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ENTOMON

Vol. 49 *September 2024* *No. 3*

Contents

Page

https://doi.org/10.33307/entomon.v49i3.1250 295
Molecular identification of ecologically relevant hoverflies (Diptera, Syrphidae) from eastern India
Oishik Kar, Debjani Ghosh, Arka Mukherjee, Koustav Mukherjee, Debdeep Pramanik, Saikat Sarkar, Jayita Sengupta, Atanu Naskar and Dhriti Banerjee

https://doi.org/10.33307/entomon.v49i3.1251 309
A checklist of butterflies of Sálim Ali Centre for Ornithology and Natural History (SACON) campus, Anaikatty, Western Ghats, India
P.P. Ashiq, S. Jeevith, M. Sri Sowmiya, J. Chaithra Shree, P. Pramod and P.R. Arun

https://doi.org/10.33307/entomon.v49i3.1252 327
Aquatic insect diversity and distribution in Maguri Beel and Khamti Guali Beel wetlands, Upper Brahmaputra Basin, Assam, India
Innifa Hasan, Diksha Rabha and Dip Jyoti Haloi

https://doi.org/10.33307/entomon.v49i3.1253 335
Species diversity and seasonal dynamics of insects in the high altitude tea estate in Uttarkhand, India
Kartik Omanakuttan, Sanjana Goswami and Manoj Kumar Arya

https://doi.org/10.33307/entomon.v49i3.1254 343
Molecular characterization and identification of insect pollinators of *Valeriana jatamansi* Jones from Shimla Hills, Western Himalaya, India
Madhu Rana, Amar Singh and Mahender Singh Thakur

https://doi.org/10.33307/entomon.v49i3.1255 355
A checklist of flesh flies (Diptera, Sarcophagidae) from Assam, India
Mayoushree Gogoi, Annu Kumari, Anurudha Chakraborty, Sangeeta Biswas and Abesh Chakraborty

https://doi.org/10.33307/entomon.v49i3.1256 363
Species composition, abundance and seasonality of dermatitis causing *Paederus* rove beetles in paddy fields of Malabar region, Kerala, India
P.M. Kavyamol, P.J. Vineesh and V.P. Vineetha

https://doi.org/10.33307/entomon.v49i3.1257 373
First record of water mite larvae, *Hydrachna* sp. (Acari, Hydrachnidae) parasitism as quiescent nymphophan on two major aquatic insects of Coleoptera and Hemiptera from West Bengal and Odisha, India
Anindita Das and Susanta Kumar Chakraborty

https://doi.org/10.33307/entomon.v49i3.1258 381
Description of nine new species of the genus *Sycophila* Walker (Chalcidoidea, Eurytomidae) from Kerala, India
Shilpa K. Satheesan and Shreevihar Santhosh

https://doi.org/10.33307/entomon.v49i3.1259 399
A checklist of moths in Bilaspur district, Himachal Pradesh, in the western Himalayan foothills, India
Paul Pop, Nivedita Pattanaik, Kuldeep Singh Barwal1, Harminder Pal Singh, Puneet Pandey, Sanjay Sondhi and Randeep Singh

https://doi.org/10.33307/entomon.v49i3.1260 417
Efficacy of bee pollen and beebread against *Salmonella typhimurium* in BALB/c mice
Rajinder Kaur and Abhinav Saxena

https://doi.org/10.33307/entomon.v49i3.1261 425
Bacillus thuringiensis israelensis VCRC B650 culture filtrate useful for mosquito oviposition attractant and larvicidal action
Jibi Lukose, Kakhuangailiu Gangmei, Bhagyashree Bora, P. Hemaladkshmi, V. Abhisubesh, Sahadiya Mandodan, K. Aneha, A. Mathivanana, I. Geetha and S. Poopathi

https://doi.org/10.33307/entomon.v49i3.1262 435
Exploring the diversity of coccinellid species: A comprehensive study on polymorphism and species-specific male genitalia identification
Gokul Krishna Dhanapati, Suprakash Pal and K. Swapna Rani

Short Commuinication

https://doi.org/10.33307/entomon.v49i3.1263 445
First record of leaf miner, *Phyllocnistis unipunctella* (Stephens, 1834) (Lepidoptera, Gracillariidae) infesting *Populus deltoides* Marsh (Salicaceae) in Jammu and Kashmir, India
Deen Mohd Bhat, Sajad Ahmad Khan and Inayat Ullah Lone

https://doi.org/10.33307/entomon.v49i3.1264 447
Predatory potential of *Coccinella undecimpunctata* L. (Coleoptera, Coccinellidae) on *Aphis craccivora* Koch. (Hemiptera, Aphididae) in Ladakh, India
Mohd Ali, Alyas Ali and Shabbir Hussain

https://doi.org/10.33307/entomon.v49i3.1265 453
First record of orange oak leaf - *Kallima inachus* (Boisd) in Uttar Pradesh, India
Jyoti Antil, Prashant Tripathi, M. Semmaran and Amita Kanaujia

https://doi.org/10.33307/entomon.v49i3.1266 455
Ant-treehopper mutualism affecting biocontrol of *Parthenium hysterophorus* by Mexican beetle, *Zygogramma bicolorata* Pallister
Anand Harshana and Debjani Dey

https://doi.org/10.33307/entomon.v49i3.1267 459
Studies on the insect pests of brinjal in Hoshiarpur District of Punjab, India
Amrit Deep Kaur, Sajad Ahmad Bhat and Kamlpred Kaur

https://doi.org/10.33307/entomon.v49i3.1268 465
Description of a new species of potter wasp (Hymenoptera, Vespidae, Eumeninae) from northeast India
E. Benny Femi, A. P. Ranjith and D.R. Priyadarsanan

471
https://doi.org/10.33307/entomon.v49i3.1269
First report of mealybug, *Crisicoccus hirsutus* (Newstead) on cocoa (Hemiptera, Peudococcidae) from India
Jayalaxmi Narayan Hegde, Indhusri Chavan, G. A. Kavya Yadav and R. Darshan

Obituary 473

Author Index 475

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Molecular identification of ecologically relevant hoverflies (Diptera, Syrphidae) from eastern India

Oishik Kar, Debjani Ghosh, Arka Mukherjee, Koustav Mukherjee, Debdeep Pramanik, Saikat Sarkar, Jayita Sengupta, Atanu Naskar* and Dhriti Banerjee

Diptera Section, Zoological Survey of India, New Alipore, Kolkata 700053, West Bengal, India. Email: atanudiptera@gmail.com

ABSTRACT: Syrphid identification keys that cover all life cycle stages of the taxonomy are insufficient, and there are problems with the morphological identification of these flies. Cytochrome oxidase I (COI) is widely used for molecular identification and phylogenetic reconstruction. The study examined the effectiveness of COI in identifying 18 specimens containing 13 agriculturally important species of syrphids collected from different geo-climatic regions of West Bengal. Phylogenetic analysis was performed using Maximum Likelihood (ML) and Bayesian (BA) trees, which were almost congruent. Barcodes were generated for *Dasysyrphus orsua* and *Eristalinus polychromata* for the first time. This is the first study to use the COI for barcoding ecologically and agriculturally relevant syrphid flies from eastern India and their phylogeny. The findings contribute to the basic understanding of the diversity of syrphids across West Bengal and the molecular characterization of hoverflies, promoting their conservation and thus leading to the augmentation of crops. © 2024 Association for Advancement of Entomology

KEY WORDS: Flower flies, phylogenetic analysis, COI gene, genetic divergence, barcodes

INTRODUCTION

Issues such as pollution, global warming, urbanization, and industrialization, as well as current farming procedures, are causing intense harm to classic supporting functions such as pollination (Klein *et al.,* 2007). Most plants, especially commercial crops, require pollination to reproduce. Many animals perform this ecological role. Numerous species of plants would be pushed to extinction if this service did not exist, and many current crops might be challenging to cultivate (Abrol, 2012). Pollination is thought to be responsible for up to 75 per cent of the production of food from agriculture (Klein *et al.,* 2007). During the last few years, there has been an enormous decline in the ratio of insect pollinators across the world (Rhodes, 2019). India has many endemic species and is ranked sixth among the world's 12 biodiversity hotspots (Singh and Chaturvedi, 2017). With over 6,000 known species worldwide, divided into 300 separate groups (Skevington *et al.,* 2019), the Syrphidae (hoverflies or flower flies) family of Diptera is one of the most diverse and well-known to the people (Courtney *et al.,* 2017). Adult hoverflies are essential for pollinating flowering plants (Free, 1993; Richards *et al.,* 1997). Syrphids are one of the most common families of flies, having significant potential as the ecosystem's first-line pollinator (Owen and Gilbert, 1989), especially in certain landscapes

* Author for correspondence

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where hymenopteran pollination competence is at risk of depletion because they rely on endothermically produced energy (Milièiæ *et al.,* 2017). Thus, there is a need for second-line pollinators like hoverflies for these types of ecosystems. Another characteristic of the syrphid flies is that their larvae consume various foods (Knutson *et al.,* 1975). Some larvae are saprotrophs, while others are insectivores, feeding on Hemipterans (aphids, adelgids, psyllids), as well as Thysanopterans (Thrips) and other plant-sucking insects (Brunetti, 1907, 1908; Thompson and Ghorpadé 1992; Tenhumberg, 1995; Rojo *et al.,* 2003; Bugg *et al.,* 2008; Sengupta *et al.,* 2018). A few syrphid larvae can reduce weed growth (Rizza *et al.,* 1988).

Flower flies are a cosmopolitan family that can be found in most biomes, except deserts, high-altitude tundra, and Antarctica (Sutherland *et al.,* 2001). Some hoverfly species have a restricted distributional range, i.e. they are indigenous to a specific habitat or location, whilst others have a wide distribution across numerous countries (Owen and Gilbert, 1989). Hoverflies are usually distinguishable from other flies by a spurious vein or Vena spuria that runs parallel to the fourth longitudinal wing vein (Vockeroth, 1992), however, there are exceptions (e.g. syrphid flies of the genera *Graptomyza* and *Paragodon*) (Thompson, 1969). Four subfamilies are currently recognized (Eristalinae, Microdontinae, Pipizinae and Syrphinae) (Mengual *et al.,* 2015), but some authors (Speight, 1987; Thompson, 1969, 1972) have split off the basal clades of Syrphidae, recognizing a separate family Microdontidae. There are 202 genera and 96 subgenera of Syrphidae currently recognized, grouped into 13 tribes and 12 subtribes (Thompson, 1972; Vockeroth, 1992; Young *et al.,* 2016). In India, 357 species from 69 genera have been identified (Ghorpadé, 2014). Several of the species are indigenous to India.

Morphological taxonomic keys require entomological expertise to identify species, as many species have a similar appearance and are difficult to distinguish (Achint and Singh, 2021). Identification markers such as wing venation, eye color pattern, color patterns of legs, setae of thorax and abdomen, and their color for specimens are frequently degraded during storage and collection techniques such that morphological identification of syrphids is difficult and time-consuming. Another disadvantage of morphological identification is the lack of keys to all life stages, although taxonomic keys for adult syrphids are well documented. Alternative strategies to address these challenges include DNA barcoding (Hogg and Hebert, 2004). A 350-700bp of the mitochondrial cytochrome c oxidase I (cox1) (Hebert *et al.,* 2003a, b) is used as a proper approach for identifying worldwide biota (Waugh, 2007). Using a standardized DNA locus for DNA barcoding has become a popular and effective way of differentiating species (Achint and Singh, 2021; Bajaj *et al.,* 2023). For the correct identification of numerous groups to the species level (http://www.ibol.org/resources/) as well as species complexes, a brief sequence of standardized COI gene mitochondrial DNA has been employed and recognized (Tyagi *et al.,* 2017). The current study aimed to test the COI gene to correctly identify these pollinating hoverflies from the different geo-climatic zones of West Bengal with respect to morphology-based identification procedures.

MATERIALS AND METHODS

Our survey for the collection was carried out in different regions of West Bengal. It was chosen as our study due to its vastness and diversity as it includes the different geo-climatic regions, namely the hilly regions, arid region, the Gangetic plains, and coastal areas (Maity *et al.,* 2016). West Bengal is also one of the leading states in terms of agricultural crop production in our country. Hence, a COI barcode database for these crop-friendly pollinators was needed for easy identification and conservation. The study used 54 specimens from 19 species of 9 genera representing distinct syrphid subfamilies. During the years 2020–2021, 18 specimens containing 13 species were collected from various districts of West Bengal (Murshidabad, Kolkata, South 24 Parganas, Kalimpong) including the vulnerable Sagar Islands (Table 1) (Fig 1.). Their DNA sequences were submitted to GenBank, while sequences for the

remaining species were acquired from the Genbank database (NCBI). We surveyed extensively in this one year, covering all three seasons, namely pre-monsoon, monsoon, and post-monsoon, where these 13 species of hoverflies were found in all the seasons. Fly specimens were collected by sweep net collection from flowering vegetation and agricultural lands. The specimens were preserved in high-grade-ethyl alcohol (70%). After that, part of the collected specimens was dried and pinned with entomological pins, and after morphological identification with stereomicroscope, specimens were deposited in the designated repository of the National Zoological Collections (NZC), Zoological Survey of India, Kolkata. The necessary specimen photographs were obtained with a Leica stereo-iso microscope M205A, a Leica DFC 500 camera, and the Leica Application Suite LASv 3.6 software. According to Systema Dipterorum, valid species names were allocated (Evenhuis and Pape, 2022).

The maps for this paper were created using Arc GIS® Desktop software (version 10.8) by ESRI after registering the geographical coordinates of the collection sites in Garmin GPS device (Fig. 1).

DNA extraction, Polymerase Chain Reaction (PCR), and DNA sequencing:

Genomic DNA (gDNA) was extracted from individual fly specimens using the QIAmp DNA extraction kit (Qiagen). The whole procedure was done according to the manufacturer's instructions. Voucher specimens were submitted in the Diptera Section of the ZSI, Kolkata. The amount of DNA was recorded on a Qubit Fluorometer (Life Technologies, USA), and the extracted DNA was kept at -20°C for subsequent analysis. Using primers- forward LCO-1490 (F) (GGT CAA CAA ATC ATA AAG ATA TTG G) and reverse HCO-2198 (R) (TAA ACT TCA GGG TGA CCA AAA AAT CA), roughly 20 ng genomic DNA was utilized

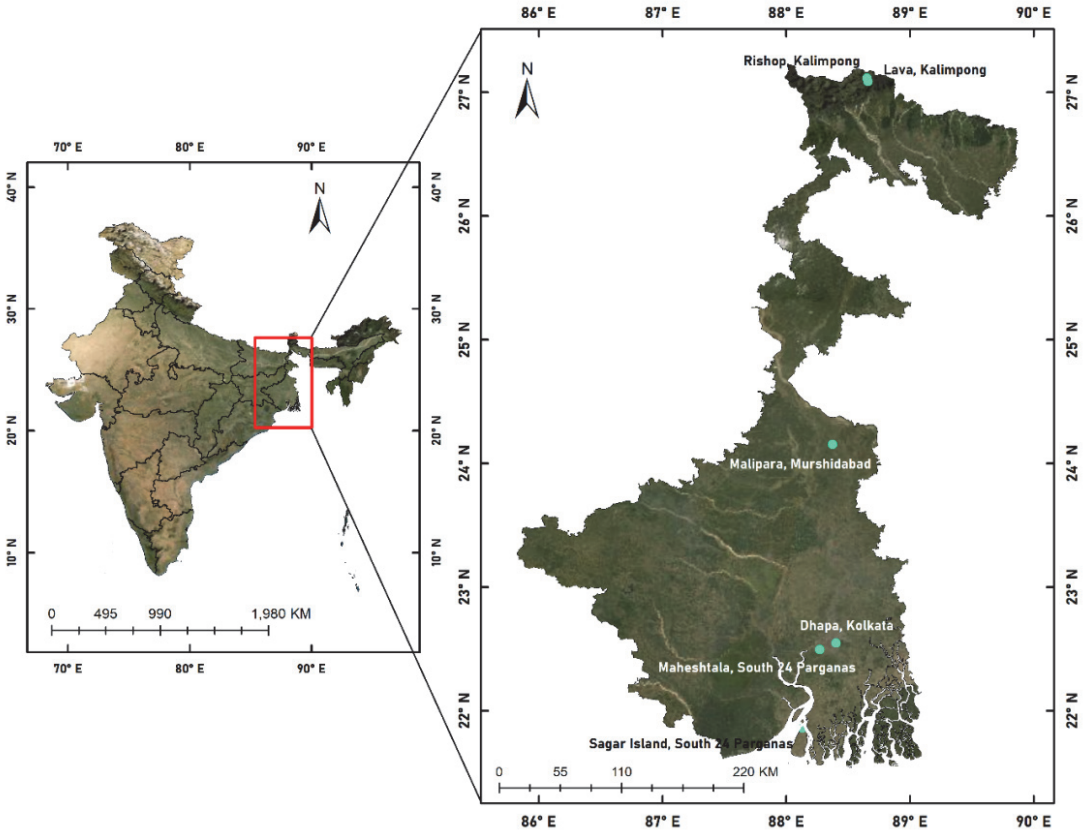

Fig 1. Sampling localities of collected syrphid flies from different geo-climatic regions of West Bengal

Table 1 Analyzed syrphid samples, with sampling locations from West Bengal, GenBank accession numbers, species names, and collection dates

No.	Locality	Accession no.	Species name	Date of collection	Coordinates
1	Murshidabad, Malipara	ON421581	*Episyrphus balteatus*	08/03/2020	24.15341 N; 88.37381 E
2	Murshidabad, Malipara	ON421583	*Ep. balteatus*	08/03/2020	24.15341 N; 88.37381 E
3	Murshidabad, Malipara	ON421584	*Episyrphus balteatus*	08/03/2020	24.15341 N; 88.37381E
4	South 24 Parganas, Maheshtala	ON210051	*Er. quinquestriatus*	21/10/2021	22.496025 N; 88.2703667 E
5	South 24 Parganas, Maheshtala	ON217545	*Er. quinquestriatus*	21/10/2021	22.496025 N; 88.2703667 E
6	South 24 Parganas, Maheshtala	ON226501	*Er. polychromata*	21/10/2021	22.496025 N; 88.2703667 E
7	Murshidabad, Malipara	ON421642	*Er. polychromata*	08/03/2020	24.15341 N; 88.37381 E
8	Kolkata, Dhapa	ON248238	*Er. arvorum*	09/11/2021	22.54769167N;88.40233611E
9	Kalimpong, Rishop	ON260958	*Eristalis cerealis*	27/11/2021	27.1123 N; 88.65324 E
10	Kalimpong, Rishop	ON248443	*Eristalis tenax*	27/11/2021	27.1123 N; 88.65324 E
11	Kalimpong, Lava	ON209555	*Ersitalis himalayensis*	18/10/2021	27.0863 N; 88.6615 E
12	Kalimpong, Lava	ON222740	*Eupeodes luniger*	18/10/2021	27.0863 N; 88.6615 E
13	Kalimpong, Lava	ON261094	*Dasysyrphus orsua*	18/10/2021	27.0863 N; 88.6615 E
14	South 24 Parganas, Sagar Island	ON422271	*Dideopsis aegrota*	05/10/2021	21.86216 N; 88.12949 E
15	Murshidabad, Malipara	ON421526	*Ischiodon scutellaris*	08/03/2020	24.15341 N; 88.37381 E
16	South 24 Parganas, Sagar Island	ON440975	*I. scutellaris*	17/02/2021	21.86216 N; 88.12949 E
17	Murshidabad, Malipara	ON421461	*Melanostoma orientale*	08/03/2020	24.15341 N; 88.37381 E
18	Murshidabad, Malipara	ON421571	*Paragus crenulatus*	08/03/2020	24.15341 N; 88.37381 E

to amplify about 700 base pairs from the 5' end of the mitochondrial cytochrome c oxidase subunit I (COI) gene (Folmer *et al.,* 1994). PCR was carried out in a 50µl total reaction volume comprising 20 Pico moles of each primer, 100 mM KCl, 20 mM Tris–HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 2.0 mM MgCl2, 0.25 mM of each dNTP, primer cocktail, and 1U of Taq polymerase (Takara BIO Inc., Japan) with the following cycling parameters: 5 min at 94^0C; followed by 40 cycles of 30 s at 94^0C, 40 s at 53^0C, 1 min at 72^0C and final extension for5 min at 72^0C. To confirm the amplicon size, the amplified products were seen in a 1 per cent agarose gel, stained with SYBR@safe DNA gel dye, and imaged on a safe gel imager (Invitrogen). The QIAquick Gel Extraction Kit (Qiagen) was used to purify the PCR-amplified products according to the manufacturer's instructions. For cycle sequencing, about 15 ng of purified PCR product was utilized. Cycle sequencing was performed on an ABI thermal cycler using the BigDye®Terminator ver. 3.1Cycle Sequencing Kit (Applied

Biosystems, Inc) with both forward and reverse PCR primers using the following parameters: 96°C for 1 min, then 25 cycles of 96°C for 10 s, 50°C for 5 s, and a final extension at 60°C for 1 min15 s. After cycle sequencing, the products were cleaned with the BigDye X-terminator kit (Applied Biosystems Inc.) and placed into an ABI 3730 capillary Genetic analyzer at the Zoological Survey of India sequencing laboratory (Banerjee *et al.,* 2015; Tyagi *et al.,* 2017; Achint and Singh, 2021).

Sequence analysis and dataset formation: MEGA X was used to manually edit the sequences from each specimen (Kumar *et al.,* 2018). All sequences were matched to identical reported sequences in the NCBI database utilizing the BLAST (https://blast.ncbi.nlm.nih.gov) algorithm (Chakraborty *et al.,* 2019). The ORF finder of NCBI (https://www.ncbi.nlm.nih.gov/orfnder/gorf.html) is used to examine the accurate amino acid codes devoid of any stop codon or indels (insertion or deletions). Each sequence was uploaded to GenBank library, and unique accession numbers were issued to each one (Table 1). A total of 54 specimens from 19 species belonging to 9 genera from different syrphid subfamilies were included in this investigation (both Indian and global sequences). The 9 genera include *Ischidon* containing 1 species, *Dasysyrphus* containing 3 species, *Eupeodes* containing 1 species, *Episyrphus* containing 1 species, *Dideopsis* containing 1 species, *Melanostoma* containing 1 species, *Paragus* containing 2 species, *Eristalis* containing 4 species, and *Eristalinus* containing 5 species. A member of the putative Syrphidae sister-group, Pipunculidae, was added as outgroup (Ståhls *et al.,* 2003). 36 sample sequences from 55 taxa were downloaded from NCBI, Genbank, including the outgroup. Those 36 samples includes *Ischiodon scutellaris* KY845775 (Pakistan), MK771152 (Bangladesh), KY846329 (Pakistan); *Dasysyrphus amalopis* JX828010 (Canada), JX828112 (Canada); *Dasysyrphus pauxillus* MZ610653 (Finland), MZ629684 (Finland); *Eupeodes luniger* KT959887 (Finland), KY834510 (Pakistan), MW077802 (France); *Episyrphus balteatus* OL765264 (India), MN973969 (India), *Dideopsis aegrota* MW473976 (Canada); *Melanostoma orientale* KY839783 (Pakistan), KY837293 (Pakistan), KT175592 (India); *Paragus serratus* MG194422 (India), KY837201 (Pakistan); *Paragus crenulatus* JN298750 (Canada), JF872389 (Canada); *Eristalis himalayensis* OL442159 (India); *Eristalis tenax* OL441830 (India), MN967351 (India), MN967352 (India); *Eristalis cerealis* OK465106 (India), OK287112 (India); *Eristalis arbustorum* JN269860 (Canada), MN868856 (Portugal); *Eristalinus arvorum* MK751019 (Germany), MK751022 (Germany), MK751021 (Germany); *Eristalinus aeneus* MW473968 (Canada); *Syrphidae sp.* KY841659 (Pakistan); *Eristalinus pari*a OK655827 (India), OK444104 (India); *Eristalinus sp.* MK771154 (Bangladesh); Pipunculidae KR506987 (Canada) (outgroup).

Genetic divergence and cluster analysis:

Initially, sequences were aligned (multiple sequence alignment) in MEGA X software via the ClustalW algorithm (Kumar *et al.,* 2018). To avoid any form of coherent outcomes, the dataset is constructed to be 663 base pairs long. The genetic divergence between and within taxonomic groups was estimated in MEGAX using the Kimura-2-parameter (Kimura, 1980; Kumar *et al.*, 2018). The best-fit nucleotide substitution model was determined using JModelTest v2.1.10 (Darriba *et al.,* 2012) through CIPRES server (Miller *et al.,* 2010) to discover a suitable evolutionary model for the syrphid flies dataset based on the Bayesian Information Criterion (BIC). Models with the lowest BIC scores (-5650.248) were considered to describe the substitution pattern the best (Nei and Kumar, 2001). The GTR+G+I model was selected for the syrphid COI dataset. MEGA X was used to investigate nucleotide substitution and nucleotide composition data (Kumar *et al.,* 2018).

The COI dataset has been used to construct the phylogenetic trees based on the Maximum Likelihood (ML) algorithm (Fig. 2). The dataset was designed and analyzed in IQ-TREE on XSEDE (2.1.2v) (Nguyen *et al.,* 2015; Minh *et al.,* 2020) via the CIPRES website (Miller *et al.,* 2010), employing 1,000 bootstrapping tests and default parameter settings (Siriwut *et al.,* 2021). The

FigTree v1.4.4 software (http://tree.bio.ed.ac.uk/software/fgtree/) was used to edit the resultant files. This offered a graphic depiction of the specimen's sequencing divergence. The Bayesian (BA) tree (Fig. 3) was generated in Mr. Bayes v3.2.7a with nst=6 for the GTR+G+I model utilizing metropolis-coupled Markov Chain Monte Carlo (MCMC) and run for 5,000,000 generations with 25 per cent burn-in and trees saved every 100 generations. The posterior probability was used to determine branch support (PP). The web-based iTOL v6 program (https://itol.embl.de/) was used to create a tree from the produced files, which aided visual display. Haplotypes calculations were done in DnaSP v5.10 (Librado and Rozas, 2009).

The PTP model (Zhang *et al.,* 2013) was utilized for species delimitation, which defines species based on the number of substitutions in the phylogenetic tree changing. For the species delimitation study, the BA tree file in Newick format was submitted to the bPTP server (https://species.h-its.org/html). The robustness of species delimitations is estimated using Bayesian support values. A higher bootstrap value at the node indicates that the terminal node is more certain to belong to a specific species. The PTP analysis was run for 500,000 MCMC (Markov Chain Monte Carlo) generations with a thinning value of 100 and a burn-in of 25 per cent, and the outgroup was excluded.

RESULTS AND DISCUSSION

DNA sequences:

The dataset includes 663bp of the cox1 gene of ecologically and agriculturally essential syrphids of 19 species under 9 genera namely *Ischidon*, *Dasysyrphus*, *Eupeodes*, *Episyrphus*, *Dideopsis*, *Melanostoma*, *Paragus*, *Eristalis*, and *Eristalinus*. The conserved, variable, and parsimoniously informative sites for the studied species were examined. The data set contains 55 sequences with 663 base pairs, 299 distinct patterns (variable sites), 201 parsimony-informative sites, 34 singleton variable sites, and 428 constant (conserved) sites. Hence, this study demonstrates that the COI gene is highly conserved.

Base composition and nucleotide substitution:

Both the nucleotide sequence and the specific nucleotide percentage were examined in this study since both characteristics are significant for evaluating variation among different species in MEGA X (Kumar *et al.,* 2018). The nucleotide base composition of the sequenced fly species collected by us showed that ON421583 (*Episyrphus balteatus*) and ON421581 (*Episyrphus balteatus*) have the highest (AT) percentage (70.6%), while ON260958 (*Eristalis cerealis*) had highest (GC) percentage (31.7%). On the other hand, ON421642 (*Eristalinus polychromata*) had the lowest (AT) percentage (67.9%) and ON421583 (*Episyrphus balteatus*) and ON421581 (*Episyrphus balteatus*) have lowest (GC) percentage (29.4%) (Table 2). The average nucleotide frequencies are 30.53 (A), 38.64 (T/U), 14.72 (C), and 16.11 per cent (G). This clearly reveals that in the nucleotide sequences (A + T) content was higher than (G + C). Thus it proves that insect mtDNA has a higher (A + T) frequency (Lunt and Hyman, 1997).

Evolutionary analyses and divergences were examined in MEGA X (Kumar *et al.,* 2018). To visualize the characterization of genetic variations of different species, sequences were downloaded from GenBank in FASTA format (Achint and Singh, 2021). Transition/transversion rate ratios were k1 = 2.002 (purines) and k2 = 1.915 (pyrimidines). The overall transition/transversion bias was R = 0.833, {where R = [A*G*k1 + T*C*k2]/[(A+G) * (T+C)]} as calculated by the Maximum Composite Likelihood method in MEGA X. This result shows that the rate of transitions was higher than the rate of transversions. Nucleotide Substitution patterns were calculated (Table 3). Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option) (Tamura *et al.,* 2004; Kumar *et al.*, 2018; Ghosh *et al.,* 2022).

Haplotypes and haplotype diversity: Further research indicated the presence of 16 haplotypes in 18 sampled syrphid fly specimens, representing a significantly high degree of haplotype diversity. Most of samples showed unique haplotypes (Table 4).

Sequence divergence: To ensure that the COI gene is reliable for identification, intraspecific divergences should not exceed 3 per cent and interspecific divergences should be greater than 3 per cent (Wells and Sperling, 2001). A method of DNA barcode analysis for species discrimination has been developed that contrasts intraspecific and interspecific genetic divergence (bar-coding gap) (Hebert *et al.,* 2004; Ghosh *et al.,* 2022). For species recognition in insects, a 3 per cent interspecific genetic divergence limit was advised to close the barcoding gap (Hebert *et al.,* 2003a). Later, it was proposed that intraspecific and interspecific genetic divergences vary between taxa, and that a universal cutoff does not exist (Meyer and Paulay, 2005). Intraspecific divergence was found to be 0.00-5.00 per cent. The highest intraspecific divergence was seen in *Eristalinus polychromata* (5%) and *Eristalis himalayensis* (5%) while lowest intraspecific divergence was shown by *Ischiodon scutellaris* (0%), *Eristalinus arvorum* (0%), *Eristalinus paria* (0%), *Eristalinus arbustorum* (0%), *Eristalis tenax* (0%), *Dideopsis aegrota* (0%), *Episyrphus balteatus* (0%), *Paragus crenulatus* (0%), *Melanostoma orientale* (0%), *Eupeodes luniger* (0%), *Dasysyrphus pauxillus* (0%), and *Dasysyrphus amalopis* (0%). The intraspecific divergence for *Eristalinus quinquestriatus* was 1 and *Eristalis cerealis* was 2 per cent. The intraspecific genetic distance of *Eristalinus polychromata* and *Eristalis himalayensis* showed a value of more than 3 per cent, indicating a possible overlap of intra and interspecific divergences. The interspecific divergence ranged from 0.8 to 19.6 per cent. The lowest interspecific divergence (0.8%) was recorded between *Dasysyrphus pauxillus* and *Dasysyrphus amalopis* (Meyer and Paulay, 2005). Interspecific divergences less than 3 per cent in Diptera have previously been considered as evidence of species complexes or cryptic species. Additional evidence regarding the delimitation of *Dasysyrphus amalopis* and *Dasysyrphus pauxillus* is needed to be investigated, including genes other than COI (Banerjee *et al.,* 2015).

Phylogenetic analysis:

In the Maximum Likelihood (ML) tree (Fig. 2), the two main clades correspond to the subfamilies Eristalinae and Syrphinae. The first clade of the Eristalinae subfamily contained species of the genera *Eristalinus* and *Eristalis*, while in the second clade of subfamily Syrphinae, species of the genera *Paragus*, *Melanostoma*, *Dideopsis*, *Episyrphus*, *Eupeodes*, *Dasysyrphus*, and *Ischiodon* were present. The intraspecific genetic distance of *Eristalinus quinquestriatus* was less than 3 per cent (1%) and the global sequence MK771154 *Eristalinus sp.* formed the same clade with ON210051 *Eristalinus quinquestriatus* and ON217545 *Eristalinus quinquestriatus*. MK77154 is a likely sequence of Bangladesh sample of *Eristalinus quinquestriatus* probably. Sequence ON260958 fell in the same clade as OK465106 *Eristalis cerealis* and OK287112 *Eristalis cerealis*, both from India. The bootstrap value of the clade was 100 per cent. We conclude that ON260958 is *Eristalis cerealis* although morphological identification of the specimen was complicated by its poor condition. Clades with >90% bootstrap support are considered as strongly supported. The genera *Ischiodon, Melanostoma* and *Paragus* are strongly supported with 100 per cent bootstrap support. Some species developed their unique conspecific cluster due to geographical differences in the collected samples.

In the Bayesian (BA) tree (Fig. 3), it is evident that the two subfamilies distinguished from one another with a very high support value of 0.99 at the deep node, showing complete congruence with the ML tree and the observations done by other researchers (Mengual *et al.,* 2015; Mengual, 2020; Moran *et al.*, 2022) done on Palaearctic and Nearctic regions respectively. The deep node value supporting each of the subfamilies show Eristalinae to be monophyletic (0.99) but Syrphinae to be a non-monophyletic clade (0.716) which is supported by the work done by Mengual *et al.* (2008).

There are five described tribes under the subfamily Syrphinae: Bacchini, Melanostomini, Paragini, Syrphini, and Toxomerini. The studied sample set

Table 2 The nucleotide base composition of the sequenced fly species using MEGAX

Species	Position	T	C	A	G	AT (%)	GC (%)
ON440975 *Ischiodon scutellaris*	1st	49.5	1.0	48.1	1.5	69.9	30.1
	2nd	26.6	15.5	27.5	30.4		
	3rd	43.0	25.6	15.0	16.4		
ON421526 *I. scutellaris*	1st	49.4	1.3	48.8	0.6	69.4	30.5
	2nd	28.3	14.5	27.7	29.6		
	3rd	41.5	28.3	12.6	17.6		
ON261094 *Dasysyrphus orsua*	1st	49.1	1.8	49.1	0.0	69.4	30.6
	2nd	27.7	16.9	26.5	28.9		
	3rd	42.2	27.1	13.9	16.9		
ON222740 *Eupeodes luniger*	1st	54.5	1.9	42.7	0.9	69.6	30.4
	2nd	27.4	15.6	26.4	30.7		
	3rd	43.4	25.0	14.6	17.0		
ON421583 *Episyrphus balteatus*	1st	50.7	0.9	48.3	0.0	70.6	29.4
	2nd	28.9	14.7	25.6	30.8		
	3rd	43.6	25.1	14.7	16.6		
ON421584 *Ep. balteatus*	1st	48.8	0.6	50.6	0.0	70.4	29.6
	2nd	31.2	14.7	24.7	29.4		
	3rd	42.4	26.5	13.5	17.6		
ON421581 *Ep. balteatus*	1st	49.1	0.6	50.3	0.0	70.6	29.4
	2nd	31.4	14.8	24.9	29.0		
	3rd	42.6	26.6	13.6	17.2		
ON422271 *Dideopsis aegrota*	1st	54.2	0.0	45.8	0.0	70.4	29.5
	2nd	28.3	13.3	29.2	29.2		
	3rd	41.2	26.1	12.6	20.2		
ON421461 *Melanostoma orientale*	1st	49.7	2.7	47.0	0.7	68.9	31.1
	2nd	26.8	16.8	28.9	27.5		
	3rd	43.0	28.9	11.4	16.8		
ON421571 *Paragus crenulatus*	1st	47.1	0.8	51.3	0.8	69.4	30.6
	2nd	26.1	16.0	29.4	28.6		
	3rd	40.7	27.1	13.6	18.6		
ON209555 *Eristalis himalayensis*	1st	46.4	5.3	47.4	1.0	69.1	30.9
	2nd	27.3	15.3	28.7	28.7		
	3rd	42.6	25.4	14.8	17.2		
ON248443 *Er. tenax*	1st	42.2	6.2	51.7	0.0	69.1	30.8
	2nd	27.8	15.1	28.3	28.8		
	3rd	42.9	25.0	14.6	17.5		
ON260958 *Er. cerealis*	1st	42.6	5.9	51.0	0.5	68.3	31.7
	2nd	26.0	14.7	28.9	30.4		
	3rd	43.9	24.9	12.7	18.5		
ON248238 *Er. arvorum*	1st	44.9	5.3	49.8	0.0	69.8	30.3
	2nd	29.0	14.5	27.5	29.0		
	3rd	43.0	25.1	15.0	16.9		

ON421642 *Er. polychromata*	1st 2nd 3rd	40.6 28.2 41.8	6.5 17.1 27.1	52.4 27.1 13.5	0.6 27.6 17.6	67.9	32.2
ON226501 *Er. polychromata*	1st 2nd 3rd	46.2 29.6 41.4	3.0 16.0 27.2	50.9 27.2 13.6	0.0 27.2 17.8	69.7	30.4
ON217545 *Er. quinquestriatus*	1st 2nd 3rd	45.6 30.2 41.4	2.4 15.4 27.2	51.5 27.2 13.6	0.6 27.2 17.8	69.9	30.2
ON210051 *Er. quinquestriatus*	1st 2nd 3rd	46.9 27.4 42.9	2.4 15.6 25.5	49.8 27.4 14.6	0.9 29.7 17.0	69.7	30.4

(All frequencies are given in percentage)

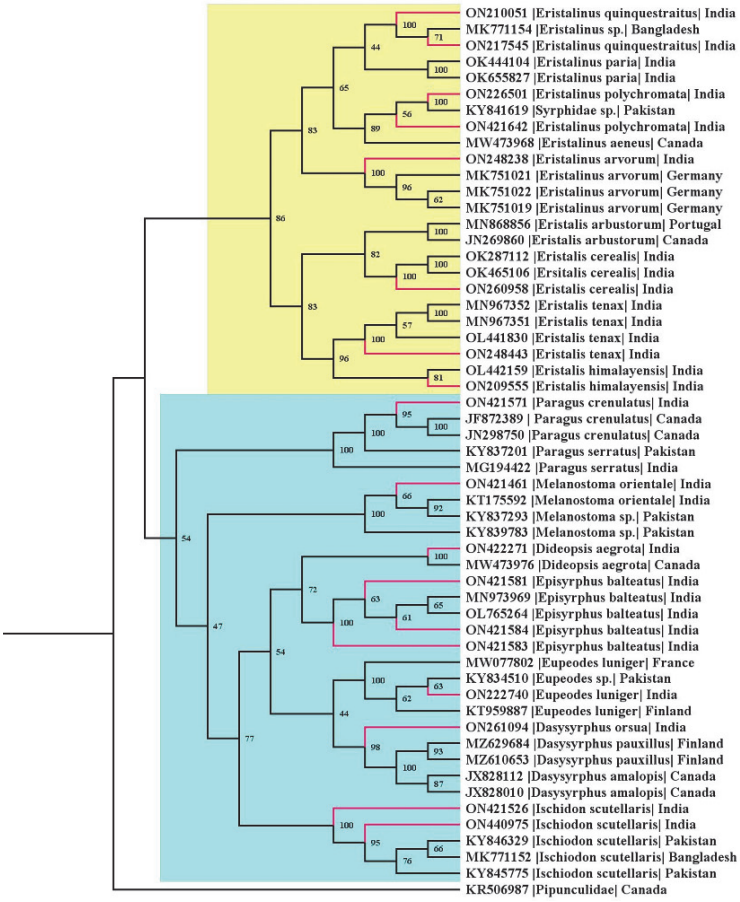

Fig. 2 Phylogenetic Tree (Maximum-likelihood) from collected syrphid COI (pink branch) dataset. IQ-TREE on XSEDE (v2.1.2) generated 1000 bootstrapped ML (GTR + G+I) tree of syrphid flies based on COI gene. Numbers indicate bootstrap values from ML analysis. Pipunculidae (Diptera) was used as an out-group

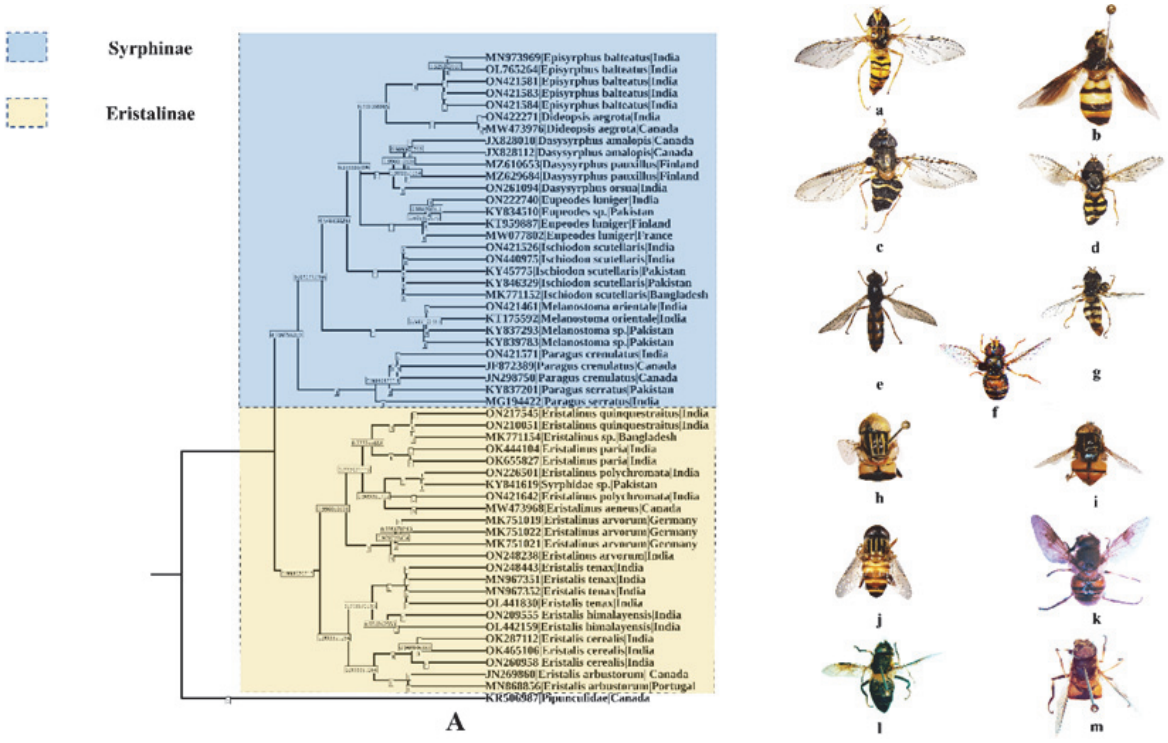

Fig. 3 The Bayesian (BA) Tree of 54 hoverflies sequences with posterior probabilities from Bayesian analysis. Pipunculidae has been used as outgroup. The different species are shown in this figure namely- a. *Episyrphus balteatus*, b. *Dideopsis aegrota*, c. *Dasysyrphus orsua*, d. *Eupeodes luniger*, e. *Melanostoma orientale*, and f. *Paragus crenulatus*. g. *Ischiodon scutellaris*, h. *Eristalinus quinquestriatus*, i. *Eristalinus polychromata*, j. *Eristalinus arvorum*, k. *Eristalis tenax*, l. *Eristalis himalayensis,* m. *Eristalis cerealis*

contains taxon sampling under three of these tribes: Melanostomini, Paragini, and Syrphini.

Melanostomini comprises one genus in our dataset with *Melanostoma orientale* that forms a sister clade with the tribe Paragini which is a monotypic taxon with only one genus *Paragus*. Under the tribe, Syrphini, *Episyrphus*, and *Dideopsis* form a sister clade with 0.8 BI (Bayesian Inference) support, whereas, *Dasysyrphus* and *Eupeodes* form a large polytomy with the previous group. The lower value at the support branch in the deep nodes creates confusion about the exact relationship of the said groups from an oriental perspective.

The three species of the sample set under the genus *Dasysyrphus* are resolved together, showing prominent monophyly, although the genus shows significant variation in male genitalia and larval character (Mengual *et al.,* 2008).

Under the subfamily Eristalinae, there are multiple tribes, and our studied taxons fall under the tribe Eristalini and subtribe Eristalina. In the BA tree, the two genera under consideration form definitive monophyly with a branch support of 0.99, whereas

Table 3. Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution

	A	T	G	C
A	-	9.77	3.72	**8.16**
T	7.72	-	**7.13**	4.07
G	7.72	**18.7**	-	4.07
C	**15.45**	9.77	3.72	-

Each entry shows the probability of substitution (r) from one base (row) to another base (column).
For simplicity, the sum of r values is made equal to 100.
Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics.

each of the genera also forms monophyly at the species level, having a branch support of 0.996 for the genus *Eristalinus* and 0.993 for genus *Eristalis*. At the species level, *Eristalis cerealis* and *Eristalis arbustorum* form monophyly with each other and are of the closest proximation. On the other hand, *Eristalis tenax* and *Eristalis himalayensis* form another monophyly with a support of 0.9. The other sister clade shows subclades formed by *Eristalinus quinquestriatus* and *Eristalinus paria* and *Eristalinus polychromata* and *Eristalinus aeneus*. The previous shows non-monophyly at the recent divergence, whereas the latter shows monophyly. The whole subclade forms a monophyletic clade with *Eristalis arvorum*.

At the species level, several taxa show branching and variation with low BI values, like *Episyrphus balteatus*, *Eupeodes luniger*, and *Melanostoma orientale*, so it is hard to assign a definitive relationship between these taxa and their conspecifics. According to the bPTP analysis, there are 42 putative species in the study set, which can be due to the varied geographical distribution and the presence of haplotypes.

The COI gene can be used to identify Indian syrphid flies and to determine their phylogeny. Because of its variety, the barcoding gene COI is an excellent marker for phylogenetic study and confirming geographical population distribution patterns. It is crucial to identify and segregate the data of the agriculturally important syrphid flies to understand their effect in the agricultural and food industries. We anticipate that the barcode data provided by this research may aid in rapidly identifying these syrphid flies in both their adult and larval stages. This is the first-ever study that provided the barcoding of the syrphid flies from West Bengal, along with the first-ever barcoding of the COI gene of *Dasysyrphus orsua* and *Eristalinus polychromata*. It is critical to accurately quantify the pollinator diversity to uphold existing variety and prevent further reduction in their population, and barcoding them allows us to identify them even if skilled taxonomists are not present. Correctly identifying these pollinating insects is critical in developing methods for conserving them and augmentation programs in crops to increase production. Hence, this study and the combination of additional molecular markers and morphological and ecological data would be helpful in better characterization and understanding them in the study site on a larger scale in the future.

Table 4. Number of specimens and mitochondrial haplotypes in collected syrphid flies

No.	Species	specimens	haplotypes
1	*Ischiodon scutellaris*	2	2
2	*Dasysyrphus orsua*	1	1
3	*Eupeodes luniger*	1	1
4	*Episyrphus balteatus*	3	1
5	*Dideopsis aegrota*	1	1
6	*Melanostoma orientale*	1	1
7	*Paragus crenulatus*	1	1
8	*Ersitalis himalayensis*	1	1
9	*Eristalis tenax*	1	1
10	*Eristalis cerealis.*	1	1
11	*Eristalinus arvorum*	1	1
12	*Eristalinus polychromata*	2	2
13	*Eristalinus quinquestriatus*	2	2

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A checklist of butterflies of Sálim Ali Centre for Ornithology and Natural History (SACON) campus, Anaikatty, Western Ghats, India

P.P. Ashiq[1*], S. Jeevith[1,2*], M. Sri Sowmiya[1,3], J. Chaithra Shree[1,4], P. Pramod[1] and P.R. Arun[1]

[1]Sálim Ali Centre for Ornithology and Natural History, South India Centre of Wildlife Institute of India, Anaikatty, Coimbatore 641108, Tamil Nadu, India.
[2]The Wynter-Blyth Association, 7D Plains View Garden, Tiger Hill, Coonoor, The Nilgiris 643101, Tamil Nadu, India.
[3]Department of Zoology, Kongunadu Arts and Science College, Coimbatore 641029, Tamil Nadu, India.
[4]Siddarth Foundation, No 26, Abbas Garden, TVS Nagar, Coimbatore 641025, Tamil Nadu, India.
Email: ashiqleo7@gmail.com; jeevithbotany@gmail.com; srimsowmiya@gmail.com; chaithrashree89@gmail.com; neosacon@gmail.com; eiasacon@gmail.com

ABSTRACT: An updated checklist of butterfly species recorded from the Sálim Ali Centre for Ornithology and Natural History (SACON) campus, constitute a total of 178 species of butterflies, including previous records of 101 species. The butterfly species represent Papilionidae (17), Pieridae (22), Nymphalidae (55), Lycaenidae (54), Riodinidae (1) and Hesperiidae (29). Seventy-seven species are new additions. Among the 178 species, 56 (31.46%) were found to be very common, 74 (41.57%) were moderately common, 42 (23.60%) were rare and 6 (3.37%) as very rare in sightings. Ninety plant species belonging to 33 families as larval host plants of 160 butterfly species were also recorded in the present study. Host plants of 18 species of butterflies were not available in the study area.
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KEY WORDS: Lepidoptera-Rhopalocera, species, updated, new additions, larval host plants, abundance

INTRODUCTION

Butterflies are one of the major taxonomic groups belonging to the insect order Lepidoptera, and one of the most important components of biodiversity. They play vital ecological functions in pollination, forest food chain as well as urban ecosystems (Mac and Fleishman, 2004). Globally, around 19,238 species of butterflies are described under seven families and account for about 12 per cent of the known Lepidopteran species (Heppner, 1998). In India, there are numerous studies on the diversity and distribution of butterflies, which account for one fifth of the global butterfly species (Wynter-Blyth, 1957). About 1,501 species of butterflies have been reported from India (Kunte, 2000), out of which 335 species have been reported in the

* Author for correspondence

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Western Ghats (Gaonkar, 1996), and 327 species in Tamil Nadu (Pavendhan *et al*., 2022).

The campus of Sálim Ali Centre for Ornithology and Natural History (SACON), in Anaikatty hills, Coimbatore (11^{0}05'30.9"N; 76^{0}47'36.2"E), is located towards the north of Palghat gap on the eastern slopes of Western Ghats at 600 m ASL. SACON campus was established in early 2000. The area surrounded by degraded forest land with rocky slopes, where planting of native and ornamental species in and around the campus in later years enhanced the vegetation and attracted several pollinators as well as numerous wildlife. This region experiences annual temperature of minimum 20^{o}C and maximum 41^{o}C, and the area receive both southwest and northeast monsoon, with annual rainfall up to 700 mm. SACON campus is surrounded by reserve forest covering 180 sq.km with southern tropical mixed deciduous and southern tropical dry mixed deciduous forest (Champion and Seth, 1968), and grassland patches at the hills of nearby Dumanur village. The campus is rich in floristic diversity with 402 species of flowering plants belonging to 84 families (Balasubramanian *et al*., 2015). A total of 93 species of butterflies were recorded in the study area (Eswaran and Pramod, 2005; Eswaran, 2006) and in 2014 recorded 91 butterfly species along with 13 new additions compared to the previous studies in the SACON campus (Kumar and Arun, 2014) making the butterfly diversity of the campus to 106 species. There has been no long-term monitoring of insect populations, their diversity and distribution in India even for species like butterflies that are given more importance in citizen science (Sondhi and Kunte, 2020). Since it has been almost a decade after the last checklist, more species have been reported from the region and there is a need to update the information on the butterfly diversity of the area. In this context, an updated checklist with new additions to the butterflies in the area becomes significant and will help in collating and providing a database of the diversity of butterflies and larval host plants for the region.

MATERIALS AND METHODS

The field data on butterflies recorded by authors from the Sálim Ali Centre were compiled and updated with new additions (Fig. 1), Regular fortnight surveys were carried out from 2016 to 2022 on fixed routes with different zones inside campus and adjoining areas using standard methods (Pollard, 1977). The butterflies were observed and recorded directly in the field. Sampling was done between 09 00-11 00 h and 15 00-17 00 h, and more than 100 aggregate visits were made for collection of butterfly data. The species were identified using literature and standard keys (Yates, 1935; Wynter-Blyth, 1957), field guides (Kehimkar, 2016; Bhakare and Ogale, 2018; Kunte *et al*., 2024). Based on their abundance and sighting frequency in SACON campus, the species were classified into four categories; Very Common (VC), Common (C), Rare (R) and Very Rare (VR) (Tiple and Khurad, 2009). Doubtful species were excluded from the list. The photographs of butterflies were taken using Canon 70D with Tamron 180mm macro lens and Nikon D3500 with 70-300mm lens. In addition, butterfly larval host plants, migration, and congregation were also recorded during the study period.

RESULTS AND DISCUSSION

A total of 178 butterfly species belonging to six families were recorded, and among these, 77 species are new additions to SACON campus. The 178 species reported from the study comprise family Papilionidae with 17 species, Pieridae with 22, Nymphalidae with 55, Lycaenidae with 54, Riodinidae with one, and Hesperiidae with 29 (Table 1). Based on the frequency of occurrence, 56 (31.46%) species were VC, 74 (41.57%) were C, 42 (23.60%) as R and 6 (3.37%) VR in the SACON campus. *Charaxes psaphon*, *Phaedyma columella*, *Zinaspa todara*, *Horaga onyx*, *Bibasis sena*, and *Burara jaina* were found to be very rare in the campus. In addition to the occurrence of rare endemic species from Western Ghats parts of Kerala and Tamil Nadu, some endemic records and range extensions have also occurred in the study area. Eight out of the 178 recorded species in SACON campus are endemic to Western Ghats, such as *Troides minos*, *Pachliopta pandiyana*, *Papilio dravidarum*,

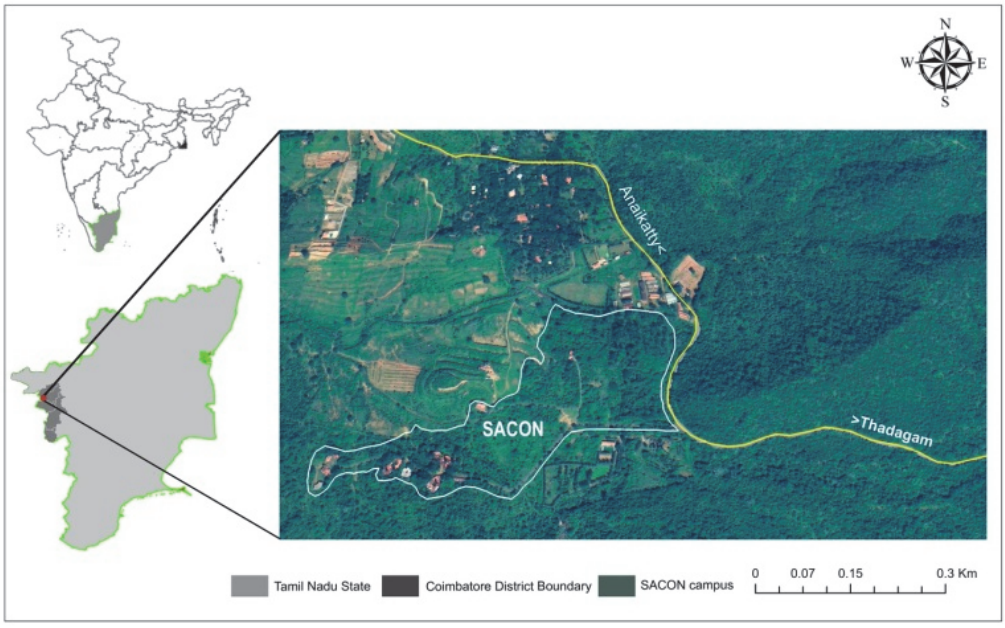

Fig. 1 Location of the campus - Sálim Ali Centre for Ornithology and Natural History (SACON), Coimbatore District, Tamil Nadu

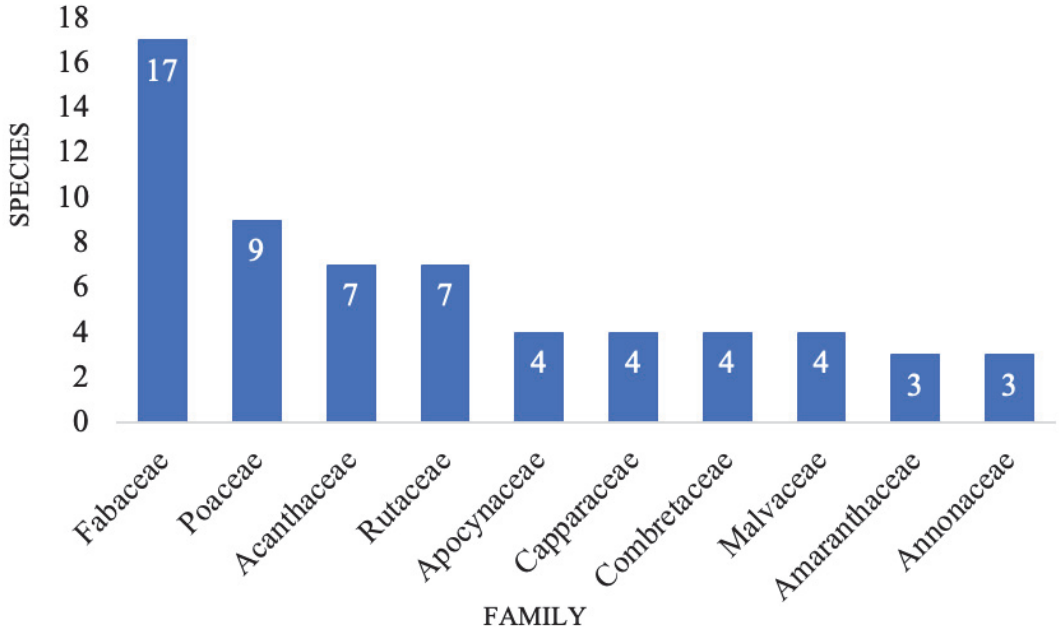

Fig. 2 Dominant family of butterfly larval host plants in SACON campus

Fig. 3 Some selected butterflies of SACON; A. Malabar Banded Peacock, B. Malabar Raven (Papilionidae); C. Pioneer, D. Chocolate Albatross (Pieridae); E. Chestnut Streaked Sailer, F. Commander, G. Bamboo Tree Brown, H. Dark Evening Brown (Nymphalidae). (Photographs of A, B © P.P. Ashiq, and other images, © S. Jeevith)

Papilio buddha, *Pareronia ceylanica*, *Parantica nilgiriensis*, *Ancema sudica* and *Hypolycaena nilgirica*. The records of new additions in the recent survey are as follows; *Papilio paris* is the new addition among the 17 species in family Papilionidae. In Pieridae, 7 out of 22 species were new additions namely, *Eurema brigitta*, *Cepora nadina*, *Colotis fausta*, *Colotis danae*, *Colotis etrida*, and *Colotis aurora*, and *Pareronia ceylanica*.

