonartha birmanicum* (Jacoby) from the tribe Aliticini, is reported on mango for the first time. The chrysomelid beetles were found in abundance; nearly around 60 to 90 numbers on a panicle in full bloom stage. The beetle population was spotted particularly during the month of November, on the panicles in full bloom. These are bluish-black minute beetles and were noticed feeding mainly on pollen.
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KEY WORDS: New report**,** bloom panicles, floral pollen

Mango is one among the predominant fruits of twenty first century. The delicious fruit is renowned for its aroma and flavor with blended nutritional benefits (Dhenge *et al.,* 2022). Mango production and cultivation has turned out to be a hopeful venture in the global scenario. India with a share of more than 54 per cent is one of the leading mango producers (Tharanathan *et al.,* 2006). The crop suffers heavy incidence of invasion from insect and non insect species of varying orders comprising Coleoptera, Lepidoptera, Thysanoptera and Hemiptera (Kannan *et al*., 2002).

Mango panicles/ inflorescence samples were collected from Athiyannur block (8° 25' 21. 71'' N; 76° 58'37. 23" E) of Thiruvananthapuram district in Kerala. As a part of it, insect species from different orders were recorded and documented. Copious amount of minute bluish black beetles, of around 60 to 90 beetles from a full bloom panicle/ inflorescence were noticed from the collected samples. They were mainly feeding on the floral pollen. The insect was identified as *Nonartha birmanicum* (Jacoby), belonging to the tribe Aliticini of Chrysomelidae under Coleoptera. The beetle population was spotted particularly during the month of November, on the panicles in full bloom.

The incidence of *N. birmanicum* on mango as a host plant was noticed for the first time. There were reports of *N. cyaneum* on host plants of *Besella rubra, Hibiscus syriacus, Prunus tomentosa, Pyracantha angustifolia, Rosa borboniana, Spiraea thunbergii, Itea parviflora, Lythrum anceps, Swida stlonifera, Syringa vulgaris, Vitex cannabifolia, Cirsium nipponica, Baccharis trimera, Stenactis annuus* and *Liriope platyphylla* in Japan. They were primarily considered as floral visitors in these hosts (Kakutani *et al*., 1990). *N. patkia* reported in India was under the Wildlife protection Act (1972) (Kriti and Sidhu, 2015).

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Evergestis forficalis (L.) (Lepidoptera, Crambidae), a pest of cruciferous crops in the UT of Jammu and Kashmir, India

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ABSTRACT: Detailed studies on the pest biology, identification, nature and extent of damage, host plants and natural enemies of *Evergestis forficalis* (L.) (Lepidoptera, Crambidae) on cruciferous crops in Kashmir valley, India is reported. The biology of the pest on *Brassica oleracea* var. *acephala* is documented. The pest was found to be active in the field from July to September and inflicted serious damage to some economically important cruciferous plants in Kashmir Valley. One ichneuomonid parasitoid, *Chorinaeus* sp. has been recorded on the pest. © 2023 Association for Advancement of Entomology

KEY WORDS: Biology, extent of damage, host plants, parasitoid, *Chorinaeus, Brassica oleracea*

Evergestis forficalis (L.) (Lepidoptera, Crambidae), the garden pebble, is found in Europe, the Palearctic and North America and some parts of Asia. The moth has been reported as a pest on several crucifers/ Brassicaceae vegetable crops in India (Gupta, 1994; Bhat *et al.,* 2011; Bhat and Ahanger, 2018; Chandra *et al*., 2019; Anonymous, 2023). However, after a thorough literature study, indicated no detailed study on *E. forficaluis*, biology, host-range, nature and extent of damage and its natural enemies in Jammu and Kashmir. The present study encompasses the various aspects of the pest under taken.

