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#### **ENTOMON**

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ENTOMON acknowledges the AAE web site Manager, Professor K. Madhavan Nair, for his yeoman service. M/s SB Press, Thiruvananthapuram ably supports in bringing out ENTOMON issues on time. Albeit the work load of experts and peer reviewers, they have responded to our requests for their critical evaluation and suggestions on the manuscripts. It is our bounden duty to extend our sincere and profound thankfulness to them.

It is gratifying to inform that all the issues of ENTOMON are as per schedule. Herewith ENTOMON volume 49, issue 1 (March, 2024) is presented before you.

With warmest of regards

Regards

Dr M.S. Palaniswami

Chief Editor

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#### Redescription of *Pomponia cyanea* Fraser, 1948 (Hemiptera, Cicadidae) from the Western Ghats, with a note on its junior synonym *P. zebra* Bliven, 1964, *syn. nov.*, and *Terpnosia polei* (Henry, 1931), from Sri Lanka

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**ABSTRACT**: *Pomponia cyanea* Fraser, 1948 from the southern Western Ghats, of peninsular India is redescribed based on the types and fresh specimens with additional data on distribution, morphometrics, and structure of male genitalia. The validity of *P. zebra* Bliven, 1964, originally described from the Anamalai Hills is discussed. A study of fresh specimens and holotype images of *P. zebra* revealed no difference in general morphology, wing venation, or structure of male genitalia so as to separate it from *P. cyanea*, and hence *P. zebra* is treated as a junior synonym of *P. cyanea* Fraser, 1948, **syn. nov.** The taxonomic status of *Terpnosia polei* (Henry, 1931) from Sri Lanka is revised based on male morphology and is transferred to its original genus as *Pomponia polei* Henry, 1931, **comb. nov.** A key to males of known species of *Pomponia* of Western Ghats is provided. © 2024 Association for Advancement of Entomology

KEY WORDS: Cicada, Auchenorrhyncha, Psithyristriini, Kerala, morphometric index

#### **INTRODUCTION**

The genus *Pomponia* Stål, 1866 is a speciose group of cicadas distributed in the Oriental and Palearctic regions (Pham *et al.*, 2015). Ten species of *Pomponia* have been recorded from the Indian territory (Price *et al.*, 2016). Of these, *P. cyanea* Fraser, 1948; *P. zebra* Bliven, 1964; and *P. linearis* (Walker, 1850) were reported from the Western Ghats of Peninsular India (Distant 1906; Price *et al.*, 2016). Sadasivan (2021), established that the records of *P. linearis* from the Western Ghats, were erroneous, and this species in south India was an undescribed one and was recently described as *P. pseudolinearis* Sadasivan 2021. *Pomponia cyanea* Fraser, 1948, was collected by F.C. Fraser from Coorg (Karnataka State) and Munnar Hills (Kerala State), in the Western Ghats. The original description was very brief and there was no information on the structure of the male genitalia. *Pomponia zebra* Bliven, 1964, was a species described from Kadamparai, in Anamalai Hills, Madras State (Tamil Nadu), just north of Munnar (type locality of *P. cyanea*).

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During the field survey of *Pomponia* in the Anamalai Hills, collected specimens from type localities of *P. cyanea* and *P. zebra*. A careful analysis of the morphology of type specimens of the two taxa, and that of fresh field specimens collected, revealed no character to separate them, and thus the two species are here synonymised. *Pomponia cyanea* is redescribed based on fresh male specimens with additional data on morphometrics and male genitalia. During our study, it was noted that the holotype of *Terpnosia polei* (Henry, 1931) from Sri Lanka was similar in appearance to *P. cyanea* and subsequent examination indicated that the former should be returned to its original genus, *Pomponia*.

#### **MATERIALS AND METHODS**

Three morphotypes of Pomponia were collected from the Anamalais and Agasthyamalais in the Western Ghats of Kerala State, in southern India (Fig. 1). The taxon from the low elevation (< 1200 m ASL) was described as P. pseudolinearis Sadasivan 2021. Of the high elevation morphotypes collected from near Valparai and high ranges of Munnar, some individuals matched the description of P. zebra, while others matched with that of P. cyanea. A detailed study of the internal morphology and morphometrics of the specimens was done, and compared with the holotypes and paratypes at the Natural History Museum, London (BMNH) London, and the California Academy of Sciences (CAS), California. Photographs were taken with a Canon 7D Digital SLR, 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 description follows that of Moulds (2005). The taxonomy and placement follows Hill *et al.* (2021) and Dmitriev *et al.* (2021). Morphometric measurements follow Sarkar (2019) and Sadasivan (2021). Illustrations were hand-drawn by KS using the stereomicroscope and then digitalized. The orientation of spines is referred to as 'erect', 'semi-erect', 'semi-decumbent', 'decumbent', and 'appressed'. 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. The original descriptions, specimens, and field photographs were analysed for comparison.

Measurements (in millimetres taken in the dorsal view, unless specified) and indices used in descriptions as per Sadasivan (2021) are as follows-

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 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  $\times$  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

#### **Systematics**

**Family** Cicadidae Latreille, 1802; **Subfamily** Cicadinae Latreille, 1802; **Tribe** Psithyristriini Distant, 1905

#### Genus Pomponia Stål, 1866

Diagnosis. Vertex of head narrower and somewhat equal to the distance between eyes; posterior pronotal collar broad; broad pale transverse band across the postclypeus; lateral margin of pronotum dentate. Wings hyaline; forewing basal vein of apical cell 1 extremely short; forewing apex sharp; costal margin of forewing hardly concave. Metanotum entirely concealed at the midline. Male operculum small, scale-like; male opercula wider than long and nearly contiguous to each other; male operculum broader than long, nearly touching each other. The larger part of the timbal concealed with the timbal covering. Large-sized, male body longer than 35mm; male abdomen gradually tapering to apex; lateral surfaces of male 3rd and 4th abdominal sterna without tubercle-like projections; male 8th abdominal tergum with no or little white pollinosity. Acute lateral pygofer lobes, a trapezoid uncus with medial incision suggesting the fusion of two short, broad lobes, and a pair of claspers, each with two spines protruding from below the uncus and distinctly protruding paramedian basal pygofer lobes or lobes that are placed laterally adjacent to the sides of the pygofer (Distant, 1906; Lee and Hayashi 2003; Duffels and Hayashi 2006; Pham *et al.*, 2015).

#### Pomponia cyanea Fraser, 1948 (Figs. 2-7)

*Pomponia cyanea* Fraser, 1948: 184–185 (Original Description); Duffels & Hayashi 2006: 197 (brief note on species group and male genitalia).

**Material examined (type locality of** *P. cyanea*) (n = six males). Three males, Rajappara, Munnar, Idukky District, Kerala, May 2019, 1800m ASL, THRG 0035 (Coll. Kalesh Sadasivan); Two males, Mangulam Reserve Forest, Idukki District, Kerala, 900 m ASL May 2022 (KS); and one male, Shantanpara, Idukky District, Kerala, 1200 m ASL, 19<sup>th</sup> May 2012 (Coll. Prathapan K.D). Two male specimens each will be subsequently deposited in National Centre for Biological Sciences, Bengaluru, Karnataka, and Zoological Survey of India, Calicut, Kerala.

**Fresh material collected from Anamalai Hills** (type locality of *P. zebra*): 4 Males, Valparai slope of High Range, Munnar, Idukky District, 2200m ASL, May 2022 (KS).

**Type specimens studied:** Five males. BMNH (E) #1009395, BMNH (E) #1009396, BMNH (E) #1009397, BMNH (E) #1009398 (all male syntypes of *P. cyanea*) Munnar, Anamalai Hills, Travancore (Coll. Fraser F.C., 1933), and BMNH (E) #1009394 male, Munnar, Anamalai Hills, Travancore (Coll. Fraser F.C., date of collection unknown).

**Field observations** (Not collected). *Agasthyamalais*: 5 males and 3 females, Shendurney Wildlife Sanctuary, Kollam District, 1200m ASL, May 2018 (KS & AS). *Anamalais*: 15 males and 3 females, Pampadum Shola National Park, Idukky District, 2300 m ASL, May 2019 (KS & AS); 5 males and 3 females, Eravikulam National Park, Idukky District, 2200 m ASL, May 2019 (KS & AS); 6 males and 2 females, Shantanpara, Idukky District, Kerala, 1300m ASL May 2019; 3 males and 2 females (KS & AS); Mangulam Reserve Forest, Idukky District, Kerala, 900m ASL May 2022 (KS); 3 males, Valparai slopes, Eravikulam National Park, Idukky District, 2200m ASL, May 2019 (KS & AS).

**Measurements from fresh material collected from Anamalai Hills from the type locality of** *P. cyanea*): Males (n=six): FWL-50.75±5.06; FWW-16.50±1.29; HWL-27.75±2.22; HL-3.13±0.63; HW-9.75±0.96; EL-2.94±0.13; PL-4.88±0.25; PW-13.50±1.00; ML-10.00±0.82; MW-10.50±1.29; AL-24.50±2.52; AW-16.75 ±2.06; OPL-6.44±0.66; RL-11.00±1.41; ABL-18.00±1.47; TL-42.50±3.54; CI-317.08±37.28; OI-30.29±2.48; PI-276±6.67; MI-105.05±10.07; OPI-40.5±6.17; API-73.85±7.19; RI-61.32±8.75; FAR-307.61±19.80; TI-282.69±29.02; IWR-182.70±4.95, GI-68.41±5.49.

**Measurements from fresh material collected from Anamalai Hills near the type locality of** *P. zebra*: Males (n=2 males): FWL–50.75±5.06; FWW–16.50±1.29; HWL–27.75±2.22; HL– 3.13±0.63; HW–9.75±0.96; EL–2.94±0.13; PL– 4.88±0.25; PW–13.50±1.00; ML–10.00±0.82; MW–10.50±1.29; AL–24.50±2.52; AW–16.75 ±2.06; OPL–6.44±0.66; RL–11.00±1.41; ABL– 18.00±1.47; TL–42.50±3.54; CI–317.08±37.28; OI–30.29±2.48; PI–276±6.67; MI–105.05±10.07; OPI–40.5±6.17; API–73.85±7.19; RI–61.32±8.75; FAR–307.61±19.80; TI–282.69±29.02; IWR– 182.70±4.95, GI–68.41±5.49.

#### Description of the male (Figs. 2-7)

*Head* (Figs. 2–3, 6–7). In dorsal view, head is small, triangular, postclypeus anterior margin rounded, but not prominently protruding anteriorly; head much wider than long (CI–317.08±37.28); head bright green with brown markings; ocelli bright pink and ocular tubercles surrounding the ocelli greenishbrown; distance between lateral ocelli and medial margin of eyes twice the distance between the two lateral ocelli; postocular long golden hairs present, medial part of epicranial suture brown and its anterior arms inconspicuous; eyes dark amber

brown; pedicel and rest of the flagellum amber brown; frons green; supra-antennal plate dark greenish-black; frontoclypeal suture bordered with brown; dorsum of postclypeus dark green with transverse rudimentary dark greenish-black lines in the transverse grooves. In anterior view, eyes prominent, its inferior edge bordered in chrome yellow; scape of antenna greenish-brown; postclypeus squarish, swollen, and its inferior aspect triangular and tapering towards the anteclypeus; postclypeus fully green without any broad pale greenish-yellow transverse band; genae turquoise green; groove below supra-antennal plate green; lorum yellowish green; anteclypeus wholly green with its basal tip yellow; whole of the rostrum bluishwhite with the brown central groove and distal-most tip (one-eighth) black; labrum bordered with brown and mentum tipped with brown; labium pale brown with median groove dark brown, rostrum reaches distal border of sternite I, at the level of the distal margin of the operculum (RI $-61.32\pm8.75$ ).

Pronotum (Figs. 2-3, 6-7). Pronotal width almost thrice its length (PI-276±6.67); lateral margin of pronotum dentate; lateral angle of pronotal collar broad and rounded and its postero-lateral margin well-developed; rest of the collar thinner. The anterior borders of pronotum with the head bear a thin dark greenish-black band, which joins median hourglass-shaped green mark; paramedian and lateral pronotal lobes prominent and coloured bluishgreen. The general colour of the pronotum is dark green; paramedian and lateral fissures not conspicuously marked; ambient fissure marked in black; the pronotal collar green, the region of the lateral spine greenish-brown, a small median black line on the dorsum present as a continuation of the hourglass mark.

**Mesonotum** (Figs. 2–3, 6–7A, B). In dorsal view, the mesonotum is marginally wider than its length (MI–105.05 $\pm$ 10.07); submedian sigillae (ssig), bluish-green to violet-blue with regular borders, much shorter than half of the length of the mesonotum, black, and bordered with dark brown laterally; lateral sigillae (lsig) greenish-blue, with regular borders, and distal end reaches the anterior arms of the scutellum; distal ends of sigillae are not



Fig. 1 Map showing the type locality and spot records of *Pomponia cyanea* and *P. zebra* from the Western Ghats

connected; scutal depression is black and this region has prominent golden pilosity; (scutellum) sap green and lateral depressions greenish-blue. In ventral view, green with pollinosity mostly in the mid-ventral aspect; meracanthus (mc) very short and reaches distal border of hind coxae; wing grooves and postero-lateral aspect of mesonotum are turquoise blue.

**Operculum** (Fig. 3C). Broad, short (OPI– $40.5\pm6.17$ ), almost semi-circular with the medial margin angulated; operculum does not meet the opposite one; distally it reaches the sternite posterior margin of II. Colour is light opalescent green with pollinosity on its surface.

*Wings* (Figs. 2, 4A, 7A). Wings hyaline, forewing long and apex sharp; transparent with an amber tint all over, with infuscations as follows – basal veins of all apical segments except distal half of spaces 3, 6, and 8; at nodal line intersection and intersections of CuA<sub>2</sub>; distal half of all veins RA<sub>2</sub>, RP,  $M_1-M_4$  and CuA<sub>2</sub>; Pterostigma and wing margins faintly infuscated; veins bordering the clavus and cubital cell of forewing line by red; rest of the veins yellow, but lined in black around the joints; anterior wing margin till the node is dark

bluish brown to black. Hindwing with 6 apical spaces; and 10 minor transparent veinlets/folds in the anal lobe space between veins 3A and 2A on magnification.

*Legs* (Figs. 3D, 7A, C). Forelegs with coxae dark green; femur pale green with black lower third; primary (long, blunt-tipped almost appressed to the femoral surface) and secondary spines (long as the primary, but sharp) present and small spine one-third of the primary spine present distal to secondary spine; tibiofemoral joint black, tibia shiny black; meso-metatarsus, pretarsus, and claws black; middle legs with same color scheme as forelegs; hind legs with coxae paler green, femur greenish; upper tibia black and rest of it pale green; tibiotarsal joint region very pale brown; tibial spines and combs brown; claws black at the tip.

**Abdomen** (Figs. 2, 3A, D, 6, 7A). Slightly longer than head and thorax put together (API– $73.85\pm7.19$ ); widest at the distal end of the sternite II; sides uniformly tapering till 8; color bright greenish-blue to blue with posterior third of each segment thinly lined with black and the adjoining part of the tergite violet-blue for about its distal third.



Fig. 2 *Pomponia cyanea* Fraser, 1948 male from the type locality Munnar, Anamalais. A–dorsal view of pinned insect; B–ventral view of pinned insect; C–ventroposterior view of anal appendage; D–lateral view of the anal appendage. Images by Kalesh Sadasivan

The middle third violet-blue; anterior aspect of each segment on its mid-dorsum has a thin violet-blue transverse margin, which is thicker mid-dorsum. On the second and third tergite, the anterior and posterior violet margins are linked by a central dorsal band, which is thick in segment 2 and much thinner in segment 3. All tergite bears on its dorsolateral aspect a small dark violet-blue spot, this may be absent in some specimens on the basal segments. No tubercles.

*Genitalia* (Fig. 5). The lateral lobe of the pygofer acute, with the apex sharp and directed posteriorly, its inferior margin irregularly curved; pygofer near



Fig. 3 *Pomponia cyanea* Fraser, 1948. Images of dry pinned insect from the type locality Munnar, Anamalais. A–dorsal close-up of the head and body; B–dorsal close-up of the head, pronotum, and mesonotum; C–ventral view of operculum; D–lateral view of the body. Images by Kalesh Sadasivan

the apex bears sparse hairs; medial lobe of the uncus trapezoid with a median incision at its tip suggests a fusion of lobes, tip notched at the exit of the aedeagus; clasper protruding from below uncus broad rounded; upper basal lobe of pygofer welldeveloped and prominent than basal lobes; distinctly protruding short paramedian pygofer basal lobes with apices directed posterolaterally; gutter between the lower basal lobes on each side is U-shaped, deep and divergent (Fig. 5A); aedeagus thick with its proximal half wider, rest is gently curved, tapering finely to its tip. Basal plate as shown in figure 8D. On, the dorsal view the apex of the lateral lobe of the pygofer reaches height of anal styles; dorsal beak is well-developed and extends as a small lingula (Fig. 5D).

**Variation.** There was significant variation in the size and color of the specimens studied. While some were bright blue, others were turquoise green (Figs. 6A-D). Dry preserved specimens had shades of browns instead of blues (Fig. 2). The total length of the insect varies with a range of  $42.00\pm4.34$  mm. The wing lengths were slightly variable (FWL- $50.75\pm5.06$ ), while the rostral lengths were relatively constant (RL- $11.00\pm1.41$ ). The dorsal beak of the lateral pygofer lobe in male genitalia is well-developed as a lingular extension, but the length may vary.

**Current distribution.** Coorg, Karnataka (Fraser 1948); Munnar (Fraser 1948), Mangulam Reserve Forest, Idukky District, Kerala, 900m ASL May 2022 (KS). Eravikulam (KS & AS), Mathikettan Shola National Park (KDP), Pampadum Shola National Park (KS & AS), in Idukky District Kerala, Shendurney Wildlife Sanctuary, Kollam District, Kerala (KS & AS); Valparai and Indira Gandhi Wildlife Sanctuary, Tamilnadu (KS & AS). Hence, the species is endemic to the Western Ghats South of Coorg (Fig. 1).

**Ecological Notes.** The species is a resident of the temperate sholas and subtropical evergreen forests of the southern Western Ghats (600–2300m ASL). They call mostly throughout the day, especially when overcast and the sun is down. The calls come as crops and the individuals may be seen moving from one tree trunk to another as a loose group inside the sholas. They seem to be present on tree trunks from the base up to 8–10m. The single call is a quick crescendo followed by a series of notes in decrescendo.

### Remarks on the synonymy of *P. zebra* Bliven, **1964** (Fig. 9)

*Pomponia zebra* Bliven, 1964: 99–100, Fig. 6 (Original Description); Sanborn (2014): 349 (mentions about the type material listed); Price *et al.* 2016: 94 (Type locality and distribution in Southern India).

Material examined (from images): Holotype *Pomponia zebra* Bliven, 1964, CASTYPE13809,

labeled 'S. India, Madras State, Anamalai Hills, Kadamparai 3000'. P.Susai Nathan, v. 63'.

*Pomponia cyanea* Fraser, 1948, was described from Coorg (Karnataka State) and Munnar Hills, Travancore (Kerala State), in the Western Ghats, with a very brief original description, and with no details about the structure of the male genitalia. *Pomponia zebra* Bliven, 1964, was described from Kadamparai, in Anamalai Hills, Madras State (Tamil Nadu). The type locality of the latter is just north of Munnar (type locality of *P. cyanea*) in the same landscape (Fig. 1). This species has not been reported since its very short original description and vague illustration of the male genitalia in Bliven (1964). Comparison of original descriptions of *P.* 



Fig. 4 *Pomponia cyanea* Fraser 1948, male genitalia treated with 10% KOH overnight, cleaned, and preserved in glycerol, darker shades represent sclerotized parts. A–ventroposterior view; B–lateral view; C–dorsal view and D–close-up of aedeagus. Illustration by Kalesh Sadasivan

No.	Character	P. cyanea	P. zebra
1	Size(TBL)mm	36.50-47.00	42.00-45.00
2	Head Width (HW) mm	8.00–11.00	10.25
3	Forewing length (FWL) mm	45.00-57.00	57.00
4	Forewing width (FWW) mm	15.00–18.00	17.00
5	Cephalic Index (CI)	275.00-360.00	360.00
6	Ocular Index (OI)	27.27–33.33	27.77
7	Pronotal Index (PI)	266.67–280.00	260.00
8	Mesonotal Index (MI)	90.00–111.11	110.00
9	Opercular Index (OPI)	27.27–33.33	27.77
10	Anteroposterior Index (API)	66.00-83.33	70.00
11	Rostral Index (RI)	51.43-72.73	57.14
12	Forewings (FAR)	288.89-335.29	329.80
13	Tegmen Index (TI)	257.14-316.67	300.00
14	Inter-Wing Index/Ratio(IWR)	179.31–190.00	184.21
15	Gastral Index (GI)	62.96–76.00	64.00

Table 1. Comparison of morphometric characters of P. zebra based on Bliven (1964) and study of type specimensand P. cyanea based on Fraser (1948) as well as fresh specimens from the field

*zebra* in Bliven (1964) and *P. cyanea* from Fraser (1948), they appeared to be very similar in most aspects. Moreover, both species were described from the same mountain range, elevation, and habitat, which demanded revalidation with the comparison of types and the structure of male genitalia.

According to Bliven (1964), *P. zebra* differs from *P. cyanea by* the following features 'larger size', 'normally sized head', 'strongly dentate margins of pronotum', 'remarkably narrow forewings', and 'white banded abdomen'. On careful study of the types and freshly collected specimens we found that the morphometrics of *P. zebra* falls within the range for those of *P. cyanea* (Table 1). The head is relatively of the same size range, forewings are narrow and long in both and the abdomen has each segment base marked with a white pruinose fascia, which is variable amongst individual specimens in its strength, and fades on preservation. Detailed comments are given below.

Head: As per the original description by Bliven (1964), P. zebra has a head including eyes subequal in width to the base of the mesonotum, while P. cvanea has it small and much narrower than the base of the thorax (prothorax), as seen in Fig. 1 in Fraser (1948), but the Travancore population of P. cvanea has relatively larger head than Coorg ones as per Fraser (1948). The original description (OD) states that the "head is very small, much narrower than the base of the thorax and quite out of proportion to the rest of the body". In the type specimens, of both P. zebra and P. cyanea in our examination, the width of the head including the eyes is smaller than the width of the base of the mesonotum, and the lateral border of the eyes reaches short of the level of the lateral margin of the lateral lobes of the pronotum on a dorsal view (Fraser, 1948). The rostrum extends well beyond the posterior coxae in P. cyanea, the extent of the rostrum is not mentioned in the OD of P. zebra. However, the examination of types at CAS revealed



Fig. 5 *Pomponia cyanea* Fraser, 1948, field images of live male insects. A–dorsal view of *P. cyanea* male from Munnar, Anamalais; B–lateral view of *P. cyanea* male from Munnar, Anamalais; C–dorsal view of *P. cyanea* male from Shendurney, Agasthyamalais; D–lateral view of *P. cyanea* male from Shendurney, Agasthyamalais. Images A&B by Kalesh Sadasivan, and C&D by Manoj.P

that the rostrum extends well beyond the posterior coxae in *P. zebra*, as seen in *P. cyanea*.

**Pronotum:** Bliven (1964), states that *P. zebra* has pronotum humeral angles rectangular, sub-truncate, posterior margin sinuate, lateral margins strongly toothed, and anterior angles prominent as per the OD. Fraser (1948), wrote that the pronotum has the lateral border notched, posterior margin straight and outer angles rounded in *P. cyanea*. Examination of type specimens revealed that both species have the pronotal angles rectangular with rounded edges, posterior margin shallowly sinuate, and lateral margins toothed. This is reaffirmed on the examination of fresh field specimens as well.



Fig. 6 *Pomponia cyanea* Fraser, 1948. Images of live male insect from the type locality Munnar, Anamalais. A–dorsolateral view of the whole insect; B–dorsal close-up view of the head, pronotum, and mesonotum; C–anterior view of head and postclypeus; D–posterior view of anal appendages; E–lateral view of anal appendages. Images by Kalesh Sadasivan

*Wings:* Forewings fusiform in *P. zebra*, more than three times as long as wide, without any hint of the anal angle. Fraser (1948), did not mention this character but an examination of the types in BMNH revealed that the wings in *P. cyanea*, were almost three times as long as their width. Bliven (1964), stated 'remarkably narrow forewings' for *P. zebra* while stating the differences from *P. cyanea*. But it is not the case, The FAR high aspect ratio indicates long, narrow wings and a low aspect ratio indicates short, wide wings as per Sadasivan (2021). *P. zebra* type specimen has a similar forewing (FAR 329.80) as *P. cyanea* (FAR 288.89–335.29) (Table1).





Fig. 7 *Pomponia cyanea* Fraser, 1948. Images of dry pinned insect from Valparai Slopes near the type locality of *P. zebra*, High Ranges of Munnar, Anamalai Hills. A–dorsal close-up of the wings; B–anterio-ventral view of head and postclypeus; C– inferolateral view of operculum; D–dorso-lateral view of the head, pronotum, mesonotum, and anterior abdomen; E–ventro-posterior view of anal appendages; E–lateral view of anal appendages. Images by Kalesh Sadasivan

**Coloration:** The head of *P. zebra* is light olive green with linear fuscous spots, bearing long golden hairs, behind each eye (Bliven, 1964). It was described as olive green in *P. cyanea* by Fraser (1948). In both, the head was light olive green with long postocular golden hairs and ocular tubercles surrounding the ocelli being greenish-brown. Pronotum light olive green with anterior border infuscated and a large oval patch on either side covering the region of lateral fissures, greyish

Fig. 8 *Pomponia cyanea* Fraser, 1948. Images of syntype BMNH (E) #1009397, Munnar, Anamalai Hills, Travancore. A-dorsal view of the whole insect; B-ventral view of head and postclypeus, and operculum; C-lateral view of anal appendages. Images © The Trustees of the Natural History Museum, London

pruinose in *P. zebra* (Bliven, 1964). Disc and the anterior border (the latter more noticeable) are sparsely clothed with deciduous linear golden scales. The extreme edge of the posterior margin is infuscated (Bliven 1964). Pronotum olive green as per Fraser (1948). Mesonotum light green of a slightly darker shade than head and pronotum, without, definite maculation, the usual obconical spots being evident only as vague discolorations (Bliven, 1964). Mesonotum with four purplish-

brown triangular spots (ssig & lsig), posterior border bright verdigris (greenish-blue), and with bright golden hairs (Fraser 1948). Examination of type specimens revealed no difference in coloration except those arising due to drying and fading on preservation. The color is greenish-blue with scattered short golden hairs in both species with the submedian sigillae and lateral sigillae. Abdomen and opercula bright verdigris (greenish-blue) with diffuse and poorly defined blackish-brown markings on dorsum. Each segment with a narrow mid-dorsal fascia that splays out posteriorly along the apical border, beneath hyaline and transparent (Fraser 1948). Abdomen castaneous above becomes green laterally with a series of ovoid fuscous spots on either side. Posterior margins of segments are narrowly black, the anterior margin of each segment marked by a conspicuous white pruinose fascia. Abdomen entirely whitish pruinose, apically and sparsely covered with short golden linear scales which tend to aggregate along the posterior border of each tergite except the terminal ones' (Bliven 1964).

Analysis of type and fresh specimens revealed no difference in colour between them. The colour was bluish-green with ovoid fuscous spots on the lateral aspect, each segment in mid-dorsum with the brown fascia laterally on the apical border, each segment base with conspicuous white pruinose fascia, and sparsely covered with short golden linear scales which tend to aggregate along the posterior border. The white pruinose fascia may lose prominence in preserved specimens. As per Bliven (1964), 'basal membranes, tympanum covers, and foliaceous postero-lateral margins of mesonotum greyish, spotted with small flecks of green pigment; basal membranes appearing pale blue by transmitted light. Thoracic region and opercula whitish pruinose'. But, examination of the types and fresh specimens of P. cvanea, offered no significant difference, except for the loss of bluish-green color in preservation. The legs are green, front and middle pair with a brownish suffusion on coxae, trochanters, and femora with tibiae, distally and tarsi, apically infuscated. Hind legs, including tarsi, are almost entirely pale green (Bliven, 1964). In P. cyanea legs are ochreous (Fraser, 1948). In the observation, the legs are marked in green and brown with green on the flexor aspect and brown on the extensor aspect. On preservation the green colour fade to light brown and brown becomes ochraceous.

*Venation:* The venation and infuscations are the same in *P. cyanea* and type of *P. zebra*. No appreciable difference noted in them (see above for a detailed description of venation).

*Genitalia:* Fraser (1948) and Bliven (1964), do not provide any detailed descriptions of male genitalia, however, the latter has a basic and non-informative lateral view far from reality. A comparison of the male genitalia of types of *P. cyanea* and *P. zebra* revealed no difference in the structure (see above for a detailed description).

#### DISCUSSION

*Pomponia cyanea* Fraser, 1948, syntypes from Anamalai Hills, Travancore at BMNH were studied and compared with images of *P. zebra* Bliven, 1964 (holotype CASTYPE13809) Kadamparai, Anamalai Hills at CAS. This detailed morphological study of *P. cyanea* and *P. zebra* revealed no difference in general morphology, wing venation, or structure of male genitalia, hence *P. zebra* is proposed as a junior synonym of *P. cyanea* Fraser, 1948, **syn. nov**. A key to males of known species of *Pomponia* of Western Ghats is provided.

The taxonomic findings of the paper highlights the need to study the male genitalia in cicadas for species determination. The morphological indices developed by Sadasivan (2021) were found to be useful in species comparison. The name Pomponia linearis group is proposed by Duffels and Hyashi (2006) for the *Pomponia* species with the following characteristics: 1) a broad pale transverse band across the postclypeus, 2) acute lateral pygofer lobes, 3) a trapezoid uncus with medial incision suggesting the fusion of two short, broad lobes, and 4) a pair of claspers, each with two spines, protruding from below the uncus. Within the linearis group Duffels and Hayashi (2006) distinguished the *linearis* species complex with the distinctly protruding paramedian basal pygofer lobes. Pomponia cyanea has only two features out of



Fig. 9 Holotype images of *Pomponia zebra* Bliven, 1964. Holotype CASTYPE13809 labeled S. India, Madras State, Anamalai Hills, Kadamparai. A–dorsal view of the whole insect; B–ventral view of head and postclypeus, and operculum; C–lateral view of anal appendages. Images by Christopher C. Grinter

the four described for the *P. linearis* species group, and it lacks broad pale transverse band across the postclypeus and claspers with spines. Thus, *P. cyanea* is an aberrant inside the *P. linearis* species group and might eventually need a separate species group for placement.

## On the generic placement of *Pomponia polei* Henry, 1931, comb. nov.

*Pomponia polei* Henry, 1931: 118–120, Plate XXVII. *Terpnosia polei* (Henry), Lee, 2012: 257.

The species *Pomponia polei* Henry, 1931 was described from Sri Lanka, and later transferred to *Terpnosia* Distant, 1892 by Lee (1912). During this work, examined the holotype male of *Terpnosia* 



Fig. 10 *Pomponia polei* Henry, 1931. Images of holotype BMNH(E)#1009399. A–dorsal view of the whole insect; B–ventral view of head and postclypeus, operculum, and abdomen; C–ventral view of anal appendages.; D–lateral view of pygofer. Images © The Trustees of the Natural History Museum, London

*polei* (Henry, 1931) at the Natural History Museum, London, BMNH(E) # 1009399 (Figs. 10 A–D).

Lee (2012), established that *Terpnosia* is possibly polyphyletic based on morphology and redefined *Terpnosia* by the following characters: pronotum lateral margin dentate; forewing basal portion of vein RA2 extremely short; forewing with broad, transparent infuscations on crossveins r, r-m, and m; elliptical infuscation present on each hind margin of veins RA2, RP, M1–4, and CuA1; male operculum broader than long with apex roundish, reaching or extending just beyond posterior margin of sternite II; male abdomen cylindrical, long, much longer than head and thorax together; timbal cover well-developed, covering most of timbal; male abdominal sternites without tubercle-like projections; male pygofer with a pair of triangular claspers behind uncal lobes; uncal lobes bifurcate, long; and distal shoulder of pygofer rounded, not acutely pointed. Lee (2012), based on the OD and illustrations of *P. polei*, transferred it to the genus *Terpnosia*.

The authors examined the type specimen of P. polei, and the OD in Henry (1931) and observed that the species is closely allied to P. cvanea from the adjacent Western Ghats of India and has the following differences from Terpnosia: forewing apex comparatively acute (Fig. 10A), not roundish as in Terpnosia; forewing infuscations smudged, with no distinctive borderline as in Terpnosia (Fig. 10A); uncal lobes of male genitalia fused (Fig. 10C), not bifurcate and long as in Terpnosia; distal shoulder of male pygofer broad, prominent and acutely pointed (Fig. 10D), not rounded as in Terpnosia. All the above characters of P. polei are in agreement with the findings in *P. cyanea* as well. Hence, in the light of the above findings, we return Terpnosia polei (Henry, 1931) to its original genus Pomponia reinstating the original combination as Pomponia polei Henry, 1931, comb. nov.

### Key to *Pomponia* Stål, 1866 of Western Ghats of India

1. Both sexes with postclypeus bearing a pale transverse band; operculum triangular with medial angle produced to distinctly overlap the opposite operculum across the midline; male genitalia with each clasper bearing a pair of spines and of them the medial spine distinctly longer than lateral .....*P. pseudolinearis* Sadasivan, 2021

The taxonomic confusion that existed in two species

of Pomponia of the Western Ghats of India is resolved. The taxon P. zebra is synonymised with P. cyanea based on the study of the original descriptions, types, and freshly collected specimens from type localities. The species P. cyanea is redescribed with additional morphometric data and its male genitalia illustration. Thus, as far as it is known, the Western Ghats has only two valid species of Pomponia, namely P. cyanea Fraser, 1948, and P. pseudolinearis Sadasivan 2021. It is hoped that the resolution of the taxonomic confusion in Pomponia of Western Ghats would lead to the description of new taxa based on the detailed study of morphometrics and male genitalia. In addition, the taxonomic status of Terpnosia polei (Henry, 1931), from Sri Lanka, is revised based on male morphology and is transferred back to its original placement as Pomponia polei Henry, 1931.

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#### DNA barcoding, life history and taxonomy of lablab pod borer, *Adisura atkinsoni* Moore (Lepidoptera, Noctuidae)

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**ABSTRACT:** DNA barcoding and morphological characters together with thorough bioecological investigations to diagnose field bean pod borer, *Adisura atkinsoni* Moore, 1881 an important pest was carried out. *A. atkinsoni* has a limited host range. The incidence of *Adisura* was higher on local varieties (photo sensitive) compared to HA4 hybrid (photo-insensitive). The field bean pod borer appeared during middle of October and continue to March. The undefined taxa were identified using both female and male genitalia. But since they could be differentiated from their morphologically closest relative, they were marked as sensu lato. While doing BLAST analysis, the mitochondrial COI Sequence of *Adisura* specimens collected from Bengaluru showed similarity to *A. bella*. In the phylogenetic tree, these two samples Sp1 and Sp2 separate new sub-clade which stands separately from the rest of the *Adisura* species. It was identified that the lepidopteran samples collected from Bengaluru are *Adisura* and the DNA sequences did not match with any of the existing species of *Adisura*. The shift in this pest's prevalence has been discussed. © 2024 Association for Advancement of Entomology

KEY WORDS: Field bean pod borer, molecular phylogenetics, bioecology, prevalence

#### **INTRODUCTION**

The field bean pod borer, *Adisura atkinsoni* Moore, is a noctuid moth first described in 1881. *Adisura leucanioides* Moore, 1881; *A. pallida* Moore, 1881 and *A. atcinsoni* Hampson, 1903 are the synonyms (Savela, 2023). Hampson (1903) and Lefroy (1909) recorded *A. atkinsoni* with notes on their distribution and larval plants from India. Kishida (2011) mentioned brief morphological description, distribution, developmental period, food plants and sporadic outbreaks of *A. atkinsoni* in Japan. Also, there are several mentions of *A. atkinsoni* (Moore, 1881) in checklists and online databases (India Biodiversity Portal, 2016; ICAR-NBAIR, 2016; Encyclopedia of Life, 2018). *A. atkinsoni* is a specific, locally adapted, economically important pod borer on *Lablab* beans (*Lablab purpureus*). In Karnataka, the life cycle of the pod borer appears to have co-evolved with the life cycle of the local cultivars. *A. atkinsoni* is attracted to the specific odor that emanates from

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the local cultivar and its parts. Local cultivar is highly susceptible to this pod borer (Krishnamurthy and Appanna, 1948). In some Lablab varieties, which secrete fragrant oil on the surface of pods is preferred for consumption by humans and insects as well. The pod exudate consists of homologous fatty acids and their methyl esters-42 in all from C-11 through C-24, including odd carbon chain compounds. Apart from trans-2-dodecenoic and trans-2-tetradecenoic acids, which constitute the major percentage of the oil, other homologous Ä2enoic acids and saturated acids and esters are also found (Fernandes and Nagendrappa, 1979). Local cultivar is photo-sensitive and blooms under restricted photoperiod range. The seeds of local cultivar have specific odor and taste which local people relish. A. arkinsoni is the dominant pod borer on local cultivar and persists on the crop from the beginning to the end (July-August to January-February) in Karnataka (Mallikarjunappa, 1989). This species is distributed across Asia and Africa (Hampson, 1894; Hampson, 1903; India Biodiversity Portal, 2016; ICAR-NBAIR, 2016 and CABI Datasheet, 2019). In present study, species identification using morphology and DNA barcoding along with bioecology of A. atkinsoni were undertaken. Also carried out phylogenetic relationship of A. atkinsoni with three other Adisura species and Helicoverpa armigera (Hubner) as outgroup.

#### **MATERIALS AND METHODS**

Field experiments were carried out at Zonal Agricultural Research Station (ZARS) of UAS, Bengaluru and ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India during 2016-2020. Geographically, ZARS, Bengaluru is located at 12°58' N; 77°35' E and an altitude of 930m above sea level. The annual average rainfall ranges from 679.1 to 888.9mm. The local photo-sensitive variety (local avare) was grown under field conditions for maintenance of laboratory culture, bioecological and life table studies. The recommended agronomic practices were followed to raise a healthy crop, except crop protection chemicals. The initial culture of Adisura was obtained by collecting larvae from farmer's fields in and around areas of Doddaballapur, Bengaluru Rural district (13.29° N; 77.54° E) and maintained in laboratory at ICAR-Indian Institute of Horticulture Research during 2018-2020. The larvae were reared in the glass jars (41cm height and 30cm diameter). Fresh buds of local lablab bean were provided daily as food for larvae till pupation. Freshly formed pupae were kept individually in plastic jar along with sand, the top of which was covered with muslin cloth and secured firmly with rubber bands. On emergence of moths, they were fed with diluted (5%) honey solution. The emerged adults were paired and allowed to mate to get gravid females and were used for further studies. The moths were maintained in the laboratory (at  $27 \pm 2^{\circ}$ C and 40%relative humidity).

Host Range: To test the host range of A. atkinsoni in laboratory, fresh blooms containing bud, flowers and tender pods of L. niger (Field bean), Cajanus cajan (Pigeon pea), Vigna unguiculata (Cow pea), Cicer arietinum (Chickpea) were offered to ovipositing moths in a wooden-wire-mesh cage  $(0.3 \text{ m}^3)$  in the laboratory under no-choice conditions. Two pairs of moths were released in to each cage and the experiment was repeated twice. To test the larval feeding response, fresh tender pods of L. niger, C. cajan, V. unguiculata and C. arietinum were offered to third instar larvae in petri dishes (9cm diameter), separately. Host acceptability was based on the orientation behavior and palpation and initiation of feeding. The experiment was repeated twice.

**Species description:** Different life stages were collected from the laboratory culture and stored in ethanol (10%) at the constant climatic parameters as stated above. Adults were dried, stretched, pinned and maintained in wooden insect boxes. A digital camera lucida was used for measurement. The adult moths were separated into males and females after identification of the specimens under laboratory conditions. All stages of *A. atkinsoni* were examined under an AO microscope i.e, Stereo Zoom microscope (Olympus) and mounted for species identification. All measurements like adult

and larval body length and width in adults, paired wings; length and width of thorax, abdomen and size of pupae, etc. were measured in mm and photographs were taken using Leica DFC 425 mounted on a Leica M205C.

For genitalia preparation, the abdomen of male and female moths were separated using a pair of micro scissors and placed in a test tube containing was KOH (10%) solution for overnight. Genitalia then transferred to a cavity block containing water and washed repeatedly to remove excess KOH. Then, genitalia was placed in glycerol on a slide for dissection and examination. Genitalia were taken out from the abdomen under a stereo binocular microscope. After examination, the parts of the specimens were transferred to a micro vial (10ml) containing glycerol (98% pure) and the vial was pinned below the specimen. The terminologies of Klots (1965) were adopted to describe the genitalia. Hampson (1894), Kristensen (2003) and Keegan et al. (2021) terms were used as the basis for identification of specimens.

DNA Barcoding: Sample collection and DNA Extraction: Third instar Adisura larvae were collected on local Lablab cultivar pods and stored in ethanol (95%). A total of six samples were collected on Lablab host plant species from three different locations of Karnataka (India) and preserved immediately in ethanol (95%) and stored at 4°C for future use. From all six samples, Adisura larval specimens were collected each for the marker analysis. Total genomic DNA was extracted from individual larvae. The specimens were washed brieflyin sterile distilled water to remove alcohol prior to homogenization. Genomic DNA was extracted using MN- Genomic DNA from tissue kit (Macherey-Nagel, Germany) and then stored the DNA at -20°C for future use.