Family Nymphalidae is represented with 55 species, with 20 new additions; *Melanitis phedima*, *Melanitis zitenius*, *Lethe rohria*, *Lethe drypetis*, *Mycalesis mineus*, *Mycalesis subdita*, *Ypthima baldus*, *Ypthima asterope*, *Ariadne ariadne*, *Byblia ilithyia*, *Charaxes agrarius*, *Moduza procris*, *Neptis jumbah*, *Junonia atlites*, *Pantoporia sandaka*, *Euthalia aconthea*, *Euploea klugii*, *Tirumala limniace*, *Tirumala septentrionis*, and *Parantica aglea*. Thirty-one new species were added to the Family Lycaenidae making the total species count as 54 species. The additional species recorded are, *Spalgis epius*, *Anthene lycaenina*, *Acytolepis puspa*, *Caleta decidia*, *Tarucus ananda*, *Everes lacturnus*, *Chilades parrhasius*, *Freyeria putli*, *Prosotas nora*, *Prosotas dubiosa*, *Petrelaea dana*, *Pseudozizeeria maha*, *Zizula hylax*, *Azanus ubaldus*, *Azanus jeso*us, *Surendra quercetorum*, *Zinaspa todara*, *Spindasis lohita*, *Spindasis schistacea*, *Spindasis ictis*, *Horaga onyx*, *Ancema sudica*, *Hypolycaena nilgirica*, *Virachola isocrates*, *Virachola perse*, *Deudorix epijarbas*, *Catochrysops strabo*, *Amblypodia anita*, *Zesius chrysomallus* and *Curetis acuta*.

In Western Ghats, only two species belonging to family Riodinidae are reported (Sadasivan *et al*., 2023; Kunte *et al*., 2024), and among them only *Abisara bifasciata* was recorded in the study area. Family Hesperiidae was represented by 29 species in total from the study area and 17 species were newly recorded in the campus and they are *Bibasis sena*, *Burara jaina*, *Hasora vitta, Sarangesa dasahara*, *Coladenia indrani*, *Tagiades japetus*, *Celaenorrhinus ambareesa*, *Ampittia dioscorides*, *Gangara thyrsis*, *Matapa aria*, *Taractrocera maevius*, *Cephrenes acalle*, *Oriens* gola, *Oriens goloides*, *Halpe hindu*, and *Borbo cinnara*. Eswaran and Pramod (2005) and Eswaran (2006) reported 93 species of butterflies in and around the SACON campus, followed by 106 species with 13 new additions recorded by Kumar and Arun (2014), and 81 species by Monica Sri (2019). Sony and Arun (2015) studied butterfly road kills along a stretch of road in Anaikatty hills and reported 12 species, out of the 135 individuals of butterfly road kills. In 1995, 104 butterfly species were reported from Coimbatore district (Gunathilagaraj *et al*., 1997) and 75 species from Siruvani forests (Arun, 2003). Recently, 170 butterfly species were reported in a three days annual butterfly survey at Coimbatore region conducted by the Tamil Nadu Forest Department with TNBS, CNS, WWF (Anonymous, 2022).

The geographical area of campus is close to Silent Valley National Park with an aerial distance of 40 km towards the north of Palghat gap and with 80 kms from Nilgiris. All of these areas are major hotspots, which attract a huge diversity of butterfly species in different seasons including their local migration period. The earlier records of butterfly species in Nilgiris were 260 species recorded by Hampson (1888), followed by 282 species reported by Yates (1935), 290 species by Wynter-Blyth (1957) and later Larsen (1987a, b, c, 1988) reported 300 species from the district. Mathew and Rahamathulla (1993) reported 100 species from Silent Valley National Park and recently, Sadasivan *et al.* (2023) reported 290 species, which includes high altitude grassland species and Western Ghats endemicity after two decades. In this survey, larval host plants present in the SACON campus were also identified and included in addition to butterfly checklist (Table 1). The plant species were identified by utilizing vegetative and reproductive characters along with utilizing regional floras and field guides (Balasubramanian *et al.*, 2015; Nitin *et al.,* 2018), and the database POWO (https://powo.science.kew.org). A total of 90 plant species belonging to 33 families were recorded as larval host plants for the 160 species, whereas host plants were either not available or unknown in the campus surroundings for the remaining 18 species. Among plants, Fabaceae dominated with 17 species followed by Poaceae with nine, and Acanthaceae and Rutaceae with seven each (Fig. 2).

Table 1. Checklist of Butterflies in SACON campus, Anaikatty, Western Ghats

No	Common Name - Scientific Name	Occ	Rec	Larvae Host plant in the campus
Family: Papilionidae				
1	Southern Birdwing - *Troides minos* (Cramer, 1779)*	C	A	*Aristolochia indica* L (Aristolochiaceae)
2	Common Rose - *Pachliopta aristolochiae* (Fab, 1775)	VC	A	*Aristolochia indica* L (Aristolochiaceae)
3	Crimson Rose - *Pachliopta hector* (Linnaeus, 1758)	VC	A	*Aristolochia indica* L (Aristolochiaceae)
4	Malabar Rose - *Pachliopta pandiyana* (Moore, 1881)*	R	A	Not available
5	Tailed Jay - *Graphium agamemnon* (Linn, 1758)	C	A	*Annona squamosa* L.; *A. muricata* L. (Annonaceae)
6	Common Jay - *Graphium doson* (C. & R. Felder, 1864)	C	A	*Annona squamosa* L.; *A. muricata* L. (Annonaceae)
7	Spot Swordtail - *Graphium nomius* (Esper, 1799)	C	A	*Monoon longifolium* (Sonn.) B.Xue & R.M.K.Saunders (Annonaceae)
8	Common Bluebottle - *Graphium sarpedon* (Linnaeus, 1758)	VC	A	*Magnolia champaca* (L.) Baill. ex Pierre (Magnoliaceae)
9	Lime Swallowtail - *Papilio demoleus* (Linnaeus, 1758)	VC	A	*Glycosmis pentaphylla* (Retz.) DC., *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen (Rutaceae)
10	Malabar Raven - *Papilio dravidarum* Wood-Mason, 1880*	R	EP, RS	*Glycosmis pentaphylla* (Retz.) DC., *Citrus maxima*
11	Red Helen - *Papilio helenus* Linnaeus, 1758	C	A	*Glycosmis pentaphylla* (Retz.) DC.; *Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen (Rutaceae)
12	Common Mormon - *Papilio polytes* (Linnaeus, 1758)	VC	A	*Bergera koenigii* L.; *Murraya paniculata* (L.) Jack (Rutaceae)
13	Blue Mormon - *Papilio polymnestor* (Cramer, 1775)	VC	A	*Atalantia monophylla* DC.; Bergera koenigii L. (Rutaceae)
14	Common Mime - *Papilio clytia* Linnaeus, 1758	C	A	Not available
15	Common Banded Peacock - *Papilio crin*o (Fabricius, 1792)	VC	A	*Chloroxylon swietenia* DC. (Rutaceae)
16	Malabar Banded Peacock - *Papilio buddha* (Westwood, 1872)*	R	EP, RS	Not available
17	Paris Peacock - *Papilio paris* Linnaeus, 1758	R	RS	*Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen (Rutaceae)
Family: Pieridae				
18	Common Emigrant - *Catopsilia pomona* (Fabricius, 1775)	VC	A	*Cassia fistula* L.; *Senna tora* (L.) Roxb. (Fabaceae)

19	Mottled Emigrant - *Catopsilia pyranthe* (Latreille, 1758)	VC	A	*Cassia fistula* L.; *Senna tora* (L.) Roxb. (Fabaceae)
20	Common Grass Yellow - *Eurema hecabe* (Linnaeus, 1758)	VC	A	*Cassia fistula* L.; *Moullava spicata* (Dalzell ex Wight) Nicolson (Fabaceae)
21	Three-spot Grass Yellow - *Eurema blanda* (Boisduval, 1836)	VC	A	*Cassia fistula* L.; *Moullava spicata* (Dalzell ex Wight) Nicolson (Fabaceae)
22	Small Grass Yellow - *Eurema brigitta* (Stoll, 1780)	C	RS	Not available
23	Common Jezebel - *Delias eucharis* (Drury, 1773)	VC	A	*Butea monosperma* (Lam.) Kuntze (Fabaceae); *Loranthus* sp. (Loranthaceae)
24	Common Gull - *Cepora nerissa* (Fabricius, 1775)	VC	A	*Capparis grandiflora* Wall. ex Hook.f. & Thomson*; C. zeylanica* L. (Capparaceae)
25	Lesser Gull - *Cepora nadina* (Lucas, 1852)	R	RS	*Capparis divaricata* Lam. (Capparaceae)
26	Pioneer - *Belenois aurota* (Fabricius, 1793)	C	A	*Capparis divaricata* Lam. (Capparaceae)
27	Common Albatross - *Appias albina* (Boisduval, 1836)	C	A	Not available
28	Chocolate Albatross - *Appias lyncida* (Cramer, 1777)	R	EP, RS	*Bombax ceiba* L. (Malvaceae); *Capparis zeylanica* L. (Capparaceae)
29	Psyche - *Leptosia nina* (Fabricius, 1793)	VC	A	*Cleome viscosa* L. (Cleomaceae)
30	Large Salmon Arab - *Colotis fausta* (Olivier, 1804)	C	RS	*Capparis grandiflora* Wall. ex Hook.f. & Thomson (Capparaceae)
31	Small Salmon Arab - *Colotis amata* (Fabricius, 1775)	C	A	*Zanthoxylum asiaticum* (L.) Appelhans, Groppo & J.Wen (Rutaceae)
32	Crimson-tip - *Colotis danae* (Fabricius, 1775)	C	RS	*Capparis divaricata* Lam. (Capparaceae)
33	Small Orange-tip - *Colotis etrida* (Boisduval, 1836)	C	RS	*Capparis grandiflora* Wall. ex Hook.f. & Thomson*; C. zeylanica* L. (Capparaceae)
34	Plain Orange-tip - *Colotis aurora* (Cramer, 1780)	C	RS	*Cadaba fruticosa* (L.) Druce (Capparaceae)
35	White Orange-tip - *Ixias marianne* (Cramer, 1779)	VC	A	*Capparis grandiflora* Wall. ex Hook.f. & Thomson*; C. zeylanica* L. (Capparaceae)
36	Yellow Orange-tip - *Ixias pyrene* (Linnaeus, 1764)	VC	A	*Capparis grandiflora* Wall. ex Hook.f. & Thomson*; C. zeylanica* L. (Capparaceae)
37	Great Orange-tip - *Hebomoia glaucippe* (Linnaeus, 1758)	VC	A	*Capparis grandiflora* Wall. ex Hook.f. & Thomson.; *C. zeylanica* L. (Capparaceae)
38	Common Wanderer - *Pareronia hippia* (Fabricius, 1787)	VC	A	*Capparis divaricata Lam.*; *C. zeylanica* L. (Capparaceae)
39	Dark Wanderer - *Pareronia ceylanic*a (C. & R. Felder, 1865)*	C	RS	*Capparis divaricata Lam.*; *C. zeylanica* L. (Capparaceae)

Family: Nymphalidae				
40	Common Evening Brown - *Melanitis leda* (Linnaeus, 1758)	VC	A	*Bambusa bambos* (L.) Voss; (Poaceae)
41	Dark Evening Brown - *Melanitis phedima* (Cramer, 1780)	C	RS	*Bambusa bambos* (L.) Voss; *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Poaceae)
42	Great Evening Brown - *Melanitis zitenius* (Herbst, 1796)	R	RS	*Bambusa bambos* (L.) Voss; *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Poaceae)
43	Common Treebrown - *Lethe rohria* (Fabricius, 1787)	C	RS	*Bambusa bambos* (L.) Voss; *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Poaceae)
44	Tamil Treebrown - *Lethe drypetis* (Hewitson, 1863)	R	RS	*Bambusa bambos* (L.) Voss (Poaceae)
45	Bamboo Treebrown - *Lethe europa* (Fabricius, 1775)	C	A	*Bambusa bambos* (L.) Voss; *Bambusa vulgaris* Schrad. ex J.C.Wendl. (Poaceae)
46	Gladeye Bushbrown - *Mycalesis junonia* Butler, 1868	C	A	Not available
47	Common Bushbrown - *Mycalesis perseus* (Fabricius, 1775)	VC	A	*Echinochloa colonum* (L.) Link (Poaceae)
48	Dark-branded Bushbrown - *Mycalesis mineus* (Linnaeus, 1758)	R	RS	*Echinochloa colonum* (L.) Link (Poaceae)
49	Tamil Bushbrown - *Mycalesis subdita* (Moore, 1890)	R	RS	*Echinochloa colonum* (L.) Link (Poaceae)
50	Common Five-ring - *Ypthima baldus* (Fabricius, 1775)	VC	RS	*Imperata cylindrica* (L.) Raeusch.; *Setaria barbata* (Lam.) Kunth (Poaceae)
51	Common Four-ring - *Ypthima huebneri* Kirby, 1871	VC	A	*Axonopus compressus* (Sw.) P.Beauv. (Poaceae)
52	Common Three-ring - *Ypthima asterope* (Klug, 1832)	C	RS	*Setaria barbata* (Lam.) Kunth (Poaceae)
53	White Four-ring - *Ypthima ceylonica* Hewitson, (1865)	VC	A	*Cynodon dactylon* (L.) Pers. (Poaceae)
54	Common Castor - *Ariadne merione* (Cramer, 1777)	VC	A	*Ricinus communis* L. (Euphorbiaceae)
55	Angled Castor - *Ariadne ariadne* Linnaeus, 1763	VC	RS	*Ricinus communis* L. (Euphorbiaceae)
56	Joker - *Byblia ilithyia* (Drury, 1773)	R	RS	*Tragia involucrata* L. (Euphorbiaceae)
57	Common Nawab - *Charaxes bharata* C. & R. Felder, (1867)	C	A	*Vachellia farnesiana* (L.) Wight & Arn (Fabaceae)
58	Anomalous Nawab - *Charaxes agrarius* Swinhoe, (1887)	R	RS	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
59	Black Rajah - *Charaxes solon* (Fabricius, 1793)	R	EP, RS	*Tamarindus indica* L. (Fabaceae)

60	Tawny Rajah – *Charaxes psaphon* Westwood, 1847	VR	KA, RS	*Tamarindus indica* L. (Fabaceae)
61	Common Map - *Cyrestis thyodamas* Doyère, (1840)	R	EP, RS	*Ficus benghalensis* L.; *Ficus religiosa* L. (Moraceae)
62	Common Leopard - *Phalanta phalantha* (Drury, 1773)	VC	A	Not available
63	Baronet - *Symphaedra nais* (Forster, 1771)	R	EP, RS	*Mangifera indica* L. (Anacardiaceae)
64	Lobed Beak - *Libythea laius* Trimen, 1879	C	A	Not available
65	Tamil Yeoman - *Cirrochroa thais* (Fabricius, 1787)	R	A	Not available
66	Commander - *Moduza procris* (Cramer, 1777)	R	RS	*Neolamarckia cadamba* (Roxb.) Bosser (Rubiaceae)
67	Common Sailer - *Neptis hylas* (Linnaeus, 1758)	VC	A	*Dalbergia latifolia* Roxb. (Fabaceae)
68	Chestnut-streaked Sailer - *Neptis jumbah* Moore, (1858)	C	RS	*Dalbergia latifolia* Roxb. (Fabaceae)
69	Short-banded Sailer - *Phaedyma columella* (Cramer, 1780)	VR	KA, RS	*Dalbergia latifolia* Roxb. (Fabaceae)
70	Great Eggfly - *Hypolimnas bolina* (Linnaeus, 1758)	VC	A	*Sida rhombifolia* L. (Malvaceae)
71	Danaid eggfly - *Hypolimnas misippus* (Linnaeus, 1764)	VC	A	*Barleria cristata* L. (Acanthaceae)
72	Blue Pansy - *Junonia orithya* (Linnaeus, 1758)	C	A	*Barleria mysorensis* Roth; *Rostellularia procumben*s (L.) Nees (Acanthaceae)
73	Lemon Pansy - *Junonia lemonias* (Linnaeus, 1758)	C	A	*Mimosa pudica* L. (Fabaceae), *Hygrophila auriculata* (Schumach.) Heine (Acanthaceae)
74	Peacock Pansy - *Junonia almana* (Linnaeus, 1758)	C	A	*Hygrophila auriculata* (Schumach.) Heine (Acanthaceae)
75	Grey Pansy - *Junonia atlites* (Linnaeus, 1763)	R	RS	*Hygrophila auriculata* (Schumach.) Heine (Acanthaceae)
76	Chocolate Pansy - *Junonia iphita* (Cramer, 1779)	VC	A	*Ruellia simplex* C.Wright (Acanthaceae)
77	Yellow Pansy - *Junonia hierta* (Fabricius, 1798)	VC	A	*Ruellia simplex* C.Wright (Acanthaceae)
78	Painted Lady - *Vanessa cardui* (Linnaeus, 1758)	R	EP, RS	*Argemone mexicana* L. (Papaveraceae)
79	Tawny Coaster - *Acraea terpsicore* (Linnaeus, 1758)	VC	A	*Passiflora subpeltata* Ortega (Passifloraceae)

80	Tailed Palmfly - *Elymnias caudata* Butler, 1871	C	A	*Cocos nucifera* L. (Arecaceae)
81	Common Lascar - *Pantoporia hordonia* (Stoll, 1790)	VC	A	*Vachellia farnesiana* (L.) Wight & Arn (Fabaceae)
82	Extra Lascar - *Pantoporia sandaka* (Butler, 1892)	R	RS	*Vachellia farnesiana* (L.) Wight & Arn (Fabaceae)
83	Rustic - *Cupha erymanthis* (Drury, 1773)	C	A	Not available
84	Common Baron - *Euthalia aconthea* (Cramer, 1777)	C	RS	*Mangifera indica* L. (Anacardiaceae)
85	Clipper - *Parthenos sylvia* (Cramer, 1775)	R	EP, RS	Not available
86	Common Crow - *Euploea core* (Cramer, 1780)	VC	A	*Ficus benghalensis* L. (Moraceae); *Asclepias curassavica* L. (Apocynaceae)
87	Double-branded Crow - *Euploea sylvester* (Fabricius, 1793)	C	A	*Ficus benghalensis* L. (Moraceae); *Asclepias curassavica* L. (Apocynaceae)
88	Brown King Corw - *Euploea klugii* Moore, (1858)	R	RS	*Streblus asper* Lour. (Moraceae)
89	Blue Tiger - *Tirumala limniac*e (Cramer, 1775)	VC	RS	*Asclepias curassavica* L.; *Calotropis gigantea* (L.) W.T.Aiton (Apocynaceae)
90	Dark Blue Tiger - *Tirumala septentrionis* (Butler, 1874)	VC	RS	*Wattakaka volubilis* (L.f.) Stapf (Apocynaceae)
91	Plain Tiger - *Danaus chrysippus* (Linnaeus, 1758)	VC	A	*Asclepias curassavica* L.; *Calotropis gigantea* (L.) W.T.Aiton (Apocynaceae)
92	Striped Tiger - *Danaus genutia* (Cramer, 1779)	VC	A	*Asclepias curassavica* L.; *Calotropis gigantea* (L.) W.T.Aiton (Apocynaceae)
93	Glassy Tiger - *Parantica aglea* (Stoll, 1782)	C	RS	*Asclepias curassavica* L.; *Calotropis gigantea* (L.) W.T.Aiton (Apocynaceae)
94	Nilgiri Tiger - *Parantica nilgiriensis* (Moore, 1877)*	R	EP, RS	*Vincetoxicum indicum* (Burm.f.) Mabb. (Apocynaceae)
Family: Lycaenidae				
95	Apefly - *Spalgis epius* (Westwood, 1851)	C	RS	Unknown / larvae feeds on mealy bugs
96	Pointed Ciliate Blue - *Anthene lycaenina* (R. Felder, 1868)	C	RS	*Leucaena leucocephala* (Lam.) de Wit; *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
97	Common Hedge Blue - *Acytolepis puspa* (Horsfield, 1828)	R	RS	*Peltophorum pterocarpum* (DC.) Backer ex K.Heyne (Fabaceae)
98	Common Pierrot - *Castalius rosimon* (Fabricius, 1775)	VC	A	*Ziziphus mauritiana* Lam.; *Ziziphus oenopolia* (L.) Mill. (Rhamnaceae)
99	Angled Pierrot - *Caleta decidia* (Hewitson, 1876)	VC	RS	*Ziziphus oenopolia* (L.) Mill. (Rhamnaceae)

100	Banded Blue Pierrot - *Discolampa ethion* (Westwood, 1851)	C	A	*Ziziphus mauritiana* Lam.; *Ziziphus oenopolia* (L.) Mill. (Rhamnaceae)
101	Stripped Pierrot - *Tarucus nara* (Kollar, 1848)	C	A	*Ziziphus mauritiana* Lam.; *Ziziphus oenopolia* (L.) Mill. (Rhamnaceae)
102	Dark Pierrot - *Tarucus ananda* (de Nicéville, 1884)	R	RS	*Ziziphus oenopolia* (L.) Mill. (Rhamnaceae); *Loranthus* sp. (Loranthaceae)
103	Lime Blue - *Chilades lajus* (Stoll, 1780)	VC	A	*Citrus* × *limon* (L.) Osbeck (Rutaceae)
104	Plains Cupid - *Chilades pandav*a (Horsfield, 1829)	C	A	*Cycas revoluta* Thunb. (Cycadaceae)
105	Small Cupid - *Chilades parrhasius* (Fabricius, 1793)	C	RS	*Dichrostachys cinerea* (L.) Wight & Arn. (Fabaceae)
106	Indian Cupid - *Everes lacturnus* (Godart, 1824)	C	RS	*Dichrostachys cinerea* (L.) Wight & Arn. (Fabaceae)
107	Gram Blue - *Euchrysops cnejus* (Fabricius, 1798)	C	A	*Crotalaria pallida* Aiton (Fabaceae)
108	Grass Jewel - *Freyeria trochylus* (Freyer, 1845)	C	A	*Trichodesma indicum* (L.) Sm. (Boraginaceae)
109	Oriental Grass Jewel - *Freyeria putli* (Kollar, 1844)	VC	RS	*Trichodesma indicum* (L.) Sm. (Boraginaceae)
110	Common Cerulean - *Jamides celeno* (Cramer, 1775)	VC	A	*Pongamia pinnata* (L.) Pierre (Fabaceae)
111	Dark Cerulean - *Jamides bochus* (Stoll, 1782)	C	A	*Pongamia pinnata* (L.) Pierre; *Gliricidia sepium* (Jacq.) Kunth (Fabaceae)
112	Metallic Cerulean - *Jamides alecto* (C. Felder, 1860)	R	A	*Pongamia pinnata* (L.) Pierre (Fabaceae)
113	Pea Blue - *Lampides boeticus* (Linnaeus, 1767)	VC	A	*Gliricidia sepium* (Jacq.) Kunth (Fabaceae)
114	Zebra Blue - *Leptotes plinius* (Fabricius, 1793)	VC	A	*Plumbago zeylanica* L. (Plumbaginaceae)
115	Common Lineblue - *Prosotas nora* (C. Felder, 1860)	VC	RS	*Mimosa pudica* L.; *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
116	Tailless Lineblue - *Prosotas dubiosa* (Semper, 1879)	VC	RS	*Mimosa pudica* L. (Fabaceae)
117	Dingy Lineblue - *Petrelaea dana* (de Nicéville, 1884)	VC	RS	*Terminalia catappa* L. (Combretaceae)
118	Dark Grass Blue - *Zizeeria karsandra* (Moore, 1865)	C	A	*Amaranthus spinosus* L.; *A. viridis* L. (Amaranthaceae)
119	Pale Grass Blue - *Pseudozizeeria maha* (Kollar, 1848)	C	RS	*Oxalis corniculata* L. (Oxalidaceae)
120	Lesser Grass Blue - *Zizina otis* (Fabricius, 1787)	C	A	*Oxalis corniculata* L. (Oxalidaceae); *Amaranthus spinosus* L. (Amaranthaceae)

121	Tiny Grass Blue - *Zizula hylax* (Fabricius, 1775)	VC	RS	*Tribulus terrestris* L. (Zygophyllaceae)
122	Bright Babul Blue - *Azanus ubaldus* (Stoll, 1782)	C	RS	*Mimosa pudica* L.; *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
123	African Babul Blue - *Azanus jesous* (Guérin-Méneville, 1849)	C	RS	*Mimosa pudica* L.; *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
124	Common Acacia Blue - *Surendra quercetorum* (Moore, 1858)	R	RS	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
125	Silver Streaked Acacia Blue - *Zinaspa todara* (Moore, 1884)	VR	RS	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
126	Common Silverline - *Spindasis vulcanus* (Fabricius, 1775)	C	A	*Cassia fistula L.* (Fabaceae); *Ziziphus mauritiana* Lam. (Rhamnaceae);
127	Long-banded Silverline - *Spindasis lohita* (Horsfield, [1829])	R	RS	*Senna siamea* (Lam.) H.S.Irwin & Barneby (Fabaceae); *Terminalia paniculata* B.Heyne ex Roth (Combretaceae)
128	Plumbeous Silverline - *Spindasis schistacea* (Moore, 1881)	R	RS	*Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)
129	Common Shot Silverline - *Spindasis ictis* (Hewitson, 1865)	R	RS	*Senna siamea* (Lam.) H.S.Irwin & Barneby (Fabaceae); *Terminalia paniculata* B.Heyne ex Roth (Combretaceae)
130	Red Pierrot - *Talicada nyseus* (Guérin-Méneville, 1843)	VC	A	*Kalanchoe delagoensis* Eckl. & Zeyh. (Crassulaceae)
131	Common Onyx - *Horaga onyx* (Moore, 1858)	VR	RS	*Mangifera indica* L. (Anacardiaceae)
132	Blue-bordered Plane - *Bindahara moorei* Fruhstorfer, 1904	C	A	*Salacia fruticosa* Wall. ex M.A.Lawson (Celastraceae)
133	Yam Fly - *Loxura atymnus* (Stoll, 1780)	C	A	*Dioscorea pentaphylla* L. (Dioscoreaceae)
134	Monkey Puzzle - *Rathinda amor* (Fabricius, 1775)	VC	A	*Mangifera indica L.* (Anacardiaceae); *Ixora coccinea* L. (Rubiaceae)
135	Peacock Royal - *Tajuria cippus* (Fabricius, 1798)	C	A	*Loranthus sp*. (Loranthaceae)
136	Silver Royal - *Ancema sudica* (Evans, 1926)*	R	RS	*Loranthus sp*. (Loranthaceae)
137	Nilgiri Tit - *Hypolycaena nilgirica* Moore, (1884)*	R	RS	*Eulophia graminea* Lindl. (Orchidaceae)
138	Common Guava Blue - *Virachola isocrates* (Fabricius, 1793)	R	RS	*Psidium guajava* L. (Myrtaceae)
139	Large Guava Blue - *Virachola perse* (Hewitson, 1863)	R	RS	*Psidium guajava* L. (Myrtaceae)
140	Cornelian - *Deudorix epijarbas* (Moore, 1858)	R	RS	*Sapindus emarginatus* Vahl (Sapindaceae)

141	Slate Flash - *Rapala manea* (Hewitson, 1863)	C	KA, RS	*Mangifera indica* L. (Anacardiaceae); *Senegalia torta* (Roxb.) Maslin, Seigler & Ebinger (Fabaceae)
142	Forget-me-not - *Catochrysops strabo* (Fabricius, 1793)	C	RS	*Pongamia pinnata* (L.) Pierre (Fabaceae)
143	Purple Leaf Blue - *Amblypodia anita* (Hewitson, 1862)	R	RS	Not available
144	Common Quaker - *Neopithecops zalmora* (Butler, 1870)	VC	EP, RS	*Glycosmis pentaphylla* (Retz.) DC. (Rutaceae)
145	Redspot - *Zesius chrysomallus* Hübner, (1819)	R	RS	Not available
146	Large Oakblue - *Arhopala amantes* (Hewitson, 1862)	R	RS	*Terminalia paniculata* B.Heyne ex Roth (Combretaceae)
147	Indian Sunbeam - *Curetis thetis* (Drury, 1773)	C	KA, RS	*Pongamia pinnata* (L.) Pierre (Fabaceae)
148	Acute Sunbeam - *Curetis acuta* Moore, 1877	C	RS	*Pongamia pinnata* (L.) Pierre (Fabaceae)
Family: Riodinidae				
149	Double-banded Judy - *Abisara bifasciata* Moore, 1877	R	RS	Not available
Family: Hesperiidae				
150	Orange-tailed Awl - *Bibasis sena* (Moore, 1866)	VR	RS	*Combretum* sp. (Combretaceae)
151	Common Orange Awlet - *Burara jaina* (Moore, 1866)	VR	RS	Not available
152	Common Banded Awl - *Hasora chromus* (Cramer, 1780)	C	A	*Pongamia pinnata* (L.) Pierre (Fabaceae)
153	Plain Banded Awl - *Hasora vitta* (Butler, 1870)	C	RS	*Pongamia pinnata* (L.) Pierre (Fabaceae)
154	White-banded Awl - *Hasora taminatus* (Hübner, 1818)	C	A	*Pongamia pinnata* (L.) Pierre (Fabaceae)
155	Brown Awl - *Badamia exclamationis* (Fabricius, 1775)	C	A	*Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae)
156	Common Small Flat - *Sarangesa dasahara* (Moore, 1866)	C	RS	*Lepidagathis cuspidata* Nees (Acanthaceae)
157	Fulvous Pied Flat - *Pseudocoladenia dan* (Fabricius, 1787)	C	A	*Achyranthes aspera* L. (Amaranthaceae)
158	Tricolour Pied Flat - *Coladenia indrani* (Moore, 1866)	R	RS	*Dalbergia latifolia* Roxb. (Fabaceae)
159	Water Snow Flat - *Tagiades litigiosa* Möschler, 1878	VC	KA, RS	*Dioscorea pentaphylla* L. (Dioscoreaceae)

160	Common Snow Flat - *Tagiades japetus* (Stoll, 1781)	VC	RS	*Dioscorea pentaphylla* L. (Dioscoreaceae)
161	Malabar Spotted Flat - *Celaenorrhinus ambareesa* (Moore, 1866)	C	RS	*Strobilanthes* sp. (Acanthaceae)
162	Suffused Snow Flat - *Tagiades gana* (Moore, 1866)	R	A	*Dioscorea pentaphylla* L. (Dioscoreaceae)
163	Golden Angle - *Caprona ransonnettii* (R. Felder, 1868)	C	KA, RS	*Triumfetta rhomboidea* Jacq. (Malvaceae)
164	Indian Bush Hopper - *Ampittia dioscorides* (Fabricius, 1793)	C	RS	*Imperata cylindrica* (L.) Raeusch. (Poaceae)
165	Chestnut Bob - *Iambrix salsala* (Moore, 1866)	VC	EP, RS	*Bambusa bambos* (L.) Voss (Poaceae)
166	Giant Redeye - *Gangara thyrsis* (Fabricius, 1775)	C	RS	*Bambusa bambos* (L.) Voss (Poaceae)
167	Common Branded Redeye - *Matapa aria* (Moore, 1866)	C	RS	*Bambusa bambos* (L.) Voss (Poaceae)
168	Grass Demon - *Udaspes folus* (Cramer, 1775)	VC	RS	Not available
169	Common Grass Dart - *Taractrocera maevius* (Fabricius, 1793)	C	RS	Not available
170	Plain Palm Dart - *Cephrenes acalle* (Höpffer, 1874)	C	RS	*Bambusa bambos* (L.) Voss (Poaceae)
171	Dark Palm Dart - *Telicota bambusae* (Moore, 1878)	C	KA, RS	*Bambusa bambos* (L.) Voss (Poaceae)
172	Common Dartlet - *Oriens gola* (Moore, 1877)	C	RS	*Oplismenus compositus* (L.) P.Beauv. (Poaceae)
173	Smaller Dartlet - *Oriens goloides* (Moore, 1881)	C	RS	*Oplismenus compositus* (L.) P.Beauv. (Poaceae)
174	Indian Grizzled Skipper - *Spialia galba* (Fabricius, 1793)	C	A	*Sida rhombifolia* L. (Malvaceae)
175	African Mar bled Skipper - *Gomalia elma* (Trimen, 1862)	C	KA, RS	*Abutilon indicum* (L.) Sweet (Malvaceae)
176	Indian Ace - *Halpe hindu* Evans, 1937	C	RS	*Bambusa bambos* (L.) Voss (Poaceae)
177	Small Branded Swift - *Pelopidas mathias* (Fabricius, 1798)	R	KA, RS	*Imperata cylindrica* (L.) Raeusch. (Poaceae)
178	Rice Swift - *Borbo cinnara* (Wallace, 1866)	C	RS	*Setaria verticillata* (L.) P.Beauv. (Poaceae)

Note: Occ-Occurrence in SACON campus; Rec-Records; (*) Endemic to Western Ghats;
VC-Very Common; C-Common; R-Rare; VR-Very Rare;
EP-Eswaran and Pramod (2005); KA-Kumar and Arun (2014); A-All authors; RS - Recent Survey.

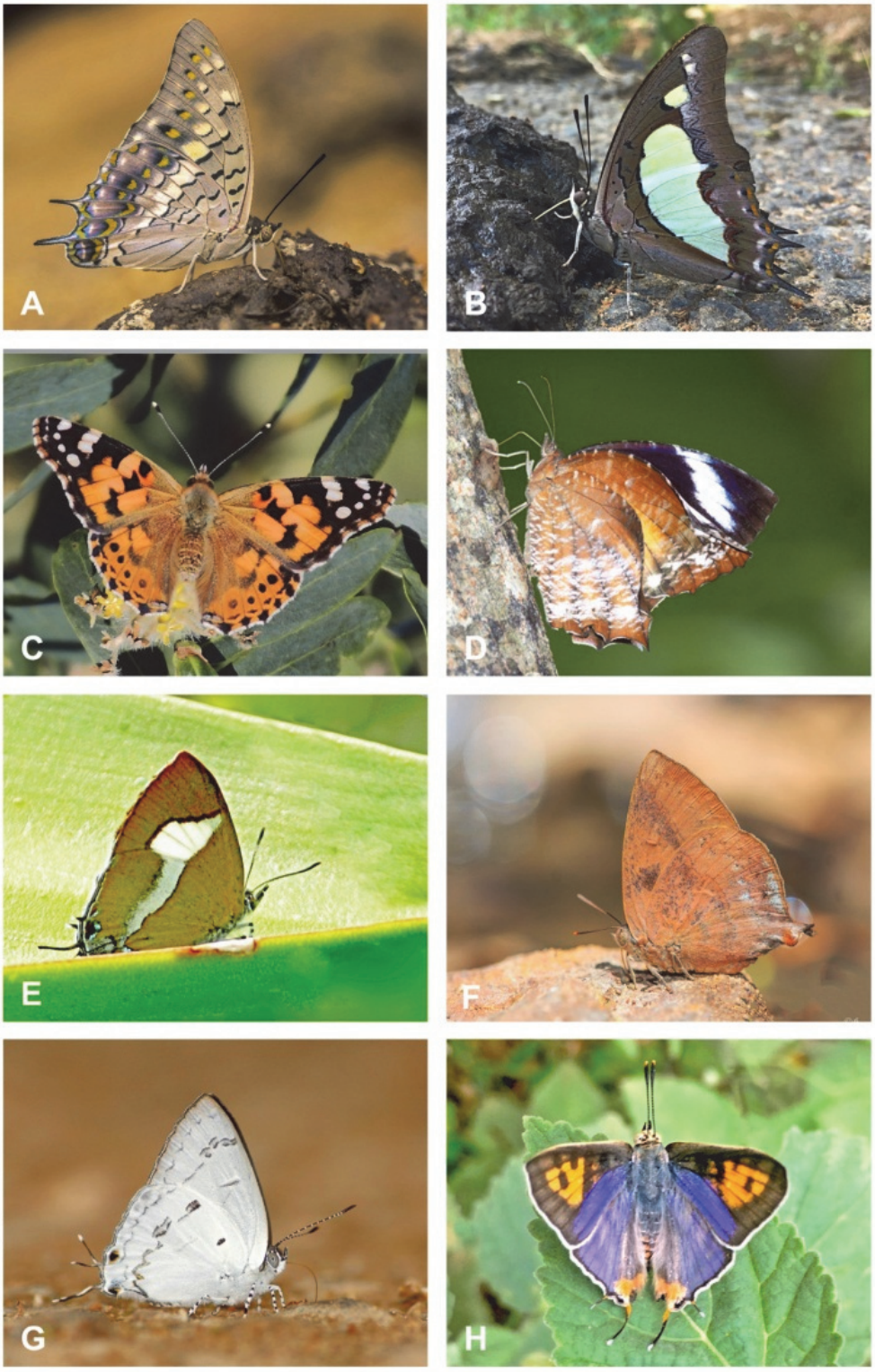

Fig. 4 Some selected butterflies in SACON; A. Black Rajah, B. Anomalous Nawab, C. Painted Lady, D. Tailed Palmfly (Nymphalidae); E. Common Onyx; F. Purple Leaf Blue; G. Nilgiri Tit; H. Common Shot Silverline (Lycaenidae). (All images © S. Jeevith)

Fig. 5 Some selected butterflies in SACON; A. Silver Streaked Acacia Blue, B. Common Acacia Blue, C. Dark Pierrot (Lycaenidae); D. Orange-tail Awl; E. Common Orange Awlet; F. Tricoloured Flat, G. Rice Swift, H. Suffused Snow Flat (Hesperiidae). (Photographs of D, G - ©Chaithra Shree, and other images © S. Jeevith)

Looking at the habit-wise representation of larval host plants, both herbs and trees were represented with 32 species each, followed by 19 shrubs and seven climbers. The campus is rich in plant diversity with a reported 402 plants belonging to 84 families as reported by Balasubramanian *et al*. (2015), and Prakash *et al.* (2022) reported 98 invasive alien plant species from Anaikatty hills, of which, 26 species of invasive plants served as nectar food plant for 42 butterfly species (Monica Sri, 2019).

The present study provides detailed information about the species composition and a significant increase in the species richness of butterflies indicate the success of habitat conservation efforts by SACON and the resultant improvement in floral and faunal diversity over the years. The selected images of butterfly species are given in figures 3 to 5. The information regarding the butterfly host plants in the study area will also help to understand the vegetation structure of the area which will further the conservation efforts to conserve these biodiversity rich habitat zones.

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Aquatic insect diversity and distribution in Maguri Beel and Khamti Guali Beel wetlands, Upper Brahmaputra Basin, Assam, India

Innifa Hasan, Diksha Rabha and Dip Jyoti Haloi*

Department of Zoology, Handique Girls' College, Panbazar, Guwahati 781001, Assam, India.
Email: diphaloi1979@gmail.com

ABSTRACT: The investigation revealed the presence of a total of 16 species of aquatic insects in Maguri Beel and Khamti Guali Beel, two freshwater ecosystems, in Assam, India. The distribution and diversity of species present in the two wetlands with an account of physico-chemical water analysis and macrophyte distribution are discussed. Variation in the diversity indices of both the wetlands, illustrated key differences in diversity and distribution of aquatic insects in Maguri and Khamti Guali Beel.
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KEY WORDS: Aquatic ecosystems, physico-chemical water analysis, macrophytes, diversity indices

INTRODUCTION

Insects, occupying a dominant position as the largest group in the animal kingdom, are the most conspicuous forms of life in the aquatic ecosystem (Sharma and Agrawal, 2012). Aquatic insects in particular serve as excellent bioindicators and are essential for the proper functioning of an aquatic ecosystem. By virtue of their taxonomic diversity, varied abundance and tropical significance, aquatic insects can be found in almost every type of aquatic habitat throughout the world such as lakes, ponds, rivers, torrential streams, coastal water and estuaries, highly saline pools, groundwater, acid peat swamps, hot springs and even pools of crude oil seeping from the ground (Yule and Sen, 2004). Aquatic insect communities can vary greatly within and among habitats and these communities play significant roles within the freshwater ecosystems through the cycling of nutrients or through their overall contribution to secondary production. Intriguing in structure and biology, some of these insects are of significant importance to public health as well as aquaculture of inland waters. In consequence, aquatic insects are considered model organisms among the freshwater animal taxa, because of their high abundance, high birth rate coupled with short generation time, large biomass and rapid colonization of freshwater habitats for studying and analyzing the structure and functioning of inland water systems (Sharma and Agrawal, 2012).

Inland waters, particularly freshwaters, cover less than one per cent of the Earth's surface area; however, they are known to harbour 10 per cent of known fauna, of which 60 per cent comprise of aquatic insects. This diversity is represented by 12

* Author for correspondence

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orders of living aquatic insects, of which, the following species have larvae which are aquatic and adults which are terrestrial in nature: mayflies (Ephemeroptera), dragonflies and damselflies (Odonata), stoneflies (Plecoptera), alderflies (Megaloptera), lacewings (Neuroptera), flies (Diptera), caddisflies (Trichoptera), moths (Lepidoptera) and wasps (Hymenoptera). Aquatic beetles (Coleoptera) and bugs (Hemiptera) larval or nymphal and adult stages are fully aquatic (Subramanian and Sivaramakrishnan, 2007). Thus life cycle stages play a major role in the occupancy of water habitats by insects. Trichoptera, Ephemeroptera, and Plecoptera are pollution-sensitive insect orders that are widely utilised in aquatic insect biomonitoring projects. Given that these taxa are often intolerant, many bio monitoring programmes believe species diversity to be more vulnerable to stress than total number of taxa (Abhijna *et al.* 2013). Along with biomonitoring, aquatic insect biodiversity provides a variety of additional services like as food for many fish species, nutrient retention, litter decomposition, and noxious weed management, and many more.

Global estimates suggest that around 45,000 of aquatic insect species are derived from the fauna of North America, Australia, Asia and Europe of which around 5,000 species are estimated to inhabit inland wetlands of India. These 5,000 aquatic species of India are predominantly represented by mayflies (Ephemeroptera), dragonflies (Odonata), and caddisflies (Trichoptera) (Subramanian and Sivaramakrishnan, 2007). The northeast India bio-geographic zone holds much significance as it represents the transition zone between the Indian, Indo-Malay and Indo-Chinese bio geographic regions as well as the point of confluence of Himalayan mountains and peninsular India (Barman and Gupta, 2015). Despite this and the fact that the north-eastern region of India has been identified as a biodiversity hotspot by the World Conservation Monitoring Centre (WCMC, 1998), the aquatic insect fauna has been poorly documented compared to the studies conducted in peninsular India (Choudhury and Gupta, 2015). The Brahmaputra drainage system of the Northeast India is one of the largest river systems in the world. Aside from 47 major tributaries, the river basin has around 3000 flood plain lakes known locally as Beels (Biswas, 2014). One such freshwater ecosystem is Maguri Beel, one of the major wetlands of the upper Brahmaputra basin, situated in the Tinsukia district of Assam. This wetland is located on the outskirts of the Dibru- Saikhowa National Park and is a part of Dibru- Saikhowa Important Bird Area (Kardong *et al.,*2020). Another freshwater ecosystem is the Khamti Guali Beel located in the 'buffer zone' of the Dibru- Saikhowa Biosphere Reserve (DSBR). This research is intended to shed light on the current biodiversity in these areas, as well as how biotic and abiotic characteristics influence the distribution of aquatic insects.

MATERIALS AND METHODS

The field work is carried out on the two freshwater bodies in Tinsukia district, Assam. The freshwater ecosystems taken under study are Maguri Beel and Khamti Guali Beel. The Maguri Motapung Beel is a wetland lake located near the Dibru- Saikhowa National Park and Motapung Village of Tinsukia district of Assam (situated between 27°34'49.92"N to 27°34'34.98"N and 95°21'7.68"E to 95°24'11.39"E, with an altitude of 96.1m above sea level and area of 119ha). This wetland is lake-like with static water formed by inundation of low-lying lands during flooding, with water trapped even after floodwaters recede. Another freshwater ecosystem is that of the Khamti Guali Beel that connects the Maguri Beel to the River Dibru. It is situated between 27º34'23.4"N to 27º34'26.0"N and 95°20'27.4"E to 95°20'53.8"E. It is located at an altitude of 97.4m above sea level and covers an area of 11ha. Three stations were visited on both the study locations twice a month during the day.

The current study was conducted over a five-month period, from January 2023 to May 2023 (pre-monsoon). Water and insect samples were taken from both bodies of water from the 3 sampling sites. As many aquatic insects migrate to deeper water during the late hours of the day, habitat sampling of the insects and water was conducted during the early hours of the day (6am - 9am). Aquatic insects were gathered from locations using the 'Kick' approach (Brittain, 1974), which involved disturbing

Table 1. Different species of aquatic insects in Maguri Beel (M) and Khamti Guali Beel (K)

No.	Order	Family	Species	No.	
				M	K
1.	Hemiptera	Belostomatidae	*Diplonychus annulatus*	05	04
2.			*D.rusticus*	12	09
3.		Nepidae	*Ranatra filiformes*	15	
4.			*Laccotrephes sp.*	07	
5.	Coleoptera	Dytiscidae	*Laccophilus sp.*	19	10
6.		Hydrophilidae	*Hydrophilus sp.*	04	
7.	Odonata	Libellulidae	*Urothemis signata*	23	17
8.			*Tramea basilaris*	08	03
9.			*Trithemis sp.*	04	
10.		Macromiidae	*Macromia sp.*	03	05
11.		Gomphidae	*Paragomphus sp.*	14	09
12.			*Macrogomphus sp.*	19	
13.			*Ictinogomphus sp.*	16	14
14.		Aeshnidae	*Anax guttatus*	11	12
15.	Diptera	Culicidae	*Culex sp.*	06	07
16.			*Aedes sp.*	07	08
Total				173	98

the plant and dragging a net over it for a unit period. A sample was made up of three such drags. Three replicate samples were collected, after which the insects were separated, numbered, and stored in 70 per cent ethyl alcohol. They were later identified using advanced dissection or stereo zoom microscope (10X or above) with the help of standard keys and identified using taxonomic literature (Bouchard, 2004).

Water samples were obtained from a depth of (at least) 40cm using a 1.5 litre plastic jar. Water samples were collected and stored in clean stoppered plastic bottles for later testing. Physico-chemical parameters of water such as air temperature (AT), water temperature (WT), and pH of water sample were analyzed using a mercury bulb thermometer, pH meter (Model: pH Digital meter) and TDS with TDS digital meter respectively. Dissolved oxygen (DO), free carbon dioxide (CO_2), calcium, magnesium, total hardness, and chloride of water sample were analyzed by standard titrimetric methods (APHA, 2017). Aquatic macrophyte species, present in the area were collected and documented to construct herbarium. Species diversity indices of the collected aquatic insects such as Shannon–Wiener diversity index (1949), Simpson's Index of Diversity, Pielou's Evenness Index (Pielou, 1966), Margalef's Index (Margalef, 1958), and Berger-Parker (Berger and Parker, 1970) indices were computed to study and understand the biotic community of each area.

RESULTS AND DISCUSSION

The physicochemical parameters of the water at Maguri Beel and Khamti Guali Beel indicated no marked differences for the water parameters.

Although the physiochemical analysis revealed only minor variations, particularly in dissolved oxygen, chloride, and calcium concentrations, these factors collectively impacted aquatic insect diversity and distribution in both the ecosystems. Dissolved oxygen (DO) is one of the most essential parameters for indicating water quality and determining the distribution of diverse aquatic insect groups (Wahizatul *et al.,* 2011). In the investigation, the dissolved oxygen in Maguri Beel and Khamti Guali Beel was 12 mgL^{-1} and 10.9 mgL^{-1} respectively (Fig. 2). The dissolved oxygen was observed to be less in Khamti Guali Beel compared to Maguri Beel, which might be due to polluted water and eutrophication. This could be correlated to the lesser diversity and abundance of species reported in Khamti Guali Beel in comparison to its counterpart, Maguri Beel.

Chloride concentration, which can be attributed to the dissolution of salt deposits, discharges of effluents from chemical industries, oil well operations, sewage discharges, and many more pollutants, was found to be 12.43 mgL^{-1} and 14.67 mgL^{-1} in Maguri Beel and Khamti Guali Beel respectively, with values for Khamti Guali Beel being higher representing increase chloride levels in water. Furthermore, the Calcium concentration was also higher in Khamti Guali Beel with a value of 20 mgL^{-1} as opposed to 15.7 mgL^{-1} in Maguri Beel (Fig. 1)**.**

Four species of macrophytes were found in the two water bodies. 1) Submerged suspended, perennial *Ceratophyllum demersum* belonging to Cerratophyllaceae family; 2) Free floating, annual *Pistia stratiotes*belonging to Araceae; 3) Free floating, perennial *Eichhornia crassipes* belonging to Pontedariaceae and 4) Emergent anchored, perennial *Alternanthera philoxeroides* belonging to Amaranthaceae.

A total of 16 aquatic insect species were recorded belonging to four orders (Hemiptera, Coleoptera, Odonata, and Diptera). Order Odonata represents the highest number of species (8) followed by order Hemiptera (4) and other orders such as Coleoptera and Diptera (2 each). Plecoptera and Tricoptera, two significant water insect species, were completely absent from the study area. In contrast, the orders Hemiptera, Coleoptera, Odonata, and Diptera had a great species richness and abundance of insects (Table 1).

In Maguri Beel there were Odonata (50%), Hemiptera (25%), Diptera (13%) and Coleoptera (12%). In Khamti Guali Beel it was Odonata (55%), Hemiptera (18%), Diptera (18%), and Coleoptera (9%). Maguri Beel displayed a higher diversity of species and abundance, with 16 species from nine families and a total of 173 insects. The species diversity in Khamti Guali Beel was restricted, with 11 species from seven families, among the 98 total insects collected. Insects collected throughout each month of January, February, March, April, and May, only four species were observed, from both aquatic environments during the five months. These species were *Diplonychus rusticus* (Hemiptera), *Laccophilus sp.* (Coleoptera), *Urothemis signata* (Odonata), and *Culex sp.* (Diptera).

The values of Shannon diversity at the two water ecosystems of Maguri Beel and Khamti Guali Beel are 2.61461 and 2.292607 respectively, which were in the normal range of 1.5-3.5 (Türkmen and Kazanci, 2010). The slightly higher index value of Maguri Beel indicates a greater diversity of species found. In other terms, higher values of H' is a representative of more diverse communities in the ecosystem. The Simpson index of diversity (1-D) values for Maguri and Khamti Guali Beel were 0.92324 and 0.89943, respectively. This result was comparable to Shannon index values, with higher values for Maguri Beel suggesting greater sample diversity. In this scenario, this index indicates the likelihood that two individuals chosen at random from a sample will belong to different species, and so may be used to calculate dominance. The Pielou's diversity index (e) value for Maguri Beel is 0.943, whereas Khamti Guali Beel has a rating of 0.956. The values fall within the proper range of 0 to 1, with values closer to 1 suggesting that individuals are dispersed equally, implying that the species of aquatic insects in Khamti Guali Beel are scattered more evenly than in Maguri Beel**.** The Margalef

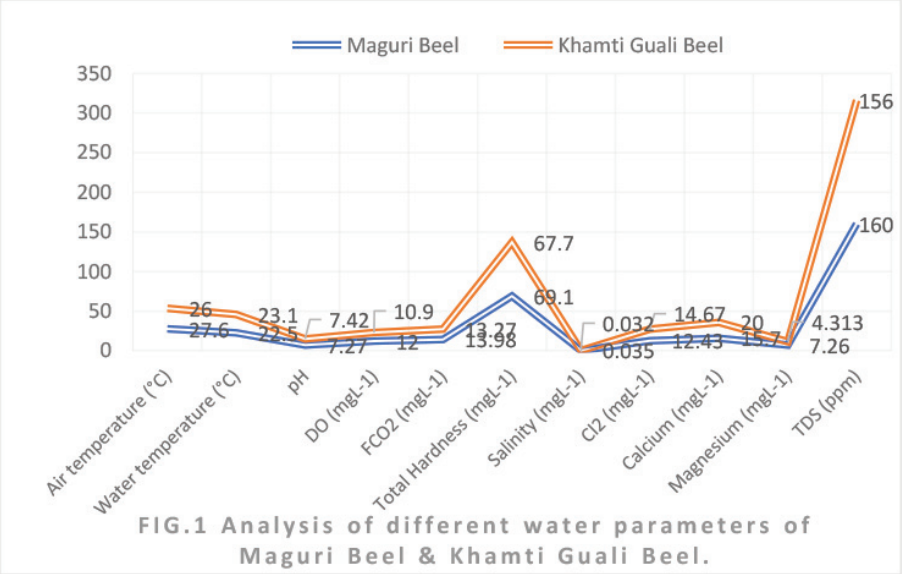

FIG.1 Analysis of different water parameters of Maguri Beel & Khamti Guali Beel.