A weekly extensive field survey was conducted at two study sites in two districts of Kashmir Valley during the year 2021 viz., vegetable field at Danderkhah Batmalo Srinagar District (34.0687°N; 74.7783°E) and another vegetable field at Serch Chowdrybagh in Ganderbal District (34.2165° N; 74.7719° E). The vegetable farms were surveyed. One plot was selected for sampling from each of the study site in above mentioned study areas. Samples were collected by hand picking method by using gloves. The sampling procedure was standardized by way of direct count technique where in number of larvae per plant was counted from at least 10 plants in four corners of the plot. The immature stages (larvae/caterpillars/pupae) of the pest were searched on the host plants and were collected and brought to the laboratory. The field data of the pest regarding number of eggs, larvae on each searched plant and nature and extent of damage of host plants, was recorded. The immature stages were reared in rearing containers in the laboratory to rear them as adults simultaneously observing for emergence of parasitoids, if any. The rearing was done at normal room temperature (around 30-32 ^{0}C) and in dry condition with proper hygiene and care maintained in rearing room. The larvae, during rearing, were frequently supplied with fresh leaves of the host plant. The debris and

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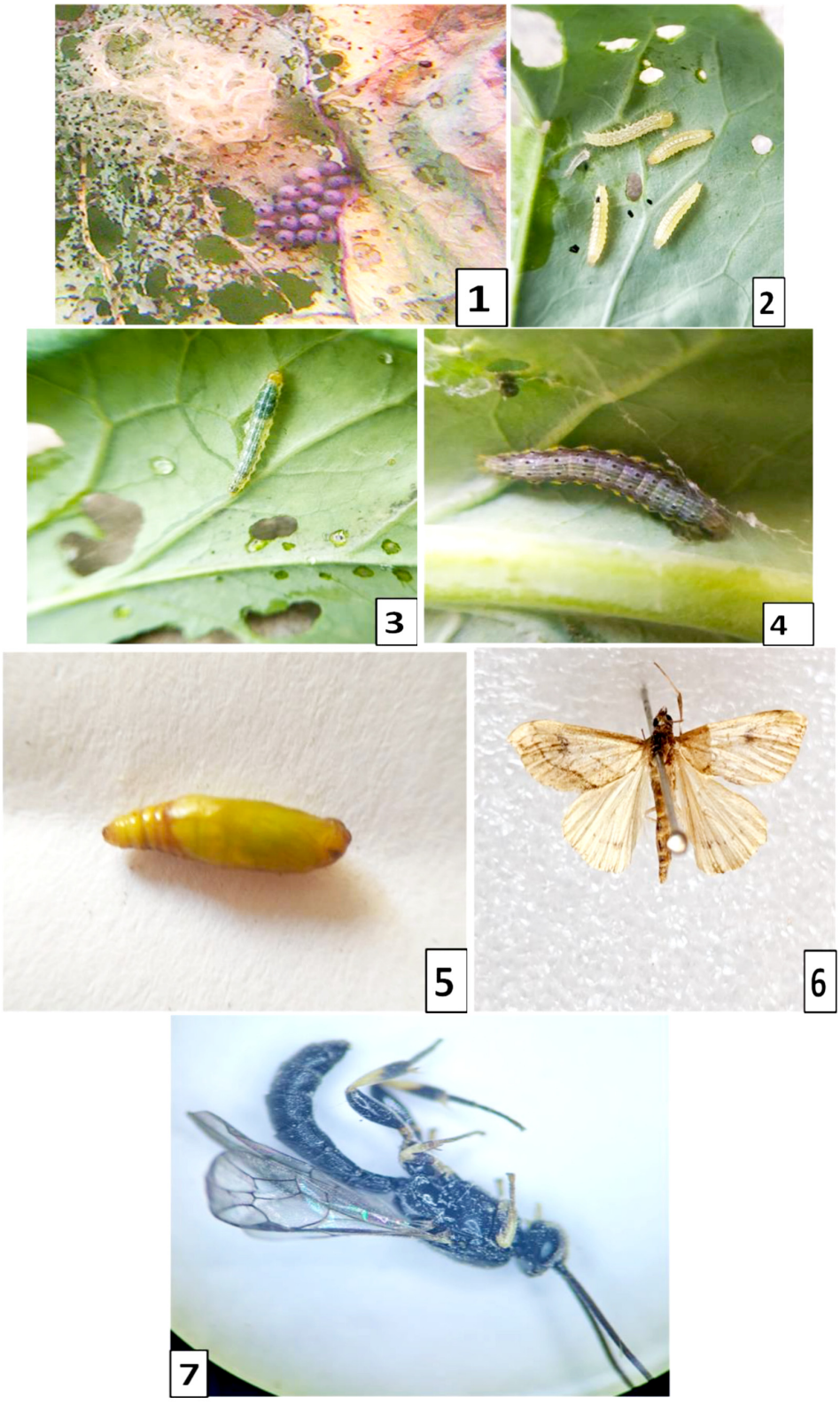