**Mitochondrial COI gene amplification:** A portion of mitochondrial COXI gene (approx 658 bp) fragment was amplified in a 25-µl reaction mix, containing 1X PCR buffer (10 mM Tris-HCl pH 8.0), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 10 p mole each of Forward and reverse primers [LCO-1490 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-

3'; HCO-2198 5'- TAAACTTCAGGGTGA CCAAAAAATCA-3'], 1.25 UTaq DNA polymerase (Genei, Bangalore) and 200 ng of DNA template. PCR was performed in Veriti 96 well thermo-cycler (Life technologies-AB, USA) according to the following cycling condition, initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec; annealing temperature 54°C for 40 sec; extension at 72°C for 50 sec, and final extension at 72°C for 10 min to extend the incomplete fragment.

PCR products were visualized in agarose gel (1.2%) and the band was eluted using PCR clean-up gel extraction kit (Macherey-Nagel, Germany). Purified PCR products were cloned using pTZ57-T/A plasmid vector system and the vector was transformed into Escherichia coli DH5á according to the manufacture protocol (Fermentas Life science, USA). The plasmid DNA was isolated from three randomly selected clones selected from each samples using Gene JET Plasmid mini prep kit (Fermentas Life Science, USA). Presence of the insert was checked by colony PCR using gene specific primer and visualized in agarose gel(1.2%). The sequencing was performed using M13 universal primers at Eurofins MWG Operon, India. The sequence homology was determined using BLASTn (http://www.ncbi.nlm.nih.gov), and the selected sequences were edited by manual using the sequence alignment editor 'BioEdit'version7.0.

**Phylogenetic analyses:** Phylogenetic analyses were carried out for mtCOI marker. The sequences were aligned separately in CLUSTAL W program. Independent alignment was carried out for each taxon sample, resulting in marker dataset. Neighbor-Joining method was used to construct phylogenetic tree with kimura-2-parameter model with South American pin worm, *Tuta absoluta* as out-group. In addition, the number of substitutions, Transition (Ti)/Transversion (Tv) ratio, and nucleotide compositions for mitochondrial COI were also determined using MEGA version 7.0.

**Biology:** Laboratory studies on *A. atkinsoni* were carried out at the Department of Entomology, University of Agricultural Sciences, G.K.V.K.,

Bengaluru. The insect culture in laboratory was maintained at  $25 \pm 2^{\circ}$ C,  $70\pm5$  RH, and photoperiod of 14:10 (L:D) at constant regimes of climatic parameters. Dates of pre-oviposition, oviposition and post- oviposition were recorded. All the plant parts were examined for eggs and counted. The total number of eggs laid by each female during life-span was counted. Such ten females were observed for the eggs laid. Observations on incubation, larval and pupal periods were recorded as per the rearing procedure in Govindan (1974) and Chakravarthy (1977).

**Seasonal incidence:** Field observations were recorded at the Zonal Agricultural Research Station, UAS, during September 2019 to February 2020. Seeds of local variety and HA-4 hybrid of *lablab* bean were sown during third week August 2019 in 800 m<sup>2</sup> area to record the seasonal incidence of *Adisura*. The study area was divided into four quadrates. Observations on the number of larvae per 10 plants at each quadrate were made at weekly intervals commencing from 50 per cent flowering (50 days after sowing) to pod maturity stage. ANOVA was carried out by treating quadrates as replications and dates of observations as treatments to know the differences among dates of observations.

#### **RESULTS AND DISCUSSION**

Host range: A. atkinsoni has a limited host range. Fletcher (1919) recorded on Blumea sp at Pusa; Cajanus cajan (L.) and Dolichos lablab (L.) at Coimbatore and on Cicer arietinum L.; Lens esculenta Moench at Indore, Rattan Dhâr, Ujjain, Hoshangabad, Betur and Bilaspur districts of Madhya Pradesh (Bhatia, 1962). According to Mujtaba (1918) the caterpillar was found feeding on the leaves and buds of Blumea sp. Adisura larvae have been recorded on C. cajanin Delhi (Issac, 1946). However, none of the above workers reared A. atkinsoni on plants, they reported and these plants may serve as larval plants only, not host plants. Therefore, Lablab is the only established host plant for A. atkinsoni. According to Gardner (1946), the caterpillar of this insect was found feeding on the Hibiscus mutabilis at

Dehradun (Uttarkand). Adisura has been reported to be boring into the pods of Dolichos in Mysore state (Krishnamurthy and Appanna, 1948). Bhatia (1962) reported that this species was found in abundance on chickpea, pigeon pea and lentil. Blumea sp. belong to Asteraceae and Hibiscus sp. mutabilis belong to Malvaceae. All other plants belong to Leguminaceae. Interestingly, Thontadarya et al. (1982) recorded A. marginalis (Walker) feeding on pods of redgram from the campus of the University of Agricultural Sciences, Bengaluru for the first time from south India. Preliminary experiments in laboratory revealed that while larvae of A. atkinsoni made directed movements towards Lablab bean pods, they randomly nibbled on the pods of other plants offered in the laboratory. Moths of A. atkinsoni did not oviposit on blooms of other legumes in laboratory, but preferred only the blooms of field beans. Thus, A. Atkinsoni is the only pod borer of field beans which is monophagous, while other pod borers are oligo or polyphagous in Karnataka.

**Biology:** The moths emerged in mid-October from the pupae of previous season, when the local cultivar was in the initial reproductive phase. The pupae were formed inside the soil. During the season, insect completed three generations and fourth generation caterpillars pupated in the soil and remained till the next season of the crop. The total lifecycle depended on the total and average rainfall and accompanying temperature and their distribution in a year.

**Egg:** Iridescent white spherical eggs were laid singly either on the pods or on the flower buds. The incubation period on an average lasted for three to four days  $(3.84\pm0.24)$ . Tender leaves were preferred for oviposition only when flower buds and pods were not offered in laboratory. Both in field and lab, gravid *A. atkinsoni* moths did not oviposit on pigeon pea, chick-pea and cowpea leaves, flower buds, and pods observed. Ramachandra Rao (1918) and Krishnamurthy and Appanna (1948) earlier recorded similar observations.

**Larva:** *Adisura* underwent five larval instars, required I instar  $3.20 \pm 0.30$ , II instar  $1.94 \pm 0.48$ ,

DNA barcoding, life history and taxonomy of lablab pod borer, Adisura atkinsoni Moore



Fig.1 A - Morphological variations in *Adisura atkinsoni* larvae; B - Earthen pupal cell of *Adisura atkinsoni*; C - Adult Female and D - Male of *Adisura atkinsoni* 



Fig. 2 C - Male genitalia, D - Adeagus, E - Female genitalia



Fig. 3 Phylogenetic tree constructed for Adisura sp. using mitochondrial COI gene sequence

III instar  $3.04 \pm 0.32$ , IV instar  $3.48 \pm 0.28$  and V instar  $6.15 \pm 0.57$  days respectively (n=28) to complete the life-stages. A fully grown 5<sup>th</sup> instar caterpillar measured 27-28mm long. A great variation was recorded in the colour of the later stages of the larvae, especially in the third generation (Fig.1A). Larvae showed cannibalistic tendency when reared in cages. These observations are in agreement with the observations of Ramchandra Rao (1918) and Krishnamurthy and Appanna (1948). According to Steven Passoa (2007) larvae of *A. atkinsoni* can be distinguished from other species of Heliothinae by spinner spatulate and crochets bifurcate at their tip.

**Pupa:** The matured caterpillar entered the soil, formed an oval earthen chamber underground (Fig.1B) and pupated. However, even in the absence of soil, the larvae pupated in individual receptacles. The pupae measured 18mm long and appeared thick and red-brown. The pupal period varied from 15 to 18 days on an average of  $17.08\pm$  2.26 days in the first two generations to over nine months in the third generation. Govindan (1974) and

Chakravarthy (1977) too recorded observations in concurrence with the above results.

The moth completed the life cycle on an average in  $38.73\pm3.25$  days (n=3). Longevity of male is 13.5  $\pm$  1.78 and female is 10.8  $\pm$  1.12 days. The fecundity of Adisura moths varied from 150 to 180 eggs, on an average (n=20). Each female moth oviposited for two to nine days in the laboratory. Mujtaba (1918), Krishnamurthy and Appanna (1951), Chakravarthy (1977), Chakravarthy (1983), Chakravarthy and Lingappa (1984, 1988), Chakravarthy (1988), Thontadarya et al. (1982), and Mallikarjunappa (1989) and Chakravarthy and Rajendra Prasad (2016) recorded bioecology of A. atkinsoni from different locations in different periods in Karnataka, south India. There were slight variations in the life history depending on weather conditions but broadly conformed to the results obtained in this study.

**Seasonal incidence:** Field observations revealed that the pod borer, *A. atkinsoni* was the dominant pest of field bean occurring from October to March

under field conditions. Its appearance in the field coincided with the initiation of flowering and pod formation stages of the local variety of lablab beans and the borer population disappeared with the local cultivar attaining senescence stage. It was found specifically feeding on flowers and pods of Lablab. The field bean pod borer appeared during middle of October and the per cent infestation on an average (n=100 blooms) varied from 24.50 to 60.00. The incidence of Adisura was higher on local varieties (photo sensitive) compared to HA4 hybrid (photo-insensitive). The pod infestation started from October through March and ranged up to 39.2 per cent on local cultivar. The peak Adisura borer infestation was recorded during November-January on local cultivar. The incidence on HA 4 hybrid was as low as 1.2 to 4.0 per cent. So, A. atkinsoni was the only dominant borer whose life-cycles coincided and completed with the local Lablab cultivar.

Adult: A. atkinsoni head and thorax were with a vestibule consisting of greyish brown hair - like scales. Abdomen covered with straw coloured, less dense scales. Palpi dark brownish with dense brownish scales on the outer side than the mesal area and proboscis well developed. Antennae setaceous; forewing greyish brown with black irregularly distributed scales; costal margin tinged with deep coppery brown; two or more less black circular spots, present on basal area of median space; an oblique series of black points present just below the sub-terminal area. Hind wing is light brownish with black suffusion on the terminal area narrowing from inner margin to the apex; fringe whitish throughout. The orbicular spot towards the base of the forewing is prominent. The male and female forewings measured on an average (n=20) about 30.73mm and 30.92mm, respectively (Figs.1C, D). According to Mathews (1991), Adisura has unique form of coiling of the female appendix bursae and the male vesical and dopa decarboxylase (DDC) sequences permitted Adisura to be treated as sister group.

**Male genitalia:** Uncus evenly elongated, pointed tip; tegumen small, subtriangular; valva narrow band; cucullus is well separated from other part of

valva by more or less narrow neck; succulus strongly sclerotized, broader base narrowing towards apex; vinculum U-shaped. Aedeagus long nearly uniform width, sinuate medially with apically pointed (Figs. 2C, D, E).

**Female genitalia:** Papillae analis sclerotized, setose plate, two times as long as wide; posterior apophysis as long as anterior apophysis; ostium sclerotized, cup shaped; ductus bursae membranous sac like, almost equal length to corpus bursae and sclerotized distally; corpus burse membranous with a sclerotized irregular band signum.

Ecology: The pupae of A. atkinsoni of the previous year were maintained under  $70 \pm 2.0$  per cent relative humidity with light: dark (13:11 h/day) in the laboratory under ordinary light and regime periods 24±2.0 for eight and half months. Diapause of A. atkinsoni could not be broken under above conditions. Workers have observed that under Bangalore conditions, a combination of high humidity of the north-east monsoon season and heavy rainfall or heavy mist in the morning and bright sunshine during the day appeared favourable for normal appearance, multiplication and optimum activity of the A. atkinsoni moths and the caterpillars. It was observed that failure of the monsoon and even a poor monsoon acted adversely on the emergence of the moths early in the season and a large number of pupae of the previous season got desiccated in the soil and a number of moths even after eclosion from many pupae failed to travel upwards to the soil surface and emerged successfully. These adverse weather conditions affected growth and development of local cultivar under field conditions (Ramachandra Rao, 1918; Govindan, 1974; Chakravarthy and Rajendra Prasad, 2016).

**Phylogenetic studies:** The species included many of those in the catalog by Hampson (1894) and also some undefined morpho- species. These undefined taxa were identified using both female and male genitalia. But since they could be differentiated from their morphologically closest relative, they were marked as *sensu lato*; further studies will be necessary to name and validate these taxa. While

doing BLAST analysis, the mitochondrial COI Sequence of Adisura specimens collected from Bengaluru showed 95 per cent similarity to A. bella and 94 per cent to A. dulcis, while A. litraga and A. marginalis showed less similarity. So, A. bella was most closely associated with A. atkinsoni. In the phylogenetic tree, these two samples Sp1 and Sp2 separate new sub-clade which stands separately from the rest of the Adisura species. According to the above-mentioned results and the results from classical taxonomic studies, it was identified that these lepidopteran samples collected from Bengaluru are Adisura and the DNA sequences did not match with any of the existing species of Adisura (Table 1). Therefore, it was confirmed that the samples belonged to A. atkinsoni (Fig. 3). Cho et al. (1995) and (2008); Mitchell and Gopurenko (2016) conducted studies on DNA barcoding and molecular phylogenetics of heliothine moths including species of Adisura without A. atkinsoni. The above workers showed phylogenetic relationship of A. litraga, A. bella, A. marginalis and A. purgata with 26 other heliothine moth species. Cho et al. (2008) confirmed monophyletic nature of Adisura having all genes allied to the Heliothis group.

Table 1. Database of nucleotide sequence of Adisuraspp. in BOLD system

Species	Specimens	Sequences	Barcodes >500bp
Adisura aerugo	2	2	2
A. bella	2	2	2
A. callima	1	1	1
A. dulcis	12	11	11
A. litarga	9	9	5
A. marginalis	21	20	20
A. parva	2	2	2
A. purgata	1	1	1
Adisura sp.	28	28	16

http://www.boldsystems.org/index.php/Taxonpage/ SpeciesSummary?taxid=53549 Hardwick (1965) suggested Heliothentinae is the correct spelling to replace Heliothidinae or Heliothinae. Some of the world's most destructive pests belong to the noctuid subfamily Heliothinae. The subfamily Heliothinae is well-defined, comprising about 400 species of small to mediumsized noctuid moths: antennae in both sexes are filiform; palpi short, pressed; proboscis well developed; fronsconvex, sometimes with sclerotized comb; in most genera tibia of all legs armed with spines. The monophyly of Heliothinae is supported by two apomorphies. First, the larval integument is covered in conical granules each bearing aminute apical spine. Spinose skin also occurs in Herminiinae, Cuculliinae and Plusiinae (Kitching, 1984) but these conditions are non-homologous. For instance, the spinules in Plusiinae are fine and hair-like (Lafontaine and Poole, 1991). Secondly, in most noctuid larvae, seta L1 on the prothorax is vertically above seta L2, as it is in early instars of Heliothinae. But in mature heliothinae larvae L2 is positioned directly posterior to L1. The COI barcode region is for species identification, and has been used to not only corroborate morphologically defined species but also to define the species in the Adisura complex.

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#### A new marine littoral species of *Oudemansia* Schött (Collembola, Neanuridae) from Lakshadweep Island, India with a key to world species of the genus

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**ABSTRACT:** A new species, *Oudemansia dhritiae* **sp. nov.** is described from Lakshadweep Island, India. It is characterised by unguis with a single internal tooth and without any lateral teeth; mandibles with 11 teeth, six dental setae, and antennae IV with 6 sensilla. Identification key to the world species of *Oudemansia* Schött, 1893 is provided. © 2024 Association for Advancement of Entomology

KEY WORDS: Taxonomy, chaetotaxy, springtails, Pseudachorutinae, anal spines

#### **INTRODUCTION**

The Collembolans have a very diverse distribution occurring in all parts of the world, inhabiting a wide range of ecological niches and in any climatic region. Commonly, 'Springtails' are soil and litter dwelling, often preferring wet or damp surroundings. Collembolan may be found in mosses, in soil, under stones, in caves, in ant-nest and in termite mound. They are also present in the intertidal zone of the coast, on the surface of lakes and ponds, and on snowfields (Cheng, 1976). The strong water repellent body cuticle of Collembola has made them possible to live in aquatic habitat (Noble-Nesbitt, 1963). Littoral species live in sand or in the small crevices of rocks under algae (Hopkin, 1997). Globally, family Neanuridae comprises 1608 species under 180 genera (Bellinger et al., 1996-2023). In India, the family Neanuridae comprises 55 species under 24 genera (Mandal, 2018). According to Deharveng et al. (2008), a total of 525 water dependent species of Collembola have been recognized worldwide, out of which 103 live in freshwater and 109 are linked to marine habitats; among them are 8 species of Neanuridae of the genus Oudemansia. Prabhoo (1970) reported O. subcoerulea Denis, 1948, from the coast of Kanyakumari (Cape Comorin) in India, later synonymized with O. coerulea Schött, 1893. A second species, new to science, has been collected in the Lakshadweep archipelago, an Indian territory that comprises 36 islands in the Arabian Sea. Agatti Island is one of its atolls, which hosts of a diverse flora and fauna favored by local environmental conditions, such as temperature range of 25–35°C, humidity 70-76 per cent and an average annual rainfall of 1600mm. The new species Oudemansia

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*dhritiae* **sp. nov.** is described from the marine littoral zone of this island.

#### **MATERIALS AND METHODS**

Specimens were collected from rock crevices near sea-shore using entomological aspirator and were preserved in 70 per cent ethanol, and later on mounted in Hoyer's solution (Krantz, 1978). Four specimens were de-pigmented using Nesbitt's solution (Krantz, 1978) and mounted in Hoyer's medium on slides for the study of chaetotaxy. The slides were dried on a hot plate for 48 hours and slide coverslips were sealed. Mounted slides were photographed using Leica DM 2500 binocular microscope attached to an image capturing device namely Leica DFC 295. Chaetotaxy and other morphological parts were digitally drawn using CorelDRAW Suite 2021 version 23.1.0.389. Some specimens were photographed for further details using Scanning Electron Microscope (SEM), model - ZEISS EVO 18 special edition.

**Material Deposition: ZSI** Zoological Survey of India, New Alipore, Kolkata, India.

Abbreviations used in descriptions and figures: a—setae of anterior row; A–G, d–f—labial setae according to Massoud (1967), Deharveng (1979, 1981, 1983), D'Haese (2003).

Abd.—Abdominal segment; Ant.—antennal segment; d—cephalic dorsal setae; lb-labium; LSlabral sclerotization; m—setae of median row; ms—microsensillum; Md—mandible; Mx—maxilla; or—subapical organite; Oc—Ocular setae; p setae of posterior row; PAO—postantennal organ; pl—prelabral setae; pso—pseudocellum, pseudocelli; S—cylindrical sensillum on Ant. IV; sd—subdorsal cephalic seta; Sgd—dorsal guard sensillum; Sgv—ventral guard sensillum; ss—body sensorial seta; Th. —thoracic segment; Tita tibiotarsus; VT—ventral tube.

#### **RESULTS AND DISCUSSION**

#### **Systematics**

Poduromorpha Börner, 1913;

Neanuridae Börner, 1901 sensu Yosii, 1956;

Pseudachorutinae Börner, 1906;

Genus Oudemansia Schött, 1893

Type species. Oudemansia coerulea Schött, 1893

Diagnosis: Body deep blue to bluish black in colour, 0.8-2.0mm in length, without any digitations. Head bears 8+8 eyes and lacks PAO. Cephalic and abdominal pseudocelli are well developed. A trilobed apical bulb present on Ant. IV. Beak like structure of the labrum with long distal and short proximal setae, total 11-13 labral setae, labral sclerotification is the characteristic of the genus. Forked hypopharynx is well developed and placed above the truncate labium. Long, well-formed sensorial setae present on Th.II and III and on the abdominal segments. Abd.VI with 2-4 distinguishable spines or spiniform setae. Unguiculus absent and unguis is with or without any internal or lateral tooth. Tenent hair usually absent from tibiotarsi. Furcula strongly developed and with a moderately long mucro. 3+3 teeth present on retinaculum. All the representatives under this genus are usually from marine or littoral habitat.

**Distribution.** Vietnam, New Caledonia, Japan, Madagascar, Indonesia, Africa, Australia, China, North America and India.

#### Oudemansia dhritiae sp. nov. Figs. 1-24

LSIDurn:lsid:zoobank.org:act:FE172B45-73CF-4759-876D-8F5DB736E934

**Type Locality.** India: Lakshadweep, Kattupallikad, near mosque, Agatti Island, (10°51' 98'' N; 72°11'92''E), 70m above sea level.

**Type material.** India: Lakshadweep, Kattupallikad, near mosque, Agatti Island, G.P. Mandal leg., 23 February, 2019. Holotype, female adult (Reg. No. 3222/H14) and 11 paratypes: 1 male and 5 females mounted on slides (with Reg. No. 3223/H14), and 4 specimens in ethyl alcohol (Reg. No. 2812/H14).

**Description:** Adult body length excluding appendages up to 1.05–1.42 mm (Fig.1). Body colour dark purple. Abdomen with intersegmental areas devoid of pigment. Body colour is not even, but as scattered patches of pigment (Fig. 2) all over

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Figs. 1-2 *Oudemansia dhritiae* **sp. nov.** 1 - SEM photograph of habitus, 2 - dorsal view of full body with colour pattern

the body. Cuticular granulations are prominent and with conspicuous pseudocelli.

Antennae. Antennal segment ratio- Ant. I: Ant. II: Ant. III: Ant. IV = 1: 1.08: 1.06: 1.2. Ant. IV with trilobed apical bulb placed distally (Fig. 9), subapical organite present in a groove. Antennae and head subequal. S1 and S2 are slight thin and 4 well differentiated sensilla (S3, S4, S7, S8) which are very prominent, present on Ant. IV (Fig. 9). Ant. III organ with slightly bent conspicuous Sgv with ms nearby, relatively short Sgd (Fig. 10). Ant. I and Ant. II with 7 and 11 setae, respectively (Fig.11).

**Mouthparts.** Labrum long, setae arranged in four rows (Fig.14), four prelabral setae and nine labral setae present; labral formula 4/3,4,2. Labium proximally truncate with 4+4 distal setae arranged in a cascade, setae A and C quite long (Fig. 15). The mandible contains two strong apical teeth, and 9 small teeth placed below (Fig. 18). Maxillae styliform and labral sclerotification present (Figs. 16, 17). Hypopharynx cylindrically placed above mandible with rough edges.

**Head.** Eyes with 8+8 ommatidia in three groups, one with A, B, C and D other with E, F, G and last one with H (Fig. 12). Oc1–3 setae present, Oc3 is



Figs. 3-8 *Oudemansia dhritiae* **sp. nov.** 3 - setae and sensilla of Ant. IV, 4 - fore leg, 5 - Abd. VI with anal spine, 6 - furcula, 7 - labral sclerotification, 8 - genital setae, 3-6 - SEM photographs, 7-8 - microscopic photographs
smaller, Oc2 larger than Oc1. PAO absent. Cephalic pso present, five dorsal cephalic setae are observed (d1–d5). Head with 4+4 sub dorsal setae (sd2–5) and 3+3 posterior setae (p1–p3), p2 smaller and thinner, c row setae absent (Fig. 13).

**Chaetotaxy.** (Fig. 21) Body setae composed of ordinary mesosetae usually smooth or unilaterally ciliated and long sensorial setae. Sensorial setae formula by half tergite: 022/11111. Th.I with 2+2 m setae. Th.II and Th.III with 3+3 anterior (a1, a4, a5), 1+1 medial (m6=ss) and 5+5 posterior setae (p1, p2, p4–p6; p5=ss). Abd. I–IV with 3+3 anterior (a1, a4, a5) and 5+5 posterior setae (p1, p2, p4–p6; p5=ss). Abd. V with 2+2 anterior setae (a1, a4) and 3+3 posterior setae (p1, p3, p4; p3=ss). Abd. VI with 2+2 anal spines a1 are slightly larger and curved than a2. Abd. VI with 1+1 anterior (a3), and 2+2 medial ordinary setae (m1, m2). Pseudocelli present on Abd. I and Abd. III–IV.

**Legs.** Without tenent hair. Ratio of Tita: Unguis is 1.27: 1. Legs I-III show chaetotaxy as follows:

Coxa-3,6,7; Trochanter-6,6,6; Femur-12,11,11; Tibiotarsus-18,18,17 (Figs. 20, 22). Length of Tita I-III 54, 57, 64im. respectively. Unguis with one internal and devoid of any lateral teeth (Figs. 4, 22). Unguiculus absent.

**Ventral chaetotaxy.** Thorax devoid of setae. VT with 2+2 lateral setae. Tenaculum well developed and usually with 3+3 teeth (Fig. 19). Abd.II and III with 2+2 ventro-internal setae. Abd. IV with 4+4 ventro-lateral setae. Lateral anal valves with 15+15 setae.

**Furcula.** Complete and well developed; manubrium with 7+7 setae. Dens roughly granulated with 6 setae (Fig. 23). Mucro with two lateral lamellae, slightly larger than one-third of dens (Fig. 23). Ratio of mucro: dens = 1: 2.5 (range-2.18-2.51; n=4).

**Female genital plate** with 7 circumgenital, 2 small eugenital setae and 3+3 pregenital setae (Fig. 24).

**Etymology.** The species name is dedicated to Dr. Dhriti Banerjee, the first woman Director of





Figs. 9-12 *Oudemansia dhritiae* **sp. nov.** 9 - dorsal view of Ant III and IV, 10 - ventral view of Ant III and IV, 11 - dorsal view of Ant I and II, 12 - ocular area

Figs. 13-15 *Oudemansia dhritiae* **sp. nov.** 13 - head chaetotaxy, 14 - labrum, 15 - labial setae with labral sclerotization

Species	Country	Unguis teeth	Ant. IV sensilla number	Dental setae number	Dens posterior granulation	Ratio dens: mucro	Abd. VI spines	Mandi bular teeth	Colour	Size (mm)
O. schoetti	Vietnam New Caledonia	one internal, no lateral	2	6	coarse	2.10	2 acuminate and slightly curved	Several in one row	Black	0.85
O. esakii	Japan	one internal, no lateral	6-8	6	coarse	2.5-3.0	Spiniform setae	20 in one row	Blue violet, indigo	2.50
O. georgia	North America	one internal, no lateral	6 (5?)	6	?	2.0–2.5	Spiniform setae	13 in one row	Blue	1.60
O. petiti	Madagascar	without internal, one small lateral	4	6	coarse	2.50	4 short, acuminate and straight	many in two rows	Red violet	1.25
O. dhritiae sp. nov.	India	one internal, no lateral	6	6	coarse	2.1–2.5	4 strong, blunt and straight	11 in one row	Deep purple	1.05- 1.4
O. coerulea	Indonesia	without internal or lateral	4-5(?)	6	coarse	2.0-3.0	4 long, acuminate and curved	9 in one row (after Y & S 1997)	Blue	1.50
O. barnardi	Australia Africa,	without internal or lateral	4-5	6(5)	coarse	4.0	4 short, blunt and straight	?	Blue- black	1.40
O. subcoerulea	Vietnam	one internal, no lateral	?	6	fine	2.15	4 acuminate and slightly curved	?	Black	1.10
O. dubia	Madagascar	one internal, no lateral	5	6	coarse	2.60	4 acuminate and slightly curved	15 in one row	Black	1.25
O. chenorum	China	one internal, no lateral	4	6	coarse	2.60	4 Short, blunt and straight	14 in one row	Blue- black	1.24

Table 1. Differential characters of world species of Oudemansia



Figs. 16-20 *Oudemansia dhritiae* **sp. nov.** 16 - mouthparts with maxillae and mandible, 17 - magnified picture of maxilla, 18 - magnified picture of mandible, 19 - tenaculum, 20 - fore leg

Zoological Survey of India, for her contribution towards eminence in entomology and to the knowledge of Dipteran Taxonomy.

**Distribution.** Only known from the type locality.

**Remarks:** Oudemansia dhritiae **sp. nov.** is characterized by having six dental setae (as all species of the genus) and one internal tooth on unguis (as the other 6 species of the genus). The new species has 6 sensilla on Ant. IV and a single row of 11 teeth (two strong apical and below 9 small ones) on its mandible which is different from other species of the genus. The unguis of *O. dhritiae* **sp. nov.** is similar to that of *O. dubia* 

Denis, 1947, however differing from it in the number of sensilla on Ant. IV. The new species shows significant resemblance with *O. chenorum* on the basis of setae pattern in female genital plate and Ant. IV sensilla number (Palacios-Vargas and Bu 2020), on the other hand *O. dhritiae* **sp. nov.** is dissimilar in ratio of dens: mucro and mandibular teeth number. *O. petiti* Delamare Debouteville & Massoud, 1964 and the new species has resemblance in sensilla number, conversely the former species has one small lateral tooth on unguis. Prabhoo (1970) first time reported *O. subcoerulea* Denis, 1948 from Kanyakumari, India and he mentioned 3 apical and 2 basal sense rods (or



Figs. 21 *Oudemansia dhririae* **sp. nov.** gorsal body chaetotaxy

sensilla) on Ant IV and termed Sgv as guard sensilla of Ant. III organ, however the new species has 6 sensilla in Ant IV and differs from the previous one in mandibular teeth no. The main diagnostic characters of the world species of *Oudemansia* are summarized (Table 1).

Key to the world species of the genus *Oudemansia* (Modified from Massoud, 1967 and Palacios-Vargas and Bu, 2020)

1.	Abd.	VI	with	2	anal	spines
			O.	scho	etti Der	nis, 1948
–Ab seta	od. VI wit e	h more	than 2 a	nal sp	oines or s	piniform
2. A	bd. VI o	nly wit	th spinifo	orm s	etae	3
–Ab	d. VI wit	h 4 true	e anal spi	nes w	ell defin	ed4
3. N 2.5-	/andible 3.0	with 2	20 teeth. <i>O. es</i>	Rati akii (	o dens: Kinoshi	mucro = ta, 1932)



Figs. 22-24 *Oudemansia dhritiae* **sp. nov.** 22 - tibiotarsus of hind leg with internal tooth on unguis, 23 - furcula, 24 - female genital chaetotaxy

–Mandible with 13 teeth. Ratio dens: mucro = 2.0- 2.5 georgia Christiansen & Bellinger, 1980
4. Unguis without internal tooth 5
–Unguis with one internal tooth7
5. Unguis with small lateral teeth. Mandible with many teeth in two rows <i>O. petiti</i> Delamare Debouteville & Massoud, 1964
–Unguis without lateral teeth. Mandible with teeth in one row6
6. With 4 acuminate and curved anal spines on individual papillae
-With 4 blunt and straight anal spines without individual papillaeO. barnardi (Womersley, 1934)
7. Posterior surface of dens with fine granulations; Ungues III: mucro = 1 to 1.05

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.....O. subcoerulea Denis, 1948

-Posterior surface of dens with coarse granulations; Ungues III: mucro = 1 to 1.4......8

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### Ant and spider diversity of Karuvatta, a coastal island in Vembanad, Kerala, India

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**ABSTRACT:** Ants and spiders are good indicators of ecosystem health; therefore, the present work aims to understand the ant and spider diversity of region Karuvatta, a coastal island in Vembanad wetland ecosystem of Kerala. For the purpose of this study, the island was divided into Karamuttu and Naluchira regions. The study conducted by different collection methods like litter sifting, umbrella method, and handpicking, revealed, 72 species of spiders and 36 species of ants. Diversity indices, species accumulation curves, functional group analyses, and PCA of soil factors were worked out. The results showed that Naluchira had better diversity than Karamuttu. © 2024 Association for Advancement of Entomology

**KEY WORDS:** Biodiversity, wetland ecosystem, diversity indices, species accumulation curves, functional group analyses, PCA of soil factors

#### **INTRODUCTION**

The presence of ants (Hymenoptera, Formicidae) and spiders (Arachnida, Araneae) in a natural environment indicates a healthy ecosystem. Their action towards the environment is essential to the well-being of the habitats in which they live. They are recurrently described as "ecosystem engineers" because they perform many vital ecological services. According to Gadagkar *et al.* (1993), ants are one of the most significant ecological invertebrates in the terrestrial ecosystem because of their enormous biomass, species composition, trophic interactions, mutualistic relationships, and symbiotic relationships that influence the biotic and abiotic community interaction matrix (Dash, 2004). Since changes in ant assemblages are usually associated with changes in other invertebrate assemblages, they have been used in evaluating management conservation practices (Folgarait, 1998) and as a focal group in insect biodiversity studies (Dobson *et al.*, 2006).

In the animal kingdom, spiders are among the most prevalent kinds of predatory organisms (Riechert and Lockley, 1984). Also, they are great candidates for land conservation research because they typically have strict humidity and temperature preferences that confine them to locations within the ranges of their physiological tolerances (Riechert and Lockley, 1974). In addition to this, spiders are valuable indicators of the overall species richness and health

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of the ecosystem (Noss, 1990). Despite their ecological roles as important biocontrol agents, (Riechert and Lockley, 1984), regulating decomposer population (Clarke and Grant, 1968) and influencing ecosystem functioning (Lawrence and Wise, 2000), they have received high threats and little attention from conservation communities (Skerl, 1999). The present study of ant and spider diversity is conducted in Karuvatta Island of Vembanad wetland ecosystem in Alappuzha district of Kerala. The evergreen island, home to diverse flora and fauna, predominantly forms habitats for a variety of ants and spiders. Due to their abundance, which allowed for a high sample size and relative ease of sampling compared to large creatures, the two invertebrates, ants and spiders, have been the subject of the current study. This study will shed some light into the abundance of spider and ant diversity in this island ecosystem, and its outcome will also throw light to make a benchmark of Vembanad island in terms of effective conservation and habitat management.

#### **MATERIALS AND METHODS**

The study was carried out in Karuvatta village in Alappuzha District of Kerala, India. It is an island surrounded by backwaters and lies in between Haripad and Thottappally in Alappuzha (Fig. 1). The total geographical area of the village is 1440ha ha and the elevation of the study area is 4m above sea level. Sampling was done using the standard transect method. Two sites were selected as the representative of the area and were named as site 1 and 2. Site 1 is Naluchira, a village in Purakkadu panchayath and Site 2 is Karamuttu, a village in Karuvatta panchayath.

Site 1 (Naluchira): Naluchira (Situated at  $9.32289^{\circ}$ E; 76.40091°N) is a human inhabitant area comprising houses, roads, and canals. The site possesses wide variety of habitats, which include paddy fields, grasslands, and marshy lands. For the study purposes the site 1 is again divided in to 3 sub sites, 1a - cashew plantation site, 1b - grass lands and 1c - marshy land.

Site 2 (Karamuttu): Karamuttu (Situated at 9.3347°E; 76.4095°N) is the eastern part of the

Karuvatta island. It is a human inhabitant area comprising houses, roads and canals. The site possesses wide variety of habitats, with paddy fields, grasslands and marshy lands. It also comprises many flowering plants and trees such as acacia, bamboo trees etc. For the study purposes the site 2 is again divided in to 3 sub sites, 2a marshy land, 2b - bamboo trees and 2c - acacia trees.

Spiders and ants were sampled from May to July 2022 (for a period of three months) between 6am to 11am. Sampling methods included bait traps (used to attract foragers), beating low vegetation, litter sifting, handpicking, sweep net (for only spiders) and visual search. Ant specimens were also collected using subterranean traps (plastic recipients with 8cm diameter and 12cm height with four radial holes to allow ants to access the interior of the trap). Collected specimens were preserved in 70 per cent alcohol for further analyses.

Detailed examination of each spider and ants were done using the Labomed CZM4 Stereomicroscope. The epigynum of female adult spiders were dissected, cleared in KOH (10%), mounted on a temporary slide and observed under a compound microscope (Leica DM1000 LED) to study the internal structures. Adult male spiders were identified by observing their palp. Measurements of the legs and palps were taken using the Leica S8APO version 4.2. Spiders were identified using literature (Sebastian and Peter 2009; Sudhikumar, 2007) and taxonomic keys of Bingham (1903) and Bolton's (1994) Catalogue of Ants of the World. All identified specimens were deposited in the Zoological Museum of the Department of Zoology, University of Kerala, Kariavattom for reference.

The checklists of the specimens of ants and spiders were prepared. The percentage compositions of the different families at different sites were calculated and graphically plotted. The diversity indices were calculated using R environment. Dominance index was estimated using *Estimate Dominance* package and other indices were analysed using *vegan* package in R Core Team, 2021. Principal Coordinate Analysis of different sites was done using *vegan* package in R (Oksanen, 2022). The PCA between the soil parameters and the sites were done using Base R. Species accumulation curves were plotted using *iNEXT* package (Chao *et al.*, 2014; Hsieh *et al.*, 2016). Normality of the data was checked with the Shapiro-Wilkins Test. Normalization of data was done where needed. ANOVA of the species diversity, indices, and soil parameters was done using Base R package. The post-hoc analysis was done using Tukey's HSD.

Soil collected from all the subsites were analysed for organic carbon content by Walkley and Black rapid titration method. The pH was measured using a pH meter. Moisture was measured by gravimetric method.

Landscape analysis was done using QGIS Tisler 3.28 version. The images were obtained from USGS Landsat 8 data (USGS, 2023).

#### RESULTS

A total of 36 species of ants were collected from five subfamilies and 18 genera. Subfamily Formicinae had the highest abundance and the most number of species. A checklist of the ants collected is given (Table 1). Their diversity indices were calculated (Table 2). *Camponotus rufoglaucus, Diacamma rugosum, Odontomachus simmillimus*  and *Technomyrmex albipes* were found in all sites. Site 1a showed the highest diversity while Site 2a was with least diverse.

The species accumulation curve was found to have reached an asymptote for the island (Fig. 2).

#### Functional Group Analysis:

The ants were divided into 9 functional groups Opportunists, Specialist predators, Subordinate camponotinii, Tropical Climate Specialists, Hot Climate Specialists, Cold Climate Specialists, Generalised Myrmicinae, Cryptic species, Small Sized Hypogaeic Generalist Foragers (Fig. 3). Hot Climate Specialists were found only in Site 3 while, Cold Climate specialists, Cryptic species and Small-Sized Hypogaeic Generalist Foragers were found only in Site 2.

Total 72 species of spiders belonging to 15 families were reported from both Karamuttu and Naluchira Islands of Karuvatta village, Alappuzha, during the period of three months (May 2022-Jul 2022) study. A checklist of the spiders collected (Table 3), revealed that, the dominant family was Salticidae (19), followed by Araneidae (17), Tetragnathidae (7), Theridiidae, Lycosidae and Thomisidae (4), Clubionidae (3), Liocranidae (3), Corrinidae (2), Hersiliidae (2) Uloboridae and Oxyopidae (2). The



Fig. 1 Collection sites in Karuvatta, Kerala; Red circle- Naluchira and Yellow square- Karamuttu



Fig. 2 Species Accumulation Curve of ants



Fig. 3 Functional Group Composition of Ants

least common families are Cheiracanthidae, Pisauridae and Sparassidae represented with single species.

Diversity indices of total number of spider species from both sites (Table 4), indicated that form subsite of site 1, Cashew Plantation Site (S1a) has the most diversity with Shannon-Weiner Index 3.263. A good diversity in S1a and S1c of S1 Naluchira is due to the presence of vegetation with the abundance of shrubs and bushes in these subsites. The lowest diversity is observed from the Grassland land subsite (S1b) of site 1. Unlike site 1, in site 2 Salticidae is the dominant family with eight species. The family Araneidae ranks second with five species. Considering the diversity of spiders listed under each subsite of site 2, the Marshy land (S2a) has the most diversity with Shannon-Weiner Index and Dominance diversity index as 2.998, 0.06011



Fig. 4 Species Accumulation Curve of spiders



Fig. 5 Guild composition of spiders.

respectively. A wide variety of shrubs and small trees in this area provide spiders an ideal habitat for assemblage, mating and capturing small insects as prey. The lowest diversity is observed from Bamboo tree subsite (2b) of site 2. Diversity indices of site 1a was found to be highest, and evenly distributed and least dominant. Site 2b was most dominant. Species accumulation curve has reached an asymptote (Fig. 4).

Guild structure: In the present study six guild structure of the spiders were observed namely stalkers, orb-web weavers, foliage and ground runners, space web builders and ambushers based on foraging behaviour and ecological characteristics (Uetz *et al.*, 1999; Hofer *et al.*, 2001; Young and Edwards, 1990). Among the spider families collected, the spiders belonging to the guild structure orb-web builders were the dominant group

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Table 1. Checklist of ants of Karuvatta

No.	Species
	Subfamily: Dolichoderinae
1.	Dolichoderus sp.
2.	Tapinoma indicum (Forel, 1895)
3.	Ta. melanocephalum (Fabricius, 1793)
4.	Technomyrmex albipes (Smith, F., 1861)
5.	Te. vitiensis (Mann, 1921)
	Subfamily: Formicinae
6.	Anoplolepis gracilipes (Smith, F., 1857)
7.	Camponotus irritans (Smith, F., 1857)
8.	Camponotus sp.
9.	C. rufoglaucus (Jerdon, 1851)
10.	Nylanderia birmana (Forel, 1902)
11.	N. bourbonica (Forel, 1886)
12.	N. taylori (Forel, 1894)
13.	N. yerburi (Forel, 1894)
14.	Oecophylla smaragdina (Fab, 1775)
15.	Paratrechina longicornis (Latreille, 1802)
16.	Polyrhachis tibialis Smith F., 1858
	Subfamily Myrmicinae
17.	Carebara sp.