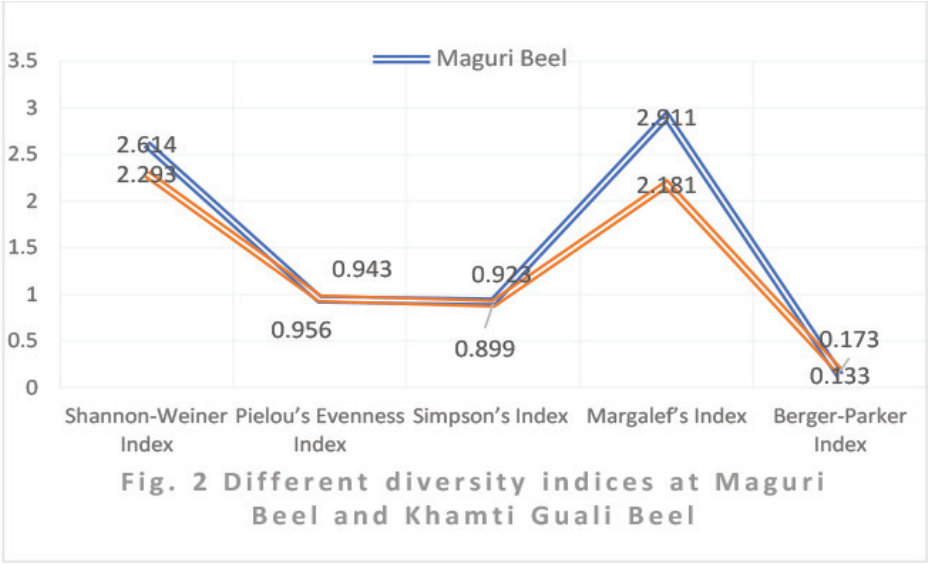

Fig. 2 Different diversity indices at Maguri Beel and Khamti Guali Beel

diversity index shows variation depending on the number of species, that is, the richness or variety of taxa/species/types present in an assemblage or community. It thus has a different purpose of usage from other indices. However, it showed similar results with the other indices as the vales for Maguri Beel was higher (2.911) than that of Khamti Guali Beel (2.181) with moderately polluted waters in both ecosystems. Finally, the Berger-Parker index of dominance value for the dominant species *Urothemi signata,* which was the most abundant species in both locations, was found to be 0.1329 in Maguri Beel and 0.1735 in Khamti Guali Beel. This index expresses the proportional importance of the most abundant type (Fig. 2)**.** The results of the different diversity indices that were used to examine the community structure of both water ecosystems show that the Khamti Guali Beel's aquatic fauna is more evenly distributed and contains more dominant taxa, while the Maguri Beel's aquatic insect fauna is more diverse, richer, and distinct in terms of taxa.

While the physicochemical properties of both water ecosystems were quite comparable, with just minor

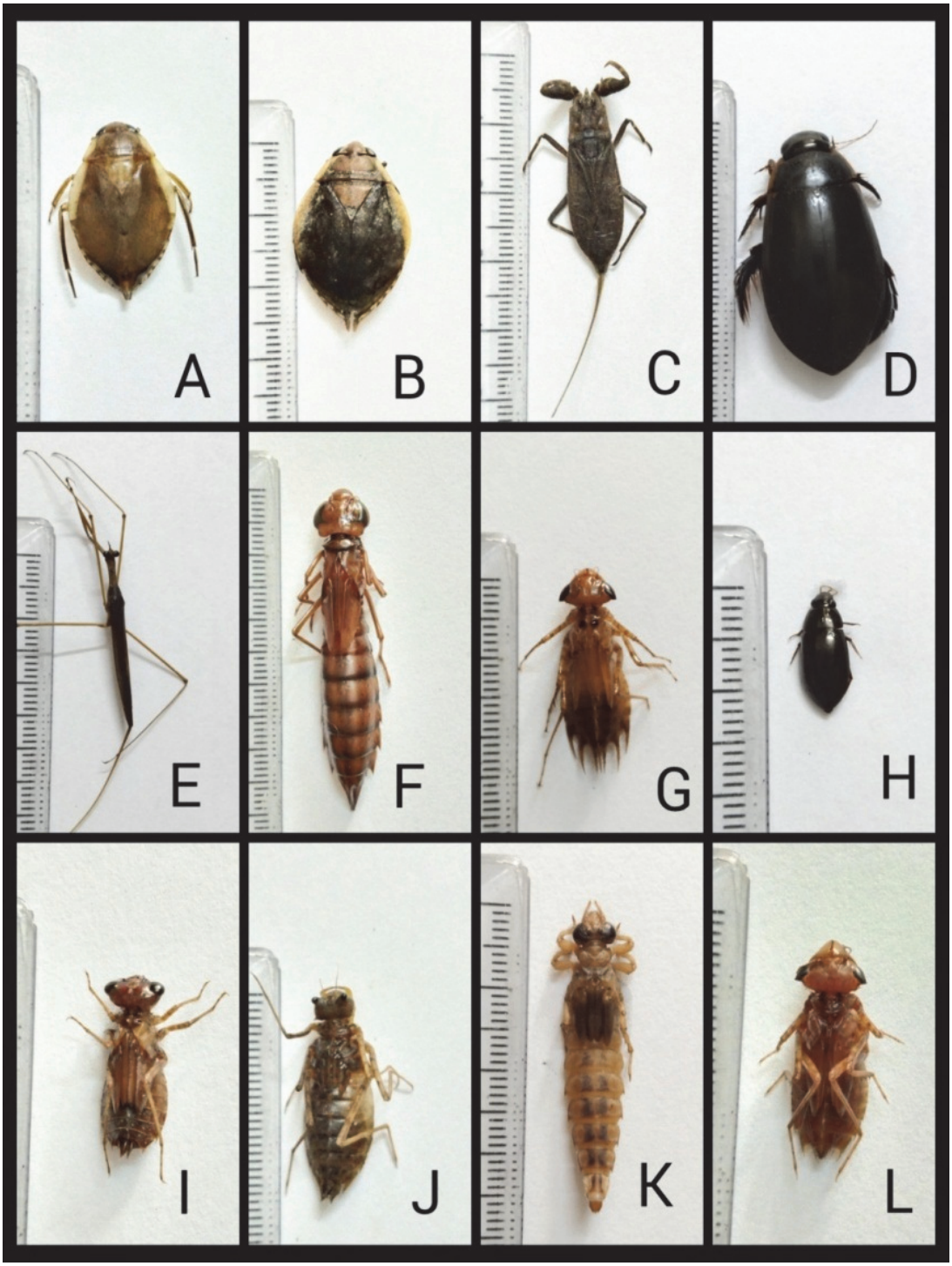

Fig. 3 Aquatic insects collected from study sites: A. *Diplonychus rusticus,* B. *Diplonychus annulatus*, C. *Laccotrephes sp.,* D. *Laccophilus sp.,* E. *Ranatra filiformes*, F. *Anax guttatus*, G. *Tramea basilaris*, H. *Hydrophilus* sp., I. *Trithemis sp.,* J. *Macromia* sp., K. *Paragomphus sp.,* L. *Urothemis signata* *(Scales in the photographs are expressed in Centimeters)*

changes in some parameters (DO, calcium, and chloride concentrations), the specific conditions in Maguri Beel may have provided for a more conducive environment for the existence of a broader range of aquatic insects. The diversity indices corroborate the conclusion that Maguri Beel harbors a richer variety of aquatic insect populations compared to Khamti Guali Beel, which has a more uniform distribution of its relatively fewer species.

Diplonychus, Laccophilus, Urothemis, Macromia, Tramea, Anax, Paragomphus, Macrogomphus, Culex, and *Aedes* were among the species studied in this study for the two water bodies under inquiry (Fig. 3). In this study, 16 species were identified from two separate aquatic environments, and the number of species and abundance differed across the two pools. The dominance of the orders Odonata and Hemiptera indicates that the water bodies are ecologically healthy. Despite anthropogenic perturbations, the Maguri Beel ecosystem remains rich and diversified, according to this study. Khamti Guali Beel, on the other hand, demonstrated evenness in taxonomic distribution based on the numerous diversity indices. As a result, a monitoring strategy and the use of a number of diversity and biotic indices could shed light on the state of health of a water ecosystem and influence public attitudes and policies towards water body conservation in both protected and unprotected regions.

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Species diversity and seasonal dynamics of insects in the high altitude tea estate in Uttarkhand, India

Kartik Omanakuttan, Sanjana Goswami and Manoj Kumar Arya*

Department of Zoology, D.S.B. Campus, Kumaun University, Nainital 263001, Uttarakhand, India.
Email: dr.manojkumar19@rediffmail.com

ABSTRACT: The study explored the insect communities and their seasonality within tea plantations micro-climate and finds the relatedness of these insects with the environment variables of the terroir. The study carried out at the Ghorakhal Tea Estates of Uttarakhand, collected a total of 2195 insect individuals, belonging to seven orders and 15 families and their population dynamics were measured along with the environmental variables in the estate. The Shannon-Weiver Index (H) indicated moderate species richness (2.94). The most abundant Order was Hymenoptera (30%), followed by Lepidoptera (27%). Family of pollinator bees (24%) and Pierid butterflies (19%) were dominant in the tea plantation area. The highest diversity and evenness of insect communities was observed during the summer season. The study highlights the influence of temperature, air quality, and humidity on the insect population. Canonical Correspondence Analysis suggests a significant relationship between abiotic environmental factors and insect species' abundance. Distribution of 69.4 per cent was explained by the temperature and humidity, and 30.5 per cent by the air quality. Insect diversity was positively correlated to temperature (0.85) and humidity (0.93) and negatively correlated with air quality (-0.89).
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KEY WORDS: Biodiversity, climate dependence, Shannon-Weiver Index, Canonical Correspondence Analysis

INTRODUCTION

Insects play a crucial role in the functioning of ecosystems and provide a wide range of benefits to humans (Scudder, 2017). They have played unique role in the Tea culture, which is an integral part of many societies. Xu (2013) explored the history of insect tea (made from the faeces of insects) and its cultural significance made from insects that feed on tea leaves. *Camellia sinensis* is a species of evergreen shrub or small tree in the flowering plant family Theaceae, whose leaves and leaf buds are used to produce the popular beverage tea (Graham, 1992). It requires proper rainfall, drainage, and slightly acidic soil (Graham, 1992, 1999). Green Tea phenolic compounds are predominately composed of catechin derivatives, although other compounds, such as flavonols and phenolic acids, are also in lower proportion (Chan *et al*., 2007). It is grown in India and Sri Lanka at altitudes up to 2000m above sea level (Graham, 1999). In India, Tea cultivation began in the Darjeeling district in the early 1850s, with thousands of acres of land being cleared and numerous nurseries being established with China Jat (Atkinson, 1980). In 2018-19, the plantation sector

* Author for correspondence

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in India had a gross output value of $31475.14 billion, which accounted for approximately two per cent of the country's total agricultural output value (Viswanathan, 2021). This highlights the crucial contribution of Tea plantation sector to the country's inclusive development due to its significant role (Joseph and Viswanathan, 2016). A major land area contributes to tea plantation in Uttarakhand. The state is divided into two regions, Garhwal and Kumaon, with 13 districts. The climate ranges from subtropical in the southern foothills to warm temperate in the middle Himalayan valleys. In Uttarakhand, 572.19 ha of inorganic farming is done under the Uttarakhand Tea Development Board, while 593.81 ha of Organic farming is done (UTDB, 2023). The Ghorakhal Tea Estate (GTE) is the second-largest Organic farm in the state that covers 112.26 ha. The Tea is being cultivated over 105 ha for 30 years (UTDB, 2023). The region remains isolated from the urban town and has a unique ecosystem that provides optimum conditions for excellent growth of flora and fauna (Arti, 2018). The Tea estates are essential sources of national income and require much care; thus, identifying factors that have beneficial or detrimental effects on its growth becomes necessary. Many pests are responsible for damaging the tea plant. Rai (2004) reported that various pests cause heavy damage to the plant. Previous records of insect fauna in or around GTE are scarce (Smetacek, 2010, 2011) which highlights the entomofaunal research gap in the region, thus making a survey primarily important.

Tea terroirs offer a unique micro-environment that affects its associated biodiversity unlike any other landscape, which consequently affects its own growth (Mattson and Addy, 1975; Suba and Bhattacharya, 2024). Also, Insects are sensitive to climate and act as nature's indicator organisms. In light to this knowledge, we hypothesised that these environment variables would have a positive correlation with the insect population dynamics. We also assumed a strong effect of the seasons on the alpha diversity variables of the insects exclusive to the Tea ecosystem. Accordingly, the objectives of this study were designed to (i) Measure the three environment variables (temperature, air quality and humidity) inside the tea estate throughout the study period; (ii) Monitor the insect communities and their status in the estate; (iii) Calculate the seasonality of insects using standardized methods; and (iv) Statistically elucidate the relationship between the environment variables with the distribution of these insect communities.

MATERIALS AND METHODS

This study was conducted for one year, from May 2022 to June 2023, in GTE. It is situated in the Nainital district of Uttrakhand The geographic latitude and longitude are 29º38 N to 79º28' E at an altitude of 1740m, and the closest town (Bhowali) is about three kilometres away from the terroirs. The site has even distribution of tea cultivars with fragmented distribution of Pine cover. The overall climate in the plantation area is warmer than the downtown area. Three environmental parameters were recorded using standardised digital instruments, i.e., mean temperature of the month, mean humidity and air quality index (AQI). All the meteorological parameters were compared daily from the Accuweather Weather Database for standard error following Sadeghian *et al*. (2022). AQI was obtained from the UPCB database (UPCB, 2023). Ten transects of 20x20 metre were observed for one hour each, inside the plantation area. Two transects were monitored on each visit and their mean values were used to summarise the results for the Tea estate. Butterflies, bees and some flying insects were observed using the traditional Pollard walk method (Pollard 1977). Bug and beetles were observed by sweep net sampling method and beating plant foliage. Flies were sampled using a Malaise trap, following Nejati *et al*. (2020).

Insects were preserved using cotton-soaked fumigants in the Jar (Chloroform and 10% Ethyl Acetate) and then preserved in the lab by stretching and pinning the insects following Upton and Mantle (2010). Furthermore, insect diversity and abundance were studied through observational approaches. Identification of insects was done using a Stereomicroscope, after bringing the collected insects to the laboratory, and compared to the reference collections in the Insect

Biodiversity Laboratory at the Department of Zoology, D.S.B. Campus, Kumaun University, Nainital. Identification was based on key descriptions. Those that could not be identified at the species level were identified at the genus level. Those not readily identified were sent to the Northern Regional Station Zoological Survey of India, Dehradun. Seasons were distributed according to the method used by Farooq *et al*. (2021), i.e., Summer = S (March, April, and May), Rainy = R (June, July, and August), Autumn = A (September, October, and November), Winter = W (December, January and February). Insects were classified on the basis of the number of sightings according to Farooq *et al.* as Very Common (VC) >=70 sightings, Common (C) =30-69, Occasional = (O) = 10-29, Rare = (R) =<=9. All the statistical analysis was performed using Microsoft Excel and PAST 4.13 software, including significance tests, correlation, and multivariate test. The CCA correlation score was further used to construct correlation matrix.

RESULTS AND DISCUSSION

A total of 2195 insect individuals of 26 species were observed during the study, belonging to seven insect orders and 15 families. Among the abundance of various orders, Hymenoptera recorded maximum (30%), followed by Lepidoptera (27%) and Diptera (23%). Orthoptera (3%) and Dermaptera (2%) were in lower abundance. With respect to abundance of families, the most abundant family was Apidae (24%), followed by Pieridae (18%), Culicidae (13%), Nymphalidae (7.5%), Coccinellid*ae* and Vespidae (each 6%), Lebelullidae (5.4%), Sarcophagidae (5.1%), Calopterigidae (3.2%), Calliphoridae (2.8%), Acrididae (2.7%), Asilidae (2.1%), Forficulidae (1.9%), Lycaenidae (0.8%) and Erebidae (0.4%) . Overall, the Shannon Weiver Index (H) was 2.94, whereas Simpson's Dominance (D) was 0.06, and the Gini-Simpson Index (or 1-D) was 0.93, concluding a moderate richness of species during the study. The Pielou's evenness (e^H/S) was high (0.7), signifying the even distribution of most species at the study site. Menhinick Index was 0.53, whereas the Margalef Index was 3.12 (Table 1).

Table 1. Distribution, relative abundance and status of insects observed at the GTE

Order/ family/ Species	Distribution during seasons				RA	Status
	Winter	Summer	Rainy	Autumn		
Dermaptera						
Forficulidae						
Forficula spp. (Linnaeus)	-	+	+	-	0.02	C
Hymenoptera						
Apidae						
Apis dorsata (Fabricius)	-	+	+	-	0.06	VC
Apis cerana (Fabricius)	+	+	+	+	0.16	VC
Bombus haemorrhoidalis (Smith)	+	+	-	+	0.02	C
Vespidae						
Vespa tropica (Linnaeus)	-	-	+	+	0.03	VC
Polistes olivaceus (DeGeer)	+	+	+	-	0.03	VC
Odonata						
Calopterigidae						
Neurobasis chinensis (Linnaeus)	+	+	+	-	0.03	VC

Lebelullidae						
Crocothermis servilla (Drury)	+	+	+	+	0.05	VC
Diptera						
Calliphoridae						
Calliphora vomitoria (Linnaeus)	+	+	+	-	0.03	VC
Asilidae						
Neoitamus spp. (Osten-Sacken)	-	+	+	-	0.02	C
Culicidae						
Culex pipiens (Linnaeus)	-	+	+	+	0.04	VC
Uranotaenia sp. (Lynch Arribjlzga)	-	+	+	+	0.09	VC
Sarcophagidaea						
Sarcophaga argyrostoma (Robineau-Desvoidy)	+	+	+	-	0.05	VC
Orthoptera						
Acrididae						
Acrida exaltata (Walker)	-	+	+	-	0.03	VC
Coleoptera						
Coccinellidae						
Coccinella sempunctata (Linnaeus)	-	+	+	-	0.06	VC
Lepidoptera						
Nymphalidae						
Vanesssa indica (Herbst)	-	+	+	-	0.01	R
Danaus chrysippus (Linnaeus)	+	+	+	-	0.01	O
Ypthilma baldus (Fabricius)	+	+	+	+	0.06	VC
Pieridae						
Colias fieldii (Ménétriés)	+	+	+	-	0.01	R
Belenois aurota (Fabricius)	+	+	+	+	0.02	V.C.
Eurema hecabe (Linnaeus)	-	+	+	+	0.03	V.C.
Pieris brassicae (Linnaeus)	+	+	+	-	0.08	VC
Leptosia nina (Fabricius)	+	+	+	-	0.03	VC
Catopsila pyranthe (Linnaeus)	-	+	+	-	0.01	R
Lycaenidae						
Lampides boeticus (Linnaeus)	-	+	+	-	0.01	R
Erebidae						
Amata bicincta (Kollar)	-	+	+	-	0.02	R

(+)= present, (-)= absent, VC= Very Common, C= Common, O= Occasional, R= Rare); RA- Relative abundance

Of the 26 insect species, 17 were very common, three were commonly seen, one was an occasional visitor and five were rarely observed. *Colias fieldii* (Ménétriés), *Catopsila pyranthe* (Linnaeus), *Lampides boeticus* (Linnaeus) and *Vanesssa indica* (Herbst) were the rare species at the site. *Danaus chrysippus* (Linnaeus) was an occasional visitor. *Neoitamus* spp. (Osten-Sacken), *Bombus haemorrhoidalis* (Smith) and *Forficula* spp. (L.) were common, while the remaining 17 species were very common (Table 1).

The highest Shannon diversity index was observed in Summer (2.9), followed by the Rainy season (2.8). A similar trend for evenness can be seen during the two seasons as 0.8 and 0.7 Pielou's evenness, respectively. The least Shannon diversity and evenness can be seen in the Autumn season. Insects were most evenly distributed during the Summer (Fig. 1). The rainy season shows the highest Margalef Index value (3.41). The alpha diversity variables, tallied seasonally, confirm a significant effect of seasonal variations on insect assemblage (Dominance, Shannon, Simpson, Evenness, Margalef, and Fisher alpha) (2 way ANOVA, $p<0.01$, Sum of squares=27.91, f=10.76) which supports our second hypothesis.

The Individual Rarefaction Curve for various seasons (Fig. 2) estimates the collection effort efficiency. The plot shows the formation of an early asymptote for the winter and autumn seasons,

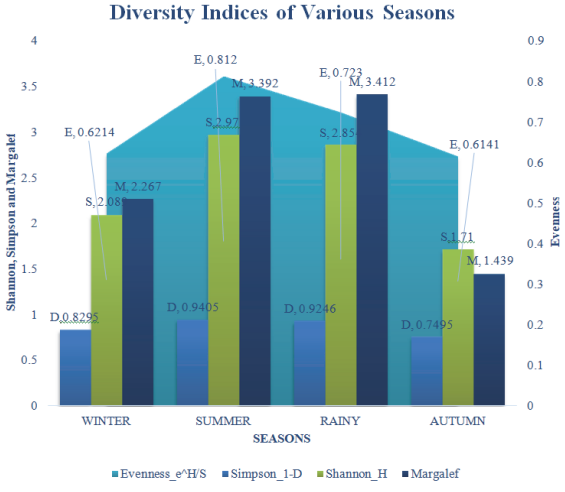

Fig. 1 Seasonal diversity of Insects

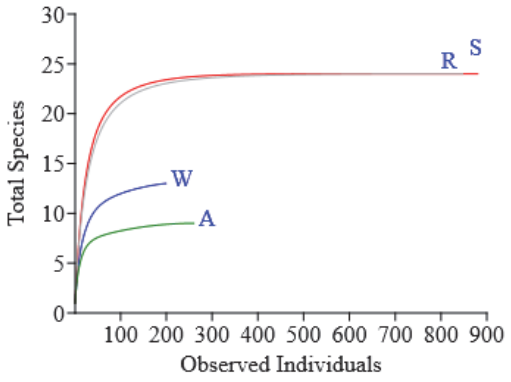

Fig. 2 Seasonal Individual Rarefaction Curve

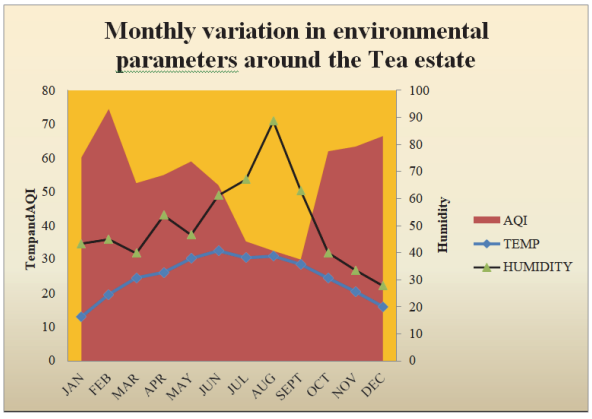

Fig. 3 Monthly variations in the environmental parameters

whereas both summer and rainy seasons form a late asymptote curve due to the relatively high number of species found in the rainy and summer seasons.

Among the monthly variation of environmental parameters (Air Quality Index, Temperature and Humidity), the highest humidity in the Tea estate was recorded in August. The average Air Quality Index (AQI) was much lower and fair (53.6). The highest AQI was recorded from November to February, rising above 63. The month of February saw the highest AQI at 74.5 (Fig. 3).

The C.C.A. analysis determined the relationship between insect abundance and environmental variables. The *p*-value of 0.017, suggests a statistically significant relationship between the environmental variables and species abundance data. Axis 1 and 2 demonstrates the statistically

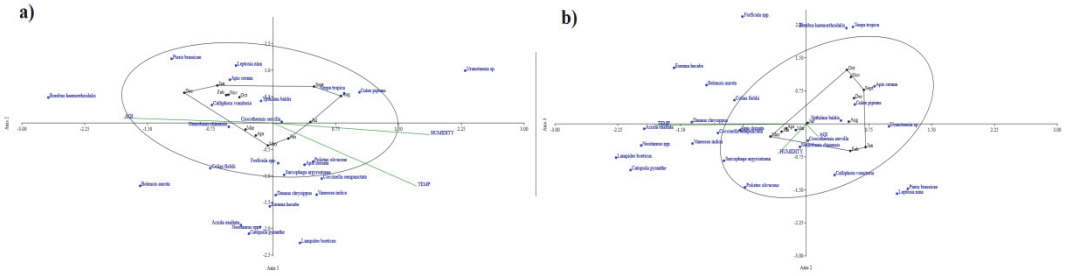

Fig. 4 (a, b): Relationship of insects with the environment variables. a) CCA between Axis 1 vs. 2 b) CCA between Axis 2 vs. 3.

significant relatedness of species on independent variables (Fig. 4a). Axis 3, on the other hand, has a non-significant *p*-value (0.733), explains no significant variations. The insect communities had a positive correlation with Temperature (0.85) and Humidity (0.93), and a negative correlation with the air quality (-0.89) (Fig. 5). Overall correlation of the insect assemblage was noted with these variables.

Distribution of *Bombus haemorrhoidalis* was negatively correlated with temperature (-2.04), which is in correlation with several previous findings (Peat and Goulson, 2005; Ghimire and Bhusal, 2021). The insect community was negatively correlated with air quality, i.e. poor air quality reduced the overall alpha diversity which is in alliance with the disturbance hypothesis and other recent studies (Dial and Roughgarden, 1998; Meléndez-Jaramillo *et al*, 2021). *V. tropica*, *C. pipiens*. and *Uranotaenia sp.* were positively correlated to Air quality, which may be due to their lower sample size, infact their distribution was greatly explained by temperature and humidity collectively (correlation>78%). Axis 1 and 2 explain 69.41 and 30.58 per cent of the variations in the species distribution respectively, which can be attributed to the environmental variables (Fig. 4b). These findings also correlate with previous reporting (Buchori *et al*., 2018; Hamid *et al*., 2014) and further support our first hypothesis that the environmental conditions of the tea estate do affect the population dynamics of its associated insect communities. While most of the species at Ghorakhal Tea Estate show high association with temperature and humidity, butterflies, bees and dragonflies show moderate association with air quality, suggesting their behaviour as indicator species. *Uranotaenia sp.* and other flies show a high association with temperature. This is also certain because temperature highly affects the population dynamics of blood-feeding flies (Yamana and Eltahir, 2013).

In the present study most of the recorded species of insects were locally abundant (Sondhi and Kunte, 2018). *Ceryx wallengren* which was previously identified in this region by Smetacek (2010) was not recorded in our study, instead another member of subfamily Arctiinae, *Amata bicincta* was identified which is not a local endemic species (Majumdar, 2010). The present study does not monitor the insect communities outside the perimeters of the tea estate, and therefore we could not obtain a comparative data with the surrounding biodiversity, which is a major limitation in this study. Neither does the study present temporal data beyond a year, which underscores our understanding of the long-term effect of climate change in this micro-environment. Most studies associated with tea also highlight the associated pest activity, which is an understudied aspect of this work

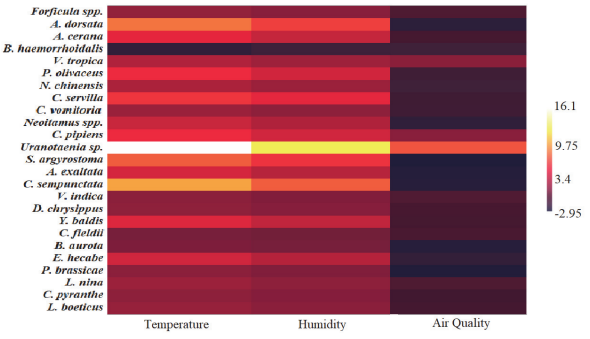

Fig. 5 Correlation of Insect assemblage in Tea estate with environment variables

(Chhetri 2007; 2010). Higher abundance of Hymenoptera (30%) and diversity in the order Lepidoptera was noted from the GTE, which corresponds to the reporting of various studies from the western Himalaya (Goswami *et al*., 2013; Arya *et al*., 2020; Chandra *et al*., 2023). Among the pollinators, Apidae was the most abundant (24%), followed by Pieridae (18%). Diversity and evenness for both autumn and monsoon seasons were found to be highest. Chhetri (2007; 2010) measured the insect diversity for two seasons, autumn and monsoon, in the Teesta valley tea garden, with Shannon diversity indices of 1.62 and 1.73, respectively, and evenness indices of 0.90 and 0.88, respectively. Several studies have reported high seasonal diversity of insects during the spring with low abundance in autumn which correlates with our findings (Bhusal *et al*., 2019). Das (2021) reported 39 species of Orthoptera in the tea agroecosystem of West Bengal. The study found orthopteran insects as significant species in the tea agroecosystem.

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Molecular characterization and identification of insect pollinators of *Valeriana jatamansi* Jones from Shimla Hills, Western Himalaya, India

Madhu Rana[1], Amar Singh[2] and Mahender Singh Thakur[3*]

[1,3]Department of Biosciences, Himachal Pradesh University, Shimla, Himachal Pradesh, India.
[2]Department of Dermatology, Venereology and Leprosy, Pt. Jawahar Lal Nehru Government Medical College, Chamba, Himachal Pradesh, India.
Email: madhuamar08@gmail.com; drmahender74@gmail.com

ABSTRACT: *Valeriana jatamansi* Jones a perennial medicinal herb belongs to the family Caprifoliaceae and highly pollinated by wild insect pollinators. In present investigation a total of 51 species of flower visitors were reported on *V. jatamansi* at altitude range of 1,927 to 2,850 m, in different localities of Shimla Hills, Western Himalaya. The collected insect pollinator species were taxonomically identified and 29 of them were molecularly characterized and identified using the cytochrome c oxidase subunit 1 (COI) sequence from mitochondrial DNA (mtDNA). BLAST analysis revealed 98 to 100 per cent similarity with the existing GenBank sequences. The average AT content was found to be significantly higher (69.8%) compared to the GC content (30.0%), indicating AT bias in all sequences. Phylogenetic analysis of 29 different species of insect 'pollinator of *V. jatamansi* revealed two clades, one shows phylogenetic relationship between 28 species which belongs to four orders Diptera, Hymenoptera, Hemiptera and Lepidoptera and the other shows the one species which belongs to order Coleoptera. This study contributes to increasing the number of published accounts of NCBI and helps to accurately distinguish the pollinator fauna of *Valeriana jatamansi*. © 2024 Association for Advancement of Entomology

KEY WORDS: Medicinal herb, BLAST analysis, phylogenetic analysis, mtCOI sequences

INTRODUCTION

Pollination is a vital process that promotes biodiversity, agricultural productivity, wild plant reproduction, ecosystem health, and food security. It involves the interaction of plants and animals, and affects human well-being. They have a reciprocal link in which the survival of one of them benefits the other. Animals provide food, shelter, and space in exchange for being the primary pollinators of plants (Nepi *et al.,* 2018). Insects are one of the most significant types of pollinating organism. Insect pollination is vital to the reproduction and survival of several wild plants (Ollerton *et al*., 2011) including *Valeriana jatamansi*. In India, *V. jatamansi* belonging to the family Caprifoliaceae, is on the verge of extinction due to the over-exploitation of its roots/rhizomes from its natural habitat to meet the burgeoning industrial demand. It has become threatened and endangered plant species and is included in the endangered category by IUCN (Reveal and Chase,

* Author for correspondence

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2011). This plant is commonly known as Indian valerian (English), Mushkbala (Kashmiri), Sughanthdawal, or Tagar (Sanskrit), is a native of Himalaya with extent distribution from Afghanistan to southwest China, India, Nepal, Bhutan, Myanmar and Burma. It is an important medicinal herb being exploited for its roots and rhizomes which contain valepotriates and is highly effective against leprosy epilepsy and hysteria (Maurya and Agnihotri, 2024), Parkinsons disease, Lewy body dementia (Bagchi and Hooper, 2011) and have anxiolytic properties (You *et al*., 2012). Cytotoxity of valepotriates has been reported for potential anti-tumor properties (Diapher and Hindwarch, 2004). It has a relatively high level of genetic diversity and therefore has greater environmental adaptability. The adaptability is also demonstrated by the distribution of this species, which extends between a wide altitudinal range of 1000 to 3000m elevation, and is generally preferred to grow on hill slopes, moist places, damp woods, ditches, and along streams (Rather *et al*., 2012; Jugran *et al*., 2013). The flowering and fruiting time for the species is March–June (Jugran *et al*., 2013).

The identification of insects via the conventional approach is fairly challenging because of the morphological alterations due to seasonal and regional variances. Organisms adapt themselves physiologically and morphologically to flourish in difficult environments. According to Jalali *et al*. (2015), genetic studies are subsequently essential to the identification and phylogenetic study of organisms at the species level. While morphological data are generally time-consuming and require expertise, DNA barcoding methods offer a standardized and practical means of species identification for insects. Molecular identification and showing phylogenetic connections employing COX 1 of the mitochondrial area as species identification markers are considered effective since mitochondrial DNA changes fast as compared to nuclear DNA. Present-day mitochondrial DNA (mtDNA) serves as among the most widely utilized molecular markers. Since mtDNA is maternally inherited, it changes quite quickly, and most of the nucleotide modifications occur at neutral sites. With relation to this genetic marker, the intra and inter-phylogenetic links have been studied employing the sequencing data acquired from the COX 1 marker gene amplification (Jalali *et al.,* 2015).

The purpose of this study was to explore the molecular characterization and identification of insect pollinators of *Valeriana jatamansi* using mitochondrial DNA sequences of the COI barcode dataset. The main goals of this study were to examine the utility and effectiveness of the mtDNA (COI) marker for identifying insect species, compared to traditional taxonomic identification methods. This research aims to demonstrate the practical use of the mtDNA marker for species identification in insect pollinators of *V. jatamansi*, given the medicinal significance and endangered status of the plant. Precisely identifying the pollinator fauna of *V. jatamansi* could help protect wild pollinators and improve plant yields.

MATERIALS AND METHODS

The present investigation was carried out on insect pollinators of *V. jatamansi* in eight localities of Shimla Hills, Western Himalayas, viz., Tara Devi (1,927m; 31°04’06"N: 77°07’24"E), Dhalli (2,155m; 31°06'39"N: 77°11'53"E), Chaura Maidan (2,100m; 31°06'34"N: 77°08'34"E), Fagu (2,576m; 31°05'22"N: 77°18'05"E), Matiyana (2,419m; 31°12'36"N: 77°24'11"E), Kufri (2,609m; 31°06'01"N: 77°16'02"E), Kharapather (2,703m; 31°07'13"N: 77°37'37"E) and Kupper (2,850m; 31°06'00"N: 77°36'13"E). Collection of insect pollinators were done during the flowering season i.e., from March to May, in the years 2018 to 2021 and during this period collected specimens were preserved at -80^{0}C in deep freezer for molecular analysis. During study the relative abundance of different insect pollinators on *V. jatamansi* was determined in terms of their visit per 500 flowers/10 minutes. The observation was recorded during 0900–1000, 1200–1300, and 1500–1600 hours of a day, and the average count at these hours gives an abundance of insect pollinators for that particular day (Mattu and Kumar, 2016). In order to ascertain whether pollen grains were present, each specimen was put into a distinct vial, frozen and then rinsed in 0.1ml of 70 per cent ethanol. Pollen was cleaned onto a glass slide and then stained with carbol fuchsin. Pollen grains were

identified by comparing them with pollen removed from flowers harvested from the plant. All the preserved specimens of insect pollinators from different altitudinal population of Shimla Hills, Himachal Pradesh were identified based on the diagnostic morphological features, wing venation and genital characteristics. Identification was done with the help of earlier records of the Entomology and Biodiversity Laboratory of the Department of Biosciences, Himachal Pradesh University, High Altitude Regional Centre, Zoological Survey of India, Solan, Zoological Survey of India, Kolkata and also by consulting literature.

Genetic analysis: DNA was extracted from the legs or thorax of the insect specimen by using DNeasy blood and tissue Qiagen kit method and extracted DNA was preserved in the -20^{0}C for further use. Integrity and presence of DNA was checked on 1.2% agarose gel electrophoresis and DNA was visualized under UV transilluminator as bright bands. Target DNA from mitochondrial gene, i.e., Cytochrome Oxidase subunit I was amplified by using a pair of forward primers LCO1490 5'-GGT-CAA-CAA-ATC-ATA-AAG-ATA-TTG-G-3' and reverse primer HCO2198 5'- TAA-ACT-TCA-GGG-TGACCA-AAA-AAT-CA-3' (Folmer *et al.,* 1994). Polymerase chain reaction (PCR) was performed in 96-well plates with 20 ml reaction volume (1mL DNA template; 1 mL forward primer; 1 mL reverse primer; 5 mL distilled water; 12 mL emerald PCR master mix) in a C1000 thermal cycler. The amplified product was analyzed on a 1.2% agarose gel electrophoresis and checked under UV light and documented. The amplified DNA fragments were extracted from agarose gels and purified using DNA/RNA purification Qiagen kit method. The primers used were the same primers used in PCR amplification and sequencing was done in Big dye terminator version 3.1" cycle sequencing kit with sequencing machine (ABI 3500xL Genetic analyzer). After completion of sequencing, the results were analyzed by using MEGA 11 software (Kumar *et al.,* 2018). Analyses were performed on 1000 bootstrapped data sets generated by the program (Felsenstein, 1985).

Sequences and Phylogenetic analysis: All fasta format sequences obtained by Sanger sequencing were used for BLAST search in NCBI. All the sequences were edited and aligned by using bio edit (7.2.5 software) sequence alignment editor software. All the gaps and mismatched data were removed and sequences were submitted in the NCBI GenBank for accession number. The pairwise analysis of 29 sequences of different insect pollinators species obtained by using the Neighbor-joining bootstrap method and the Kimura-2 parameter in MEGA11 software. The number of base substitutions per site was analyzed between all sequences. Codon positions included were 1st+2nd+3rd+non-coding. All positions containing gaps and missing data were eliminated from the dataset. All sequences A, T, G, C, AT and GC content were obtained using MEGA 11 software. The AT per cent at three codon positions was calculated using the same program. Sequences were aligned using the MEGA 11 software package.

RESULTS AND DISCUSSION

Fifty-one species of insect pollinators belonging to 5 orders and 12 families of the class Insecta were reported on *Valeriana jatamansi* during the study period. Thirty three species belonged to order Diptera, nine to Lepidoptera, four to Hymenoptera, three to Coleoptera, and two to Hemiptera (Table 1). Diptera was most dominant order represented by 33 (64.71%) insect pollinator species belonging to four families, viz., Syrphidae (26), Tachinidae (4), calliphoridae (2) and bombyliidae (1) with syrphidae (51%) being the most dominant family. Relative abundance study shows that dipterans were the most abundant insect pollinators in all eight localities i.e. Tara Devi (80.14%), Dhalli (84.97%), Chaura Maidan (87.59%), Fagu (91.10%), Matiyana (63.2%), Kufri (92.62%), Kharapather (82.73%), and Kupper (82.91%), followed by hymenopterans, lepidopterans, hemipterans and coleopterans. Among all the insect pollinators, *Sphaerophoria indiana* was the most abundant insect visitor on *V. jatamansi* flowers in all localities [Tara Devi (32.98%), Dhalli (23.39%), Chaura Maidan (19.38%), Fagu (19.70%), Matiyana (13.51%), Kufri (9.06%), Kharapather (11.05%) and Kupper

Table 1. Systematic list of insect pollinators of *Valeriana jatamansi* Jones from different localities of Shimla Hills, western Himalaya

Order	Family	Fauna/ Insect species
Diptera	Syrphidae	*Eristalis tenax* (Linnaeus, 1758)
		Eristalis cerealis Fabricius,1805
		Eristalis himalayensis Brunetti, 1908
		Eristalinus arvorum (Fabricius 1787)
		Eristalinus megacephalus (Rossi, 1794)
		Eristalinus paria (Bigot, 1880)
		Episyrphus balteatus (De Geer, 1776)
		Syrphus vitripennis Meigen, 1822
		Syrphus torvus (Osten Sacken, 1875)
		Melanostoma mellinum (Linnaeus, 1758)
		Melanostoma scalare (Fabricius, 1794)
		Melanostoma orientale (Wiedermann, 1824)
		Sphaerophoria indiana Bigot, 1884
		Syritta pipiens (Linnaeus, 1758)
		Eupeodes luniger (Meigen, 1822)
		Eupeodes latifasciatus (Macquart, 1829)
		Parasyrphus lineolus (Zetterstedt, 1843)
		Chrysotoxum baphyrus Walker, 1849
		Scaeva pyrastri (Linnaeus, 1758)
		Meliscaevia cinctella (Zetterstedt, 1843)
		Rhingia laticincta Brunetti, 1907
		Eumerus aurifrons (Wiedermann, 1824)
		Dasysyrphus lenensis Bagatshanova, 1980
		Platycheirus nigrofemoratus (Kanervo, 1934)
		Platycheirus albimanus (Fabricius, 1781)
		Brachyopa notata Osten Sacken, 1875
	Tachinidae	*Gymnosoma sylvaticum* (Zimin, 1966)
		Nowickia marklini (Zetterstedt, 1838)
		Tachina fera (Linnaeus, 1761)
		Estheria petiolata (Bonsdorff, 1866)
	Calliphoridae	*Calliphora vomitoria* (Linnaeus, 1758)
		Lucilia papuensis Macquart, 1843
	Bombylidae	*Bombylius major* Linnaeus, 1758

Hymenoptera	Apidae	*Apis cerana* Fabricius, 1793
		Apis mellifera Linnaeus, 1758
	Halictidae	*Lasioglossum minutissimum* (Kirby, 1802)
	Megachilidae	*Megachilidae* sp.
Coleoptera	Coccinellidae	*Coccinella septempunctata* (Linnaeus, 1758)
		Hippodamia variegate (Goeze, 1777)
		Oenopia sexareata (Mulsant, 1853)
Hemiptera	Miridae	*Pinalitus rubricatus* (Fallen, 1807)
		Orthops scutellatus Uhler, 1877
Lepidoptera	Pieridae	*Pieris canidia* (Sparrman, 1768)
		Colias electo fieldi Ménétriés, 1855
		Eurema laeta Boisduval, 1836
		Eurema hecabe (Linnaeus, 1758)
	Nymphalidae	*Neptis mahendra* Moore, 1872
	Lycaenidae	*Heliophorus sena* (Kollar, 1844)
		Heliophorus androcles (Westwood, 1851)
		Celastrina lavendularis (Moore, 1877)
		Celastrina huegeli (Moore, 1882)

(16.00%)].

Jugran *et al*. (2013) conducted a similar experiment on *V. jatamansi*, discovered that 13 species of dipterans were the most dominant visitors to its blooms. Hymenopterans, lepidopterans, and other minority groups were also flower visitors. The present outcome is supported by the recent finding of Katoch and Thakur (2022); they detected 29 pollinator species from the orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera, on two plants *Bergenia ciliata* (Haw.) Sternb. and *Vinca major* in the Western Himalayas. Kumari *et al*. (2021) studied how a reduction in flower size influenced the pollination and reproduction of *V. wallichii*, an endangered medicinal plant and observed that entomophily was the method of pollination.

All 51 species are taxonomically characterized during study which belongs to five orders i.e. Diptera, Hymenoptera, Lepidoptera, Coleoptera and Hemiptera. Dipterans are characterized by having one pair of forewings and hindwings are modified into halteres. Hymenopterans are small and medium sized insects with two pairs of wings and a narrow waist that set off abdomen from the thorax. Lepidopterans forewings and hind wings have large surface area, body and wings are mostly covered with tiny colored scales. Coleopterans are distinguished on the basis of sclerotized forewings called elytra and a pair of membranous hind wings. Hemipteran wings are partly membranous and partly harden known as hemelytra. Dipterans showed similar wing venation patterns but showed differences in vein R_{4+5} in different species. Species of family Syrphidae differentiated on the bases of their wings. They have a unique vein vena spuria which is absent in other dipterans. In *Eristalis tenax* spurs were present in the bm-cu region. Tachinid are minute to very large flies with extremely diverse appearance, often extremely bristled. Nearly all adult tachinids have a distinct bend in M vein and absence of vena spuria. All dipterans have a large

Table 2. Sequencing with accession number and BLASTN analysis on 29 insect pollinators on *Valeriana jatamansi* Jones

Order	Family	Samples with accession no.	BLASTN results	Query cover (%)	Identical (%)
Diptera	Syrphidae	*Eristalis tenax* OK598005	MN967352	100	100
		Eristalis cerealis OL454830	OL440713	100	100
		Eristalis himalayensis OL442159	MW307783	100	100
		Eristalis arbustorum OP393912	MN868881	100	100
		Episyrphus balteatus OP847796	OL405702	100	100
		Melanostoma scalare OK639012	MN481519	100	98.85
		Syrphus torvus OP363961	KT959674	100	99.84
		Syrphus vitripennis OL305851	KR657522	100	100
		Eupeodes luniger OP363960	KY834510	100	100
		Syritta pipienes OL454816	MN868864	100	100
		Parasyrphus lineolus OP380744	MZ609220	100	100
		Scaeva pyrastri OP788181	HQ944919	100	100
		Dasysyrphus lenensis OP363531	KM930046	98	100
		Platycheirus nigrofemoratus OP363759	HQ577938	99	100
		Platycheirus albimanus OP363294	NC056282	100	99.05
	Tachinidae	*Gymnosoma sylvaticum* OP379750	MT048383	100	100
		Nowickia marklini OP393894	HM861393	100	100
		Tachina fera OL445006	LR999969	100	100
Hymenoptera	Apidae	*Apis mellifera* OP847793	MW428265	100	99.56
	Halictidae	*Lasioglossum minutissimum* OP393898	KT164664	100	100
Coleoptera	Coccinellidae	*Coccinella septempunctata* OL539601	XM898241	100	100
Hemiptera	Miridae	*Pinalitus rubricatus* OP393894	KM022967	100	100
		Orthops scutellatus OP380608	KR032953	100	99.61
Lepidoptera	Pieridae	*Pieris canidia* OP788182	MT935585	100	100
		Eurema laeta OK639008	GU372560	100	99.55
		Eurema hecabe OP393888	OL343184	100	99.51
	Nymphalidae	*Neptis mahendra* OP788197	OK342272	100	100
	Lycaenidae	*Heliophorus sena* OK639007	KC755862	99	100
		Heliophorus androcles OP363632	KT236373	100	99.81

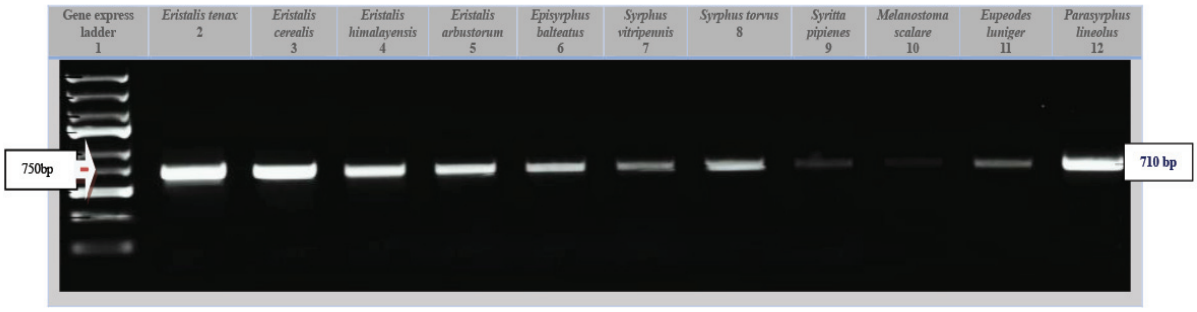

Fig. 1 Analysis of amplified PCR product in 1.2% agarose; Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5, 6, 7, 8, 9, 10 11, 12: 710 bp size mtCOI gene

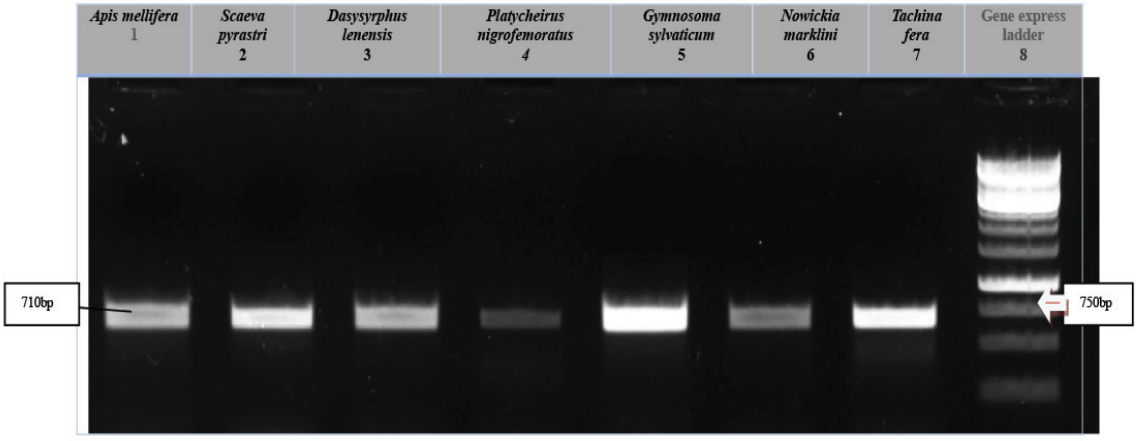

Fig. 2 Analysis of amplified PCR product in 1.2% agarose; Lane 8: Gene ruler express DNA ladder, Lane 1,2, 3, 4, 5, 6, 7: 710 bp size mtCOI gene

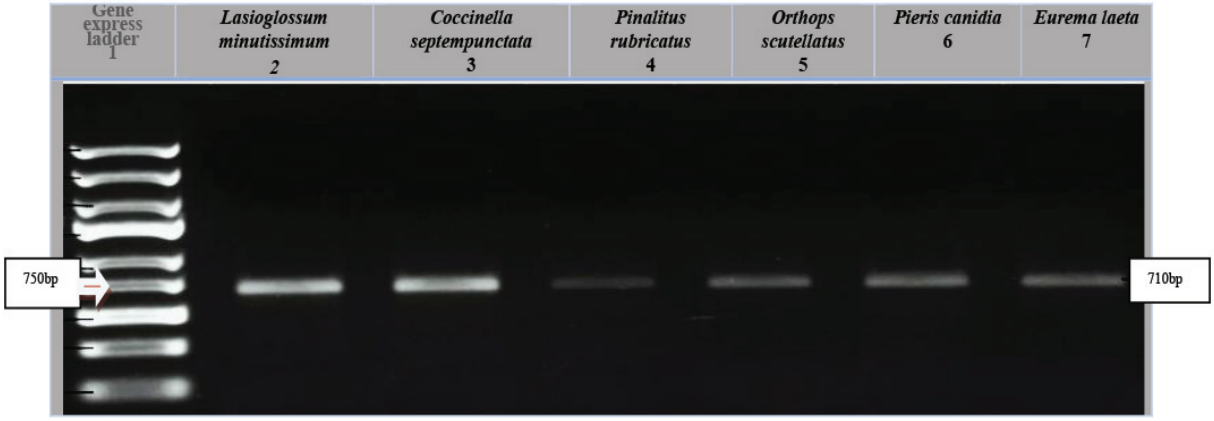

Fig. 3 Analysis of amplified PCR product in 1.2% agarose; Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5, 6, 7: 710 bp size mtCOI gene

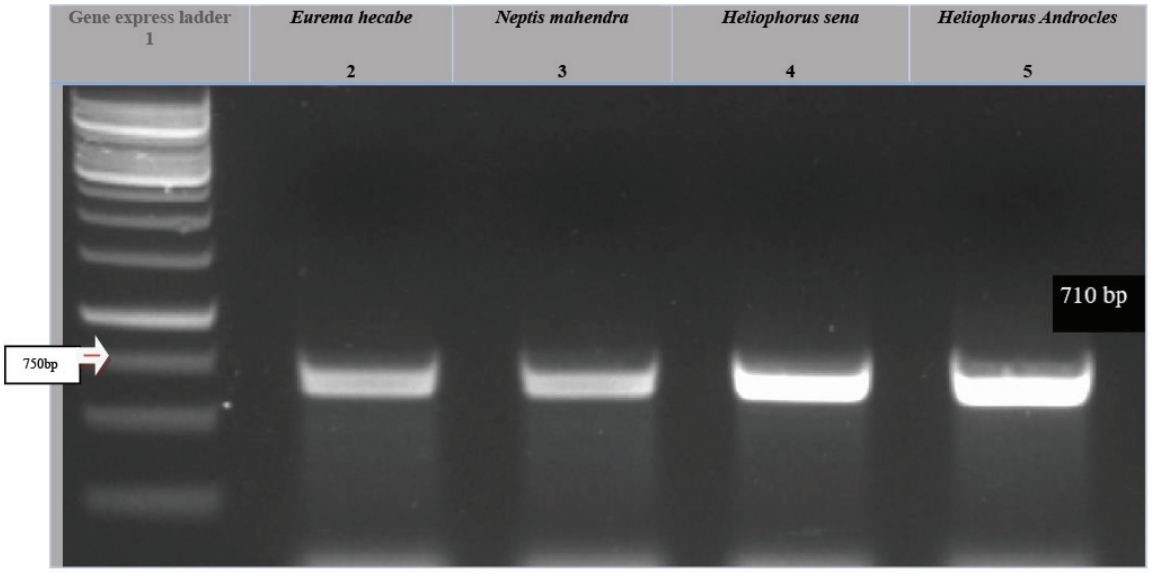

Fig. 4 Analysis of amplified PCR product in 1.2% agarose; Lane 1: Gene ruler express DNA ladder, Lane 2, 3, 4, 5: 710 bp size mtCOI gene

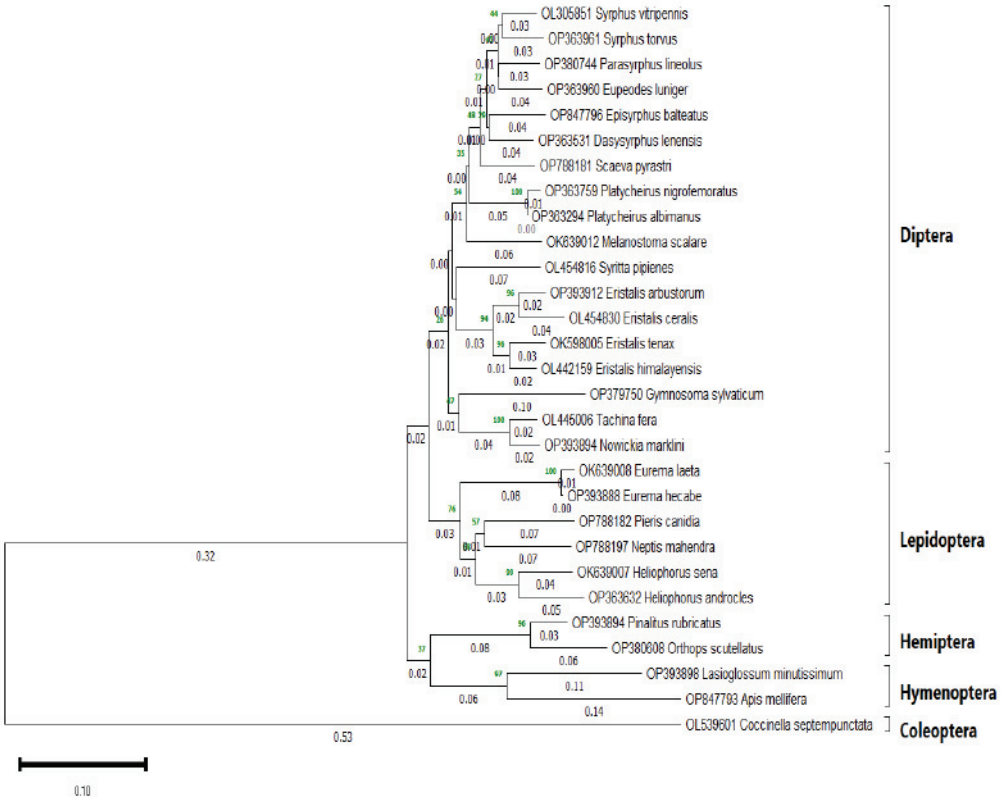

Fig. 5 Phylogenetic tree for 29 insect pollinators of insect pollinator species showing genetic relationship derived from COI sequences by using Neighbor-Joining (NJ) method of MEGA 11 Software

genital tergite located apico-ventrally, partially surrounded by a pair of lobular structures called cerci pair of hairy surstyli, and a large, typically thick superior lobe. While collecting the hymenopterans, most of specimens were workers and it has been observed that genitalia are modified into sting apparatus. Sting apparatus in *Apis mellifera, A. cerana*, *Bombus ardens,* and *Lasioglossum minutissimum* contains a barbed lancet.

When it comes to dipterans, the present findings are similar to the research conducted by Miranda *et al.* (2013), who created an interactive photographic key that covers all genera of Syrphidae in the Nearctic Region. Miranda and Moran (2017) also studied the female abdomen and genitalia of Syrphidae and examined the presence of sclerotized areas on the intersegmental membrane that appears in Rhingiini. Sengupta *et al.* (2018) discovered four new records from Himachal Pradesh's cold, dry zones when they investigated the taxonomic account of hover flies. They described eighteen species of hoverflies from fourteen genera and two subfamilies found in the Himalayan cold and dry zone. The present study is identical with the study of Belyaev and Farisenkova (2019) on the allometry of wing shape and wing venation in dipterans and they observed that the arrangement of veins varied significantly between different families. Rana and Thakur (2019) conducted a comprehensive experiment focusing on the biodiversity of butterflies in the Dharampur region, located in the Mandi district of Himachal Pradesh. Their study involved the collection and analysis of 33 distinct butterfly species, which were classified into 25 genera, spread across six families and two superfamilies.