Fig. 1 Egg stage of *E. forficalis,* Fig. 2-4 larval stage of *E. forficalis,* Fig. 5 Pupal stage of *E. forficalis* Fig. 6 Adult stage of *E. forficalis*, Fig.7 Parasitoid, *Chorinaeus* sp. recorded on *E. forficalis*

excreta were regularly removed from rearing jars. In order to reduce the congestion, only 4-5 larvae were reared in one container. The bottoms of the rearing containers were filled with dry soil, so that, the final instars could get substratum for undergoing pupation. The adult moths emerged were preserved and identified (Hampson, 1896; https://www.mothsofindia.org/evergestis-forficalis). Similarly, the parasitoids (natural enemies) recovered during rearing were preserved and identified.

Adult moths (Fig. 6) were 10 to 14 mm long and brown with dark eyes and blotched markings on the tips of the wings with wingspan of around 25 mm. Forewings were straw-colored with olive to purplish-brown markings and transverse lines and hind wings were whitish with a dark margin.

The eggs were light pinkish and were laid in small clusters (10-28) on the under surface of leaves and were 1.5 mm x 1.00 mm each (Fig.1).The first instars after hatching were cream in color (Fig. 2), with subsequent instars turning greenish (Fig. 3). The fully grown caterpillars were having purple and white stripes across the body, two dots on each segment of the abdomen, and yellow lines that extend the length of the body on both sides (Fig. 4). Larvae developed through 4 instars. The last instar was around 18-20 mm long and had a bluish-gray coloring along the back with numerous transverse black bands.

During the present study, only 1-2 generation of the pest were observed in a year. Under the rearing conditions at room temperature (30^{o}C), the duration time from egg to adult was around 40 days. The egg took 4-8 days to hatch. Larval development was around 20-22 days (with fist and second instars 5-7 days, third instar 5-6 days and fourth instar 10-12 days). The pupal stage lasted 10-13 days. In the present study, the pupation was observed underneath leaves or in soil at the bottom of rearing containers. Pupae were brownish in colour and around 12 mm long (Fig. 5). The newly emerged adults upon emergence died within 5-6 days (under rearing conditions).

The young larvae after hatching from eggs scratched soft parts of leaves and fed gregariously. The later instars dispersed and fed in isolation, punching holes or causing more extensive defoliation of young and mature leaves. Caterpillars created irregular- shaped holes in leaves when feeding leaving only the veins causing skeltonization symptoms in the leaves. Under severe infestation, 3-4 leaves of each plant were found to be heavily skeltonized.

In Kashmir, the infestation of the host plants was recorded from the month of July to September, with highest activity in the month of August as observed during the study. The pest was observed feeding on some Brassicaceae plants and mostly infesting Kale (*Brassica oleracea* var. *acephala*), knol-khol (*B. o.* var. *gongylodes*), turnip (*Brassica rapa*) and cabbage (*B. o.*var. *capitata*).

One species of parasitoid *Chorinaeus* sp. (Ichneuomonidae) was recovered during rearing of *E. forficalis* larvae. However, the percentage of the parasitisation was very low, (1-2 %). The colour of the parasitoid was black (Fig 7). Face, clypeus, malar space, mandible, palpi, ventral part of scape, tegula, fore and mid legs, hind trochanters, and hind tibia to tarsus yellow. Antenna with 29 flagellomeres.