18.	Crematogaster dohrni Mayr, 1879
19.	C. rothneyi Mayr, 1879
20.	Meranoplus bicolor (Guérin-Méneville, 1844)
21.	Monomorium indicum Forel, 1902
22.	M. orientale Mayr, 1879
23.	M. pharaonic (Linnaeus, 1758)
24.	Pheidole sp.
25.	P. vulcan Fischer & Fisher, 2013
26.	Solenopsis geminata (Fabricius, 1804)
27.	Tetramorium bicarinatum (Nylander, 1846)
28.	T. pacificum Mayr, 1870
29.	T. rossi (Bolton, 1976)
	Subfamily Ponerinae
30.	Anochetus validus Bharti & Wachkoo, 2013
31.	Diaccamma rugosum (Le Guillou, 1842)
32.	Odontomachus simmillimus Smith F 1858
	Ouoniomaenas simminias Sinini, 1., 1050
33.	Plathytyrea parallela (Smith F., 1859)
33.	Plathytyrea parallela (Smith F., 1859)         Subfamily Pseudomyrmicinae
33. 34.	Plathytyrea parallela (Smith F., 1859)         Subfamily Pseudomyrmicinae         Tetraponera nigra (Jerdon, 1851)
<ul><li>33.</li><li>34.</li><li>35.</li></ul>	Plathytyrea parallela (Smith F., 1859)         Subfamily Pseudomyrmicinae         Tetraponera nigra (Jerdon, 1851)         T. periyarensis Bharti & Akbar, 2014

(comprising of 39%) followed by the stalkers (29%) (Table 1, Fig. 5).

The PCA analysis for soil parameters explains 85% of the variation in the values (Fig. 6). The Summary of the soil data is given (Table 6). The subsites 1

and 2 showed positive relation with pH and negative relation to the Organic Carbon. Subsite 4 showed a positive relation to pH. Subsite 3 and 4 showed a positive relation to moisture as they are both marshy lands. Subsite 1, 2, and 5 showed a negative relation to moisture. Subsite 1 and 2 seem to be related.

Indices	la	1b	1c	2a	2b	2c	Total
Shannon_H	2.491	2.453	2.353	1.964	2.256	2.156	2.822
Evenness_e^H/S	0.8045	0.6117	0.7516	0.7918	0.6819	0.7196	0.5421
Dominance_D	0.09497	0.1163	0.1182	0.1576	0.1284	0.14	0.07662

Table 2. Diversity indices of Ants

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No.	Species	No.	Species
	Araneidae Clerck, 1757		Salticidae Blackwall, 1841
1	Anepsion maritatum (O.P Cambridge, 1877)	36	Asemonea tenuipes (O.P Cambridge, 1869)
2	Araneus ellipticus (Tikader & Bal, 1981)	37	Bianor angulosus (Karsch, 1879)
3	Argiope aemula (Walckenaer, 1841)	38	Brettus cingulatus Thorell, 1895
4	A. anasuja Thorell, 1887	39	Carrhotus viduus (C.L. Koch, 1846)
5	A. catenulata (Doleschall, 1859)	40	Menemerus bivittatus (Dufour, 1831)
6	Chorizopes khanjanes Tikader, 1965	41	Myrmaplata plataleoides (O.P Cambridge, 1869)
7	Cyclosa confraga (Thorell, 1892)	42	Phintella vittata (C. L. Koch, 1846)
8	Cyrtophora cicatrosa (Stoliczka, 1869)	43	<i>Phintelloides undulatus</i> (Caleb & Karthikeyani, 2015)
9	C. citricola (Forsskål, 1775)	44	Plexippus paykulli (Audouin, 1826)
10	Eriovixia laglaizei (Simon, 1877)	45	P. petersi (Karsch, 1878)
11	Gasteracantha geminata (Fabricius, 1798)	46	Rhene flavicomans Simon, 1902
12	Guizygiella sp. Zhu, Kim & Song, 1997	47	R. flavigera (C.L. Koch, 1846)
13	Herennia multipuncta (Doleschall, 1859)	48	Telamonia dimidiata (Simon, 1899)
14	Neoscona bengalensis Tikader & Bal, 1981	49	Myrmarachne spissa
15	Neoscona nautica (L. Koch, 1875)	50	Marengo sachintendulkar
16	N. vigilans (Blackwall, 1865)	51	Myrmarachne ramunni
17	Neoscona sp. Simon, 1864	52	M. melanocephala
	Cheirancanthiidae Wagner, 1887	53	M. uniseriata
18	Chericanthium melanostomum Thorell 1895	54	Myrmarachne sp.
	Clubionidae Wagner, 1887		Sparassidae Bertkau, 1872
19	Clubiona drassodes O.P Cambridge, 1874	55	Olios sp.
20	C. filicata O.P Cambridge, 1874		Tetragnathidae Menge, 1866
21	C. tridentata Dhali, Roy, Saha & 2016	56	Leucauge granulata (Walckenaer, 1841)
	Corinnidae Karsch, 1880	57	Tetragnatha ceylonica O. P Cambridge, 1869
22	Castianeira zetes Simon, 1897	58	T. cochinensis Gravely, 1921
23	Corinnomma severum (Thorell, 1877)	59	T. mandibulata Walckenaer, 1841
	Hersiliidae Thorell, 1869	60	Tetragnatha viridorufa Gravely, 1921
24	Hersilia savignyi Lucas, 1836	61	Tylorida striata (Thorell, 1877)
25	H. tibialis	62	T. ventralis (Thorell, 1877)

Table 3. Checklist of spider fauna collected from Karuvatta island

	Liocranidae Simon, 1897		Theridiidae Sundevall, 1833
26	Oedignatha scrobiculata Thorell, 1881	63	Argyrodes argentatus O.P Cambridge, 1880
27	Oedignatha sp.2 Thorell, 1881	64	Nihonhimea indicum (Tikader, 1977)
28	Sphingius barkudensis Gravely, 1931	65	Parasteatoda celsabdomina (Zhu, 1998)
	Lycosidae Sundevall, 1833	66	Theridion manjithar Tikader, 1970
29	Pardosa pseudoannulata (Bösenberg & Strand, 1906)		Thomisidae Sundevall, 1833
30	P. sumatrana (Thorell, 1890)	67	Camaricus formosus Thorell, 1887
31	P. parathompsoni Wang & Zhang, 2014	68	Thomisus projectus
32	P. oriens (Chamberlin, 1924)	69	Thomisus sp.
	Oxyopidae Thorell, 1869	70	Amyciaea forticeps
33	Oxyopes javanus Thorell, 1887		Uloboridae Thorell, 1869
34	O. birmanicus	71	Philoponella feroka (Bradoo, 1979)
	Pisauridae Simon, 1890	72	Uloborus krishnae Tikader, 1970
35	Dendrolycosa gitae (Tikader, 1970)		



Fig. 6 PCA of soil factors

The satellite image of Karuvatta (Fig. 7) and habitat utilization (Fig. 8) represents a large area of the island covered by paddy field. Also as few habitation areas around the paddy fields.

#### DISCUSSION

Ants and spiders are very important for ecosystem resilience and maintaining the system in equilibrium

regarding edaphic and biological factors. The Karuvatta island in Kuttanad is dominated by paddy fields and both spiders and ants act as biocontrol agents in paddy fields as reported by Way *et al.* (2002) and ants are also known to influence soil properties in paddy fields (Jouquet *et al.*, 2008). Hence, it is highly necessary to assess the richness of these two categories of organisms in an ecosystem.



Fig. 7 Satellite Image of Karuvatta Island



Fig. 8 Habitat Utilization of Karuvatta Island

Diversity Indices	1a	1b	1c	2a	2b	2c	Total
Shannon_H	3.263	3.231	3.239	2.998	2.891	2.979	3.67
Evenness_e^H/S	0.7914	0.7444	0.7731	0.7157	0.7202	0.7284	0.6039
Dominance_D	0.04435	0.04736	0.04688	0.06011	0.06592	0.06355	0.03635

Table 4. Diversity indices of Spiders

Ants are important components of ecosystems because they constitute a great part of the animal biomass and, they act as ecosystem engineers. This study documents the diversity of ants in Karuvatta Island in Vembanad wetland ecosystem, where 36 species of ants belonging to 18 genera and five subfamilies were identified. The subfamily Formicinae accounted for the highest number of species (11 species), followed by subfamily Myrmicinae with 10 species. A similar study was conducted at Kuttanad region of Kerala, India where 25 species of ants belonging to five subfamilies distributed among 17 genera were reported (Rabeesh et al., 2017). In a study at Chorao Island, Goa, India a total of 38 ant species belonging to 24 genera and six subfamilies were collected (Pai et al., 2009). In another study at Havelock Island in the Andaman Islands, a total of 50 species of ants belonging to 25 genera were identified (Agavekar et al., 2019).

Ant diversity studies have also been conducted in various terrestrial ecosystems in India. From Silent Valley National Park, Western Ghats, Kerala, 30 genera representing 40 species and six subfamilies were recorded (Sabitha *et al.*, 2018). In a study at selected sites of Aralam Wildlife Sanctuary, Kerala, a total of 19 species of ants were collected and identified by Joseph and Thomas (2021). From Amravati City of Maharashtra, 34 species of ants belonging to five subfamilies were identified (Chavhan and Pawar, 2011). The Udupi District, Karnataka recorded 31 species of ants under 17 genera and five subfamilies (Cunha and Nair, 2013).

Table 5. Guild structure of spiders and number of species

No.	Guild structure	No. of species
1	Orb- web builders	27
2	Foliage runners	5
3	Ground dwellers	9
4	Ambushers	7
5	Stalkers	20
6	Space web builders	4
	Total	72

#### Table 6. Soil data summary

Naluchira							
	pН	OC	Moisture	Temperature			
Mean	3.628	1.977	13.603	24.22			
SD	0.115	0.535	11.511	1.92			
Minimum	3.52	1.2	5.3	21			
Maximum	3.9	2.5	29.1	27			
		Kara	muttu				
Mean	3.614	1.178	16.7	26			
SD	0.564	0.555	2.60	1			
Minimum	3	0.5	14.2	25			
Maximum	4.5	1.95	20.12	27			

The Functional group analysis showed that the opportunists and subordinate Camponotini are present in large numbers in all sites. Their general resilience to change and generalist nature has enabled them to conquer most sites. Small-hypogeic generalist foragers should have shown better distribution, but their hypogaeic nature makes them difficult to collect and their representation not accurately possible. More number of species seems to have an association to Naluchira than Karamuttu. Overall, the Naluchira has a better ant diversity and lower dominance and therefore, it is an ecologically balanced habitat.

Spiders are important predatory arthropods that play a crucial role in maintaining the ecological balance. The present observation documents 72 species of spiders belonging to 15 families in Karuvatta village in India. The dominant families were Salticidae and Araneidae, with 19 and 17 species, respectively. A similar study was conducted at Pathiramanal Island in the same wetland ecosystem, where 147 species of spiders belonging to 26 families under 92 genera were documented (Malamel and Sudhikumar, 2020). In Kuruva Island of the Wayanad district in Kerala, 19 spider species belonging to 10 families were identified (Andrews and Jose, 2021). Another study in the St. Estevam Island in Goa documented spiders belonging to eight families, 19 genera, and 29 species (Halarnkar and

Pai, 2018). In the selected islands of the Gulf of Kutch, 123 species of spiders belonging to 81 genera under 25 families were identified (Parmar et al., 2015). In the Andaman and Nicobar Islands of the Indian Ocean, 58 species of spiders contained in 41 genera and distributed in 20 families were documented, 26 of which were new to science (Tikader, 1977). In addition, study on diversity of family Tetragnathidae was also conducted in Kuttanad by Babu and Prasad (2022). The dominant family of spiders varies across different studies and locations. In Karuvatta, on of the dominant families was Araneidae similar to that of Pathiramanal Island (Malamel and Sudhikumar, 2020) and Kuruva Island (Andrews and Jose, 2021). The variation in the distribution patterns of spiders may be due to the influence of microhabitat types on species distribution. Many factors determine species composition. This may be related to the changes in the vegetation structure of the habitat. The stalkers (wanderers) and the web-building spiders rely on vegetation for some part of their lives to find food, retreat, or build webs (Sanders and Platner 2007). Therefore, the vegetation structure of different types of plantations in the present study area might influence the diversity of Salticidae and Araneidae compared to other families. Reports are also available on the species diversity and evenness from various localities of the world. In Karuvatta, Naluchira is having a good population of spiders than Karamuttu. The soil analysis of the sub sites in Naluchira and Karamuttu showed different relations to pH, organic carbon, and moisture content. Naluchira had a higher diversity of species and a greater number of associated species compared to Karamuttu. The guilds of the spider families in Karuvatta village were dominated by web spiders, followed by stalkers. Previous studies have shown conflicting results on the prevalence of different types of spiders in different habitats, with some studies finding a higher proportion of jumping spiders and others finding a higher proportion of hunters. These differences may be due to variations in habitat and other factors that affect the distribution and diversity of spiders. Further research is needed to understand the specific factors that influence the distribution and diversity of spider species

Naluchira had better species richness than Karamuttu perhaps because of the larger habitation area in Karamuttu. Naluchira is dominated by paddy fields in terms of area and hence this provides a much lower disturbance than habitation sites.

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# Influence of weather parameters on the population of *Bactrocera* spp. (Diptera, Tephritidae) in the mango orchards of Padanakkad, Kerala, India

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**ABSTRACT:** An investigation was conducted to study the seasonal occurrence of *Bactrocera* spp. (Diptera, Tephritidae) in the mango orchards of College of Agriculture, Kerala Agricultural University, Padannakkad. Surveillance of fruit flies was conducted using bottle traps with methyl eugenol lure. A total of 10,546 fruit flies were trapped with the predominant species being *Bactrocera dorsalis*. Four species *viz., B. dorsalis, Zeugodacus tau, B. zonata* and *Z. cucurbitae* were identified from the population collected in that order of dominance. Out of fruit flies captured, *B. dorsalis*, accounted for 97.97 per cent. The highest weekly population of fruit flies was recorded in the 14<sup>th</sup> standard meteorological week (SMW) of 2022 from April 2 to 8, 2022, with 793 fruit flies, while the lowest population in 50<sup>th</sup> SMW from December 10 to 16, 2021 with only one fruit fly. The monthly average population was highest in May with 667.5 flies per month and lowest in December with 16 flies per month. During the period of host availability (April to June), the population of fruit flies exhibited significant positive correlation with minimum temperature (0.805), and a significant negative correlation with soil temperature (-0.512). There was a negative correlation with maximum temperature (-0.329) and wind speed (-0.192) while a positive correlation was observed with rainfall (+0.204). © 2024 Association for Advancement of Entomology

**KEY WORDS:** Surveillance, *Bactrocera dorsalis, B. zonata, Zeugodacus tau* and *Z. cucurbitae*, weather parameter, population dynamics

#### **INTRODUCTION**

Mango (*Mangifera indica* L.) holds immense commercial importance as the foremost fruit crop in India, contributing to over 54 per cent of global mango production (Tharanathan *et al.*, 2006). India, the world's largest producer, producing around 21

million metric tons of mango in 2022 (Statista, 2023), accounting for 44 per cent of worldwide production. The productivity of mango in Kerala in the year 2020-21 was reported to be 6206 kg ha<sup>-1</sup> (FIB, 2023). However, India's global market share is limited to just 15 per cent (Sahithi, 2022), due to various insect pests, especially fruit flies (Diptera,

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Tephritidae), which infest both ripe and unripe fruits (Choudhary et al., 2018). The Oriental fruit fly, Bactrocera dorsalis (Hendel) is a major pest of mango causing both quantitative and qualitative losses as well as export barriers (Hossain et al., 2020). It is widely recognized as a highly invasive species, with documented populations in over 60 countries, primarily concentrated in Asia and Africa. Fruit flies have been identified as one among the ten most severe threat to crop production due to their polyphagous nature and severity in infestation that causes 2.5 to 100 per cent of damage and significant economic loss (Verghese et al., 2004). Apart from the immediate harm caused to the fruit, such as its softening, rotting, decay, and subsequent premature dropping to the ground before harvesting, there are also trade-related concerns and trade relationship issues arising from quarantine restrictions imposed because of fruit fly infestations. These are considered as high priority quarantine pests. Being polyphagous pests with high reproductive potential, wide host range, adaptability to climate and overlapping of generations, their management is rather difficult (Agarwal and Kumar, 1999). The key to successful control is effective monitoring. Prior to creating an insect pest management plan tailored to a particular agroecosystem, it is vital to possess fundamental data about pest prevalence in relation to weather parameters. This information aids in determining the right timing for intervention and selecting the most effective control methods. Monitoring the pest population throughout the year stands as a crucial foundational element in implementing the Integrated Pest Management (IPM) approach for management of fruit flies in mango ecosystem.

#### **MATERIALS AND METHODS**

**Surveillance of population:** Surveillance was conducted in the mango orchards of Instructional Farm I (IF I) at College of Agriculture, Padanakkad of Kerala Agricultural University within the district of Kasaragod (Kerala, India) (12.25°N; 75.11°E), at an altitude of 8.73m above sea level. The mango orchard spans 6.64 ha and consists diverse range of mango varieties, including Alphonso, Bennet Alphonso, Bangalora, Banganappalli, Neelum,

Himayuddin X Neelum, Kalapady, Gomanga, Mundappa, Prior, Phirangiladuva, Karpooram, and more. Notably, no insecticidal sprays were applied during the entire observation period. Surveillance was carried out from September 2021 to September 2022, *i.e.*, from 38<sup>th</sup> standard meteorological week (SMW) of 2021 to 37<sup>th</sup> SMW of 2022. Four standardised Methyl Eugenol (ME) baited bottle traps were installed in the Instructional Farm - 1 of College of Agriculture, Padannakkad at a height of 1.5m from the ground in a shady place in the mango orchard. The lure blocks were replaced at monthly interval and the trapped flies were removed and counted on a weekly basis. Weekly population of fruit flies were monitored and average monthly population was computed for the surveillance period.

**Dominance of fruit fly species:** Dominance of fruit fly species was assessed by counting the number of each species. The fruit flies were identified based on the keys specified by David and Ramani (2011). The calculation of the diversity of fruit fly species was carried out for each species using the Shannon-Wiener diversity, Simpson dominance index and Margalef's Species richness index and Species evenness was also assessed.

> The Shannon Weiner index (H') is a quantitative measure that reflects how many different species are there in a dataset, and accounts how evenly the basic entities (such as individuals) are distributed among those types.

$$H^1 = -\sum_{i=0}^s Pi \ln Pi$$

where: s = number of species in the community

 $p_i$  = proportion of total abundance represented by  $i^{th}$  species

Simpson's Dominance Index (D) is a measure of diversity which takes into account both richness (the number of species per sample) and evenness (abundance of the different species making up the richness of an area).

$$D = 1 - (\sum (pi)^2)$$

Margalef's Species Richness Index (d)-

Simplest measure of biodiversity and is a count of the number of different species in a given area calculated using the formula:

$$d = \frac{S-1}{\ln N}$$

Where: S = number of species, N = total number

Species evenness - Indicate the measure of how similar the abundance of different species, species evenness was calculated to estimate the equitability component of diversity.

 $J = H' / \ln s$ 

**Influence of weather parameters with population of fruit flies:** Weekly population data of fruit flies was correlated (Pearson's simple correlation) with weather parameters like minimum temperature, maximum temperature, relative humidity, rainfall, wind speed and soil temperature. These meteorological observations were collected from the records of the Regional Agricultural Research Station, Pilicode, Kasaragod, Kerala.

#### **RESULTS AND DISCUSSION**

**Surveillance of population:** Fruit fly populations monitored throughout showed that the weekly catch in trap varied from 1.00 to 793.00 fruit flies. In total, 10546 adult fruit flies were trapped during the year from September, 2021 to September, 2022. Maximum population was recorded in the 14<sup>th</sup> SMW of 2022, while the lowest population was recorded during 50<sup>th</sup> SMW (Fig. 1). A sudden increase in the population of fruit flies was observed in the first week of April after the summer showers in late March. Conversely, a gradual decline in the population of fruit flies was noticed from the first week of July as the season of mangoes came to an end and also coincided with the heavy rainfall.

The average monthly population recorded was highest in May followed by June, while lowest monthly population was noted in December. A higher population of fruit flies was mainly reported from April to June which coincided with host availability and suitable weather parameters since the population of fruit flies is mainly influenced by host availability and suitable weather parameters. The population of fruit flies trapped was significantly lower when mango was unavailable since the study area is deprived of other suitable hosts like guava and banana.

The seasonal pattern of population fluctuation was similar to that of the reports of Akhila (2015) that population build-up was noticed from April and attained a peak in May to June in Kerala. Begam *et al.* (2021) also reported a similar trend from Tamil Nadu where the population was found to gradually increase from the first fortnight of April and reached its first peak during first fortnight of July. Vignesh *et al.* (2020) observed the peak incidence of fruit flies in August and least in December in Tamil Nadu and Sumathi *et al.* (2019) reported less fruit fly population in traps from January to April in Tamil Nadu.

**Dominance of fruit fly species:** *Bactrocera dorsalis* was found to be the predominant species. Out of the total fruit flies captured (10,546), an over whelming majority were *B. dorsalis* (10,332), accounting for 97.97 per cent. Other species constituted only a small proportion *viz., Zeugodacus cucurbitae* (Coquillett) (199 fruit flies, sharing1.89%), *B. zonata* (Saunders) (9 fruit flies, accounting 0.09%) and *Z. tau* (Walker) (six fruit flies, accounting 0.06%).

The species richness value determined was 0.32 at IF I of CoA, directly indicating fruit fly species composition responding to methyl eugenol in ME traps. The Simpson Dominance Index (D) showed a value of 0.04 in the overall range of 1 to 4. The low value indicates a low species diversity in the community with a few dominant species. From the surveillance study, only four species of fruit flies were captured, with *B. dorsalis* being the most



Fig. 1 The weekly population of fruit flies collected in Instructional Farm 1, CoA Padannakkad



Fig. 2 Influence of weekly weather parameters on population of fruit flies during host availability period



Fig. 3 Correlogram presentation of influence of weather parameters on population of fruit flies



Fig. 4a Influence of minimum temperature on population (r=+0.81)



Fig. 4b Influence of soil temperature on population (r=-0.52)

prevalent and dominant species. Shannon-Wiener Diversity Index (H) determined was 0.11 in the range of 0 to 1.38. The low value of H, indicates a lower diversity of species in the community. Additionally, the Evenness Index (J) estimated was 0.42, within its range of 0 to 1. The low value suggests that there is an unequal distribution of individuals among the species within the community.

A similar dominance trend was also reported from the field experiments of Mariadoss *et al.* (2020) from the surveillance study in Telangana during 2018 and 2019. Ebi *et al.* (2020) also trapped fruit flies where more than 90 per cent were *B. dorsalis*. Among the three species of *Bactrocera*, the highest population trapped was *B. dorsalis* followed by *B. zonata* and *B. correcta* (Kumar *et al.*, 2021). Roy *et al.* (2022) also trapped a higher population of *B. dorsalis* (85.41%) from the surveillance from April to June 2020 in Bangladesh.

Influence of weather parameters on fruit fly population: The fluctuation in fruit fly population may be attributed to prevalence of congenial environmental conditions and/or fruiting and flowering time of the hosts as suggested by Laskar and Chatterjee (2010). Weekly weather parameters during mango fruit availability *i.e.*, from 26-02-2022 to 01-07-2022, which coincided with fruit maturing and ripening stage, were analysed and correlated with the population of fruit flies during the same period (Fig. 2). The maximum temperature ranged from 29.66 to 35.64°C and the minimum temperature ranged from 20.36 to 24.67°C, indicating warm to hot climate during this period. Relative humidity fluctuated between 72.07 to 92.07 per cent, suggesting a moderate to high moisture level in the air. There are periods with no recorded rainfall and some weeks with varying amounts, ranging from 4.50 to 274.60mm. This indicates a

mix of dry and wet periods, with some weeks experiencing significant rainfall. Wind speed varied from 0.57 to 2.61 km h<sup>-1</sup>, suggesting generally calm to light breezes during this period. The soil temperature ranged from 29.71 to 45.50 °C indicating consistently warm soil conditions throughout the period.

Population of fruit flies showed significant positive correlation (+0.81) with the minimum temperature, significant negative correlation (-0.52) with soil temperature at a depth of 5cm, negative correlation (-0.33) with the maximum temperature, minute positive correlation (0.07) with relative humidity, positive correlation (0.20) with rainfall and a negative correlation (-0.19) with wind speed (Figs. 3, 4a, b).

Cai *et al.* (2023) stated that development and reproduction of fruit flies is in the range of 15 to  $34^{\circ}$ C with an optimum range of 20 to  $28^{\circ}$ C. Minimum temperature recorded during the study period lies in the optimum range for development and reproduction of fruit flies. Positive and highly significant correlation of *B. dorsalis* incidence with minimum temperature was recorded earlier by Bana *et al.* (2017) in Gujarat, Abro *et al.* (2021) in Hyderabad and Larkana, Kumar *et al.* (2021) in Meerut, Kumar *et al.* (2022) in Ayodhya, and Amur *et al.* (2022) in Pakistan.

Fruit flies pupate in the soil at a depth of one to five centimetres (Dimou et al., 2003). Soil temperature is a crucial determinant to ensure successful pupation and for the subsequent emergence of adult fruit flies. Unfavourable soil temperatures, such as being too high or too low, can negatively impact pupal survival and reduce the overall population size. In a surveillance study by Barma et al. (2013) in West Bengal, they reported a higher population of fruit flies in an optimum range of 27 to 30°C. Since the soil temperature recorded during the present study period was mostly in the range of 30 to 45°C, which was beyond the threshold limit of optimum temperature for the lifecycle of fruit flies, surveillance data recorded a negative correlation of population with soil temperature. A similar negative correlation of population with maximum temperature is also reported by Ganie *et al.* (2013) in Kashmir and Dale and Patel (2010) in Gujarat.

While relative humidity contributes to the overall suitability of the environment for fruit fly survival and reproduction, its minute-scale fluctuations alone may not be the primary determinant of population abundance. So, a similar correlation has been reported by Konyak *et al.* (2023), Kumar *et al.* (2021) and Bana *et al.* (2017), where pest population was not influenced by relative humidity.

Population build-up evidently happens after the receipt of summer showers which favours the emergence of fruit flies from the soil. Moist environments created by rain and increased relative humidity may enhance larval survival rates and accelerate their growth, potentially increasing the population size. Bateman (1968) cited by Drew et al. (1984) suggested that ample moisture has a significant impact on the abundance of fruit flies. This influence might be attributed to the fact that the availability of moisture in the air and soil promotes the emergence of pupae. And this might be the reason for the higher population of fruit flies followed by the receipt of summer showers. Similar reports of positive correlation between rainfall and fruit fly population were reported by Mouly et al. (2017) in Karnataka, Jena et al. (2022) in Gujarat, and Amur et al. (2022) in Pakistan. Higher wind speed can make it more challenging for fruit flies to locate and assess suitable host plants. Similar negative correlation of population of flies with wind speed was reported by Draz (2016).

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## Phytochemical profiling of ethanolic extract of bee pollen using Gas Chromatography Mass Spectroscopy and its *in silico* analysis of hypocholesterolemic activity

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**ABSTRACT:** Bee pollen, a bee product, is considered as one of the valuable products in natural medicine, for its excellent therapeutic properties. Cold maceration technique was employed to extract bee pollen and the phytochemical constituents were analysed. The preliminary phytochemical analysis showed the presence of acids, proteins, carbohydrates, phenol, flavonoids, glycosides, anthraquinone, quinone, resins, saponin, tannin, and terpenoids. The advanced phytochemical screening of volatile chemical constituents analyzed using Gas Chromatography and Mass Spectrometry showed 40 volatile chemical compounds from the bee pollen extract. Among those, the hypocholesterolemic effect-related compound of bee pollen was analyzed by in silico docking method, where Oxidosqualene: Ianosterolcyclase (OSC) was taken as a target enzyme which helps in cholesterol biosynthesis. Hexadecanoic acid, Pentadecanoic acid, Pyrroloindole, and Alpha amyrin selected as ligands, and Ro 48-8071 fumarate used as a standard enzyme inhibitor for molecular docking analysis, showed that all the ligands efficiently bound with the target enzyme (-13.37 kJ mole<sup>-1</sup>). The OSC enzyme inhibition activity may be responsible for the hypocholesterolemic effect and weight-reducing property of bee pollen.

**KEY WORDS:** Cold maceration, Ro 48-8071 fumarate, hypocholesterolemic, Oxidosqualene: Ianosterolcyclase, molecular docking analysis, Alpha amyrin

#### **INTRODUCTION**

Honey bee pollen contains numerous essential nutrients, including carbohydrates, unrefined fibers, proteins, and lipids. Furthermore, minor constituents, encompassing amino acids, minerals, trace elements, vitamins, carotenoids, phenolic compounds, flavonoids, sterols, and terpenes also constitute bee pollen (Linskens and Jorde, 1997; Kostić *et al.*, 2015; Li *et al.*, 2018). The composition of bee pollen is influenced by various factors, including atmospheric conditions, soil

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characteristics, and the foraging behaviour of bees (Liolios et al., 2019; Mayda et al., 2020). Bee pollen is collected using special collecting tray called pollen trap. Pollen from flowers is collected by worker bees and stored as pellets in the pollen baskets in the corbiculae of the legs. When the bees enter into the hive, the pellets of pollen can be scrapped from the legs and collected in the pollen trap (Somerville, 2012). Recent studies have proven the economic importance of bee products due to several bioactive potential, such as antimicrobial, antiviral, antitumor, and anti-inflammatory properties (Alvarez-Suarez, 2017). The components of bee pollen is of great interest to the pharmaceutical industry especially flavonoids and phenolic acids, as they are reportedly used in therapeutics against many diseases related to oxidative damage such as cardiovascular and neurodegenerative disorders (Alimoglu et al., 2021). The present work describes the phytochemical constituents in the cold macerated ethanolic extract of bee pollen, quantified through GCMS. Additionally, the hypocholesterolemic effect of bee pollen was determined using in silico molecular docking method (Campos et al., 1997; Mãrgãoan et al., 2019; Morris and Lim-Wilby, 2008).

#### **MATERIALS AND METHODS**

Bee pollen and chemicals: Apis indica bee pollen was collected using pollen traps from the Nilgiri Biosphere Nature Park (NBNP), Coimbatore, Tamil Nadu, maintained by the Zoological Parks Association of India at PSGR Krishnammal College for Women. Test reagents and solvents were obtained from Sigma Aldrich and Hi-Media. Cold maceration was selected as the extraction process, much like cold pressing, employs low extraction temperature and retains the odour of the source material without degrading the thermolabile compounds present in the fraction (Wu et al., 2015; Sankeshwari et al., 2018). GCMS help us to identify different substances within a test sample (Prabhakar Joshi and Dayaram Wagh, 2018). Molecular docking is a technique used to predict the best match between two molecules when they are bound to each other in order to generate a stable complex (Kumar, 2019). This technique, utilized in various domains provides a comprehensive approach to study the molecular systems of small chemical systems up to large biological molecules and material assemblies (Lengauer and Rareyt, 1996).

**Extraction of sample**: Powdered bee pollen 100g was soaked in ethanol in a stoppered container at room temperature for three days. The mixture was then strained and filtered to obtain a complete extract following the method described by Nurdianah *et al.* (2016). The solvent ethanol was allowed to evaporate, resulting in a semi-solid extract that was stored for further analysis (Li *et al.*, 2019).

Preliminary phytochemical analysis: Tests were carried out on the ethanolic extract of Indian bee pollen to identify the presence of tannins, saponins, flavonoids, alkaloids, anthraquinones, terpenoids, glycosides, proteins, carbohydrates, resins, and acids (Kaur et al., 2013; Sundarraju et al., 2014). The cold macerated ethanolic extract was subjected to GCMS analysis to identify the phytochemical constituents present in it. An autosampler system (7693) was equipped for the sample injection process. Helium was used as the carrier gas, with a post-run total flow of 2 minutes at 25 ml/min. The splitless flow rate was maintained at 1 ml/min. and the constant flow rate was maintained at 1 ml/ min. An Agilent DB5MS capillary column of length 30m, an internal diameter 0.25mm, and a thickness 0.25 microns was used. The total run time was approximately 28 minutes, starting with an injector volume of 1µl. The pressure varied from 7.6522 psi, and the detector scan ranged between 50 and 500 with a 0.5s interval. The split ratio was 100:1, and the split flow was 1 ml/min. The acquired spectra of the chromatogram were crossreferenced with a mass spectral library (National Institute of Standards and Technology (NIST) version 14.0) to identify the eluted chemical compounds in the bee pollen extract using retention time and peak area.

*In silico* molecular docking: Preparation of ligands: The phytochemical constituents, such as Hexadecanoic acid, Pentadecanoic acid,

Pyrroloindole and Alpha amyrin, were selected from the GC-MS chromatogram of the cold ethanolic bee pollen extract. Ro 48-8071 fumarate (an Oxidosqualene cyclase (OSC) inhibitor) was taken as the standard and compared with the binding energy of the phytochemicals. The 2D chemical structures of the chosen phytochemical compounds were retrieved from the PubChem database at the National Center for Biotechnology Information (NCBI) (https://pubchem.ncbi.nlm.nih.gov), and the 3D structure of the ligands was drawn using ChemSketch software. The ligands were prepared by energy minimization and the addition of hydrogen atoms using the ChemSketch software building tool. The 3D structures were saved in Protein Data Bank (PDB) format and prepared for the docking analysis using ChemSketch software. The protein molecular target was obtained from the protein database. The structure of human OSC (1W6J) was chosen for molecular docking studies based on literature reviews.

**Cavity prediction and binding site analysis:** Computed Atlas of Surface Topography of Proteins (CASTP), an online tool, is employed for the precise identification, delineation, and quantitative assessment of the geometric and topological attributes of target protein structures (Binkowski *et al.*, 2003).

**Visualization of target proteins and ligands:** The atomic charges of the amino acid residues were fixed, and energy minimization was carried out. The prepared target protein structures, H-bond, and nonbond interactions of ligands with the active site residues were analyzed using the UCSF Chimera software to obtain high resolution images (Narayanaswamy *et al.*, 2014).

**Docking:** Geometrical optimization of the input compounds was performed using the Arguslab software to obtain a stable structure of the prepared compounds. After preparing the ligand and target protein structures, molecular docking was performed using Autodock 4.0 software. The standard operational protocol for ligand-protein (enzyme) docking was followed (Jemal, 2019).

#### **RESULTS AND DISCUSSION**

The qualitative phytochemical analysis showed presence of various bioactive compounds (viz., acids, anthraquinone, alkaloids, carbohydrates, flavonoids, glycosides, phenol, proteins, quinone, resins, saponin, steroids, tannin and terpenoids) in the bee pollen extract. The results of GCMS with the standards of acquired chromatogram were compared with NIST and diverse compounds were recognized. A total number of 40 volatile phytochemical compounds were noted in the extract by considering retention time, peak number and peak area with percentage (Fig. 1). The initial peak detected was 1, 3, 5 - benzenetriol (1.02 %) at the time of 6.9 minutes. Other compounds such as 4HPyran-4-one, 2, 3 diHydro-3,5 dihydro 6methyl (1.93%), methyl salicylate (1.12%), 4 mercaptophenol (9.89%), pyridine 2 fluro (9.89%), thiophene 2-propyl (9.89%), glucuronolactone (1.14%), 2-heptane isothiocyanate (1.14%), D mannoheptulose (0.71%), hexadecanoic acid (16.5%), pentadecanoic acid (4.34%), alpha amyrin (4.10%), pyrroloindole (4.10%), oleic acid (1.23%), dodecanol (1.23%), taraxa sterol (2.23%), lupeol (2.23%), 9,12,15 octadecatrienoic acid (1.58%), and squalene (0.66%) were also detected. The ultimate peak was ethyl vanillin (0.85%) at the retention time of 26.5 minutes. These compounds (Table 1, 2) are reported for certain biological values (Aragão et al., 2006; Mujeeb et al., 2014; Manjal et al., 2019; Alam et al., 2021).

*In silico* molecular docking: The five selected phytochemical constituents of cold macerated bee pollen extract were selected and their molecular structures were obtained from PubChem Database for chemical compounds at NCBI and the 3D structures were obtained from UC SF chimera (chimera). The selected active compounds of extraction were subjected to molecular *in silico* docking with an Oxidosqualene: Ianosterolcyclase enzyme to show the hypocholesterolemic activity of bee pollen. The protein structures of target enzyme Oxidosqualene: Ianosterolcyclase was obtained from Protein data bank and analysed using CASTP web server for the characterization of



Fig. 1 GCMS Analysis of cold macerated ethanolic extract of bee pollen



Fig. 2 Stick model image of docking of phytocompounds with Oxidosqualene: Ianosterolcyclase enzyme a) hexadecanoic acid, b) Pentadecanoic Acid, c) Pyrroloindole, d) Alpha Amyrin, d) Ro 48-8071 fumarate

No	Compound	Molecularformula	Mol.wt (g/mol)	Retention time (mins)	Peak area (%)
1	Hexadecanoic Acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256.43	18.230	16.50
2	4 Mercaptophenol	C6H6OS	126.18	09.531	9.89
3	Pyridine, two fluro	C5H4FN	097.09	09.531	9.89
4	Thiophene 2-propyl	C7H10S	126.22	09.531	9.89
5	PentadecanoicAcid	C15H30O2	242.40	20.107	4.34
6	Alpha Amyrin	С30Н50О	426.70	21.762	4.10
7	Pyrroloindole	C12H12N2	154.17	21.762	4.10
8	Lupeol	С30Н50О	426.70	23.540	2.23
9	Taraxasterol	С3Н50О	426.70	23.540	2.23
10	4HPyran-4-one, 2, 3diHydro- 3,5 dihydro 6methyl	С6Н8О4	144.12	08.053	1.93
11	9,12,15 octadecatrienoic acid	C18H30O2	278.40	24.642	1.58
12	Oleic Acid	C18H34O2	282.00	23.462	1.23
13	Dodecanol	C20H42O	186.33	23.462	1.23
14	Glucuronolactone	C6H8O6	176.12	13.574	1.14
15	Methyl Salicylate	C8H8O3	152.15	08.842	1.12
16	D Mannoheptulose	C7H14O7	210.18	14.863	0.71
17	Squalene	C30H50	410.70	25.273	0.66

Table 1. GCMS analysis in cold macerated ethanol extract of bee pollen

active sites. The docking results of Oxidosqualene: Ianosterolcyclase and phytocompounds showed a significant interaction between ligand and target enzyme. All the compounds bound to the target enzyme with single hydrogen bond. The hexadecenoic acid interacted with histidine residue and pentadecanoic acid with tryptophan residue, pyrroloindole and alpha amyrin with aspartic acid residue and Ro 48-8071 fumarate with tyrosine residue of the target enzyme with a binding energy of -5.01 Kcal/mol, -6.55 Kcal/mol, -6.00 Kcal/mol, -13.37 Kcal/mol, and-10.21 Kcal/mol, respectively (Table 3, Fig. 2).

A commonly used technique for determining the botanical origin of pollen loads is the microscopic pollen analysis since, the size, shape and surface properties of pollen grains are characteristic to particular plant species (Kieliszek et al., 2018). Pollen is usually marketed after drying, but freezing and lyophilization are also acceptable techniques for preservation (Thakur and Nanda, 2020). Bee pollen is consumed as a food supplement for its varied health benefits. The technique of cold maceration is employed to obtain ethanolic extract of bee pollen and the total yield of cold macerated ethanolic bee pollen extraction was 5 per cent. Pollen extracts have been documented to show hypolipidemic activity, effectively reducing the levels of total lipids, triacylglycerol and cholesterol. Consequently, they exhibit potential effects to address cardiovascular diseases (Polanski et al., 1998). The GCMS analysis exhibits the presence of various compounds with their own retention time,

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No	Name	Nature	Biological activity	
1.	Hexadecanoic Acid	Saturated fatty acids	Anti-inflammatory, Anti-hypoxic Antipruritic, Antithrombotic, Antinociceptive, Antiparasitic	
2.	4 Mercaptophenol	Phenol	Anti-cancer agents against melanoma and breast cancer cell lines	
3.	Pyridine, two fluro	Inositol	Kinase 1 inhibitor, Useful in clinical oncology	
4.	Thiophene 2-propyl	Hetro cyclic	Anti-microbial, Anti-cancer, Anti-inflammatory, Anti- hypertensive, Analgesic	
5.	Pentadecanoic Acid	Saturated fatty Acids	Antibacterial	
6.	Alpha Amyrin	Triterpene	Anti-tumor, anti-inflammatory, anxiolytic	
7.	Pyrroloindole	Amine	Muscle relaxant, antifungal, antitumor, and antibiotic	
8.	Lupeol	Triterpenoid	anti-inflammatory, anti-cancerous, cardioprotective, Anti-Diabetic, skin protective, antimicrobial agents, antiprotozoal agent, nephroprotective agent	
9.	Taraxasterol	Triterpene	inflammatory diseases	
10.	4H- Pyran-4-one, Hydro-3,5 dihydro, 6 methy	Flavonoid	Hyaluronic acid production, Melanin production 2,3 di linhibitor	
11.	9, 12, 15 octadecatrienoic acid	Linolenic Acid	Anti-inflammatory, hypocholesterolemia cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge	
12.	Oleic Acid	Acids	antibacterial activity, antifungal activity	
13.	Dodecanol	Long-chain fatty acids	antibacterial activity	
14.	Glucuronolactone	Glucuronic acid	anti-inflammatory effect for the skin, and lowering abnormally high plasma concentrations of cholesterol	
15.	Methyl Salicylate	Ester	Anti-inflammatory and analgesic agent	
16.	D Mannoheptulose	Heptose	Breast cancer and to suppress the D-glucose induced insulin release	
17.	Squalene	Triterpene	Antioxidant, Antitumor activities	

Table 2. Biological activity of the compounds present in the ethanolic extract of bee pollen

Source: Aragão et al., 2006; Mujeeb et al., 2014; Manjal et al., 2019; Alam et al., 2021

area and concentration. These compounds were reported for their therapeutic efficiency. Hexadecanoic acid (16.5%) (saturated fatty acids) has potential anti-inflammatory, antiallopactic and antioxidant properties (Cupido *et al.*, 2022). Mercaptophenol known for its anti-cancer properties against melanoma and breast cancer cell lines (Ruzza *et al.*, 2009; Shpakovsky *et al.*, 2014). Pyridine 2 fluoro (9.89%) is used as Kinase 1 inhibitor and in clinical oncology (Laha Roy *et al.*,

No	Compounds	Binding energy (Kcal/mol)	Amino acid interaction residues
1.	Hexadecanoic acid	-5.01	1W6J:A:HIS232:ND2—O:UNL1
2.	Pentadecanoic Acid	-6.55	1W6J:A:TRP581:O—O:UNL1
3.	Pyrroloindole	-6.00	1W6J:A:ASP455:OD2—O:UNL1
4.	Alpha Amyrin	-13.37	1W6J:A:ASP455:OD2—H:UNL1
5.	Ro 48-8071 fumarate	-10.21	1W6J:A:TYR704:OH——C:UNL1

Table 3. In silico molecular docking analysis in the cold macerated extract of bee pollen

Note: No. of Hydrogen is one in all compound bonds

2018). Thiophene derivatives show antimicrobial (Tehranchian *et al.*, 2005), analgesic and antiinflammatory (Pillai *et al.*, 2005), antihypertensive (Russell *et al.*, 1988), and antitumor activity (Chen *et al.*, 2015).