Rego *et al.* (2022) represents a pictorial key for the identification of 26 species of hoverflies. Pathania and colleagues (2022) conducted a comprehensive study on the physical attributes of the queen, workers, and drones of the *A. mellifera* species in the Kangra district of Himachal Pradesh. They meticulously measured and analyzed 10 distinct features of these *A. mellifera*.

Among all the insect pollinators attempted to sequence during molecular analysis, only 29 insect species of pollinators were able to successfully sequence using Sanger sequencing with mtDNA markers. These markers included the cytochrome oxidase subunit sequence I (COI) (Table 4). All the sequenced genes showed 98 to 100% similarity with the existing GenBank sequences in the BLAST analysis (Table 2). The CO1 region in almost all the samples was in the range of 710bp (Fig. 1– 4). All sequences were submitted in the NCBI GenBank for accession number.

Nucleotide composition of COI gene sequences: The nucleotide content (A,T,G,C) and the total C+G and A+T at first, second and third codon position of all the samples revealed that the average AT content was significantly higher by 69.8 per cent than the GC content of 30per cent. Sequences were deeply AT-biased due to 3rd codon position, which is expected in insect mtDNA. The high numbers of polymorphic sites were uniformly distributed throughout the third codon position in the COI gene. For all codon locations in this area, the A+T bias was very strong. In *V. jatamansi* there were a total of 684 positions in the final dataset. Evolutionary analyses were conducted in MEGA11software. Average genetic distances between the diverse groups of insects used in this study showed higher values at the third codon position (93.2%), indicating that further research of the third codon location for insects could disclose possible evolutionary information between these closely related groups of insects (Table 3).

Phylogenetic analysis: Phylogenetic tree for DNA sequences of 29 species of insect pollinators collected on *Valeriana jatamansi* was constructed by the Neighbor-joining method. The tree was divided into two clades, one shows the phylogenetic relationship between Diptera, Hymenoptera, Hemiptera and Lepidoptera and the other clade shows the phylogenetic relationship between Coleoptera. Out of 29 species first clade consists of 28 species, 18 belongs to Diptera, two to Hemiptera, six to Lepidoptera and two to Hymenoptera and second clade constitute only one species which belonged to order Coleoptera. Phylogenetic analysis of Diptera formed two

distinct groups which included family Syrphidae in one group and Tachinidae in another group. In Lepidoptera, two groups were formed included species belonging to families Pieridae, Nymphalidae and Lycaenidae. Hemipterans formed one distinct group of two species from family Miridae and Hymenoptera showed one species each from family Apidae and Halictidae. The Phylogenetic tree had a total branch length of 2.62 base substitutions per site. The study concluded that dipterans, hymenopterans, hemipterans and lepidopterans arise from common ancestor as compare to coleopterans (Fig. 5).

Molecular characterization of 29 insect pollinators species collected on *Valeriana jatamansi* Jones have been performed by mtCOI sequences. The average AT content was 69.8 per cent, while the GC content was 30 per cent. The sequences exhibited a strong bias towards AT in the third codon position, which is in line with expectations for insect mtDNA. Average genetic distances across the numerous groups of insects employed in this research revealed greater values at the third codon position, suggesting that future examination of the third codon site for insects could offer probable evolutionary evidence among this closely related group of species. All the species were phylogenetically very closely related to each other. Above results are in accordance with the earlier findings of Jalali *et al.* (2015) identified agriculturally important insects using DNA barcoding. They found that AT content was significantly higher than GC content, and phylogenetic analysis showed two clades, one consisting of hymenopteran insects and the other consisting of other orders.

Mitochondrial DNA analysis of COI sequences is a standardized, accurate, efficient, and time-saving method to illustrate and identify insect pollinator species, as compared to conventional taxonomic identification which is time consuming and require expertise. Molecular biologists around the world have been using mitochondrial cytochrome oxidase subunit I (COI) to identify insect species. Kumar *et al.* (2012) identified medically important insects in India through DNA barcoding, identifying seven morphologically identified species of *Phlebotomus* and *Sergentomyia*. Prabhakar *et al.* (2013) analyzed the population genetic structure of the pumpkin fruit fly, *Bactrocera tau*, using mitochondrial cytochrome oxidase I (mtCOI) gene sequences. Karthika *et al.* (2016) assessed the

Table 3. AT % at the First, Second and Third Codon of different insect species of *Valeriana jatamansi* Jones

Samples	1st	2nd	3rd	Total	
	AT	AT	AT	C+G	A+T
Eristalis tenax	56.5	56.2	93.9	30.9	68.8
Eristalis cerealis	56.9	54.2	92.9	31.8	68.0
Eristalis himalayensis	56.9	55.7	95.8	30.3	69.5
Eristalis arbustorum	56.9	54.7	97.2	30.2	69.7
Episyrphus balteatus	58.0	54	98.9	29.5	70.3
Melanostoma scalare	56.8	53.6	96.7	30.8	69.0
Syrphus vitripennis	57.3	54.1	99.0	29.7	68.8
Syrphus torvus	57.1	53.8	95.6	31.0	70.1
Eupeodes luniger	57.3	53.3	97.5	30.5	69.3
Syritta pipienes	59.9	52.1	92.1	31.8	68.0
Parasyrphus lineolus	57.0	53.1	96.5	31.0	68.8
Scaeva pyrastri	57.5	53.0	96.6	30.7	69.1
Dasysyrphus lenensis	57.6	52.6	97.2	30.7	69.1
Platycheirus nigrofemoratus	56.7	54.6	93.8	31.5	68.4
Platycheirus albimanus	56.9	52.6	99.0	30.3	69.5
Gymnosoma sylvaticum	58.0	58.3	91.6	30.6	69.2
Nowickia marklini	57.0	53.2	96.1	31.0	68.8
Tachina fera	57.9	54.3	96.0	30.4	69.3
Apis mellifera	61.6	65.8	95.9	25.5	74.5
Lasioglossum minutissimum	60.7	66.2	90.6	27.3	72.6
Coccinella septempunctata	58.0	62.9	85.2	31.0	68.8
Pinalitus rubricatus	59.3	59.7	88.1	30.7	69.1
Orthops scutellatus	57.8	57.8	82.3	33.8	66.0
Pieris canidia	58.1	58.3	90.5	30.9	69.0
Eurema laeta	58.2	57.5	88.1	31.7	68.0
Eurema hecabe	56.1	57.6	86.7	33.1	66.8
Neptis Mahendra	57.9	57.9	91.0	30.8	68.9
Heliophorus sena	59.5	61.3	91.6	29.0	70.8
Heliophorus androcles	59.1	62.8	89.2	29.4	70.4
Average	57.8	56.5	93.2	30.5	69.2

DNA barcoding and evolutionary ancestry of 15 insect pests of agricultural crops in South India, finding high divergence among insect pests. Khullar *et al*. (2016) studied six forensically relevant blowfly species from India, using mitochondrial cytochrome oxidase subunit I (COI) DNA as an identifying marker. Kaur and Singh (2020) described the evolutionary significance of the pentatomid insect using mitochondrial COI gene sequences, finding an A+T concentration of 65.8 per cent and a R value of 1.39. Molecular Characterization of Pollinators in cotton ecosystem done by Bajaj *et al*. (2023) revealed that specimens collected in cotton belong to the Hymenoptera and Diptera orders.

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A checklist of flesh flies (Diptera, Sarcophagidae) from Assam, India

Mayoushree Gogoi, Annu Kumari, Anurudha Chakraborty, Sangeeta Biswas and Abesh Chakraborty*

Dept. of Zoology, Royal School of Life Science, The Assam Royal Global University, Betkuchi, Guwahati 781035, Assam, India.
Email: achakraborty9@rgu.ac

ABSTRACT: A comprehensive revised checklist pertaining to taxonomy and bionomics of Sarcophagidae of Assam is prepared. The current study revealed the presence of 23 species, including four endemic and 19 non-endemic from Assam, in comparison to elsewhere in the world.
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KEY WORD: Revised, endemic, forensic, taxonomy, bionomics

INTRODUCTION

The family Sarcophagidae, better known as the flesh flies, consists of 3079 species and 133 genera worldwide (Roskov *et al*., 2016). In India, Sarcophagidae comprises of 2 sub families (Miltogrammatinae and Sarcophaginae), 17 genus and 126 species, till date. The confirmatory identifying characteristics of this group are the three stripes that run along the dorsal side of the thorax (Chakraborty *et al.,* 2017). The family Sarcophagidae previously known as Sarcophaginae was included in the sub-family of Calliphoridae. It was later separated from Calliphoridae and was included in a separate family namely Sarcophagidae; the taxonomic criteria for separation of Sarcophaginae (now Sarcophagidae) was the absence of setulae on the posterior side of the stem-vein of the wing and four notopleurals on the hind leg (Senior-White, 1940). This paved the way for the taxonomic revision of this subfamily Sarcophaginae and reclassifying them into the family we now know as Sarcophagidae (Nandi, 1979) and later it was revised to its current taxonomic status by Chakraborty *et al.* (2016, 2017). There is a paucity of information pertaining to the species inventory in the state level for Assam's Sarcophagidae. Although some fragmented attempts were made by some previous authors (Senior- White, 1940), (Nandi, 1977a, b, 1978, 1979a, b, c), which described only 15 species from this region. Chakraborty (2019) highlights the pivotal role of meat flies, in ecosystems. Meat flies are crucial decomposers, environmental indicators, and research subjects, contributing to ecosystem balance, maintaining nutrient levels, and have applications in forensics and medicine. A comprehensive revised checklist pertaining to taxonomy and bionomics of Sarcophagidae of Assam is discussed.

* Author for correspondence

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MATERIALS AND METHODS

Taxonomic nomenclature used for the checklist follows (Evenhius, 2023). The Indian distribution and elsewhere are also given, along with the synonyms of the species. The study is based on the available literature, rather than on extensive new taxonomic work. Most of the names of the species presented here are in accordance with the most recent scheme of classification following (Evenhius, 2023). Dipterans stored in the repository of National Zoological Collection in the Zoological Survey of India, H.Q. Kolkata, were studied and utilized for the preparation of the checklist. The national zoological collection and general Diptera collection was also consulted from the central entomological labs of Zoological Survey of India, H.Q of registration numbers 5333/H6 to 8301/H6.

Taxonomic literatures were reviewed for extracting out Indian species of the medico-legally important dipterans from internet sources and other relevant literature searches, like Catalogue of Life (updated on September, 2023), *Systema Dipterorum* (updated on September, 2023) (Evenhuis,2023), *Oriental catalog* (Mercedes and Hardy, 1977), *Fauna of British India* (White, 1940) and *Catalog of Diptera from Australasian and Oceania regions* (Evenhius, 2016), *Zoo records series* (2016 to 2023) and *State fauna series* and Open internet search for papers on the family Sarcophagidae (1940 to 2023).

Numerical taxonomy was done with the help of M.S. excel where the various per cent of the species abundance was given in Assam. The per cent of the various bionomics is also shown here. This was achieved by dividing on criteria basis and utilizing graphs to visualize data (Sokal, 1963). At first, found out the sub-families per cent, endemic species criteria are those species which has only been recorded from a certain specified historic geographic range. Dividing the no. of indiviuals from sub-families *Miltogrammatinae* (Enderlein 1928) and *Sarcophaginae* (Townsend, 1917) by total no. of species. The same way the bionomics of the various species is also done. The list is arranged till subgenus and genus level and alphabetically thereafter, to make the search easier for a given taxon. Main references to the original distribution and host preference are listed. The acronyms used for collections follow the standard of the *Systema Dipterorum* (Evenhuis, 2023).

RESULTS AND DISCUSSION

There are 23 species in total from Assam. Among them 20 (86.95%) are from the sub-family *Miltogrammatinae* (Enderlein 1928) and 3 (13.04%) are from Sarcophaginae (Townsend, 1917). The species bionomics study shows *Sarcophaga (Sarcorohdendorfia) antilope* (Bottcher, 1913) (4.34%) seems to be parasitoid of Lepidoptera. The comprehensive analysis of the flesh fly (Diptera, Sarcophagidae) species found in Assam, India provides valuable insights into the diversity and ecological roles of this important group of flies in the region. The key findings indicate that the Sarcophagid fauna of Assam is dominated by species from the subfamily Miltogramminae, accounting for 86.95 per cent of the 23 total species identified (Table 1, 2).

The diverse bionomics exhibited by these flesh flies highlights their adaptability to a range of ecological niches. The preference of certain species for bushes, nests, symbiotic relationships, carrion, and feces suggests they play important roles in nutrient cycling, decomposition, and potentially even as vectors of pathogens. This information is crucial for understanding the broader ecosystem functions and public health implications of Sarcophagids in Assam. Further research is needed to fully elucidate the ecological interactions, seasonal dynamics, and potential forensic applications of these flesh fly species. Expanding the survey efforts to other regions of Assam and northeastern India could also uncover additional undocumented species, providing a more comprehensive picture of Sarcophagid diversity in the state.

Miltogramma angustifrons* and *Phylloteles hyalipennis: These two Miltogrammatinae flies are found in the Doom Dooma and Cachar/Rashan regions of Assam, respectively, and are not recorded elsewhere in India. They breed in the nests of various Hymenoptera insects like Vespidae, Sphecidae, Apidae, Eumenidae and Trypoxylionidae.

Table 1. Check list of Sarcophagidae species, bionomics and distribution in Assam

No.	Species	Sub Family	Bionomics	Distribution	Distribution in India
1	*Miltogramma angustifrons* (Townsend, 1933)	Miltogrammatinae Enderlein 1928	Breed in the nests of Vespidae, Sphecidae, Apidae, Eumenidae and Trypoxylionidae. Some of the adult flies are attracted to nectar and damaged fruits of different kinds of trees	Doom Dooma	Not recorded elsewhere in India.
2	*Phylloteles hyalipennis* (Baranov, 1934)	Miltogrammatinae Enderlein 1929	Some larvae are pedators or guests in the nests of Hymenoptera, Orthoptera and Isoptera.	Cachar, Rashan	Not recorded elsewhere in India.
3	*Senotainia navigatrix* (Meijere, 1910)	Miltogrammatinae Enderlein 1930	Sphecidae /Apidae nest	Silchar	Bihar (Pusa), Kerala (Midigare), Karnataka (Trivandrum),Tamil Nadu (Cinchona).
4	*Sarcophaga* (*Bercaea*) *africa* (Wiedemann, 1824)	Sarcophaginae Townsend, 1917	Bait of raw fish	Assam	Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Kerala, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, Uttar Pradesh, WestBengal, Bihar (Several localities).
5	*Sarcophaga* (*Fengia*) *ostindicae* (Senior-White, 1924)	Sarcophaginae Townsend, 1917	Bushes	Assam	Mizoram: Aizawal P.U. College campus,1,100m, Aizwal,Bonkon,1050m, Kolasib,800m and Uttar Pradesh (Dehradun)
6	*Sarcophaga* (*Harpagophalla*) *kempi* (Senior-White, 1924)	Sarcophaginae Townsend, 1917	Dead sphingid larva	Barpathar, Dibrughar, Jorhat	Arunachal Pradesh (Tipi), Bihar (Deoghar, Giridih, Mahaudanga, Pusa), Himachal Pradesh (Dharmasala), Sikkim (Gangtok), Kerala (Trivancore), Manipur (D.M. College campus), Meghalaya (Shilong, Singimari), Mizoram Aizwal, Kolasib, Lunglei), Tripura (Kanchanpur, Sipahijala), Nagaland (Dimapur) and West Bengal (Arabariforest, Bhatpur, Calcutta, Chandrapur forest, Gorumara forest, Kenduah, Ketka, Susunia forest), Karnataka, Madhya Pradesh, Uttar Pradesh
7	*Sarcophaga* (*Iranihindia*) *futilis* (Senior-White, 1924)	Sarcophaginae Townsend, 1917	In bushes, grasses and flowering plants	Silchar	Arunachal Pradesh (Mangalagiri), Bihar (Chapra, Pusa,Chota Nagpur, Deoghar, Dumraon, Giridhi water falls, Hazaribagh, Patna, Raja Rappa water falls, Rajgir),

					Jharkhand (Ranchi, Titalgarh), Gujrat (Ahmedabad, Bet Dwarka, Dwarka, Gir santuary), Karnataka (Bannerghata National park, Banglore, Kellar, Mysore), Kerala (Calicut, Wilingdonisl.), Maharashtra (Lonavale, Nagpur), Madhya Pradesh (Artham, Jonk river), Nagaland (Dimapur), Mizoram (Aizwal, Bonkon), Orissa (Ashanput,Balugaon, Hirakund), Tamil Nadu (Coimbatore, Chennai, Parvatipuram), Tripura (Matabari, Pratia forest), Uttar Pradesh (Dehradun), West Bengal (Bhatpur, Bishnupur, Calcutta, Dulmi West, Kachujur, Sahebbundh, Sasankali, Purulia), Andhra Pradesh, Karnataka, Orissa; Barkuda isl., Tripura
8	*Sarcophaga.* (*Iranihindia*) *indica* Nandi, 1979	Sarcophaginae Townsend, 1917	Bushes in forest area	Assam	Andra Pradesh, Kerala, Karnataka, Maharashtra, Tamil Nadu WestBengal (Birbhum; Panchubaga, Midnapore; Salboni), Bihar (Hazaribagh National Park: 300m, Palamau National Park: 320m, Madhudanga, Netarhat).
9	*Sarcophaga* (*Iranihindia*) *martellata* (Senior-White, 1924)	Sarcophaginae Townsend, 1917	Spoiled beef as bait	Silchar	Andhra Pradesh, Arunachal Pradesh (Manalagiri), Bihar (Chota Nagpur, Deoghar, Dumraon, Giridhi water falls, Hazaribagh, Patna, Raja Rappa water falls, Rajgir), Jharkhand (Titalgarh), Gujrat (Ahmedabad, Bet Dwarka, Dwarka, Gir Santuary), Karnataka (Bannerghata National Park, Bangalore, Kellar, Mysore), Kerala (Calicut, Willingdon isl.), Maharashtra (Lonavale, Nagpur), Madhya Pradesh (Artham, Jonk river), Nagaland (Dimapur), Mizoram (Aizwal, Bonkon), Orissa (Ashanput, Balugaon, Hirakund), Tamil Nadu (Coimbatore, Chennai, Parvatipuram), Tripura

					(Matabari, Pratia forest), Uttar Pradesh (Dehradun), West Bengal (Bhatpur, Bishnupur, Calcutta, Dulmi West, Kachujur, Sahebbundh, Sasankali
10	*Sarcophaga* (*Liopygia*) *ruficornis*(Fabricius, 1794)	Sarcophaginae Townsend, 1917	Baits of decayed carrion, rabbit, fish, liver, and chicken.Cause myiasis in Dogs	Assam	Andhra Pradesh, Bihar, Delhi, Meghalaya, Mizoram, Goa, Gujrat, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Nagaland, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal and Union territories of Andaman and Nicobar Isl. Dadra and Nagar Haveli, Delhi, Lakshdweep and Pondicherry
11	*Sarcophaga* (*Liosarcophaga*) *brevicornis* Ho, 1934	Sarcophaginae Townsend, 1917	Pig carcasses	Assam	Andhra Pradesh, Arunachal Pradesh, Bihar, Gujrat, Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Rajasthan, Tripura and West Bengal.
12	*Sarcophaga* (*Liosarcophaga*) *dux*(Thomson, 1869)	Sarcophaginae Townsend, 1917	Carcass of Chicken, Toad, Fish, Rat, Lizard	Assam	Andhra Pradesh, Arunachal Pradesh, Bihar, Delhi, Jammu and Kashmir, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Punjab, Rajasthan, Tamil Nadu,Tripura, Uttar Pradesh, West Bengal and the union territories of Andaman and Nicobar Isl. and Lakshadweep.
13	*Sarcophaga* (*Liosarcophaga*) *sarupi*(Nandi, 1979)	Sarcophaginae Townsend, 1917	Vertebrate carcasses	Champhai Assam rifle area	Uttar Pradesh; Nainital, 1230m, Uttar Pradesh; Kaushani, 1890m, Mizoram; Champhai, P .W.D. Campus, 1600m, 1615m and Meghalaya (Barapani)
14	*Sarcophaga* (*Liosarcophaga*) *scopariiformis* (Senior-White, 1927)	Sarcophaginae Townsend, 1917	Dead bodies and carcasses	Dibrughar, Nowgong	Karnataka (Chikmagalore), Kerala (Walayarforest), Manipur (Imphal), Mizoram (Aizwal), Nagaland (Dimapur), Tripura (Kanchanpur, Matabari),

					Tamil Nadu (Tranquebar) and West Bengal (Daimond harbour, Gour, Rudranagar, Sankrail.
15	*Sarcophaga* (*Pandelleisca*) *assamensis* (Nandi & Ray, 1982)	Sarcophaginae Townsend, 1917	Human excrement	Jorhat	Manipur; D.M.College campus.
16	*Sarcophaga* (*Pandelleisca*) *bainbriggei* (Senior-White, 1925)	Sarcophaginae Townsend, 1917	Human excrement	Silchar	Bihar (Deoghar, Pusa), Himachal Pradesh (Solan), Kerala (Kurumbagram), Orissa (Balugaon, Nandankanan, Taptapani), Tripura (Trishna),Tamil Nadu (Coimbatore, Chennai, Tranguebar) and West Bengal (Baharampur, Bishnupur, Calcutta, Gour Suri)
17	*Sarcophaga* (*Parasarcophaga*) *albiceps* (Meigen, 1826)	Sarcophaginae Townsend, 1917	Decaying larvipost of Mutton	Assam	Andhra pradesh, Arunachal Pradesh, Bihar, Chandigarh, Delhi, Goa, Gujrat, Harayana, Himachal Pradesh; Kullu; 6,000ft, Mizoram, Nagaland, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal, Andaman and Nicobar, Delhi, Karnataka, Kerala, Madhya Pradesh, Manipur, Maharashtra, Orissa, Panjab, Chandighar, Daman Diu, Pondicherry.
18	*Sarcophaga* (*Parasarcophaga*) *misera* (Walker,1849)	Sarcophaginae Townsend, 1917	Decayed carrion-baits, human faeces, carcasses and dead fish	Assam	Andhra Pradesh, Arunachal Pradesh, Bihar, Chandigarh, Delhi, Goa,Gujrat, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Punjab, Rajasthan, Tamil Nadu, Tripura
19	*Sarcophaga* (*Parasarcophaga*) *taenionota* (Wiedemann, 1819)	Sarcophaginae Townsend, 1917	Human and cow faeces along with dead animals	Assam	Andhra Pradesh, Arunachal Pradesh, Assam, Bihar; Banhar, Delhi, Goa,Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttar

					Pradesh,West Bengal.
20	*Sarcophaga* (*Prionophalla*) *peregrina*(Robineau-Desvoidy, 1830)	Sarcophaginae Townsend, 1917	Breed in chicken manure	Assam	Bihar, Himachal Pradesh, Kerala, Maharashtra, Madhya Pradesh, Manipur, Mizoram, Nagaland, Sikkim, Tamil Nadu, Tripura, Uttar Pradesh,West Bengal, Andaman and Nicobar Isl. Dadra and Nagar Haveli and Pondicherry
21	*Sarcophaga* (*Sarcorohdendorfia*) *antilope* (Bottcher, 1913)	Sarcophaginae Townsend, 1917	Internal parasites of lepidoptera insects	Margherita and Sadiya	Not recorded elsewhere in India.
22	*Sarcophaga* (*Sarcorohdendorfia*) froggatti (Taylor, 1917)	Sarcophaginae Townsend, 1917	Decayed carrion-baits	Assam	Not recorded elsewhere in India.
23	*Sarcophaga* (*Seniorwhitea*) *princeps* (Wiedemann, 1830)	Sarcophaginae Townsend, 1917	Feed and develop on vertebrates' carcasses	Assam	Arunachal Pradesh, Andhra Pradesh, Assam; several localities, Bihar; Chapra, Gujrat, Harayana, Himachal Pradesh,, Karnataka, Kerala, Madhya Pradesh, Maharashtra; Mumbai, Manipur, Mizoram, Nagaland, Orissa;Barkuda isl., Pondicherry, Sikkim, Tamil Nadu; several localities, Tripura, Uttar Pradesh; Dehradun, West Bengal and Andaman.

Reference: Nandi, 2022, Chakraborty *et al.*, 2017, Chakraborty, 2019

Table 2. Species bionomics

Species	No.	%	Species in Table1
Carrion, carcass, dead, meat baits	9	39.13	9, 10, 11, 12, 13, 14, 17, 22, 23
Bushes	3	13.04	5,7,8
Symbiotic relationships	2	8.69	1,2
Kleptoparasitic	3	13.04	3,4,6
Parasitoids	1	4.34	21
Faeces	5	21.73	15, 16, 18, 19, 20

No. = Number of Individuals; Species representation in Table 1: %= percentage to total

Senotainia navigatrix: This Miltogrammatinae fly is found in Silchar, Bihar, Kerala, Karnataka, and Tamil Nadu, breeding in the nests of Sphecidae and Apidae.

Several Sarcophaginae flies are reported from Assam, including *S. (Bercaea) africa, S. (Fengia) ostindicae, S. (Harpagophalla) kempi, S. (Iranihindia) futilis, S. (Iranihindia) indica, S. (Iranihindia) martellata, S. (Liopygia) ruficornis, S. (Liosarcophaga) brevicornis, S. (Liosarcophaga) dux, S. (Liosarcophaga) sarupi, S. (Liosarcophaga) scopariiformis, S. (Pandelleisca) assamensis, S. (Pandelleisca) bainbriggei, S. (Parasarcophaga) albiceps, S. (Parasarcophaga) misera, S. (Parasarcophaga) taenionota, S. (Prionophalla) peregrina, S. (Sarcorohdendorfia) antilope, S. (Sarcorohdendorfia) froggatti,* and *S. (Seniorwhitea) princeps.* These Sarcophaga flies have diverse bionomics, with some breeding in the nests of Hymenoptera, others feeding on decaying carrion, human/animal feces, and even causing

myiasis in dogs. Their distributions range across multiple states in India, with a few species like *S. (Sarcorohdendorfia) antilope* and *S. (Sarcorohdendorfia) froggatti* being restricted to Assam. Among the 23 there are four endemic (17.39%) and 19 (82.61%) non-endemic.

The results of the study provide a comprehensive taxonomic and ecological inventory of fly species found in the Assam region, highlighting their unique bionomics and distribution patterns within India.

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Species composition, abundance and seasonality of dermatitis causing *Paederus* rove beetles in paddy fields of Malabar region, Kerala, India

P.M. Kavyamol, P.J. Vineesh* and V.P. Vineetha

PG and Research Department of Zoology, St. Joseph's College (Autonomous), Devagiri, Kozhikode, Kerala, India.
Email: kavyamohan5540@gmail.com; vineeshpj@gmail.com, vineethavpnambiar@gmail.com

ABSRACT: Species composition, abundance, and seasonality of *Paederus* in the paddy fields of the Malabar region, Kerala, India, are analysed. Among the five species of *Paederus* beetles collected from Malabar, four are known to cause dermatitis. *Paederus sondaicus* Fauvel 1895 was the dominant species in the paddy fields of the Malabar region. Regional variation in the species composition, abundance, and seasonality of species was observed. *P. sondaicus* was dominant in Wayanad and Palakkad, *P. fuscipes* Curtis, 1826 in Malappuram and Kozhikode and *P. extraneus* Wiedemann, 1823 in Kannur. A modified taxonomic key for identification of *Paederus* beetles in Kerala is provided.
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KEY WORDS: Rove beetles, regional variation, rice ecosystem, pederin, linear dermatitis

INTRODUCTION

Paederus Fabricius, 1775 is a genus of Rove beetle belonging to the subfamily Paederinae of the family Staphylinidae, with more than 622 species distributed in all continents except Antarctica (Zargari *et al.,* 2003; Mammino, 2011). In general, species of *Paederus* inhabit moist environments such as marshes, edges of freshwater lakes, river banks, and crop fields (Frank and Kanamitsu, 1987). *Paederus* species are nocturnal in habit, remain under bark, stones, soil, litter during day time (Frank and Kanamitsu, 1987; Nasir *et al*., 2012; Bong *et al.,* 2012). There are predators on soft bodied insects; soil nematodes and hence act as biological control agents (Frank and Kanamitsu, 1987, Bong *et al*., 2015, Maruthadurai *et al*., 2022). Large populations of *Paederus* have been recorded from agricultural habitats which make them beneficial due to their feeding on insect pests of major crops and fodders (Devi *et al*., 2003), particularly insect pests like *Heliothis armigera* Hübner, 1808; *Aphis gossypii* Glover, 1877; *Earias vittella* Fabricius, 1794; *Spodoptera litura* Fabricius, 1775; *Marasmia patnalis* Bradley, 1981; *Aphis glycines* Matsumura, 1917 and many dipterous and other arthropods (Berglind *et al*., 1997; Krakerb *et al*., 2000; Devi *et al*., 2003). However, the benefits provided by species of *Paederus* are annulled by the problems they cause for humans (Bong *et al*., 2015). When a beetle is brushed vigorously over the skin or crushed, a toxic haemolymph containing Pederin is released those leads to localized blistering (Kitisin and Sukphopetch, 2021) and inflammation

* Author for correspondence

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referred as *Paederus* mediated dermatitis also called linear dermatitis, dermatitis linearis, Nairobi fly dermatitis, spider-lick, whiplash, Blister beetle dermatitis (Zargari *et al*., 2003; Mullen and Durden, 2009). *P. fuscipes* is reported to cause Paederus dermatitis in Kerala (Ramakrishnan *et al.,* 2019). Eleven species of *Paederus* are recorded till now from Kerala including three species recorded by Cameron (1931) and Kavyamol *et al*. (2023), and there is no recent taxonomic analysis of the *Paederus* beetles in the paddy fields of the Malabar region. Present study was undertaken to identify the species of *Paederus* beetles in Malabar region and to generate baseline ecological data of *Paederus* beetles in Malabar region.

MATERIALS AND METHODS

Sampling for ecological studies: During 2018–2019 light traps (SAFS ltrap 01 B) were used to sample *Paederus* beetles. Ten light traps were placed at a distance of 100 meters within a paddy field and monthly collections were made from Palakkad (10.6726° N, 76.7531° E), Malappuram (10.9015° N, 76.1904° E), Wayanad (11.6165°N, 76.2140°E), Kozhikode (11.4146°N, 75.9363°E) and Kannur (11.7481°N, 75.4929°E). Thus, thirty samples were collected from each site during Pre-summer (December, January, February/Pre-harvesting), Summer (March, April, May/ Harvesting) and Monsoon (September, October, November/ Post-harvesting) seasons. Traps were operated from 6pm to 7am the next day. Trapped beetles were collected and transferred into vials containing 70% alcohol. Beetles collected were examined under a Stereo Zoom Trinocular Microscope (LABOMED – 200 MAR, CODE:-ZM 45 TM). Specimens were identified with the help of keys provided by Cameron (1931). Photographs were taken with a Leica MC170HD camera attached to a Leica M205C stereomicroscope.

Species abundance data was tested for normality with Anderson-Darling test and opted parametric tests for further analysis. Shannon diversity index was calculated to analyse the diversity of *Paederus* beetles in different seasons at different collection sites. Two- way ANOVA followed by Tukey's test was done to compare the species abundance between habitats and seasons. All statistical analyses were done using PAST software version 3.15 (Hammer *et al.,* 2001). For all analyses, significance was determined at $P<0.05$.

RESULTS AND DISCUSSION

Overall Species composition, abundance and distribution: Five species of *Paederus* were recorded in the paddy fields of Malabar region (Plate 1). Among these, *P. sondaicus* Fauvel 1895 was the dominant ($F= 97.72$; $P< 0.0001$) (Fig. 1). Tukey's comparison examined whether there is any possible difference between the mean of all possible pairs and found that, *P. sondaicus* showed difference in abundance with that of *P. alternans* Walker, 1858, *P. extraneus* Wiedemann, 1823, *P. nigricornis* Bernhauer, 1911 and *P. fuscipes* Curtis, 1826 (Table 1).

Site wise Species composition, abundance and distribution: Variation in species composition and abundance in different localities was recorded. Highest diversity was recorded in Wayanad. Five species were recorded from Wayanad and *P. sondaicus* as the dominant there ($F= 89.07$; $P< 0.0001$). Comparison of the abundance of species collected from Wayanad found that *P. fuscipes* showed difference in abundance with that of *P. sondaicus* ($P< 0.05$), *P. alternans* ($P< 0.0001$), *P. extraneus* ($P< 0.0001$), *P. nigricornis* ($P< 0.0001$); *P. sondaicus* showed difference in abundance with that of *P. alternans* ($P<0.0001$), *P. extraneus* ($P<0.0001$), *P. nigricornis* ($P< 0.0001$) (Table 2, 3).

Three species of *Paederus* were recorded from Malappuram and *P. fuscipes* was dominant here ($F= 125.8$; $P< 0.0001$). Tukey's comparision indicates that in Malappuram, *P. fuscipes* showed difference in abundance with *P. sondaicus* ($P< 0.0001$) and *P. alternans* ($P< 0.0001$); *P. sondaicus* showed difference in abundance with that of *P. alternans* ($P< 0.0001$). Two species of *Paederus* were recorded from Palakkad and *P. sondaicus* was the dominant species ($F=691.9$; $P< 0.0001$). Tukey's test compared the abundance of that species and found that in this site, there is a difference in abundance of *P. fuscipes* with that of

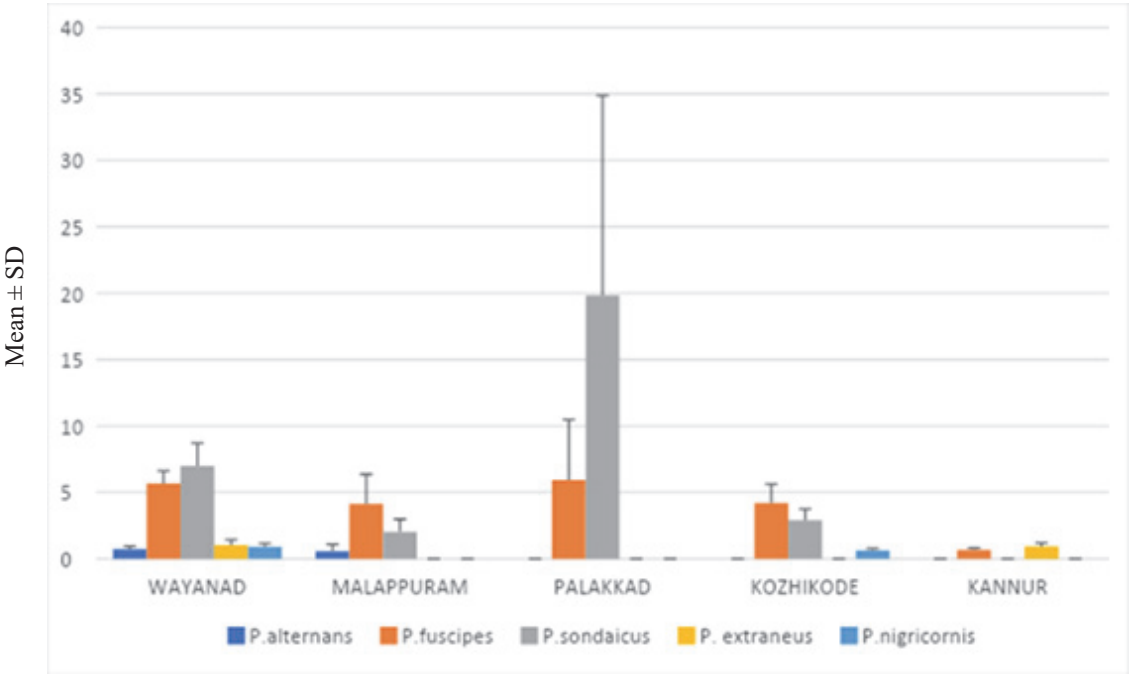

Fig. 1 Abundance of *Paederus* beetles collected from Malabar region

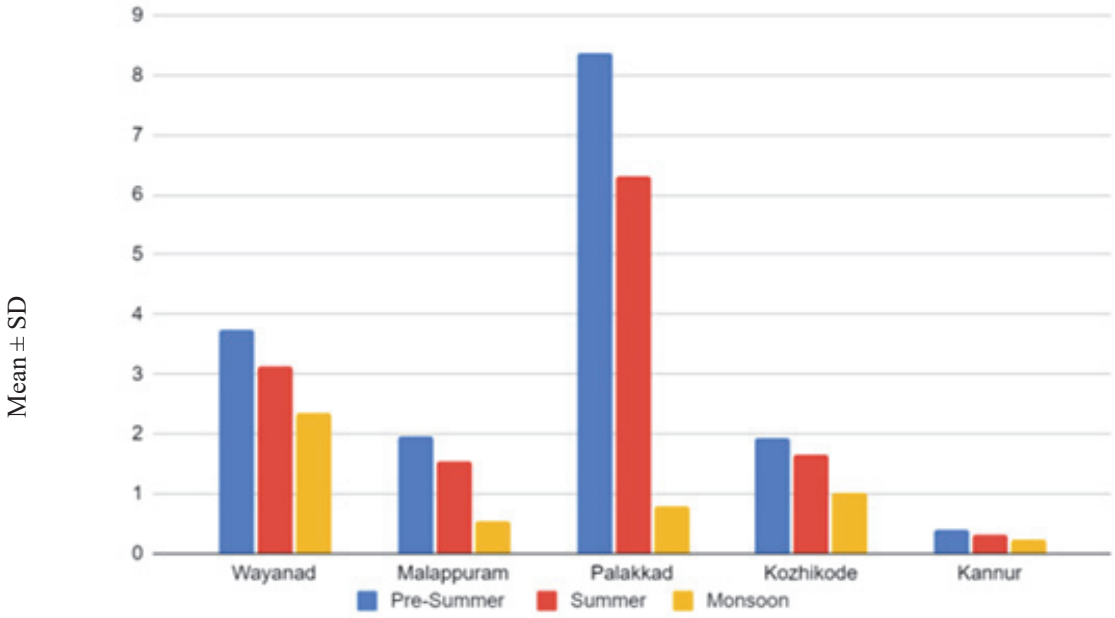

Fig. 2 Seasonality of *Paederus* beetles collected from Malabar region Pre-summer (December, January, February); Summer (March, April, May); Monsoon (September, October, November)

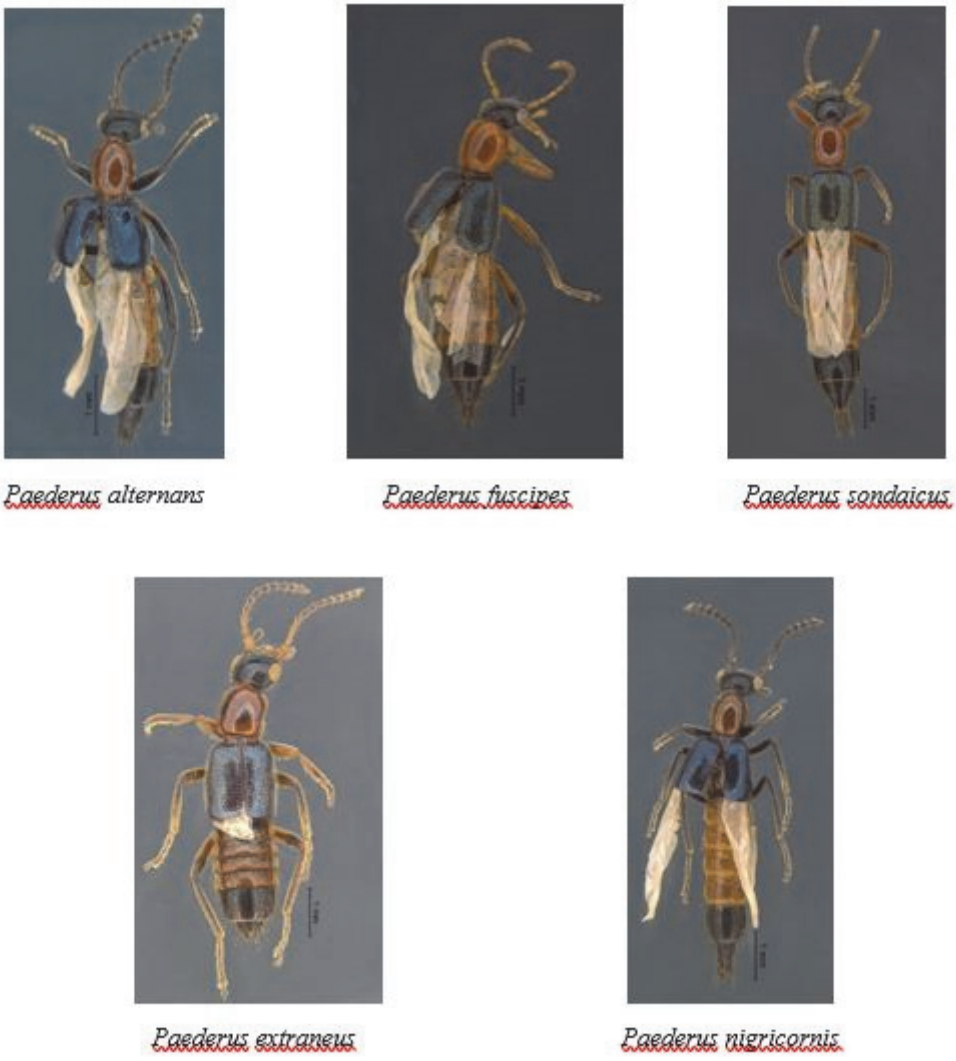

Plate 1. Spicies recorded in paddy fieds of Malabar region

P. sondaicus (P< 0.0001). Three species of *Paederus* were recorded from Kozhikode and *P. fuscipes* was the dominant species (F=167.8; P<0.0001). Tukey's comparision results that, *P. fuscipes* has shown a difference in abundance with *P. sondaicus* (P< 0.0001), *P. nigricornis* (P< 0.0001); *P. sondaicus* has shown a difference in abundance with *P. nigricornis* (P< 0.0001). Two species of *Paederus* were recorded from Kannur with *P. extraneus* as the dominant one (F= 5.987; P< 0.05). Comparison of abundance of species collected from Kannur indicated that there is a difference in abundance between *P. extraneus* and *P. fuscipes* (P< 0.05).

All species were not recorded from all collection sites. *P. alternans* was recorded only from Wayanad and Malappuram; *P. fuscipes* were captured from all the five collection sites *i.e.*, Palakkad, Malappuram, Kozhikode, Kannur and Wayanad; *P. sondaicus* from all the collection sites except Kannur region; *P. extraneus* from Wayanad and Kannur; *P. nigricornis* from Kozhikode and Wayanad. The highest overall abundance (Mean±SD) of the total *Paederus* beetles was recorded at Palakkad followed by Wayanad.

Overall Seasonality: *P. fuscipes* (P< 0.0001), *P. sondaicus* (P< 0.0001), and *P. alternans* (P<

0.001) showed high dominance during pre- summer (December, January and February) in Malabar region. Tukey's test for seasonal comparison of the dominant *Paederus* species indicated that, there was a significant variation of the abundance of *P. fuscipes* between all the three seasons (Table 4).

Site-wise Seasonality: *Paederus* beetles showed seasonality in abundance with high dominance during pre- summer season in all the collection sites (Fig. 2). *P. sondaicus* (P< 0.05), *P. extraneus* (P< 0.001) and *P. nigricornis* (P< 0.01) showed seasonality with high abundance during the pre-summer in Wayanad. Likewise, among the three species recorded from Malappuram, *P. fuscipes* (P< 0.0001) and *P. sondaicus* (P< 0.001) showed seasonality in abundance. The average number of all species collected from Palakkad has shown significant seasonal variation *i. e., P. fuscipes* (P<0.0001) and *P. sondaicus* (P<0.0001) have shown seasonality in abundance. Similarly, in Kozhikode *P. fuscipes* (P< 0.0001) and *P. sondaicus* (P< 0.0001) showed seasonality in abundance. From Kannur, *P. extraneus* showed seasonality in abundance (P< 0.05). Two-way ANOVA was done to compare the abundance of *Paederus* beetles irrespective of species, between different habitat (collection sites) and seasons. There was a significant variation in *Paederus* beetle abundance between different habitats (F=4.41; P<0.05) but there was no significant variation between the seasons (F=2.87; P>0.05).

As per literature references, a total of 11 species of *Paederus* are reported till now from Kerala (Table 5). A toxonomic key for the identification of these species is prepared.

Key to the *Paederus* species of Kerala (modified from Cameron, 1931)

1 Unicolorous species with reddish yellow colour……..…….....….*P. pallidus*

Species multicolored.....2

2 Elytra reddish.....*P. mussardi*

Elytra bluish or blackish.....3

3 Elytra vertically impressed near lateral margins……..…...……*P. loebli*

Elytra without vertical impression.....4

4 Apterous, head red.....*P. hingstoni*
Winged, head black, blue or blueblack.....5

5 Head black.....7

Head blueblack.....6

6 Legs including the coxae entirely black.....*P. kuluensis*

Legs with at least the coxae reddish brown.....*P. variicornis*

7 Legs wholly dark brownish black including the coxae.....8

Legs in part at least testaceous.....9

8 Post- ocular region almost rounded.....*P. extraneus*

Post-ocular region straightly converging to the neck.....*P. nigricornis*

9 Legs black, the coxae and extreme base of the femora reddish yellow.....*P. alternans*

Legs almost yellowish and anterior femora entirely testaceous.....10

10 Larger (>9mm). Last joint of antennae testaceous..... *P. sondaicus*

Table 1. Tukey's table (Studentized range statistic) the comparison of total *Paederus* species collected from Malabar region

Species name	Q value	P value
P. fuscipes/P. sondaicus	7.87	<0.0001
P. fuscipes /P. alternans	13.56	<0.0001
P. fuscipes /P.extraneus	13.15	<0.0001
P. fuscipes /P. nigricornis	13.44	<0.0001
P. sondaicus /P. alternans	21.43	<0.0001
P.sondaicus /P. extraneus	21.02	<0.0001
P. sondaicus/P. nigricornis	21.31	<0.0001
P. alternans/P.extraneus	0.42	>0.05
P. alternans/P. nigricornis	0.12	>00.5
P. extraneus/P. nigricornis	0.29	>0.05

Smaller (<7.5mm). Last joint of antennae concolorous.....*P. fuscipes*

The high abundance of *Paederus* beetles, in paddy fields, is closely linked to their role as natural predators that coexist with numerous agricultural pests (Bong *et al*., 2015). Specifically, proximity to paddy fields is a pivotal factor in the outbreak of *Paederus* beetle dermatitis (Coondoo and Nandy, 2013). The current study further revealed that paddy fields serve as the optimal habitat for *Paederus* beetles (Frank and Kanamitsu, 1987; Bong *et al*., 2015), indicating their preference for this particular ecological niche.

Among the five species of *Paederus* collected from paddy fields of Malabar region, the following species are known to cause dermatitis in different parts of the world: *P. fuscipes* (Frank and Kanamistu 1987; Verma and Agarwal 2006; Toppo *et al*., 2013; Ramakrishnan *et al* 2019); *P. alternans* (Frank and Kanamistu, 1987), *P. extraneus* (Taneja *et al*., 2013; Gopal 2014) and *P. nigricornis* (Nikbakhtzadeh and Tirgari, 2008).

Of the five different study areas of Malabar, the highest abundance was recorded in Palakkad and the highest diversity was recorded in Wayanad, as these two places have the largest area under paddy cultivation as compared to other collection sites (Agricultural Statistics, 2020; Jankielsohn, 2023). According to the Kerala Water Resource Information System (KWRIS 2018-2019), the annual rainfall of Palakkad and Wayanad is less compared to Kannur, Kozhikode and Malappuram, which is the second possible reason for the high abundance of *Paederus* beetles in Palakkad and high diversity in Wayanad since heavy rainfall adversely affect the survival of *Paederus* beetles (Nasir *et al*., 2012).

During the pre-summer season with mild and moderate weather, there was a noticeable rise in the number of *Paederus* beetles (personal observation). The amount of rainfall significantly affects the *Paederus* beetle population. There is a noticeable decrease in *Paederus* beetles in summer and monsoon with extreme climatic conditions. Intense heat and dryness were noticed during the summer season, which lead to low abundance of *Paederus* beetles in this period of collection. Previous study results from Pakistan, recorded that, heavy rainfall in the monsoon season causes suffocation of larvae and pupae in the soil, and it reduces the population of *Paederus* beetles during the months of heavy rain (Nasir *et al*., 2012). This study results also substantiate the negative influence of heavy rainfall on abundance of *Paederus* beetles.

The abundance of *Paederus* beetles was strongly influenced by the various stages of Paddy cultivation (Maryam *et al*., 2017). Similarly, this study reaffirmed that the different paddy cultivation stages significantly impact the number of *Paederus* beetles, as they were prevalent during the milky grain stage of rice crop (pre-summer season), when insect pests like planthoppers and leafhoppers pose a significant threat to rice plant. *Paederus* beetles are predators of these insect pests (Frank and Kanamistu 1987; Kartohardjono,1988) and the availability of their food sources increased their population density at this

Table 2. Seasonal Abundance data (mean ± SD) of *Paederus* species from the study sites

Species name	WAYANAD			MALAPPURAM			PALAKKAD			KOZHIKODE			KANNUR		
	PS	S	M	PS	S	M	PS	S	M	PS	S	M	PS	S	M
P. alternans	0.9±0.7	0.8±0.7	0.5±0.6	1±0.9	0.7±0.6	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
P. fuscipes	6.7±4.0	5.5±3.7	4.8±3.2	5.8±2.8	5±2.2	1.6±0.9	10.1±3.4	6.6±2.3	1.1±1.1	5.3±1.9	4.7±1.5	2.6±1.3	0.8±0.5	0.7±0.5	0.5±0.5
P. sondaicus	8.6±5.1	7.2±4.2	5.2±3.7	3±2.21	2±1.4	1.1±0.7	31.7±3.4	24.9±4.3	2.9±1.7	3.7±1.2	3±1.4	2±1.1	0±0	0±0	0±0
P. extraneus	1.4±0.9	1.1±0.6	0.6±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	1.2±0.7	0.9±0.7	0.7±0.5
P. nigricornis	1.1±0.6	1±0.5	0.6±0.5	0±0	0±0	0±0	0±0	0±0	0±0	0.8±06	0.6±0.5	0.5±0.5	0±0	0±0	0±0

PS= Pre- summer, S= Summer and M=Monsoon

Table 3. Shannon Diversity Index of different sites in different seasons

Seasons	Wayanad	Malappuram	Palakkad	Kozhikode	Kannur
Overall	1.219	0.8709	0.5403	0.9071	0.6809
Pre-summer	1.229	0.9044	0.5529	0.9013	0.6768
Summer	1.239	0.8551	0.5141	0.8854	0.6829
Monsoon	1.167	0.673	0.5939	0.947	0.684

stage (Bong *et al*., 2013). The lowest number of *Paederus* beetles during the monsoon season may be due to the aftermath of the Kerala flood in August 2018. *P. fuscipes* was the only species found in each of the five collection sites. According to Frank and Kanamistu 1987, *P. fuscipes* is a widely distributed species, and from central Asia its range extends west to the British Isles, east to Japan, and southeast to Australia. Its habitats range from cultivated, irrigated fields to marshes and riverbanks. The results of this study are consistent with the especially wide distribution of *P. fuscipes* and its adaptability to nearly every environment. *P. fuscipes, P. sondaicus and P. alternans* have shown seasonality in abundance in Malabar region, however, *P. nigricornis* and *P. extraneus* showed no seasonality. It might be because of the fewer number of *P. nigricornis* and *P. extraneus* in the collection.

Table 4. Tukey's table showing the seasonal comparison of species collected from Malabar

Species	[1]Seasons	Q value	P value
P. fuscipes	Pre-Summer/Summer	4.677	<0.01
	Pre-Summer/Monsoon	13.75	<0.0001
	Summer/Monsoon	9.07	<0.0001
P. sondaicus	Pre-Summer/Summer	2.69	>0.05
	Pre-Summer/Monsoon	9.77	<0.0001
	Summer/Monsoon	7.08	<0.0001
P. alternans	Pre-Summer/Summer	1.51	>0.05
	Pre-Summer/Monsoon	5.76	<0.001
	Summer/Monsoon	4.26	<0.01

Pre-summer (December, January, February); Summer (March, April, May); Monsoon (September, October, November)

According to reports (Singh and Ali, 2007; Kumaraguru *et al*., 2022) *P. melampus* is the most common species found in India. Contrary to the former report, *P. sondaicus* was found to be the predominant species in the Malabar region. Although *P. sondaicus* was already reported from Silent Valley, Kerala (Biswas, 1986), additional research on the abundance and seasonality of *Paederus* species need to be conducted in Kerala.

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Table 5. Table showing the distribution of *Paederus* species in Kerala, India.

No	Species name	Distribution	Reference	Authors' observation
1	*P. alternans* Walker, 1858	Tamil nadu: Nilgiri Hills; Karnataka: Kanara; Himalayas	Cameron, 1931; Biswas & Sengupta, 1982	Kerala: Malappuram, Wayanad
2	*P. fuscipes* Curtis, 1826	Central Kerala; Tamil Nadu: Annamalainagar, Chidambaram; Karnataka; Uttar Pradesh: Rampur, Ballia, Mirzapur, Allahabad; Gujarat; Uttarakhand; Himachal Pradesh; Bihar; Kashmir, Madhya Pradesh; West Bengal: Sikkim, Darjeeling District; Meghalaya and Tripura	Cameron, 1931; Kamaladasa *et al*.,1997; Varma and Agarwal, 2006; Toppo *et al*., 2013; Sar and Hedge, 2015; Ramakrishnan *et al*., 2019; Vineesh *et al*., 2023	Kerala: Palakkad, Malappuram, Kozhikode, Kannur, Wayanad
3	*P. sondaicus* Fauvel, 1895	Kerala: Silent Valley (Palakkad); Tamil nadu: Nilgiri Hills; Karnataka: Belgaum, Nagargali, Khanapur, Sampgaon; Meghalaya: Khasia Hills, Maharashtra	Cameron, 1931; Biswas, 1986	Kerala: Palakkad, Malappuram, Kozhikode, Wayanad
4	*P. extraneus* Wiedemann, 1825	Karnataka: Manipal; Andra Pradesh; Bengal	Cameron, 1931; Taneja *et al*., 2013;Gopal, 2014	Kerala: Wayanad, Kannur
5	*P. nigricornis* Bernhauer, 1911	Uttar Pradesh: Allahabad- Ramghat; Mizoram; Uttaranchal; Uttarakhand: Chakrata district Garhwal hills; West Bengal: Darjeeling, Nurbong and Mahanadi Valleys; Sikkim; Himachal Pradesh: Simla Hills	Cameron, 1931; Sar and Hedge, 2015; Sar and Ilango, 2016	Kerala: Kozhikode, Wayanad
6	*P. mussardi* Biswas and Gupta, 1982	Kerala: Silent Valley (Palakkad); Peermade, Munnar	Biswas & Sengupta, 1982; Biswas, 1986	-
7	*P. pallidus* Scheerpeltz, 1933	Kerala: Munnar; Tamil nadu: Madura, Palani Hills, Kodaicanal, Madras	Cameron, 1931; Biswas & Sengupta, 1982	-
8	*P. loebli* Biswas and Gupta, 1982	Kerala: Munnar	Biswas & Sengupta, 1982	-
9	*P. variicornis* Fauvel, 1903	Kerala: Silent valley (Palakkad); Tamil nadu: Nilgiris: Coonoor. Ghozeh. Madura; Karnataka: Kanara).	Cameron, 1931; Biswas and Sengupta, 1982; Biswas, 1986	-
10	*P. kuluensis* Bernhauer, 1911	Kerala: Silent Valley; Himachal Pradesh	Cameron, 1931; Biswas, 1986	-
11	*P. hingstoni* Cameron, 1928	Kerala: Calicut; Sikkim: Darjeeling	Cameron, 1931; Sreevidhya and Sebastian, 2020	-

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First record of water mite larvae, *Hydrachna* sp. (Acari, Hydrachnidae) parasitism as quiescent nymphophan on two major aquatic insects of Coleoptera and Hemiptera from West Bengal and Odisha, India

Anindita Das*[1], and Susanta Kumar Chakraborty[2]

[1]*Department of Zoology, Vidyasagar University, Midnapore 721102, West Bengal, India.*
[2]*Department of Zoology, Raja Narendra Lal Khan Women's College (Autonomous), Midnapore 721102, West Bengal, India.*
Email: aninditazoology1993@gmail.com

ABSTRACT: Occurrence of quiescent nymphophan of *Hydrachna* sp. attaching to the sternites and tergites of thorax and abdomen of water beetle (*Hydrophilus* sp.) and giant water bug (*Lethocerus* sp.) is reported for the first time from West Bengal and Odisha of India. After feeding from one to five weeks as a parasitic larva on its host, *Hydrachna* sp, stops feeding and enters a quiescent nymphophan (nymphochrysalid) stage of development in which the larva squeezes into its exoskeleton and forms a sac-like structure where metamorphosis occurs. By the means of gnathoma, it remains attached to the host body, casts off its exoskeleton, and within a short time, the developing nymph can be seen within it. The nymph comes out of a slit in the exoskeleton and assumes a free-living existence. These nymphochrysalids ranged in length from 682.17 to 2112.45µm with lateral stripes adorning their external integuments. Body appeared to be bottle shaped with pointed or rounded posterior end. Preferences of water mites for insect host body parts and seasons, infection intensity and prevalence were reported.
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KEY WORDS: *Hydrachna*, water beetle, *Hydrophilus*, water bug, *Lethocerus,* morphometrics, infection intensity

INTRODUCTION

The Hydrachnidia (true water mites) are found in almost all freshwater ecosystems (Zawal, 2003a). In the life cycle of water mites, the larva, deutonymph and adult are the active stages (Di Sabatino *et al*., 2000; Zawal, 2008). While the larval stages of almost all species of water mite remain as ectoparasites on some specific aquatic insect orders, the other active stages are free living predators that are attached with the eggs and larvae of aquatic insects and micro crustacea (Reilly and Mccarthy, 1993; Di Sabatino *et al*., 2000; Smith *et al*., 2001; Fairn *et al.,* 2008). Depending on the species, larval water mites tend to attach to their hosts from 4 days to 6 weeks (Ihle and McCreadie, 2003; Zawal, 2003b). The host provides nutrients to the larval mites and triggers high dispersal capability to the mite population (Zawal, 2003a).