The observations recorded, during the present study on the host plants, nature and extent of damage and biology of *E. forficaluis*, are in line with the observations made by Meyrick, (1895), Thunisen *et al*. (1985), Gupta (1994), Gratwick (1992), Bhat *et al.* (2011) and Chandra *et al.* (2019). Moreover, the parasitoid, *Chorinaeus* sp. (Ichneuomonidae) is the first report on *E. forficalis.*

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Evaluation of cauliflower genotypes and eco-friendly molecules for management of Diamond back moth, *Plutella xylostella* (Linnaeus) (Lepidoptera, Yponemeutidae) and their safety to natural enemies

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ABSTRACT: Four late season cauliflower genotypes namely Pusa Snowball 1 (PSB 1), Pusa Snowball K1 (PSB K1), Pusa Snowball K25 (PSB K25) and Plantsman Snowball (PMS) were evaluated for their resistance to Diamond Back Moth (DBM), *Plutella xylostella* (Linnaeus), during 2018-19. Among the genotypes screened PSB1 and PMS were comparatively less susceptible. Biochemical analyses of leaves from 85 days old healthy plants showed that PMS had higher phenol content (89.9 mg $100g^{-1}$) which on par with PSB-1 with 81.43 mg $100g^{-1}$ of leaf. This was followed by PSB K25 and PSB K1 with 69.5 and 54.23 mg $100g^{-1}$ respectively. The varieties with higher phenol content offered more resistance to DBM. Protein content of the plant showed no correlation with DBM infestation. Foliar application of spinosad (45 % SC @ 56.25 g ai ha^{-1} +NSKE 2.5 %) recorded a higher reduction (94.40 %) of DBM population over control, with comparatively higher number of syrphids and coccinellids indicating its safety to natural enemies.
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KEY WORDS: Resistance, phenol, spinosad, NSKE, syrphids, coccinellids

Cauliflower, *Brassica oleracea* var *botrytis* (n=9), belonging to Brassicaceae family is a winter vegetable of global importance due to its nutritive and economic value. Lo Scalzo *et al.* (2013) evidenced that cauliflower produced organically contained high amount of carotenoids, polyphenols and ascorbic acid and showed increased antioxidant activity. India is the second largest producer of cauliflower and one of the largest exporters of cauliflower as well. Punjab is one of the major cauliflower producing Indian states (Department of Horticulture, Govt. of Punjab, 2021). Cauliflower cultivation is challenged by nearly 40 species of insect pests. Diamondback moth (*Plutella xylostella* L.), cabbage white butterfly (*Pieris brassicae* L.), cabbage head borer (*Hellula undalis* F.), cabbage webworm (*Crocidolomia binotalis* Zeller), tobacco caterpillar (*Spodoptera litura* F.), mustard saw fly (*Athalia lugens proxima* Klug.), pea leaf miner (*Chromatomyia horticola* Goureau), green peach aphids (*Myzus persicae* Sulzer), mustard aphid (*Lipaphis erysimi* Kaltenbach) and painted bug (*Bagrada hilaris* Burmeister) are major insect pests for cauliflower in India (Ahuja *et al*., 2015; Bhushan and Pathma, 2019). Diamond back moth (DBM), *Plutella*

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Table 1. Effect of genotypes and plant age on DBM infestation in cauliflower

Genotypes	Number of DBM per plant*					
	74	81	88	95	102	109
PSB 1	0.00 (0.70)[a]	0.00 (0.70)[a]	0.27 (0.87)[a]	1.30 (1.34)[a]	0.30 (0.9)[b]	0.30 (0.9)[ab]
PSB K1	0.03 (0.72)[a]	0.03 (0.72)[a]	0.17 (0.81)[a]	1.73 (1.49)[a]	0.67 (1.08)[c]	0.73 (1.10)[b]
PSB K25	0.07 (0.75)[a]	0.03 (0.72)[a]	0.27 (0.87)[a]	1.73 (1.49)[a]	0.50 (1.00)[bc]	0.77 (1.12)[b]
PMS	0.00 (0.70)[a]	0.00 (0.70)[a]	0.30 (0.9)[a]	1.07 (1.25)[a]	0.00 (0.70)[a]	0.17 (0.81)[a]

DAS – Days After Transplanting, * Mean of six replicates

Figures in parentheses are $\sqrt{x+0.5}$ transformed values

In column, means followed by a common letter are not significantly different by DMRT (P=0.05)

xylostella (L.) (Lepidoptera, Yponemeutidae) is one of the most destructive pests damaging brassica crop worldwide. Management of this noxious pest is of global concern owing to its short life cycle, high migratory potential, evolutionary traits and gut microbiota which had enabled them to develop resistance to almost all known classes of synthetic insecticides as well as to *Bacillus thuringiensis* (Furlong *et al.*, 2013; Dotasara *et al*., 2017; Gautam *et al*., 2018; Li *et al*., 2018; Qin *et al*., 2018; Xia *et al*., 2018; Liao *et al.*, 2019; Wang *et al.*, 2020).