In animals, the OSC enzyme is primarily produced and catalyzed with the help of lanosterol, which is involved in the cholesterol biosynthesis pathway. Cholesterol is crucial for body temperature regulation and is a precursor for testosterone in males and oestradiol in females (Liang et al., 2014). In this study, the docking results showed that the active compounds in the ethanolic extract of bee pollen strongly bound with the target OSC enzyme. The binding interaction between alpha amyrin and OSC enzyme was comparatively higher than other compounds, including standard drugs. Alpha amyrin is one of the effective compounds with antioxidant, anti-inflammatory and hypoglycemic properties (Gunnam and Nangia, 2019), and the binding energy of the alpha amyrin is high (-13). Ro 48-8071 fumarate acts as an inhibitor of OSC with IC<sub>50</sub> of approximately 6.5nM (Mallick and Dighe, 2014). Ro 48-8071 fumarate has a binding energy of -10.21 which is lower than alpha amyrin. Ro 48-8071 fumarate is a 2, 3-Oxidosqualene cyclase (OSC) inhibitor (IC<sub>50</sub> = 6.5 nM); blocks cholesterol synthesis in HepG2 cells (Morand et al., 1997). Since it can withstand the enzyme for a longer duration of time, all these four compounds are present in high percentage in the bee pollen ethanolic extract. These compounds significantly interact with the OSC enzyme and serve the enzyme inhibitory action. Targeting the OSC enzyme to reduce cholesterol is an alternative way to discover a new hypercholesteroemic drug. This *in silico* molecular docking study showed the efficiency of compounds present in the bee pollen extract in inhibiting cholesterol biosynthesis through OSC inhibition.

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# A checklist of hover flies (Diptera, Syrphidae) of Kerala, India

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**ABSTRACT:** A checklist of the hover flies from Kerala belonging to two subfamilies is provided. Among the 59 species listed, the subfamily Syrphinae shares maximum number of species (34), followed by the subfamily Eristalinae (25). © 2024 Association for Advancement of Entomology

KEY WORDS: Flower flies, Syrphinae, Eristalinae, species

### **INTRODUCTION**

The family Syrphidae, commonly called hover flies or flower flies is regarded as the most anthophilous flies in the order Diptera (Larson et al., 2001). Hover flies have recently gained research interest because of their importance as both pollinators and biological control agents (Lucas et al., 2018). They are significant pollinators as adults, while their larvae are effective predators of many pests (Mitra et al., 2008). Most of the adults, mimic bees or wasps and are brightly coloured, may be striped, banded or spotted. India has 357 different species of hover flies (Ghorpadé, 2014; Mitra et al., 2015), out of 6,107 species under 209 genera reported from the world (Evenhuis and Pape, 2023). The first elaborated work on Indian Syrphidae was done by Brunetti (1923). The south Indian fauna of Syrphidae became enriched by Fabricius (1805), Wiedemann (1819), Macruat (1842), Bigot (1883), Brunetti (1908, 1915, 1923), Knutson et al. (1975)

and Joseph and Parui (1986). Datta and Chakraborti (1986) reported 45 species of hover flies belonging to 21 genera based on the collections (1970-1981) accumulated in the Zoological Survey of India from south India.

Recent studies on Indian Syrphidae include the checklist prepared by Ghorpadé (2014) in which 357 species under 14 tribes of three subfamilies are reported. A review of the hover flies from India by Mitra *et al.* (2015) reported 357 species of Syrphidae from India. In the book, Faunal Diversity of Biogeographic Zones of India, Banerjee *et al.* (2020) published a book chapter on the Diptera fauna of the Western Ghats biogeographic area, which reported 35 species of Syrphidae from Kerala. Sankararaman *et al.* (2022) described two new species of Syrphidae with a review of the Indian species of *Monoceromyia* Shannon. The present work is the first attempt to provide a checklist of hover flies in Kerala.

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# **MATERIALS AND METHODS**

For the preparation of the checklist, a thorough examination of published articles, books, catalogues, checklists, and other sources containing information on hover flies' distribution records was conducted. This checklist is wholly based on a literature review; no specimens were collected or studied for the same. All nomenclature and classification have been updated as per Systema Dipterorum (Evenhuis and Pape, 2023).

# **RESULTS AND DISCUSSION**

According to the current literature survey, 59 species of hover flies have been reported from Kerala to date. All species and their distribution within the state are provided with the reference.

**Subfamily Eristalinae:** The larvae of Eristalinae are mainly saprophagous and some are phytophagous. Eristalinae is the only polyphyletic subfamily under the family Syrphidae (Mengual *et al.*, 2015). Out of the 357 species reported from India, 197 belong to the subfamily Eristalinae (Sengupta *et al.*, 2016).

#### **Tribe Ceriodini**

#### Genus Monoceromyia Shannon, 1922

The members are wasp mimics and the genus can be distinguished by its scape that is at least as long as frontal prominence and with an incomplete postmetacoxal bridge. The petiole of the abdomen has short basal segments and is elliptical in shape. Antennae are elongated.

1. Monoceromyia javana (Wiedemann, 1824)

Source: Sankararaman *et al.* (2022) Distribution: Pathanamthitta

 Monoceromyia tredecimpunctata (Brunetti, 1923)
 Source: Mitra *et al.* (2008)
 Distribution: Kerala

**Remarks:** According to Mitra *et al.* (2008), *M. tredecimpunctata* is widely distributed in India

including the states of Assam, Karnataka, Kerala, Meghalaya, Sikkim, Tamil Nadu, Tripura, and West Bengal. But the subsequent Indian checklist by Ghorpadé (2014), Mitra *et al.* (2015), and Sengupta *et al.* (2016) supports that distribution of *M. tredecimpunctata* is restricted to north Indian states. So, the record from Kerala by Mitra *et al.* (2008) is suspicious.

#### **Tribe Eristalini**

#### Genus Eristalinus Rondani, 1845

Head is board as thorax. Wings with closed marginal cells and  $3^{rd}$  vein ( $R_{4+5}$ ) evidently looped downward into the first posterior cell ( $R_5$ ). Yellow to orange coloured abdomen and yellowish-brown scutellum.

3. Eristalinus arvorum (Fabricius, 1787) Subgenus: Eristalinus Source: Joseph and Parui (1986), Mathew (2004)
Distribution: Silent Valley National Park

(Palakkad)

- 4. Eristalinus aurulans (Wiedemann, 1824) Subgenus: Eristalinus Source: Brunetti (1915) Ghorpadé (2014, 2019) Mitra et al. (2015) Sengupta et al. (2016) Distribution: Travancore
- Eristalinus megacephalus (Rossi, 1794) Subgenus: Eristalinus Source: Datta and Chakraborti (1986), Shah et al. (2014), (Ghorpadé, 2019) Distribution: Chalakudy (Thrissur), Parambikulam (Palakkad)
- *Eristalinus obliquus* (Wiedemann, 1824)
   Subgenus: *Eristalinus* Source: Ghorpadé (2019)
   Distribution: Kerala
- 7. Eristalinus quinquestriatus (Fabricius 1794)

Subgenus: Eristalinus

Source: Datta and Chakraborti (1986), Mukherjee et al. (2006), Mitra et al. (2008), Shah et al. (2014), Sengupta *et al.* (2016) Distribution: Kumily (Idukki)

8. Eristalinus tristriatus (Meijere, 1911) Subgenus: Eristalinus

Source: Datta and Chakraborti (1986), Mitra *et al.* (2008, 2015), Ghorpadé (2014, 2019)

Distribution: Konnakuzhy, Chalakudy (Thrissur)

# Genus Phytomia Guerin-Meneville, 1833

Eyes are bare, with comparatively shorter antennae. Densely pubescent and thick obconical abdomen. Loop of third vein with short appendix, comparatively shorter and week legs.

9. Phytomia argyrocephala (Macquart, 1842)

Subgenus: Phytomia

Source: Brunetti (1923), Ghorpadé (2014, 2019), Mitra *et al.* (2015) Sengupta *et al.* (2016)

Distribution: Travancore, Nilgiri hills

10. Phytomia crassa (Fabricius, 1787)

Subgenus: Dolichomerus

Source: Datta and Chakraborti (1986), Joseph and Parui (1986), Brunetti (1923), Mathew (2004), Mitra *et al.* (2008), Ghorpadé (2014, 2019), Shah *et al.* (2014) Distribution: Idamalayar (Ernakulum), Silent

Valley National Park, Parambikulam (Palakkad), Madathara (Kollam)

11. Phytomia errans (Fabricius, 1787)

Subgenus: Phytomia

Source: Brunetti (1915, 1923), Datta and Chakraborti (1986), Mathew (2004) Mukherjee *et al.* (2006), Mitra *et al.* (2008, 2015), Ghorpadé (2014, 2019), Shah *et al.* (2014)

Distribution: Idamalayar (Ernakulum),

Parambikulam (Palakkad), Kumily (Idukki), Thiruvananthapuram

12. Phytomia zonata (Fabricius, 1787)

Subgenus: *Phytomia* Source: Brunetti (1923) Datta and Chakraborti (1986), Mukherjee *et al.* (2006), Mitra *et al.* (2008, 2015), Ghorpadé (2014, 2019) Distribution: Valiyaparathodu (Palakkad),

Nilgiri hills

# Tribe Merodontini

#### Genus Eumerus Meigen, 1822

The head is broader than the thorax. Slightly arched and sub quadrate thorax. Wings with widely opened marginal cells and closed 1<sup>st</sup> posterior cell. Long abdomen, nearly always having three pairs of pale lunules.

- 13. Eumerus figurans Walker, 1859
  Source: Sandhya et al. (2016), Ghorpadé (2019)
  Distribution: Thrissur, Palakkad
- 14. Eumerus nicobarensis Schiner, 1868Source: Brunetti (1923)Distribution: Palode (Thiruvananthapuram)

### Genus Psilota Meigen, 1822

The head with pilose eyes and face without tubercles. Slightly concave or straight face with an anteriorly projecting lower facial margin. The wings have a straight  $R_{4+5}$  vein and an oblique  $M_1$  vein.

15. Psilota shewelli Thompson, 2012Source: Thompson (2012)Distribution: Vellayani (Thiruvananthapuram)

#### **Tribe Milesiini**

#### Genus Milesia Latreille, 1804

*Milesia* is one of the diverse genera of hover flies. More than eighty species in this genus mimic social wasps. They have a hairy post-pronotal lobe, and their eyes and arista are bare. Wings with straight third vein and oblique cross vein within apical half of discal cell. Presence of pale pubescence on the abdomen.

- 16. Milesia caesarea Hippa, 1990
  Source: Hippa (1990), Ghorpadé (2014, 2019), Mitra et al. (2015)
  Distribution: Ponmudi range (Thiruvananthapuram), Anamalai hills
- 17. Milesia cinnamomea Hippa, 1990
  Source: Hippa (1990), Ghorpadé (2014, 2019), Mitra et al. (2015)
  Distribution: Chembra peak (Wayanad), Ponmudi range (Thiruvananthapuram)
- 18. Milesia mima Hippa, 1990
  Source: Hippa (1990), Ghorpadé (2014, 2019), Mitra et al. (2015)
  Distribution: Ponmudi range

(Thiruvananthapuram), Anamalai hills

19. Milesia sexmaculata Brunetti, 1915

Source: Brunetti (1915, 1923), Hippa (1990) Mathew (2004), Ghorpadé (2014, 2019), Mitra *et al.* (2015), Sengupta *et al.* (2016) Distribution: Thiruvananthapuram

# Genus *Syritta* Le Peletier and Audinet-Serville, 1828

Pale triangular spots present behind the head. Metasternum without hairs and patch of fine hairs on metepisternum. Separate dorsal and ventral hair patches are present on Katepisternum. Greatly enlarged hind femur and apical third with an anteroventral spinose ridge.

20. Syritta indica (Wiedemann, 1824)

Source: Datta and Chakraborti (1986), Van Steenis (2010), Ghorpadé (2014, 2019), Shah *et al.* (2014), Mitra *et al.* (2015) Distribution: Chalakudy (Thrissur), Ponmudi range (Thiruvananthapuram)

21. Syritta orientalis Macquart, 1842 Source: Mathew *et al.* (1987), Mathew (2004), Distribution: Nilambur (Malappuram), Peechi (Thrissur)

- 22. Syritta proximata Lyneborg and Barkemeyer, 2005Source: Ghorpadé (2014, 2019)Distribution: Anamalai hills
- 23. Syritta stylata Lyneborg and Barkemeyer, 2005
  Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016)
  Distribution: Chembra peak (Wayanad)

## Genus Xylota Meigen, 1822

Head elliptical in anterior view and marginally wider than thorax. Elongate abdomen. Wings with anterior cross vein situated beyond middle of discal cell. Hind femur thickened and serrated below.

24. Xylota bistriata Brunetti, 1915
Source: Brunetti (1915, 1923), Mathew (2004), (Ghorpadé, 2014, 2019), Mitra et al. (2015), Sengupta et al. (2016)
Distribution: Parambikulam (Palakkad)

# **Tribe Volucellini**

### Genus Graptomyza Wiedemann, 1820

*Graptomyza* is the only genus of Syrphidae having wings without the "Spurious vein." The head is broader than the thorax. Mouth having elongated and thin proboscis. Antennae have short 1<sup>st</sup> and 2<sup>nd</sup> segments, whereas the 3<sup>rd</sup> joint is very elongated.

25. Graptomyza brevirostris Wiedemann, 1820
Source: Brunetti (1923), Ghorpadé, (2019)
Distribution: Thaliparamba (Kannur),

Erattupetta (Kottayam)

### **Subfamily Syrphinae**

The larvae of the subfamily Syrphinae are mainly predacious that prey on soft-bodied insects, mostly Hemiptera (Rojo *et al.*, 2003). The group contains more than 1,600 species worldwide (Evenhuis and

Pape, 2023) and is monophyletic (Mengual *et al.*, 2015). Out of the 357 species reported from India, 143 belong to the subfamily Syrphinae (Sengupta *et al.*, 2016).

#### **Tribe Bacchini**

### Genus Melanostoma Schiner, 1860

Face and Scutellum completely black in background colour. Microscopic pubescence present on anterior anepisternum. Un-marginated abdomen with bare eyes and metasternum. Slender legs without bristles, hair tufts, or modified hair in males.

26. Melanostoma orientale (Wiedemann, 1824)

Source: Joseph and Parui (1986), Ghorpadé (2019)

Distribution: Silent Valley National Park (Palakkad)

27. Melanostoma univittatum (Wiedemann, 1824)

Source: Brunetti (1915, 1923), Datta and Chakraborti (1986), Joseph and Parui (1986), Mathew (2004) Mitra *et al.* (2008, 2015), Ghorpadé (2014, 2019), Shah *et al.* (2014)

Distribution: Kumily (Idukki), Eluppara (Kottayam), Silent Valley National Park (Palakkad), Nedumangad (Thiruvananthapuram)

#### **Tribe Paragini**

#### Genus Paragus Latreille, 1804

They are small sized hover flies with length 7.5 mm or less. Distinctly haired eyes with vertical bands of contrasting colour. Unmarked wings except for stigmal darkening. Metasternum bare, well developed tergite I.

28. Paragus crenulatus Thomson, 1869 Subgenus: Paragus

Source: Thompson and Ghorpadé (1992) Ghorpadé (2014, 2019), Mitra *et al.* (2015), Sengupta *et al.* (2016) Distribution: Chalakudy (Thrissur), Idamalayar (Ernakulum), Parambikulam, Walayar forest (Palakkad)

- 29. Paragus politus Wiedemann, 1830
  Subgenus: Pandasyopthalmus
  Source: Brunetti (1908, 1915, 1923), Datta and Chakraborti (1986), Mathew (2004), Shah et al. (2014)
  Distribution: Tenmalai (Kollam), Chalakudy (Thrissur)
- 30. Paragus rufocinctus (Brunetti, 1908)
  Subgenus: Pandasyopthalmus
  Source: Datta and Chakraborti, 1986),
  Thompson and Ghorpadé (1992) Ghorpadé (2014, 2019), Mitra et al. (2015)
  Distribution: Chalakudy (Thrissur), Meppadi (Wayanad), Kaikatty (Palakkad)
- 31. Paragus serratus (Fabricius, 1805)
  Subgenus: Paragus
  Source: Datta and Chakraborti (1986),
  Joseph and Parui (1986), Brunetti (1923),
  Mathew (2004), Mitra et al. (2008),
  Mukherjee et al. (2006), Shah et al. (2014),
  Sengupta et al. (2019)
  Distribution: Chalakudy (Thrissur),
  Parambikulam (Palakkad), Silent Valley
  National Park (Palakkad), Travancore,
  Ernakulum
- 32. Paragus tibialis (Fallén, 1817)
  Subgenus: Pandasyopthalmus
  Source: Mitra et al. (2008), Mukherjee et al. (2006), Shah et al. (2014)
  Distribution: Kerala
- 33. Paragus yerburiensis Stuckenberg, 1954
  Subgenus: Paragus
  Source: Thompson and Ghorpadé (1992),
  Ghorpadé (2014, 2019), Shah et al. (2014),
  Mitra et al. (2015), Sengupta et al. (2016)
  Distribution: Walayar forest (Palakkad)

**Tribe Syrphini** 

Genus Allobaccha Curran, 1928

Many species of this genus have an extended abdomen that mimics wasps. Head with bare eyes. Thorax with pilose post-pronotum and incomplete post-metacoxal bridge. Abdomen petiolate in shape.

- 34. Allobaccha amphithoe (Walker, 1849)
  Subgenus: Allobaccha
  Source: Ghorpadé (2014, 2019), Shah et al. (2014), Mitra et al. (2015), Sengupta et al. (2016), Sengupta et al. (2019)
  Distribution: Kerala
- 35. Allobaccha apicalis (Loew, 1858) Subgenus: Allobaccha

Source: Ghorpadé (2014, 2019), Shah *et al.* (2014), Mitra *et al.* (2015), Sengupta *et al.* (2016)

Distribution: Nilgiri hills

36. Allobaccha oldroydi Ghorpadé, 1994
Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016)
Distribution: Nedumkayam (Malappuram)

### Genus Allograpta Osten Sacken, 1875

The genus exhibits variations in morphological characters, including colour pattern and shape of the head. Yellow-faced head with or without a medial black vitta. Oval to slightly elongate basoflagellomere. Thorax with at least partially yellow scutellum. Moderately dense and complete subscutellar fringe. Hypopleuron usually bare.

37. Allograpta javana (Wiedemann, 1824)

Subgenus: Allograpta

Source: Ghorpadé (2014, 2019), Shah *et al.* (2014), Mitra et al. (2015) Sengupta *et al.* (2016)

Distribution: Kerala

38. Allograpta maculipleura (Brunetti, 1913)
Subgenus: Allograpta
Source: Ghorpadé (2014, 2019), Mitra et al.

(2015), Sengupta *et al.* (2016)

Distribution: Munnar (Idukki)

## Genus Asarkina Macquart, 1842

The humerus and latero-tergites are completely bare. A noticeable face bump is present. Abdomen flat and broader. Widened sub marginal cell at middle of the wing. Wings completely hyaline or differently, variably, darkened.

- 39. Asarkina ayyari Ghorpadé, 1994
  Source: Ghorpadé (2014, 2019) Mitra et al. (2015) Sengupta et al. (2016)
  Distribution: Meppadi (Wayanad), Malabar
- 40. Asarkina ericetorum (Fabricius, 1781) Subgenus: Asarkina Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016), Sengupta et al. (2019) Distribution: Marayur (Idukki), Kaikatty (Palakkad), Pathanamthitta
- 41. Asarkina hema Ghorpadé, 1994
  Source: Ghorpadé (2014, 2019) Mitra et al. (2015) Sengupta et al. (2016)
  Distribution: Thekkady (Idukki), Walayar forest (Palakkad)
- 42. Asarkina pitambara Ghorpadé, 1994Source: Ghorpadé (2019)Distribution: Taliparamba (Kannur)

# Genus Asiobaccha Violovich, 1976

Head with bare eyes. Thorax with microtrichose anatergum, pilous anterior an-episternum, and metaepisternum. The post-metacoxal bridge is incomplete. Sclerotized black dots on the posterior wing margin. The petiolate abdomen without an abdominal margin.

43. Asiobaccha nubilipennis (Austen, 1893)
Source: Brunetti (1923), Mengual (2016), Mathew (2004), Ghorpadé (2014, 2019) Mitra et al. (2015) Sengupta et al. (2016)

Distribution: Thiruvananthapuram

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#### Genus Betasyrphus Matsumura, 1917

Densely haired eyes. Densely and uniformly trichose wing membrane at least beyond level of end of false vein or spurious vein. Eyes of male without evident demarked area of larger facets above. Tergum 2 with narrow yellow or grey fascia which may be interrupted in centre in some specimens.

- 44. Betasyrphus fletcheri Ghorpadé,1994
  Source: Ghorpadé (2014, 2019) Mitra et al. (2015) Sengupta et al. (2016)
  Distribution: Mananthavady (Wayanad), Kaikatty (Palakkad), Munnar (Idukki)
- 45. Betasyrphus linga Ghorpadé, 1994
  Source: Ghorpadé (2014, 2019) Mitra et al. (2015) Sengupta et al, (2016)
  Distribution: Kerala

#### Genus Citrogramma Vockeroth, 1969

Posteriorly joined sterno-pleural patches of hair. Bright yellow coloured lateral mesonotal margin. Presence of bright yellow areas within pleuron. Scutellum simple with dark sub-scutellar fringe. Pilose metasternum with some long hairs.

46. Citrogramma chola Ghorpadé, 1994 Source: Mengual (2012)

Distribution: Munnar (Idukki), Kaikatty (Palakkad)

47. Citrogramma flavigena Wyatt, 1991
Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016)
Distribution: Munnar (Idukki)

**Remarks:** According to Ghorpadé (2019), *Citrogramma chola* is treated as a junior synonym of *Citrogramma flavigenum*. But following the classification scheme of Systema Dipterorum (Evenhuis and Pape, 2023) both are considered separate valid species.

### Genus Dasysyrphus Enderlein, 1938

Partially bare wings. Eyes with yellow maculae.

Distinctly marginated abdomen. Tergum 2 black in colour with yellow spots. Entirely trichose wing membrane. Presence of undivided or medially divided yellow bands of tergites.

48. Dasysyrphus rossi Ghorpadé, 1994
Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016)
Distribution: Munnar (Idukki)

#### Genus Dideopsis Matsumura, 1917

This genus is distinguished by a unique banding pattern on the wing that is typically characterised by the presence of a dark median brown band. Apical third of wing is hyaline. Bare hypopleuron.

49. Dideopsis aegrota (Fabricius, 1805)

Source: Datta and Chakraborti (1986), Mukherjee *et al.* (2006), Ghorpadé (2014, 2019), Shah *et al.* (2014), Mitra *et al.* (2015), Sengupta *et al.* (2016)

Distribution: Idamalayar (Ernakulum), Konnakuzhi, Chalakudy (Thrissur)

# Genus *Episyrphus* Matsumura and Adachi, 1917

Larvae are predators that often feed on aphids. Metasternum pilose and haired. Short stout tooth apically within the superior lobe of male genitalia. Abdomen petiolate to sub-oval shaped.

50. Episyrphus balteatus (De Geer 1776) Subgenus: Episyrphus

Source: Datta and Chakraborti, 1986), Mukherjee *et al.* (2006), Mitra *et al.* (2008), Shah *et al.* (2014)

Distribution: Vazhachal (Thrissur), Parambikulam (Palakkad), Anamalai Hills

51. Episyrphus viridaureus (Wiedemann, 1824) Subgenus: Episyrphus
Source: Ghorpadé (2014, 2019), Shah et al. (2014), Mitra et al. (2015), Sengupta et al. (2016)

Distribution: Nelliyampathy hills (Palakkad)

#### Genus Eosphaerophoria Frey, 1946

Small-sized hoverflies with lengths ranging from 4.9 mm to 6.8 mm. Head with bare eyes and slightly broadened face. The short antenna with a length less than the width of the head. Oval to slightly elongate baso-flagellomere. Thorax with black Scutum having narrow yellow postsutural stripe.

52. Eosphaerophoria dentiscutellata (Keiser, 1958)Source: Mengual (2013)Distribution: Anamalai hills

#### Genus Ischiodon Sack, 1913

A slender tooth is located on the underside of the hind trochanters in both sexes. Bright yellow lateral mesonotal margin and posteriorly separated sternopleural hair patches. The abdomen is dorsally slightly convex or flattened.

53. Ischiodon scutellaris (Fabricius, 1805)
Source: Mitra *et al.* (2008, 2015), Ghorpadé (2014, 2019), Shah *et al.* (2014) Sengupta *et al.* (2016), Sengupta *et al.* (2019)
Distribution: Kerala

#### Genus Meliscaeva Frey, 1946

Eyes, metasternum, and metapisternum are all bare, and the anterior anepisternum is usually pilose. Presence of minute series of closely spaced black maculae on the posterior wing margin. Oval to parallel sided abdomen. Terga pale yellow in colour.

54. Meliscaeva mathisi Ghorpadé, 1994
Source: Ghorpadé (2014, 2019), Mitra et al. (2015)
Distribution Kanda

Distribution: Kerala

### Genus Rhinobaccha Meijere, 1908

The scutellum has a curved posterior margin and a normal subscutellum. No facial tubercle. Wings anal lobe reduced . Lower face is strongly produced into the snout.

55. Rhinobaccha krishna Ghorpadé, 1994 Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016) Distribution: Munnar (Idukki)

56. Rhinobaccha peterseni Ghorpadé, 1994Source: Ghorpadé (2014), Mitra et al. (2015), Sengupta et al. (2016)Distribution: Kerala

# Genus *Sphaerophoria* Lepeletier and Serville, 1828

They are smaller but more slender species. Males have a 5-segmented abdomen plus the genitalia, while females have a seven- to 8-segmented abdomen. The anterior cross vein always appears before the middle of the discal cell, and the third longitudinal vein is usually straight.

- 57. Sphaerophoria indiana Bigot, 1884
  Subgenus: Sphaerophoria
  Source: Datta and Chakraborti (1986), Mukherjee et al. (2006), Mitra et al. (2008), Shah et al. (2014), Sengupta et al. (2018)
  Distribution: Eluppara (Kottayam)
- 58. Sphaerophoria knutsoni Ghorpadé, 1994
  Source: Ghorpadé (2014, 2019), Mitra et al. (2015)
  Distribution: Nilgiri hills
- 59. Sphaerophoria macrogaster (Thomson, 1869)
  Subgenus: Sphaerophoria
  Source: Ghorpadé (2014, 2019), Mitra et al. (2015), Sengupta et al. (2016)
  Distribution: Kerala

According to literature, 59 hover flies of the subfamilies Syrphinae and Eristalinae are reported from Kerala. The distributional records of the species within Kerala are biased because many of the reports come from single records. The literature survey also shows no extensive field surveys were conducted in Kerala exclusively for Syrphidae. Some hover flies are also reported from the field surveys at Shendurney Wildlife Sanctuary, Kollam (Mathew *et al.*, 2004a), Peechi-Vazhani Wildlife

Sanctuary, Thrissur (Mathew *et al.*, 2005) and Peppara Wildlife Sanctuary, Thiruvanthapuram (Mathew *et al.*, 2004b), but they are only identified up to family level. The unidentified Syrphidae reports also show the insufficiency of Syrphidae taxonomists in Kerala. Further extensive field surveys are needed to reveal a clearer picture of the distributional record of the reported species within the state.

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# Diet and body size modulate the remating behaviour of a predaceous ladybird, *Coccinella transversalis* (Fabricius) (Coleoptera, Coccinellidae)

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**ABSTRACT:** The nutritional condition and body size influence the mating and female remating behaviour of a predaceous ladybird, *Coccinella transversalis*. When well-fed males were provided with females of three different dietary conditions, *viz*. (i) well-fed, (ii) food-deprived and (iii) honey-fed, the well-fed ones were most fecund with highest percentage of egg-viability and least preoviposition period and remating refusals, while food-deprived ones showed vice-versa. However, honey-fed females laid unfertile eggs after coercively mating with males, and resisted the most to remate, which gets strengthened in the second mating trial. This indicates females' nutritional condition modulates the females' mating behaviour and post-mating outcomes. The adult body size was directly proportional to reproductive output with heavier females showing high fecundity and percentage of egg viability with least preoviposition period than the lighter ones. Large females resisted the least to remate with larger males than with smaller males, while large males coercively mating trial. Both diet and adult body size modulate the re-mating behaviour of female *C. transversalis*, as the food-deprived and large females greatly resisted to re-mate with smaller males. © 2024 Association for Advancement of Entomology

KEY WORDS: Reproduction, dietary conditions, fecundity, egg viability, aphidophagous

#### **INTRODUCTION**

Food gives direction to sexual selection by affecting sexual development, adult phenotype, and reproduction during the early development stage (Richardson and Smiseth, 2019). Both quality and quantity of food affect the growth, development and reproduction, at individual, species, and interspecific levels (Yuan *et al.*, 2020). Stressful food conditions may allow adult survival with hampered reproduction (Dmitriew and Rowe, 2007), while an enriched early diet may lead to quantitative and fitter progeny (Li *et al.*, 2020). Mating is associated with high energy consumption, where males expend energy in mate-search (Evans, 2003) and ejaculate production (Shandilya *et al.*, 2021), while females in egg production (Perry, 2011). Thereby, the females can modify their nutrition acquisition as per the energy demand (Camus *et al.*, 2018), sometimes by modulating the mating

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duration during nuptial feeding (Monalisa *et al.*, 2020) or by increasing forage that enhances their nutritional state to increase fecundity (Fox and Moya-larano, 2009). As reproduction is affected by the dietary state during adult development, the food-limited environment can reduce feeding potential that directly reduces offspring production with mate choice and remating frequency (Auer *et al.*, 2010).

It is widely held that body size influences the reproductive success of predaceous ladybirds (Pervez and Singh, 2013; Singh et al., 2021). It indicates individual fitness (Beukeboom, 2018), as larger adults have higher fitness levels (Singh et al., 2021). Larger body size also supports sexual coercion (Wallen et al., 2016). The female ladybirds mate more though the behavioural resistance towards remating is also prevalent (Obata, 1988; Perry et al., 2009; Pervez et al., 2022). This female reluctance to remating could be associated with heavy costs, like reduced foraging opportunities (Perry and Rowe, 2015), risk of physical damage (Ronn et al., 2007), elevated mortality (Ronn et al., 2007; Perry and Rowe, 2015), and increased risk of sexually transmitted diseases (Fiedler and Nedved, 2019). There are also benefits, like improved offspring fitness when potential mates are of high quality (Perry et al., 2009), however this female choosiness may create sexual conflict (Burke et al., 2021).

Coccinella transversalis (Fabricius) is an aphidophagous ladybird (Coleoptera, Coccinellidae) of the Oriental region with a wide prey range (Omkar and James, 2004) and biocontrol prospects (Michaud et al., 2013). It suppresses the population of aphid, Hysteroneura setariae (Thomas) (Pervez and Sharma, 2021), and can survive on non-aphid foods during aphid scarcity (Maurice et al., 2011). During aphid scarcity, females have pressure to optimally forage (Kindlmann and Dixon, 1993) and males to search for potential mates (Dixon, 2000), which probably depends on bodysize. Considering the wide distribution and biocontrol potential of C. transversalis, we investigated the influence of dietary conditions and body size on the females' remating and reproductive behaviour.

### **MATERIALS AND METHODS**

#### Stock culture

Adults of C. transversalis were collected from the agricultural fields near the suburbs of Kashipur, India (29.2104° N; 78.9619° E) and brought to the laboratory. They were paired in separate Petri dishes (2.0cm x 9.0cm) containing ad libitum quantity of aphids, Aphis craccivora (Koch) infested on cowpea, Lablab purpureus (L.) twigs. These Petri dishes were then kept in an Environmental Test Chamber (Remi, Remi Instruments) maintained (at 25±1°C; 65±5 % R.H.; 12L: 12D photoperiod). The adults mated and the females laid fertile eggs, which were reared from egg-hatch till adult emergence on the above diet, and F<sub>1</sub> virgin adults thus obtained were sexually identified by carefully examining their genitalia under Stereoscopic trinocular (Lyzer) and were isolated. These F, adults were used in the experiment carried out from 10:00 to 18:00 hours.

# (i) Effect of nutritional conditions on female re-mating resistance

To find out the effect of nutritional condition on female resistance towards first and second mating, one hundred individuals of C. transversalis were reared from egg-hatch to adult-emergence on a sufficient quantity of A. craccivora in hundred Petri dishes (size and prey as above; one larva per Petri dish). After emergence, the adult ladybirds were sexed by carefully examining the genitalia under a spectroscopic trinocular. The newly emerged adult males continued on the same diet. However, the newly emerged females were split into three different groups, viz., (i) excess aphid diet (Foodsatiated females), (ii) one-tenth of aphid diet (Fooddeprived females) and (iii) non-aphid sufficient quantity of honey-diet for the next five days (Honeyfed females). It is known that adult males and females become sexually mature in less than five days after emergence (Pervez et al., 2022). Five day-old females were paired with respective 5-dayold males fed ad libitum aphids in ten replicates (n=10) in dietary statuses. The entire behavioural activities including mating refusal incidences, the time required to commence mating, latent period

(*i.e.*, duration between the establishment of genital contact and first mating bout), number of bouts, and mating duration, were observed using stereoscopic trinocular at 40X and 100X magnifications with computer attachment. After the mating was terminated, the adult male and female ladybirds were taken out and kept in different Petri dishes (size and food, as above). These were again paired on the next day to record the same parameters in the second mating trial. Thereafter, the females were isolated in Petri dishes (size and food, as above) and monitored for oviposition for the next five days to record their pre-oviposition period, fecundity, and percent egg viability.

#### (ii) Effect of adult body size of both sexes on the female re-mating resistance

To find out the effect of small and large adult body size on the mating behaviour and refusals, the adult males and females were isolated in the Petri dishes (size as above) containing ad libitum A. craccivora infested on the twigs of L. purpureus immediately after emergence. Thereafter, these adults were weighed using an electronic balance (SHIMADZU, Model ATX-224 at 0.1mg precision) and segregated into two categories (small and big) in accordance with their body size (*i.e.* large female  $\sim 27.0$ -28.0mg, small female  $\sim 17.0$  - 18.0mg, large male  $\sim 20.0 - 21.0$ mg, small male  $\sim 15.0 - 16.0$ mg). After 5 days post-emergence, these adults were grouped into four mating-pair groups, viz. (i) large male  $\times$  large female, (ii) large male  $\times$  small female, (iii) small male × small female, and (iv) small male  $\times$  large female, and were allowed to mate. The mating behaviour was observed using stereoscopic trinocular, as above and the time of mating commencement, latent period, bouts in copula, mating duration, and steps taken by the adult females showing mating refusals were recorded (space and food as above). The mating pair was isolated if mating did not commence and they were again re-paired at 10:00h on the next day until the mating commenced. After mating was terminated, the adult male and female ladybirds were isolated and again paired on the next day to record the same parameters in the second mating trial. After the two mating trials, the females were isolated and observed for the next five days to record the preoviposition period, fecundity, and percent egg viability. The experiment was replicated ten times.

The data of both experiments were subjected to the Kolmogorov–Smirnov test for the normality distribution check and Bartlett's test for the homogeneity of variances using statistical software (SAS 9.0, 2002). The data on mating refusals, mating commencement duration, latent period, number of bouts, mating duration, post-oviposition period, fecundity, and percent egg-viability were subjected to one-way ANOVA and means were compared using Tukey HSD on SAS 9.0 (2002). All the studied mating parameters were further subjected to two-sample t-test using SAS 9.0 (2002) to determine the effect of first and second mating on them.

### **RESULTS AND DISCUSSION**

# (i) Effect of nutritional conditions on female re-mating resistance

The nutritional condition during mating significantly affects the female remating behaviour, as the first and second mating commenced earlier by a foodsatiated female than food-deprived and honey-fed females with fewer mating refusals (Fig. 1). The honey-fed females mated coercively and longer in first and second trials than satiated and fooddeprived females (Table 1). Latent periods varied significantly in both first and second mating trials. Similarly, bouts also varied significantly in first and second trials. The males forcefully tried to mount on food-deprived females and latter responded by frequently bending their abdomens downwards and dislodging the males, thereby displaying refusals. These males moved away after a few attempts (5 -10) and thereafter showed no interest in mating. Food-satiated females exhibited significantly the least number of mating refusals  $(0.9\pm1.10)$ compared to food-deprived  $(2.1\pm1.37)$  and honeyfed (2.2±1.14) females during the first trial. The mating refusals by food-satiated  $(4.20\pm1.48)$ , fooddeprived (9.10±3.07) and honey-fed (2.2±1.14) females increased significantly during the second trial (Table 1).

Mating trial	Nutritional state	Duration (in minutes)	Commencement (in minutes)	Latent period (in seconds)	Bouts (no.)
First	Satiated	18.23±1.8 <sup>b</sup>	2.80±1.14 <sup>b</sup>	3.7±0.82ª	238.70±26.80ª
	Food deprived	15.62±1.35°	$6.60 \pm 7.69^{ab}$	2.1±1.52 <sup>b</sup>	214.50±14.55
	Honey fed	25.57±1.24ª	9.50±5.48ª	3.4±1.17 <sup>a</sup>	221.40±7.76 <sup>ab</sup>
Second	Satiated	18.43±2.08 <sup>b</sup>	1.90±1.45 <sup>b</sup>	3.4±1.17ª	221.40±7.76 b
	Food deprived	15.06±2.06°	11.90±4.86ª	2.30±0.48 <sup>b</sup>	245.60±27.19 <sup>b</sup>
	Honey fed	28.27±1.70ª	14.20±3.71ª	3.70±0.82ª	289.40±25.60ª

 Table 1. Mating duration, mating commencement, latent period and bouts in copula of females of C. transversalis at different dietary statuses

Data are Mean  $\pm$  S.D.; Tukey's Range = 3.51; d.f. = 2, 27; Different letters in the column denote that data is

significantly different

The mating refusals were significantly greater in second trials in food satiated (t = -5.67; P < 0.0001; d.f. = 16), deprived (t = -6.58; P < 0.0001; d.f. = 12), and honey-fed females (t = -9.25; P < 0.0001; d.f. = 11). Similarly, the mating commencement (t = -2.25; P = 0.040; d.f. = 15) and mating duration were significantly increased in honey-fed females (t = -4.05; P = 0.001; d.f. = 16). However, this increase was not significant in satiated (t = -0.24; P = 0.812; d.f. = 17 and t = 1.55; P = 0.141; d.f. = 17) and in deprived females (t = 0.72; P = 0.480; d.f. = 15 and t = -1.84; P = 0.085; d. f. = 15). The bouts in food-deprived (t = -3.19; P = 0.007; d.f. = 13) and honey-fed females (t = -8.04; P < 0.0001; d.f. = 10) also increased significantly in second trials. The pre-oviposition period of C. transversalis after two copulations was significantly shorter in satiated females than food-deprived and honey-fed females. The fecundity and egg viability were significantly greater in satiated females than fooddeprived and honey-fed females (Table 2).

# (ii) Effect of body weight of both sexes on the female remating resistance

The body size significantly affected the mating to post-mating parameters of *C. transversalis*. The time of mating commencement was greater when smaller males were used in the first and second trials. This time to commence mating in all four mating groups decreased significantly in the second trial. Similarly, latent periods of smaller males were significantly greater in both the first and second mating trials. The latent period in all four mating groups decreased significantly in the second trial. The smaller-sized males and females copulated for significantly longer duration in the first and second trials. However, the number of bouts was significantly greater when larger male and female copulated in both trials (Table 3).