* Author for correspondence

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Water mites are made up of 300 genera containing more than 5000 species but descriptions of their larvae and deutonymphs are rare found from the literature (Di Sabatino *et al*., 2000; Zawal, 2008). It has long been known that the host association of *Hydrachna* species is extended, that the protonymph (old terminology: nymphochrysalis, nymphophan) is spent attached to the host, and that the host groups for this genus are aquatic Coleoptera and aquatic Hemiptera (Soar and Williamson, 1925). The water mites of Hydrachnidiae have a cosmopolitan distribution and inhabits standing waters, in addition to aquatic hemipterans, it parasitizes aquatic coleopterans. The larvae of the water mites of the genus *Hydrachna* are observed as ectoparasites that parasitize on Noteridae, Dytiscidae, Heteroceridae, Hydrophilidae, Gyrinidae of Coleoptera (Davis and Brown, 1969; Biesiadka and Cichocka, 1994; Fairn *et al*., 2008; Hajizadeh and Hosseini, 2022), as well as Corixidae, Nepidae, Veliidae, Belostomatidae of Hemiptera (Davids, 1972; Hajizadeh and Hosseini, 2019; Zawal *et al*., 2013; Perez *et al.,* 2014; Abe *et al.,* 2015; Gerecke *et al.,* 2020) and they have strong selection power for their hosts preferring to attach to selected sites on the host's body, i.e. sternites and tergites of thorax and abdomen (Wainstein, 1980; Reilly and McCarthy, 1993; Biesiadka and Cichocka, 1994; Cichocka, 1995; Zawal, 2002, 2003a, b; Sánchez *et al*., 2015; Céspedes *et al*., 2019).

The present study is an attempt to highlight the host-parasite relationship of water scavenger beetle, *Hydrophilus* sp. and electric light bug, *Lethocerus* sp. with water mite larvae, *Hydrachna* sp. From the selected water body at the buffer zone of Kuldiha Wildlife Sanctuary Odisha and an aquatic body of Haldia industrial belt, West Bengal, India.

MATERIALS AND METHODS

Study area and study period: Water beetles belonging to the family Hydrophilidae and giant water bugs belonging to the family Belostomatidae were collected from Rissia dam, Kuldiha Wildlife Sanctuary, Odisha, and aquatic body of Haldia, West Bengal, India (Fig. 1). A total of six samplings in a gap of four months have been done, two of them from post-monsoon, another two from pre-monsoon and another two from monsoon season (December 2021 to August 2023).

Sample collection and fixation: A net with a mesh size of 0.5mm was used to collect specimens. The collected specimens were fixed and preserved in situ with ethyl alcohol 70 per cent solution. In the laboratory, they were cleaned with a small paint brush and each specimen was closely observed under microscope (Nikon SMZ 745T) for study of parasite larvae attached to different body parts. The site specific occurrences and the number of parasitic mites on the host body have been also recorded. Ectoparasites were separated from their host bodies and mounted in glycerine jelly for detailed morphological studies. All the morphometric measurements and photographs of parasites were made using Carl Zeiss Axiovert A1.Mat Inverted Advance Binocular Research Microscope.

RESULTS AND DISCUSSION

Description of parasite life stages: There are seven distinct stages in the life cycle of *Hydrachna* water mites, that are: i) eggs, ii) active larval stage, iii) parasitic larva, iv) quiescent nymphophan, v) nymph, vi) quiescent telescophan, and vii) adult (David Lou Kass, 1962). Free-living larvae emerge from the eggs. Following a brief period of free-swimming, the larvae become parasitic when they connect themselves to aquatic insects by the means of gnathosoma. They develop from the parasitic larvae to nymphophans that stay affixed to the host insects and undergo a metamorphosis process into nymphs during this period. The nymphs soon break off their sac like container and become visible as free living nymphs. After a short period, according to Crowell (1957), the nymph attaches to algae or any substrates, and a second pupal stage, quiescent teliphan, appears in which the final adult characteristics develop. Once the quiescent teliphan stage ends, the sexually mature adult beign to emerge.

Morphological description of quiescent nymphophan: The organism stop feeding after one to five weeks as a parasitic larva on its host, and enter a quiescent nymphophan (nymphochrysalid)

Table 1. Occurrences of water mite larvae *Hydrachna* sp. on Coleopteran and Hemipteran hosts

Month	Host no.		Infested host no.		Prevalence (%)		Parasite no.		Average intensity		Body location	
	Hy	Le	Hy	Le	Hy	Le	Hy	Le	Hy	Le	Hy	Le
December, 2021	8	0	1	0	12.5	-	1	0	1	-	Hind tarsus (right)	-
April, 2022	13	1	3	1	23	100	6	98	0.46	98	Foreleg coxa, pronotum, metasternal process	Scutellum, wings, prosternum, coxal region
August, 2022	2	0	0	0	0	-	0	0	0	-	-	-
December, 2022	9	0	0	0	0	-	0	0	0	-	-	-
April, 2023	9	0	2	0	22.2	-	4	0	0.44	-	Hind tarsus (left), foreleg coxa, prosternum	-
August, 2023	1	0	0	0	0	-	0	0	0	-	-	-

Hy – *Hydrophilus*, Le – *Lethocerus*

Table 2. Morphometric measurements of parasitic larvae (in µm) (n=5)

Characters	Range	Mean	SD
Total Body Length	682.17-2112.45	1418.98	616.15
Gnathosoma length	215.71-242.52	230.35	11.42
Idiosoma length	466.46-1869.93	1188.62	604.87
Gnathosoma width at sucker	70.46-72.61	71.29	0.86
Maximum width of Gnathosoma	121.36-141.60	130.82	7.99
Maximum width of idiosoma	227.38-685.78	431.86	177.11
Width of idiosoma at posterior most region	122.96-242.70	183.31	46.47
Eye length	30.07-48.04	39.70	7.30
Eye width	18.06-35.17	26.89	6.68
Total length of eye and anterior eye plate	45.15-80.58	64.02	14.23

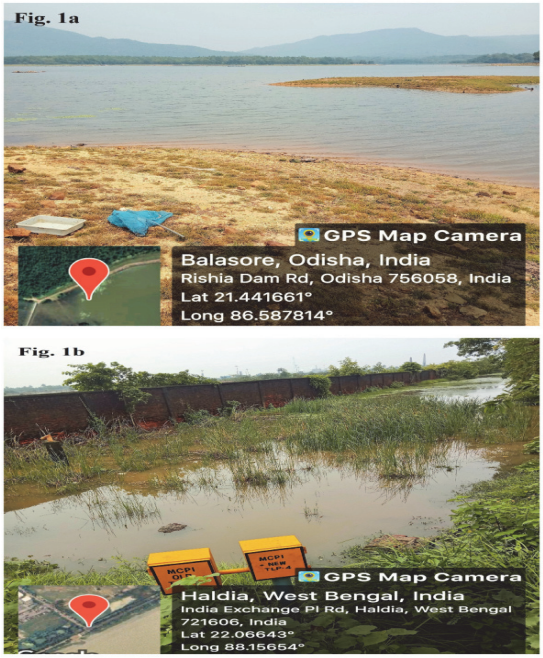

Fig. 1 The study area: a) Rissia Dam, Kuldiha WLS, Odisha and b) aquatic habitat at Haldia Industrial Belt, Purba Medinipur, West Bengal, India

stage of development, where in the larva shrink into its exoskeleton and forms a sac-like structure where metamorphosis takes place. By the use of gnathosoma, they eling to their host and cast off theor exoskeleton eventually revealing the growing nymphs inside. The nymph comes out of an opening in the exoskeleton and appear a free-living existence. These nymphochrysalids measured from 682.17 to 2112.45µm. in length and the external integuments of the larvae are ornamented with lateral stripes. Body appeard to be bottle shaped with pointed or rounded posterior end (Fig. 4).

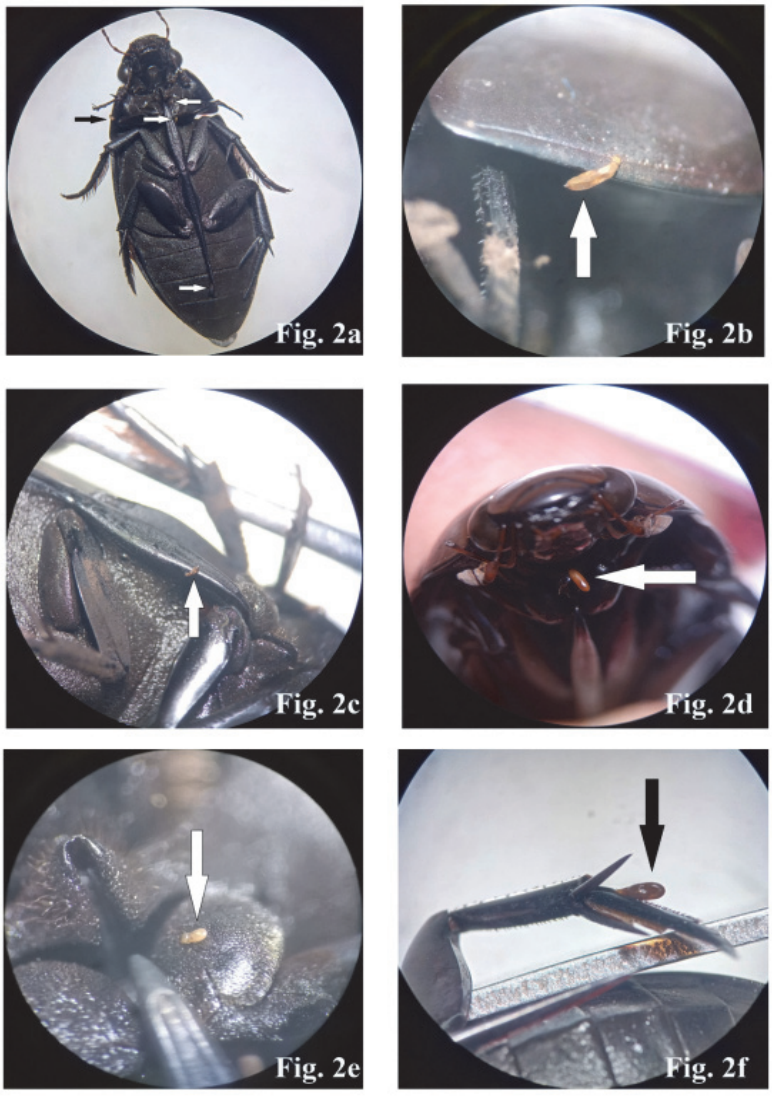

Fig. 2 a) *Hydrophilus* sp. with parasites *Hydrachna* sp.; b)-f) Different positions of *Hydrachna* larva on host (*Hydrophilus* sp.) body parts: b) edge of pronotum, c) anterior portion of metasternal process, d) raised portion of prosternum, e) another position of prosternum, and f) hind tarsus

Occurrences of water mites on host bodies: Water mite parasitism on aquatic insects hosts belonging to the family Hydrophilidae (Coleoptera) and Belostomatidae (Hemiptera) was investigated and they were all in resting stage (nymphochrysalis). A total of 42 beetle specimens of Hydrophilidae and one bug specimen of Belostomatidae were collected, among which six were infested with 11 parasites that were attached on the surfaces of the foreleg coxa, prosternum, pronotum, metasternal process, hind tarsus of the beetle body and one bug was infested with 98 parasites that were attached on dorsal side of wings and ventral side of head and thorax (Table 1, Figs. 2, 3).

Morphometric measurement: Total body length (gnathosoma length: 215.71-242.52µm and idiosoma length: 466.46-1869.93µm) ranges from 682.17-2112.45µm; maximum width of gnathosoma ranges from 121.36-141.60µm and minimum width of gnathosoma (sucker area) ranges from 70.46-72.61µm; maximum width of idiosoma ranges from 227.38 to 685.78µm and minimum width of idiosoma

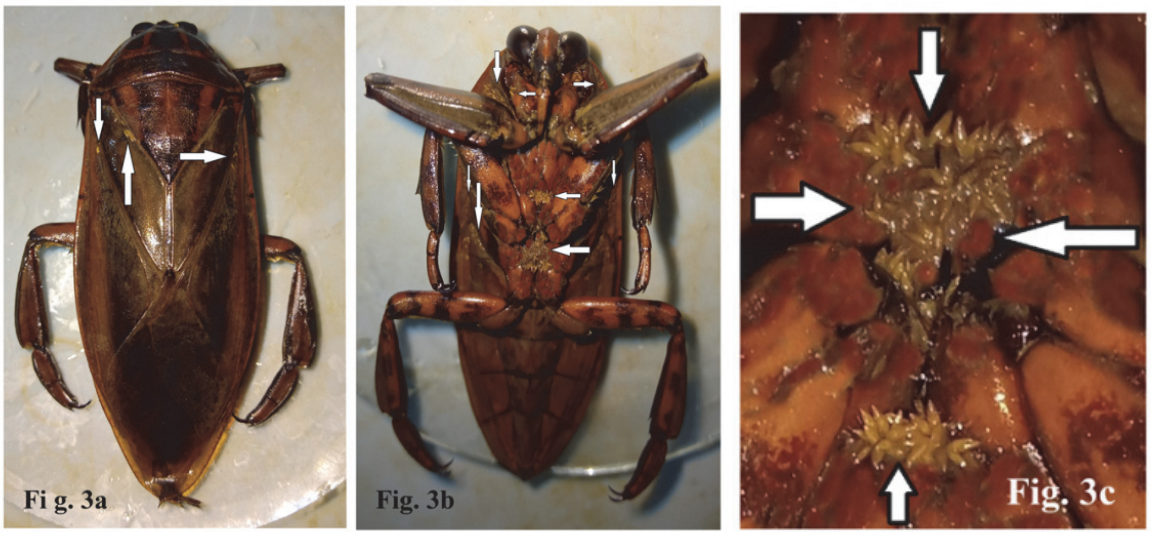

Fig. 3 *Lethocerus* sp. with parasites *Hydrachna* sp. a) Dorsal side: junction between scutellum and wing and on the wing (hemelytra), b) Ventral side: maximum parasites were found in the coxal region of mid and hind leg, some were found in prosternum and some were in the ventral side of hemelytra, and c) focused view of parasites in coxal portion

(posterior most region) ranges from 122.96 to 242.70µm; eye length ranges from 30.07-48.04µm; eye width ranges from 18.06 to 35.17µm; total length of eye with anterior eye plate ranges from 45.15 to 80.58µm (Table 2).

Preferences of water mites for host body parts: The prevalence of *Hydrachna* sp. on the head, prothorax, meso- and metathoraxes, abdomen, fore legs, mid legs and hind legs for six infested adult *Hydrophilus* sp. and one *Lethocerus* sp. were calculated (Table 1). In case of *Hydrophilus* sp., among 11 parasites, three were found in forelegs, three on hind legs, two on pronotum, one on prosternum, and two on metasternal process. In case of *Lethocerus* sp., 17 parasites were found on dorsal side (on wings) and 81 were found on ventral side (head and thorax) (Fig. 3). Preference for a particular attachment site on a host aquatic insect has been noted for the mites' larvae. Lanciani (1970) enumerated the attachment sites on several genera under the families Dytiscidae and Hydrophilidae. In this study, it was observed that maximum number of parasites were attached to the ventral parts of the beetles and minimum to the dorsal sides. This fact is also true for the family Belostomatidae.

Parasite load of a single beetle indicates that mites may be deliberately selecting unparasitized hosts or hosts with only a few parasites. Comparisons of the frequency distribution of the number of mites per host revealed that attachment was not random. Work done by Nielsen and Davids (1975) indicates that mites actively select the sites on a host. Data in Table 1 offer one explanation for such selectivity.

Preferences of water mites for season: The highest abundance of aquatic beetles and parasites was seen in pre-monsoon season and the prevalence was recorded (23 and 22.2% respectively for April 2022 and April 2023). Post-monsoon season shows the medium abundance of beetles and very few parasites and prevalence (12.5 and 0% respectively for December 2021 and December 2022). During monsoon season, although got very few beetles, but there were no parasites. On the other hand, one *Lethocerus*, that is called giant water bug (8cm long), shows the host for much higher parasite prevalence (100%) in pre-monsoon of 2022. Greatest parasitism rates were found in the spring and early summer, when the beetle and bug populations are high or increasing (Aiken and Wilkinson, 1985; Aiken, 1985a), affording the mite

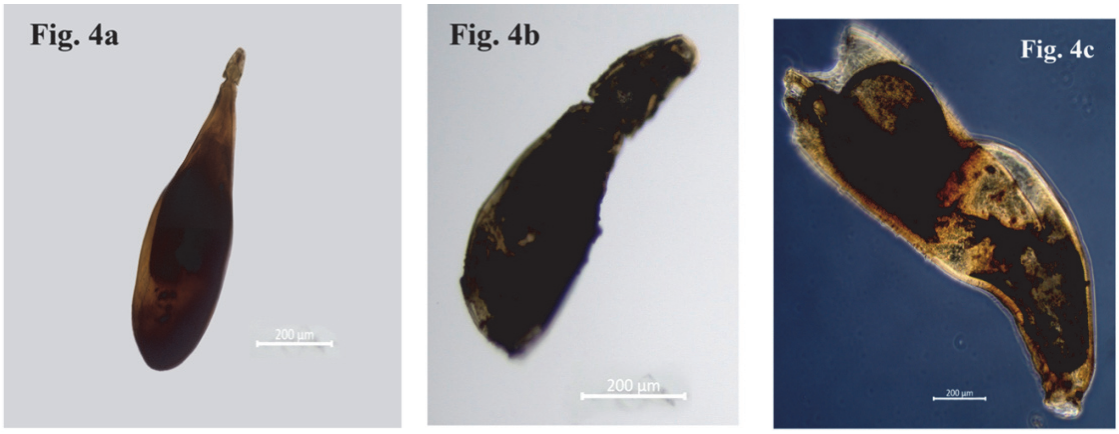

Fig. 4 Different positions of the isolated *Hydrachna* parasites: a) dorsal view with gnathosoma and idiosoma, b) lateral view with distinct gnathosoma and idiosoma, and c) lateral view with prominent sucker. Scale bars: 200µm.

Fig. 5 Microscopic view of: a) gnathosoma with sucker, four pairs of limb buds and internal structures, and b) position of eye and anterior eye plate. Scale bars: 50µm.

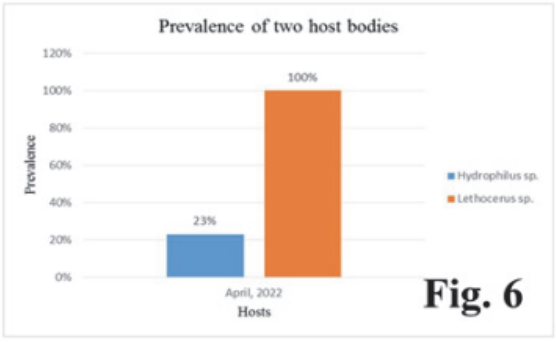

Fig. 6 Comparison of prevalence in water beetle, *Hydrophilus* sp. and giant water bug, *Lethocerus* sp.

higher numbers of potential hosts. When hosts are scarce in monsoon (July and August), most mites completed their larval growth and are in nonparasitic stages (Table 1). The study reveals the same fact that the higher parasitism (most of the hosts and parasites) was found in pre-monsoon season (April 2022 and 2023), lower in post-monsoon (December 2021 and 2022) and none in monsoon (August 2022 and 2023) season.

Infection intensity and prevalence: The infection prevalence of this study is somehow low in *Hydrophilus* sp. of Coleoptera and much higher in *Lethocerus* sp. of Hemiptera (Fig. 4, Table 1). Previous studies showed that the host specificity varies according to the species considered: *Hydrachna geographica* and *H. inermis* were found only on Dytiscidae, *Hydrachna leegei* and *H. incognita* only to species of Hydrophilidae whereas *Hydrachna crassipalpis* parasitizes beetles belonging to the both families. The number

of larvae, the intensity of infection and the prevalence of parasitism were higher in Dytiscidae than in Hydrophilidae of Coleoptera. This was the result of the different strategy of infection (Zawal, 2002). But this study reveals the infection of *Hydrachna* larvae on *Hydrophilus* sp. of Hydrophilidae, along with *Lethocerus* sp. of Belostomatidae.

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Description of nine new species of the genus *Sycophila* Walker (Chalcidoidea, Eurytomidae) from Kerala, India

Shilpa K. Satheesan and Shreevihar Santhosh*

Systematic Entomology Laboratory, Malabar Christian College, Kozhikode, Affiliated to University of Calicut, Kozhikode, India.
Email: shilpasatheesan175@gmail.com; sant@mccclt.ac.in

ABSTRACT: Nine new species *viz., Sycophila drupacea* **sp. nov**., *S. arnottiana* **sp. nov**., *S. religiosa* **sp. nov.,** *S. virens* **sp. nov**., *S. infectoria* **sp. nov**., *S. wayanadensis* **sp. nov**., *S. batheri* **sp. nov**., *S. tinctoria* **sp. nov**. and *S. gibbose* **sp. nov.** were described and reported from five different fig species, *Ficus drupacea*, *F. arnottiana*, *F. religiosa, F. virens* and *F, tinctoria*, from Wayanad regions of Kerala, India.
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KEY WORDS: Fig wasp, Hymenoptera, taxonomy, Wayanad, non-pollinator

INTRODUCTION

The genus *Sycophila* was described for the first time by Walker in 1871 with *S. decatomoides* Walker, 1871 reared from the fruits of *Ficus benghalensis* L. as the type species. The major publication on fig associated *Sycophila* in India was Joseph and Abdurahiman, 1968, describing six *Sycophila* species. An extensive study of this genus of oriental region was published by Narendran in 1994. There are around 119 species of *Sycophila* in the world (Noyes, 2019) out of which 31 are from oriental region (Noyes, 2019). *Sycophila* species that are associated with figs are around 20 in number, in the world; 11 in oriental region and 8 in India (Noyes, 2019). Although more than 100 different host species are reported for *Sycophila* (Noyes, 2019), including parasitoids on species of Hymenoptera, Diptera, Lepidoptera and Hemiptera, genus *Sycophila* reared from syconia of *Ficus* are actually inquilines in Epichrysomallinae galls (Lotfalizadeh *et al*., 2007). In this study, new species of genus *Sycophila,* associated with five different *Ficus* species namely *F. drupacea*, *F. arnottiana*, *F. religiosa*, *F. virens* and *F. tinctoria,* are described from Kerala, India.

MATERIALS AND METHODS

Nearly mature D-phase figs (syconia) of different *Ficus* sp. were collected during 2019 – 2021 and brought them to the laboratory and divided the figs into parts and observed the fig wasp's emergence. Later the fig wasps were transferred to alcohol (70 %), labelled and then the specimens were transferred to alcohol (90 %) for storage. The specimens were card mounted on triangular cards (14×5mm) after passing through alcohol series (95 and 100 per cent) and then drying in HMDS (hexamethyldisilazane) solution. The labelled and card mounted specimens were later studied, identified and described using LEICA M205 stereo zoom microscope and imaged with an attached

* Author for correspondence

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LEICA DFC 2900 digital camera. Measurements were obtained using Leica LAS software (Leica Application Suite V3.80) and images taken at varying focal depths were stacked using LAS. Final illustrations were improved for contrast and brightness using Adobe® Photoshop® CS5 (Version 12.0 x 64) software. The type species is deposited in the entomological collections of Systematic Entomology Laboratory, Malabar Christian College, Kozhikode and will be deposited also in the 'National Zoological Collection' of Zoological Survey of India, Western Ghat Regional Centre, Kozhikode (ZSIK).

The general abbreviations of the terms are as follows, POL – distance between posterior ocelli; OOL – distance between posterior ocellus and eye margin; SMV – submarginal vein; MV – marginal vein; STV – stigma vein; PMV – post marginal vein; T_1 – T_6 – gaster tergal segments. All lengths are measured medially and widths are measured at the maximum wider area, unless mentioned otherwise. The total range are taken from all specimens while the ratios mentioned are the mode values taken from multiple measurements from a single (a holotype and single paratype male) specimens.

RESULTS AND DISCUSSION

Key to species of fig associated *Sycophila* of India, modified from Narendran, 1994

1 Antenna pedicel as long as F1 or shorter than F1 2

– Antenna pedicel always longer than F1.....12

2 Pedicel as long as F1 3

– Pedicel shorter then F1.....6

3 POL < OOL; MV d" 2× STV 4

– POL > OOL; MV > 2× STV 5

4 Median length of pronotum distinctly shorter than half the median length of scutellum; MV 2× STV; SMV having 12 bristles in row .....***S. tinctoria* sp. nov.**

– Median length of pronotum distinctly longer than half the median length of scutellum; MV 1.3× STV; SMV having 9 bristles in row .....***S. gibbosa* sp. nov.**

5 Anterior width of head 1.35× length; pterostigmal area 2× as wide as long; MV 2.75× STV; T4 longest ..... ***S. dharwarensis*** (Joseph & Abdurahiman)

– Anterior width of head 3.8× length; pterostigmal area a little longer than wide; MV 3× STV; T3 longest.....***S. fici*** (Joseph)

6 F1 distinctly longer than pedicel (F1 >1.5× pedicel); median length of mesoscutum and scutellum subequal; T3 or T4 longest 7

– F1 a little longer than pedicel; median length of mesoscutum longer or shorter than the median length of scutellum; T4 longest 10

7 POL almost equal to OOL; F1 ≥ 2× pedicel; T3 longest.....8

– POL ≥ 1.5× OOL; F1< 2× pedicel; T3 or T4 longest 9

8 Anterior width of head 1.45× length; length of F1 > 2× length of pedicel; pterostigmal area wider than long; propodeum with a median smooth fovea, sides weakly reticulate ……***S. karnatakensis*** (Joseph & Abdurahiman)

– Anterior width of head 1.7× length; length of F1 2× length of pedicel; pterostigmal area longer than wide; median area of propodeum concave with narrow median groove interrupted by a number of transverse carinae .....***S. taprobanica*** (Westwood)

9 Anterior width of head 1.4× length; pterostigmal area 1.3× wide as long; T4 longest; POL 1.8× OOL; propodeum with a median fovea, sides of fovea punctate with large number of macrochaetae on either side .....***S. pilosa*** (Joseph & Abdurahiman)

– Anterior width of head 1.18× length; pterostigmal area wider than long; T3 longest; POL 1.5× OOL; propodeum with broad depressed fovea, its bounded laterally by carinae, surface of fovea smooth and shiny ..... ***S. decatomoides*** Walker

10 Median length of mesoscutum distinctly longer than the median length of scutellum; SMV having 20 bristles in a row; pterostigmal area longer than wide ..... ***S. robusta*** (Joseph & Abdurahiman)

6 Median length of mesoscutum little shorter than the median length of scutellum; SMV with less than 15 bristles in a row; pterostigmal area wider than long 11

11 Anterior width of head 1.45× length; POL 2× OOL; SMV with 14 bristles in a row; propodeum with a median smooth fovea, bounded by lateral carinae, anteriorly delimited by oblique cross carinae..... ***S. drupacea*** **sp. nov.**

– Anterior width of head 1.7× length; POL 1.2× OOL; SMV with 10 bristles in a row; propodeum with a median fovea, bounded by lateral carinae, surface of the fovea rugulose and imbricate medially .....***S. virens*** **sp. nov.**

12 POL < OOL distance; Anterior width of head 1.6× length.....***S. infectoria*** **sp. nov.**

– POL > OOL distance; Anterior width of head ≥ 1.6× length 13

13 Eye length less than 2× malar space; POL < 2× OOL 14

– Eye length more than or equal to 2× malar space; POL > 2× OOL 16

14 POL 1.5× OOL; scape 2.4× pedicel; Anterior width of head1.4× length ..... ***S. benghalensis*** (Joseph & Abdurahiman)

– POL almost equal to OOL; Other characters also different 15

15 Pronotum distinctly shorter than half the median length of scutellum; T5 longest .....***S. batheri*** **sp. nov.**

– Median length of pronotum longer than the median length of scutellum; T3 longest .....***S. wayanadensis*** **sp. nov.**

16 Median length of pronotum shorter than the median length of scutellum; T4 longest; MV 2× STV; SMV with 15 bristles in a row .....***S. arnottiana*** **sp. nov.**

– Median length of pronotum longer than the median length of scutellum; T5 longest; MV 1.6× STV; SMV with 5 bristles in a row .....***S. religiosa*** **sp. nov.**

SYCOPHILA Walker

Sycophila Walker, 1871. Type species *Sycophila decatomoides* Walker.

Decatomidea Ashmead, 1888. Type species *Decatomidea xanthochroa* Ashmead.

Eudecatoma Ashmead, 1888. Type species *Decatoma batatoides* Ashmead.

Isanisa Walker, 1875. Type species: *Sycophila decatomoides* Walker, by monotypy.

Pseudisa Walker, 1875. Type species: *Pseudisa smicroides* Walker, by monotypy.

Tineomyza Rondani, 1872. Type species: *Tineomyza pistacina* Rondani, by monotypy.

Diagnosis. Antennal formula 11153; MV enlarged, with big stigma, with dark brown shading below MV, PMV absent or short, hind femur with enlarged in the middle, gaster petiolate and mostly compressed laterally; propodeum with cris-cross carina medially adhering to the anterior margin.

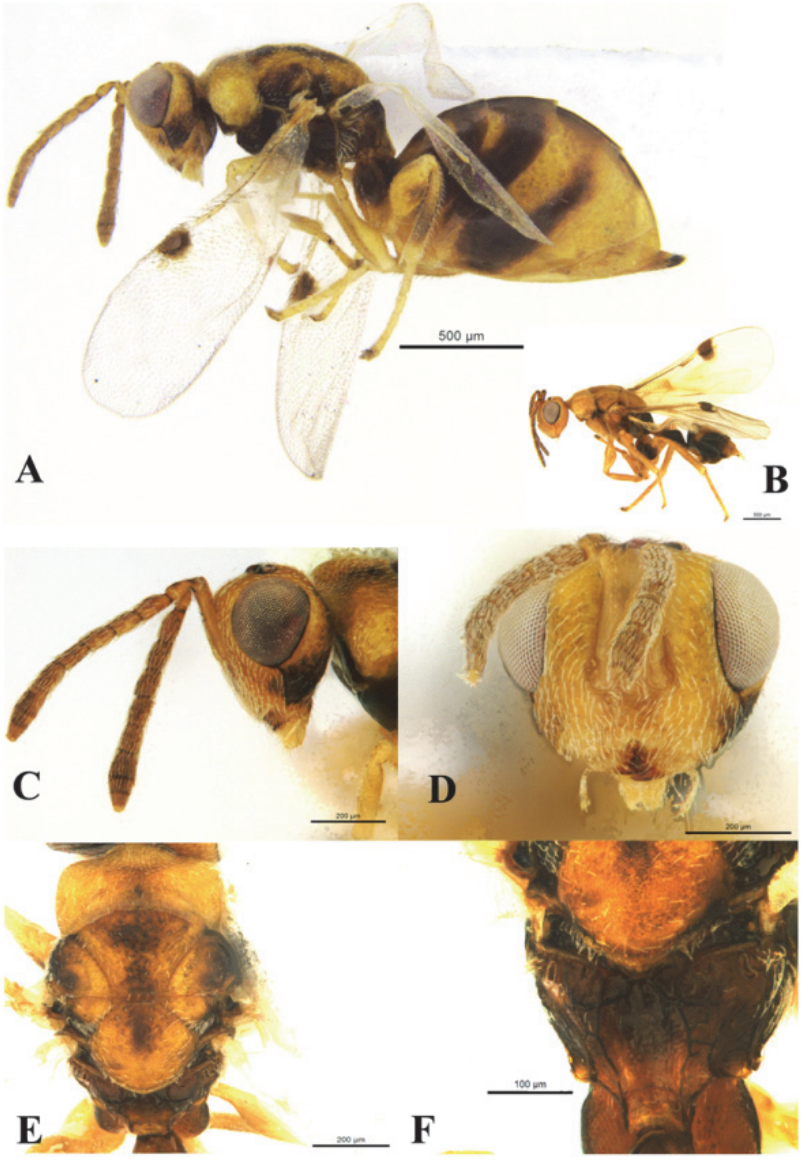
Sycophila drupacea sp. nov.

LSIDurn:lsid:zoobank.org:act:815604E9-5E9B-4551-9A96-C57648FCFF51

(Plate 1, Figs. 1A – F)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, 11.82098 N 76.09564 E, 19.iv.2019, collected by Shilpa K. Satheesan, ex *Ficus*

Plate 1. *Sycophila drupaceae* **sp. nov.**



Figs. 1A–1F. *Sycophila drupacea* **sp. nov.** Holotype ♀ 1A. Habitus, lateral view; 1B. ♂ Habitus, lateral view; 1C. Head, lateral view; 1D. Head, frontal view; 1E. Thorax, dorsal view; 1F. Propodeum, dorsal view

drupacea Thunb., Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Paratype: Male specimen with same collection data as holotype.

Diagnosis: Length 2.7mm. General colour yellow. Head width (anteriorly) 1.45× distance between front ocellus and clypeal margin; POL 1.9× OOL. Eyes as long as wide. F1 longer than pedicel. Median length of pronotum distinctly shorter than half the median length of scutellum. T4 longest.

Description: **Female**. *Holotype.* Length 2.7mm. General colour yellow; lateral lobe of mesoscutum and propodeum, mostly black. The posterior 3/4 of T1, T2, T3 and lateral side of T4 black. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infuscation; Antennal scape and pedicel yellow and funicle brownish yellow.

Head (Figs. 1C, D): Head alveolate with pubescence; Head width (anteriorly) 1.45× distance between front ocellus and clypeal margin; POL 1.9× OOL. Eyes as long as wide. Malar space 0.135mm. Scrobe deep, not reaching front ocellus; Scrobe is 0.015mm from front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11153; scape reaching front ocellus, not reaching level of vertex; length of scape 3.45× length of pedicel; F1 longer than pedicel. Flagellum plus pedicel is 0.63mm. Pedicel is 1.7 times as long as broad while F1 is 1.6 times as long as broad. Flagellar segments stout-filiform.

Mesosoma (Figs. 1A, E, F): Dorsum of thorax with irregular areolae sculpturing and pubescent; Median length of pronotum distinctly shorter than half the median length of scutellum; mesoscutum little shorter than scutellum; scutellum as wide as long. Notauli complete. Propodeum declining sharply, with a median shallow depression, which is carinate on sides, anteriorly delimited by oblique cross carinae diverging from the middle of base. Surface of depression rugulose with short carinae at lateral third; area lateral to fovea mostly rugulose with a short carinae or short costula, plica present, spiracle bean shaped having setae rising from black pits to the lateral side. Forewing length 2.4× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having 14 bristles in row; MV broad and distinctly longer than STV. MV 1.48 times STV. Rusty infuscation extends beyond the posterior margin of MV. The infuscation is wider than long. Hind coxa with 14 bristles on ventral side; Hind tibia with a series of setae and 2 prominent spurs at apex, tarsal segments in ratio 9:5:4:3 :4.

Metasoma (Fig. 1A): Petiole as long as broad but distinctly less than length of hind coxa; gaster strongly compressed, its surface smooth and shiny; T4 longest. Gaster little longer than thorax. Hypopygium ending shortly before middle of gaster body.

Male: Length 2.8mm. Similar to female in general except in having: antenna with four funicular segments and one club with four fused segments; gaster small with sooty brown to black patches; petiole longer than gaster with a characteristic hump dorsally.

Host: Syconia galls of *F. drupacea* Thunb.

Etymology: 'drupacea' derived from the name of host plant *F. drupacea*

Discussion: *S. drupacea* **sp. nov.** differs from all other *Sycophila* species' in having these combination of characters – First funicular segment (F1) a little longer than pedicel; Median length of mesoscutum little shorter than the median length of scutellum; pterostigmal area wider than long. Anterior width of head 1.45× length between front ocellus and clypeal margin; POL 2× OOL; SMV with 14 bristles in a row; propodeum with a median smooth fovea, bounded by lateral carinae, anteriorly delimited by oblique cross carinae.

S. drupacea **sp. nov**. is similar to *S. virens* **sp. nov**., in having similar shorter pedicel than F1; length ratios of scape and pedicel; length of scutellum larger than length of mesoscutum but differs in *S. drupacea* sp. nov. having anterior width of head 1.45× its length (*S. virens* **sp. nov**. having width 1.7× length); POL 2× OOL (POL 1.2× OOL in *S. virens* **sp. nov**.) and having propodeal

fovea smooth (fovea imbricate and rugulose in *S. virens* **sp. nov**.).

Remarks: Colour variation on the propodeum from black to dark rusty brown is seen. Dark patches on thorax are also seen in lighter shades.

***Sycophila arnottiana* sp. nov.**

LSIDurn:lsid:zoobank.org:act:0C29FBDD-9D63-461B-B336-5C83B5AE3B37

(Plate 2, Figs. 2A – F)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, Lakkidi 11°30.7962 N, 76°01.0892 E, 23.iv.2019, collected by Shilpa K. Satheesan, ex *Ficus arnottiana* (Miq.) Miq., Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Paratype: Male specimen with same collection data as holotype.

Diagnosis: Length 1.4mm. General colour yellowish brown or honey brown. Head width (anteriorly) 1.44× distance between front ocellus and clypeal margin; POL 2.8× OOL. Eyes as long as wide. Pedicel is longer than F1. Median length of pronotum shorter than half the median length of scutellum. T4 longest.

Description: **Female**. Length 1.4mm. General colour yellowish brown or honey brown. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining pterostigma; Antenna yellowish brown.

Head (Figs. 2A, E): Head width (anteriorly) 1.44× distance between front ocellus and clypeal margin; POL 2.8× OOL. Eyes as long as wide. Malar space 0.110mm. Scrobe deep, almost reaching front ocellus; Scrobe is 0.070mm from front ocellus; Para scrobal space smooth; Median ocellus red and lateral ocelli white; eyes glaborous. clypeal margin bilobed; Malar groove complete. Malar groove moderately carinate at the distal half Antennal formula 11153; scape not reaching front ocellus; length of scape 2.8× length of pedicel; pedicel is longer than F1. Pedicel is 2.2 times as long as broad while F1 is 1.75× as broad as long.

Mesosoma (Figs. 2C, D, F): Pronotum with faint or weak areola extending as a weak rugulae laterally. Mesoscutum and scutellum imbricate with scattered white setae. Median length of pronotum shorter than half the median length of scutellum; mesoscutum shorter than scutellum; scutellum as wide as long. Notauli complete, Propodeum declining sharply, with a broad depressed median fovea bounded laterally by carinae, surface of fovea smooth; area lateral to fovea rugulose with a plica present, spiracle bean shaped having five setae rising from black pits to the lateral side. Forewing length 2.3× length of SMV; PMV absent; costal cell (CC)with minute pilosity; basal 1/3 of forewing bare. SMV having 15 bristles in row; MV broad and distinctly longer than STV. STV 2× MV. Pterostigma a wider than long. Hind tibia with a series of setae and one prominent bifid spur at apex, tarsal segments in ratio 3:2:3.3:2.

Metasoma (Fig. 2A): Petiole distinctly less than length of hind coxa; gaster strongly compressed, its surface smooth and shiny; T4 longest. Gaster longer than thorax. Hypopygium ending before middle of gaster body.

Male: Similar to female in general except in having: antenna with four funicular segments and one club with four fused segments; gaster small with yellowish colour; petiole longer than gaster.

Host: Syconia galls of *F. arnottiana* (Miq.) Miq.

Etymology: 'arnottiana' derived from the name of host plant *F. arnottiana*

Discussion: *S. arnottiana* **sp. nov**. differs from all other *Sycophila* species' in having these combination of characters – Antenna pedicel always longer than F1; Median length of pronotum shorter than the median length of scutellum; T4 longest; MV 2× STV; SMV with 15 bristles in a row. Eye length 2.1× malar space; POL 2.8× OOL; Anterior width of head 1.44× length between front ocellus and clypeal margin.

S. arnottiana **sp. nov**. is similar to *S. religiosa* **sp. nov**. in having longer pedicel than F1; similar eye length to malar space ratio, POL – OOL ratio,

head length to width ratio but differs in having length of pronotum shorter than scutellum in *S. arnottiana* **sp. nov**. where as in *S. religiosa* sp. nov. length of pronotum is longer than scutellum; MV 2× STV and SMV with 15 bristles in a row in *S. arnottiana* **sp. nov**. whereas MV 1.6× STV and SMV with 5 bristles in a row in *S. religiosa* **sp. nov**.; In *S. arnottiana* **sp. nov**. fourth tergite is the longest whereas in *S. religiosa* **sp. nov**. fifth tergite is longest; propodeal median fovea smooth, slightly rugulose in *S. arnottiana* **sp. nov**. whereas propodeal median fovea is imbricate with carinae in *S. religiosa* **sp. nov**.

***Sycophila religiosa* sp. nov.**

LSIDurn:lsid:zoobank.org:act:CCBEB4FC-C03C-47BF-A478-64DCD965814E

(Plate 2, Fig. 2G – L)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, 11.71986N 76.32443E, 14.i.2019, collected by Shilpa K. Satheesan, ex *F. religiosa* L. Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Paratype: Male specimen with same collection data as holotype.

Diagnosis: Length 1.6mm. General colour yellowish brown or honey brown. Head width (anteriorly) 1.4× distance between front ocellus and clypeal margin; POL 2.8× OOL. Eyes as long as wide. Pedicel is longer than F1. Median length of pronotum longer than half the median length of scutellum; mesoscutum shorter than scutellum. T5 longest.

Description: Female *Holotype*. Length 1.6mm. General colour yellowish brown or honey brown. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining pterostigma; Antenna yellowish brown.

Head (Figs. 2G, K): Head width (anteriorly) 1.4× distance between front ocellus and clypeal margin; POL 2.8× OOL. Eyes as long as wide. Malar space 0.110mm. Scrobe not reaching front ocellus; Scrobe is 0.07mm from front ocellus; Para scrobal space smooth; Median ocellus and lateral ocelli white; eyes glaborous. clypeal margin bilobed; Malar groove complete. Malar groove moderately carinate at the distal half Antennal formula 11153; scape not reaching front ocellus; length of scape 2.7× length of pedicel; pedicel is longer than F1. Pedicel is 2.2 times as long as broad while F1 is 1.75× as broad as long.

Mesosoma (Figs. 2I, J, L): Pronotum with weak areola extending as a weak rugulae laterally. Mesoscutum strigulated anteriorly posterior mesoscutum and scutellum imbricate with scattered white setae. Median length of pronotum longer than half the median length of scutellum; mesoscutum shorter than scutellum; scutellum wider than long. Notauli complete, Propodeum declining sharply, with a broad depressed median fovea bounded laterally by carinae, surface of fovea imbricate rugulose; area lateral to fovea with numerous carinae, spiracle bean shaped. Forewing length 2.23× length of SMV; PMV absent; costal cell (CC)with minute pilosity; basal 1/3 of forewing bare. SMV having 5 bristles in row; MV broad and distinctly longer than STV. MV 1.6 × STV. Pterostigma a wider than long. Hind tibia with a series of setae and one prominent bifid spur at apex, tarsal segments in ratio 13:7:5:6:4.

Metasoma (Fig. 2G): Petiole distinctly less than length of hind coxa; gaster strongly compressed, its surface smooth and shiny; T5 longest. Gaster longer than thorax. Hypopygium ending before middle of gaster body.

Male: Similar to female in general except in having: antenna with four funicular segments and one club with three fused segments; gaster small with yellowish colour; petiole longer than gaster.

Host: Syconia galls of *F. religiosa* L.

Etymology: 'religiosa' derived from the name of host plant *F. religiosa*.

Discussion: *S. religiosa* **sp. nov**. is similar to *S. arnottiana* **sp. nov**. but differs from it and all other *Sycophila* species' in having these combination of characters – Antenna pedicel always longer than F1; Median length of pronotum longer than the median length of scutellum; T5 longest; MV 1.6× STV; SMV with 5 bristles in a row; propodeal

median fovea imbricate rugulose; Eye length 2.1× malar space; POL 2.8× OOL; Anterior width of head 1.41× length between front ocellus and clypeal margin.

***Sycophila virens* sp. nov.**

LSIDurn:lsid:zoobank.org:act:7D29C166-4E27-4E17-80EF-F0765A324AD8

(Plate 3, Figs. 3A – E)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, 11.887N 76.0687E, 18.ii.2021, collected by Shilpa K. Satheesan, ex *F. virens* Aiton. Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Diagnosis: Length 2mm. General colour yellow. Head width (anteriorly) 1.7× distance between front ocellus and clypeal margin, with POL 1.2× OOL. Eyes slightly longer than wide. F1 longer than pedicel. Median length of pronotum distinctly shorter than half the median length of scutellum. T5 slightly longer than T4.

Description: **Female**. *Holotype.* Length 2mm. General colour yellow; lateral lobe of mesoscutum and propodeum, mostly black. The posterior 3/4 of T1, T2, T3 and lateral side of T4 black. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infuscation; Antennal scape and pedicel yellow and funicle brownish yellow.

Head (Figs. 3B, C): Head alveolate with pubescence; Head width (anteriorly) 1.7× distance between front ocellus and clypeal margin, with POL 1.2× OOL. Eyes slightly longer than wide. Malar space 0.112mm. Scrobe deep, almost reaching front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11153; scape reaching front ocellus; length of scape 3.3× length of pedicel. F1 longer than pedicel. Pedicel is 1.7 times as long as broad while F1 is 1.8 times as long as broad. Flagellar segments stout-filiform.

Mesosoma (Figs. 3C, D, E): Pronotum faintly coriaceous; Median length of pronotum distinctly shorter than half the median length of scutellum; mesoscutum little shorter than scutellum; scutellum slightly wider than long. Notauli complete. Propodeum declining, with a median area carinate on sides. Surface of depression rugulose; area lateral to fovea mostly rugulose with a short carinae or short costula, plica present, spiracle bean shaped having setae rising from black pits to the lateral side. Forewing length 2.2× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having 10 bristles in row; MV broad and distinctly longer than STV. MV 1.27 times STV. Rusty infuscation extends beyond the posterior margin of MV. The infuscation is wider than long. Hind tibia with a series of setae with prominent spurs at apex, tarsal segments in ratio 15:8:5:4:5.

Metasoma (Fig. 3A): Petiole as length 1.67× times wide; gaster strongly compressed, its surface smooth and shiny; T5 slightly longer than T4. Gaster little longer than thorax. Hypopygium ending shortly before middle of gaster body.

Male: Unknown; not represented in collection.

Host: Syconia galls of *F. virens* Aiton.

Etymology: 'virens' derived from the name of host plant *F. virens*.

Discussion: *S. virens* **sp. nov**. is similar to *S. drupacea* **sp. nov**. but differs from it and all other *Sycophila* species' in having these combination of characters – First funicular segment (F1) of antenna a little longer than pedicel; Median length of mesoscutum little shorter than the median length of scutellum; pterostigmal area wider than long; anterior width of head 1.7× length between front ocellus and clypeal margin; POL 1.2× OOL; SMV with 10 bristles in a row; propodeum with a median fovea imbricate and rugulose, bounded by lateral carinae, anteriorly not delimited by oblique cross carinae.

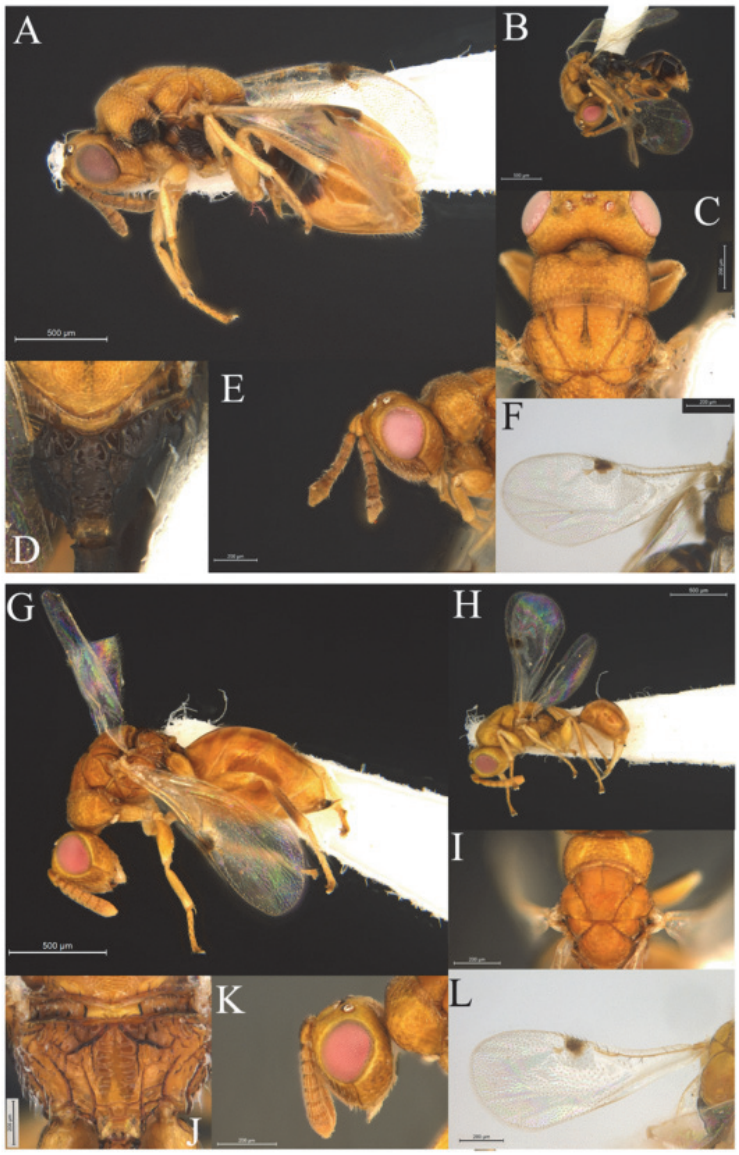
***Sycophila infectoria* sp. nov.**

LSIDurn:lsid:zoobank.org:act:89EE55C3-8CB8-4EE7-9779-5B33A48B58A3

(Plate 3, Figs. 3F – J)

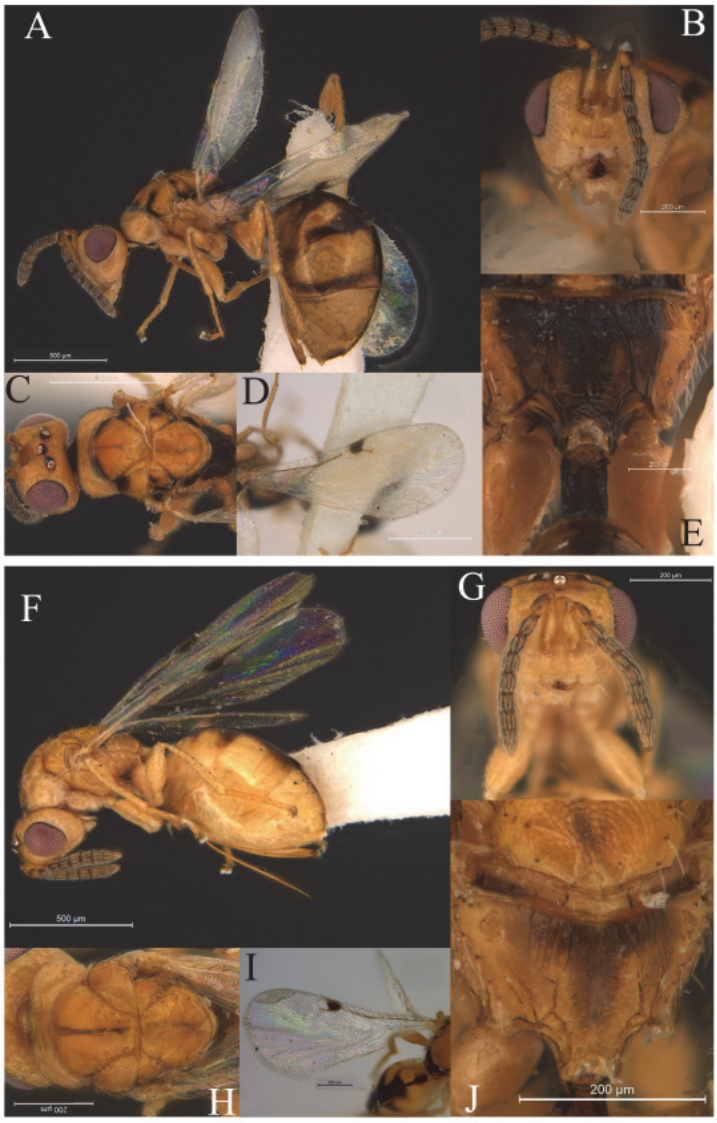
Type material. Holotype ♀: INDIA, Wayanad, Kerala, 11.887N 76.0687E, 18.ii.2021, collected by Shilpa K. Satheesan, ex *Ficus virens* Aiton.,

Plate 2. *S. arnottiana* **sp. nov.** and *S. religiosa* sp. nov.



Figs. 2A–2F. *Sycophila arnottiana* **sp. nov.** Holotype ♀ 2A. Habitus, lateral view; 2B. ♂ Habitus, lateral view; 2C. Thorax, dorsal view; 2D. Propodeum, dorsal view; 2E. Head, lateral view; 2F. Fore wing
Figs. 2G–2L. *Sycophila religiosa* **sp. nov.** Holotype ♀ 2G. Habitus, lateral view; 2H. ♂ Habitus, lateral view; 2I. Thorax, dorsal view; 2J. Propodeum, dorsal view; 2K. Head, lateral view; 2L. Fore wing

Plate 3. *S. virens* **sp. nov.** *and S. infectoria* sp. nov.