Under Indian scenario DBM cause a significant damage (31-100%) and heavy economic loss (Talekar and Shelton, 1993; Lingappa *et al*., 2006; Uthamasamy *et al.,* 2011; Imran, 2018). Numerous pesticides have been used to control DBM as well as other pest complex on cauliflower and its threatening to understand that majority of the cauliflower samples tested elsewhere globally showed increased levels of pesticide residues than the permissible limits (IARI, 2009; Panhwar and Sheikh, 2013; Pujeri *et al.*, 2015; Prodhan *et al*., 2016). A study was formulated to assess a few popular Indian cauliflower genotypes suitable for late season cultivation in Kapurthala, Punjab, India for its degree of resistance as well as evaluate eco-friendly molecules and their combinations with neem seed kernel extract against *P. xylostella* with minimum impact on non-target organisms.

The experiments were carried out in research farm of Lovely Professional University (LPU), Phagwara, Punjab, India (31° 15' 47" N; 75° 41' 20" E). For both the trials cauliflower plants were grown with a spacing of 60×45 cm in Randomized block design (RBD) in plots of size 17.28 m^2 (3.6 × 4.8m). All the cultivation practices except crop protection were followed as per the recommendation**.** Seedlings were raised in the hi-tech poly-house facility in LPU and were transplanted on main field on 40 days after sowing (DAS). Four popular Indian late season cauliflower genotypes *viz*., Pusa Snowball 1 (PSB1), Pusa Snowball K1 (PSB K1), Pusa Snowball (PSB K25) and Plantsman snowball (PMS) were used in the study with six replications. Observations were made at weekly intervals on the incidence of DBM on different cauliflower genotypes. Morphological features such as seedling colour, leaf size, leaf colour, leaf shape, surface wax, curd color, curd compactness etc., of different cauliflower genotypes were studied. Total phenol (Zieslin and Ben-Zaken, 1993) and total proteins (Lowry *et al.,* 1951) of the genotypes were estimated using standard protocol in order to assess the influence of the plant biochemistry on incidence of DBM.

To study the bio-efficacy of synthetic and natural insecticides and their combination in half their recommended doses on management of DBM, a total of eight treatments with three replications were maintained. The treatments (Table 3) were imposed 85 DAS. Cauliflower variety PSB K1 which showed resistance to black rot but showed

Table 2. Morphological characters of different cauliflower genotypes

Parts	PSB 1	PSB K 1	PSB K 25	PMS
		Leaf		
Attitude	Erect	Semi-erect	Erect	Erect
Length	Medium	Large (>50 cm)	Medium	Large (>50 cm)
Width	Medium (15-25cm)	Medium (15-25cm)	Medium (15-25cm)	Broad > 25cm
Shape	Narrow elliptic	Broad elliptic	Broad elliptic	Broad elliptic
Crimping near main vein	Medium	Strong	Strong	Medium
Colour	Light	Light	Light	Dark
Profile (dorsal)	Flat type leaf	Convex	Concave	Concave
Puckering	Absent	Medium	Strong	Medium
Maturity Group				
Curd maturity	Late	Late	Late	Late
Curd initiation	Late (>100)	Late (>100)	Late (>100)	Late (>100)
Curd colour	Creamy white	White	White	Creamy white
Surface wax				
Waxiness	Light leaf wax	Light leaf wax	Strong leaf wax	Stronger leaf wax
Seedling colour				
Anthocyanin colouration of hypocotyl	Present	Present	Present	Absent
Colour	Light violet	Light violet	Light violet	Light green

increased susceptibility to DBM was used. Observations were made on 0, 1, 3, 5, 7, 9 and 14 days after treatment (DAT) and the mean pest population and percent reduction over control were estimated. Data on natural enemies present in the ecosystem were also recorded to understand the impact of pesticides used on them.