The larger females resisted more to the mating advances of smaller sized males in both first

Nutritional state	Pre-oviposition (in days)	Fecundity (no. of eggs)	Egg viability(%)
Satiated	$5.00\pm0.00^\circ$	337.80±8.65ª	$92.87 \pm \! 5.48^a$
Deprived	$5.70\pm0.48^{\rm b}$	110.50±9.70 <sup>b</sup>	$82.80 \pm 5.02^{b}$
Honey fed	$8.20\pm1.03^{\rm a}$	3.00±1.41°	0.00±0.00°

Table 2. Reproductive output of females of C. transversalis maintained at different dietary levels

Data are Mean  $\pm$  S.D.; Tukey's Range = 3.51; d.f. = 2, 27; Different letters in the column denote that data is significantly different

(F=32.02; P < 0.0001; d.f. = 3, 36) and second (F=21.40; P < 0.0001; d.f. = 3, 36) trials as compared to other mating groups (Fig. 2). The first and second mating trials were compared using a two-sample ttest. The mating duration and mating refusals increased, while mating commencement decreased significantly in all four groups of second trials (Table 3). The fecundity (F = 37.41; P < 0.001; d.f. = 3, 36) and percent egg-viability (F = 81.83; P < 0.0001; d.f. = 3, 36) of larger females were significantly greater than those of smaller females, irrespective of male body size (Table 4). Similarly, the preovipositional periods of larger females were significantly (F = 21.60; P < 0.001; d.f. = 3, 36) shorter than those of smaller females, irrespective of male body size.

The nutritional conditions and body size influenced the reproductive behaviour of *C. transversalis*, including the female remating resistance. As expected, well-fed females readily accepted male copulatory attempts, copulated for a longer duration with higher fecundity and egg viability, and least resisted remating during both mating trials. However, food-deprived and honey-fed females took more time in accepting male copulatory attempts, mated for both longer and shorter durations, and resisted more to re-mate with lower fecundity and egg viability. The early mating commencement in well-fed females could be attributed to their satiated condition which makes them highly receptive to copulation, and increases the probability to select potential mates who have better sperm quality with accessory gland proteins and oviposition stimulants that results in better offspring (Albo *et al.*, 2012; Mirhosseini *et al.*, 2014). On the other hand, delay in mating commencement in food-deprived and honey-fed females indicates females' poor nutritional status hinders the mating process, as courting males need to invest more time and effort (Singh *et al.*, 2021).

Food-deprived females copulated for a shorter duration than the well-fed ones, as also reported in a ladybird, Menochilus sexmaculatus (Fabricius) (Singh et al., 2021). The food conditions during the adult stage exert an impact on reproductive behaviour and restricted food availability could act as a limiting factor by modulating mating duration to its shortest period. Nevertheless, the honey-fed females copulated for a longer duration than the other two females, which indicates their poor nutritional state leading to being easily overpowered and coerced by the males. Under coercive mating with honey-fed females, males modulated mating duration by inflicting themselves upon females and forcing them into copulation resulting in a longer mating duration. As observed in coercive mating,



Figure-1: Mating refusals by female, *C. transversalis* during first and second mating trials with respect to the female's nutritional status. Different letters denote that data is significantly different. Data is Mean  $\pm$  S.D.

Mating trial	Combinations	Duration (in minutes)	Commencement (in minutes)	Latent period (in seconds)	Bouts (numbers)
	Large Male x Large Female	20.85±1.05 <sup>b</sup>	4.90±0.73 <sup>b</sup>	4.00±0.82 <sup>b</sup>	325.40±8.96ª
First	Large Male x Small Female	17.56±0.73°	4.80±0.63 <sup>b</sup>	3.80±0.92 <sup>b</sup>	322.70±29.96ª
	Small Male x Large Female	16.05±0.71°	8.10±1.19ª	4.40±0.52 <sup>b</sup>	228.30±5.25 <sup>b</sup>
	Small Male x Small Female	24.64±1.09ª	5.20±0.63 <sup>b</sup>	6.20±1.13ª	234.10±7.08 <sup>b</sup>
Second Large	Male x Large Female	23.16±0.79 <sup>bc</sup>	2.80±0.63 <sup>b</sup>	3.30±0.67 <sup>b</sup>	329.50±16.26ª
	Large Male x Small Female	24.86±1.40 <sup>b</sup>	3.00±0.82 <sup>b</sup>	3.50±0.53 <sup>b</sup>	327.60±5.46ª
	Small Male x Large Female	21.55±1.53°	4.40±0.52ª	4.90±0.99ª	297.30±11.38 <sup>b</sup>
	Small Male x Small	25.70±2.16ª	3.50±0.53 <sup>ab</sup>	4.60±0.69ª	269.40±52.20°

 Table 3. Mating duration, time of mating commencement and latent period and bouts in copula of C. transversalis

 during first and second mating trials

Data are Mean  $\pm$  S.D.; Tukey's Range = 3.81; d.f. = 3, 36; Different letters in the column denote significantly difference



Figure-2: Mating refusals by female, *C. transversalis* during first and second mating trials with respect to the adult body-size. Different letters denote that data is significantly different. Data is Mean ± S.D.

males may harm the females, override their resistance by forced copulation and try to copulate with immature females (Peretti and Aisenberg, 2011). The latent period and bouts in a copula were male dependent and as the dietary condition was only applied to females and males were reared on *ad libitum* food the latent period was almost similar when copulation occurred with females of different dietary conditions under both mating tests. The number of bouts was mating duration-dependent, reported more under longer mating. However, if applied to both sexes the post-emergence nutritional conditions could influence mating performance due to increased latent period resulting in decreased vitality (Agarwala *et al.*, 2008).

The findings support the hypothesis that the females under stressful food conditions resist more male copulatory attempts compared to those reared in favourable food condition. This could be attributed to the reproductive costs associated with mating, avoidance of superfluous mating, hindrance in female foraging capacity, increased risk of predation, increased risk of sexually transmitted diseases, and physical damage leading to declining fecundity (Ronn et al., 2007; Fox and Moya-larano, 2009; Perry et al., 2009). The energy requirements vary in both sexes, as males expend energy in copulatory and pre-copulatory processes, while females invest energy in search of suitable oviposition sites, laying eggs, controlling oviposition timing and using maternal effects to tailor progeny (Sipos et al., 2012; Mirhosseini et al., 2014) hence lower nutritional state (Perry et al., 2009) and sexual immaturity of females (Khan, 2020) also trigger re-mating resistance.

Nutritional status was an important determinant of post-mating response, more fecundity with shorter pre-oviposition and a higher percentage of egg viability were observed when mating was reported in well-fed females. However, the deprived and honey-fed females had a more preoviposition period with lesser fecundity and the lowest percentage of egg viability. The higher energy consumption during the early stage enhances the reproductive capacity and females reared on a favourable diet from egg to adult stage have higher fecundity with more egg hatchability and early oviposition in comparison to the females who had a restricted diet after their emergence (Li et al., 2020). The higher hatching success in well-fed females is attributed to the suitable feeding conditions of both sexes, having more available resources to invest in reproduction (Ernande et al., 2004). Thus, egg viability is determined by both female nutritional status and male ejaculate quality and quantity with the accessory gland protein (Pervez et al., 2004; Perry and Rowe, 2008; Vargas et al., 2012; Michaud et al., 2013; Singh et al., 2016). Reduced fecundity in food-deprived and honey-fed females was attributed to their poor nutrition state negatively affected egg development and oogenesis acceleration (Behmer and Nes, 2003) and showed oosporation by reallocating resources for survival instead of reproduction (Moore and Attisano, 2011). The restricted diet may also limit female reproductive output by decreasing egg number, downregulating immune response, and reducing longevity (French et al., 2007; Karl et al., 2007) thereby to attain maximum egg viability abundant food conditions should be mandatory for all life stages of an individual. The body size significantly influenced the mating behaviour and female remating refusals. When mating was reported the large males took the least time to commence mating than the smaller males. This shows the effect of (i) mating urge in which large males vigorously court and force females for copulation (Partridge and Farquhar, 1983) and (ii) advantage of large body proportion that provides males higher fitness for more offspring production (Dubey et al., 2016). However, smaller males who work more sneakily invest more time in mating attempt thereby their persistence in mating lead females to engage in copulation that increases their reproductive success and produces more offspring (Watters, 2005).

The mating duration was significantly influenced by the body size of the mating pairs. The smaller males mated for a longer duration than large males. The prolonged mating duration in smaller males reflects their lower probability of being selected as mates, investing more time in a mating that for a period reduces the chances of their female partner engaging in copulation with another male and

Mating combination	Pre-oviposition (days)	Fecundity (no. eggs)	Egg viability (%)
Large Male x Large Female	5.00±0.00°	333.10±1.66ª	91.89±1.05ª
Large Male x Small Female	5.30±0.48 <sup>b</sup>	247.20±2.44 <sup>b</sup>	85.84±2.92 <sup>b</sup>
Small Male x Large Female	5.00±0.00°	327.90±1.19ª	90.90±2.20ª
Small Male x Small Female	5.90±0.32ª	241.00±0.82 <sup>b</sup>	83.40±1.76 <sup>b</sup>

Table 4. Reproductive output of C. transversalis in different mating combinations

Data are Mean  $\pm$  S.D.; Tukey's Range = 3.81; d.f. = 3, 36; Different letters in the column denote significant difference

provides more time to small male for inseminating female with more sperms for fertilizing more eggs leading to enhancing their probability to gain more fatherhood by attaining more offspring assurance (Holwell et al., 2016). The longer copulation results in higher male harassing behaviour towards smaller females to engage them in longer copulation (Ryan et al., 2001). From the earlier study, it is been known that large males copulate for a longer mating duration (Lupold et al., 2011; Pervez and Singh, 2013). However, a shorter mating duration in larger males when they mated with large and small females could be related to their better ejaculate size, higher quality and quantity of sperm with accessory gland products that enhance their postcopulatory reproductive success (Avila et al., 2011), thereby the larger males have an advantage over smaller males (Pervez and Singh, 2013).

Multiple matings are common in insects, including ladybirds. The males and females can enhance their reproductive success through multiple mating despite female remating refusals (Obata, 1988; Perry et al., 2009; Pervez et al., 2020). In the present study, remating resistance was significantly influenced by the body size of both mating partners. This could be associated with the benefits of mating with larger males because (i) large males have higher fitness and give rise to progeny with higher survival success (ii) large males can overcome female resistance by scaring females for further harassment (Pilastro et al., 2003; Muller et al., 2007). However, the remating resistance occurred more when small males courted large females because the females discriminate the male mating success, though the females mate with smaller males, they still prefer large males for attaining higher reproductive success (Dubey *et al.*, 2016). Thereby, the mating success does not solely depend on the males (Bretman *et al.*, 2013) though males indiscriminately and persistently attempt to mate for more fatherhood, females show more choosy behaviour and avoid those mating who are superfluous and exert some relevant costs, and this scenario leads to a sexual conflict where the mating rate works as a strong driving force in the evolution of reproductive strategies in both sexes (Rowe *et al.*, 2020).

Higher fecundity with a shorter preoviposition period was found when large females copulated than the small females, as also reported earlier (Vargas et al., 2012; Dubey et al., 2016). Large females provide more space to accommodate developing eggs, have a greater number of ovarioles, and can produce more eggs by allocating more energy resulting in higher offspring numbers (Osawa, 2005; Dixon, 2007; Singh et al., 2021). Smaller females were less fecund with a greater preoviposition period and lesser egg viability. Further mating with smaller males reduced their post-mating output because large males under copulation contribute more sperm with accessory gland proteins that stimulate egg production and lead to enhanced fecundity compared to small males (Mirhosseini et al., 2014). Thereby, the body size of both sexes shapes the reproductive success in ladybirds not only male size as stated earlier (Bista and Omkar, 2013).

The nutritional conditions and body size modulate the mating behaviour and female remating resistance in C. transversalis, and the study suggests that (i) nutritional state post-emergence shapes female reproductive behaviour, (ii) well-fed females have better reproductive output with more fecundity, percentage of egg viability and emit least remating resistance that increases in subsequent matings, (iii) poor nutritional females (deprived and honey fed) emit more remating resistance with a greater preoviposition period, lesser fecundity and percentage of egg viability, (iv) C. transversalis shows the size-dependent mating success and both males and females contribute to output, (v) copulation in larger pairs results into more fecundity, a higher percentage of egg viability and emit least remating resistance, (vi) small pairs show lower fecundity with least percentage of egg viability and (vii) large males took advantage of body size and harass smaller females by coercive mating, while large females show more remating refusals when paired with smaller males.

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# Species composition and diversity of Odonata fauna in Cauvery River of Mettur dam, Salem district, Tamil Nadu, India

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**ABSTRACT:** Odonata diversity study was carried out from September 2020 to October 2022 in the Mettur Dam, Salem District. Among the total 40 different species, 27 species were in Anisoptera (dragonflies) and 13 species in Zygoptera (damselflies) under five families. Libellulidae was the largest family recorded (16 species), followed by Coenagrionidae (11), Aeshnidae (2), Lestidae (2) and Gomphidae (1). A migratory species, *Pantala flavescens*, was the most dominant in numbers throughout the year. Maximum number of individuals was found during September 2020 to September 2021. Odonata diversity was higher in the first season of 2021 than in next second season. Both dragonflies and damselflies mainly used wetlands of the dam ecosystem and agricultural fields rather than shrubland.

KEY WORDS: Pantala flavescens, damselflies, dragon flies, richness, wetlands

#### **INTRODUCTION**

Dragonflies and damselflies are important components of biodiversity, and in the food web of ecosystems, they act as effective carnivores and detritivores (Das *et al.*, 2012; Siregar and Bakti, 2016). In all of their life-cycle stages, dragonflies act as predators and eat a wide variety of insects and other organisms. Dragonfly nymphs are predators in the aquatic ecosystem, while adult dragonflies are predators of agricultural crop pests as natural pest control agents (Kandibane *et al.*, 2005). Adult Dragonflies and damselflies are also used as bio-indicators of forest environment and water quality (Corbet, 1999; Dolny *et al.*, 2011; Das *et al.*, 2012). Dragonflies are important biological control agents for mosquito larvae (Spencer *et al.*, 1999; Mandal *et al.*, 2008). Around 6,000 species and subspecies of Odonata have been described under 630 genera in 28 families throughout the world (Tsuda, 1991). In India, 499 species, 139 genera, and 17 families of dragonflies and damselflies have been documented (Prasad and Varshney, 1995; Sharma, 2010). Odonata diversity has been extensively studied in different forest areas. (Emiliyamma, 2005) has recorded 31 species of dragonflies and damselflies from the southern Western Ghats in the Kottayam district of Kerala. Gunathilagaraj *et al.* (1999), Kandibane *et al.* (2005) and Anbalagan *et al.* (2013) have studied the Odonata diversity in agricultural fields.

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Odonata is freshwater insects because the females lay eggs on water or submerged plants and the larval development occurs under water (Hornug and Rice, 2003). Unlike the larva, the adults are aerial. Odonata assemblage is higher in aquatic habitats (Oppel, 2005). Besides water reservoirs, Odonata diversity varies in different climatic zones. They occur worldwide in varied ecological niches extending from seashores, brackish, marshy areas, and mangroves to semi-arid areas (Kalkman et al.,2008). The majority of the species are highly sensitive to changes in habitat quality (Smith et al.,2007; Silva et al.,2010). Besides, these insects also play an important role as prey-predator of natural ecosystems (Das et al., 2021). The Dragonflies are used for food and medical resources at a local scale (Kalkman et al. 2008; Shantibala et al.,2012). A study was conducted on the species composition and diversity of Odonata fauna in the agricultural areas and water bodies of the villages in Cauvery River of Mettur dam, Salem district, Tamil Nadu, India.

#### **MATERIALS AND METHODS**

Dragonflies and damselflies were recorded in Mettur Dam, Kolathur and Madhanyankuttai of Salem district. The geocordination of Mettur Dam is 11°47'59.99"N; 77°47'59.99"E with elevation 1,341m. The study was conducted in the agricultural areas and water bodies of the villages adjacent to Mettur town (Kolathur, Madhanyankuttai and Thottilpatt). In each village, dragonflies and damselflies were observed in three different locations by quadrate method. Quadrates of 25m x 10m size were laid down with threads inside grasses. Totally five quadrates were put in each village area. Perched dragonflies and damselflies found inside the quadrates were collected by sweep net (25cm in diameter) during day times (between 10.00 AM to 3.00 PM). Flying Odonates inside the quadrate area were also caught with a sweep net. Sampling was done weekly once a month from September 2020 to October 2022. Specimens from replications were pooled together. The species diversity from all these habitats was recorded and species composition of significant differences of diversity among the three parts of the area studied.

The specimens were identified using taxonomic keys provided by Fraser (1933) and Subramanian (2009). After identification and counting the total number of specimens, a few specimens from each taxa were retained and others were left behind alive in the field. Specimens that were not identified in the field were brought to the laboratory for identification. Collected species were sorted out into families, genera, and specimens. A total number of individuals collected under each family were used for diversity analysis. Species richness Menhinick (R1), Margalef (R2), evenness, and diversity indices such as Shannon's index and Simpson index were calculated by using PAST software.

#### **RESULTS AND DISCUSSION**

A total of 40 species of Odonata belonging to five families were recorded. Among the five families, Libellulidae was the dominant member (60%) followed by the Coenagrionidae (28%), Lestidae (5%) and Aeshnidae (5%). Out of the 40 species, 27 species belong to the suborder Anisoptera (dragonflies) and 13 to the suborder Zygoptera (damselflies). Libellulidae family recorded maximum of 24 species and two species each in Aeshnidae and Lestidae, and one under Gomphidae. Diplacodes trivialis (Rambur) was abundant under Anisoptera and Pseudagrion microcephalum (Rambur) among Zygoptera in Mettur dam (Table 1). The dragonflies, Crocothemis servilia (Drury), Diplacodes trivialis (Rambur), and Orthetrum sabina (Drury) (Libellulidae) were recorded from all three villages. Only Ceriagrion coromandelianum (F) (Coenagrionidae) among Zygoptera was present in all three villages. Totally nine species of Anisoptera viz., Brachythemis contaminata, Bradinopyga geminata, and Crocothemis servillia, Diplocodes trivialis, Orthetrum pruinosum, O. sabina, Pantala flavescens, Trithemis aurora and T. festiva, and among the Zygoptera Agriocnemis femina, Ceriagrion coromandelianum, Ischnura aurora and I. rubilio were present in all three habitations. Ceriagrion coromandelianum and Agriocnemis femina were confined to Mettur dam and Madhanyankuttai and Kolathur Lakes. The abundance of Libellulidae dragonflies and



Fig. 2 Odanates collected from Mettur Dam, Salem District

Coenagrionidae damselflies in the present study might be due to their shorter life cycle and widespread distribution (Norma-Rashid *et al.*, 2001) and tolerant to a wide range of habitats (Gentry *et al.*, 1975; Samways, 1989).

Odonata recorded were categorized family wise into four on the basis of their abundance such as VC- very common (70-100%), C-common (40-70%), R-rare (20-40%) and VR-very rare (below 20%) (Table1). The total number of individuals recorded in Mettur Dam was 1513. Maximum total abundance (878) was recorded from September 2020 to September 2021. Maximum Shannon-Wiener diversity index (1.49) and evenness (0.89) are recorded during the first season of 2021. Odonata diversity in the first season was higher than in the second season. The Margalef index (0.61) was calculated as (0.59) for each study year. Among the five families encountered during the study period, the maximum dominance and contribution of the species diversity accounted for 24 species in Libellulidae followed by Coenagrionidae which contributed 11 species. The minimum species was recorded to belong to two families each two species Lestidae, Aeshnidae and Gomphidae only one species respectively. In the study, the distribution and abundance of dragonfly species have higher dominance than damselfly species. The abundance of dragonflies in Mettur Dam could be attributed to the presence of shade over the habitat from the trees and shrubs present in the water bodies and to the presence of aquatic

No.	Species	Common name	Status		
	Sub order: Anisoptera; Family: Libellulidae				
1.	Diplacodes trivialis (Rambur)	Ground skimmer	VC		
2.	Tholymis tillarga (F)	Coral-tailed cloud wing	С		
3.	Pantala flavescens (F)	Wandering glider	С		
4.	Crocothemis servilia(Drury)	Ruddy marsh skimmer	С		
5.	Brachythemis contaminate (F)	Dithch jewel	VC		
6.	B.chalybea (Brauer)	Rufous-backed Marsh	С		
7.	D. nebulosa (F)	Black tipped ground skimmer	VC		
8.	D. trivialis (Rambur)	Ground skimmer	R		
9.	Bradinopyga geminate (Rambur)	Granite ghost	R		
10.	Orthetrum sabina (Drury)	Green marsh hawk	С		
11.	O. glaucum (Brauer)	Common Blue Skimmer	R		
12.	O. testaceum(Burmeister)	Scarlet Skimmer	VR		
13.	Sympetrum flaveolum (Linn)	Yellow-winged darter	VR		
14.	S. vulgatum flavum (Bartenef)	Southern Migrant Hawker	С		
15.	O. pruinosum (Burmeister)	Crimson-tailed marsh hawk	R		
16.	Neurothemis tullia (Drury)	Pied paddy skimmer	С		
17.	Rhodothemis rufa(Rambur)	Common red bolt	С		
18.	Hylaeothemis indica (Fraser)	Blue hawkelet	R		
19.	O. chrysis(Selys)	Brownbacked red marsh hawk	С		
20.	Tetrathemis platyptera (Selys)	Pigmy skimmer	VR		
21.	Trithemis aurora (Burmeister)	Crimson marsh glider	С		
22.	T. festiva (Rambur)	Black stream glider	R		
23.	T. pallidinervis (Kirby)	Long-legged Marsh Glider	R		
24.	Rhyothemis variegate(Linn)	Common picture wing	R		
	Family: Aesh	nidae			
25.	Anaciaeschna jaspidea (Burmeister)	Rusty darner	VR		
26.	Anax immculifrons (Rambur)	Magnificent emperor	R		
	Family: Gom	phidae			
27.	Gomphus vulgatissimus(Linn)	Club-tail	VR		
	Sub order: Zygoptera; Family: Coenagrionidae				
28.	Ischnura aurora(Brauer)	Golden Dartlet	С		
29.	Ceriagrion coromandelianum (F)	Coromandel marsh dart	VR		
30.	Agriocnemis pygmaea (Rambur)	Pigmy dartlet	С		

Table1. Odonata species recorded during September2020 to October-2022 in Mettur Dam

31.	A. splendidissima (Laidlaw)	Splendid dartlet	С		
32.	I. senegalensis (Rambur)	Senegal golden dartlet	R		
33.	Paracercion malayanum (Selys)	Malay illy squatter	R		
34.	Agriocnemis femina (Brauer)	Pinhead wisp	С		
35.	I. rubilio (Selys)	Western golden dartlet	VC		
36.	Pseudagrion microcephalum (Rambur)	Blue sprite	С		
37.	P. decorum(Rambur)	Elegant sprite	С		
38.	P. indicum(Fraser)	Yellow striped blue dart	R		
	Family: Lestidae				
39.	Lestes elatus (Selys)	Emerald spread wing	R		
40.	L. viridulus(Rambur)	Emerald striped spread wing	VR		

\*(VC-Very common; C- common; R- Rare; VR- Very rare)

vegetation. This is confirmed by the findings of Fraser (1933) and Subramanian (2005) who revealed that shade and aquatic vegetation could favour Zygoptera more than Anisoptera. In the three areas of the study in Mettur area has the maximum richness and abundance of the dragonfly species followed by Kolathur and Madhanyankuttai. Arulprakash and Gunathilagaraj (2010) recorded twenty-one species of Odonata (14 species of Anisoptera and seven species of Zygoptera) belonging to 17 genera under four families from 13 temporary water bodies of Coimbatore and Salem districts in Tamil Nadu.

There were 635 individuals in the first year, while it was 878 in the second year. Shannon-Wiener Diversity Index(H) was 1.493 and 1.488 respectively for first and second year. Simpson1-D was 0.7517 and 0.7489 during the first and second year. Margalef Index (R1) was 0.6198 in the first year, while it was 0.5902 in the second year. Menhinick (R2) showed marked difference in the first year (0.1984) and in the second year (0.1687). Evenness (I year = 0.8902 and II year - 0.8856) and Berger-Parker Index (I year = 0.8902 and II year = 0.8856) showed a small difference between the two years. Odonata diversity was higher during 2020 - 2021 than in 2021-2022. The diversity of Odonata species was highest in the monsoon period. The abundance of dragonflies and damselflies was widely distributed all over the month, November month was more active, and the lower proportion was during January. The Odonata are mainly seen in the pond ecosystem and agricultural lands because the availability of food is higher in the surface of water bodies mainly in the standing waters.

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# Butterfly fauna of Dhansiri Reserve Forest, Karbi Anglong, Assam, India

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**ABSTRACT:** The field survey carried out to document the baseline data of butterfly fauna of Dhansiri Reserve Forest of Karbi Anglong District, Assam, recorded 106 species belonging to six families. *Graphium sarpedon, Appias albina, Spindasis lohita, Charaxes marmax, Athyma ranga* and *Tanaecia lepidae, Hypolycaena othona and Euploea mulciber* are legally protected species under the Indian Wild Life (Protection) Amendment Act, 2022. *Eurema andersonii, Appias albina, Appias galba, Charaxes marmax* and *Athyma ranga* are some of the rare species recorded during the study. Record of rare and endemic species from this Reserve Forest, and presence of species legally protected under the Indian Wildlife (Protection) Amendment Act, marks its importance as an area for butterfly conservation. © 2024 Association for Advancement of Entomology

KEY WORDS: Lepidoptera, species richness, conservation, rare species

#### **INTRODUCTION**

Among insects, butterflies occupy an important position in the ecosystem (Ghazoul, 2002) and are considered to be flagship species for conservation (Thomas, 2005). Butterflies act as biological indicators of habitat quality, environmental changes and anthropogenic disturbance (Talbot, 1939; Pandhye *et al.*, 2012; Tiple, 2012; Kocher and Williams, 2000; Kunte 2000, 2023; Bhowmik, 2021; Das *et al.*, 2023). North East India is a part of the Eastern Himalayas Biodiversity hotspot, and is known as Biodiversity Hotspot for Butterfly fauna. The Eastern Himalaya is one of the richest areas of butterfly fauna (Saikia, 2011). In India the number of butterfly species is 1431 as per Kunte (2023), while Das *et al.* (2023) checklists 1379 butterfly species. Evans (1932) recorded 962 species of butterflies in Northeast India, while Das *et al.* (2023) reported 745 species in Eastern Himalaya and NE India.

Butterfly studies were earlier done in Cachar Hills (Wood-Mason and de Niceville 1887), Manipur and Naga Hills (Tytler, 1915), and Khasi and Jayantia Hills (Parsons and Cantlie, 1948), but there was no mention of Mikir Hills. Karbi Anglong District of Assam (earlier known as Mikir Hills district) is an area where scanty work has been done on butterfly taxonomic work. A few studies were done in Kaziranga-Karbi Hills (Gogoi, 2013, 2015), Garampani and Nambor Wildlife Sanctuary (Bawri *et al.*, 2014) and Nambor-Doigrung Wildlife Sanctuary (Mudai *et al.*, 2015). Goswami and

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Reddy (2021) reported *Appias galba* from Dhansiri Reserve Forest which is stated as rare according to Evans (1932). The species was earlier recorded by Parsons and Cantlie (1948) from Lumding, Upper Assam. Karbi Anglong harbors a rich floral and faunal diversity. But a comprehensive account of butterfly fauna in the protected areas and non-protected areas of Karbi Anglong is not available. The present work is an attempt to document the baseline data on butterfly fauna of Dhansiri Reserve Forest of Karbi Anglong District of Assam.

## **MATERIALS AND METHODS**

The study was carried out in Dhansiri Reserve Forest of Assam. Dhansiri Reserve Forest is located in Karbi Anglong District of Assam. Dhansiri is the second largest reserve forest of Assam with an area of 770.38 km<sup>2</sup>, bordering Intanki National Park of Nagaland. It is a part of Dhansiri-Lumding Elephant Reserve. The area consisting of undulating plains and low hills is a part of Karbi Plateau. Dhansiri river is the major river flowing through Dhansiri Reserve Forest. The area experiences tropical monsoon climate. The area falls in the rainshadow zone of Northeast India. Dhansiri Reserve Forest comprises of Tropical Moist Deciduous and Tropical Semi-evergreen type of forest (Choudhary, 1993). Birdlife International, a global partnership for conservation organization, has recognized Dhansiri Reserve Forest as Important Bird Area.

Field survey was conducted during September 2015 to December 2019 between 8.00 to 16.00h in all seasons i.e., pre-monsoon, monsoon, retreating monsoon and winter. Data of butterfly fauna was collected by random survey. The species were photographed and identified using field guide of Kehimkar (2008) and Sondhi *et al.* (2013). All butterfly species were identified up to species level. No specimen was collected for the study. Classification and nomenclature of butterflies were done according to the website of Indian Butterflies (https://www.ifoundbutterflies.org).

#### **RESULTS AND DISCUSSION**

A total of 106 species belonging to six families were

recorded from Dhansiri Reserve Forest (Plate I, IITable 1). Out of 106 species, eight species [Graphium sarpedon, Appias albina, Spindasis lohita, Charaxes marmax, Athyma ranga and Tanaecia lepidae, Hypolycaena othona and Euploea mulciber] are legally protected under various Schedules of the Indian Wildlife (Protection) Amendment Act (IWLPA Act), 2022 (IWLPA, 2022). Species richness was maximum in the family Nymphalidae comprising of 49 species, followed by Lycaenidae (22 species), Pieridae (16 species), Papilionidae (11 species), Hesperiidae (7 species) and Riodinidae (1 species).

Some of the 'rare' species recorded from the Reserve Forest were Eurema andersonii, Appias albina, A. galba, Charaxes marmax and Athyma ranga. Species which are 'endemic' to Northeastern and Eastern Himalayas viz., Psolos fuligo, Papilio nephelus, Gandaca harina, Delias descombesi and Kaniska canace were also recorded from the study area. Butterflies Arhopala eumolphus, Appias galba, Hypolycaena othona, Zizula hylax, Ticherra acte and Pseudergolis wedah were very rare and sighted only once during the study period.

In Nambor - Doigrung Wildlife Sanctuary of North East India, Mudai et al. (2015) check listed 224 butterfly species belonging to 137 genera representing five families, of which Nymphalidae was the dominating family with 84 species, followed by 19 species of Papilionidae, 19 Pieridae, 62 Lycaenidae and 40 Hesperiidae. Some very important species encountered were Lasippa viraja viraja, Lamproptera curius curius, Capila zennara, Cupitha purreea and Bibasis sena sena; all are included in the IWLPA. Singh (2020) annotated a list of 375 species of butterflies so far recorded from eastern Assam that includes a large number of very rare species. Bhowmik (2021) recorded 51 butterfly species as additions to the fauna of Tripura, North-east India. In soraipung range of Dehing Patkai National Park, Assam, a total of 92 butterfly species belonging to five families were recorded of which 13 species were listed as protected under various schedules of the IWLPA Act (Gogoi et al., 2023).

Plate I



a. Psolos fuligo, b. Notocrypta curvifascia, c. Iambrix salsala, d. Tagiades japetus, e. Sarangesa dasahara, f. Graphium sarpedon, g. Papilio nephelus, h. Papilio polytes, i. Zemeros flegyas

Plate II



(J) Delias pasithoe, (k) Eurema blanda, (l) Appias indra, (m) Arhopala eumolphus, (n) Surendra quercetorum, (o) Jamides alecto, (p) Prosotas nora, (q) Discolampa ethion, (r) Hypolycaena erylus, (s) Hypolycaena othona, (t) Nacaduba beroe, (u) Chersonesia risa

Table 1 Check list	of butterflies	of Dhansiri	Reserve Forest
Tuble 1. Check hot	of outter me	o or Diffution i	

No	Common Name	Scientific Name	Status		
Family: Hesperiidae					
1	Chestnut Angle	Odontoptilum angulatum (Fedler, 1862)	Not rare		
2	Common Small Flat	Sarangesa dasahara (Moore, [1866])	Common		
3	Common Snow Flat	Tagiades japetus (Stoll, [1781])	Common		
4	Coon	Psolos fuligo (Mabille, 1876)	Common		
5	Fulvous Pied Flat	Pseudocoladenia dan (Evan, 1949)	Common		
6	Chestnut Bob	Iambrix salsala (Moore, [1866])	Common		
7	Restricted Demon	Notocrypta curvifascia (C. & R. Felder, 1862)	Common		
		Family: Papilionidae			
8	Common Bluebottle *	Graphium sarpedon (Linnaeus, 1758)	Common		
9	Great Jay	G. eurypylus (Linnaeus, 1758)	Not rare		
10	Common Jay	G doson (C. & R. Fedler, 1864)	Common		
11	Common Mime	Papilio clytia (Linnaeus, 1758)	Common		
12	Common Mormon	P. polytes Linnaeus 1758	Common		
13	Red Helen	P. helenus Linnaeus 1758	Common		
14	Yellow Helen	P. nephelus Boisduval, 1836	Common		
15	Great Mormon	P. memnon Linnaeus 1758	Common		
16	Common Batwing	Atrophaneura varuna White 1842	Not rare		
17	Lime Butterfly	P. demoleus Linnaeus 1758	Very common		
18	Common Birdwing #	Troides helena Linnaeus, 1758	Not rare		
		Family: Pieridae			
19	One Spot Grass Yellow	<i>Eurema andersonii</i> (Moore, 1886). This species is distributed in Andaman Islands only	Rare		
20	Three Spot Grass Yellow	E. blanda (Boisduval, 1836)	Common		
21	Common Grass Yellow	E. hecabe (Linnaeus, 1758)	Very common		
22	Tree Yellow	Gandaca harina (Horsfield, [1829])	Not rare		
23	Common Emigrant	Catopsilia pomona (Fabricius, 1775)	Common		
24	Mottled Emigrant	C. pyranthe (Linnaeus, 1758)	Common		
25	Yellow Orange Tip	Ixias pyrene (Linnaeus, 1764)	Common		

26	Chocolate Albatross	Appias lyncida (Cramer, [1777])	Common
27	Common Albatross*	A. albina (Boisduval, 1836)	Rare
28	Orange Albatross	A. galba (Wallace, 1867)	Rare
29	Lesser Gull	Cepora nadina (Lucas, 1852)	Not rare
30	Psyche	Leptosia nina (Fabricius, 1793)	Common
31	Red-base Jezebel	Delias pasithoe (Linnaeus, 1767)	Not rare
32	Red-spot Jezebel	D. descombesi (Boisduval, 1836)	Not rare
33	Plain Puffin	A. indra (Moore,[1858])	
34	Indian Cabbage White	Pieris canidia (Linnaeus, 1768)	Very common
		Family: Lycaenidae	
35	Western Centaur Oakblue	Arhopala centaurus (Fabricius, 1775)	Not rare
36	Green Oakblue	Arhopala eumolphus (Cramer, [1780])	Common
37	Common Acacia Blue	Surendra quercetorum (Moore,[1858])	Common
38	Yamfly	Loxura atymnus (Stoll, 1780)	Common
39	Common Tit	Hypolycaena erylus (Godart, [1824])	Not rare
40	Fluffy Tit	Zeltus amasa (Hewitson, 1865)	Common
41	Orchid Tit*	H.othona Hewitson, [1865]	Not rare
42	Long-banded Silverline*	Spindasis lohita (Horsfield, [1829])	Common
43	Purple Sapphire	Heliophorus epicles (Godart, [1824])	Common
44	Common Ciliate Blue	Anthene emolus (Godart, [1824])	Common
45	Common Pierrot	Castalius rosimon (Fabricius, 1775)	Common
46	Banded Blue Pierrot	Discolampa ethion (Westwood, [1851])	Not rare
47	Tailless Lineblue	P. dubiosa (Semper, [1879])	Common
48	Brown Lineblue	Prosotas lutea (Martin, 1895)	Common
49	Dark Grass Blue	Zizeeria karsandra (Moore, 1865)	Common
50	Tiny Grass Blue	Zizula hylax (Fabricius, 1775)	Common
51	Common Lineblue	Prosotas nora (C. Felder, 1860)	Common
52	Slate Flash	Rapala manea (Hewitson, 1863)	Common
53	Blue Imperial	Ticherra acte acte (Moore, [1858])	Not rare
54	Opaque Six-Lineblue	Nacaduba beroe (C. & R. Felder, [1865])	Not rare
55	Transparent Six-Lineblue	N. kurava (Moore, [1858])	Common

56	Metallic cerulean	Jamides alecto (C. Felder, 1860)	Common			
	Family: Riodinidae					
57	Punchinello	Zemeros flegyas (Cramer, [1780])	Common			
		Family: Nymphalidae				
58	Glassy Tiger	Parantica aglea (Stoll, [1782])	Common			
59	Plain Tiger	Danaus chrysippus (Linnaeus, 1758)	Very common			
60	Striped Tiger	D. genutia (Cramer, [1779])	Very common			
61	Dark Blue Tiger	Tirumala septentrionis (Butler, 1874)	Not rare			
62	Common Crow	Euploea core (Cramer, [1780])	Common			
63	Striped Blue Crow*	E.mulciber (Cramer, [1777])	Common			
64	Magpie Crow	E. radamanthus (Fabricius, 1793)	Not rare			
65	Indian Nawab	Charaxes bharata C & R Felder[1867]	Common			
66	Tawny Rajah	C. bernardus (Fabricius, 1793)	Common			
67	Yellow Rajah*	C. marmax Westwood, 1847	Rare			
68	Common Evening Brown	Melanitis leda (Linnaeus, 1758)	Very common			
69	Angled Red Forester	Lethe chandica (Moore, [1858])	Not rare			
70	Common Palmfly	Elymnias hypermnestra (Linnaeus, 1763)	Common			
71	Common Bushbrown	Mycalesis perseus (Fabricius, 1775)	Very common			
72	Dark-branded Bushbrown	M. mineus (Linnaeus, 1758)	Very common			
73	Nigger	Orsotriaena medus (Fabricius, 1775)	Common			
74	Common Five-ring	Ypthima baldus (Fabricius, 1775)	Very common			
75	Common Four-ring	Y. huebneri Kirby, 1871	Very common			
76	Leopard Lacewing	Cethosia cyane (Drury, [1773])	Not rare			
77	Cruiser	Vindula erota (Fabricius,1793)	Not rare			
78	Large Yeoman	Cirrochroa aoris Doubleday, [1847]	Not rare			
79	Common Yeoman	<i>C. tyche</i> C. & R. Felder, 1861	Common			
80	Rustic	Cupha erymanthis (Drury, [1773])	Common			
81	Vagrant	Vagrans egista (Cramer, [1780])	Not rare			
82	Common Leopard	Phalanta phalantha (Drury, [1773])	Common			
83	Commander	Moduza procris (Cramer, [1777])	Common			
84	Common Sergeant	Athyma perius (Linnaeus, 1758)	Common			

85	Blackvein Sergeant*	Athyma ranga Moore, [1858]	Rare
86	Colour Sergeant	A. inara Westwood, 1850	Not rare
87	Common Lascar	Pantoporia hordonia (Stoll, [1790])	Common
88	Common Sailer	Neptis hylas (Linnaeus, 1758)	Very common
89	Short-banded Sailer	Phaedyma columella (Cramer, [1780])	Not rare
90	Plain Sailer	N. cartica Moore, 1872	Not rare
91	Knight	Lebadea martha (Fabricius, 1787)	Not rare
92	Grey Count*	Tanaecia lepidae (Butler, 1868)	Not rare
93	Common Map	Cyrestis thyodamas Doyère, [1840]	Common
94	Common Maplet	Chersonesia risa (Doubleday, [1848])	Not rare
95	Angled Castor	Ariadne ariadne (Linnaeus, 1763)	Common
96	Common Castor	Ariadne merione (Cramer, [1777])	Common
97	Black Prince	Rohana parisatis (Westwood, [1851])	Not rare
98	Common Jester	Symbrenthia lilaea (Hewitson, 1864)	Common
99	Peacock Pansy	Junonia almana (Linnaeus, 1758)	Common
100	Chocolate Pansy	J. iphita (Cramer, [1779])	Common
101	Grey Pansy	J. atlites (Linnaeus, 1763)	Not rare
102	Blue Pansy	J. orithya (Linnaeus, 1758)	Very common
103	Great Eggfly	Hypolimnas bolina (Linnaeus, 1758)	Common
104	Tawny Coster	Acraea terpsicore (Linnaeus, 1758)	Common
105	Blue Admiral	Kaniska canace (Linnaeus, 1763)	-
106	Tabby	Pseudergolis wedah (Kollar, [1844])	Not rare

\* Represents species protected under the Indian Wildlife (Protection) Act, 2022

# Represents species listed in CITES Appendix I

The present study is a first time investigation to document the baseline data of butterfly fauna Dhansiri Reserve Forest of Karbi Anglong District. Dhansiri Reserve Forest was already recognized as an Important Bird Area. Now, noteworthy record of rare and endemic species from this Reserve Forest, and presence of species legally protected under the IWLA Act, 2022, marks its importance as an area for butterfly conservation. A recent study stated that forest fragmentation is increasing in Dhansiri Reserve Forest and there is an alteration in forest area due to anthropogenic activities (Chowdhury *et al.*, 2017). Habitat fragmentation and deterioration of habitat quality are considered to be two major threats to biodiversity in recent time (Rosin *et al.*, 2012; Sarma *et al.*, 2012). Butterflies being ecological indicators can be used to monitor any alteration in forest habitat and can
thus play a key role in protection of biodiversity. Therefore, conservation efforts should be taken up in the Reserve Forest towards protection of butterfly fauna and also for conservation of flora and fauna.