Figs. 3A–3E. *Sycophila virens* **sp. nov.** Holotype ♀3A. Habitus, lateral view; 3B. Head, frontal view; 3C. Thorax, dorsal view; 3D. Fore wing; 3E. Propodeum, dorsal view
Figs. 3F–3J. *Sycophila infectoria* **sp. nov.** Holotype ♀3F. Habitus, lateral view; 3G. Head, frontal view; 3H. Thorax, dorsal view; 3I. Fore wing; 3J. Propodeum, dorsal view

Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Diagnosis: Length 1.3mm. General colour yellow. Head width (anteriorly) 1.6× distance between front ocellus and clypeal margin, with POL 0.73× OOL. Eyes 1.2× longer than wide. Pedicel longer than F1. Median length of pronotum distinctly shorter than half the median length of scutellum. T4 longest.

Description: Female. *Holotype.* Length 1.3mm. General colour yellow. The median portion of T1, T2, T3 and T4 black. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infuscation; Antennal scape and pedicel yellow and funicle brownish yellow.

Head (Fig. 3F, G): Head weakly coriaceous with pubescence; Head width (anteriorly) 1.6× distance between front ocellus and clypeal margin, with POL 0.73× OOL. Eyes 1.2× longer than wide. Malar space 0.115mm. Scrobe deep, not reaching front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11153; scape not reaching front ocellus; scape length is 2.2× length of pedicel. Pedicel longer than F1. Pedicel is 1.8 times as long as broad while F1 is 1.32 times as long as broad. Flagellar segments stout-filiform; Inter-torular distance 1.3× each torulus width.

Mesosoma (Figs. 3H, I, J): Pronotum faintly coriaceous; Median length of pronotum distinctly shorter than half the median length of scutellum; mesoscutum little longer than scutellum; scutellum slightly wider than long, with four pair of setae. Notauli complete. Propodeum declining, with a median area carinate on sides on the posterior half. Surface of depression wrinkled and glaborous; area lateral to fovea mostly smooth, pale yellowish with small brown shades at the anterior part mostly in the median area; an inverted Y shaped carinae lateral side just above the neck of the propodeum, laterally; spiracle bean shaped having setae rising from black pits to the lateral side. Forewing length 2.12× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having 10 bristles in row; MV broad and distinctly longer than STV. MV 1.5 times STV. Rusty infuscation extends beyond the posterior margin of MV. The infuscation is longer than wide. Hind tibia with a series of setae with prominent spur at apex, tarsal segments in ratio 4:3:2:1:2.

Metasoma (Fig. 3F): Petiole as long as wide; gaster strongly compressed, its surface smooth and shiny; T4 longest. Gaster longer than thorax.

Male: Unknown; not represented in collection.

Host: Syconia galls of *F. virens* Aiton.

Etymology: 'infectoria' derived from the synonymised name of host *F. arnottiana*.

Discussion: Antenna pedicel always longer than F1; POL 0.73× OOL distance; Anterior width of head 1.6× length; Eyes 1.2× longer than wide; scape length is 2.2× length of pedicel; median length of pronotum distinctly shorter than half the median length of scutellum; Propodeal fovea wrinkled and glaborous; an inverted Y shaped carinae just above the neck of the propodeum, laterally. MV 1.5 times STV. T4 longest.

S. infectoria **sp. nov**. is different from all the other *Sycophila* species with above mentioned combination of characters. It shares similar character with *S. benghalensis* in having antennal pedicel always longer than F1; median length of pronotum distinctly shorter than half the median length of scutellum and differs from it in having these combination of characters, like, POL 0.73× OOL distance (POL 1.5× OOL in *S. benghalensis*) ; Anterior width of head 1.6× length (head width is 1.4× length in *S. benghalensis*); scape length is 2.2× length of pedicel (2.44× in *S. benghalensis*); propodeum with inverted Y shaped carinae just above the neck of the propodeum (propodeum without inverted Y shaped carinae in *S. benghalensis*).

Sycophila wayanadensis sp. nov.

LSIDurn:lsid:zoobank.org:act:37F680E1-9D8B-44F2-A18A-C0EF9C2B800B

(Plate 4, Figs. 4A – E)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, 11.887N 76.0687E, 18.ii.2021, collected by Shilpa K. Satheesan, ex *F. virens* Aiton. Deposited

in Zoological Survey of India, Western Ghats Regional Centre, Calicut

Paratype: Male specimen with same collection data as holotype.

Diagnosis: Length 1.4mm. Brownish yellow colour thorax, head; pale yellow legs except coxa; later side of gens below the eye, lateral lobe of mesoscutum and propodeum, hind coxa black. Head width (anteriorly) 1.4× distance between front ocellus and clypeal margin, with POL 1.02× OOL. Eyes slightly longer than wide. F1 shorter than pedicel. Median length of pronotum longer than median length of scutellum. T3 longest.

Description: **Female**. *Holotype.* Length 1.4mm. Brownish yellow colour thorax, head; pale yellow legs except coxa; later side of gens below the eye, lateral lobe of mesoscutum and propodeum, hind coxa black. The dorsal side of tergites black and ventral side tergites mostly black with yellow patches. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infestation; Antennal scape and pedicel yellow and funicle honey yellow.

Head (Figs. 4C, D): Head faintly strigulate with pubescence; Head width (anteriorly) 1.4× distance between front ocellus and clypeal margin, with POL 1.02× OOL. Eyes slightly longer than wide. Scrobe deep, reaching front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11143; scape not reaching front ocellus; length of scape is 2× length of pedicel. F1 shorter than pedicel. Pedicel is 2.1 times as long as broad while F1 is 1.4 times as long as broad. Flagellar segments stout-filiform.

Mesosoma (Figs. 4C, E): Pronotum coriaceous and mesosoma and scutellum imbricate strigulate. Median length of pronotum longer than median length of scutellum; mesoscutum longer than pronotum and scutellum; scutellum as wide as long. Notauli complete. Propodeum declining sharply, with a median shallow depression, anteriorly delimited by oblique cross carinae diverging from the middle of base. Surface of depression rugulose with each rugae boarded by short wavey or circular carinae; area above the fovea rugulose with a short carinae bordering each rugae, spiracle round shaped having white setae rising from pits to the lateral side. Forewing length 1.86× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having 15 bristles in row; MV broad and distinctly longer than STV. MV 2.2 times STV. Rusty infuscation extends beyond the posterior margin of MV. The infuscation is wider than long. Hind tibia with a series of setae and prominent spur at apex, tarsal segments in ratio 7: 4: 3: 2: 2.

Metasoma (Fig. 4A): Gaster strongly compressed, its surface smooth and shiny; T3 longest. Gaster shorter than thorax.

Male: Length 1.5mm. Similar to female in general except in having: yellow colour; antenna with four funicular segments and one club; gaster small with sooty brown to black patches medially on T1 and T4; setaceous with setae raising from dark pits. 13 bristles on SMV and four round sensilla on stigma vein; petiole 4.4× longer than broad.

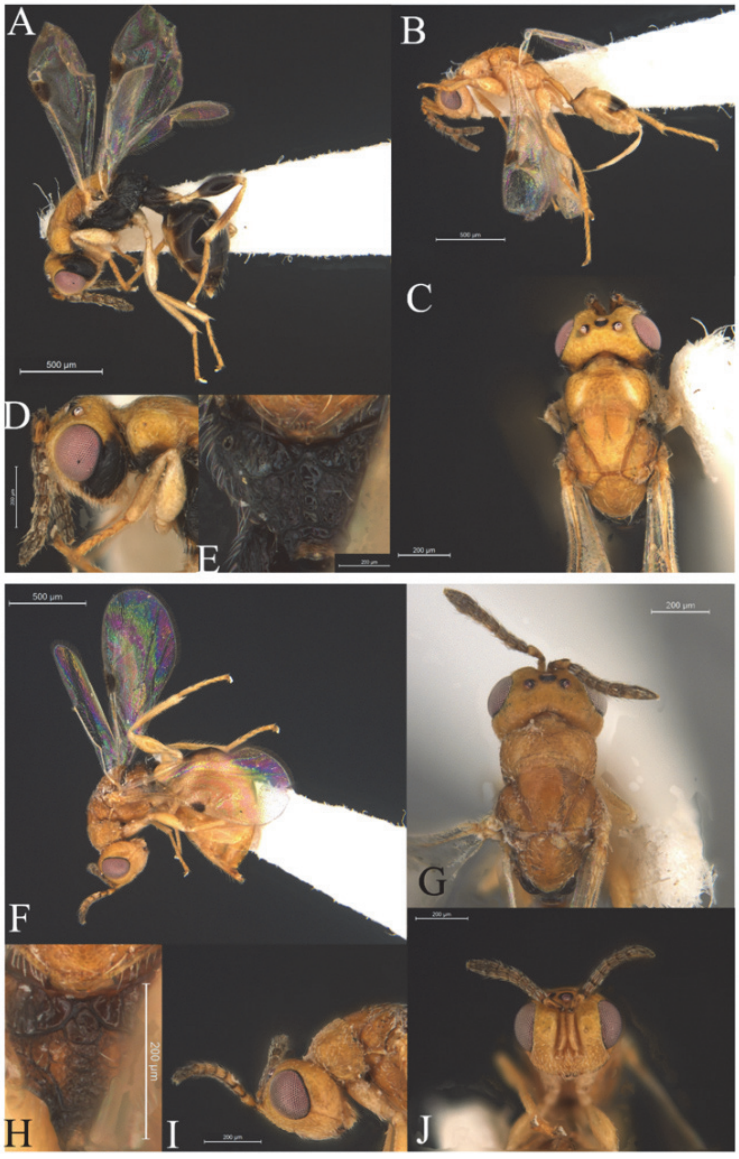
Host: Syconia galls of *F. virens* Aiton.

Etymology: 'wayanadensis' derived from the name of the place of collection of the specimen, Wayanad.

Discussion: Antenna pedicel always longer than F1; POL 1.02× OOL distance; Anterior width of head 1.4× length; Eyes slightly longer than wide; scape length is 2× length of pedicel; median length of pronotum longer than the median length of scutellum; Propodeum anteriorly delimited by oblique cross carinae diverging from the middle of base. Surface of propodeal fovea rugulose with each rugae boarded by short wavey or circular carinae. MV 2.2 times STV. T3 longest.

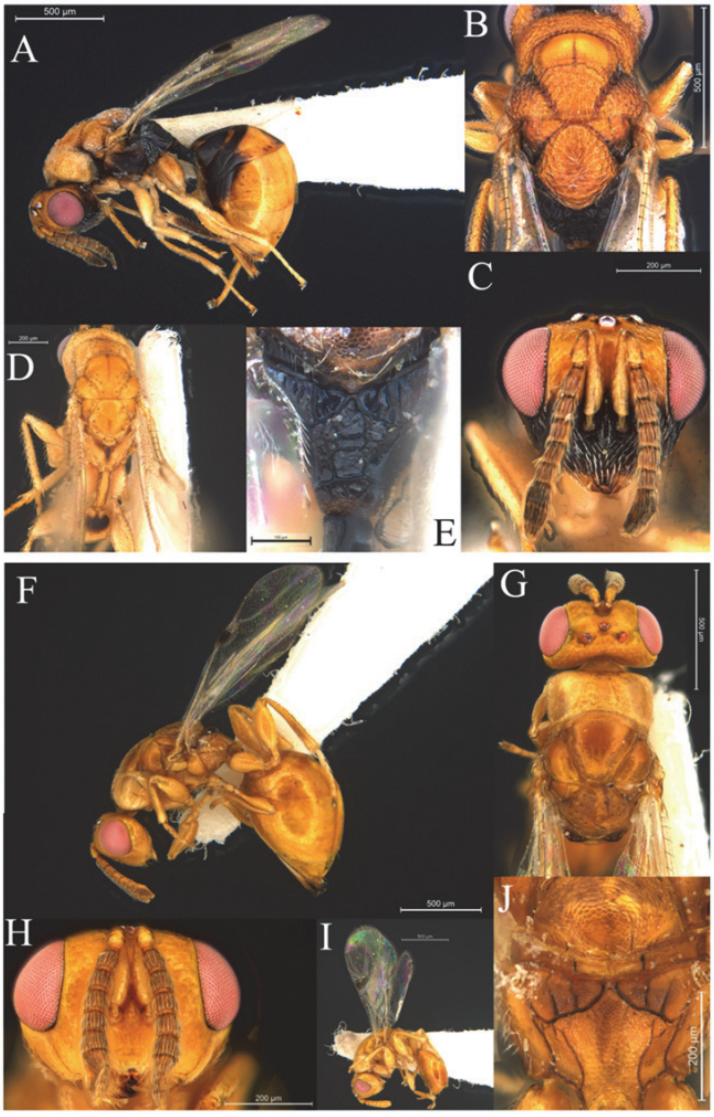
S. wayanadensis **sp. nov**. is different from all the other *Sycophila* species with above mentioned combination of characters. It shares similar character with *S. benghalensis* in having antennal pedicel always longer than F1; Anterior width of head 1.4× length and differs from it in having these combination of characters, like, POL 1.02× OOL distance (POL 1.5× OOL in *S. benghalensis*); scape length is 2× length of pedicel (2.44× in *S.*

Plate 4. *S. wayanadensis* **sp. nov.** and *S. batheri* **sp. nov.**



Figs. 4A–4E. *Sycophila wayanadensis* **sp. nov.** Holotype ♀4A. Habitus, lateral view; 4B. ♂ Habitus, lateral view; 4C. Thorax, dorsal view; 4D. Head, lateral view; 4E. Propodeum, dorsal view
Figs. 4F–4J. *Sycophila batheri* **sp. nov.** Holotype ♀4F. Habitus, lateral view; 4G. Thorax, dorsal view; 4H. Propodeum, dorsal view; 4I. Head, lateral view; 4J. Head, frontal view

Plate 5. *S. tintoria* **sp. nov.**, *S. gibbosa* **sp. nov.**



Figs. 5A–5E. *Sycophila tinctoria* **sp. nov.** Holotype ♀ 5A. Habitus, lateral view; 5B. Thorax, dorsal view; 5C. Head, frontal view; 5D. ♂ Thorax, dorsal view; 5E. Propodeum, dorsal view
Figs. 5F–5J. *Sycophila gibbosa* **sp. nov.** Holotype ♀ 5F. Habitus, lateral view; 5G. Thorax, dorsal view; 5H. Head, frontal view; 5I. ♂ Habitus, lateral view; 5J. Propodeum, dorsal view

benghalensis); median length of pronotum larger than the median length of scutellum (length of pronotum shorter than length of scutellum in *S. benghalensis*); Surface of propodeal fovea rugulose with each rugae boarded by short wavey or circular carinae (Surface of propodeal fovea smooth in *S. benghalensis*).

***Sycophila batheri* sp. nov.**

LSIDurn:lsid:zoobank.org:act:6815D2B1-D443-48D4-9D64-06D00FE47BE2

(Plate 4, Figs. 4F – J)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, 11.7439N 76.2271E, 19.ii.2021, collected by Shilpa K. Satheesan, ex *F. virens* Aiton., Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Diagnosis: Length 1.7mm. General colour brownish yellow. Head width (anteriorly) 1.43× distance between front ocellus and clypeal margin, with POL 1.01× OOL. Eyes 1.15× longer than wide. Pedicel longer than F1. Median length of pronotum distinctly shorter than half the median length of scutellum. T5 longest.

Description: Female. *Holotype.* Length 1.7mm. General colour brownish yellow; with propodeum and median area of gaster brown. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infuscation; Antennal scape and pedicel yellow and funicle brownish yellow.

Head (Figs. 4G, I, J): Head faintly imbricate strigulate with pubescence; Head width (anteriorly) 1.43× distance between front ocellus and clypeal margin, with POL 1.01× OOL. Eyes 1.15× longer than wide. Malar space 0.118mm. Scrobe deep, not reaching front ocellus; Scrobe is 0.031mm from front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11153; scape not reaching front ocellus; length of scape is 2.4× length of pedicel. Pedicel longer than F1. Pedicel is 1.8 times as long as broad while F1 is 1.2 times as long as broad. Flagellar segments stout-filiform.

Mesosoma (Figs. 4F, G, H): Dorsum of thorax with faintly imbricate strigulate and pubescent; Median length of pronotum distinctly shorter than half the median length of scutellum; mesoscutum little shorter than scutellum; scutellum longer than wide. Notauli complete. Propodeum declining sharply, with a median shallow depression, which is carinate on sides, anteriorly delimited by oblique cross carinae diverging from the middle of base. Surface of depression slightly rugulose with several short carinae and depressions; area lateral to fovea mostly rugulose, spiracle circular having setae to the lateral side. Forewing length 2.2× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having 12 bristles in row; MV broad and distinctly longer than STV. MV 1.6 times STV. Rusty infestation extends beyond the posterior margin of MV. The infestation is wider than long; tarsal segments in ratio 6: 4: 2: 2: 3.

Metasoma (Fig. 4F): Gaster strongly compressed, its surface smooth and shiny; T5 longest. Gaster distinctly longer than thorax.

Male: Unknown

Host: Syconia galls of *F. virens* Aiton.

Etymology: 'batheri' derived from the name of the place of collection of the specimen, Sulthan Bathery.

Discussion: Antenna pedicel always longer than F1; POL 1.01× OOL distance; Anterior width of head 1.5× length; Eyes 1.15× longer than wide; scape length is 2.4× length of pedicel; median length of pronotum distinctly shorter than half the median length of scutellum; Propodeal fovea slightly rugulose with several short carinae and depressions. MV 1.6 times STV. T5 longest.

S. batheri sp. nov. is different from all the other *Sycophila* species with above mentioned combination of characters. It shares similar character with *S. benghalensis* in having antennal pedicel always longer than F1; median length of pronotum distinctly shorter than half the median length of scutellum; scape length is 2.4× length of pedicel and differs from it in having these combination of characters, like, POL 1.01× OOL distance (POL 1.5× OOL in *S. benghalensis*) ; Anterior width of head 1.5× length (head width is

1.4× length in *S. benghalensis*); MV 1.6 times STV (MV 3.75× STV in *S. benghalensis*); Propodeal fovea slightly rugulose with several short carinae and depressions (Surface of propodeal fovea smooth in *S. benghalensis*).

***Sycophila tinctoria* sp. nov.**

LSIDurn:lsid:zoobank.org:act:39D05281-D665-41FC-ABA1-826B9D7BD8F8

(Plate 5, Figs. 5A – E)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, N 11° 38.617' E 76° 18.087', 17.ii. 2021, collected by Shilpa K. Satheesan, ex *F. tinctoria* G. Forst., Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Paratype: Male specimen with same collection data as holotype.

Diagnosis: Length 1.9mm. General colour yellow; Head width (anteriorly) 1.38× distance between front ocellus and clypeal margin, with POL 0.92× OOL. Eyes 1.1× longer than wide. Pedicel almost equal to F1. Median length of pronotum distinctly shorter than half the median length of scutellum. T4 longest.

Description: Female. *Holotype.* Length 1.9mm. General colour yellow; anterior part of head, lateral side of thorax, propodeum, petiole, T1, T2 and lateral and median portion of T3 black. Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infestation; antennal scape and pedicel yellow and funicle brownish yellow to greyish black at the ends.

Head (Figs. 5A, C): Head faintly strigulate with pubescence; Head width (anteriorly) 1.38× distance between front ocellus and clypeal margin, with POL 0.92× OOL. Eyes 1.1× longer than wide. Malar space 0.103mm. Scrobe deep, not reaching front ocellus; Scrobe is 0.029mm from front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11153; scape almost reaching front ocellus; scape length is 2.64× length of pedicel. Pedicel almost equal to F1. Pedicel is 1.95 times as long as broad while F1 is 1.94 times as long as broad. Flagellar segments stout-filiform.

Mesosoma (Figs. 5B, E): Dorsum of thorax with irregular areolae sculpturing and pubescent; Median length of pronotum distinctly shorter than half the median length of scutellum; mesoscutum little longer than scutellum; scutellum longer than wide. Notauli complete. Propodeum declining sharply, with a median shallow depression, anteriorly delimited by oblique cross carinae diverging from the middle of base. Surface of depression large irregular reticulations with carinae bordering these reticulations; area above the fovea mostly imbricate with a short carinae, spiracle circular having setae rising from lateral side. Forewing length 2.06× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having 12 bristles in row; MV broad and distinctly longer than STV. MV 2.1 times STV. STV with four sensilla. Rusty infuscation extends beyond the posterior margin of MV. The infuscation is longer than wide; tarsal segments in ratio 11: 6: 7: 5: 5.

Metasoma (Fig. 5A): Petiole as long as broad; gaster strongly compressed, its surface smooth and shiny; T4 longest. Petiole 1.12× wider than long; Gaster as long as thorax.

Male: Length 1.2mm. Colour yellow, T4 posteriorly black and T5 anteriorly black. Numerous setae coving the body. Similar to female in general except in having: antenna with four funicular segments and one club with three fused segments; petiole 3.26× longer than wide.

Host: Syconia galls of *F. tinctoria* G. Forst.

Etymology: 'tinctoria' derived from the name of host plant *F. tinctoria*.

Discussion: *S. tinctoria* sp. nov. differs from all other *Sycophila* species' in having these combination of characters – Pedicel as long as F1; POL 0.92× OOL; Anterior width of head 1.38× length between front ocellus and clypeal margin; median length of pronotum distinctly shorter than half the median length of scutellum; MV 2× STV; SMV having 12 bristles in row; pterostigma is longer than wide; propodeal fovea with large irregular reticulations each delimited with carinae.

S. tinctoria sp. nov. is similar to *S. gibbosa* **sp. nov**. in having Pedicel as long as F1; POL < OOL; similar eye length to malar space ratio, but differs in having anterior width of head 1.38× length between front ocellus and clypeal margin (head width 1.68× length in *S. gibbosa* sp. nov.); median length of pronotum distinctly shorter than half the median length of scutellum (length of pronotum distinctly longer than half the length of scutellum in *S. gibbosa* sp. nov.); MV 2× STV (MV 1.3× STV in *S. gibbosa* **sp. nov**.); SMV having 12 bristles in row (SMV having nine bristles in *S. gibbosa* **sp. nov**.); pterostigma is longer than wide (pterostigma wider than long in *S. gibbosa* **sp. nov**.); propodeal fovea with large irregular reticulations each delimited with carinae (propodeal fovea imbricate-reticulate and smooth in *S. gibbosa* **sp. nov**.).

Remarks: Colour variation of fully yellow-coloured individuals with black petiole observed. Dark patches on T1, T2 and T3 are also seen in yellow colour.

***Sycophila gibbosa* sp. nov.**

LSIDurn:lsid:zoobank.org:act:CF215287-96B6-4F4C-903F-0C04627E3E65

(Plate 5, Figs. 5F – J)

Type material. Holotype ♀: INDIA, Wayanad, Kerala, N 11° 38.617' E 76° 18.087', 17.ii. 2021, collected by Shilpa K. Satheesan, ex *F. tinctoria* G. Forst Deposited in Zoological Survey of India, Western Ghats Regional Centre, Calicut.

Paratype: Male specimen with same collection data as holotype.

Diagnosis: Length 1.6mm. General colour yellow. Head faintly imbricate; Head is 1.68× as wide as length between front ocellus and clypeal margin, with POL 0.96× OOL. Eyes 1.08× longer than wide. Pedicel almost equal to F1. Median length of pronotum distinctly longer than half the median length of scutellum. T4 distinctly longest. Gaster little longer than thorax.

Description: Female. *Holotype.* Length 1.6mm. General colour yellow; Wings semi hyaline with area below costal cell bare, veins brown with rusty brown patch adjoining the dark infestation; antennal scape and pedicel yellow and funicle yellow.

Head (Fig. 5G & 5H): Head faintly imbricate; Head is 1.68× as wide as length between front ocellus and clypeal margin, with POL 0.96× OOL. Eyes 1.08× longer than wide. Malar space 0.113mm. Scrobe not reaching front ocellus; Scrobe is 0.036mm from front ocellus; clypeal margin bilobed; Malar groove complete. Antennal formula 11153; scape not reaching front ocellus; scape length is 2.45× length of pedicel. Pedicel almost equal to F1. Pedicel is 1.52 times as long as broad while F1 is 1.13 times as long as broad. Flagellar segments stout-filiform.

Mesosoma (Figs. 5F, G, J): Dorsum of thorax coriaceous and imbricate; Median length of pronotum distinctly longer than half the median length of scutellum; mesoscutum longer than scutellum; scutellum wider than long. Notauli complete. Propodeum declining sharply, with a median shallow depression, which is carinate on sides, anteriorly delimited by oblique cross carinae diverging from the middle of base. Surface of depression reticulate and smooth; area lateral to fovea reticulate with a short carinae or short costula, plica present, spiracle bean shaped having setae rising from black pits to the lateral side. Forewing length 2.26× length of SMV; PMV absent; costal cell (CC) with minute pilosity; basal 1/3 of forewing bare. SMV having nine bristles in row; MV broad and distinctly longer than STV. MV 1.31 times STV. Rusty infuscation extends beyond the posterior margin of MV. The infestation is wider than long; tarsal segments in ratio 10: 9: 7: 5: 6.

Metasoma (Fig. 5F): Petiole as 1.77× wider than long; gaster strongly compressed, its surface smooth and shiny; T4 distinctly longest. Gaster little longer than thorax.

Male: Length 1.2mm. Colour yellow. Similar to female in general except in having: antenna with four funicular segments and one club with three fused segments; SMV with 10 setae and four sensilla at the end of stigma vein.

Host: Syconia galls of *F. tinctoria* G. Forst.

Etymology: 'gibbosa' derived from the name of

the variety gibbose of the host *F. tinctoria*.

Discussion: *S. gibbosa* **sp. nov.** is similar to *S. tinctoria* **sp. nov**. but differs from it and all other *Sycophila* species' in having these combination of characters – Pedicel as long as F1; POL 0.96× OOL; Anterior width of head 1.68× length between front ocellus and clypeal margin; median length of pronotum distinctly longer than half the median length of scutellum; MV 1.3× STV; SMV having nine bristles in row; pterostigma is wider than long; propodeal fovea with imbricate-reticulate and smooth.

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A checklist of moths in Bilaspur district, Himachal Pradesh, in the western Himalayan foothills, India

Paul Pop[1*], Nivedita Pattanaik[2], Kuldeep Singh Barwal[1], Harminder Pal Singh[3], Puneet Pandey[4], Sanjay Sondhi[5] and Randeep Singh[6]

[1]Zoology Department, Government College Bilaspur, Kosrian Sector, Bilaspur 174001, Himachal Pradesh, India.
[2]Institute of Science, Banaras Hindu University, Ajagara, Varanasi 221005, Uttar Pradesh, India.
[3]Department of Environment Studies, Panjab University, Sector 14, Chandigarh 160014, India.
[4]Conservation Genome Resource Bank for Korean Wildlife (CGRB), ResearchInstitute for Veterinary Science and College of Veterinary Medicine, SeoulNational University, Seoul, Republic of Korea.
[5]Titli Trust, 49 Rajpur Road Enclave, Dhoran Khas, near IT Park, P.O. Gujrada, Dehradun 248001, Uttarakhand, India.
[6]Amity Institute of Forestry and Wildlife (AIFW), Amity University, Sector 125, Gautam Buddha Nagar, Noida 201313, Uttar Pradesh, India.
Email: paulvpop@gmail.com

ABSTRACT: In the first ever enumeration of moth diversity of Bilaspur district, Himachal Pradesh, India, located mostly in the Shivalik range, 82 species/morphospecies were reported, at least 22 of which are new records for Himachal Pradesh, and five are new records for Western Himalayas. In addition to a list of moths for the district supplemented with photographs, identification keys for similar species, larval host plants for species, and a near exhaustive dataset of distribution of the species/genera within and outside India are also provided. © 2024 Association for Advancement of Entomology

KEY WORDS: Heterocera, Shivalik range, morphotaxonomy, distribution, surveys, morphospecies

INTRODUCTION

Moths are used for individual conservation management as well as indicators of environmental and vegetation changes (Dey *et al.*, 2015). For conservation of such assemblages, species diversity analysis is significantly important. Moth diversity and allied studies have been conducted across the Himalayan ranges. For example, in the Eastern Himalayas, there has been a study which enumerated settling moths in sites within the Eastern Himalayas (Sikkim, North Bengal and Arunachal Pradesh), recording 140 species (Singh *et al.*, 2022). In the Central Himalayas (Nepal), diversity of moths have been enumerated over the years from 1992-2000 (Haruta, 1992, 1993, 1994, 1995, 1998, 2000). Moth diversity studies in the Western Himalayas seem to be higher compared to the other parts. Certain regions of Western Himalayas have been explored starting from Cotes and Swinhoe (1887) in 'A catalogue of moths of India' and Hampson in 'Fauna of British India: Moths. Vol I' (1892).

* Author for correspondence

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In the state of Uttarakhand, there have been a number of studies such as the one in Nanda Devi Biosphere Reserve, spread across the districts of Chamoli, Pithoragarh and Bageshwar in Garhwal Himalayas (Dey *et al.*, 2017). Due to extensive studies, a checklist for the whole of Jammu and Kashmir is available (Dar *et al.*, 2020). Some studies were done in landscapes that straddle between two main administrative units, such as in the Tons Valley in the high-altitude region shared between the states of Himachal Pradesh and Uttarakhand (Bhardwaj *et al.*, 2012). Some studies spanned all three main administrative units in the W. Himalayas such as the study of diversity of Lymantriidae family (tussock moths) (Kaleka, 2012). While there have been several studies on moth diversity across the Himalayas, several parts remain completely unexplored, especially at the finer level biodiversity preservation efforts are geared towards (by the forest departments), such as at the district level. Some among them are the districts falling under the Shivalik range in Himachal Pradesh. One in particular with no moth studies till date is Bilaspur district. While a recent study has thoroughly documented moths across the Shivalik range, it was largely restricted to Uttarakhand (Singh and Lekhendra, 2023), and did not cover Bilaspur district. The aim of the current study is to document moth biodiversity of this district, in the Lower Himalaya biogeographical zone.

MATERIALS AND METHODS

The study area is Bilaspur district, which is largely located in the Lower Himalaya biogeographical zone (in the biogeographical province of 2B: West Himalaya), with a minor part in the Middle Himalaya (in the 2A: North-West Himalaya province), in the state of Himachal Pradesh (Rodgers and Panwar, 1988; Chauhan *et al.*, 2020). Most of the district is part of the Shivalik range of the Himalayas (Yadav *et al.*, 2015). The elevation varies quite considerably between ~300 m asl to ~1800 m asl, resulting in a wide range of habitats and climatic conditions.

As part of surveys for the project 'High resolution spatial mapping of bird phenology as an indicator of ecosystem health in relation to climate change in Himalaya', two circular plots each of 25 m radius within 34 stratified random hexagons (with sides of approx. 500 m) were intensively studied, with at least two survey efforts each (except one hexagon), separated temporally by 26-120 days. Within each such circular plot, 1 to 3.5 hours of intensive sampling of invertebrates were carried out using methods of visual encounter surveys and temporarily flushing the ground litter using boots or sticks. All invertebrates sighted were noted down and photographed for identification and/or documentation. Since all moths were unidentifiable at that time, they were repeatedly photographed in every plot. Moths were also photographed opportunistically when sighted along the route to survey plots or at the basecamps in Bilaspur town. Since the surveys were diurnal, most of the moth species inventoried are diurnal. However, several nocturnal or crepuscular species were also recorded in the basecamps. The sampling was done from 16th March 2020 to 1st November 2021. Moths were identified through morphotaxonomy with the help of the following resources: i) Citizen science websites like Moths of India (Sondhi *et al.*, 2024), iNaturalist (iNaturalist, 2024) and India Biodiversity Portal (Vattakaven *et al.*, 2016), ii) books like Fauna of British India: Moths Vol I-IV (Hampson, 1892, 1894, 1895, 1896) and Moths of Borneo (Holloway, 2024).

RESULTS AND DISCUSSION

A total of 78 moth species/morphospecies were recorded during our study (Plates 1-7). An additional four species were found from the citizen science platform iNaturalist, which were not recorded during our study (Kohli, 2021a, 2021b, 2021c; Mujumdar, 2023). These 82 species in Bilaspur district belong to nine super families and 12 families (including 45 identified to species level, 25 to genus level, and 12 to higher taxonomic levels). The checklist contains the details of the species, along with known larval host plants and identification keys for species which have morphologically similar counterparts. The larval host plants are the ones recorded in India, gathered from available publications (from Robinson *et al.*, 2010 unless specified) and all may not be found in Bilaspur

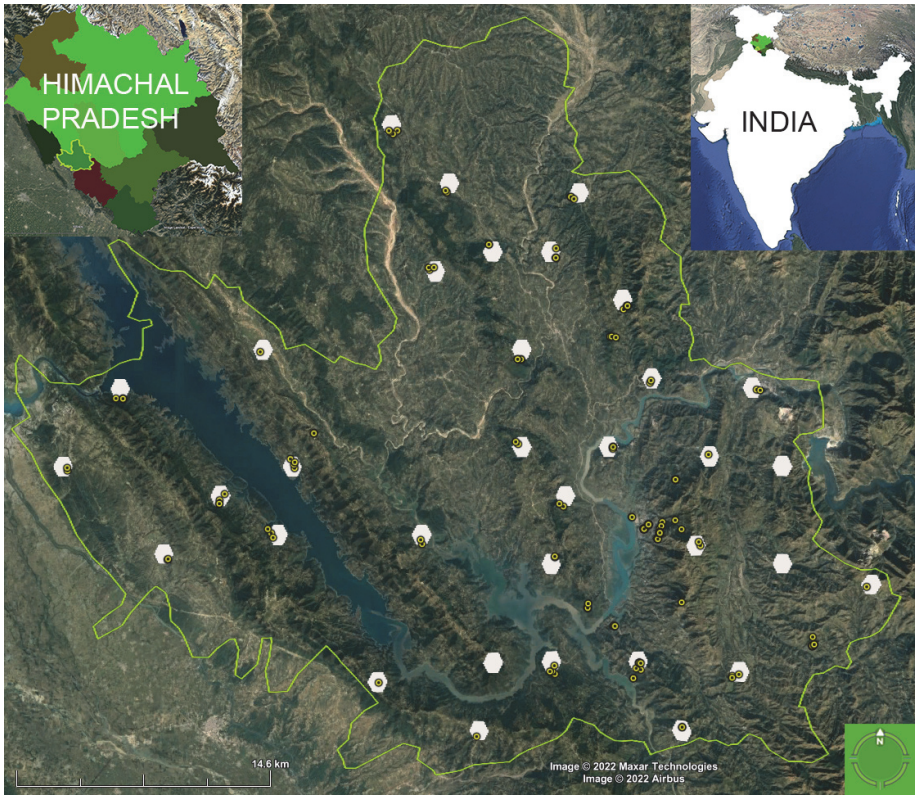


Fig.1 Study area of Bilaspur district. Hexagons represent the stratified random sampling cells and the circles represent the locations in which moths were observed

district (and only for moths identified to the species level). Distribution of the species around the world as well as within India has been given in the supplementary dataset (Zenodo: https://doi.org/10.5281/zenodo.13248751).

Superfamily Bombycoidea; Family Sphingidae; Subfamily Macroglossinae; Tribe Hemarini

1. *Cephonodes hylas* (Linnaeus, 1771): The dark pink band on the dorsal side of the abdomen has two prominent thick black bands on either side (which is faint and thin in a similar species *C. picus*). **Host plants**: *Xylia xylocarpa* (Roxb.) W.Theob. (Fabaceae), *Tectona grandis* L.f. (Lamiaceae), *Catunaregam spinosa* Thunb., Tirveng., *Gardenia* J.Ellis sp., *Haldina cordifolia* (Roxb.) Ridsdale, *Hymenodictyon obovatum* Wall., *Hymenodictyon orixense* (Roxb.) Mabb., *Mitragyna diversifolia* (Wall. ex G.Don) Havil., *Mitragyna parvifolia* (Roxb.) Korth, (Rubiaceae)

Superfamily Choreutoidea; Subfamily Brenthiinae; Family Choreutidae; Tribe Choreutini

2. *Brenthia* Clemens, 1860 sp.

Superfamily Gelechioidea

3. *Morphospecies A*

Superfamily Geometroidea; Family Geometridae; Subfamily Desmobathrinae; Tribe Eumeleini

4. *Eumelea* cf. *rosalia* (Stoll, [1781]): Prominent, continuous crimson bands on both dorsal and ventral sides, which is discontinuous in similar species *E. ludovicata*. **Host plant**: *Mallotus* Lour. sp. (Euphorbiaceae)

Subfamily Ennominae; Tribe Abraxini

5. *Abraxas* Leach, 1815 sp.

1. *Cephonodes hylas*, 2. *Brenthia* sp., 3. *Hyposada hydrocampata*, 4. *Morphospecies A*, 5. *Eumelea cf. rosalia*, 6. *Hyperythra lutea*, 7. *Chiasmia* sp., 8. *Chiasmia eleonara*, 9. *Chiasmia* cf. *fidoniata*, 10. *Chiasmia perfusaria*, 11. *Isturgia* sp., 12. *Morphospecies B*

Tribe Caberini

6. *Hyperythra lutea* (Stoll, [1781])

Host plants: *Ziziphus oenoplia* (L.) Mill., *Gouania leptostachya* DC. (Rhamnaceae)

Tribe Macariini

7. *Chiasmia* Hübner, 1823 sp.

8. *Chiasmia eleonora* (Cramer, [1780]): Unlike *C. eleonora*, *C. nora* is suffused with black, especially beyond the medial band of both wings. In *C. nora*, forewing has a black speck at end of cell, and the black patches in the hindwing beyond the band are more numerous, with a white patch on the outer area below vein 4 (Hampson, 1895).

9. *Chiasmia* cf. *fidoniata* Guenée, 1858

10. *Chiasmia perfusaria* (Walker, 1866)

11. *Isturgia* Hübner, 1823 sp.: As per morphology, current distribution, and species listed under the genus in India, *I. disputaria* is the only option, but requires more taxonomic studies before this species can be confirmed, as it's an African species, and may not occur in India.

12. *Morphospecies B*

Subfamily Geometrinae

13. *Agathia* Guenée, 1858 sp.

Subfamily Sterrhinae; Tribe Cosymbiini

14. *Traminda mundissima* (Walker, 1861)

Host plants: *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb., *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (Fabaceae)

Tribe Cyllopodini

15. *Rhodostrophia stigmatica* Butler, 1889: Similar to *R. vibicaria* but description given in Butler (Ed.) (1889) matches more with *R. stigmatica*. Although the discocellular spots don't look black as described, it looks darker than the red for *R. vibicaria*. The spot also looks more oblong for *R. vibicaria* as shown in Rennwald (Ed.) (2019). The second line in this individual looks narrower than *R. vibicaria*.

Tribe Rhodometrini

16. *Rhodometra sacraria* (Linnaeus, 1767)

Host plant: *Rumex vesicarius* L. (Polygonaceae)

Tribe Scopulini

17. *Problepsis vulgaris* Butler, 1889

18. *Scopula* Schrank, 1802 sp.: There could be two species of *Scopula* amongst the photographed *Scopula* spp. given the morphological variations. But we are considering only one species due to lack of clarity (note that the morphospecies *Scopula* sp. 17 and sp. 18 in Plate 2 could be the same).

Family Uraniidae; Subfamily Epipleminae

19. *Orudiza protheclaria* Walker, 1861

Host plants: *Bajanella sp., Oroxylum indicum* Vent. (Bignoniaceae) (Smetacek & Smetacek, 2011)

Superfamily Lasiocampoidea; Family Lasiocampidae; Subfamily Lasiocampinae; Tribe Pinarini

20. *Lebeda nobilis* ssp. *nobilis* Walker, 1855: Only one similar species in India - *Lebeda trifascia* Walker, 1855, which has nearly parallel lines on the dorsal region, compared to the curved and spreading lines in *L. nobilis*. **Host plants**: *Casuarina equisetifolia* L. (Casuarinaceae), *Cupressus* L. (Cupressaceae), *Pteridium aquilinum (L.) Kuhn* (Dennstaedtiaceae), *Quercus* L. sp. (Fagaceae), *Myrica rubra* Siebold & Zucc. (Myricaceae), *Pinus kesiya* Royle ex Gordon (Pinaceae), *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda (Poaceae), *Rubus* L. sp. (Rosaceae), *Camellia* L. (Theaceae).

Superfamily Noctuoidea; Family Erebidae

21. *Morphospecies C* (Caterpillar): Individuals with two pairs of functional abdominal prolegs and anal claspers belongs with a high degree of certainty, to the family Erebidae. In contrast, individuals in the

family Geometridae only have one pair of functional abdominal prolegs.

22. *Morphospecies D*

Subfamily Aganainae

23. *Asota plaginota* (Butler, 1875)

Host plants: Millets sp. (Poaceae) (Kalaisekar *et al.*, 2016)

Subfamily Anobinae; Tribe Anobini

24. *Anoba* Walker, 1858 sp.

25. *Plecoptera* Burmeister, 1839 sp.

Subfamily Arctiinae; Tribe Lithosiini

26. *Siccia* Walker, 1854 (= *Aemene* Walker, 1854) sp.

27. *Cyana* cf. *chrysopeleia* N.Singh, Volynkin, Kirti & Datta, 2020

28. *Morphospecies E* (Caterpillar)

29. *Morphospecies F*: *Wittia sororcula* (Hufnagel, 1766) or Lithosiini-genera sp. or *Eilema* sp. Or *Katha* sp. Morphological characters are similar in all genera, except for variations in coloration which would not provide the correct identification.

Subtribe Nudariina

30. *Miltochrista* cf. *undulata* (Swinhoe, 1903)

Tribe Arctiini; Subtribe Spilosomina

31. *Morphospecies G* (Caterpillar)

Subfamily Boletobiinae; Tribe Aventiini

32. *Cerynea punctilinealis* Walker, 1865: two black spots on costa, which is single in similar species *C. ustula*.

33. *Hyposada hydrocampata* (Guenée, 1857): *Hyposada hydrocampata* has large black dots on the forewings unlike *Phalacra* spp..

Tribe Eublemmini

34. *Eublemma cochylioides* (Guenée, 1852)

Host plants: *Vigna unguiculata* (L.) Walp. (Fabaceae); *Lactuca sativa* L., *Elephantopus scaber* L. (Asteraceae)

Tribe Phytometrini

35. *Rhesala* Walker, 1858 sp.

Subfamily Calpinae

36. *Fodina pallula* Guenée, 1852

Host plant: *Vallaris solanacea* (Apocynaceae)

Tribe Calpini

37. *Oraesia emarginata* (Fabricius, 1794): Both adult and caterpillar stages of *Oraesia* were recorded during the study. The fore wing pattern (transverse dark brown bands with pale white striations) are as described in literature, but the hindwing pattern is necessary confirm the species. However, *Oraesia argyrosigna* is the only other similar species found nearby, and they are darker.

Subfamily Erebinae

38. *Morphospecies H* (Caterpillar)

Either in the tribe Ophiusini or Poaphilini

Tribe Acantholipini

39. *Acantholipes trajecta* (Walker, 1865): Hind wings have a dark brown patch, above which a discontinuous band is present, which is absent in similar-looking *A. circumdata*.

Tribe Poaphilini

40. *Dysgonia torrida* (Guenée, 1852): Compared to the similar-looking *D. algira* whose middle band on the forewing is grey and has angular lines (especially the inner line) on the narrowest part, *D. torrida* has a middle band that is clear to white and has rounded lines on the narrowest part (Demerges and Grandmaire, 2014).

Tribe Erebini

41. *Erebus hieroglyphica* (Drury, 1773)

Tribe Hypopyrini

42. *Hypopyra* Guenee, 1852 sp. or *Spirama*

Guenée, 1852 sp.: While the individual looks like *Hypopyra*, individuals of the genus *Spirama* sometimes only have part of the spiral present like the discal stigma in *Hypopyra*.

Tribe Euclidiini

43. *Mocis frugalis* (Fabricius, 1775)

Host plants: *Cyperus rotundus* L. (Cyperaceae); *Vigna radiata* (L.) R. Wilczek (Fabaceae); *Sorghum bicolor* (L.) Moench, *Oryza sativa* L., *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs, *Saccharum officinarum* L. (Poaceae); Zingiberaceae sp.

44. *Mocis undata* (Fabricius, 1775)

Host plants: *Phaseolus* Hennig, 1932 (Chlorophyceae); *Shorea robusta* Roth (Dipterocarpaceae); H*evea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (Euphorbiaceae); *Butea monosperma* (Lam.) Taub., *Cajanus cajan* (L.) Millsp., *Dalbergia latifolia* Roxb., *Glycine max* (L.) Merr., *Indigofera* L., *Ougeinia oojeinensis* (Roxb.) Hochr., *Rhynchosia minima* (L.) DC., *Vigna mungo* (L.) Hepper, *Vigna trilobata* (L.) Verdc., *Vigna unguiculata* (L.) Walp. (Fabaceae); *Gossypium* L. (Malvaceae); *Solanum tuberosum* L. (Solanaceae)

Subfamily Herminiinae

45. *Herminia undulata* (Moore, 1882): Submarginal line have a white lining unlike other *Herminia* spp. occuring in Asia, such as the *Herminia kurukoi*. The rest of features matches the description by Moore (1879).

46. *Hydrillodes* Guenée, 1854 sp.

Subfamily Hypeninae

47. *Rhynchina* Guenée, 1854 sp.

48. *Dichromia sagitta* (Fabricius, 1775)

Host plants: *Stephanotis volubilis* (L.fil.) S.Reuss, Liede & Meve, *Tylophora asthmatica* (L.fil.) Wight & Arn., *Vincetoxicum indicum* (Burm.fil.) Mabb., *Vincetoxicum lindleyi* A.Kidyoo, (Apocynaceae) (Gole & Das, 2011; National Bureau of Agriculturally Important Insects, 2013), Asclepiadaceae sp. (Swafvan & Sureshan, 2022)

Subfamily Lymantriinae; Tribe Nygmiini

49. *Morphospecies I*

Subfamily Rivulinae

50. *Bocula* Guenée, 1852 sp.

51. *Rivula* Guenée [1845] sp.: *Rivula basalis* or *R. simulatrix*: This individual is likely *Rivula basalis* but these two species can only be confidently be separated by features in their hind-wing and abdomen, which is not visible in the image taken.

Subfamily Tinoliinae

52. *Calesia haemorrhoa* Guenée, 1852: The wing pattern is similar to *C. fuscicorpus*, but *C. dasypterus* has a distinct reddish abdomen. Females of *C. dasyptera* look similar but have prominent white dots on the forewing. The frons and palpi is orangish (clearly contrasting the reddish abdomen) in *C. haemorrhoa*, instead of bright red like individuals of *C. dasyptera*. *C. haemorrhoa* show 3 prominent squiggly dark lines over the greyish-black or greyish-brown dorsal side, whereas the females of *C. dasyptera* has only one prominent dark line running through the mid-dorsal portion of all the wings, like a necklace. **Host plants**: *Barleria cristata* L., *Justicia adhatoda* L., *Justicia wynaadensis* (Nees) B.Heyne, *Eranthemum purpurascens* Wight ex Nees (Acanthaceae)

Family Noctuidae; Subfamily Agaristinae

53. *Episteme* Hübner, 1820 sp.

Subfamily Condicinae; Tribe Condicini

54. *Condica* Walker, 1856 sp.: Proportionately bigger and bulkier thorax compared to the similar-looking *Amyna* spp.

Subfamily Eriopinae

55. *Callopistria* Hübner, [1821] sp.

Subfamily Eustrotiinae

56. *Amyna* Guenée, 1852 sp.

57. *Maliattha signifera* (Walker, [1858])

Host plant: *Oryza sativa* L. (Poaceae)

Subfamily Heliothinae

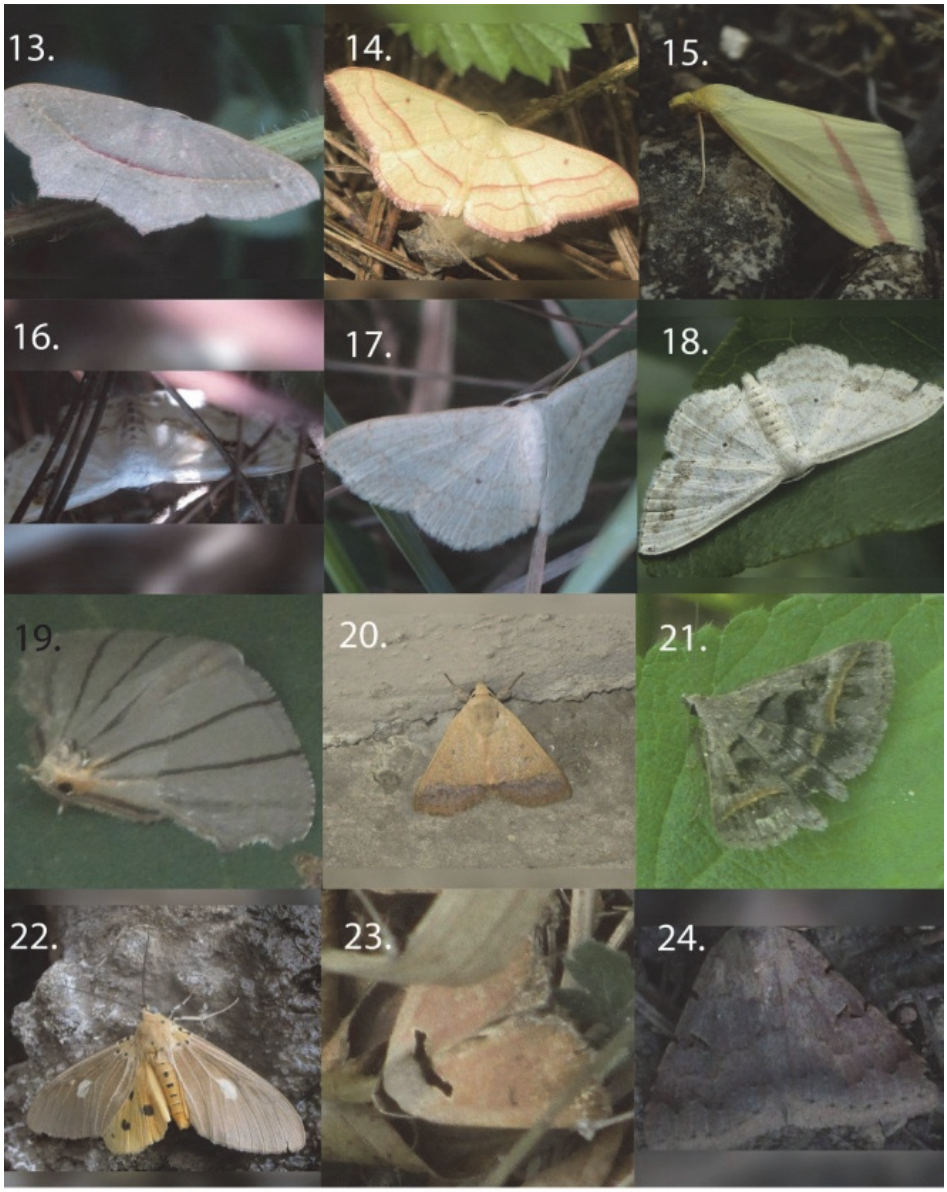
58. *Helicoverpa armigera* Hübner, [1809]

Host plants: *Allium cepa* L. (Amaryllidaceae); *Cannabis sativa* L. (Cannabaceae), *Dianthus caryophyllus* L. (Caryophyllaceae); *Ricinus communis* L. (Euphorbiaceae); *Carthamus tinctorius* L., *Guizotia abyssinica* (L.fil.) Cass., *Lipschitziella heteromalla* (D.Don) Kasana & A.K.Pandey, *Zinnia violacea* Cav. (Compositae); *Avena sativa* L., *Cenchrus americanus* (L.) Morrone, *Oryza sativa* L., *Sorghum bicolor* (L.) Moench (Poaceae), *Albizia procera* (Roxb.) Benth., *Arachis hypogaea* L., *Cajanus cajan* (L.) Millsp., *Crotalaria juncea* L., *Dalbergia sissoo* Roxb. ex DC., *Medicago sativa* L., *Pisum sativum* L., *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb. (Fabaceae); *Linum usitatissimum* L. (Linaceae); *Abelmoschus esculentus* (L.) Moench, *Alcea rosea* L., *Gossypium hirsutum* L., *Hibiscus mutabilis* L. (Malvaceae); *Platanus orientalis* L. (Platanaceae); *Citrus* × *sinensis* (L.) Osbeck (Rutaceae); *Populus ilicifolia* (Engl.) Rouleau, *Salix tetrasperma* Roxb. (Salicaceae); *Antirrhinum majus* L. (Plantaginaceae); *Datura stramonium* L., *Hyoscyamus niger* L., *Solanum lycopersicum* L., *Solanum tuberosum* L. (Solanaceae)

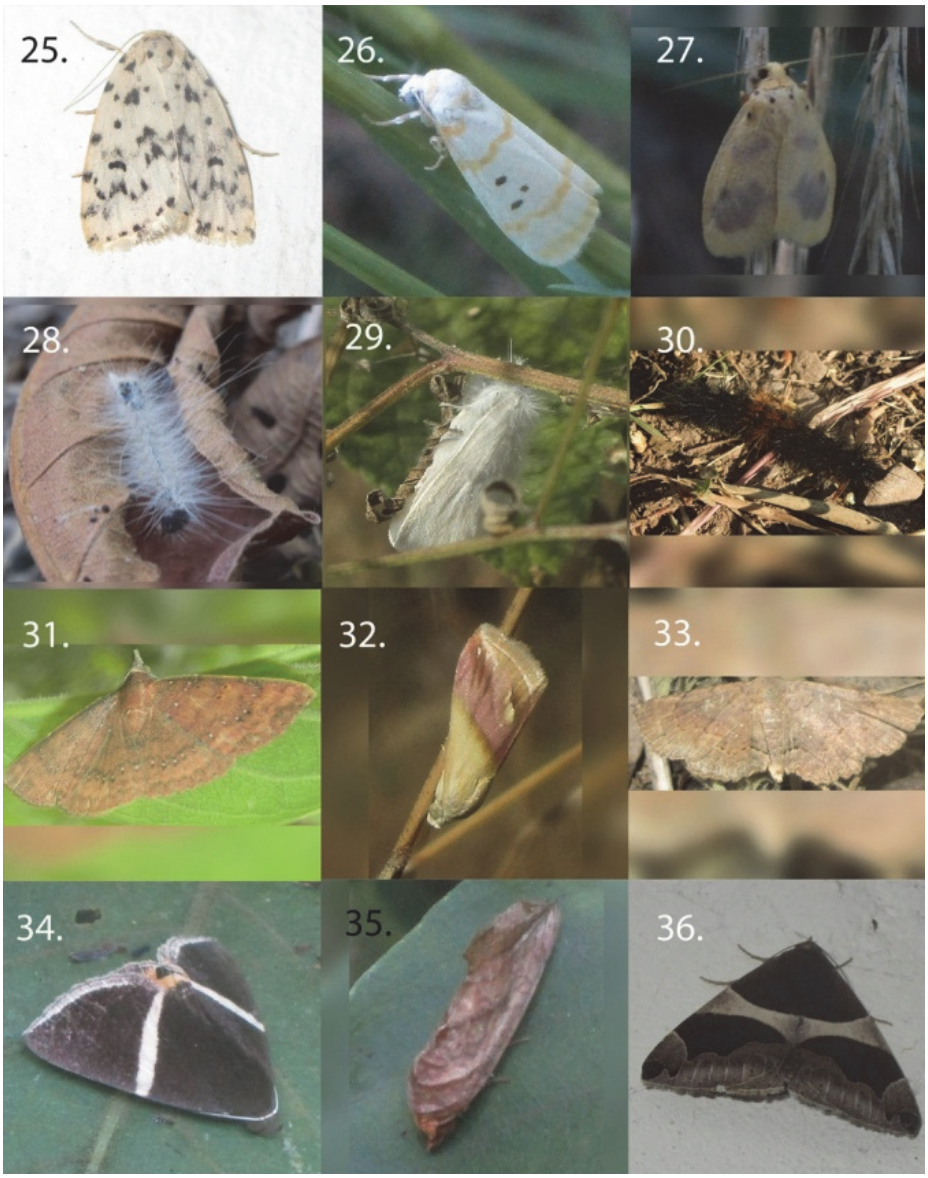
Subfamily Noctuinae; Tribe Prodeniini

59. *Spodoptera litura* (Fabricius, 1775)

Host plants: *Beta vulgaris* L., *Chenopodium album* L., *Spinacia oleracea* L. (Amaranthaceae); *Allium cepa* L. (Amaryllidaceae); *Mangifera indica* L. (Anacardiaceae); *Annona squamosa* L. (Annonaceae); *Apium graveolens* L. (Apiaceae); *Carissa carandas* L. (Apocynaceae); *Typhonium trilobatum* (L.) Schott, *Colocasia* Schott sp. (Araceae); *Cordia macleodii* Hook.fil. & Thomson (Boraginaceae); *Brassica oleracea* L., *Raphanus raphanistrum ssp. sativus* (L.) Domin (Brassicaceae); *Carica papaya* L. (Caricaceae); *Casuarina equisetifolia* L. (Casuarinaceae); *Terminalia corrugata* (Ducke) Gere & Boatwr. (Combretaceae); *Carthamus tinctorius* L., *Chrysanthemum* L. sp., *Guizotia abyssinica* (L.fil.) Cass., *Helianthus annuus* L., *Lactuca sativa* L. (Compositae); *Ipomoea batatas* (L.) Lam. (Convolvulaceae); *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Momordica dioica* Roxb. ex Willd. (Cucurbitaceae); *Diospyros montana* Roxb. (Ebenaceae); *Ricinus communis* L. (Euphorbiaceae); *Senna obtusifolia* (L.) H.S.Irwin & Barneby, *Acacia nilotica Vachellia nilotica* (L.) P.J.H.Hurter & Mabb., *Arachis hypogaea* L., *Cajanus cajan* (L.) Millsp., *Clitoria ternatea* L., *Glycine max* (L.) Merr., *Lathyrus sativus* L., *Phaseolus vulgaris* L., *Pisum sativum* L., *Sesbania grandiflora* (L.) Poir., *Trigonella foenum-graecum* L., *Vigna mungo* (L.) Hepper (Fabaceae); *Tectona grandis* L.f. (Lamiaceae); *Abelmoschus esculentus* (L.) Moench, *Corchorus capsularis* L., *Corchorus olitorius* L., *Hibiscus* L. sp. (Malvaceae); *Ficus carica* L., *Ficus religiosa* L., *Morus alba* L., *Morus nigra* L. (Moraceae); *Moringa* Adans. sp. (Moringaceae); *Musa acuminata* Colla (Musaceae); *Syzygium malaccense* (L.) Merr. & L.M.Perry, *Psidium guajava* L. (Myrtaceae); *Argemone mexicana* L., *Papaver somniferum* L. (Papaveraceae); *Sorghum bicolor* (L.) Moench, *Oryza sativa* L., *Saccharum officinarum* L., *Triticum aestivum* L., *Zea mays* L. (Poaceae); *Malus domestica* (Suckow) Borkh, *Malus sylvestris* Mill, *Prunus domestica* L. (Rosaceae); *Catunaregam spinosa* Thunb., Tirveng., *Tamilnadia uliginosa* (Retz.) Tirveng. & Sastre (Rubiaceae); *Citrus grandis* (L.) Osbeck (Rutaceae); *Capsicum annuum* L., *Cestrum nocturnum* L., *Solanum lycopersicum* L., *Nicandra physalodes* (L.) Gaertn., *Nicotiana tabacum* L., *Solanum violaceum* Ortega, *Solanum torvum* Sw., *Solanum tuberosum* L. (Solanaceae); *Camellia sinensis* (L.) Kuntze (Theaceae); *Lantana camara* L. (Verbenaceae); *Vitis vinifera* L. (Vitaceae)



13. *Traminda mundissima*, 14. *Rhodostrophia stigmatica*, 15. *Rhodometra sacraria*, 16. *Problepsis vugaris*, 17. *Scopula* sp., 18. *Scopula* sp., 19. *Orudiza protheclaria*, 20. *Bocula* sp., 21. *Acantholipes trajecta*, 22. *Asota plaginota*, 23. *Anoba* sp., 24. *Plecoptera* sp.