The data were subjected to analysis of variance (ANOVA). The means were separated by Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984). For all statistical analysis SPSS version 22.0 was used.

DBM incidence was observed by the last week of February and reached peak by the fourth week of March (1.46 DBM per plant). Observations on DBM incidence on the four different cauliflower cultivars showed that all the varieties showed similar infestation levels until 95 days after transplanting. At 102 DAT, PMS showed less infestation followed by PSB 1 and PSB K25 which were on par with each other while PSB K1 showed higher infestation levels. When the crop was ready to harvest at 109 DAT PMS and PSB 1 showed lower levels of infestation and were statistically similar while PSB K1 and PSB K25 showed increased levels of infestation by DBM (Table 1). Observations on morphological traits of genotypes showed no significant difference however, PMS, which showed least suscelibility to DBM infestation, had dark green leaves with stronger surface wax as compared to the other genotypes (Table 2).

The total phenol as well as the total protein content significantly varied among the genotypes. PMS showed higher phenol content (89.9 mg per 100 g of leaves) followed by PSB1, PSB K25 and PSB K1 with 81.43, 69.50 and 54.23 mg per 100 g of leaves respectively. PSB K1 recorded higher total proteins (154.26 mg/g) followed by PSB1 (136.69 mg g^{-1}), PMS (111.73 mg g^{-1}) and PSB K25 recorded the lowest total protein content of 32 mg/g of leaf sample.

In the bioefficacy of the treatments against DBM, all the treatments except control were found equally effective on 1, 3, 5, 7, 9, and 14 DAT against DBM.

Table 3. Efficacy of insecticides and NSKE and their combination against DBM on cauliflower

Treatment	No. of DBM / per plant								% Reduction
	PTC	1DAT	3DAT	5DAT	7DAT	9DAT	14DAT	Mean	
T1 - Spinosad 45 % SC 112.5 g ai/ha	1.80 (1.51)^{ab}	0.93 (1.19)^{a}	0.33 (0.91)^{a}	0.20 (0.83)^{a}	0.07 (0.75)^{ab}	0.20 (0.83)^{a}	0.13 (0.79)^{a}	0.31	86.63
T2 - Chlorantraniliprole 18.5% SC 40 g ai/ha	2.33 (1.68)^{ab}	0.40 (0.94)^{a}	0.33 (0.91)^{a}	0.20 (0.83)^{a}	0.20 (0.83)^{abc}	0.20 (0.83)^{a}	0.07 (0.75)^{a}	0.23	90.08
T3 - Acetamiprid 20% SP 20 g ai/ha	2.73 (1.80)^{ab}	0.27 (0.87)^{a}	0.40 (0.94)^{a}	0.13 (0.79)^{a}	0.00 (0.70)^{a}	0.27 (0.87)^{a}	0.00 (0.70)^{a}	0.18	92.24
T4 - NSKE 5 %	1.27 (1.33)^{ab}	0.40 (0.94)^{a}	0.07 (0.75)^{a}	0.20 (0.83)^{a}	0.33 (0.91)^{c}	0.20 (0.83)^{a}	0.40 (0.94)^{a}	0.27	88.36
T5 - Spinosad 45 % SC 56.25 g ai/ha +NSKE 2.5%	1.80 (1.51)^{ab}	0.13 (0.79)^{a}	0.47 (0.98)^{a}	0.07 (0.75)^{a}	0.07 (0.75)^{ab}	0.00 (0.70)^{a}	0.07 (0.75)^{a}	0.13	94.40
T6 – Chlorantraniliprole 18.5% SC 20 g ai/ha +NSKE 2.5 %	0.53 (1.01)^{a}	0.47 (0.98)^{a}	0.53 (1.01)^{a}	0.13 (0.79)^{a}	0.13 (0.79)^{abc}	0.20 (0.83)^{a}	0.40 (0.94)^{a}	0.31	86.63
T7 - Acetamiprid 20% SP 10 g ai/ha +NSKE 2.5 %	1.80 (1.51)^{ab}	0.33 (0.91)^{a}	0.40 (0.94)^{a}	0.07 (0.75)^{a}	0.27 (0.87)^{bc}	0.27 (0.87)^{a}	0.20 (0.83)^{a}	0.26	88.80
T8 - Control (water)	3.60 (2.02)^{b}	3.20 (1.92)^{b}	4.00 (2.12)^{b}	2.60 (1.76)^{b}	1.60 (1.44)^{d}	1.40 (1.37)^{b}	1.13 (1.28)^{b}	2.32	