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# Pollinators diversity and pollination effects on yield attributes of sunflower (*Helianthus annuus* Linnaeus) in Odisha, India

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**ABSTRACT:** In the study on the diversity of pollinators and their pollination efficiency in sunflower (*Helianthus annuus* L.) under field condition of Odisha during 2021-22, recorded 18 species of pollinators. Indian honeybee, *Apis cerana indica* F. (Hymenoptera, Apidae) was found to be the major one among the insect pollinators. The experiment conducted for two years with three different treatments *viz.*, open pollination (OP), managed *A. cerana indica* pollination (HB) and pollinator exclusion (PE) for better yield and quality revealed that both quantitative and qualitative parameters were significantly higher in OP sunflower crops followed by crops pollinated by *A. cerana indica*. Significantly higher seed yield, 1000 seed weight, percentage of seed filling and number of seed per capitulum were recorded in these treatments. These findings indicate OP and HB increase 33 per cent more yield in sunflower. © 2024 Association for Advancement of Entomology

KEY WORDS: Species, Apis cerana indica, open pollination, seed yield, seed filling, capitulum

#### **INTRODUCTION**

Healthy population of pollinators is essential to maintain biodiversity and healthy natural ecosystems. They are vital for the pollination of cultivated plants including agricultural crops and horticultural plants. Furthermore, the plants and wildlife supported directly and indirectly through diversity of pollinators and provide other ecosystem services. Sunflower (*Helianthus annuus* L.) is an important edible oilseed crop, being a diploid having chromosome number, 2n = 34 belong to the family Asteraceae (Compositae). Presently, sunflower is cultivated in India in an area of 2.240 lakh ha with

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a production of 2.045 lakh tonnes and yield of 913 kg ha<sup>-1</sup> (Directorate of Oilseed Development, 2020). The crop is having desirable attributes such as short photoperiod insensitivity, duration, drought tolerance, low seed rate, high multiplication ratio, and highquality edible oil having high degree of polyunsaturated fatty acid content for which it has been grown in large by farmers. It is highly essential to enhance the productivity of oilseed crops like sunflower to bridge the gap of edible oil availability and demand. Improper pollination is a reason for poor seed set and filling (Free et al., 1964; Seetharam et al., 1976) where as Seetharam and Kusuma Kumari (1974) has shown shorter pollen viability as the constraint of poor seed setting. Inadequacy of pollinators and their activity to pollinate all the florets in sunflower capitulum is the major cause for poor seed setting in sunflower. The problem of poor seed setting is mostly due to lack of sufficient number of pollinators and their activity in field conditions (Seetharam et al., 1976 and Singh et al., 1977). Therefore, it is essential to know the possibility of utilization of honey bee colonies for the purpose of hybrid seed production in Odisha ecosystem. Therefore, this study was undertaken.

#### **MATERIALS AND METHODS**

The present study was undertaken during two cropping seasons *i.e.* first season (September-December, 2021) and second season (January-April, 2022) in the Experimental Station of Entomology located in the upland area of Experimental Research Field, M.S. Swaminathan School of Agriculture, Paralakhemundi, Centurion University of Technology and Management, Odisha.

The seeds of HYV sunflower cv. HY SUNFLOWER MSFH-17 were sown during 20<sup>th</sup> September, 2021 and 13<sup>th</sup> of January of Rabi 2022. The inter and intra row spacing were maintained at 45 cm and 25 cm respectively and the plot size of 4 m×5 m was maintained. A recommended dose of N<sub>2</sub>: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O at 120:60:60 kg ha<sup>-1</sup> was applied. Necessary agronomic practices were followed to maintain proper plant population and normal growth of plants. A prophylactic spraying of the insecticide Profenophos @ 2ml L<sup>-1</sup> against the infestation of early season pests at 15 and 30 days after sowing prior to onset of flowering was implemented to keep the crop away from pest and diseases infestation. The experiments were held in Randomized Block Design with three treatments replicated seven times. The performance of pollinators assessed by comparing the yields of Open Pollination (OP), Apis cerana indica pollination (HB) and Pollinator Exclusion (PE) treatments. The OP treatments were allowed for open pollination of the flowers without any restriction. The HB treatments were restricted with pollination of sunflowers only with managed A. cerana indica colonies inside the replications. The PE treatments were restricted completely from pollinators to prevent pollination by the pollinators.

Keen observation on OP treatments was taken during flowering period of sunflower where diversity of pollinators was recorded. The identification of insects was done from a large number of samples by following fixed plot survey in selected experimental sites. The collected adult insects were killed and dry preserved in the laboratory of Department of Entomology, MSSSoA (Borror *et al.*, 1981) and identified referring the identified specimen maintained in collections of AICRP on Honeybees and pollinators, Odisha University of Agriculture and Technology, Bhubaneswar. The common name, scientific name, family, order, habitat of the specimens were recorded with their foraging behaviour.

The observations on effect of the pollinators on yield attributing characters of sunflower crop *viz.*, plant height (cm), disc diameter (cm), number of seeds per capitulum, thousand (1000) seed weight (g), seed yield of plots (kg ha<sup>-1</sup>), oil content (%) and germination (%) of sunflower were recorded. The heights and disc diameters were counted by measuring tape. The number of seeds per capitulum and germination percentage was counted manually. For analyzing the 1000 seed weight, the mechanical device was used followed by weighing in weighing balance. The oil content was analyzed by Soxhlet extraction method. After getting the value in ml they were converted to percentage value for proper value representation.

#### **RESULTS AND DISCUSSION**

The results of the study revealed that the activity of different pollinators started at 10 per cent flowering stage coinciding with 45 DAS and the activity continued till the late flowering stage i.e. 84 DAS in the first season, whereas pollinators started arriving at 10 per cent flowering stage coinciding with 48 DAS and the activity continued till the late flowering stage *i.e.* 88 DAS in the second season. A great majority of sunflower plants flowered between 65 DAS to 80 DAS and the diversified activities of pollinators mostly observed during the period were recorded. Studies on pollinator diversity during both the seasons at different locations of Gajapati District, Odisha, revealed that the crop was visited by 18 types of pollinators belonging to order Hymenoptera and Lepidoptera (Apidae 66%, Nymphalidae 21%, Crambidae 7% and piridae 6%). Hymenopterans were the dominant pollinators. Adult lepidopteran pollinators also visited the plants to fulfill their dietary nectar requirement.

Stray populations of ants and true flies were also observed visiting the sunflower for their dietary requirements. The pollinators include, rock bees (Apis dorsata), Indian honeybees (Apis cerana indica), European bees (A. mellifera), little bees (A. florea), stingless bee (Tetragonula iridipennis), two species of carpenter bee (Xylocopa latipes and X. aestuans), digger bee (Amegilla zonata), two species under family vespidae i.e. Oriental hornet (Vespa orientalis) and a wasp species (V. tropica). Apart from the hymenopteran pollinators, some lepidopteran pollinators were also found visiting sunflower flowers. The butterflies viz., tawny coster (Acraea terpsicore), common crow (Euploea core), grey pansy (Junonia atlites), blue glassy tiger (Idiopsis vulgaris), plain tiger (Danaus chrysippus), lemon pansy (Junonia lemonias), common/lemon emigrant (Catopsilia pomona), and cucumber moth (Diaphania indica), were recorded during the present investigation. Similarly major pollinators observed by Nayak et al. (2021) in Odisha were two honey bee species (Apis dorsata and A. cerana indica) and one Carpenter bee (Xylocopa sp) associated with sunflower crop. Similar observations were recorded by Yasmeen *et al.* (2021) in Tamil Nadu found a total of eight species of pollinators (*Apis mellifera* Linnaeus, *A. dorsata*, *A. cerana indica* F., *Trigona iridipennis* Smith, *Vespa tropica* L. and a Hesperidae species).

Effect of pollination treatments on different yield attributing characters of sunflowers analyzed by imposing OP, HB and PE condition over two seasons revealed significant results (Table 1). The number of seeds per capitulum of sunflower showed significant variation among the treatments. The number of seeds per capitulum was maximum in the OP (398.11) followed by HB (371.71) and it was low in the PE (358.98). There was an overall increase of 10.90 per cent in OP and 3.54 per cent in HB against PE. Hemanth Kumar *et al.* (2020) also reported similar findings.

Similarly, the percentage of seed filling was highest in OP (92.98%) followed by HB (89.18%) and PE (86.71%) leaving the central area unfilled. An overall increase of 7.23 per cent in OP and 2.85 per cent in bee pollination against pollination exclusion plot was recorded. The 1000 seed weight of sunflower showed significantly higher variation among the treatments. The seed weight of 1000 seeds were more in the OP plot (53.42g) followed by the HB plot (51.48g) and PE plot (45.40g). An overall increase of 17.66 per cent in OP and 13.37 per cent in HB against PE was recorded. Similar observations were recorded by Hemanth Kumar et al. (2020) and he has revealed that Open pollinated sunflower recorded significantly higher seed index (100 seed weight) (5.82g) which was at par with sunflower pollinated with A. cerana indica (5.62g) and the lowest was reported from sunflower enclosed to avoid pollinators (5.27g). Similarly, Mehmood et al. (2018) revealed that weight of 100 seeds was maximum in case of open pollinated heads (5.04g) followed by A. mellifera pollination (4.63g). Likewise, Basavaraj et al. (2016) revealed that seed index was higher in unbagged plants of DRSF-108 (7.77g/ 100 seeds) as compared to the bagged ones (5.07g/100 seeds).

The seed yield of sunflower during both the seasons showed significantly higher variation among the treatments. OP recorded highest yield (1735.06 kg

Parameters	Year/Treatment	Open Pollination	Bee pollination	Pollination exclusion	SE(m)±	CD (0.05)
Plant height (cm)	2021	104.62	102.99	102.48	0.67	NS
	2022	107.21	105.12	104.9	0.95	NS
	Increase over Pollination exclusion (%)	2.14	0.35			
Disc diameter (cm)	2021	12.41	11.25	11.16	0.45	NS
	2022	14.21	13.62	13.02	0.4	NS
	Increase over Pollination exclusion (%)	10.09	2.81			
Number of	2021	205.01	2(0.02	256 75	(7	20.77
seeds per capitulum	2021	395.01	368.92	356./5	6./	20.67
	2022	401.21	374.49	361.21	6.04	18.62
	Pollination exclusion (%)	10.9	3.54			
1000 Seed eight (g)	2021	53.02	51.32	45.28	0.59	1.82
	2022	53.82	51.63	45.53	0.87	2.68
	Increase over Pollination exclusion (%)	17.66	13.37			
Total yield (kg/ha)	2021	1713.15	1577.62	1290.32	24.18	74.5
	2022	1756.97	1605.01	1309.18	22.11	68.12
	Increase over Pollination exclusion (%)	33.49	22.43			
Oil Content (%)	2021	39.71	35.14	34.57	2.07	NS
	2022	40.57	35.14	34.57	1.56	NS
	Increase over Pollination exclusion (%)	10.7	0.85			
Germination (%)	2021	78.57	77.43	76.29	1.79	NS
	2022	80	79.71	76.86	1.49	NS
	Increase over Pollination exclusion (%)	3.55	2.61			

Table 1. Effect of pollination treatments on different parameters of sunflower in both the seasons

ha<sup>-1</sup>) followed by HB plot (1591.32 kg ha<sup>-1</sup>) and PE plot (1299.75 kg ha<sup>-1</sup>). An overall increase of 33.49 per cent in OP and 22.43 per cent in HB against PE plot. Thomas et al. (2018) revealed that pollinators played a major role in increasing sunflower yield up to 40 per cent. Basavaraj et al. (2016) revealed that seed yield was higher in open pollinated DRSF-108 plants (0.95 kg/34 plants) as compared to the bagged plants (0.07 kg/34 plants). Nderitu et al. (2008) also revealed that the plots where insect visitors had access produced on average 53 per cent more seed yield compared with plots where insect visitors were excluded. Mehmood et al. (2007) have recorded bee pollination increased sunflower seed number by 59 per cent. Significantly highest seed yield of sunflower with honey bee colonies at Dharwad was recorded by Patil (2013). Suryanarayana et al. (1987) reported significant increase in seed yield, number of filled seeds and percent seed set due to pollination by honey bees.

Effects of pollination treatments on plant height of sunflower and disc diameter of the capitulum was done by covering the respective treatment plots with nylon mesh but the plants of different treatments were not having any significant differences among the treatments. Oil content and germination percentage also showed no significant variation among the pollination treatments. The oil content percentage was recorded highest in the open pollination plot (40.14%) followed by the bee pollination plot (36.58%) and pollination exclusion plot (36.26%). Hemanth Kumar et al. (2020) reported similar observations on sunflower hybrid KBSH-44 with no significant difference in oil content. Though Basavaraj et al. (2016) recorded higher oil content in the unbagged DRSF108 plants (40.41%) as compared to the bagged plants (38.81%) but they were at par statistically.

The germination percentage among different treatments showed no significant variation where it was recorded highest in the open pollination plot with 79.29 per cent germination followed by the bee pollination plot (78.57%) and pollination exclusion plot (76.57%). These finding are in corroboration with the findings of Nderitu *et al.* 

(2008). Hemanth Kumar *et al.* (2020) recorded similar trend in germination percent. These finding are in corroboration with the findings of Nderitu *et al.* (2008).

The oil seed crop sunflower is an important source of pollinators' dietary requirement attracting 18 numbers of pollinators. The production capacity of sunflowers can be easily increased by a minimum of 33 per cent by conserving the natural pollinators. Pollination by the honeybees showed positive impact on increasing the number of seeds per capitulum, seed filling percentage, test weight and seed yield of sunflower. The Indian honeybees, *Apis cerana indica* colonies can be recommended to utilize effectively inside the sunflower ecosystem to enhance the productivity of the crop.

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# Influence of rice grain traits on susceptibility to the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae)

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**ABSTRACT:** Rice grains of 20 varieties were tested for their physical and biochemical basis of resistance against lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae). Physical parameters such as grain length x breadth, thousand seed weight, and grain hardness exhibited significant differences among tested varieties. Grain hardness conferred resistance significantly by resulting in delayed adult emergence (-0.519), and weight loss per cent (-0.603). Biochemical parameters such as total carbohydrates and proteins showed significant differences among the varieties. Total carbohydrate was positive and significantly correlated with adult emergence, but with median development period negatively correlated with the median development period. © 2024 Association for Advancement of Entomology

**KEY WORDS**: Adult emergence, development period, susceptibility index, physical-biochemical factors

#### **INTRODUCTION**

Rice is the most commonly grown cereal with high food value worldwide. Rice grains are stored for various reasons, including food safety, seed purpose, and trading to make monetary gains. In tropical agriculture conditions, lack of suitable storage facilities and the high humidity, losses of cereal grains can exceed 30 per cent due to insect pests (Ramputh *et al.*, 1999). *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae) infestation alone can diminish the weight of brown rice by around 40 per cent (Smith, 1989). Globally in cereal grains, *R. dominica* is one of the important stored pests,

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and it reduces the kernels to the pericarp by feeding (Winterbottom 1922; Cambell and Sinha 1976). Eggs are laid on the surface of the grain by female and first instar larvae enter into the kernels, choosing broken grains or germ portion. Larvae and adults of R. dominica spend the majority of their time inside the kernel, feeding on the endosperm and germ, which directly affects the physicochemical characteristics of the grain and results in damage (Edde, 2012). The host-plant variety selection that hinders insect growth could be a substitute for treatment with insecticides for any cereal crop that suffer from post-harvest insect pests. A study was undertaken to assess a selection of rice varieties for host-plant resistance to R. dominica and determine any specific physical or chemical characteristics of the grains that may contribute to this resistance.

### **MATERIALS AND METHODS**

The experiment was carried out with 20 rice varieties (Table 1) in the Department of Entomology, MSSSoA, Parlakhemundi from 2021 to 2022. The selected varieties of rice were obtained from ICAR - National Rice Research Institute, Cuttack; while RNR-15048 was obtained from the Seed Unit at Centurion University. These varieties were kept in the oven for disinfestation at a temperature of 55°C for 4 hours to eliminate the hidden infestation if any, without affecting the viability of the seeds (Singh, 1989). The grains were kept in a desiccator with KOH solution (51g of KOH per 100 ml of water) for 21 days after disinfection to raise the moisture content near equilibrium (Solomon, 1951). This pre-conditioned seed material was used for screening.

The culture of *R. dominica* was obtained from the storage Entomology laboratory, Centurion University. Beetles were mass multiplied throughout the experimental period in the laboratory on rice variety, RNR-15048 and maintained (at  $27\pm2^{\circ}$ C and  $65\pm5\%$  RH). Five pairs of newly emerging adults of *R. dominica* were released into each plastic jar of 20g healthy seeds from each variety and replicated thrice in a Complete Randomized Design. Muslin cloth was used to cover the jar

mouth. The insects were removed after seven days of oviposition, and the jars were then maintained under the same experimental conditions. Data were analysed using the SPSS software version 16.0. The following parameters were used to compare the susceptibility of different rice varieties to *R. dominica*.

**Developmental parameters:** The development of beetle was indirectly determined by the index of susceptibility. It was an important characteristic which was calculated using the method of Dobie (1974) where 0–3 (resistant), 4–7 (moderately resistant), 8–10 (susceptible) and>11 (highly susceptible). The growth index a key factor to determine host suitability was calculated (Tripathi *et al.*, 2012; Soumia *et al.*, 2015) and grain weight loss per cent was done by count and weight method (Gwinner and Harnish, 1996).

**Physical parameters of rice varieties:** The length and breadth of grain measured using digital vernier callipers were expressed in millimetres (mm). Likewise, thousand seed weight (TSW) was also recorded for each variety and was counted and weighed per replication with the help of electronic balance. The hardness of the grains was determined by the pressure exertion method using a texture analyser (Brookfield, Model: CT3 10K, USA). On a flat plate, a single kernel was compressed using a 12mm flat probe at a crosshead speed of 50mm per min. Hardness (N) a measurement of the force necessary to rupture (first break) a grain of rice was recorded, in three replications (Taghinezhad *et al.*, 2016).

**Biochemical Properties of rice varieties:** Standard and widely accepted methods were used to estimate the amount of soluble protein (Lowry *et al.*, 1951). Similarly, the estimation of carbohydrates (also known as sugars) was carried out as per Hedge and Hofreiter (1962).

### **RESULTS AND DISCUSSION**

**Insect development characters:** In test varieties of rice, there were significant differences in adult emergence, median developmental period, susceptibility index, growth index, weight losses per cent towards insect response (Table 1). Maximum adult emergence was found in RNR-15048 (24.67) whereas the least emergence was found in Sneha (2.33) followed by CR Dhan 206 (3.00) and CR Dhan 201(3.67). The highest developmental period was observed in CR Dhan 201(46.67days) and the minimum developmental period was observed in CR Dhan 205 (35 days) followed by RNR-15048(35.33 days). The susceptibility index ranged from 1.94 (Sneha) to 9.08 (RNR-15048). Out of twenty rice varieties, Sneha, CR Dhan 206 and CR Dhan 201 were found to be resistant whereas RNR-15048 variety is susceptible as per Dobie (1974). Resistance/ susceptibility reaction among the varieties was observed as per growth index. It ranged from 0.05 for variety Sneha and 0.70 for RNR-15048. The highest per cent of weight loss was found in variety RNR-15048 (15.75), while Sneha registered minimum weight loss (1.47).

**Physical parameters of rice varieties:** The thousand seed weight of the test varieties varied from 13.13 to 25.20g. The highest thousand seed weight was noticed in CR Dhan209 (25.20g)

Rice varieties	Adult emergence (No)	MD	SI	GI	Weight loss
CR Dhan 40	4.67 *(2.27) <sup>jkl</sup>	43.00 <sup>b</sup>	3.57 <sup>h</sup>	0.11 <sup>def</sup>	2.88 *(1.84) <sup>j</sup>
CR Dhan 101	6.33 (2.61) <sup>ghij</sup>	40.67 <sup>de</sup>	4.54 <sup>fg</sup>	0.16 <sup>cdef</sup>	$5.27(2.40)^{i}$
CR Dhan 200	10.00 (3.24) <sup>cde</sup>	39.67 <sup>ef</sup>	5.81 <sup>cd</sup>	0.25 <sup>bcdef</sup>	8.24 (2.96) <sup>ef</sup>
CR Dhan 201	3.67 (2.04) <sup>klmn</sup>	46.67ª	2.76 <sup>i</sup>	0.08 <sup>ef</sup>	2.08 (1.60) <sup>k</sup>
CR Dhan 202	7.33 (2.80) <sup>fgh</sup>	36.00 <sup>h</sup>	5.53 <sup>de</sup>	0.2 <sup>cdef</sup>	8.14 (2.94) <sup>efg</sup>
CR Dhan 203	4.67 (2.27) <sup>jklm</sup>	38.00 <sup>g</sup>	4.04 <sup>gh</sup>	0.12 <sup>def</sup>	3.44 (1.99) <sup>j</sup>
CR Dhan 204	6.67 (2.68) <sup>fghij</sup>	37.67 <sup>g</sup>	5.03 <sup>ef</sup>	0.18 <sup>cdef</sup>	7.98 (2.91) <sup>efg</sup>
CR Dhan 205	12.00 (3.54)°	35.00 <sup>h</sup>	7.1 <sup>b</sup>	0.34 <sup>bc</sup>	10.85 (3.37) <sup>b</sup>
CR Dhan 206	3.00 (1.87) <sup>1n</sup>	40.00 <sup>e</sup>	2.75 <sup>i</sup>	0.08 <sup>ef</sup>	1.93 (1.56) <sup>k</sup>
CR Dhan 207	8.67 (3.03) <sup>def</sup>	38.67 <sup>fg</sup>	5.58 <sup>de</sup>	0.22 <sup>cdef</sup>	8.38 (2.98) <sup>de</sup>
CR Dhan 209	11.00 (3.39) <sup>cd</sup>	38.00 <sup>g</sup>	6.31°	0.29 <sup>bcd</sup>	9.86 (3.22) <sup>bcd</sup>
Virender	$7.00(2.74)^{\text{fghi}}$	41.67 <sup>cd</sup>	4.67 <sup>fg</sup>	$0.17^{\text{cdef}}$	6.85 (2.71) <sup>fgh</sup>
Hazari dhan	5.67 (2.48) <sup>hij</sup>	41.33 <sup>d</sup>	4.19 <sup>gh</sup>	0.14 <sup>def</sup>	5.61 (2.47) <sup>hi</sup>
Santha bhima	5.67 (2.48) <sup>hij</sup>	39.67 <sup>ef</sup>	4.37 <sup>fg</sup>	0.14 <sup>def</sup>	6.79 (2.70) <sup>gh</sup>
Sneha	2.33 (1.68) <sup>n</sup>	42.67 <sup>bc</sup>	1.94 <sup>j</sup>	0.05 <sup>f</sup>	1.47(1.40) <sup>k</sup>
Sadabahar	16.00 (4.06) <sup>b</sup>	37.67 <sup>g</sup>	7.36 <sup>b</sup>	0.42 <sup>b</sup>	10.38 (3.30) <sup>bc</sup>
IR-64 DIRT	8.33 (2.97) <sup>efg</sup>	35.67 <sup>h</sup>	5.94 <sup>cd</sup>	0.23 <sup>cdef</sup>	8.79 (3.05) <sup>cde</sup>
Anjali	5.00 (2.35) <sup>ijk</sup>	37.67 <sup>g</sup>	4.27 <sup>g</sup>	0.13 <sup>def</sup>	6.19 (2.59) <sup>hi</sup>
Abhishek	10.33 (3.29) <sup>cde</sup>	37.67 <sup>g</sup>	6.2 <sup>cd</sup>	0.27 <sup>bcde</sup>	9.55 (3.17) <sup>bcde</sup>
RNR-15048	24.67 (5.02) <sup>a</sup>	35.33 <sup>h</sup>	9.08ª	0.70ª	15.75 (4.03) <sup>a</sup>

Table 1. Development parameters of R. dominica in grains of rice varieties

The values in parentheses are transformed \*  $\sqrt{x + 0.5}$  value; DAS: Days after storage; Any two means having a common letter are not significantly different at the 5% level of significance by DMRT. MD - Median development (days); SI - Susceptibility index; G I - Growth Index; Weight loss per cent at 120DAS

followed by Hazari dhan (25.07g) and Anjali (24.37g) whereas the lowest seed weight was noticed in RNR-15048 (13.13g) followed by Sneha (16.9g) and CR Dhan 40 (18.21g) (Table 2). Thousand seed weight was found to have a non-significant and negative effect on susceptibility index (r = -0.283), growth index (r = -0.514), weight loss per cent (r = -0.264), and adult emergence (r = -0.494), but non-significant and positive response with median development period (r = 0.171) (Table 3).

The grain length x breadth ranged from 14.62 to 26.27mm. The maximum length x breadth was noticed in CR Dhan 200 (26.27mm) followed by Santha Bhima (25.99mm) and CR Dhan 207 (24.33mm). Minimum was noticed in RNR-15048 (14.62mm) followed by Abhishek (17.41mm) and CR Dhan 40 (18.20mm) (Table 2). The adult emergence (r=-0.341), susceptibility index (r=-0.162), growth index (r = -0.361), and weight loss per cent (r = -0.123) exhibited negative non-significant relation with grain length x breadth; But non-significant and positive correlation with the median development period (r = 0.089) (Table 3).

The hardness of tested varieties ranged from 128.00 (Sneha) to 83.67 N (CR Dhan204). The highest grain hardness was noticed in Sneha (128.00 N), CR Dhan 206 (126.33N) and CR Dhan 201 (125.33N), whereas the lowest grain hardness was noticed in CR Dhan 204 (83.67N) followed by RNR-15048 (101.33N) and Sadabahar (102.33N) (Table 2). Grain hardness showed significant negative correlation with susceptibility index (r=-0.577), adult emergence (r=-0.519), growth index (r=-0.522), and weight loss per cent (r=-0.603) whereas positive and non-significant relation with median development period (r=0.499) (Table 3).

**Biochemical parameters of rice varieties:** The carbohydrates in different rice varieties ranged from 64.32 to 78.03 per cent with significant differences. The highest carbohydrates were noticed in RNR-15048 (78.03%) followed by Virender (76.14%), Sadabahar (74.67%), and CR Dhan209 (73.24%), whereas the lowest carbohydrates were in Sneha (64.32%) followed by CR Dhan 200 (65.30%) (Table 2). Total carbohydrates were positive and significantly correlated with susceptibility index

(r = 0.691), growth index (r = 0.751), weight loss per cent (r = 0.682), and adult emergence (r = 0.765) whereas negative and non-significant relation with median development period (r = -0.274).

Protein content in test rice varieties ranged from 9.3 to 6.2 per cent. The varieties CR Dhan 206 (6.2%) and CR Dhan205 (6.5%) had the lowest protein per cent, while CR Dhan205 had the greatest protein per cent (9.3%), followed by Sadabahar (8.5%) and RNR-15048 (8.3%). All the insect developmental characters *viz*, adult emergence (r = 0.611), susceptibility index (r = 0.606), growth index (r=0.604), and weight loss per cent (r = 0.557) were positively correlated with protein content except median development period (r = -0.272) which was non-significant and negatively related (Table 3).

These findings are in line with Singh *et al.* (1984) who reported that the number of adult emergences determines the extent of their damage and subsequently grains permitting more rapid adult emergence will be more extensively damaged. Samyal et al. (2006) and Sayed et al. (2006) stated that the maximum population, per cent loss in weight was supported by the highly susceptible variety, whereas, the lowest population and minimum loss had the least susceptible variety. According to Swamy et al. (2022) in BPT2411 R. dominica emergence was the least in under-free choice (13.00 adults/100g) and non-choice (16.33 adults/ 100g) conditions. Singh et al. (2001) observed that the developmental period of R. dominica varied from 39.82-43.29 days on different wheat varieties. Bhargava and Hussain (2014) reported that grain damage per cent and weight loss per cent in wheat varieties varies against R. dominica to the extent of 17.32 to 45.79 and 6.15 to 18.50, respectively.

Prasad *et al.* (2015) reported that seed weight and *Sitophilus* emergence were negatively correlated in sorghum grains in accordance with the current results. Stejskal and Kucerova (1996) and Prasad *et al.* (2015) corroborated the current findings that the size of the grain in wheat and sorghum is negatively correlated with weevil emergence. In contrast to several of the earlier studies, Segrove (1951), Haine (1991) and Campbell (2002) reported

Variety	1000 seed weight (g)	Grain length X breadth (mm)	Grain x hardness (N)	Carb (%)	Protein (%)
CR Dhan 40	18.21 <sup>i</sup>	18.20 <sup>k</sup>	117.67 <sup>cde</sup>	66.69 <sup>jkl</sup>	7.0 <sup>ef</sup>
CR Dhan 101	21.93 <sup>def</sup>	20.47 <sup>fgh</sup>	118.33 <sup>cd</sup>	68.66 <sup>g</sup>	7.9 <sup>cd</sup>
CR Dhan 200	22.97 <sup>bcd</sup>	26.27ª	116.67 <sup>e</sup>	66.07 <sup>lmn</sup>	7.4 <sup>de</sup>
CR Dhan 201	23.33 <sup>bcd</sup>	22.32 <sup>d</sup>	125.33 <sup>b</sup>	68.42 <sup>gh</sup>	7.6 <sup>d</sup>
CR Dhan 202	22.07 <sup>cdef</sup>	23.34°	114.65 <sup>f</sup>	65.3 <sup>n</sup>	7.8 <sup>d</sup>
CR Dhan 203	22.00 <sup>cdef</sup>	19.39 <sup>ij</sup>	117.33 <sup>cde</sup>	65.75 <sup>mn</sup>	6.8 <sup>f</sup>
CR Dhan 204	20.17 <sup>gh</sup>	21.08 <sup>efg</sup>	83.67 <sup>i</sup>	66.89 <sup>jk</sup>	6.5 <sup>fg</sup>
CR Dhan 205	18.42 <sup>i</sup>	21.44 <sup>def</sup>	117.67 <sup>cde</sup>	71.29°	9.3ª
CR Dhan 206	20.77 <sup>fg</sup>	19.92 <sup>hij</sup>	126.33 <sup>b</sup>	68.32 <sup>gh</sup>	6.2 <sup>g</sup>
CR Dhan 207	23.00 <sup>bcd</sup>	24.33 <sup>b</sup>	115.00 <sup>f</sup>	70.27 <sup>f</sup>	6.6 <sup>fg</sup>
CR Dhan 209	25.20ª	21.84 <sup>de</sup>	113.33 <sup>g</sup>	73.24 <sup>d</sup>	7.6 <sup>d</sup>
Virender	22.40 <sup>cde</sup>	20.16 <sup>ghi</sup>	118.67°	76.14 <sup>b</sup>	7.6 <sup>d</sup>
Hazari dhan	25.07ª	20.46 <sup>fgh</sup>	118.00 <sup>cde</sup>	67.82 <sup>hi</sup>	6.5 <sup>fg</sup>
Santha Bhima	21.87 <sup>def</sup>	25.99ª	115.33 <sup>f</sup>	67.14 <sup>ij</sup>	7.5 <sup>d</sup>
Sneha	16.90 <sup>j</sup>	19.11 <sup>j</sup>	128.00ª	64.32°	6.8 <sup>f</sup>
Sadabahar	19.03 <sup>hi</sup>	20.13 <sup>ghi</sup>	102.33 <sup>h</sup>	74.67°	8.5 <sup>b</sup>
IR-64 DIRT	23.45 <sup>bc</sup>	21.13 <sup>ef</sup>	114.33 <sup>fg</sup>	69.02 <sup>g</sup>	6.5 <sup>fg</sup>
Anjali	24.37 <sup>ab</sup>	21.2 <sup>ef</sup>	117.00 <sup>de</sup>	66.22 <sup>klm</sup>	6.8 <sup>f</sup>
Abhishek	21.21 <sup>efg</sup>	17.41 <sup>k</sup>	115.33 <sup>f</sup>	72.58 <sup>d</sup>	7.5 <sup>d</sup>
RNR-15048	13.13 <sup>k</sup>	14.62 <sup>1</sup>	101.33 <sup>h</sup>	78.03ª	8.3 <sup>bc</sup>

Table 2. Physical and biochemical characteristics of grains of rice varieties

Any two means having a common letter are not significantly different at 5% level of significance by DMRT.

that larger grains have more progeny and better larval survival than small grains. The current findings concur with Swamy *et al.* (2022) who reported that rice variety BPT 2411 had the highest kernel hardness (7.28 kgf) and least favoured grain for the stored insects tested, including the lesser grain borer and angoumois grain moth, in rough rice. Insect fitness was found to be inversely correlated with kernel hardness (Morallo-Rejesus *et al.*, 1982). According to Astuti *et al.* (2013), in rice kernels grain hardness, amylose content, nonchalkiness, and phenol content all are associated to the variety's resistance to stored rice insects. Grain hardness is one of the characteristics that influence *R. dominica* infestation, according to Keskin and Ozkaya (2013). Mechanical structures of the rice grain varieties have been related to the resistance (Lale and Yusuf, 2001; Ashamo, 2001 and Lale and Kartay, 2006).

The results of the present experiments are in line with that of Kumar *et al.* (2020) who observed that developmental features of *R. dominica* had a significant positive correlation with ash, carbohydrate, and fat contents whereas significant negative association of surface wax, total phenol,

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Character	AE	MDP	SI	GI	WL	GLB	TSW	GH	Carbohydrate	Protein
AE	1.000									
MDP	-0.587**	1.000								
SI	0.934**	-0.754**	1.000							
GI	0.998**	-0.616**	0.933**	1.000						
WL	0.912**	-0.754**	0.977**	0.914**	1.000					
GLB	-0.341 <sup>NS</sup>	0.089 <sup>NS</sup>	-0.162 <sup>NS</sup>	-0.361 <sup>NS</sup>	-0.123 <sup>NS</sup>	1.000				
TSW	-0.494*	0.171 <sup>NS</sup>	-0.283 <sup>NS</sup>	-0.514*	-0.264 <sup>NS</sup>	0.599**	1.000			
GH	-0.519*	0.499*	-0.577**	-0.522*	-0.603**	0.124 <sup>NS</sup>	0.251 <sup>NS</sup>	1.000		
Carb	0.765**	-0.274 <sup>NS</sup>	0.691**	0.751**	0.682**	-0.428 <sup>NS</sup>	-0.287 <sup>NS</sup>	-0.289 <sup>NS</sup>	1.000	
Protein	0.611**	-0.272 <sup>NS</sup>	0.606**	0.604**	0.553*	-0.072 <sup>NS</sup>	-0.376 <sup>NS</sup>	-0.112 <sup>NS</sup>	0.522*	1.000

 Table 3. Correlation between physical and biochemical characteristics of rice varieties and development of *R. dominica*

AE = Adult emergence, MDP = Median developmental period, SI= Susceptibility index, GI= Growth index, WL =Weight loss per cent, GLB =Grain length x breadth, TSW= Thousand seed weight, GH= grain hardness; \*Significant at 0.05 level, \*\*Significant at 0.01 level

and total hull per cent in brown rice. The current findings concur with that of Rekha et al. (2017), who found a positive association between growth index (0.354), adult emergence (0.384), oviposition (0.394), pod damage (0.522), and weight loss (0.819) of Carvedon serratus with total soluble sugars of pods. The present findings corroborate with Swamy et al. (2022), who observed that the grain hardness and low protein content of variety BPT 2411 might be the key reasons for its least fitness for the progeny development of lesser grain borer, angoumois grain moth in rough rice, and red flour beetle in milled rice. The current results are also consistent with those of Swamy et al. (2022) who reported the least progeny development in stored insects such as lesser grain borer (13.0 adults/100g), and red flour beetle (23.33 adults/ 100g) were found due to grain hardness and low protein content in BPT 2411cultivar under freechoice conditions. Additionally, our results are in good agreement with Nemati et al. (2018) that the adult longevity and fecundity of R. dominica fed on barley cultivars were positively correlated to the protein content, showing that these parameters have a significant influence on the fitness of this insect. The results of the present study are in conformity with Sahoo and Sahoo (2016) who found that the protein content was positively correlated with grain damage per cent and adult emergence 0.878 and 0.827 respectively by *Sitophilus oryzae*. Additionally, our findings closely align with those of Murad and Batool (2017) who reported that high protein content varieties were highly susceptible to *Sitotroga cerealella and Sitophilus oryzae* (Soujanya *et al.*, 2017).

The experimental results suggest that resistant varieties have the least adult emergence, grain damage per cent and weight loss per cent and prolonged median developmental period. Physiochemical parameters such as grain hardness contributed to resistance while carbohydrates and proteins have a positive effect on *R. dominica* development.

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# Effects of sublethal concentration of Imidacloprid on the enzyme activity of sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera, Brentidae)

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**ABSTRACT:** Application of sublethal (LC<sub>10</sub> and LC<sub>30</sub>) dose of Imidacloprid on sweet potato weevil was found to have inhibitory effect on its enzymes *viz.*, glutathione reductase (GR), glutathione peroxidase (GPx), reduced glutathione (GSH) and glutathione S-transferase (GST), while activity of superoxide dismutase (SOD) and lipid peroxidase (LPx) was up regulated when compared to control. The weevil's expression of SOD increased by 13.53 and 69.44 and LPx by 67.38 and 73.04 per cent respectively, when the sublethal dose was raised from LC<sub>10</sub> to LC<sub>30</sub>. Although GST and GPX did not alter considerably after exposure to the sublethal doses of imidacloprid, weevil activity of GR (65.5-78.1%) and GSH (42.2 and 61.6%) decreased significantly. © 2024 Association for Advancement of Entomology

**KEY WORDS:** Glutathione reductase, glutathione peroxidase, reduced glutathione, glutathione Stransferase, superoxide dismutase, lipid peroxidase

#### INTRODUCTION

Sweet potato, (*Ipomoea batatas*), as it is grown by subsistence farmers, is known as the "poor man's crop", and it is ranked as the seventh largest food crop in the world after wheat, rice, maize, potato, barely and cassava (Narayan *et al.*, 2022). Cultivation of sweet potato is prevalent in all most the states of India; however, majority of the nation's supply is from Odisha, Kerala, West Bengal, and Uttar Pradesh (Palaniswami *et al.*, 1991; Prakash *et al.*, 2020). Sweet potato weevil (SPW), *Cylas formicarius* (Fabricius) (Coleoptera, Brentidae), is considered to be the deadliest insect pest, inflicting significant damage to sweet potato tubers that can occasionally reach 100 per cent (Palaniswami and Chattopadhyay, 2005; Prasad *et al.*, 2022). Grub of SPW excavates tunnels and feeds, while the adult feeds petioles and leaves. Chemical pesticides have historically been used to suppress SPW (Palaniswami and Mohandas, 1996; Zhang *et al.*, 2013). When the neonicotinoid imidacloprid comes in contact with an insect pest, its central nervous system is damaged and its nicotinic acetylcholine receptors are disturbed (Jeschke *et al.*, 2011; Le Goff and Giraudo, 2019). According to Elbert *et al.* (2008) Imidacloprid has a systemic action that helps to a variety of piercing-sucking insect pests, chewing pests, and soil-dwelling arthropods.

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Neonicotinoid pesticides impact the lifespan, feeding activity, larval duration, reproduction, and activity of the detoxifying enzymes of exposed insects at sublethal doses (Tan et al., 2012; De Franca et al., 2017). Insects may occasionally develop resistance to insecticides when subjected to sublethal concentrations of these chemicals. Sublethal doses of insecticides have an impact on the activity of detoxifying enzyme in a variety of insects (Jing et al., 2011; He et al., 2013; Lu et al., 2016); nevertheless, the literature review did not yield the same as in the case of C. formicarius. The current work ascertains that the sublethal exposure of imidacloprid to SPW activates several enzymes, including super oxide dismutase, lipid peroxidase, but reduces the activity of glutathione peroxidase, glutathione reductase, glutathione S-transferase and reduced glutathione.

#### **MATERIALS AND METHODS**

Sweet potato tubers infected with SPW collected from the markets and fields of the ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram were stored in one-litre plastic containers. Muslin cloth was used to cover the container's mouth. The container was filled with newspaper scraps to absorb the water that was released by tubers. As the adults emerged, they were collected, and their culture was maintained on fresh tubers (at 32°C and 75% RH).

Imidacloprid was diluted to five different concentrations 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1 and 0.5 per cent in ordinary water. Using a micro applicator,  $50\mu$ l of the aliquot was topically delivered to each of the 20 adults of 2-week-old. Three replications were kept for each treatment. In the control group, Imidacloprid was replaced with water. Mortality of the weevil was observed 24 hours after treatment (HAT), and LC<sub>10</sub>, LC<sub>30</sub> and LC<sub>50</sub> were calculated using Probit regression analysis (Finney, 1971).

Assessment of enzyme activities: The activity of six detoxifying enzymes *viz*, superoxide dismutase (SOD), lipid peroxidase (LP), glutathione reductase (GR), glutathione peroxidase (Gpx), reduced glutathione (GSH) and glutathione Stransferase (GST) were identified for the current investigation.

SOD The activity of SOD was assayed by the procedure adopted by Misra and Fridovich (1977).

LPx assay is based on the reaction of Malondialdehyde (MDA) with of Thiobarbituric acid (TBA) forming an MDA-TBA adducts (Ohkawa *et al.*, 1979).

GR was assayed by the procedure adopted by David and Richard (1983).

GPx catalyses the oxidation of reduced glutathione (GSH) to oxidized form which reacts with Nicotinamide Adenine Dinucleotide Phosphate (NADPH) and gets converted to NADP and two molecules of reduced glutathione which is measured by spectrophotometer at 340 nm (Wendel, 1980).

GSH was estimated as described by Moron *et al.* (1979).

The activity of GST in treated test insects were assayed by the procedure adopted from Mannervik (1985).

The total protein content of the SPW estimated by Lowry's method (Lowry *et al.*, 1951) using bovine serum albumin (Sigma) as a standard.

Data were subjected to analysis of variance (ANOVA) using SPSS version 17. The mean values of data were tested with Fisher's Least Significant Difference (LSD) multiple comparison tests were performed to assess the significance of Imidacloprid effects on enzyme activity (P<0.05).

#### **RESULTS AND DISCUSSION**

**Bioassay and determination of sublethal concentrations:** Mortality of adult SPW increased with an increase in the concentration of imidacloprid. Exposure at lethal and sublethal doses revealed the  $LC_{50}$  to be 0.001 ml L<sup>-1</sup>, whereas the  $LC_{10}$  and  $LC_{30}$  were 0.0001 and 0.0006 ml L<sup>-1</sup>, respectively; and these concentrations were used for further studies.