25. *Siccia (= Aemene)* sp., 26. *Cyana* cf. *chrysopeleia*, 27. *Miltochrista* cf. *undulata*, 28. *Morphospecies E*, 29. *Morphospecies I*, 30. *Morphospecies G*, 31. *Cerynea punctilinealis*, 32. *Eublemma cochylioides*, 33. *Rhesala* sp., 34. *Fodina pallula*, 35. *Oraesia emarginata*, 36. *Dysgonia torrida*

Subfamily Plusiinae; Tribe Argyrogrammatini

60. *Chrysodeixis* Hübner, [1821] sp.

61. *Thysanoplusia intermixta* (Warren, 1913): *T. intermixta* looks similar but the sub-costal forewing stigmata (the orbicular stigmata) is bilobed and grotesquely oblique, whereas in *T. orichalcea*, it is usually circular and sometimes squarish. The green area extends slightly less towards the base (i.e. towards the head region) in *T. intermixta* and is basally more blunt and rounded compared to the sharper stop of the green patch in *T. orichalcea*. But the size and length of the greenish-golden area is highly variable, and therefore the position of the orbicular stigmata is also quite variable, but usually lies right below the greenish-golden finger in the center in case of *T. orichalcea*, and lies under the semi-circular arch of the greenish-golden area in *T. intermixta.* Colour varies based on the freshness of the scales, angle of incidence of light, and other factors. So, the key based on colour i.e. forewings in *T. orichalcea* has a distinctive pale lustrous green area, whereas in *T. intermixta*, it is of somewhat yellower tone, is not very reliable. Another key which may be reliable is that *T. intermixta* has a more distinct irregular submarginal. **Host plants**: *Lactuca sativa* L. (Asteraceae), Apiaceae sp., Fabaceae sp., Rosaceae sp., Lamiaceae sp., Linaceae sp., (Hashiyama *et al.*, 2011; Kalawate *et al.*, 2023)

Family Nolidae; Subfamily Eariadinae

62. *Earias cupreoviridis* (Walker, 1862)

Host plants: *Corchorus* L., *Grewia tiliifolia* Vahl, *Hibiscus* L. sp., *Kydia calycina* Roxb., *Sida cordifolia* L., *Sida rhombifolia* L. (Malvaceae); *Oryza sativa* L. (Poaceae)

Subfamily Nolinae

63. *Nola* sp. (*Nola internella-analis* complex): The photograph could be of an individual of any of the following four species, all of which have a similar fascia and are found in India: *Nola analis* (Wileman & West, 1928), *Nola internella* (Walker, 1864), *Nola pascua* (Swinhoe, 1885), *Nola quadrimaculata* Heylaerts, 1892 (Anonymous, 2023).

Superfamily Pyraloidea; Family Crambidae; Subfamily Crambinae; Tribe Crambini

64. *Morphospecies J*

Subfamily Musotiminae

65. *Morphospecies K*

Subfamily Pyraustinae

66. *Ecpyrrhorrhoe* Hübner, 1825 sp. (previously the genus *Paliga* Moore, 1886)

Subfamily Spilomelinae

67. *Nausinoe perspectata* (Fabricius, 1775)

Host plants: *Jasminum sambac* (L.) Aiton (Oleaceae); *Nyctanthes arbor-tristis* L. (Verbenaceae)

68. *Nausinoe geometralis* (Guenée, 1854)

Host plants: *Chrysojasminum humile* (L.) Banfi, *Jasminum auriculatum* Vahl, *Jasminum flexile* Vahl, *Jasminum grandiflorum* L., *Jasminum multiflorum* (Burm. f.) Andrews, *Jasminum sambac* (L.) Aiton (Oleaceae)

Tribe Herpetogrammatini

69. *Herpetogramma* Lederer, 1863

Tribe Hymeniini

70. *Spoladea recurvalis* (Fabricius, 1775)

Host plants: *Trianthema portulacastrum* L. (Aizoceae); *Achyranthes aspera* L., *Amaranthus* L., *Beta vulgaris* L., *Celosia argentea* L., *Chenopodium album* L., *Gomphrena* L. (Amaranthaceae); *Vigna radiata* (L.) R. Wilczek (Fabaceae); *Plectranthus* L'Hér. (Lamiaceae)

Tribe Margaroniini

71. *Conogethes* Meyrick, 1884 sp.

72. *Omiodes diemenalis* (Guenée, 1854)

Host plants: *Cajanus cajan* (L.) Millsp., *Chamaecrista absus* (L.) H.S.Irwin & Barneby, *Derris elliptica* (Wall.) Benth., *Dendrolobium*

triangulare (Retz.) Schindl., *Glycine max* (L.) Merr.*, Flemingia chappar* Buch.-Ham. ex Benth., *Flemingia paniculata* Wall. ex Benth., *Ougeinia oojeinensis* (Roxb.) Hochr., *Vigna mungo* (L.) Hepper (Fabaceae)

73. *Glyphodes bicolor* (Swainson, 1821)

Host plants: *Alstonia scholaris* (L.) R.Br.*, Carissa carandas* L. (Apocynaceae); *Ougeinia oojeinensis* (Roxb.) Hochr. (Fabaceae); *Tectona grandis* L.f. (Lamiaceae); *Artocarpus integer* Merr., *Ficus benghalensis* L. (Moraceae)

Tribe Nomophilini

74. *Nomophila noctuella* (Denis & Schiffermüller, 1775)

Host plants: *Moricandia arvensis* (L.) DC. (Brassicaceae); *Glycine max* (L.) Merr., *Melilotus officinalis* (L.) Lam., *Medicago sativa* L. (Fabaceae); *Tectona grandis* L.f. (Lamiaceae); *Cenchrus americanus* (L.) Morrone, *Poa pratensis* L., *Trifolium pratense* L., *Trifolium repens* L., *Zea mays* L. (Poaceae); *Polygonum aviculare* L. (Polygonaceae); *Portulaca oleracea* L. (Portulacaceae); *Potentilla canadensis* L. (Rosaceae)

Tribe Spilomelini

75. *Cnaphalocrocis* (syn *Marasmia*) cf. *poeyalis* (Boisduval, 1833)

Host plant: *Oryza sativa* L. (Poaceae)

76. *Cnaphalocrocis medinalis* (Guenée, 1854) (syn. *rutilalis* (Walker, [1859])

77. *Cnaphalocrocis trebiusalis* (Walker, 1859)

Family Pyralidae; Subfamily Pyralinae; Tribe Pyralini

78. *Pyralis pictalis* (Curtis, 1834)

Host plants: *Millettia auriculata* Baker (Fabaceae); *Phoebe lanceolata* (Nees) Nees (Lauraceae); *Populus alba* L. (Salicaceae)

Superfamil Tortricoidea; Family Tortricidae

79. *Morphospecies L*

Superfamily Zygaenoidea; Family Zygaenidae; Subfamily Zygaeninae

80. *Praezygaena caschmirensis* (Kollar, 1844)

Subfamily Chalcosiinae

81. *Eterusia* Hope, 1841 sp. (Caterpillar)

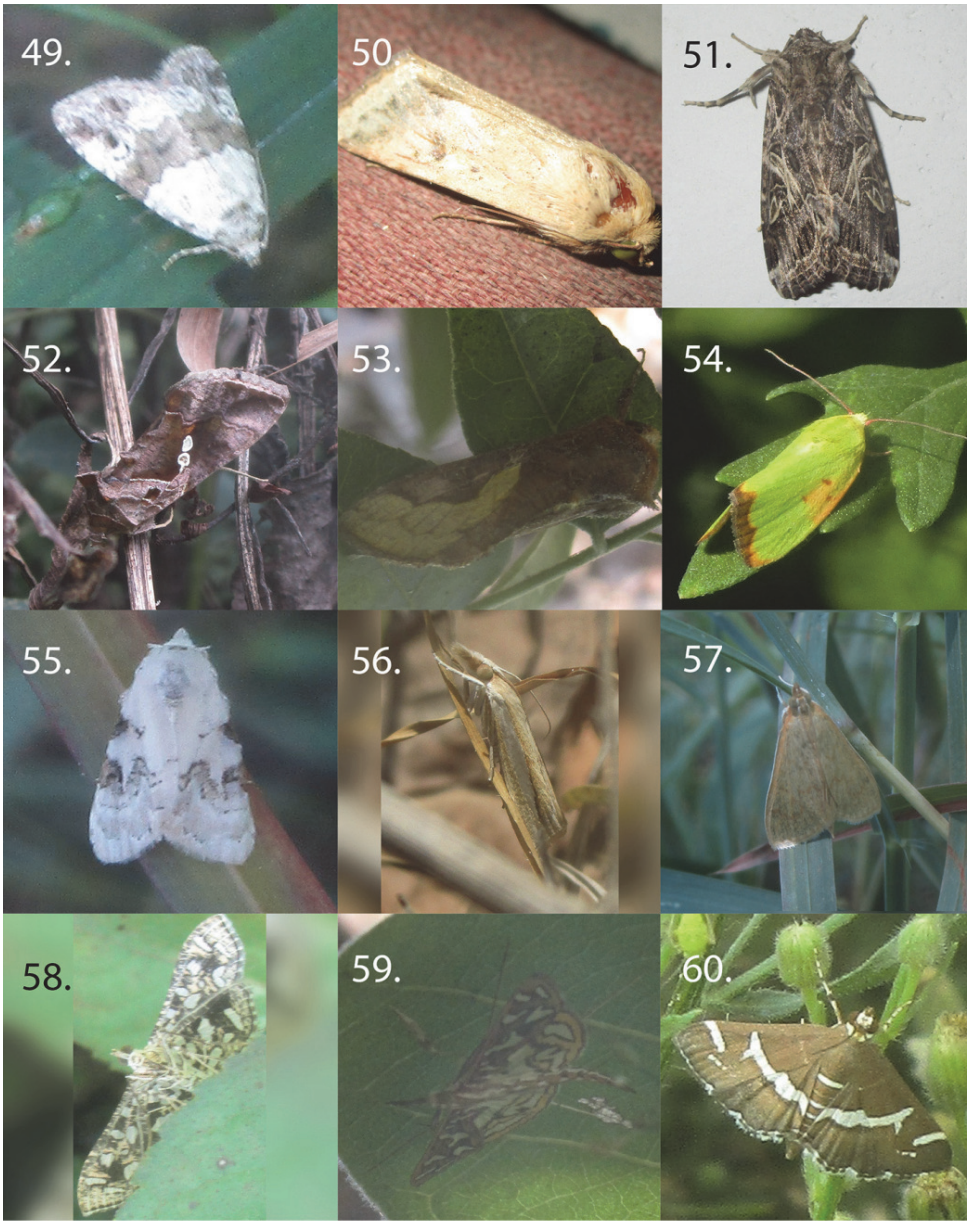
82. *Trypanophora* Kollar, 1844 sp. (Caterpillar)

In this study, at least 82 species were recorded, all of which are new records for Bilaspur district, at least 22 of which are new records for Himachal Pradesh (see supplementary dataset), and at least five of which are new records for the Western Himalayas. Note that 'at least' is used here for a couple of reasons: i) since some records are at state-level resolution with the states having Himalayan and non-Himalayan geography ii) since the identification were not possible to species level for several individuals. The new additions to the Western Himalayas are *Anoba* sp., *Brenthia* sp., *Eublemma cochylioides*, *Herminia undulata,* and *Hyposada hydrocampata*. Some of the unidentified species from this study could be new species to science, altogether. This shows the significance of our study, even though it was not a targeted study to inventorize moths, revealing that even vertebrate-targeted studies can also help in insect biodiversity assessment.

While butterfly diversity was found to be not be indicative of moth diversity at local scales in Colorado, USA (Ricketts *et al.*, 2002), moth and butterfly diversity and abundance was found to be strongly correlated in a study conducted in the Tons Valley, Western Himalayas (Bhardwaj *et al.*, 2012), which is ~150 Km from Bilaspur district (geodesic distance, from the centers of the sites). Given that Tons Valley is quite close to Bilaspur district, it may not be erroneous to assume that this correlation exists in the latter too. This can help us extrapolate or estimate the number of moth species in Bilaspur district.

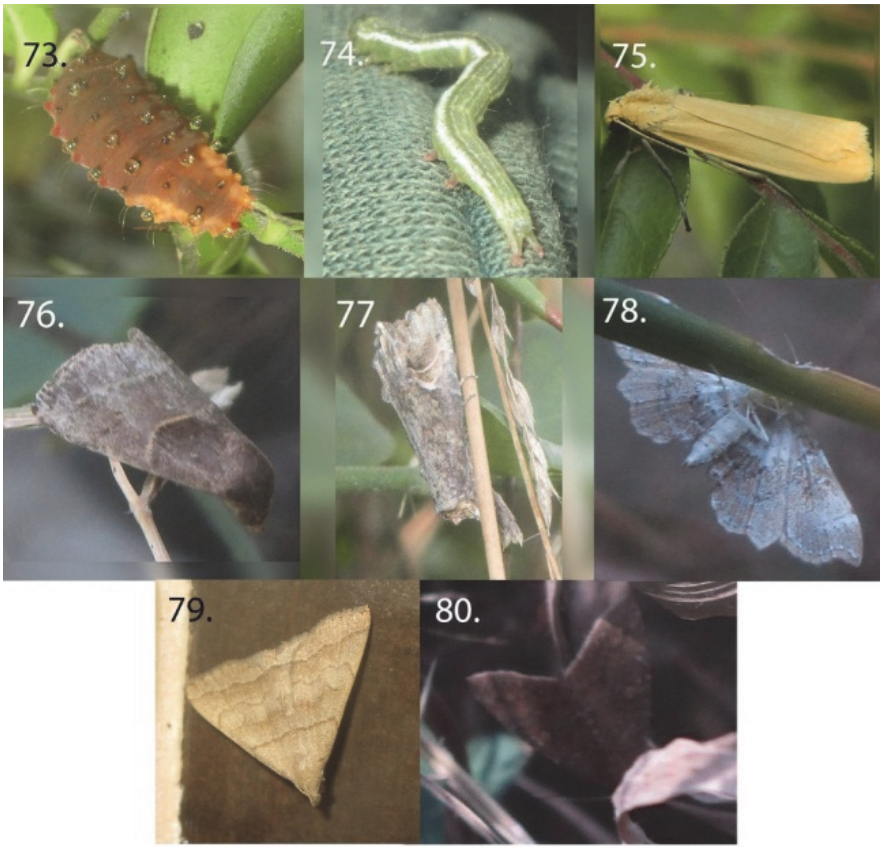
The total number of butterflies species recorded in the study of Lepidoptera in the Tons Valley was

37. *Erebus hieroglyphica*, 38. *Hypopyra or Spirama* sp., 39. *Mocis frugalis*, 40. *Mocis undata*, 41. *Morphospecies H*, 42. *Hydrillodes* sp., 43. *Rhynchina* sp., 44. *Dichromia sagitta*, 45. *Calesia haemorrhoa*, 46. *Condica* sp., 47. *Episteme* sp., 48. *Callopistria* sp.



49. *Maliattha signifera*, 50. *Helicoverpa armigera*, 51. *Spodoptera litura*, 52. *Chrysodeixis* sp., 53. *Thysanoplusia intermixta*, 54. *Earias cupreoviridis*, 55. *Nola* sp., 56. *Morphospecies J*, 57. *Ecpyrrhorrhoe* sp., 58. *Nausinoe geometralis*, 59. *Nausinoe perspectata*, 60. *Spoladea recurvalis*

61. *Conogethes* sp., 62. *Omiodes diemenalis*, 63. *Herpetogramma* sp., 64. *Glyphodes bicolor*, 65. *Nomophila noctuella*, 66. *Cnaphalocrocis* cf. *poeyalis*, 67. *Cnaphalocrocis medinalis*, 68. *Cnaphalocrocis trebiusalis*, 69. *Pyralis pictalis*, 70. *Morphospecies L*, 71. *Praezygaena caschmirensis*, 72. *Eterusia* sp.



73. *Trypanophora* sp., 74. *Morphospecies C*, 75. *Morphospecies F*, 76. *Rivul*a sp., 77. *Morphospecies D*, 78. *Morphospecies K*, 79. *Herminia undulata*, 80. *Amyna* sp.

156 (with an estimate of 163-166), while the moth diversity in terms of morphospecies was 784 (estimate: 873-891), which indicates a ratio of ~5 (range: 5.03-5.37) moth species for every butterfly species. We have recorded close to a 100 species of butterflies in Bilaspur district during the same study period, which is most likely close to the true diversity of butterflies in the district (unpublished data). Applying the same ratio for the number of moth species in Bilaspur district, we get a total of 503-537 species of moths, which means an inventory completeness of 14.1-15.1 per cent. This estimate needs to be taken with a pinch of caution since the elevational range of Bilaspur district (low to mid elevation) is different from that of Tons Valley (mid to high elevation), although there is some overlap between habitat types. This study has shone a light on how poorly studied, many parts of the ecologically sensitive biographical zone of Himalayas are. Studies like this help establish baseline data for further ecological studies.

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Efficacy of bee pollen and beebread against *Salmonella typhimurium* in BALB/c mice

Rajinder Kaur[1] and Abhinav Saxena[2*]

[1]*Department of Zoology, SGGS College, Sector- 26, Chandigarh 160071, India.*
[2]*Department of Zoology and Environmental Science, Punjabi University, Patiala, Punjab 147002, India.*
Email: abhinavsaxena.zoology105@gmail.com

ABSTRACT: The present work evaluate the antibacterial and antioxidant potential of bee pollen and beebread against the toxic changes induced by *Salmonella typhimurium* in BALB/c mice. The experiment was divided into six groups as; Gp1 was normal, Gp2 was infected with *Salmonella enterica* serovar Typhimurium at 2×10^4 CFU, Gp3 and Gp5 were administrated with bee pollen and beebread of *Helianthus annus* crop alone at 250mg kg^{-1} bw respectively; Gp4 and Gp6 were with *S. typhimurium* and bee pollen and beebread *H. annus* at 250mg kg^{-1} bw respectively. Different hematological and oxidative stress parameters were assessed in animals. It has been observed that Gp2 showed alteration in the level of all tested parameters as compared to the Gp1, which indicated the toxicity induced by bacteria, but after the treatment with bee pollen and beebread as in Gp4 and 6, their level ameliorated to near normal, which showed the effectiveness of the tested bee products against bacteria.
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KEY WORDS: Apitherapy, antibacterial, antioxidant, typhoid, *Helianthus annus* crop

INTRODUCTION

Typhoid is caused by rod shaped bacteria, *Salmonella typhi* which is transmitted through contaminated food or water. Symptoms include continued fever, rose like spots on chest, abdomen and back, spleenomegaly and inflammation of intestine. Once bacteria entered in the body through contaminants, it evades the acidic barrier and attaches itself to the epithelium of intestine and then penetrates, divides inside the mesenteric lymph nodes and finally, reaches the blood stream. Here, immune system gets activated and action of antibodies and complement system gets started against the bacteriaEverest *et al.* (2001). As a result, few of them get lysed and release endotoxins, which further cause typhoidal fever. While some cleared from the blood and come in contact with macrophages of liver, spleen, lymph nodes, bone marrow where they survive and again multiply. If untreated, some of the typhoid patients develop complications that include intestinal ulcers and perforation, further leads to bleeding, sudden rise in pulse rate, abdominal discomfort and rigidity (Butler, 2011). A number of synthetic drugs used to treat the bacterial infection. But with time, bacteria develop resistance against these drugs and this phenomenon, by which pathogenic microorganism became resistant to a group of related or unrelated drugscalled multiple drug resistance MDR (Parry*et al.*, 2002). Continue use of these drugs develop a number of undesirable effects in the human body

* Author for correspondence

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that effects the normal functioning of body organs.

Nowadays, research shift towards the development of safe and cost effective natural remedies against MDR. Natural products including bee products have gained greater popularity over widely used synthetic pharmaceuticals. Honey bee products such as honey, beeswax, bee venom, propolis, bee pollen and royal jelly, out of these three (beewax, venom and royal jelly) are chemically synthesized by the bees themselves and other three (pollen, honey and propolis) are derived from plants and are modified by the bees for their own use. All these products have amazing beneficial effects.Bee collected pollen recently drawn more attention as sources for new drug discovery as well as an additive agent to reduce negative effects of popularly used drugs. Honey bees collect pollen from different flowers of the plant in order to feed their larvae. Pollen is a good source of protein and bee required it for the proper growth and maintence of their larvae. Bees collect pollen, modify it with their own abdominal secretions and honey, further they store it in their comb cells for lactic acid fermentation. This ripened product is known as beebread. Chemically, the composition of bee pollen varies according to the age, type and nutritional status of plant species and environmental conditions of the area visited by bees (Almaraz-Abarca *et al.*, 2004). Analysis of chemical composition in previous studies revealed that it contains different types of polphenolic compounds such as flavonoids, phenolic acids, tannins and other compounds (Bonvehi *et al.*, 2001), these compounds are responsible forantibacterial (Morais *et al.*, 2011; Fatrcová-Šramková *et al.*, 2013) and antioxidant properties (Kroyer and Hegedus, 2001; Silva *et al.*, 2006; Moita *et al.*, 2013).The antibacterial and antioxidant effect of polyphenols are mainly due to their redox properties, which are helpful in scavenging free radicals, neutralizing ROS and decomposing peroxides (Nijveldt *et al.*, 2001). Besides, bee pollen also act as dietary supplement that prove beneficial to cope up with the side effects produced by drug therapies and to boost up the immunity at the time of pathological conditions (Radimer *et al.*, 2004). The present work evaluate the antibacterial and antioxidant potential of bee pollen and beebread against the toxic changes induced by *Salmonella typhimurium* in BALB/c mice.

MATERIALS AND METHODS

Bee pollen was collected by installing pollen trap at the hive entrance of the bee colonies, placed in the *Helianthus annus* crop. After that, for the collection of beebread from the comb cells, forceps and spatula were used. Collected samples were stored at -20^{o}C for further experimentations. Aqueous extracts of bee pollen and bee bread were prepared by following the protocols of Nagai *et al*. (2004) and Kaur *et al.* (2013b).

Bacterial strain of *Salmonella typhimurium* (MTCC 98) was obtained from IMTECH (Institute of Microbial Technology), Sector -39, Chandigarh and tested biochemically prior to use according to Bergey's Manual of systemic bacteriology. Further, strain was maintained in the nutrient agar and stored in the form of small aliquots at -20°C before sub culturing.

Determination of minimum inhibitory concentration (MIC): Broth dilution method was performed to test the value of MIC. A series of broth containing test tubes were prepared, to which different concentrations of extracts were added viz., 0mg ml^{-1} (negative control), 50, 100, 150, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380 and 400mg ml^{-1}. After that, inoculate the tubes with standardized suspension 2X 10^{4} CFU of test pathogen. Then incubate the tests tubes at 37°C for 24hours and next day, MIC was determined. Turbidity in the test tubes indicates the presence of bacteria and clear broth or absence of turbidity indicates as absence of pathogen. It is defined as the lowest concentration of the pollen extracts, where no visible growth of bacteria is seen in the test tubes. Determination of minimum bactericidal concentration (MBC): MBC of tested bee products analyzed by transferring aliquots of 0.1ml from MIC test tubes (means test tubes, which show no visible bacterial growth), spreading on Agar plates and incubate at 37°C for 24h. When 99.9 per cent of bacterial population is killed at the lowest concentration of the extract, is regarded as MBC.

BALB/c mice were obtained from the Central Animal House, Panjab University, Chandigarh, India. All the experimental protocols using mice were carried out strictly under the approval of the Animal Ethical Committee, Panjab University, Chandigarh. Mice weighing 20-25g and aged between 4 to 6 weeks were used in all biochemical studies. These were fed on standard pellet diet (Ashirwaad Industries, Kharar, Punjab) and water ad libitum. Animals were acclimatized for one week before the beginning of the research experiments.

BALB/c mice were divided into six groups with 6-8 animals in each. Group 1: Control (Normal mice given saline orally). Group 2: Mice were challenged intraperitoneally with *Salmonella enterica* serovar Typhimurium at 2×10^4 CFU ml^{-1}. Group 3: Animals given bee pollen (250mg kg^{-1} bw) collected from *H. annus* orally for 21 days. Group 4: *Salmonella* infected + water extract of bee pollen collected from *H. annus* (250mg kg^{-1}bw) given orally for 21 days. Group 5: Animals given beebread collected from *H. annus* (250mg kg^{-1}bw) orally for 21 days. Group 6: *Salmonella* infected + water extract of beebread collected from *H. annus* (250mg kg^{-1} bw) given orally for 21 days. Mice in group 2 were sacrificed on day 5 post infection as it was the peak day of infection. Experiments were conducted in triplicate.

Blood was aspirated from jugular vein of animals of different experimental groups in sodium salt of ethylenediaminetetraacetic acid (EDTA). After that, hemoglobin (Hb), red blood cell count (RBC), total leucocyte count (TLC), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were also calculated by following standard protocols.

Organs (Liver, Spleen and Kidney) were homogenized in an ice-cold 100mM potassium phosphate buffer (pH 7.4) containing 150 mM KCl to obtain 10 per cent homogenate (w/v). This homogenates of different tissues was divided into two portions. One portion of homogenate was used for the estimation of LPO (lipid peroxidation; Beuge and Aust, 1978) and GSH (glutathione; Moron *et al*., 1979). Other portion was subjected to cold centrifugation at 10,000g for 30 min. The pellets were discarded and supernatants regarded as post-mitochondrial supernatant, were used for further estimation of other enzymes such as SOD (superoxide dismutase; Kono, 1978) and CAT (catalase; Luck, 1971), GST (glutathione-S-transferase; Habig *et al*., 1974), GP (glutathione peroxidase; Pagila and Velentine, 1967) and GR (glutathione reductase; Carlberg and Mannervik, 1985). The quantity of protein in homogenate and supernatant of different samples was determined by the method of Lowry *et al*. (1951). The data was presented as a mean ± standard error and analyzed by ANOVA and Student t-test. p values of 0.05, 0.001 and 0.0001 were considered to be significant, very significant and extremely significant, respectively.

RESULTS AND DISCUSSION

MIC and MBC activities of bee pollen and beebread investigated to evaluate their antibacterial activity against gram negative bacteria i.e. *S. typhimurium,* revealed that both extracts were potentially effective in suppressing the bacterial growth with different potency, but beebread was the effective to retard the growth of the tested pathogenic bacteria as compared to bee pollen. Antimicrobial activities of bee pollen and beebread were 320 and 260mg ml^{-1} respectively for MIC; it was 380 and 320mg ml^{-1} respectively for MBC.

In mice, alterations in the blood parameters and antioxidant enzymes were observed in G2 as compared to the G1 but after the treatment, these alterations were ameliorated to the near normal in G4 and G6 (Table 1, 2).

The problem of multi drug resistance is growing by time therefore; actions must be taken to face this problem by introducing the safe and cost effective, natural products (Nostro *et al.*, 2000; Osman *et al.*, 2013). Apitherapy is the alternative branch of medicine that deals with the use of the honey bees and their different products. They are also known as Master alchemists as their products have many beneficial effects. The use of bee venom for the treatment of rheumatoid arthritis and joint problems and honey for the cure of common cold, cough and

Table 1: Hematological alteration with different groups due to bee products

Parameters	Gp1	Gp2	Gp3	Gp4	Gp5	Gp6
Hb	13.4 ± 0.34	9.69 ± 0.39@	13.5 ± 0.31^	12.5 ± 0.76^	13.3 ± 0.43^	13.3 ± 0.52^
RBC	8.64 ± 0.24	5.7 ± 0.54@	8.7 ± 0.43^	7.8 ± 0.73^	8.2 ± 0.97^	7.5 ± 1.35*
MCH	19.30 ± 1.22	32.91 ± 2.6@	19.59 ± 2.92^	26.94 ± 1.59#	18.70 ± 3.28^	21.94 ± 3.52^
MCV	52.46 ± 2.29	71.97 ± 3.97@	53.38 ± 2.47^	65.04 ± 2.46*	50.14 ± 4.30^	56.63 ± 2.21^
MCHC	29.73 ± 3.11	35.89 ± 1.19@	29.12 ± 2.23^	30.10 ± 3.43^	29.00 ± 4.25^	31.82 ± 2.46^
Hematocrit	39.52 ± 1.71	26.87 ± 1.03@	40.08 ± 2.59^	32.84 ± 2.09#	38.62 ± 1.39^	36.40 ± 0.80^
WBC	7767.81 ± 66.79	5620.97± 51.25@	7861.11± 48.45^	6569.57± 50.05^	7711.43±2.62^	6681.71±7.20#
Lymphocytes	67.23 ± 1.24	87.21 ± 0.70@	68.44 ± 0.71^	79.35 ± 2.69 ^	69.34 ± 1.98^	74.27 ± 1.87^
Neutrophils	25.42 ± 1.30	16.24 ± 0.58@	24.82 ± 0.63^	23.01 ± 2.74^	25.78 ± 0.75^	22.63 ± 0.60^
Eosinophils	1.00 ± 0.82	2.29 ± 0.76	0.86 ± 0.69*	1.14 ± 0.90	1.29 ± 0.95	1.14 ± 0.69

allergies are well documented in our ancient epics (Haleem *et al.*, 2015; Kaur *et al.*, 2015). With time, the therapeutic affects other bee products, also came to light (Kalia *et al.*, 2017).

In the present study, a number of blood parameters tested to investigate the effect of bee pollen and beebread against the *Salmonella*. There was extremely statistically significant (except in case of eosinophils) alterationsin the level of blood parameters were recorded in the Gp2as compared to the Gp1but after the administration of bee pollen and beebread (Gp4 and 6), these alterations were restored to the near normal, which showed the effectiveness of the bee products. This restoration was up to extremely statistically significant except in case of MCH and hematocrit, which were very statistically significant and in case of MCV, which was statistically significant in case of Gp4 and also in case of Gp6, the level of blood parameters were up to extremely statistically significant except in case of WBC, which was very statistically significant and in case of RBC, which was statistically significant. The inhibitory effects of bee pollen against bacterial infection were also reported by Aboude *et al.* (2011) and Sramkova *et al.*(2013).

Phytochemical screening of bee pollen and beebread revealed several types of secondary metabolites such as alkaloids, polyphenols, flavonoids, coumarins, saponins, tannins and steroids (Kaur *et al.*, 2013a and b). These molecules are act actively against pathogenic microorganisms, as antibacterial and antioxidant activities (Tsopmo *et al.*, 2013; Erfan and Marouf, 2019). The steroids exerts its action by forming complex with membrane lipids and thus causing leakage of enzymes and other components from cell, which in turn effect the stability of the bacterial cell (Marjorie, 1999), similarly Saponins have detergent like properties that cause leakage of proteins and important enzymes from cell (Shimada, 2006). Further, tannins present in these apicultural products have ability to react with the protein components of the cell wall of the bacteria to form a stable insoluble component, which in turn effects the functioning of the cell (Dangoggo *et al.*, 2012) while, the antibacterial effects of alkaloids are due to its ability to form interchelate with nucleic acid of both Gram positive and negative bacteria and interfere with cell division (Bukar *et al.*, 2015).

Antioxidant activity of bee pollen and beebread also assessed in liver, spleen and kidney of the mice (Table 2). In Gp2, oxidative stress was increased as compared to Gp1 but restoration was observed after the treatment with bee pollen and beebread in Gp 4 and Gp 6.Peroxidation of lipids induced due to generation of ROS and results in oxidative stress. In present study, there is an increase in the level of LPO in the infected group as compared to the normal group, which indicates the production of

Table 2. Activity of antioxidant enzymes in different groups of Liver, Spleen and Kidney

Organs	Biochemical	G1	G2	G3	G4	G5	G6
Liver	LPO	0.21 ± 0.02	0.49 ± 0.03@	0.18 ± 0.02^	0.29 ± 0.01^	0.17 ± 0.01^	0.24 ± 0.04^
	GSH	1.6 ± 0.35	0.62 ± 0.11@	1.8 ± 0.38^	1.43 ± 0.06*	1.88 ± 0.31^	1.57 ± 0.12#
	SOD	9.6 ± 2.18	3.67 ± 2.4@	12.77 ± 1.29^	7.62 ± 1.41^	13.03± 1.65^	9.47 ± 1.3^
	CAT	74.81± 2.09	46.03 ± 1.99@	75.77± 1.42^	61.37± 4.03^	76 ± 3.0^	67.48± 2.14^
	GST	0.86 ± 0.04	0.43±0.03@	0.97 ± 0.03^	0.8 ± 0.02^	1.03 ± 0.06^	0.83 ± 0.07^
	GR	49.35± 1.32	32.8 ± 1.86@	52.5 ± 1.22^	43.99± 1.33^	52.97± 1.13^	48.27± 1.56^,á
	GP	13.28± 0.89	6.43 ± 0.54@	14.92± 0.82^	10.83± 0.87^	15.21± 1.1^	12.38± 0.42^,á
Spleen	LPO	0.30 ± 0.02	0.48 ± 0.03@	0.28 ± 0.01^	0.35 ± 0.04^	0.27± 0.02^	0.33 ± 0.02^
	GSH	1.33 ± 0.15	0.67 ± 0.12@	1.5 ± 0.1^	1.08 ± 0.08*	1.56 ± 0.15^	1.26 ± 0.06^,á
	SOD	10.7± 3.27	5.5 ± 1.85@	12.67 ± 2.67^	7.99 ± 2.01#	12.97 ± 1.5^	9.76 ± 3.7^
	CAT	73.17± 1.75	27.2±1.78@	75.1 ± 5.63^	64.2± 2.69^	75.3± 4.03^	67.0± 3.83^
	GST	0.74 ± 0.04	0.23 ± 0.04@	0.90 ± 0.05^	0.69 ± 0.03^	0.92 ± 0.03^	0.73 ± 0.03^
	GR	52. 45±1.21	36.16 ±1.59@	54.44± 1.23^	45.04± 1.59^	54.94± 1.76^	48.33 ± 1.40^
	GP	9.46 ± 0.6	5.27 ± 0.32@	11.12± 0.8^	7.87 ± 0.23^	11.36± 0.32^	9.31 ± 0.56^,á
Kidney	LPO	0.11 ±0.006	0.18 ± 0.006$	0.09 ± 0.02#	0.17 ± 0.006	0.08 ± 0.06^	0.12 ± 0.001á
	GSH	1.23 ± 0.23	0.75 ± 0.05%	1.39 ± 0.01^	1.14 ± 0.05*	1.43 ± 0.13^	1.26 ± 0.09^
	SOD	10.82 ±4.25	8.2 ± 1.7@	13.0 ± 1.57^	8.17 ± 1.20	13.53 ±2.07^	9.93 ± 1.90^
	CAT	79.8 ± 2.88	60.21 ±1.05@	80.8 ± 1.19^	74.28 ± 1.11^	80.94 ±3.60^	77.9 ± 2.19^
	GST	0.71 ± 0.02	0.52 ± 0.03%	0.91 ± 0.03^	0.67 ± 0.03*	0.96 ± 0.03^	0.71 ± 0.04#
	GR	78.8 ± 1.55	75.48 ± 1.84$	79.9 ± 1.98^	71.19 ± 3.71^	80.3 ± 4.17^	75.95 ± 1.3
	GP	11.16 ±0.66	8.13 ± 0.74@	12.1 ± 0.37^	9.3 ± 0.78	12.35 ±1.02^	10.79 ± 0.23#,á

Gp 1 v/s Gp 2 $ p<0.05 (statistically significant); %:p < 0.001 (very statistically significant); @:p < 0.0001 (extremely statistically significant)

Gp 2 v/s Treated groups * : p < 0.05 (statistically significant); # : p < 0.001 (very statistically significant); ^ : p < 0.0001 (extremely statistically significant)

G4 v/s G6 (á : p < 0.05; â: p < 0.001; &: p < 0.0001)

(Gp 1: Normal mice - administered with normal saline orally. Gp 2: Infected mice - administered intraperitonelly 0.1 ml of 2×10^4 CFU/ml of *Salmonella typhimurium*. Gp 3: Normal mice administrated bee collected pollen from *Helianthus annus* orally. Gp 4: Treatment with bee collected pollen from *H. annus* (orally) in *S. typhimurium* infected mice. Gp 5: Normal mice administrated bee bread from *H. annus* orally. Gp 6:Treatment with bee bread from *H. annus* (orally) in *S. typhimurium* infected mice)

oxidative stress. Administration of bee pollen in Gp 4 and 6 significantly reduced the adverse effect induced by the bacteria. Glutathione is the most abundant cellular antioxidant, prevents damage to important cellular components by neutralizing the free radicals (Pompella *et al.*, 2003). Oxidative stress deplete the GSH level in Gp2 as compared to the Gp1, which adversely affects the cellular thiol redox balance and in turn makes the cells more susceptible to a number of internal stresses. But in treatment groups, i.e. Gp 3 and 4, the level of GSH raised to near normal, indicating the effectiveness of bee pollen and beebread. Similarly, in case of Gp2 the other level of other antioxidants such as SOD, CAT, GST, GR and GP decreased as compared to Gp1 but after the administration of

bee pollen and beebread, the level of theses enzymes ameliorate up to the normal level (Kaur *et al.*, 2018, 2020). Superoxide radicals generation occurs in the body, when oxygen gains an extra electron produced during bacterial infection and other metabolic processes by different enzymatic reactions. Superoxide dismutase helps to detoxifying the highly reactive superoxide radicals into H_2O_2 and oxygen. Here, the H_2O_2 acts as a pro-oxidant for the cells and is thus, converted into simple water and oxygen with the help of enzyme CAT and GPx using either an manganese or iron as cofactor (Chelikani *et al.*, 2004). Alone administration of bee pollen and beebread did not harm the mice (Kaur *et al.*, 2014; Kaur *et al.*, 2022, 2023). The therapeutic effects of apicultural products are a new hope to combat the dangerous threats caused by increasing evidence of antimicrobial resistance. It was investigated that bee pollen and beebread of *H. annus* showed therapeutic potential such as antibacterial and antioxidant activities against typhoid bacteria but these activities were in case of beebread as compared to bee pollen. Besides this, alone administration of these two apicultural products did not harm the animal model showing the protective effects.

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Bacillus thuringiensis israelensis VCRC B650 culture filtrate useful for mosquito oviposition attractant and larvicidal action

Jibi Lukose, Kakhuangailiu Gangmei, Bhagyashree Bora, P. Hemaladkshmi, V. Abhisubesh, Sahadiya Mandodan, K. Aneha, A. Mathivanana, I. Geetha and S. Poopathi*

Unit of Microbiology and Immunology, ICMR-Vector Control Research Centre, Department of Health Research, Ministry of Health and Family Welfare, Puducherry- 605006, India.
Email: Subbiahpoopathi@rediffmail.com

ABSTRACT: Oviposition attractants hold the potential attention for monitoring and controlling mosquitoes by enticing them to deposit eggs at specific locations. In this study, the bacterial culture filtrate of *Bacillus thuringiensis* var. *israelensis* VCRC B650, previously isolated from clay soil at an agricultural site in U.T of Puducherry, was investigated for the first time. This larvicidal bacterium was assessed for oviposition attractancy against dengue and filarial vectors, namely, gravid females of *Aedes aegypti* and *Culex quinquefasciatus*, respectively. The investigation revealed that, at 1:1 dilution of bacterial culture filtrate and water, *Cx. quinquefasciatus* exhibited significant attraction, with an oviposition activity index (OAI) value of +0.92, surpassing the standard threshold value of +0.30. The OAI of *Bti* culture filtrate at a 1:1 dilution was also compared with a standard oviposition attractant, p-cresol, at a concentration of 10ppm. It was observed that the *Bti* culture filtrate demonstrated a significant oviposition attractant effect, with 80 per cent more egg rafts laid, compared to p-cresol at 20 per cent. GC-MS analysis showed that there were 25 compounds present in the *Bti* culture supernatant, and out of those, only one compound, namely, benzaldehyde, was effective in showing oviposition attraction.
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KEY WORDS: *Bti* culture filtrate, oviposition attractant, oviposition activity index, mosquitocidal activity, *Aedes aegypti, Culex quinquefasciatus*

INTRODUCTION

Mosquito species exhibit a wide range of behaviors when selecting suitable locations to deposit their eggs. Choosing the right site is a crucial aspect of the mosquito's reproductive cycle, given the potential risks associated with factors, such as, temporary water bodies, prolonged droughts, harsh winter conditions, and insufficient nourishment for larvae. The careful selection of an egg-laying site becomes paramount for the survival and successful development of mosquito offspring. These behaviors ensure that eggs are placed in environments conducive to the thriving development of mosquito larvae, ultimately leading to the emergence of the next mosquito generation (Eitam and Blaustein,

* Author for correspondence

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2004). Understanding these diverse, oviposition behaviors is essential for devising effective strategies to monitor and control specific mosquito species that carry diseases (Wong *et al*., 2012). To produce a batch of eggs, female mosquitoes require a blood meal for the necessary protein. The breakdown of this blood meal is influenced by temperature, requiring two to three days in tropical areas and five to eight days in temperate zones. During the digestion process, byproducts like aminoacids are taken up by the fat-body, where the synthesis and release of vitellogenin (Vg), a glycophospholipoprotein, occur into the haemolymph. Subsequently, Vg is conveyed to the ovaries, where oocytes in the follicular epithelia absorb it (Wu *et al*., 2021). Various meteorological factors, such as, temperature, rainfall, relative humidity, wind, and others impact the flight and oviposition of gravid females. These females employ visual and olfactory cues while in flight to locate potential oviposition sites. The determination of a suitable oviposition site involves the use of visual, olfactory, and tactile signals (Beehler *et al.,* 1993; Day 2016). In the quest for suitable oviposition sites, mosquitoes rely on long-range infochemicals like pheromones for detecting their presence (Okal *et al*., 2013). As they approach a potential site, mosquitoes switch to short-range infochemicals to distinguish between suitable and unsuitable breeding locations for their upcoming generation. Once at an oviposition site, infochemicals become pivotal for gravid females in assessing the chemical characteristics of the potential habitats for immature stages. Mosquitoes employ contact stimuli to evaluate factors such as, water quality before engaging in oviposition (Mwingira *et al.,* 2020).

Chemical cues can elicit effective oviposition responses across mosquito species, with some species-specific cues identified in previous studies (McCall and Eaton 2001). Variations in breeding habitat preferences exist among different mosquito species, and these ecological distinctions influence the approach to their surveillance and control (Allgood and Yee, 2017). Microorganisms generate chemical cues that interact with chemosensory receptors (such as, antennae and mouth parts) and subsequently engage with other factors like pH and alkalinity when choosing locations for egg laying (Ponnusamy *et al*., 2008). The chemical cues from microorganisms may either act as repellents or stimulants for mosquito oviposition, depending on their concentration (Seenivasagan *et al*., 2014). Numerous studies have explored the potential of different plant substances, such as, cow grass, bamboo leaf infusions, barmuda grass infusion and hay infusions, to attract for oviposition (Millar *et al*., 1992; Ponnusamy *et al*., 2010). Natural attractants also include the temperature of humans, which has been identified as more appealing to mosquitoes (Schreck *et al.*, 1990; Ellwanger *et al*., 2021). The effectiveness of oviposition attractants is largely determined by the concentration of the infusion (Iyyappan *et al*., 2022). Studies indicate that bacteria obtained from hay infusion, specifically *Aerobacter aerogenes*, release volatile compounds that stimulate the oviposition of *Ae. aegypti* and *Cx. quinquefasciatus* gravid females (Hazard *et al*., 1967). Likewise, gravid *Cx. quinquefasciatus* has exhibited an attraction to protein hydrolysate solutions contaminated with bacteria (Beehler *et al*., 1994). The cell-free filtrate from *B. cereus*, *B. thuringiensi*s, and *Pseudomonas fluorescens* has demonstrated attractancy for gravid female mosquitoes of *Cx. quinquefasciatus* (Poonam *et al*., 2002). The chemical cues generated by microorganisms, in terms of quantity, compound nature, and composition, are likely to vary among bacterial species, influencing mosquito oviposition (Ponnusamy *et al*., 2015). The propensity of *Culex* mosquito species to deposit eggs in freshwater pools which are highly organic has been utilized to design effective gravid traps, for monitoring mosquito groups. Likewise, the preference of *Aedes* mosquito species for laying eggs in synthetic/plastic vessels has been leveraged to create ovitraps for monitoring and management purposes (Day, 2016).

A deeper comprehension of the diverse range of oviposition behavior of mosquitoes can enable the creation of innovative surveillance and management tools aimed at addressing other significant mosquito vectors that transmit diseases affecting both humans and animals. In the current study, explorative research was carried out to evaluate the oviposition attractancy of culture filtrate of

newly isolated *B. thuringienesis israelensis* VCRC B650 against gravid mosquitoes.

MATERIALS AND METHODS

Bacillus thuringiensis var. *israelensis* VCRC B650, a bacterial strain known for its mosquito larvicidal properties, was used for this study. Formerly, the bacterial strain was isolated from the clay soil from agricultural field of Bahour village, U.T of Puducherry, India. Nutrient Yeast Extract Mineral Salt Medium (NYSM) with the following constitutions per 100ml (wt./v %): 500mg of D-glucose, 500mg of peptone, 500mg of sodium chloride, 300mg of HM peptone, 500mg of yeast extract, and 1ml of a salt solution (containing magnesium chloride (20.3g), manganese chloride (1g), and calcium chloride (10.2g) mixed in one liter distilled water with pH adjusted to 7) was used for culturing the bacteria VCRC *Bti* B650 (Yousten *et al*., 1984; Hemaladkshmi *et al*., 2023). The bacterial culture derived from glycerol stock/agar slant was inoculated into 10 ml of the NYSM medium in a test tube using a loop. Subsequently, it was placed on a rotary shaker and incubated for 8 hours at 250-300 rpm and 28 to 30°C. There after, the bacterial culture was inoculated into a 250 ml a flask containing 50ml of NYSM medium and then incubated for a duration of 10 hours. Finally, 10ml of the inoculum was transferred to a 2 L flask containing 500ml of production medium, and it was then subjected to a 72 hour incubation period. The cell pellet was collected through centrifugation at 10,000 rpm for 10 minutes. After discarding the cell pellet, the culture filtrate was utilized as the test substance for oviposition attractancy evaluations.

Mosquito eggs collected from Rearing and Colonization Facility (RCF) in the parent institution, were placed in trays filled with dechlorinated water for hatching. The resulting mosquito larvae were raised in water and nourished with a combination of finely ground dog biscuits and yeast. After undergoing metamorphosis into pupae, they were collected and transferred to a bowl of water, before being introduced into mosquito cages measuring 23L×23B×23H cm, where the adult mosquitoes emerged. Subsequently, the adult mosquitoes were reared in cages and were provided with freshly soaked raisins as their primary diet. Later on, female mosquitoes were given a diet of fresh chicken blood on the third day after emerging. These gravid female mosquitoes were then used for the experimental procedures.

Optimal oviposition attraction: Three to five days old females of *Cx. quinquefasciatus* were fed on chicken blood and maintained on raisins soaked with water for 48 hours, at a temperature of 28 ± 2°C and a humidity level ranging from 70 to 80 per cent. Mature blood fed female mosquitoes were selected for evaluating the oviposition attraction to different substances. Various dilutions (1:1, 1:10, 1:20, and 1:50) of the study samples were concocted with water. 200 ml of each test dilution was poured into wax coated disposable paper cups with a capacity of 250ml, these cups were then placed inside a mosquito cage measuring 55L x 55B x 55Hcm. The Nutrient Yeast Extract Salt (NYSM) medium served as a control. In each cage, 100 fully gravid female mosquitoes were released. Three cages were utilized for each test and five disposable cups were consistently positioned in the cage for all the experiments. Four cups, each containing a distinct concentration of the bacterial culture filtrate, were arranged at the corners, while the cup, containing NYSM (used as a control), was positioned at the center of the cage. The cages were kept at a temperature of 28 ± 2°C and a relative humidity of 70-80 per cent. The study was initiated at 4pm (16:00 hour) and monitoring for the egg rafts, were conducted at 10am (10:00 hour), the following day. The count of egg rafts deposited in each cup was recorded, and the percentages deposited in various cups were determined based on the overall number laid, which included the control. The procedure was replicated thrice in different days, with changes in the position of disposable cups on each occasion (Geeta *et al*., 2003).

Oviposition-activity index (OAI): OAI of the test sample was assessed by positioning it inside a cage, alongside an additional cup containing NYSM as a control sample. The bacterial culture filtrate

was tested at various dilutions (1:1, 1:10, 1:20 and 1:50). As a standard oviposition attractant, p-cresol (10 ppm), was used (Bentley *et al*., 1979). The OAI was determined utilizing the following formula (Hwang *et al*., 1982):

OAI = Nt - Ns/Nt + NS

Nt represents, total-number-of -egg-rafts in-the-test-sample; Ns represents, total-number-of -egg-rafts in-the control Compounds exhibiting OAI of +0.3 and higher are categorized as attractants, whereas those with -0.3 and lower are classified as repellents (Hwang et al., 1982).

Comparative analysis with p-cresol: The attractancy of the different dilutions of bacterial culture filtrate for oviposition was individually assessed, comparing each with p-cresol by assessing them at concentrations where their attractancy is optimal. In each case, a cup with the bacterial culture filtrate and another cup with p-cresol were positioned at diagonally opposite corners of the cage. The counting and recording of the egg rafts were conducted in accordance with the previously specified procedures. The percentage of egg rafts deposited on each culture filtrate was computed in relation to the overall count, encompassing both the culture filtrate and p-cresol.

The data underwent statistical assessments using the Mann-Whitney U test to evaluate the significance of variation in oviposition attractancy. The Mann-Whitney U-test was used to evaluate the distinction between data of different groups. The level of significance (P values) less than 0.05 was considered as statistically significant. Complete statistical studies were done using STATA 14.2. These analyses aimed to determine whether there were significant variations in attractancy between the test preparations and p-cresol.

The aforementioned procedure was replicated with *Ae. aegypti* to investigate their attractancy to *Bti* VCRC B650.

Gas Chromatography- Mass Spectrometry: The bacteria culture filtrate of *Bti* VCRC B650 was lyophilized in freeze dryer until it formed dry powder. The powder obtained was then dissolved in methanol. The sample was sent for analysis for volatile component present in the filtrate which causes the mosquito to attract and enable them to oviposit. The analysis was done using gas chromatography- mass spectrometry with NIST library search in Sophisticated analytical-instrumentation facility (SAIF) at Indian-Institute-of-Technology, Madras. The components present in the culture filtrate were identified. A comprehensive literature review was conducted to identify compounds that are known to attract mosquitoes for oviposition. Through this review, a specific compound was pinpointed that has been documented to influence mosquito behavior, particularly in the context of egg-laying. This identification was crucial in understanding the potential role of this compound in guiding mosquito oviposition, thereby providing insights into its application in research or mosquito control strategies.

Mosquito larvicidal activity of the culture filtrate: Bioassay was conducted using bacterial culture filtrate at various dilutions (1:1, 1:10, 1:20, and 1:50) to evaluate its toxicity against first and second instar larvae of three mosquito species: *Cx. quinquefasciatus*, *Anopheles stephensi*, and *Ae. aegypti*. The experiment was set up in wax-coated paper cups, each containing 100 ml of the test dilutions. For comparison, a nutrient yeast salt medium (NYSM) was used as the control. In each bioassay, 25 larvae from each species were introduced into the cups containing the different culture filtrate dilutions. Four replicates were prepared for each test dilution, as well as for the control. Mortality rates of the larvae were observed and recorded at 24 hours post-exposure. The experiments were performed under controlled laboratory conditions, ensuring consistent relative humidity and temperature. To ensure the reliability of the results, the bioassay was repeated three times on different days.

Additionally, an experiment was conducted to assess the oviposition and subsequent larvicidal activity of the bacterial culture filtrate dilutions. Female mosquitoes were attracted to lay their eggs

in the various culture filtrate dilutions, once the eggs or egg rafts were deposited in the diluted culture filtrate, the containers were left undisturbed to allow the eggs to hatch naturally. The development and mortality of the larvae were closely monitored and recorded at 24 hours after the eggs had hatched.

RESULTS AND DISCUSSION

Optimum concentration for oviposition attractancy: From the initial experiment, the most effective bacterial culture filtrate dilution was found out to be 1:1 dilution in case of *Cx. quinquefasciatus* and 1:50 in case of *Ae. aegypti*. In case of *Culex* maximum number of 47 egg rafts were found in 1:1 dilution, 15 in 1:10 dilution, 7 in 1:20 dilution, 6 in 1:50 dilution and 1 in NYSM (control). In case of *Ae. aegypti* the higher oviposition was observed in 1:50 dilution with about 1862 eggs, 652 eggs in 1:20 dilution, 517 eggs in 1:10 dilution and 92 eggs in 1:1 dilution (Table 1) (Fig. 1).