* Mean of three replicates (DBM were counted on 5 randomly selected plants per replication and was expressed as no. of DBM per plant) PTC – Pre treatment count; DAT – Days after Treatment; Figures in parentheses are $\sqrt{x+0.5}$ transformed values

However, spinosad (45 % SC @ 56.25 g ai ha^{-1}) +NSKE (2.5 %) was showed maximum reduction (94.40 % over control), followed by acetamiprid (20% SP @ 20 g ai ha^{-1}) which offered 92.24 per cent, chlorantraniliprole (18.5% SC @ 40 g ai ha^{-1}) offering 90.08 per cent. Acetamiprid (20% SP @ 10 g ai ha^{-1}) + NSKE (@ 2.5 %), NSKE (@ 5 %), spinosad (45 % SC @ 112.5 g ai ha^{-1}) and chlorantraniliprole (18.5% SC @ 20 g ai ha^{-1}) +NSKE (@ 2.5 %) offering 88.80, 88.36, 86.63 and 86.63 per cent reduction over control (Table 3). Assessment of effect of pesticides under study on the natural enemies (coccinellids and syrphids) showed that there was no significant difference in coccinellid and syrphid population among the treatments imposed throughout the experimental period. Higher number of beetles were recorded on plots treated with spinosad (45 % SC @ 112.5 g ai ha^{-1}) (mean 0.07 beetles plant^{-1}) and chlorantraniliprole (18.5% SC @ 40 g ai ha^{-1}) (0.13 beetles plant^{-1}) on 3 DAT. Plots treated with spinosad (45 % SC @ 112.5 g ai ha^{-1}), Spinosad (45 % SC @ 56.25 g ai ha^{-1}) +NSKE (2.5 %), chlorantraniliprole (18.5% SC @ 20 g ai ha^{-1}) +NSKE (2.5 %) and acetamiprid (20% SP @ 10 g ai ha^{-1}) + NSKE (2.5%) recorded a mean population of 0.20, 0.07, 0.07, 0.07 beetles per plant respectively on 7 DAT. Observations on syrphid population showed that no significant difference among the treatments throughout the study period except on 7 DAT where plots treated with NSKE (5 %) and Spinosad (45 % SC @ 56.25 g ai ha^{-1}) + NSKE (2.5 %) recorded higher syrphids (0.07 and 0.13 per plant respectively).

Genotypes PSB K1 and PSB K25 showed increased levels DBM infestation while PSB1 and PMS showed comparatively lesser levels of DBM infestation. Analysis of total phenolics and total protein of the healthy leaves of 85 days old crop showed that all varieties possessed a different biochemistry which might be the potential reason behind the varying degrees of insect pest incidence at the early stages of crop growth. In the current study PMS recorded higher phenol content followed by PSB 1 which contributed to comparatively low DBM incidence on these two genotypes. PMS had dark coloured leaf with strong surface wax which could possibly influence pest incidence making it less prone to oviposition and subsequent feeding

damage by DBM. Leaf size, colour and surface wax thickness influence the pest incidence in different crops (Radcliffe and Chapman, 1966; Myers, 1985; Eigenbrode *et al.,* 1991; Talsma *et al.,* 2008). Additionally current investigation shows that host plants physiological age decides its biochemical and morphological traits thereby influencing its preference by insect pests for feeding and oviposition. The results were in line with previous investigations (Renwick and Lopes, 1999; Jankowska, 2006; Cartea *et al.,* 2009; Metspalu *et al.,* 2009, Lo Scalzo *et al.,* 2013)

Spinosad +NSKE treated plots recorded the higher number of syrphids as well as coccinellids indicating its safety to natural enemies. The results were in line with findings of Aswathi *et al.* (2013). From the investigation it is evident that use of PMS variety along with use of Spinosad (45 % SC @ 56.25 g ai ha^{-1}) +NSKE (2.5 %) will act as an effective module for management of DBM.

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