Effect of sublethal concentrations on enzyme activity: Adult SPW showed a substantial (P<0.001) variation in SOD activity between the treatment and control batches following the sublethal doses of imidacloprid administration. The SOD activity of SPW dramatically increased from its control value of 16.62±0.03 to 18.39±0.20 and 28.16±0.47, respectively, after being treated with imidacloprid at LC<sub>10</sub> and LC<sub>30</sub> (Fig. 1a) Sublethal concentrations of imidacloprid significantly (P<0.001) up regulated the activity of lipid peroxidation in SPW (Fig.1b). The MDA unit in the untreated batches of SPW was recorded to be 75.40±0.90 mg<sup>-1</sup> protein, but it significantly increased to 231.21±0.70 and 279.7±0.8 mg<sup>-1</sup>protein in the treatments of  $LC_{10}$  and  $LC_{30}$  of imidacloprid. Activity of the GPx in SPW was found significantly (P<0.001) varied in the treatment with imidacloprid (Fig.1c). The enzyme mg<sup>-1</sup>protein in the untreated SPW was  $1.56\pm0.04$ , whereas it decreased to  $0.29\pm0.01$  and  $0.14\pm0.01$ , respectively in the treatments with LC<sub>10</sub> and LC<sub>30</sub> concentrations of imidacloprid. The activity of GR was estimated by assessing the amount of NADPH utilized by the enzyme to produce reduced glutathione. The oxidation of NADPH in the untreated SPW was 1.89±0.10µmoles mg<sup>-1</sup>protein, whereas it was significantly decreased to  $0.37\pm0.03$  in LC<sub>10</sub> and  $0.45\pm0.02\mu$  moles in LC<sub>30</sub> concentrations of imidacloprid; however, this variation was statistically not significant (P<0.001) (Fig.1d). A decrease in the GST's detoxifying activity was observed in SPW when treated with sublethal concentration of Imidacloprid than the control (Fig.1e). In the case of untreated batch of SPW, the enzyme activity was  $3.25\pm0.01$  mg<sup>-1</sup> protein, whereas it was  $0.18\pm0.01$  and  $0.17\pm0.01$ , respectively when treated with  $LC_{10}$  and  $LC_{30}$ . The GSH level was significantly (P<0.001) decreased in the treated insects (Fig.1f). In the case of untreated SPW the level of GSH was 654.67±0.36 units mg<sup>-1</sup>protein and it was 480.56±0.38 and 145.07 $\pm$ 0.54, respectively when treated with LC<sub>10</sub> and LC<sub>30</sub> concentration.

Insects are exposed to a variety of xenobiotic toxins throughout their lives; some are made by plants in their natural condition, such as allelochemicals, while others take in the form of insecticides. In spite of this, insects have developed a wide range of detoxification strategies to fight the natural poisons. In some circumstances, the same mechanisms help insects resist insecticides; although the extent and type of processes vary significantly. Understanding detoxification enables one to decipher agricultural plants' chemical defence mechanisms and to choose more effective insecticides. Detoxifying enzymes play a vital role in the insect resistance mechanisms, and a variation in their activities can be seen during insecticide metabolism (Feng et al., 2018; Jin et al., 2019). Reactive oxygen species (ROS), which are produced when synthetic insecticides are applied, can cause oxidative stress in insect cells. SODs are ubiquitous enzymes that serve as an organism's first line of defence against oxygen free radicals. According to Yamamoto and Yamaguchi (2022) SOD can shield healthy cells from ROS and eliminate superoxide radicals (O2) through the process of dismutation to oxygen and hydrogen peroxide. Imidacloprid treatment at the two sublethal concentrations enhanced the activity of SOD of SPW (13.53 and 69.44%, respectively), when compared to the control. The increased rate of SOD indicates the detoxification of imidacloprid in SPW by removing the superoxide radicals  $(O_2)$ -) through the process of dismutation to oxygen and hydrogen peroxide. An increased level of SOD is an indicative of SPW's attempt to respond to an oxidative stress condition. Elevations of SOD due to the exposure of imidacloprid in different species have already been reported in insects as well as mammals. These were reports by El Gendy *et al.* (2010) in male mice, Kapoor et al. (2010) in rat, Sun et al. (2015) in Coloana cinerea, Yang et al. (2015) in Harmonia axyridis, Zhu et al. (2015) in Aphidius gifuensis, Wang et al. (2016) in Ambrostoma quadriimopressum and Balieira et al. (2018) in Apis mellifera. Whereas some studies show the inhibitory effect of imidacloprid stress in some insects. Zhou et al. (2017) noted a down regulation in SOD level in Aphidius gifuensis and Zhang et al. (2020) in Frankliniella occidentalis and F. intonsa when treated with Imidacloprid. Kolawole et al. (2014) explained this was due to the limited efficiency of SOD in some species to scavenge the accumulated O<sub>2</sub> radicals

in cell on prolonged treatment of insecticides.

Increased lipid peroxidation is a sign of the oxidative breakdown of cell membrane lipids, which results in cell damage under pesticide stress. According to Gawel et al. (2004) the presence of malondialdehyde (MDA) is a sign of LPO and, subsequently, oxidative stress. Imidacloprid exposure caused the lipid peroxidation rate in SPW to increase it by 3.3 and 5.4 times, respectively, compared to untreated batches. El-Gendy et al. (2010), Kapoor et al. (2010), Bal et al. (2012), Balieira et al. (2018), and Ndonwi et al. (2019) reported that imidacloprid treatment increases the concentration of MDA in various animal tissues. Gauthier et al. (2018) found that imidacloprid and thiamethoxam treatment increased lipid peroxidation in susceptible Apis mellifera. The presence of a significant amount of LPO in the treated SPW tissue indicated that the metabolism of imidacloprid resulted in the production of oxidative metabolites or free radicals, which may have the potential to cause progressive chain reactions. Lipid peroxidation has a pivotal role to determine the longevity of insects, when it rises above the critical level, it may culminate into the death of the insect; however, if it falls below the threshold level, the insect may live longer (Gawel *et al.*, 2004).

GSH and GPx support cellular defence by eliminating membrane phospholipid hydroperoxides. Members of the glutathione peroxidase (GPx) family play a critical role in antioxidant defense by converting organic hydroperoxides and/or hydrogen peroxide to water and/or their corresponding alcohols (Masella *et al.*, 2005). The current investigation revealed that GPx, a crucial mechanism of pesticide resistance, dropped to between 81 and 91 per cent when SPW was subjected to sublethal doses of imidacloprid, which



Fig. 1 Activities of detoxifying enzymes a- SOD, b- LPx, c- GPx, d- GR, e- GST, f- GSH in *Cylas formicarius* on the exposure to sublethal concentrations of imidacloprid

shows that GPx has a negative tolerance or resistant to imidacloprid. Contrary to the current findings, exposure to imidacloprid results in oxidative stress and resistance in a variety of different species, such as honeybees (*Apis mellifera*), rats, and mice (El-Gendy *et al.*, 2010). Che-Mendoza *et al.* (2009) reported an increase in the tolerance of mosquitoes against pyrethroids through an elevation in the expression of GPx. Bamidele *et al.* (2017) evaluated the metabolic defence mechanism by administering dichlorvos to African palm weevil larvae (*Rynchophorus phoenicis* Fabricius) found a significant increase in GPx activity.

The glutathione system, GR removes hydrogen peroxide and organic hydroperoxides such as lipid hydroperoxides on pesticide exposure (Maheshwari et al., 2011). After receiving the sublethal dosages of imidacloprid, the GR of SPW was found decreased. The decrease in enzyme activity shows that SPW is vulnerable to imidacloprid. At sublethal dosages of imidacloprid, GR activity in SPW decreased (by 65.5 and 78.1%), indicating that it is less active than the control. Bamidele et al. (2017) observed that the activity of GR decreased in response to an increase in the concentration of dichlorvos used to treat R. phoenicis larvae. According to Karadag (2019) imidacloprid and thiamethoxam doses ranging from 25 to 500mg L<sup>-1</sup> had no discernible impact on the GR enzyme activities in baker's yeast, Saccharomyces cerevisiae.

Glutathione S-transferases (GSTs) are multifunctional enzymes that are responsible for the metabolism and detoxification of both xenobiotic and physiological substances. GSTs can metabolize insecticides by facilitating their reductive dehydrochlorination or by conjugation reactions with reduced glutathione to produce water-soluble metabolites that are simpler to excrete (Hernandez et al., 2018). Imidacloprid treatment at sublethal quantities resulted in GST activity on SPW being 94% lower than control *ie*, the enzyme activity was dropped from 3.25±0.01 mg<sup>-1</sup>protein, to 0.18±0.01 and 0.17 $\pm$ 0.01, respectively when treated with LC<sub>10</sub> and LC<sub>30</sub> concentrations. As GSTs play a crucial role in the insecticide resistance, high levels of GSTs are typically observed in insects that are resistant to pesticides (Perini et al., 2021). Shojaei et al. (2017) reported the reduction of GST activity in Tribolium castaneum (Herbst) after the treatment with essential oil isolated from Artemisia dracunculus. Several reports show an increased level of GST in insects on the exposure to sublethal concentrations of imidacloprid, this include Sitobion avenae (Fabricius) and Rhopalosiphum padi (Linnaeus) (Lu et al., 2016) and Nilaparvata lugens (Yang et al., 2020). A high level of GST in the resistant strains of Culex pipiens treated with organochlorine, organophosphate, and pyrethroids was reported by Mustafa and Ek (2015). According to the current study, GSH levels were found decreased by 42.2 and 61.6 per cent in SPW when it was given sublethal dosages of imidacloprid at LC<sub>10</sub> and LC<sub>30</sub> respectively. Kapoor et al. (2010) ascertained that imidacloprid has produced a significant reduction in the GSH level in female rats. Pyrethroid exposure to a Nilaparvata lugens colony in a lab decreased the glutathione and caused an oxidative stress (Vontas et al., 2001). The current study reports that treatment with sublethal concentrations of imidacloprid caused significant impairment in the antioxidant enzyme system of SPW. The activity of SOD and LPx, increased in the treated batches of sweet potato weevil, whereas it reduced as in the case of GPx, GST, GR and GSH. Increased sublethal concentrations of imidacloprid exhibits more oxidative stress in SPW due to the over expression of SOD and LPx, while glutathione related enzymes were down regulated.

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### Araneae of *Ochlandra* reed breaks of Shendurney Wildlife Sanctuary, southern Western Ghats, Kerala, India

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**ABSTRACT:** A study of spiders of *Ochlandra* reed breaks of Shendurney Wildlife Sanctuary was conducted for a period of four seasons and revealed a total of 52 species of spiders belonging to 38 genera and 12 families. A checklist of spiders of the reed breaks is provided. *Thiania indica* Asima, Caleb and Prasad, 2023 is a new species reported from the reed breakes.

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KEY WORDS: Reed bamboo, new species, diversity, checklist, hotspot

Reeds are members of the Poaceae family and are tall, slender, shrubby and extremely productive grass (Haslam, 2010). Reed bamboo, genus Ochlandra, of the Western Ghats, India is the only species confined to the tropical zones and is one of the most ecologically and economically exploited grass species in this region (SijiMol et al., 2016). These reeds are found as large monospecific patches on hilltops and along streams or in moist pockets, intermixed with forest species. Extensive reed breaks in Kerala are seen towards the upper ghat ridges at Thiruvananthapuram division; between the Ariankavu pass and the Periyar plateau in Punalur, Konni, and Ranni divisions; and lower slopes of western flank of Anamalai in Vazhachal, Malayatoor and Kothamangalam divisions (Anonymous, 2012). In evergreen forests, reed bamboo serves as a keystone species that affects the survival of other associated species and their ecological niches (SijiMol et al., 2016).

Spiders make up a considerable portion of the animal life of this vast and diversified land. They are a highly species-rich group of invertebrates and are widespread and found in all types of habitats and occupy a few niches in virtually all the earth's biomes (Asima and Prasad, 2022). Presently, about 51,908 spider species classified in 4375 genera from 135 families are described worldwide (World Spider Catalog, 2024), while 1980 species under 500 genera from 62 families are known from India (Caleb and Sankaran, 2024). The present study documents the spiders of Ochlandra reed breaks of Shendurney Wildlife Sanctuary in the northern area of the Agasthyamalai Hills (8º 48'- 8º 57'N; 77º 4'-77º 16'E) in the southern Western Ghats. The sanctuary lies in the catchment of the Parappar Dam (Thenmalai) constructed across the Kallada River and has an expanse of 171 km<sup>2</sup>. The altitude ranges from 100m above msl at the base of the hills to 1550m on top of Alwarkurichi, the highest peak.

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Table 1. Checklist of spiders from the Ochlandrareed breaks of Shendurney Wildlife sanctuary

No.	Family/Species
	Araneidae
1.	Acusilas coccineus Simon,1895
2.	<i>Chorizopes quadrituberculata</i> Roy, Sen, Saha & Raychaudhuri, 2014
3.	Cyclosa bifida (Doleschall, 1859)
4.	C. confraga (Thorell, 1892)
5.	C. hexatuberculata Tikader, 1982
6.	C. neilensis Tikader, 1977
7.	Cyrtophora moluccensis (Doleschall, 1857)
8.	Gasteracantha sp.
9.	G. geminata (Fabricius, 1798)
10.	G. dalyi Pocock, 1900
11.	Gea sabarmata Thorell, 1890
12.	Neoscona mukerjei Tikader, 1980
13.	N. theisi (Walckenaer, 1841)
14.	N. vigilans (Blackwall, 1865)
15.	Nephila pilipes (Fabricius, 1793)
16.	Porcataraneus bengalensis (Tikader, 1975)
	Clubionidae
17.	<i>Clubiona tridentata</i> Dhali, Roy, Saha & Raychaudhuri, 2016
	Linyphiidae
18.	Neriene sundaica (Simon, 1905)
	Mimetidae
19.	Mimetus indicus Simon, 1906
	Oxyopidae
20.	Hamataliwa indica Sen & Sureshan, 2022
	Pholcidae
21.	Crossopriza lyoni (Blackwall, 1867)
22.	Pholcus medog Zhang, Zhu & Song, 2006
23.	Pholcus phalangioides (Fuesslin, 1775)

	Salticidae
24.	Chalcotropis pennata Simon, 1902
25.	Curubis tetrica Simon, 1902
26.	Indopadilla kodagura Maddison, 2020
27.	Myrmarachne prava (Karsch, 1880)
28.	Phintelloid sp.
29.	Tamigalesus munnaricus Zabka, 1988
30.	Telamonia sp.
31.	Thiania indica Asima, Caleb & Prasad, 2023
32.	<i>Vailimia jharbhari</i> Basumatary, Caleb & Das, 2020
	Sparassidae
33.	<i>Thelcticopis moolampilliensis</i> Jose & Sebastian, 2007
34.	Thelcticopis sp.1
35.	Thelcticopis sp.2
	Theridiidae
36.	<i>Chikunia nigra</i> (O. Pickard-Cambridge, 1880)
37.	Chrysso angula (Tikader, 1970)
38.	Nesticodes rufipes (Lucas, 1846)
39.	<i>Nihonhimea japonica</i> (Bösenberg & Strand, 1906)
40.	Parasteatoda celsabdomina (Zhu, 1998)
41.	Theridion hotanense Zhu & Zhou, 1993
42.	T. zonulatum Thorell, 1890
	Thomisidae
43.	Angaeus pentagonalis Pocock, 1901
44.	<i>Lycopus</i> sp.
45.	Camaricus rinkae Biswas & Roy, 2005
	Tetragnathidae
46.	Leucauge decorata (Blackwall, 1864)
47.	L. fastigata (Simon, 1877)
48.	L. tessellata (Thorell, 1887)

49.	<i>Tylorida flava</i> Sankaran, Malamel, Joseph & Sebastian, 2017
50.	Tylorida striata (Thorell, 1877)

	Uloboridae
51.	Miagrammopes extensus Simon, 1889
52.	<i>Uloborus shendurneyensis</i> Asima, Sudhikumar & Prasad, 2021



Acusilas coccineus



Gasteracantha dalyi



Cyclosa bifida



Angaeus pentagonalis



Gea sabarmata



Curubis tetrica



Cyclosa hexatuberculata



Chalcotropis pennata



Indopadilla kodagura



Leucauge decorata



Leucauge fastigata



Tylorida flava



Parasteatoda celsabdomina



Theridion zonulatum



Tamigalesus munnaricus



Vailimia jharbhari



Uloborus shendurneyensis



Thiania indica

Shendurney Wildlife Sanctuary is a part of Agasthyamalai Biosphere Reserve which is one of the richest areas of biodiversity in the Western Ghats (Anonymous, 2012). Ochlandra (reed bamboo) species are endemic to the Western Ghats of India as well as to Sri Lanka (SijiMol et al., 2016). Some of the hillocks in the Pandimotta and Alvarkurichi have dense growth of reeds sometimes growing as pure patches. Thick reed breaks are also seen in the lower valleys, along the streams and fire burnt areas. Important species of reed breaks found in the sanctuary are Ochlandra travancorica var. hirsutea, O. ebracteata, O. scriptoria and O. wightii of which O. travancorica var. hirsutea and O. scriptoria are found in riches (Anonymous, 2012).

The survey was conducted for four seasons, dry summer and Southwest monsoon, Northeast monsoon and dry winter from March 2021 to December 2022, in the Ochlandra reed breaks of Kallar and Pandimotta. Spiders were collected by beating method and, direct handpicking method. The area around the vegetation along the transect was thoroughly examined from top to bottom on leaf blades. All the collected specimens were preserved in ethyl alcohol (70%). World spider catalog (2024) and the website Araneae of India (Caleb and Sankaran, 2024) were used for the identification of spiders. Standard references such as Fauna of India, Spiders Vol I and II by Tikader (1982) and Fauna of India, Spiders, Oxyopidae by Gajbe (2008) were also used for the identification of spiders.

The present study revealed a total of 52 species of spiders belonging to 38 genera and 12 families. Families include Araneidae, Clubionidae, Linyphiidae, Mimetidae, Oxyopidae, Pholcidae, Salticidae, Sparassidae, Theridiidae, Thomisidae, Tetragnathidae and Uloboridae. The analysis of the observed species revealed that Araneidae was the dominant family. Among the 52 identified species, 16 species were belonging to Araneidae followed by Salticidae (9 species), Theridiidae (7 species), Tetragnathidae (5 species), Pholcidae and Sparassidae (3 species each), and Thomisidae and Uloboridae (2 species each). Least number of species observed in Clubionidae, Mimetidae and Oxyopidae with one species each. Genus *Cyclosa* is found to be the species rich genus with four species. *Thiania indica* Asima, Caleb and Prasad, 2023 is a new species reported from the reed breaks (Table 1, Plate 1, 2).

The Shannon-Weiner index revealed a spider diversity of 3.57 in *Ochlandra* reed breaks, suggesting a thriving and diverse spider population. This could be attributed to the minimal disturbance in this specific environment, which offers an advantageous setting for spiders to spin their webs, search for food, and build shelters. Maximum diversity was observed during dry winter (3.117) followed by southwest monsoon (3.05), and dry summer (2.88). The lowest diversity was observed during the northeast monsoon. Species richness was also higher during dry winter (6.50) followed by southwest monsoon (6.10), dry summer (4.94) and the lowest during the northeast monsoon (3.641).

Studies revealed that reed bamboo (*Ochlandra*) functions as a keystone species in evergreen forests, influencing the survival of many associated species and their ecological niches (Basha, 1991; Duckworth, 1993; Kumar *et al.*, 1999; Varma, 2001; Seshadri *et al.*, 2014; Bhagwat *et al.*, 2015). However, studies on the spider fauna of the reed breaks have not been studied in detail before. This is the pioneer attempt to identify the spider fauna of the *Ochlandra* reed breaks of the Shendurney Wildlife Sanctuary.

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# Rediscovery of Common Tinsel *Catapaecilma major* Druce, 1895 (Lepidoptera, Lycaenidae) from the Garhwal region of Uttarakhand, India

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**ABSTRACT:** During a butterfly survey in the remote village of Gailung in Chamoli district, Uttarakhand, one Common Tinsel was recorded on 27<sup>th</sup> March 2023. The butterfly was photographed and identified with the help of field guides as *Catapaecilma major* Druce, 1895 (Lepidoptera, Lycaenidae). The habitat was adjacent to local terrace farmland and was dominated by plants such as *Ageratum conyzoides*, *Oxalis* sp., *Urtica* sp., *Fluggea virosa*, and *Bauhinia variegata*. The butterfly was observed resting on the leaves of *Ageratina adenophora*. and was noted for its swift flight. The current communication highlights the first observation of Common Tinsel from the Garhwal Himalaya after 1930s. © 2024 Association for Advancement of Entomology

KEY WORDS: Butterfly, Lycaenidae, Garhwal Himalaya, Kumaon

Common Tinsel Catapaecilma major is a lycaenid butterfly with a known distribution in India, Nepal, Bhutan, Myanmar, Sri Lanka, and Southeast Asia. In India, it is found in Western Ghats (Maharashtra southwards), Odisha, Chhattisgarh, Northeastern India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland), Sikkim, and Uttarakhand (Wynter-Blyth, 1957; Kehimkar, 2008; Gasse, 2013; Anonymous, 2023). It has two subspecies in India viz., Catapaecilma major major Druce, 1895 and C. m. callone Fruhstorfer, 1915 (Sondhi and Kunte, 2018; Anonymous, 2023; Savela, 2023) whereas some authors also consider a third subspecies *i.e.*, C. m. anais Fruhstorfer, 1915 (Gasse, 2013; Varshney and Smatecek, 2015). Morphologically, its features include three tails and brownish-yellow underside with silver- black edged ochreous bands, and pale violet blue upperside in female, whereas dark violet blue in male. It has a wingspan of 28-32 mm, and altitudinal ranges of up to 1,700m (Evans, 1932; Wynter-Blyth, 1957; Kehimkar, 2008; Sondhi and Kunte, 2018). The current communication highlights the first observation of Common Tinsel from the Garhwal Himalaya after 1930s.

During a butterfly survey in the remote village of Gailung in Chamoli district, Uttarakhand, one individual of Common Tinsel was recorded on 27<sup>th</sup> March 2023 at 1544hrs (30.3195° N; 79.1506° E; Alt: 856m). The butterfly was photographed and identified with the help of field guides (Kehimkar, 2008; Sondhi and Kunte, 2018). The habitat was adjacent to local terrace farmland and was dominated by plants such as *Ageratum conyzoides*, *Oxalis* sp., *Urtica* sp., *Fluggea virosa*, and *Bauhinia variegata*. The butterfly was observed

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Figs. 1a, b - Common Tinsel photographed at the site

resting on the leaves of Ageratina adenophora. and was noted for its swift flight, however, to my surprise, it perched on the lens hood of my camera. Following a brief flight of 10-15 seconds, the butterfly returned to its original location and was seen crawling on the leaves (Figs. 1a, b). Two individuals of hill jezebel *Delias bellandona*, along with some birds such as verditer flycatcher *Eumyias thalassinus*, purple sunbird *Cinnyris asiaticus* and black bulbul *Hypsipetes leucocephalus* were also sighted in and around the same habitat. The following day, on 28<sup>th</sup> March 2023 at 1100 hrs, a search conducted at the same location failed to yield any sightings of the species.

The larval host plants are *Terminalia arjuna*, *Terminalia paniculata*, *Mallotus nudiflorus*, *Lagerstroemia parviflora*, and *Ziziphus rugosa* (Davidson *et al.* 1896; Bell, 1919; Wynter-Blyth, 1957; Nitin *et al.*, 2018). None of the aforementioned species of larval host plants were seen in the area.

There have been quite a few studies from Garhwal on the exploration of the butterfly fauna, including the earliest studies by McKinnon and DeNiceville (1899), and Ollenbach (1930). They reported the sighting of this species from Mussoorie and Dehradun. One of the most comprehensive studies was done by Singh and Sondhi (2016) where a checklist comprising 407 species was presented, which included directly recorded 349 species of butterflies from this region. However, no sightings of common tinsel were reported. It was only in 2019 that Kumar *et al.* discovered the presence of this butterfly in the Kumaon region. Finding presented in this article is noteworthy as it marks the first recorded sighting of this butterfly in the Garhwal region in 90 years.

In the recent past, owing to the extensive surveys conducted by the researchers and naturalists, various rediscoveries and addition to the butterflies of Uttarakhand came from Garhwal Himalaya, including Apefly Spalgis epeus epeus, Pale Jezebel Delias sanaca sanaca, Variegated Plushblue Flos adriana, Dark Sapphire Heliophorus indicus, Mountain Tortoiseshell Aglais rizana, Small Silverfork Lethe jalaurida, White-Ringed Meadowbrown Hyponephele davendra davendra, Dubious Five-Ring Ypthima parasakra (Singh, 2016; Sondhi, 2016; Venkatesh, 2016; Singh and Seal, 2019; Bhatt et al., 2020; Kumar et al., 2020; Singh and Singh, 2019, 2021, 2022). These records indicate that the Garhwal Himalaya region contains unexplored areas with significant potential for studying butterfly diversity and ecology. Further research is crucial to assess the status of butterflies in the Garhwal Himalaya, especially considering that many of them lack recent records.

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### Diversity and relative abundance of insect visitors to litchi inflorescence with special reference to the foraging behaviour of honeybee (*Apis mellifera* L.)

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**ABSTRACT:** The study on insect pollinators of litchi, revealed 227 specimens of insect fauna belonging to 24 species of six different orders and 15 families. Hymenoptera, (belonging to Apidae, Andrenidae, Megachilidae, Vespidae and Sphecidae) was the most dominant (72.68%), followed by Diptera (19.38%), Coleoptera (3.08%), Lepidoptera (2.2%), Hemipter (1.76%) and the lowest, Odonata (0.88%). Among Hymenopterans, honeybees were the pre-dominant insect pollinators (72.68%), *viz., Apis florea* (37%), *A. cerana* (15.41%), *A. mellifera* (7.04%) and *A. dorsata* (3.08%) on litchi bloom. Foraging activity of *A. mellifera* began early in the morning (mean 5 53h) and cessation of flight took place at evening (mean 18 01h). While the mean foraging speed of *A. mellifera* was maximum at 9 00h, the minimum foraging speed was at 17 00h. Maximum foraging rate was observed at 17 00 and minimum at 1100h. © 2024 Association for Advancement of Entomology

KEY WORDS: Pollinators, abundance, diversity, dominance index, foraging activity

*Litchi chinenesis* Sonn., a self- infertile, crosspollinated and entomophilic plant, requires different group of insects for pollination and production of fruit. Male flowers which open initially, have only stamens and produce pollen; hermaphrodite flower which function as female flower open lately, having functional pistil and fertilizable ovules but nonfunctional stamens; and the last to open was male hermaphrodite flowers, having functional stamen which release pollen but non-functional pistil without fertilizable ovule (Stern and Gazit, 1996). The opening time of male flowers in daylight was about 8 to 16 hour (Malhotra *et al.*, 2018). Different insect pollinators show diverse foraging behaviour depending on the availability of floral diversity (Bashir *et al.*, 2018; Ahmad *et al.*, 2021; Khan *et al.*, 2021; Saleh *et al.*, 2021). Researchers have zeroed upon honeybees as pollinators of litchi, following observation that they are frequent and effective flower visitors (Davenport and Stern, 2005; Abou-Shaara *et al.*, 2013). Domestic hives of Asian honeybee *A. cerana* and European honeybee *A. mellifera* have been employed to increase pollination and enhance production in some industrial litchi plantation in India and China (Davenport and Stern, 2005; Kumar and Kumar, 2014). The present study was carried out the following three objectives - to collect and identify different insects visiting litchi flower, to determine relative abundance and diversity of sampled insects

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and to study foraging behaviour of *A. mellifera* on litchi crops.

Observation was conducted in individually managed litchi orchards at two locations [26°09'09"N; 85°14'06"E, 79m msl and 26°05'46"N; 85°26'17"E, 78m msl (above mean sea level), Muzaffarpur, Bihar, India. At location one, orchard holder made independent decision about managements. During flowering period pesticide application was minimised to avoid adverse impacts on pollinators at the same time domesticated beehives were not release there. Whereas domesticated beehives put in action at location two. Samples of insect visiting litchi flowers were taken up from the start of blooming until fading of approximately 95 per cent of the flowers. Insect pollinators were monitored using sweep net, pan trap and visual observations. Collected insects were preserved in 70 per cent ethanol. All insects were subsequently identified up to their species level by using available literature and matching it with museum specimen. The number of flower visitors (insect that vosited any part of flower) was studied by direct visual observations on five randomly selected trees. Within each tree 4 inflorescence with flowers in four different direction were observed for 5 min period in morning (7 00 to 9 00h) and evening (15 00 to 17 00h) between 4-25 March 2023 at 3 days interval.

Relative abundance of pollinators visiting flowers was calculated using following formula.

Abundance (%) = Total population of flower visitor species Total population of flower visitor species

Microsoft office 2016 was used for statistical analysis of collected data at 5% level of significance. Diversity, evenness and dominance of insect visitors; Species diversity, evenness and dominance were calculated using Shannon diversity H' (Shannon, 1948), Pielou's j and Berger-Parker Dominance Index respectively.

Shannon diversity index 
$$H' = \sum_{i=1}^{R} p_i log p_i$$

Where  $P_i = Proportion of i<sup>th</sup> species, log <math>P_i =$ Natural log of  $P_i$  and R = Total number of species Pielou's  $J = \frac{H'}{\ln(S)}$ 

Where H' = Shannon-Wiener index and ln(S) = Natural log of species evenness i.e. total number of species

Berger-Parker Dominance Index d =  $\frac{n_{max}}{N}$ 

Where  $n_{max}$  = Number of individual in most abundant species and N= Total number of individual in the sample.

Foraging time of *A. mellifera* was estimated in terms of timing of initiation and cessation of flight activity. It was done by recording the time when first honeybee started its flight in the morning and the last honeybee enter into hive in the evening. Foraging behaviour was studied in terms of foraging speed and foraging rate. Time spent by *A. mellifera* on each flower within an inflorescence has been referred to foraging speed. Number of flowers visited by *A. mellifera* per minute per panicle has been referred to as foraging rate.

Litchi flowers started blooming from 1st week of March. Half of the flowering occurred in the mid-March and it continued until the last week of March. During the observation, total 227 insects were collected, belonging to 6 different orders, 15 families and 24 species (Table1). Among them, Hymenoptera was principal order comprising of 11 species from 5 different families, namely Apidae, Andrenidae, Megachilidae, Vaspidae, and Sphecidae. Hymenopterans were most dominant (72.68%) litchi flower visiting insects, followed by Diptera (19.38% with 6 species from 3 families), Coleoptera (3.08%), Lepidoptera (2.2%), Hemiptera (1.76%) and the lowest being Odonata (0.88%). Honeybees A. dorsata, A. cerana, A. florea and A. mellifera were dominant pollinators (62.55%) of total insect species visiting litchi bloom.

Honeybee abundance was in following order. *A.florea* (37%) > *A. cerana* (15.41%) >*A. mellifera* (7.04%) > *A.dorsata* (3.08%). Among various insect pollinators *A. florea* was the most


Fig. 1(a) Foraging speed (time spent in second per flower per forager) of *A. mellifera* on litchi flower(b) Foraging rate (No. of flowers visited per forager per minute) of *A. mellifera* on litchi flower

dominant species found visiting flower during the entire litchi blooming period. The Shannon diversity index of insect pollinator of litchi during the flowering period was H' = 2.33, Evenness E = 0.73and Dominance D = 0.37 showing significant results. In the present study insect diversity was moderate. According to Shannon-Wiener diversity index (low diversity (<1.5), medium diversity (>1.5) and high diversity (>2.5)). Evenness value closer to 1 shows species are evenly distributed i.e. population is dominated by less number of species. Dubey et al. (2020) found 1.07 Shannon diversity index of insect pollinator of litchi during the flowering period in Chitwan, Nepal. Kumari et al. (2023) found 1.15 Shannon diversity index of insect pollinator of litchi during the flowering period in Kangra, Himachal Pradesh.

A. mellifera started foraging as early as 5 46h (mean 5 53h) in the morning hours, while in the evening A. mellifera ceased their flight at 18 08h (mean 18 01h). Maximum foraging time was at 12 22h (mean duration 12 05h). Foraging speed of A. mellifera was observed with start of foraging activity by abundance of foragers at 7 00 – 7 30h with 4.57 seconds on a flower (Fig. 1a). Highest foraging speed was recorded at 9 00h with 5.13 seconds on a flower. Time spent on flowers gradually decreases with increase in daytime and temperature. Lowest foraging speed recorded on 17 00h with 2.40 seconds on a flower. Like foraging speed, observations on foraging rate (Fig. 1b) was

also carried out from early morning, 7 00h. Foraging rate at 7 00h were 10.63 flowers per minute per forager, which gradually declined till 11 00h (9.47 flowers per minute). Maximum foraging rate was observed at 17 00h with 12.89 flowers per minute per forager. Minimum foraging rate observed at 11 00h (with 9.47 flower per minute per forager). Bhatnagar and Karnatak (2010) observed the impact of day hours on the foraging behaviour of *A. mellifera* visiting litchi,

In present study, 24 insect species were found visiting litchi inflorescence. Srivastava et al. (2017) in Muzaffarpur Bihar, Thapa (2006) at Rampur Chitwan Nepal, Dubey el al. (2020) at Rampur Chitwan Nepal, reported 23, 21 and 23 species respectively, on litchi inflorescence. In West Bengal, Das (2019) found 13 insect visitors in Nadia district. Kumari et al. (2023) reported 75 insect species in Kangara, Himanchal Pradesh. Wide variations in insect pollinators may result due to different agro climatic regions. In the present study it has been found that hymenopterans as the most effective pollinators and among them honey bees occupy the top positon. Among the honey bees, the authors have found A.florea to be the most effective pollinators of litchi flowers, as has been found by Kumar et al. (2013), Rai et al. (2017), Srivastava et al. (2017), Dubey et al. (2020) who all found hymenopterans as the most effective pollinators and among them honey bee holding the top position. However, the findings varied as far as the particular

No	Common Name	Scientific Name	Family Order		No.	Abundance (%)
1	Rock bee	Apis dorsata	Apidae	Hymenoptera	7	3.08
2	Asiatic honey bee	A. cerana	Apidae	Hymenoptera	35	15.41
3	Red dwarf bee	A. florea	Apidae	Hymenoptera	84	37
4	European honey bee	A. mellifera	Apidae	Hymenoptera	16	7.04
5	Bumble bee	Bumbus spp.	Apidae	Hymenoptera	1	0.44
6	Carpenter bee	Xylocopa fenestrata	Apidae	Hymenoptera	3	1.32
7	Leaf cutter bee	Megchile spp.	Megchilidae	Hymenoptera	5	2.2
8	Mining bee	Andrena spp.	Andrenidae	Hymenoptera	3	1.32
9	oriental wasp	Vespa orientalis	Vespidae	Hymenoptera	3	0.88
10	Paper wasp	Polistis spp.	Vespidae	Hymenoptera	2	0.88
11	Thread waisted wasp	Sphecid spp.	Sphecidae	Hymenoptera	6	2.64
12	Damselfly	Agriochemis spp.	Coenagrionidae	Odonata	2	0.88
13	Rice bug	Leptoeorisa spp.	Alydidae	Hemiptera	1	0.44
14	Jewel bug	Scutelleridae	Scutelleridae	Hemiptera	3	1.32
15	Sandal wood defoilator	Amata parsalis	Eribidae	Lepidoptera	2	0.88
16	Cabbage butterfly	Pieris brassicae	Pieridae	Lepidoptera	1	0.44
17	Grey pansy	Jumonia atlites	Nymphalidae	Lepidoptera	2	1.32
18	House fly	Musca domistica	Muscidae	Deptera	4	1.76
19	Blue Bottle fly	Calliphora vomitoria	Calliphoridae	Deptera	7	3.08
20	Long Hoverfly	Spherophoria spp.	Syrphidae	Deptera	18	7.92
21	Hoverfly	Crystoxum festerum	Syrphidae	Deptera	3	1.32
22	Marmalade hoverfly	Episyrphus spp.	Syrphidae	Deptera	7	3.08
23	Syrphid fly	Eristalinus spp.	Syrphidae	Deptera	5	2.2
24	Lady bird beetle	Coccinella septumpunctata	Coccinellidae	Coleoptera	7	2.64
	Total collection				227	

Table 1. Diversity and relative abundance of visitor insect of litchi

species of a honey bee as the most effective pollinator is concerned. Srivastava *et al.* (2017) reported *A. mellifera* as the most effective pollinator whereas Rai *et al.* (2017), Das *et al.* (2019) and Dubey *et al.* (2020) ranked *A. dorsata.* The effectiveness of different honeybees, appear to be influenced by local factors.

Foraging activity of A. mellifera began early in the morning (mean 5 53h) and cessation of flight took place at evening (mean 18 01h). Joshi and Joshi (2010) at Uttarakhand who reported that A. mellifera started their foraging 6 17h and ceased their activity 18 38h (mean duration 12 hour 47 minute). It appears that Sunrise and Sun set times have influence on the onset and cessation of activities of A. mellifera. Since the Sunrise is delayed in Uttarakhand, the beginning of activities is also delayed. The result also highlighted that the foraging speed of A. mellifera is significantly higher at 9 00h and less at 17 00h. Mishra and Kumar (2018) reported similar result that foraging speed of A. mellifera was highest at 9 00h and minimum at 15h. The present result show that maximum foraging rate at 17 00 and minimum at 11h. Das (2019) found similar results that foraging rate of A.mellifera was maximum between 15 00 -17 00h (12.98 flower visited by a forager per minute) and minimum between 9 00 -11 00h (9.51 flower visited by a forager per minute) on litchi flower.

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# Seasonal occurrence and damage caused by Phycodes radiata Ochsenheimer (Lepidoptera, Brachodidae) on Ficus spp. in Uttar Pradesh, India

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ABSTRACT: A field study was conducted from 2019 to 2021 in the Saharanpur district of Uttar Pradesh to record the seasonal occurrence, infestation, and damage of the fig leaf roller, Phycodes radiata Ochsenheimer (Lepidoptera, Brachodidae), simultaneously on four species host plants, Ficus benjamina L., F. benghalensis L., F. glomerata L. and F. religiosa Linn. During the three years, the peak density of P. radiata larvae was observed from March to August that declined gradually till December. Maximum larval population, infestation and damage were recorded on F. religiosa followed by F. benjamina, F. glomerata, and the lowest on F. benghalensis. The incidence of P. radiata larvae was observed on F. benjamina even in the month of January, while it was nil on other three host plants. © 2024 Association for Advancement of Entomology

KEY WORDS: Ficus benjamina, F. benghalensis, F. glomerata, F. religiosa, fig leaf roller

The fig leaf roller Phycodes radiata Ochsenheimer (Lepidoptera, Brachodidae) was described by Ochsenheimer in 1808 and has been considered as a sporadic pest of Ficus species (Moraceae). Its occurrence has been recorded in Pakistan, Afghanistan, Nepal, Sri Lanka, Myanmar, and parts of India, China, and Iran (Fletcher, 1917, 1919; Beeson, 1941; Wadhi and Batra, 1964; Nair et al., 1976; Kumar and Ramamurthy, 2010; Kallies, 2004; Kallies et al., 2011; Karim et al., 2010; Dhabi et al., 2021). Bajwa and Gul (2000) reported it on Paulownia sp. from Pakistan. The fig leaf roller is a pest of Ficus spp., whose larvae fold the leaf lamina with the help of silken thread and construct a leaf tunnel to feed inside. The larvae feed from the upper epidermis, parenchyma, and mesophyll

tissues of the leaves (Karim et al., 2010; Dhabi et al., 2021). Ficus benjamina L., F. benghalensis L., F. glomerata L. and F. religiosa Linn. have various medicinal as well as spiritual values in the Indian culture. These plants are attacked by a number of insect pests and P. radiata is one of them. In the present study, seasonal abundance, infestation and damage by P. radiata has been studied simultaneously on the afore said plants in Saharanpur of western Uttar Pradesh.

A monthly survey of the gardens, roadside plantations, and residential areas was conducted for three consecutive years from 2019-2021 in Saharanpur district, U.P., India to record the seasonal abundance of P. radiata on F. benjamina, F. benghalensis, F. religiosa and F. glomerata.

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The host plants of 5-10 years of age were selected for the study, and observations were taken monthly. The larvae were sampled and counted by visual observation as well as by removing the infested leaves from each host plant. In the case of large trees such as F. religiosa and F. benghalensis, the observations were taken randomly in the east, west, north, and south directions of the trees. Ten host plants were selected for recording observations on seasonal population, percentage of infestation, and damage per leaf. The percentage of infestation on each host plant was recorded by counting the infested leaves out of the total leaves on a plant, while the damage per leaf was recorded by observing the total leaf area eaten by the larvae. The observations were taken for three years on the same host plants to confirm the results. The seasonal population, seasonal occurrence, percentage of infestation, mode of feeding and damage per leaf of P. radiata larvae were recorded on each host plant in the field. Photographs of the eggs, larvae, pupae, adults, and damage on the host plants were taken with the help of a Dewinter stereoscopic zoom microscope and digital camera. Mean values and standard errors of the collected data were calculated and compiled using Microsoft Excel. The daily meteorological data like temperatures (°C), rainfall, and relative humidity (RH) were taken from HRI and Training Center, Saharanpur.

Seasonal abundance of P. radiata: Occurrence of P. radiata on four host plants was recorded from January to December during the year 2019, 2020, and 2021. In 2019, population of P. radiata larvae was recorded to be high on F. benjamina from March to August, which declined significantly from September to December. On F. benghalensis the larval population was comparatively lower than F. benjamina, F. glomerata and F. religiosa from March to August that declined gradually from September to December. In F.glomerata the number of larvae recorded was lower than F. religiosa. The larval population was observed to be nil on three host plants, i.e., F. benghalensis, F. glomerata and F. religiosa in the month of January and February. The lowest population was observed from September to December on all the four host plants (Table 1).

During 2020, the larval population was recorded to be reduced to some extent in comparison to 2019, but the peak population was recorded from March to August on all the four host plants. The lowest population was observed from September to December, and no larvae were found from January to February on F. benghalensis, F. glomerata and F. religiosa (Table 1). In 2021, the larval population increased on the all the four host plants in comparison to 2020. The maximum number of larvae was recorded on F. religiosa followed by F. benjamina and F. glomerata. No larvae were found on F. benghalensis, F. glomerata and F. religiosa from January to February except on F. benjamina. The minimum number of larvae was observed on F. benghalensis as compared to other three host plants. In 2021, the population observed generally lowest during the months of January, February, September, October, November and December on F. benjamina and the maximum was recorded from March to September. In the months of January and February, the larval population of P. radiata was recorded to be the lowest on F. benjamina, and nil on F. benghalensis, F. glomerata and F. religiosa. Along with temperature, rainfall and relative humidity the tender leaves are also an important factor for population buildup of any insect, which are required for egg laying females and feeding for immature stags. Ficus benjamina is an evergreen plant and tender leaves are almost available throughout the year, while in case of F. glomerata, F. benghalensis and F. religiosa the tender leaves appeared in the end of March and remain till the end of December. In January and February no tender leaves were found on F. glomerata, F. benghalensis and F. religiosa, hence, the population of P. radiata observed nil during these two months. Although, temperature, humidity and rainfall also play an important role in the rise and fall of insect population. Maximum temperature is favorably and strongly associated to population abundance, but the rainfall is adversely connected to insect population reduction (Table 1).



Figs. 1- Egg, 2, 3, 4 - Final instar larva feeding on *Ficus benjamina, F. benghalensis* and *F. glomerata;* Figs. 5 - Final instar larva feeding on *F. religiosa* leaf, 6 – Cocoon, 7 – Pupa, 8 - Adult of *Phycodes radiata;* Figs. 9, 10, 11, 12 - Infested leaves of *F. benjamina, F.benghalensis, F. glomerata and F. religiosa* respectively

## Om Datta

Month N		ber of larvae per 10 host plants – 2019			Mean	Rainfall	Relative
	F. benjamina	F. benghalensis	F. glomerata	F. religiosa	temp. (°C)	(mm)	humidity (%)
January	2.11±1.02	0.00	0.00	0.00	11.5±0.89		55.90 ±8.54
February	1.57±1.01	0.00	0.00	0.00	25.1±0.68	104.77	69.35 ±8.91
March	39.80±1.28	30.20±1.25	36.80 ±1.10	40.20±1.12	31.4±0.91		53.25 ±13.16
April	40.50±1.30	33.50±1.40	38.50± 1.25	40.35±1.13	38.3±0.94		$26.36\pm\!10.23$
May	35.50±1.90	28.20±1.50	37.40±1.12	38.65±1.24	41.4±0.89	29.40	19.25±7.41
June	32.50±1.10	23.30±1.45	35.60±1.34	36.47±1.40	42.3±0.98		24.81±10.45
July	36.40±2.15	27.50±1.35	30.70±1.19	35.50±1.30	37.2±0.79		60.40±13.15
August	39.60±1.95	25.40±1.10	32.59±1.50	37.80±1.24	34.3±0.86	290.286	73.25±5.95
September	25.57±2.54	20.50±1.11	20.20±1.25	24.50±1.11	33.1±0.84		76.33±5.64
October	8.0±2.30	4.11±2.45	9.21±1.30	9.11±1.60	31.2±0.98		62.15±7.40
November	5.0.±1.02	2.31.±1.01	5.10±0.02	6.21±0.02	28.1±0.97	29	49.61±6.02
December	1.12±0.09	2.21 ±0.02	4.10±1.01	4.50±1.05	14.2±0.79		54.45±9.45
			During the year	-2020			
January	1.91±1.22	0.00	0.00	0.00	14.70±1.24		69.38±12.04
February	1.55±1.11	0.00	0.00	0.00	18.79±2.85	88.99	60.68±6.13
March	34.70±1.29	28.10±1.15	35.50 ±1.15	39.60±1.25	22.96±2.92		55.96±12.35
April	38.25±1.25	30.40±1.35	37.30± 1.27	38.95±1.30	31.7±2.40		28.16±6.25
May	33.40±1.82	25.22±1.40	35.50±1.16	37.85±1.28	37.06±2.97	38.77	22.70±9.45
June	30.31±1.15	20.20±1.31	33.90±1.24	35.90±1.45	38.23±2.84		33.13±8.89
July	35.51±2.20	22.40±1.25	29.30±1.39	33.40±1.35	35.29±3.12		52.32±11.38
August	38.82±1.87	23.50±1.15	31.69±1.41	38.70±1.28	31.32±2.18	249.143	74.32±9.33
September	27.59±2.51	17.40±1.20	22.25±1.35	23.60±1.15	31.40±1.10		62.28±9.26
October	7.21±2.25	3.10±1.16	7.80±1.25	8.19±1.50	29.67±1.46		60.83±930
November	3.51±1.11	2.80.±1.11	5.60±1.30	7.29±1.12	22.96±2.55	13.06	34.64±8.52
December	$1.10{\pm}0.08$	$1.08 \pm 0.02$	5.10±1.01	6.50±1.02	18.70±3.0.5		39.16±6.56
			During the year	-2020			
January	1.75±1.02	0.00	0.00	0.00	13.60±1.22		68.77±12.40
February	1.87±1.01	0.00	0.00	0.00	19.78±2.35	18.23	61.10±6.11
March	39.91±1.21	31.16±1.28	37.10 ±1.17	40.35±1.15	23.94±2.62		56.41±12.40
April	40.25±1.20	30.40±1.45	38.50±1.22	39.98±1.27	30.71±2.12		29.90±6.27

Table 1. Seasonal population of *Phycodes radiata* larvae on *Ficus* spp. (2019-2021)

May	36.56±1.96	25.70±1.41	37.39±1.12	37.89±1.27	36.32±2.86	78.94	23.90±9.63
June	34.53±1.15	23.30±1.45	35.80±1.44	35.84±1.34	39.35±2.25		32.22±7.79
July	37.30±2.19	25.80±1.35	28.79±1.18	36.89±1.35	34.15±3.34		53.56±11.21
August	38.70±1.82	26.30±1.15	30.69±1.42	38.83±1.28	30.41±2.31	283.88	72.90±8.42
September	23.42±2.44	19.56±1.13	22.21±1.15	24.24±1.13	28.50±1.32		61.25±8.29
October	6.71±2.21	3.11±1.05	8.91±1.25	9.15±1.21	26.47±1.55		59.81±845
November	5.5±1.12	2.31.±1.01	5.35±0.02	6.15±1.02	23.36±2.86	22.80	35.68±7.55
December	2.15±1.19	2.21 ±0.02	4.70±1.01	4.73±1.06	15.56±3.15		40.18±7.58

Mode of feeding in early and late instar larvae and damage to the host plants: The early instars are gregarious and preferred to feed on upper and lower surface of the young leaves inside the silkenweb and generally consume the entire tissue of leaf margin and leaf tip in circular way leaving a hole on the tip. Sometimes, they consume the half leaf from the tip side and fold the leaf lamina together to form a tunnel. Generally, a single larva was observed in a silken web. The second instar consumes the dorsal and ventral surfaces of the leaves, whereas the third, fourth, and fifth instars (Figs. 2-5) are solitary and forage on the dorsal side of leaves and spin a silken thread to fold the leaf lamina, and form a silken web for feeding within it. The infested leaves turned yellow, dried out, and faded away. During first year, 90 per cent infestation was recorded on an individual host plant of F. benjamina (Fig. 9) with 95 per cent damage per leaf. Maximum infestation was observed on *F. religiosa* (95±1.05% with 70.±0.955 % damage per leaf), followed by F. benjamina (90±1.20%) infestation having 95±0.89% leaf damage) and F. glomerata (80±1.15% infestation with 85±0.99 % damage per leaf). The lowest was recorded on F. benghalensis (30±1.14% with 20±0.79% damage per leaf). The larva used silken web to bind the 2-15 infested leaves into a cluster that lasted on the host plant for a long period before falling off. Whereas, the host plant F. religiosa (Fig.12) was found to be heavily infected with 95 per cent infestation per plant and 70 per cent damage per leaf. The leaf lamina was completely folded and fastened by the larva. In most cases, 2-4 folded

and unfolded leaves were found interwoven together in a silken web. *Ficus benghalensis* (Fig. 10) had the lowest infestation among the four host plants, with a 30 per cent infestation and 20 percent damage per leaf, while the larvae infested *F. glomerata* (Fig. 11) up to 80 and caused 85 percent damage per leaf, and tied 2-6 leaves together in a silken web. Singh and Kaur (2017) also reported 70-80 per cent leaf damage on fig plants caused by *P. minor* and *P. radiata* in Punjab from July to September, while, Verma and Dogra (1984) reported it 15-20 per cent damage from Solan (HP).

During second year, the percent of infestation and damaged reduced to some extent and observed to be as  $(87\pm1.10, 90\pm0.84)$ ;  $(27\pm1.14, 18\pm0.75)$ ;  $(78\pm1.15, 80\pm0.94)$ ; and  $(90\pm1.05, 68\pm0.90)$  respectively on all four host plants. During 3<sup>rd</sup> year the percent of infestation and damage was recorded as  $(93\pm1.23, 97\pm0.91)$ ;  $(34\pm1.25, 24\pm0.92)$ ;  $(84\pm1.21, 87\pm0.93)$  and  $(97\pm1.12, 75\pm0.98)$  on *F. benjamina, F. benghalensis, F. glomerata* and *F. religiosa*, respectively. The adult moths (Fig. 8) of *P. radiata* are diurnal and generally found sucking nectar on the flowers of bitter gourd and bottle gourd of family Cucurbitaceae. Similar findings were also reported by Kumar and Ramamurthy (2010).

The larvae of *P. radiata* spin a web of silken thread for shelter, feeding, pupation, and protection from natural enemies. The larvae prepare a brown cocoon for pupation (Fig. 6). The silken thread is produced by the spinneret gland of the larvae. Moreover, on being disturbed, the larvae also show wriggling movements and release a greenish or yellowish fluid (Figs. 3, 4) from its mouth to warn the predators. Wriggling movement could also help the larvae to escape from egg deposition of certain parasitoid wasps.

The study concludes that fig leaf roller *P. radiata* is a sporadic and considerable pest of Ficus benjamina, F. benghalensis, F. glomerata and F. religiosa in Saharanpur district. The activity of P. radiata commenced from fist week of March and recorded maximum up to August on all four host plants. The population was recorded on peak in the last week of each month. During the three years, the maximum population of larvae was recorded on F. religiosa, F. benjamina and F. glomerata, while the minimum was observed on F. benghalensis. The maximum percent of infestation and damage per leaf was recorded on F. religiosa and the lowest on F. benghalensis. The larvae were found throughout the year on all four host plants except in January and February on F. benghalensis, F. glomerata and F. religiosa.

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# Evaluation of carbosulfan 25 EC on the egg parasitoid, *Trichogramma chilonis* Ishii (Hymenoptera, Trichogrammatidae)

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**ABSTRACT:** Experiments to ensure the safety of carbosulfan, to *Trichogramma chilonis* Ishii in terms of adult emergence and parasitization by direct spraying on egg card technique, revealed significant adverse effect on adult emergence and parasitization. The number of adult emergence ranged from 41.96 to 58.0 and, parasitization 12.62 to 32.6 per cent at 24 HAT; while it was 44.03 to 64.63 and 18.95 to 38.8 per cent respectively at 48 HAT for all the tested three doses of carbosulfan 25 EC @ 250, 500 and 1000 g a.i ha<sup>-1</sup>. Carbosulfan 25 EC @ 250g a.i ha<sup>-1</sup>, recorded maximum adult emergence (58.00 at 24 HAT and 64.63 at 48 HAT) and maximum per cent parasitization (32.60 at 24 HAT and 38.80 at 48 HAT). Hence usage of carbosulfan 250 EC is recommended only at the low dose of 250g a.i. ha<sup>-1</sup>. © 2024 Association for Advancement of Entomology

KEY WORDS: Parasitisation, parasitoid emergence, Corcyra eggs, dose

Trichogramma species has achieved appreciable pest control success in several crop ecosystems and its role in the biological control programs is well understood (Smith, 1996; Hussain et al., 2010; Pawar et al., 2023). Trichogramma can survive in a wide range of temperature and provide successful management of lepidopteran pests in quite a lot of crops (Nadeem and Hamed, 2008, 2011; Nadeem et al., 2009, 2010). Several studies have revealed the susceptibility of Trichogramma wasps to most insecticides. Rajendran and Gopalan (1996), Sarkar et al. (1998), Charles et al. (2000), Williams and Price (2004), Preetha et al. (2009, 2010), Sattar et al. (2011) and Wang et al. (2014) indicated toxic effects of different insecticides on Trichogramma spp. Trichogramma chilonis Ishii (Hymenoptera, Trichogrammatidae) is an effective biocontrol agent in integrated pest management (Pawar *et al.*, 2023). A study was undertaken to study the safety of carbosulfan against *T. chilonis* under laboratory conditions at different doses with an objective to search for comparatively apt dose, to be incorporated in the IPM program.

Mass culture of *T. chilonis* wasps were maintained in the Biocontrol Laboratory, TNAU, Coimbatore, on the eggs of *Corcyra cephalonica* (Stainton) as per the method described by Prabhu (1991). Fresh *C. cephalonica* eggs were collected and sterilized under UV radiation of 15W capacity for 20 minutes duration at a distance of 20cm to avoid the emergence of *Corcyra* larvae. Then these eggs

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were pasted on paper cards of 21 x 30cm size having thirty 7 x 2 rectangles. These egg cards were placed in plastic bags along with the nucleus card at 6:1 ratio for parasitization. The parasitized egg cards were cut into one cm<sup>2</sup> bits and three days old hundred percent parasitized eggs (eggs appearing black and plumpy) were sprayed with insecticides at different concentrations mentioned using an atomizer. Distilled water was sprayed for untreated check. The treated egg cards were shade dried for 10 minutes and then kept in a test tube of 10 x 0.5cm size. The number of parasitoids emerged from each treatment was recorded after 24 and 48 hours of treatment and per cent emergence was worked out using the formula,

Emergence (%) = 
$$\frac{\text{No. of wasps emerged}}{\text{Total no. of eggs in 1 cm}^2} \times 100$$

Fresh eggs were provided to these parasitoids at 6:1ratio and the number of parasitized eggs (eggs appearing black and plumpy) were recorded after 24 and 48 hours of treatment and percent parasitization was worked out using the formula,

Per cent parasitization = 
$$\frac{\text{No. of parasitized eggs}}{\text{Total no. of Corcyra eggs}} \times 100$$

The data were transformed to and analysed by completely randomized design. The treatment mean values of the experiment were compared using Duncan's Multiple Range Test (Gomez and Gomez, 1984). The corrected per cent mortality for lab studies was worked out (Abbott, 1925).

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

Where, Po - Observed mortality in treatment; Pc - Observed mortality in untreated check

Corrected per cent mortality were transformed using arc sine transformation for normalization of data (Snedecor and Cochran, 1967; Steel *et al.*, 1997).

Evaluation revealed that carbosulfan @ 250, 500 and 1000g a.i. ha-1 had significant adverse effect on adult emergence after 24 HAT (41.96-58.0%) and 48 HAT (44.03-64.63%). The normal dose of carbosulfan 25 EC @ 250g a.i. ha<sup>-1</sup> recorded a safe rate of adult emergence (58.00 and 64.63% at 24 and 48 HAT respectively). But the higher doses, (a) 500 and 1000g a.i. ha<sup>-1</sup> the adult emergence was rather low (46.47 and 51.42 at 24 HAT and 41.96 and 44.03% at 48 HAT), exposing it is more toxic nature. In untreated check, adult emergence was 85.55 and 82.60 per cent after 24 and 48 hours of treatment respectively (Table 1). The results on parasitization also revealed that carbosulfan at all the doses tested affected parasitization significantly. The untreated check recorded maximum parasitization (80.66 and 83.56% at 24 and 48 HAT respectively). Of the three doses of carbosulfan tested, the recommended dose, 250g a.i. ha<sup>-1</sup> recorded better parasitization (32.6 and 38.8% at 24 and 48 HAT respectively) and was on par with the standard check, dimethoate @300g a.i. ha-1

No.	Treatments	Adult emergence (%)		Parasitization (%)	
		24 HAT	48 HAT	24 HAT	48 HAT
$T_1$	Carbosulfan 25 EC @ 250 g a.i. ha-1	58.00(42.60)°	64.63(53.51)°	32.60(34.82) <sup>c</sup>	38.80(38.53)°
T <sub>2</sub>	Carbosulfan 25 EC @ 500 g a.i. ha <sup>-1</sup>	46.47(42.96) <sup>d</sup>	51.42(45.81) <sup>d</sup>	18.45(25.44) <sup>d</sup>	22.46(28.29) <sup>d</sup>
T <sub>3</sub>	Carbosulfan 25 EC @ 1000 g a.i. ha <sup>-1</sup>	41.96(40.37) <sup>e</sup>	44.03(41.57) <sup>e</sup>	12.62(20.81) <sup>e</sup>	18.95(25.80) <sup>e</sup>
T <sub>4</sub>	Dimethoate 30 EC @ 300 g a.i. ha <sup>-1</sup>	62.02(51.95) <sup>b</sup>	65.61(54.09) <sup>b</sup>	36.98(37.45) <sup>b</sup>	40.66(39.61) <sup>b</sup>
T <sub>5</sub>	Untreated control	85.55(67.66) <sup>a</sup>	82.60(65.35) <sup>a</sup>	80.66(63.91) <sup>a</sup>	83.56(66.08) <sup>a</sup>

Table 1. Effect of carbosulfan 25 EC on the parasitoid, Trichogramma chilonis (Mean of five observations)

HAT - Hours after treatment; In a column means followed by a common letter are not significantly different by DMRT (p=0.05); Values in parentheses are arc sine transformed values

(36.98 and 40.66% at 24 and 48 HAT respectively). Higher dose of carbosulfan@ 500 and 1000g a.i. ha<sup>-1</sup> showed lower adult emergence (18.45% at 24 HAT and 22.46% at 48 HAT) and parasitization (12.62 and 18.95% respectively after 24 and 48 HAT).

The present findings corroborated the earlier reports with different insecticides against T. chilonis. Carbaryl (0.15%) and triazophos (0.15%) were more toxic to T. chilonis (Gangathara et al., 1990). Madhu et al. (2014) found toxicity of flubendiamide 20 WG against the egg parasitoid. Studies revealed that the carbamate insecticides adversely affected parasitzation of T. chilonis (Tiwari and Khan, 2002). Preetha et al. (2009) reported toxicity of imidacloprid to T. chilonis. The studies on the safety of carbosulfan on the parasitoid T. chilonis revealed substantial adverse effect on the adult emergence and parasitization, which ranged from 41.96 to 58.0 and 12.62 to 32.6 per cent at 24 HAT, but slowly rebounded to 44.03 to 64.63 and 18.95 to 38.8 per cent at 48 HAT. Therefore, the chemical is considered to have toxic effect immediately after application, but the toxicity gets reduced in time. Carbosulfan systemic insecticide, was found to be toxic to the egg parasitoid, T. chilonis, based on adult emergence and per cent parasitization. However, the recommended dose of carbosulfan 250g a.i. ha<sup>-1</sup> is found to be less toxic to the egg parasitoids and is recommended for usage in any crop, if there is a severe pest outbreak.

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# Dragonflies and damselflies (Odonata) of Silent Valley National Park, Kerala, India and its environs

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**ABSTRACT:** The odonate diversity of the Silent Valley National Park (SVNP) in the Western Ghats (WG) of Kerala state, in southern India, is discussed. A total of 111 species of odonates (41 Zygoptera and 70 Anisoptera) including 29 endemics were recorded for the SVNP region, out of the 181 species (14 families, 87 genera with 68 WG endemics). SVNP harbours 53.37 per cent of WG and 61.34 per cent of the odonate diversity of Kerala. In addition, this includes 42.64 percent endemic odonates of Kerala and 35.80 percent of WG. With respect to IUCN Red List status, there were two vulnerable, three near threatened, 84 least concerned, 17 data deficient, and five species whose status was not assessed. Family Libellulidae (40 species) dominated the diversity, followed by Coenagrionidae and Gomphidae (16 species each). None of the species listed from SVNP is protected under the Indian Wildlife (Protection) Amendment Act, 2022. © 2024 Association for Advancement of Entomology

KEY WORDS: Anisoptera, Zygoptera, checklist, Western Ghats, IUCN

Silent Valley National Park (SVNP) is located on the southwestern slopes of the Nilgiri Landscape of the Western Ghats, north of the Palghat Gap. The area lies within the latitudes 11° 2' N to11° 13' N and longitudes 76° 24' E to76° 32' E. Administratively the Silent Valley Forest division comprises the Silent Valley Range (143.52 km<sup>2</sup>) and the buffer zone of Bhavani Range (94 km<sup>2</sup>), making a total area of 237.52 km<sup>2</sup>. The terrain is undulating with steep valleys, escarpments, and hillocks. The elevation ranges from 900m to the highest point at 2,383m (Anginda peak). Both the southwest monsoon and the northeast monsoon causes rains in this area (Anonymous, 2012). The major share, however, comes from the southwest monsoon, which sets in during the first week of June. The heaviest rainfall is during the months of June, July, and August. The rainfall varies from

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7500mm per year on the northern side to 2800mm in the drier Attapady Valley. The main drainage basin is Kunthipuzha (Bharatapuzha) (Nair, 1991). The average minimum temperature ranges from 8 to 14 °C and the average maximum temperature varies from 23 to 29 °C. The major forest types known from the region based on Champion and Seth classification (1968) are Southern Hill Top Tropical Evergreen Forest, West-Coast Tropical Evergreen Forests, Cane Brakes, Wet Bamboo Brakes, West-Coast Semi-Evergreen Forests, West-Coast Secondary Evergreen Dipterocarp Forests, Southern Sub-tropical Hill Forests, Reed Brakes, South Indian Sub-tropical Hill Savannah, Southern Montane Wet Temperate Forests, Southern Montane Wet Scrub, and Southern Montane Wet Grasslands (Nair, 1991; Anonymous, 2012). The region has excellent biodiversity as exemplified by 2000 species of plants, 41 species of mammals, 97 species of birds, 42 reptiles, and 46 amphibians recorded as per Manoharan et al. (1999). There are no published records on the odonate fauna of this protected tract except that of a preliminary study conducted by Rao and Lahiri (1982), in which they reported 23 species from SVNP and the adjoining New Amarambalam Reserve Forest. There had not been any scientifically structured surveys for odonates in the SVNP, and the first one was done by the Travancore Nature History Society (TNHS) in association with Kerala Forest Department, SVNP in September 2016 with records of 35 species over a span of three days (Sadasivan and Jayakumar, 2016). In this paper, 111 species of odonates from SVNP, based on the fieldwork in the region since the year 2000, are reported.

Field data predominantly from the authors over the last two decades and the published peer-reviewed literature available on odonates of the region (Rao and Lahiri,1982; Subramanian, 2007; Emiliyamma *et al.*, 2007) were collected. The data logged in the Management Plan of SVNP, by the Kerala Forest Department (Anonymous, 2012), as well as the report on the first comprehensive invertebrate survey of SVNP done by TNHS in 2016, submitted to the Kerala Forest Department (Sadasivan and Jayakumar, 2016) were also referred. During the

fieldwork, the odonates were observed and photographed as far as possible, with special consideration to the prothorax and anal appendages. The basic taxonomy of the group follows Fraser (1933, 1934, 1936), and is updated as per the latest arrangements by Kalkman *et al.* (2020). The current Odonata checklist and distribution for the Western Ghats as well as Kerala state were critically reviewed and updated by Nair *et al.* (2021), which will be followed here. The global checklist follows Paulson *et al.* (2021) and conservation status data was derived from the IUCN site http://www.iucnredlist.org (IUCN, 2022).

Abbreviations: ASL--Above Sea Level, TORG-TNHS Odonata Research Group; SVNP-Silent Valley National Park; TNHS-Travancore Nature History Society; TIES-Tropical Institute of Ecological Sciences; IUCN-The International Union for Conservation of Nature; WG-Western Ghats, WPA- Indian Wildlife (Protection) Amendment Act, 2022

According to Nair et al. (2021) the current checklist of odonates of the Western Ghats stands at 207 species with 80 endemics. A total of 181 species belonging to 87 genera and 14 families have been compiled for Kerala state, and this includes 68 WG endemics. In SVNP 111 species of odonates including 29 endemics were recorded (Table 1). Forty-one damselflies (Zygoptera) and 70 dragonflies (Anisoptera) were recorded for the sanctuary. Family Libellulidae dominated the odonate list with 40 species, followed by Coenagrionidae and Gomphidae (with 16 species each). Fifty-four species were identified from the SVNP core zone and 103 species from the Bhavani buffer zone, and 47 were seen in both core and buffer zones of the National Park. Two races of Ceriagrion olivaceum viz., C. o. aurantiacum Fraser, 1924 (Rao and Lahiri, 1982) and C. o. olivaceum Laidlaw, 1914 (Emiliyamma et al., 2007), and Davidiodes martini Fraser, 1924 (Subramanian, 2007) are the historical records included here, with no recent sightings other than those mentioned in parenthesis.

Sheht valley Ivational Park						
End*	IUCN	Core				
		zone				
lies Family Calopterygidae						
	LC	$\checkmark$				

Table 1	Checklist of	Odonates	of Silent	Vallev	National Park
Tuble I	Checkinst of	Outonates	or onem	vancy	1 unonui 1 un

No.	Scientific name	End*	IUCN	Core zone	Buffer zone
	Suborder Zygoptera: Damse	elflies Family Calo	pterygidae		
1	Neurobasis chinensis (Linnaeus, 1758)		LC	$\checkmark$	$\checkmark$
2	Vestalis gracilis (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
3	V. apicalis Selys, 1873		LC	$\checkmark$	$\checkmark$
4	V. submontana Fraser, 1934		NA	$\checkmark$	$\checkmark$
	Family Chl	orocyphidae			
5	Heliocypha bisignata (Hagen in Selys, 1853)		LC	$\checkmark$	$\checkmark$
6	Libellago indica (Fraser, 1928)		LC	_	$\checkmark$
	Family Coe	nagrionidae			
7	Aciagrion approximans krishna Fraser, 1921	WG	LC	$\checkmark$	$\checkmark$
8	Ac. occidentale Laidlaw, 1919		LC	_	$\checkmark$
9	Agriocnemis pieris Laidlaw, 1919		LC	_	$\checkmark$
10	Ag. pygmaea (Rambur, 1842)		LC	_	$\checkmark$
11	Ag. splendidissima Laidlaw, 1919		LC	_	$\checkmark$
12	Archibasis oscillans (Selys, 1877)		LC	_	$\checkmark$
13	a) Ceriagrion olivaceum aurantiacum Fraser, 1924		LC	_	$\checkmark$
	b) C. olivaceum olivaceum Laidlaw, 1914		LC	_	_
14	C. cerinorubellum (Brauer, 1865)		LC	_	$\checkmark$
15	C. coromandelianum (Fabricius, 1798)		LC	_	$\checkmark$
16	C. rubiae Laidlaw, 1916		NA	_	$\checkmark$
17	Ischnura rubilio Selys, 1876		LC	$\checkmark$	$\checkmark$
18	I. senegalensis (Rambur, 1842)		LC	_	$\checkmark$
19	Pseudagrion indicum Fraser, 1924	WG	LC	_	$\checkmark$
20	P. malabaricum Fraser, 1924		LC	_	$\checkmark$
21	P. microcephalum (Rambur, 1872)		LC	-	$\checkmark$
22	P. rubriceps (Selys, 1876)		LC	$\checkmark$	$\checkmark$
	Family E	uphaeidae			
23	Dysphaea ethela Fraser, 1924		DD	$\checkmark$	$\checkmark$
24	Euphaea dispar (Rambur, 1842)	WG	LC	$\checkmark$	$\checkmark$
25	E. fraseri (Laidlaw, 1920)	WG	LC	$\checkmark$	$\checkmark$
	Family	Lestidae			
26	Lestes dorothea Fraser, 1924		LC	$\checkmark$	$\checkmark$
27	L. elatus Hagen in Selys, 1862		LC	-	$\checkmark$
	Family Plat	ycnemididae			
29	Caconeura ramburi (Fraser, 1922)		DD	$\checkmark$	$\checkmark$
30	Ca. risi (Fraser, 1931)	WG	DD	$\checkmark$	_

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31	Copera marginipes (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
32	Co. vittata (Selys, 1863)		LC	—	$\checkmark$
33	Esme longistyla Fraser, 1931	WG	LC	$\checkmark$	_
34	E. mudiensis Fraser, 1931	WG	DD	—	$\checkmark$
35	Onychargia atrocyana (Selys, 1865)		LC	—	$\checkmark$
36	Phylloneura westermanni (Hagen in Selys, 1860)	WG	NT	$\checkmark$	$\checkmark$
37	Prodasineura verticalis annandalei (Fraser, 1921)		LC	—	$\checkmark$
	Family Pla	tystictidae			
38	Indosticta deccanensis Laidlaw, 1915	WG	VL	_	$\checkmark$
39	Protosticta gravelyi Laidlaw, 1915	WG	LC	$\checkmark$	$\checkmark$
40	P. hearseyi Fraser, 1922	WG	DD	$\checkmark$	—
41	P. sanguinostigma Fraser, 1922	WG	VL	—	$\checkmark$
	Suborder Anisoptera: Drag	gonflies Family A	eshnidae		
42	Anax guttatus (Burmeister, 1839)		LC	$\checkmark$	$\checkmark$
43	A.indicus Leiftinck, 1942		LC	$\checkmark$	$\checkmark$
44	A. immaculifrons (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
45	Gynacantha dravida Lieftinck, 1960		DD	—	$\checkmark$
46	G. millardi Fraser, 1920		LC	$\checkmark$	$\checkmark$
	Family Chlor	ogomphidae			
47	Chlorogomphus campioni (Fraser, 1924)	WG	LC	$\checkmark$	_
	Family Co	rduliidae			
48	Hemicordulia asiatica (Selys, 1878)		LC	—	$\checkmark$
	Family Go	mphidae			
49	Burmagomphus laidlawi Fraser, 1924	WG	DD	$\checkmark$	$\checkmark$
50	B. pyramidalis Laidlaw, 1922		LC	$\checkmark$	$\checkmark$
51	Cyclogomphus flavoannulatus Rangnekar,	WG	NA	—	$\checkmark$
	Dharwadkar, Kalesh & Subramanian, 2019				
52	Davidioides martini Fraser, 1924	WG	DD	—	$\checkmark$
53	Gomphidia kodaguensis Fraser, 1923	WG	DD	—	$\checkmark$
54	Heliogomphus kalarensis Fraser, 1934	WG	DD	$\checkmark$	—
55	H. promelas (Selys, 1873)		NT	$\checkmark$	$\checkmark$
56	Ictinogomphus rapax (Rambur, 1842)		LC	—	$\checkmark$
57	Macrogomphus wynaadicus Fraser, 1924	WG	DD	_	$\checkmark$
58	Megalogomphus hannyngtoni (Fraser, 1923)		NT	_	$\checkmark$
59	Merogomphus longistigma (Fraser, 1922)	WG	DD	$\checkmark$	_
60	Me. tamaracherriensis Fraser, 1931	WG	NA	$\checkmark$	$\checkmark$
61	Microgomphus souteri Fraser, 1924	WG	LC	_	$\checkmark$
62	Melligomphus acinaces (Laidlaw, 1922)	WG	DD	_	$\checkmark$

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63	Lamelligomphus nilgiriensis Fraser, 1922	WG	LC	—	$\checkmark$
64	Paragomphus lineatus (Selys, 1850)		LC	_	$\checkmark$
	Family Lib	ellulidae			
65	Acisoma panorpoides Rambur, 1842		LC	_	$\checkmark$
66	Brachydiplax chalybea Brauer, 1868		LC	_	$\checkmark$
67	Brachythemis sobrina (Rambur, 1842)		LC	_	$\checkmark$
68	Brachythemis contaminata (Fabricius, 1793)		LC	_	$\checkmark$
69	Bradinopyga geminata (Rambur, 1842)		LC	_	$\checkmark$
70	Cratilla lineata calverti (Forster, 1903)		LC	$\checkmark$	$\checkmark$
71	Crocothemis servilia (Drury, 1770)		LC	_	$\checkmark$
72	Diplacodes nebulosa (Fabricius, 1793)		LC	_	$\checkmark$
73	Diplacodes trivialis (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
74	Epithemis mariae (Laidlaw, 1915)	WG	LC	_	$\checkmark$
75	Hydrobasileus croceus (Brauer, 1867)		LC	_	$\checkmark$
76	Hylaeothemis apicalis Fraser, 1924		DD	$\checkmark$	$\checkmark$
77	Indothemis carnatica (Fabricius, 1798)		LC	_	$\checkmark$
78	Lathrecista asiatica (Fabricius, 1798)		LC	_	$\checkmark$
79	Lyriothemis tricolor Ris, 1919		LC	_	$\checkmark$
80	Neurothemis fulvia (Drury, 1773)		LC	_	$\checkmark$
81	Neurothemis intermedia intermedia (Rambur, 1842)		LC	_	$\checkmark$
82	Neurothemis tullia (Drury, 1773)		LC	_	$\checkmark$
83	Onychothemis testacea ceylanica Ris, 1912		LC	_	$\checkmark$
84	Orthetrum chrysis (Selys, 1891)		LC	$\checkmark$	$\checkmark$
85	Orthetrum triangulare triangulare (Selys, 1878)		LC	$\checkmark$	$\checkmark$
86	Or. glaucum (Brauer, 1865)		LC	$\checkmark$	$\checkmark$
87	Or. luzonicum (Brauer, 1868)		LC	$\checkmark$	$\checkmark$
88	Or. pruinosum neglectum (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
89	Or. sabina sabina (Drury, 1770)		LC	$\checkmark$	$\checkmark$
90	Paplopleura sexmaculata (Fabricius, 1787)		NA	$\checkmark$	$\checkmark$
91	Pantala flavescens (Fabricius, 1798)		LC	$\checkmark$	$\checkmark$
92	Potamarcha congener (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
93	Rhodothemis rufa (Rambur, 1842)		LC	_	$\checkmark$
94	Rhyothemis triangularis Kirby, 1889		LC	_	$\checkmark$
95	Rh. variegata variegata (Linnaeus, 1763)		LC	_	$\checkmark$
96	Tetrathemis platyptera Selys, 1878		LC	_	$\checkmark$
97	Tholymis tillarga (Fabricius, 1798)		LC	$\checkmark$	$\checkmark$
98	Tramea basilaris (Palisot de Beauvois, 1805)		LC	$\checkmark$	$\checkmark$
99	Tramea limbata (Desjardins, 1832)		LC	$\checkmark$	$\checkmark$

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100	Trithemis aurora (Burmeister, 1839)		LC	$\checkmark$	$\checkmark$
101	Tr. pallidinervis (Kirby, 1889)		LC	_	$\checkmark$
102	Tr. festiva (Rambur, 1842)		LC	$\checkmark$	$\checkmark$
103	Zygonyx iris malabarica Fraser, 1926		LC	$\checkmark$	$\checkmark$
104	Zyxomma petiolatum Rambur, 1842		LC	$\checkmark$	$\checkmark$
	Family N	<b>Aacromiidae</b>			
105	Epophthalmia vittata vittata Burmeister, 1839		LC	$\checkmark$	$\checkmark$
106	Macromia cingulata Rambur, 1842		LC	_	$\checkmark$
107	M. ellisoni Fraser, 1924	WG	LC	$\checkmark$	$\checkmark$
108	M. flavocolorata Fraser, 1922		LC	_	$\checkmark$
	Genera i	ncertae sedis			
109	Idionyx corona Fraser, 1921	WG	DD	$\checkmark$	$\checkmark$
110	I. saffronata Fraser, 1924	WG	DD	$\checkmark$	-
111	I. travancorensis Fraser, 1931	WG	DD	$\checkmark$	$\checkmark$
	Total	29		54	103

(End\*-Endemic, WG-Western Ghats, IUCN-Red List status, LC-Least Concern, NA-Not Assessed, DD-Data Deficient,

VL-Vulnerable, NT-Near Threatened)

Suborder Zygoptera (Damselflies): All the seven damselfly families present in Kerala are represented in SVNP with 41 taxa. This includes 12 WG endemics. Family Calopterygidae includes four species distributed in two genera in the WG. All the four species of Calopterygidae present in Kerala are represented in SVNP. Vestalis submontana Fraser, 1934 was locally common in the higher reaches of SVNP from 1200m ASL. Family Chlorocyphidae with three genera with one species each in WG is represented by Heliocypha bisignata (Hagen in Selys, 1853) and Libellago indica (Fraser, 1928) in SVNP. Among the 24 species of Cenagrionidae in Kerala 16 species are present here. Both races of C. olivaceum are also found. Among the Coenagrionids present in SVNP two taxa are WG endemics namely Pseudagrion indicum Fraser, 1924, and the subspecies of Aciagrion approximans (A. a. krishna Fraser, 1921). Family Euphaeidae is represented by two genera with six species in WG. In SVNP three species are present among the four found in Kerala. Euphaea dispar (Rambur, 1842) and Euphaea fraseri (Laidlaw, 1920) are WG endemic species. Lestidae has only two species in SVNP as far as it is known, namely Lestes elatus Hagen in Selys, 1862 and *L. dorothea* Fraser, from among the 11 species occurring in Kerala. Platycnemididae is represented by 10 genera with 19 species in WG, while in SVNP nine species out of 16 are in the Kerala state. *Caconeura risi* (Fraser, 1931), *Esme longistyla* Fraser, 1931, *E. mudiensis* Fraser, 1931, and *Phylloneura westermanni* (Hagen in Selys, 1860) are WG endemics in SVNP. Platystictidae is represented by four species out of 12 in the state. They are *Indosticta deccanensis* Laidlaw, 1915, *Protosticta gravelyi* Laidlaw, 1915, *P. hearseyi* Fraser, 1922, and *P. sanguinostigma* Fraser, 1922, all the four are WG endemics.

**Suborder Anisoptera (Dragonflies):** All the seven dragonfly families seen in Kerala state are represented in SVNP with 70 species, out of 107 from Kerala. This includes 17 WG endemics. Family Aeshnidae is represented by three genera with ten species in the WG. In SVNP five species out of nine occurring in the state are reported. Chlorogomphidae has a sole representative *Chlorogomphus campioni* (Fraser, 1924), a WG endemic, and Corduliidae has a single species *Hemicordulia asiatica* (Selys, 1878). In SVNP 16 out of 22 species of Gomphidae reported

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from the state were recorded. Of these 11 taxa are endemic to WG (Table 1). Davidioides martini Fraser, 1924, Heliogomphus kalarensis Fraser, 1934, Megalogomphus hannyngtoni (Fraser, 1923), and Lamelligomphus nilgiriensis Fraser, 1922 are some of the interesting ones present here. Libellulidae is represented by 40 species out of the 52 species for Kerala and 55 in the WG. Some interesting ones include the phytotelmata breeding species -Lyriothemis tricolor Ris, 1919, and the freshwater swamp associate Epithemis mariae (Laidlaw, 1915), the latter a WG endemic. In SVNP three species of Macromiidae are found out of 11 in both Kerala state and the WG. Dragonflies belonging to the genera Macromidia and Idionyx have unclear family level affinities and hence they are treated as incertae sedis. Three species of Idionyx are found in SVNP out of nine species found in Kerala. Idionyx nadganiensis Fraser, 1924, a species described from Nadgani Ghat (Malappuram) in Nilgiri-Wayanad near SVNP in the same landscape has no recent record as in Nair et al. (2021), hence not included in the checklist.

*Endemism:* Twenty nine (24.54%) species of odonates from Silent Valley National Park were found strictly endemic to the Western Ghats (Table 1).

*IUCN Red List Status:* Regarding IUCN status, there were three Near Threatened species, two Vulnerable, eighty-four Least Concern, seventeen Data Deficient, and five species whose Red List status were not available (Table 1).

SVNP harbours a total of 111 species of odonates with 29 WG endemic species. This is the secondhighest number of odonates reported from a protected area in Kerala. Mathavan and Miller (1989) reported 36 species of odonates from Periyar Tiger reserve, Emiliyamma and Radhakrishnan (2000, 2014) reported 39 species from Parambikulam Wildlife Sanctuary, Gnanakumar *et al.* (2012) recorded 55 species from Chimmony Sanctuary; Adarsh *et al.* (2014) got 48 species from Chinnar; Varghese *et al.* (2014) got 82 species from Thattaekkad (Thattekkad) bird sanctuary; and Palot and Kiran (2016) reported 93 species from Aaralam Sanctuary. Sadasivan *et al.* (2022) reported 116 species of odonates from the Shendurney Sanctuary in Agasthyamalais. As per the findings SVNP and its buffer zone harbours 53.37 per cent of all odonates reported for WG and 61.34 per cent of the odonates from Kerala. In addition, this includes 35.80 per cent of endemic odonates of the WG and 42.64 per cent of endemics recorded from Kerala.

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I, Dr K. D. Prathapan, Secretary, Association for Advancement of Entomology, here by declare that the particulars given above are true to the best of my knowledge and belief.

Sd/-

Vellayani PO, Thiruvananthapuram 695522

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