Oviposition activity index (OAI): The analysis of OAI for *Bti* VCRC B650 bacterial culture filtrates revealed that all four dilutions exhibited oviposition attractancy against *Culex.* mosquitoes. The indices were 0.94, 0.83, 0.71, and 0.54 for dilutions 1:1, 1:10, 1:20, and 1:50, respectively (Fig. 2). These values exceeded the threshold of 0.3, indicating that these dilutions are considered attractants. However, for *Aedes*, the OAI values were 0.23, -0.26, -0.36, and -0.84, all of which were below the required threshold of 0.3 to be considered attractants (Fig. 3). Consequently, in the case of *Aedes*, the *Bti* B650 culture filtrate dilutions did not demonstrate oviposition attractancy.

Comparative analysis of test sample with p-cresol: When the oviposition attraction of culture filtrate with dilution 1:50 of *Bti* VCRC B650 was assessed against p- cresol which is a known oviposition attractant, at 10ppm the former was found to be more attracting for oviposition in case of *Cx. quinquefasciatus* with 80 per cent egg laying than the later with 20 per cent egg laying (Fig. 4). The proportion of eggs deposited in the culture filtrates for *Aedes aegypti* was lower than the quantity laid in p-cresol.

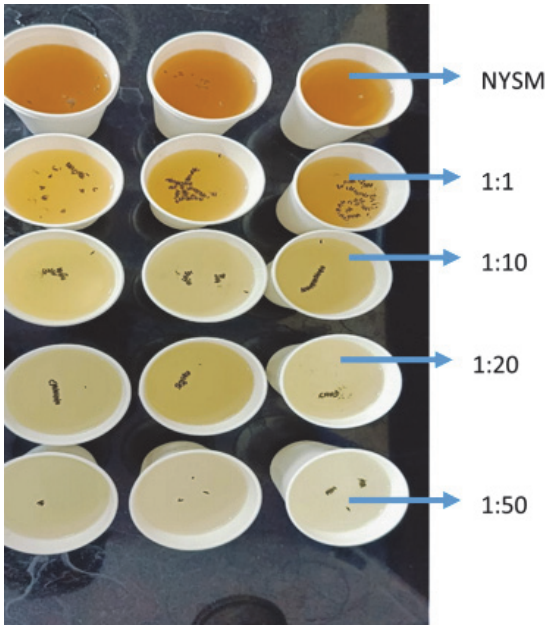

Fig. 1 *Culex quinquefasciatus* egg rafts in different concentrations of bacterial culture filtrate

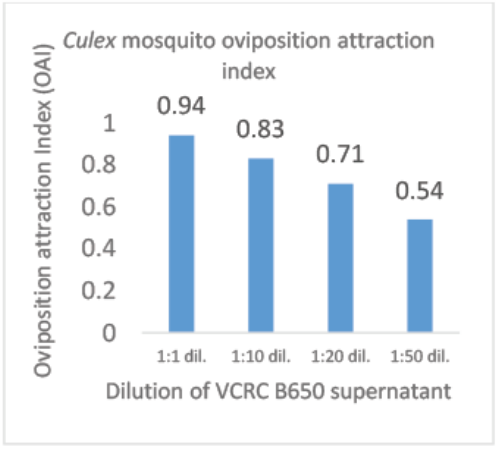

Fig. 2 Oviposition attraction index (OAI) against *Culex quinquefasciatus*

Gas chromatography- mass spectrometry: The analysis of *Bti* VCRC B650 bacteria culture filtrate through GC-MS revealed the presence of nine prominent compounds.

Through an extensive literature survey, it was determined that benzaldehyde has been documented in scientific literature as an attractant for *Aedes aegypti* and *Culex pipiens* (Dormont, 2021; Otienoburu *et al*., 2012). This finding suggests that the identified compound holds promise

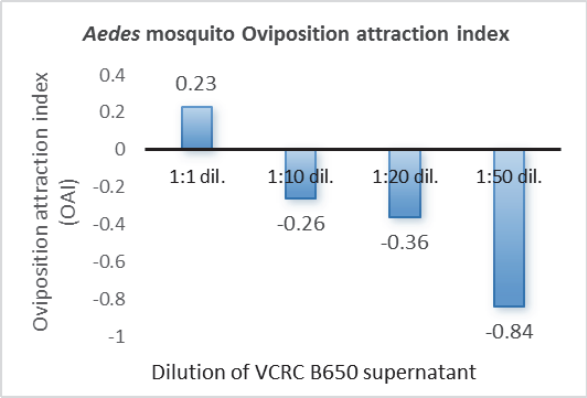

Fig. 3 Oviposition attraction index against *Aedes aegypti*

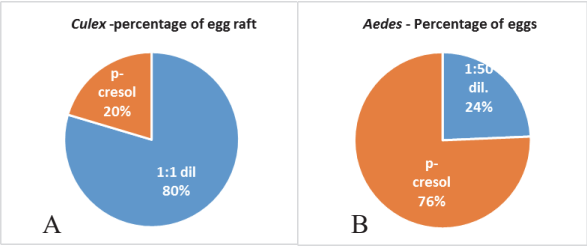

Fig. 4 Comparative analysis of the oviposition attractancy of the Bti. VCRC B650 in relation to p-cresol (A) with Culex mosquito (B) with Aedes mosquito

as a potential oviposition attractant for *Culex* species, indicating its potential for further exploration in mosquito behavior studies and control strategies, particularly in areas susceptible to vector-borne diseases (Table 3) (Fig. 5).

Mosquito larvicidal activity of the culture filtrate: The culture filtrate dilutions demonstrated significant mosquito larvicidal activity. In all dilutions tested, the larvae mortality was observed within few hours of hatching, indicating a potent mosquito larvicidal effect. In contrast, the control group (NYSM), showed no significant larval mortality, confirming that the observed lethality was directly attributable to the culture filtrate. In 1:1 dilution and 1:10 dilution the 1st instar larvae died within few hours of exposure. After 24 hours of exposure 100 per cent mortality was observed in all the bioassay cups (Table 4). The experiment demonstrated that even the lowest dilution, where 2 ml of culture filtrate was mixed with 100 ml of water, resulted in

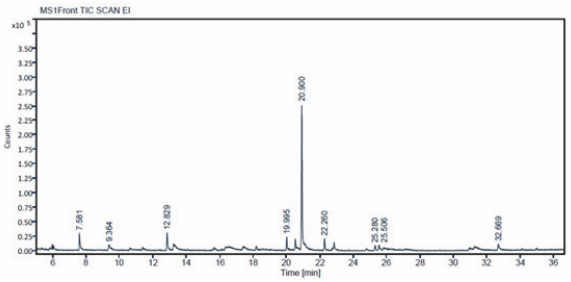

Fig. 5 Total ion chromatogram of Bti VCRC B650 culture filtrate

complete mortality of 1st and 2nd instar larvae within just 24 hours of exposure. This indicates the high potency of the culture filtrate, as it effectively caused 100% mortality at a relatively low concentration, proving its larvicidal activity against the early larval stages.

This high mortality rate among the larvae is likely due to the presence of secondary metabolites in the supernatant of the bacterial culture. These compounds, potentially produced during bacterial growth, appear to be highly effective in disrupting the development of mosquito larvae immediately after hatching. This experiment provided additional insights into the efficacy of the bacterial filtrate in preventing larval development, thus furthering the understanding of its potential as a mosquito control agent.

An attempt was made using the bacterial culture filtrate (culture supernatant), which is usually discarded as waste after harvesting the cell mass. These extracts significantly attract the gravid mosquitoes. In addition to this, the newly emerged first instar larvae died immediately. Literatures says that the chemical ecology of mosquito oviposition behavior has the potential to enhance our ecological knowledge regarding the source, function, and importance of natural organic compounds that play a role in interactions among mosquito species and their environment. Observing these interactions in their natural setting and understanding the compounds involved could pave the way for innovative strategies in the control and surveillance of mosquitoes and the diseases they transmit. Many current mosquito control methods primarily target

Table 1. Eggs deposited by *Culex quinquefasciatus* in different dilutions of *Bti.* VCRC B650 culture filtrate

Group	Number of egg rafts			P-value[1]	P-value[2]
	Mean(SD)	Median	Min – Max		
1:1	48.67(10.44)	47	41 – 75	0.0001	0.0003
1:10	14.56(3.09)	15	10 – 20		0.0003
1:20	7.44(1.51)	7	5 – 10		0.0003
1:50	4.89(2.57)	6	1 – 9		0.0037
NYSM	1.22(1.09)	1	0 – 3		Nil
Group	No. of egg rafts				P-value[2]
1:1	87.56(6.12)	85	79 – 98	0.0003	
P-cresol	22.44(2.50)	23	19 – 26	Nil	

[1]Kruskal-wallis test;[2]Mann-whitney U test

behaviors like biting and resting, and delving into the chemical ecology of oviposition could provide additional insights for more effective management approaches (Pates and Curtis, 2005). Various mosquito species demonstrate distinct preferences for laying their eggs by carefully choosing specific larval sites. Typically, mosquitoes steer clear of depositing eggs in locations already inhabited by competing species or potential predators. Their inclination is to lay eggs in environments where conspecific larvae are present, as this signifies the habitat's suitability for the survival of the upcoming generation. Consequently, mosquitoes exhibit a discerning approach when selecting sites for egg-laying, as they occupy a non-random assortment of aquatic habitats (Mwingira *et al.,* 2020). In present study, the prime aim of oviposition attractants has the potential for both surveillance and management of mosquitoes, as they entice them to deposit eggs at selected locations. Accordingly, the present study was focused on the novel mosquitocidal bacteria *Bti* VCRC B650 culture filtrate and it was tested for oviposition attractancy in gravid female of filarial and dengue vectors of *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. Among the tested concentrations, 1:1 and 1:50 dilution has shown attractancy for *Cx. quinquefasciatus* and *Ae. aegypti* respectively. When their oviposition activity index (OAI) was evaluated, the culture filtrate of *Bti* VCRC B650 exhibited oviposition attractancy with different dilution (1:1, 1:10 and 1:20) for *Cx. quinquefasciatus* and *Ae. aegypti.* For *Cx. quinquefasciatus,* the OAI was found at 1:1 dilution (0.73) which was more than 0.3 required, thus it can be considered as a potential oviposition attractant. Hence, the mosquitoes are attracted to it to lay eggs and when the egg hatches, the larvae were immediately killed, thus this bacterial culture filtrate is also mosquitocidal in nature. However, for *Ae. aegypti,* the OAI was found for 1:50 dilution to be 0.23, thus OAI is below the required threshold of 0.3, indicating that they are not considered attractants. When the attractiveness of bacterial culture filtrates for oviposition was contrasted with a known oviposition attractant (p-cresol), at 10 and 3ppm respectively, the culture filtrate of *Bti* VCRC B650 1:1, 1:10, 1:20 and 1:10 were found to be more attractant than p-cresol. From the current study, it was identified that a compound *i.e.* Benzenaldehyde, which is reported to be attractant for *Culex* spp. in the culture filtrate of *Bti* VCRC B650 that could be investigated further for its attractiveness. This could involve synthesizing the compound or obtaining the synthesized form and testing it for its ability to attract mosquito oviposition. This approach may serve as a potential control method for vector mosquitoes.

The results corroborate the pioneer workers, wherein, the *Bti* (wild and mutants) and *B. sphaericus* culture filtrates proved attractancy at 2000 ppm with OAI of 0.71, 0.59, and 0.68, respectively. The cell-free filtrate from *B. cereus*, *B. thuringiensi*s, and *Pseudomonas fluorescens* has demonstrated attractancy for gravid female mosquitoes of *Cx. quinquefasciatus* (Poonam *et al.,* 2002). While certain cues associated with bacteria prompt oviposition at specific concentrations, elevated levels of the same cues, such as, tetra decanoic acid, or other cues produced by either the same or different bacteria, like hexadecenoic acid methyl ester, act as deterrents to oviposition (Ponnusamy *et al*., 2008). Gravid mosquitoes are also known to be attracted to a variety of volatile compounds generated by microbes, which helps them to find oviposition locations for depositing eggs (Girard *et al*., 2021).

Table 2. Eggs deposited by *Aedes aegypti* in different dilutions of *Bti.* VCRC B650 culture filtrate

Group	Number of eggs			P-value[1]	P-value[2]
	Mean(SD)	Median	Min – Max		
1:1	92.89(7.75)	96	80 – 103	0.0001	0.0003
1:10	517.56(76.38)	495	425 – 658		0.0003
1:20	652.33(109.68)	629	507 – 875		0.0003
1:50	1862.11(281.51)	1725	1599 – 2445		0.0003
NYSM	1121.89(112.16)	1088	1032 – 1379		Nil

Group	Number of egg rafts			
	Mean(SD)	Median	Min – Max	P-value2
1:50	889.11(67.88)	879	802 – 976	0.0003
P-cresol	2767.11(106.95)	2780	2600 – 2889	Nil

Burgeoning literatures indicate that bacteria obtained from hay infusion, specifically *Aerobacter aerogenes*, release volatile compounds that stimulate the oviposition of *Ae. aegypti* and *Cx. quinquefasciatus* gravid females (Hazard *et al*., 1967). Similarly, gravid *Cx. quinquefasciatus* has exhibited an attraction to protein hydrolysate solutions contaminated with bacteria (Beehler *et al*., 1994). The chemical cues generated by microorganisms, in terms of quantity, compound nature, and composition, are likely to vary among bacterial species, influencing mosquito oviposition (Poopathi, 2008). The propensity of *Culex* mosquito species to deposit eggs in freshwater pools which are highly organic has been utilized to design effective gravid traps, for monitoring mosquito groups. Likewise, the preference of *Aedes* mosquito species for laying eggs in synthetic/plastic vessels has been leveraged to create ovitraps for monitoring and management purposes (Day, 2016). Hazaed *et al*. (1967) conducted a study examining the impact of chemicals on attracting mosquitoes to breeding sites and triggering egg-laying behaviors. In a laboratory setting, gravid female mosquitoes of both *Aedes* and *Culex* species were exposed to options of water and moist substrate for oviposition. The findings revealed that 66 per cent of the females showed a preference for the odor of hay infusion over distilled water, and 78 per cent favored the bacterial solution over distilled water. The results of the present study indicate that the bacterial culture supernatant lured gravid females of *Cx. quinquefasciatus* and *Ae. aegypti* to deposit eggs and this is the first report that a mosquitocidal bacteria (*Bti* VCRC B650) isolated from clay soil from Pondicherry showed substantial effect in attracting and killing the mosquitoes.

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Table 3. Compounds identified through GCMS analysis

No	Retention time	Area %	Compound Name	Class of compound	Molecular formula
1	7.581	7.2	2-Piperidinone	Piperidinones	C5H9NO
2	9.364	2.34	2-Phenylacetamide	Benzenoids	C8H9NO
3	12.829	9.47	Benzaldehyde	Benzenoids	C7H6O
4	19.995	5.55	1,4-diazabicyclo [4.3.0]nonan-2,5-dione 3-methyl	Piperazine	C8H12N2O2
5	20.900	64.01	Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	Pyrrolopyrazine	C11H18N202
6	22.260	5.29	Cyclo(L-prolyl-L-valine)	Diketopiperazine	C10H16N2O2
7	25.280	2.21	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	Piperazine	C11H18N2O2
8	25.506	2.25	5,10-Diethoxy- 2,3,7,8-tetrahydro -1H,6H dipyrrolo[1,2-a:1',2-d] pyrazine	Pyrazine	C14H22N2O2
9	32.669	1.67	2'-Hydroxy-2,3,5'-trimethoxychalcone	Chalcone	C18H18O5

Table 4. Mosquito larvicidal activity of culture filtrate dilutions

Culture filtrate dilution	Composition for 100ml	mortality after 24 hours
1:1	50 ml culture filtrate + 50 ml water	100%
1:10	10 ml culture filtrate + 40 ml water	100%
1:20	5 ml culture filtrate + 45 ml water	100%
1:50	2 ml culture filtrate + 48 ml water	100%
NYSM (control)	NYSM	No mortality

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Exploring the diversity of coccinellid species: A comprehensive study on polymorphism and species-specific male genitalia identification

Gokul Krishna Dhanapati[1], Suprakash Pal[2*] and K. Swapna Rani[3]

[1]Department of Entomology, Uttar Banga Krishi Viswavidyalaya, Pundibari 736165, West Bengal, India.
[2]Directorate of Research (RRS-TZ), Uttar Banga Krishi Viswavidyalaya, Pundibari 736165, West Bengal, India.
[3]ICAR-KVK, East Sikkim 737135, Sikkim, India.
Email: suprakash@ubkv.ac.in

ABSTRACT: In this study, the diversity of coccinellids along with their polymorphic forms is presented, by studying the structure of male genitalia and siphon. Out of the fourteen species of coccinellids listed, eleven species were predaceous on aphids. The male genitalia structures of the five most common species i.e., *Coccinella septempunctata*, *Co. transversalis*, *Cheilomenes sexmaculata*, *Micraspis discolor* and *M. yasumatsui* have been described and found to exhibit characteristic differentiating features. The melanic and non-melanic forms of *Ch. sexmaculata*, the most ubiquitous coccinellid in the Indian sub-continent, were dissected and shown to have similar male genitalia structures. Problem of misidentification due to polymorphic forms of coccinellid beetles can be mitigated by studying male genitalia and siphonal structures in addition to external morphological studies. © 2024 Association for Advancement of Entomology

KEY WORDS: Ladybird beetles, different morphs, siphonal structures

INTRODUCTION

In agricultural and horticultural crop ecosystems, coccinellids are crucial group of predators preserved and augmented to achieve both conservation and applied biological pest control. These beetles are economically valuable due to their predation on wide range of crop pests, primarily homopterans, including aphids, coccids, and other soft-bodied insects, in both their larval and adult stages (Hippa *et al*., 1978; Kring *et al*., 1985). In general, the horticultural crop ecosystems in both regions were found to have a higher richness of coccinellid species compared to agricultural crop ecosystems (Gurung *et al*., 2019). Understanding of coccinellid biodiversity enhances our knowledge of predator behaviour in relation to pest reproduction, population dynamics, and crop impact, which is critical for developing effective pest management strategies. The presence of different morphs within the same species—such as morphological variation in the elytral colour—can complicate species identification (Gullan and Cranston 2014).

* Author for correspondence

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In most insect families, the complex genitalia, which are connected to the distal segments of the abdomen, serve as the primary basis for species differentiation and aid in family identification (Dobzhanskiy, 1926). Since the male genitalia of a species are unique, morphological analysis of these structures is one of the most critical factors in taxonomic identification below the family level (Chowdary *et al.,* 2015). Unlike female genitalia, where species-specific traits are less pronounced, male genitalia are typically heavily sclerotized, rigid structures that exhibit greater variability and are often the primary focus in studies of genital evolution (Richmond *et al*., 2016). Elytral colour polymorphism is known to occur in many ladybird species, and understanding this polymorphism is crucial for accurate species identification. Since elytral colour polymorphism in coccinellids can lead to misidentification based on external morphological characters, further confirmation through the study of male genitalia is essential.

MATERIALS AND METHODS

The study was carried out at the Instructional Farm of the Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, in Pundibari, Cooch Behar, West Bengal, India. The farm is situated at $26^0$19’86"N latitude and $89^0$23’53"E longitude, with an altitude of 43m above mean sea level. The region falls within the Terai agroclimatic zone of West Bengal. The morpho-taxonomical studies of coccinellids were conducted in the Entomology lab at the Regional Research Station (Terai Zone), Directorate of Research, UBKV. Coccinellid beetle adults were randomly collected using a sweep net and by hand-picking. The specimens were dried in a hot air oven at 45–50°C for 4-6 hours after being killed with ethyl acetate in killing bottles. The collected specimens were examined using a ZEISS Stemi 508 stereo microscope (8:1 zoom) fitted with ZEISS Axiocam 105 colour camera and Carl Zeiss Zen 2.5 lite (blue edition) imaging software. In some cases, images were processed into composite images for enhanced depth and clarity using Combine ZP and edited with Microsoft Paint 3D. The coccinellids were identified at the Entomology lab, RRS (Terai Zone), using previously preserved specimens identified by an expert systematist, along with reference to literature and checklists (Poorani, 2002). Later, Dr. J. Poorani of NRC Banana, Trichy, identified and confirmed all the coccinellid specimens collected from various crop ecosystems of the UBKV campus.

Dissections were performed on the collected coccinellid beetles under a microscope to determine their sexes. The specimens were delicately positioned on a dissection tray, placed on their backs for dissection of both male and female genitalia. Using microneedles and applying gentle pressure at the thorax-abdomen intersection, the abdomen was separated from the thoracic region under a binocular microscope. To aid in the digestion of soft tissues, the separated abdomen was transferred to cavity blocks containing a few millilitres of freshly prepared 10 per cent KOH, using a camel hair brush. These cavity blocks were then left at room temperature overnight. The following day, the abdomen was carefully removed from the KOH solution using blunt needles and placed in a glass hollow dish containing distilled water. Soft tissues that had been digested were gently squeezed out. The abdomen was then placed on a glass slide with one or two drops of glycerine for genitalia dissection, after being repeatedly rinsed in distilled water. This process rendered the entire abdomen transparent, facilitating the examination of the genitalia.

The male genitalia consist of a tegmen and a sipho (Fig. 1). The tegmen comprises a basal piece, a distinct median projection or basal lobe (often incorrectly referred to as the median lobe), a pair of lateral arms or parameres, and a median basal strut or trabes. In some papers, the tegmen is collectively referred to as the aedeagus. The median lobe may be symmetrical or asymmetrical (as in the tribe Serangiini). The parameres are typically setose at their apices and / or along the outer margins. The sipho is usually elongated, tubular, and curved, with a basal siphonal capsule and various modification at the apex. It functions as the true penis or the intromittent organ. The structure of the male genitalia is species-specific and serves as the most important diagnostic feature, particularly in the absence of other reliable external diagnostic characters (Poorani, 2022).

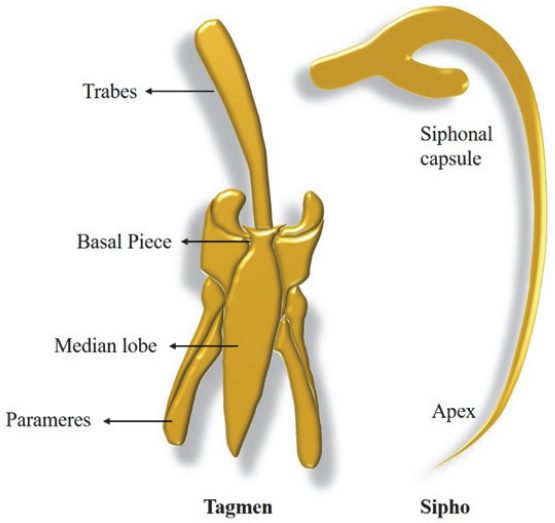

Fig.1: Structure of male genitalia

RESULTS AND DISCUSSION

Biodiversity and Identification based on external morphology of coccinellids: In the present investigation on the biodiversity and diagnostic characteristics of coccinellids collected from the sub-Himalayan Terai region of West Bengal, fourteen species belonging to nine genera and three tribes under the subfamily Coccinellinae of the family Coccinellidae were documented (Fig.2).

Sub family: Coccinellinae; Tribe: Sticholotidini (Pharini)

1. *Sticholotis* sp.

The body is slightly elongated with a glabrous outer surface. The head, pronotum, and scutellum are brownish-yellow. The elytra are also brownish-yellow, each with five black spots, for a total of ten spots across both elytra.

Tribe : Coccinellini

2. *Coccinella septempunctata* Linnaeus

The body is slightly elongated, with a black head and scutellum. The pronotum is black with orange-yellow coloration on the anterolateral sides. The elytra are yellowish-brown to reddish-brown, featuring a total of seven black spots: three on each elytron and one median black spot located on the mid-dorsal line at the junction of the elytra.

3. *Co. transversalis* Fabricius

The body is slightly elongated, with the head and scutellum black. The pronotum is black, with orange coloration on the anterolateral sides. The elytra are dull orange to yellowish-brown, featuring six stripes—three on each elytron.

4. *Harmonia octomaculata* (Fabricius)

The body is slightly elongated, with the head, pronotum, and scutellum black. The elytra are yellowish-brown, each featuring one stripe.

5. *H. dimidiata* (Fabricius)

The pronotum is orange-yellow to bright red with a pair of black spots, which are often fused into a single marking with a median emargination. The elytra are orange-yellow to bright red, featuring 13 black spots arranged across their surface.

6. *Micraspis discolor* (Fabricius)

The elytra are oval, flat underneath, convex in shape, and red in colour. The margins of the elytra form a joint median black streak along the mid-dorsal line.

7. *M. yasumatsui* Sasaji

The head, pronotum, and scutellum is light brown. The elytra are dark red and lack any spots, with no deviations along the mid-dorsal line.

8. *Cheilomenes sexmaculata* (Fabricius)

The body is oval in shape, moderately convex from the dorsal side. The elytra feature six black macular spots, including two zigzag lines. The hind pair of wings, abdomen, and eyes are yellow in colour.

9. *Propylea dissecta* (Mulsant)

The body is slightly elongated. The head is brown, the scutellum is black, and the pronotum is black

Fig. 2 a. *Coccinella septempunctata,* b. *Micraspis discolor,* c. *Illeis indica*, d. *Harmonia dimidiata,* e. *Micraspis yasumatsui,* f. *Coccinella transversalis* g. *Sticholotis* sp., h. *Synonychimorpha chittagongi,* i. *Cheilomenes sexmaculata,* j. *Harmonia octomaculata,* k. *Propylea dissecta*, l. *Henosepilachna vigintioctopunctata,* m. *Henosepilachna septima,* n. *Henosepilachna pusillanima*

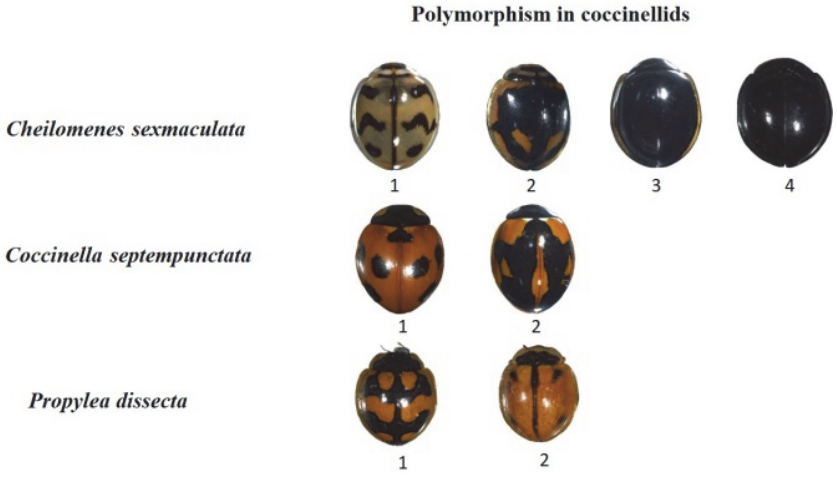

Fig. 3 Polymorphism in coccinellids found in Terai region

with half of it being pale yellow. The elytra are brownish with four black spots, two on each elytron.

10. *Illeis indica* Timberlake

The head is white, and the scutellum is black. The pronotum is pale yellow with two black spots. The elytra are creamy yellow, without any spots or stripes.

11. *Synonychimorpha chittagongi* (Vazirani)

The body is tiny and round or oval. The head, scutellum, and pronotum are yellow. The elytra are black with a broad yellow border along the outer margin, and they lack any spots or stripes.

Tribe : Epilachnini

12. *Henosepilachna vigintioctopunctata* (Fabricius)

The body is slightly elongated. The head, pronotum, and scutellum is deep red to orange. The elytra are deep red to orange with 7-14 black spots on each elytron. The anterior margin of the elytra, where it meets the pronotum, is not truncated, and the tips of the elytra are rounded.

13. *H. septima* (Dieke) Syn. *Epilachna demurili*

The body is moderately elongated. The head, pronotum, and scutellum are light copper-coloured and dull in appearance. The elytra are light copper-coloured with six black spots on each elytron. The anterior margin of the elytra, where it meets the pronotum, is truncated, and the tips of the elytra are somewhat pointed.

14. *H. pusillanima* (Mulsant) Syn. *Epilachna dodecastigma* (Wiedemann)

The body is moderately elongated. The head, pronotum, and scutellum are deep copper-coloured. The elytra are also deep copper-coloured, with six black spots on each elytron. The anterior margin of the elytra, where it meets the pronotum, is truncated, and the tips of the elytra are more rounded compared to *H. septima*.

Host plants of phytophagous coccinellids under Terai zone of West Bengal: *Henosepilachna vigintioctopunctata* and *H. pusillanima* attack various solanaceous vegetables, such as brinjal, tomato, and potato, as well as solanaceous weeds. However, in the population collected from UBKV, Pundibari, the majority individuals were *H. vigintioctopunctata* (confirmed from the specimens sent to Dr. J. Poorani for identification, which is also supported by available literature). *H. septima* exclusively feeds on cucurbitaceous vegetables and weeds.

Polymorphism in coccinellids: During the biodiversity study of the coccinellids, different morphological variations were observed in some common species of ladybird beetles. Four morphs of *C. sexmaculata* and two morphs each of *Coccinella septempunctata* and *Propylea dissecta* were identified (Fig. 3).

Among the four morphs of *C. sexmaculata*, two morphs (morph 1 and 2) are non-melanic, distinguished by physical characteristics such as ground colour, pronotum, spots, and the elytral pattern on the dorsal surface. The other two morphs (morph 3 and 4) are melanic and can only be identified by examining the male genitalia. The appearance of the male genitalia served as the primary method for confirming the species of all morphotypes. Upon examining the male genitalia, particularly sipho, of one non-melanic form (morph 1) and one melanic form (morph 4), the same siphonal structure was observed (Fig. 4).

In this way, in addition to studying external morphological characteristics, the shapes and structures of the male genitalia, particularly sipho were essential for accurately identifying the species and addressing the problem of misidentification among the morphotypes of the same species.

Male genitalia structure of common coccinellid species recorded at Pundibari:

Coccinella septempunctata (Fig. 5)

Sipho: The siphonal tube is long, bent at base, almost straight for most of its length, and the distal end

carries more or less sac-like structure. The apex appears to be distorted at three points. The siphonal capsule is bloated and dense.

Tegmen: The trabes are short and more or less uniform in thickness. The Median lobe is very broad at base, tapering gradually beyond the middle to the apex, forming a triangle-like structure. The Parameres are comparatively shorter than median lobe, covered with dense long hairs on the dorsal side except at the base.

Coccinella transversalis (Fig. 6)

Sipho: The siphonal tube is comparatively short, curved at the base, and pointed. The siphonal tube carries a transparent, bubble-like structure on the dorsal side of the sub distal portion.

Tegmen: The trabes are comparatively long, flat, and thick; the basal piece is quadrate. The median lobe is broad apically, narrowing towards the tip, and longer than the parameres. It is deeply emarginated in the distal half of its length, with the tip extending to form a tongue-like structure.

Cheilomenes sexmaculata (Fig. 7)

Sipho: The siphonal tube is strongly curved at the base; the siphonal capsule is well-developed, with the outer arm longer and pointed and inner arm shorter and rounded. The siphonal apex is thread-like.

Tegmen: The trabes are longer and straight; the median lobe is shorter than the parameres; the parameres are cylindrical and slightly bent at the apical end.

Micraspis discolor (Fig. 8)

Sipho: The siphonal tube is strongly curved at the base and up to half of its length, and straight at the apex. The siphonal capsule is well-developed, broad, and flat, with the apex of the siphon having hooked processes.

Tegmen: The tegmen has elongated, long lateral lobes with dense hairs; the median lobe is shorter than the lateral lobes or parameres, and the apex of the median lobe is pointed.

Micraspis yasumatsui (Fig. 9)

Sipho: The siphonal tube is strongly curved for most of its length. The siphonal capsule has an outer arm with a constriction, while the inner arm is short and rounded. The apex of the sipho is flattened.

Tegmen: The tegmen has elongated, long lateral lobes with dense hairs.

The siphonal tubes of above mentioned five commonly recorded coccinellids is presented in Fig-10 for better understanding of its structural differentiation between species to species.

In agriculture, accurate identification of insect pests, their bioagents, pollinators and other beneficial insects is of fundamental importance for effective, sustainable pest management and conservation of beneficial fauna (Poorani, 2022). Many researchers have conducted surveys and reported findings of coccinellid beetles from various regions worldwide (Khan *et al*., 2009; Majumder *et al*., 2013; Chowdhury *et al*., 2015; Lami *et al*., 2016; Halim *et al*., 2017; Gurung *et al*., 2019; Sharma and Joshi, 2020). The predaceous coccinellid beetles (11 species) reported in this study were collected from aphid hosts in different crop ecosystems at Pundibari. True aphidophagous coccinellids were first described by Agarwala and Ghosh (1988) as 36 species in the Indian subcontinent, and later by Poorani (2002) as 5,200 species worldwide. Coccinellid beetles in the Indian subcontinent are classified into 79 genera and 400 species. In 2020, Pervez *et al*. reported 18 predaceous coccinellid beetle species belonging to 15 genera and 3 subfamilies: Chilocorinae, Coccinellinae, and Scymninae. Polymorphism in insect groups has been a challenge for entomologists in achieving accurate species level identification. In coccinellid, this issue has garnered only limited attention, focusing on a few species (Honek, 1996). For instance, *C. sexmaculata* is the most ubiquitous coccinellid in the Indian region, yet its polymorphic forms are often misunderstood due to superficial similarities with other common but unrelated species, such as *M. discolor*, *Propylea dissecta* and *Chilocorus*

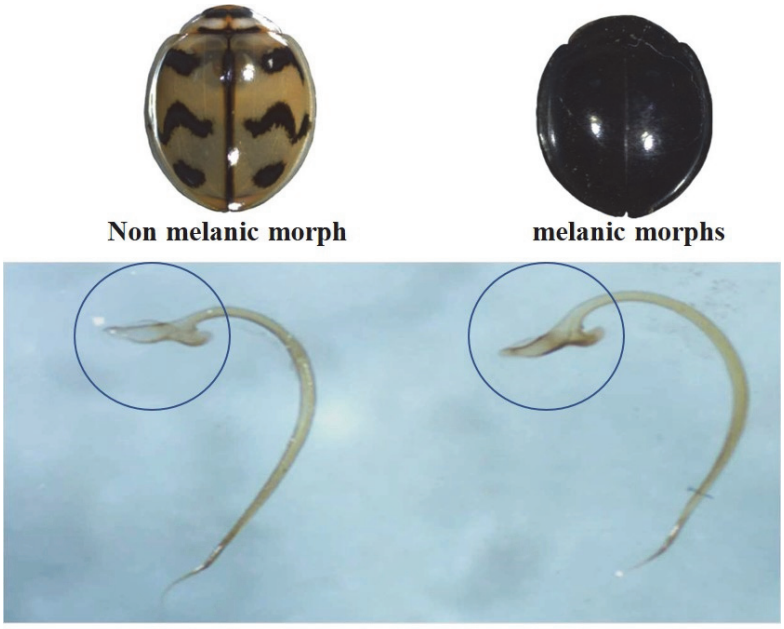

Fig. 4 Male genitalia (siphonal tube) of two morphs of *C. sexmaculata*

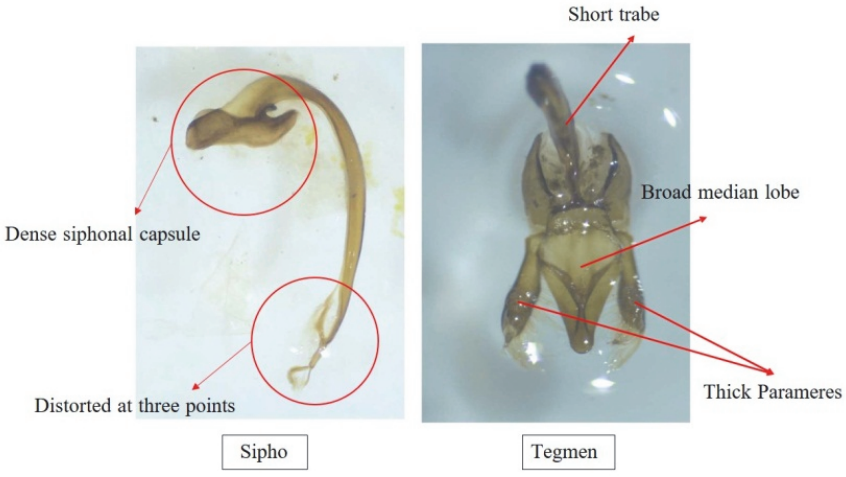

Fig. 5 Male genitalia structure of *Coccinella septempunctata*

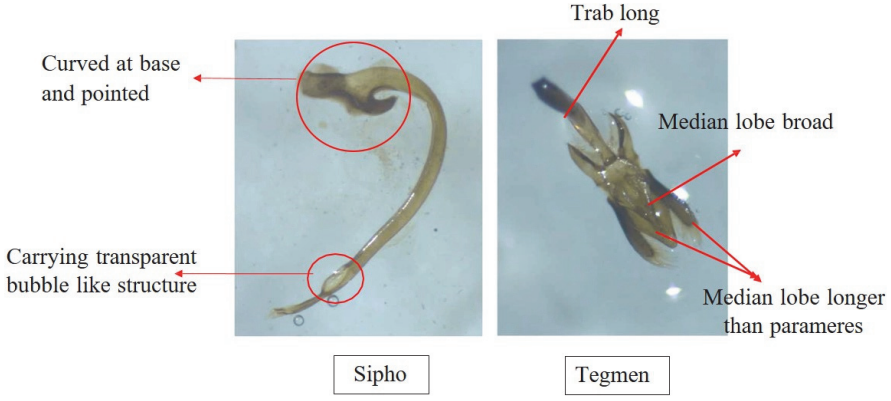

Fig. 6 Male genitalia structure of *Coccinella transversalis*

Fig. 7 Male genitalia structure of *Cheilomenes sexmaculata*

Fig. 8 Male genitalia structure of *Micraspis discolor*

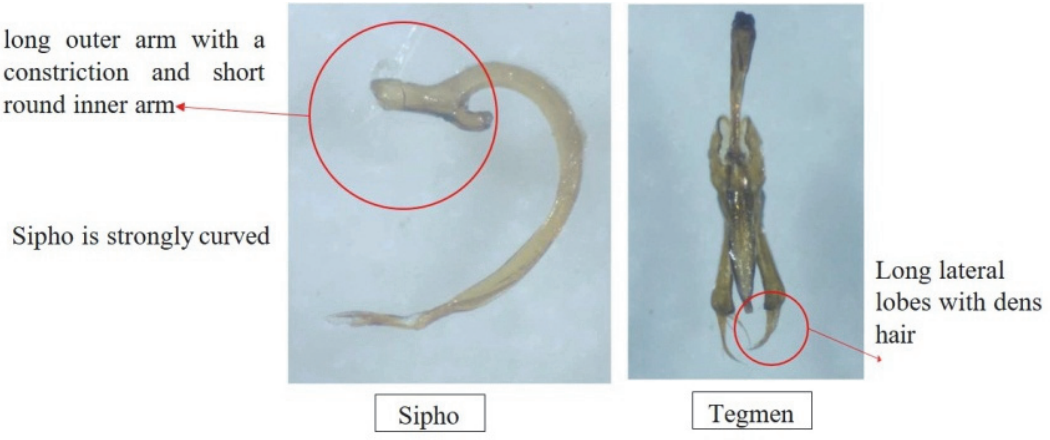

Fig. 9 Male genitalia structure of *Micraspis yasumatsui*

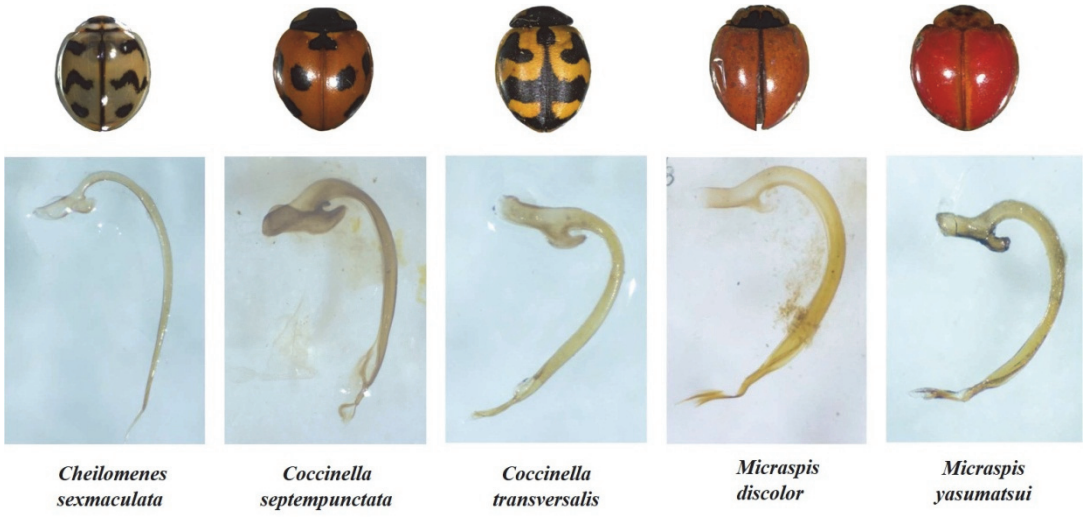

Fig. 10 Male genitalia Sipho of common coccinellids

nigrita (Poorani, 2023). In our present investigation, four different morphs of *C. sexmaculata* were recorded with the melanic forms (morph 3 and 4) being particularly confusing due to their resemblance to the external morphology of *C. nigrita*. This confusion could only be resolved by examining the male genitalia. Chazeau (1980) investigated the genetic causes of variation in the elytral colouration pattern in *Coelophora quadrivittata* in various related studies. In Chegeni region (Lorestan province, Iran), Biranvand and Shakarami (2015) identified 18 distinct morphs of *Hippodamia vareigata* Goeze based on variations in the elytra and pronotum patterns. Kawakami *et al.* (2013) documented 20 morphs of *Cheilomenes sexmaculata* from Indonesia to Japan, attributing elytral coloration to morph selection by climate. Similarly, Singh *et al*. (2016) reported six morphs of *Cheilomenes sexmaculata* in Haryana and Thamseer *et al*. (2022) found five morphs of *Coccinella septempunctata*. The genetic make-up of these forms, the varying environments to which they have been exposed, or a combination of both factors may be the cause of their variety.

In the terai sub-Himalayan region of West Bengal, eastern India, the common predaceous coccinellids encountered include *C. transversalis*, *M. discolor*, *C. sexmaculata*, *C. septempunctata*, *Brumoides suturalis*, *Propylea dissecta*, *M. yasumatsui* etc. (Gurung *et al*. 2018). The study of the male genitalia structures of these common species, revealed to give accurate identification of the coccinellid taxa at the species level.

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First record of leaf miner, *Phyllocnistis unipunctella* (Stephens, 1834) (Lepidoptera, Gracillariidae) infesting *Populus deltoides* Marsh (Salicaceae) in Jammu and Kashmir, India

Deen Mohd Bhat[1*], Sajad Ahmad Khan[2] and Inayat Ullah Lone[2]

[1]*Department of Zoology, Govt. College for Women, Cluster University, M. A. Srinagar190001, Jammu and Kashmir, India.*
[2]*Department of Zoology, Baba Gulam Shah Badshah, University, Rajouri 185234, Jammu and Kashmir, India.*
Email: bhatdm2014@gmail.com

ABSTRACT: The study reports infestation of leaf miner *Phyllocnistis unipunctella* (Stephens, 1834) (Lepidoptera, Gracillariidae) on *Populus deltoides* Marsh (Salicaceae) in the UT of Jammu and Kashmir, India. The serpentine mining of leaves by larvae caused the leaves to dry out and turn brown, which lead to premature leaf drop, especially in severe infestations. Large populations rendered a silvery hue to the appearance of infested poplars when viewed from a distance. Pupation occurred inside the mine within a silken cell. Adults emerged after a period of 10-14 days. The infestation by this moth on poplars in the field was observed from the month of July to September. © 2024 Association for Advancement of Entomology

KEY WORDS: Serpentine leaf miner, poplar species, damage, biology, occurrence

Most of the exotic poplars, especially *Populus deltoides* Marsh (Salicaceae) have been attacked by insects since their introduction in India. Over 65 insect species have been reported infesting *Populus deltoides* alone in northern India (Ahmad *et al.,* 2001; Singh *et al*., 2004). During 2023, poplar trees and polar nurseries (*P. deltoids*) were searched for the insect pest attack in the district Ganderbal (34.2165° N, 74.7719° E) of Kashmir Valley. The poplars were infested by a leaf miner. The insect larvae caused serpentine mining of the leaves on the poplar trees (Figs. 1-4). The mining of leaf tissue caused the leaves to later dry out and turn brown, which lead to premature leaf drop, especially during severely infested patches. Large population of this insect rendered a silvery hue to the appearance of infested poplars when viewed from a distance at this site. Less than 20 per cent poplar leaves were infested. Pupation occurred inside the mine within a silken cell. Adults emerged after a period of 10-14 days in the month of August 2023. The emergent moths were identified as *Phyllocnistis unipunctella* (Stephens, 1834) according to Kuznetzov and Baryshnikova (2001). These moths had a wingspan of 6mm; were narrow, lance-shaped, with white wings mottled with brown and black markings having relatively long, thread-like antennae (Figs. 5-6). The mining of the poplars was witnessed during the months from July to September.

According to Wagner *et al.* (2008), the leaf miner,

* Author for correspondence

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Fig. 1, 2 *Populus deltoids* leaves infested with leaf miner *Phyllocnistis unipunctella* (Stephens, 1834) Fig. 3, 4 Larva of leaf miner *Phyllocnistis unipunctella* (Stephens, 1834) inside the leaf tissue. Fig. 5, 6 Emergent moth, *Phyllocnistis unipunctella* (Stephens, 1834)

Phyllocnistis feeds on the contents of epidermal cells on both top (adaxial) and bottom (abaxial) surfaces of quaking aspen leaves, leaving the photosynthetic tissue of the mesophyll intact. *P. unipunctella* (Stephens, 1834) is known to attack poplars (*Populus nigra*, *P. balsamifera*, *P. nigra, P. suaveolens, P. nigra* var. *italica*) in Asian parts of Russia and Japan (Tomilova, 1973; Ermolaev, 1987; Sinev, 2008; Kobayashi and Hirowatari, 2011). Previously a sister genus of this leaf mining moth *Phyllonorycter populifoliella* (Treitschke) has been recorded on *Populus* sp. in the UT of Ladkah (Shashank *et al*., 2021), but this is the first report of *Phyllocnistis unipunctella* (Stephens, 1834*)* (Lepidoptera, Gracillariidae) infesting *P. deltoides* Marsh from Kashmir valley, India. During the field observation, *P. unipunctella* (Stephens, 1834) was found to be a moderate pest of *P. deltoides* as only less than 20 per cent leaves of the searched host trees were found infested.

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Predatory potential of *Coccinella undecimpunctata* L. (Coleoptera, Coccinellidae) on *Aphis craccivora* Koch. (Hemiptera, Aphididae) in Ladakh, India

Mohd Ali[1*], Alyas Ali[1] and Shabbir Hussain[2]

[1]Entomology Research Laboratory, Department of Zoology, University of Kashmir, Srinagar190006, Jammu and Kashmir, India.
[2]Baba Saheb Bhimrao Ambedkar University, Lucknow 226025, Uttar Pradesh, India
Email: aliarmaan256@gmail.com

ABSTRACT: Predatory potential of all instar larvae and adults of *Coccinella undecimpunctata* (Coleoptera, Coccinellidae), using *Aphis craccivora* Koch (Hemiptera, Aphididae) was assessed in laboratory settings. The study, conducted with varying aphid densities in rearing jars, showed a significant increase in prey consumption as aphid density rose. Furthermore, prey consumption heightened with larval development, with 4th instars devouring more aphids compared to earlier instars. Female adults exhibited greater aphid consumption than males. Deploying both 4th instar larvae and adults could enhance pest suppression.
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KEYWORDS: Prey consumption, fourth instar, densities

Faba bean (*Vicia faba* L.) is a highly nutritious leguminous crop known for its tolerance to cooler temperatures and a wide range of soil environments (Anil *et al.,* 2013). Faba beans, have a long history of versatile and valuable applications in both feed and food (Crepon *et al*., 2010); (Xiao *et al*., 2021). Among the significant aphid species affecting this crop, the cowpea aphid, *Aphis craccivora* Koch (Hemiptera, Aphididae) is a serious threat (Soffan and Aldawood, 2014). The economic threshold of *A. craccivora* to the bean is 8.6 aphids per plant, (Abdou *et al*., 2012). If effective management methods are not implemented during the primary infection, result in flower and leaf damage as well as a reduction in seed yield (Annan *et al.,* 2000). Regardless of the age of the pod, all levels of infection significantly reduced seed output (Ofuya, 1989).

Plants, upon infestation, emit specific volatiles that can affect the foraging behavior of insect predators targeting herbivores (Fouad, 2021). Aphid populations have the potential to proliferate significantly over time and space (Borges *et al.,* 2006; Ramzan and Khursheed 2023). Fouad (2021) discovered that adult *Coccinella undecimpunctata* utilizes plant volatiles induced by *A. craccivora* infestation, suggesting that these volatiles could serve as reliable indicators for locating prey. These responses can include changes in predation rate, prey selection (Cabral *et al*., 2013). *C. undecimpunctata* L. is an aphid predator (Smyth *et al*., 2013), and recognized as euriphagous predators (Cabral *et al*., 2009); with potential to serve as an effective biological control agent against aphids (Soares *et al*., 2003; Abd-Rabou, 2008;

* Author for correspondence

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(Fouad, 2021). This study aims to study predatory potential of various life stages of *C. undecimpunctata* on *A. craccivora* under laboratory conditions.

The experiment was conducted at LOGIC (Ladakh Organic and Green Initiative Consultancy) Agri-Farm in Kanoor, Ladakh, at an altitude of 9800ft. The faba bean seeds were initially sown in the field and later transplanted into plastic pots, and cultivated under polyhouse conditions. Nymphs and adults of *A. craccivora* were collected directly from faba bean in the field, released onto the cultivated host plants, and the culture was maintained for subsequent use. *C. undecimpunctata* adults were collected from the field and reared in plastic jars (15cm x 15cm) supplied with cowpea aphids and a faba bean twig endowed with soaked cotton. Aphids were replenished every 24 hours, and oviposition sites were provided with crumpled paper. Eggs were collected gently with the help of a camel hair brush, transferred to a new insect breeding dish (100×40mm), and allowed to hatch. Newly emerged larvae and adults were utilized for the experiments, and the culture was maintained in the laboratory until the completion of experiment (Farooq *et al*., 2018, Abbas *et al*., 2020, Marri *et al*., 2021).

The experiments consisted of an insect breeding dish/jar (100×40mm) with a moist layer of cotton and filter paper to prevent direct contact between aphids and cotton. Leaves from healthy faba bean plants were placed abaxial surface facing up on the filter paper. *A. craccivora* of various densities (10-130 aphids) were transferred onto leaves and left undisturbed for 30 minutes. 3-4 hours pre-starved *C. undecimpunctata* of different life stages were introduced in each insect-breading jar, and prey mortality was recorded every 24 hours for six replicates per treatment. Each treatment, representing a specific life stage of the predator and prey number combination. Control treatments without predators were conducted to assess natural prey mortality (Ramzan and Khursheed, 2023). For each prey density, maintained six replications. Control treatments were conducted with the abovementioned prey densities in the absence of predators to evaluate natural prey mortality unrelated to predator activity. No predator mortality was observed throughout the experiment, and similarly, no prey mortality was observed in the control treatment.

The predatory potential was calculated by using the following equation (Soares *et al.,* 2003):

$$Po = (A\text{-}a_{24})\ ra_{24}$$

Po= Number of aphids eaten; **A=** Number of aphids available; $\mathbf{a_{24}}$= Number of aphids alive after 24hrs; $\mathbf{ra_{24}}$= The ratio of aphids found alive after 24 hrs in the control treatment.

A one-way ANOVA was conducted to compare the predatory potential of all the predatory stages of *C. undecimpunctata* across different prey densities. The analysis was carried out using SPSS version 22.

Predatory potential: The findings from the current study demonstrated that with increasing prey density and developmental stages, the consumption rate of *C. undecimpunctata* rose, consistent with findings reported by Darwish (2019) and Ramzan and Khursheed (2023).

When prey density increases, the number of preys ingested by *C. undecimpunctata* larvae in their 1st, 2nd, 3rd, and 4th instars increases dramatically. Maximum values are reached at 50, 70, 70, and 90 preys given respectively, (i.e.,14±0.57, 19.33±0.33, 21.5±0.22, and 30±0.57 prey consumed, respectively). The 4th instar larvae show more voracity than those in their 1st, 2nd, and 3rd instars. This finding is consistent with other research conducted by Cabral *et al*. (2006), Moura *et al*. (2006), Cabral *et al*. (2009), and Ramzan and Khursheed (2023). Male and female voracity both rise sharply with an increase in prey density, but adult satiation occurs at greater densities as compared to larvae, i.e., when 110 prey are available (i.e., 36±0.25 and 38.66±0.42 prey consumed, respectively). It's crucial to remember that prey density determines satiation, therefore increasing prey densities does not significantly alter predatory potential. When compared to larval instars, both male and female adults of *C. undecimpunctata* showed increased predation. This increased predation in adults may be attributed to the higher

Table 1. Voracity (number of prey consumed ± SE) of *C. undecimpunctata* across its various developmental stages (1st to 4th instar larvae and adults, both females and males) when exposed to different densities of *A. craccivora* prey

Prey density	Voracity					
	1st Instar	2nd Instar	3rd Instar	4th Instar	Adult male	Adult female
10	1.66±0.21c	2.16±0.30d	4±0.25d	6.16±0.30e	8±0.36f	9±0.25f
30	6.66±0.42b	8.83±0.47c	11.16±0.30c	15.16±0.70d	16±0.25e	18±0.35e
50	14±0.57a	15.83±0.47b	17.83±0.60b	19.16±1.75c	23±0.57d	26.16±0.47d
70	14±0.44a	19.33±0.33a	21.5±0.22a	25.16±0.60b	26.66±0.55c	29.83±0.79c
90	-	19.33±0.42a	21.66±0.21a	30±0.57a	33±0.25b	35.16±0.30b
110	-	-	-	30±0.57a	36±0.25a	38.66±0.42a
130	-	-	-	-	36±0.25a	38.66±042a

In the column, values sharing the same letters are statistically non-significant at p = 0.05.

energy requirements associated with successful reproduction and mate searching. Conversely, the lower prey consumption by early larval instars may be due to their smaller size and lower energy requirements (Ramzan and Khursheed, 2023).

In the study, it was observed that female adults of *C. undecimpunctata* exhibited greater voracity compared to males, consistent with the findings of Bayoumy *et al*. (2015). This pattern aligns with the general observation in coccinellids, wherein adult females tend to be more voracious due to their larger size and increased nutrient requirements for egg production and oviposition (Omkar and Pervez, 2004). However, the results of the present study do not entirely align with the findings reported by Cabral *et al*., 2006: Moura *et al*., 2006: Cabral *et al*., 2009 and Imam 2015, who observed higher voracity in 4th instar larvae compared to adults. In this case, the discrepancy in results could be attributed to the utilization of different prey species and prey instars with varying body sizes, as previously hypothesized by Moura *et al*. (2006). The increased voracity of 4th instar larvae is a common observation in other coccinellid species as well, such as *C. transversalis* (Omkar and James, 2004), *Propylea dissecta* Mulsant (Omkar and Pervez, 2004), and *Harmonia axyridis* Pallas (Lee and Kang, 2004). This heightened voracity may be attributed to the greater energy requirements necessary for growth and achieving critical weight for pupation (Cabral *et al*., 2009).

The study demonstrated among all developmental stages, 4th instar larvae consumed the most, and adult females consumed more prey compared to adult males. Since aphidophagous ladybirds are known to lay their eggs just before the peak aphid infestation period (Hemptinne *et al*., 2000), releasing mature adults of *C. undecimpunctata* into the field early in the season may enhance as a biocontrol agent.

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First record of orange oak leaf, *Kallima inachus* (Boisd) in Uttar Pradesh, India

Jyoti Antil[1*], Prashant Tripathi[2], M. Semmaran[3] and Amita Kanaujia[4]

[1,2]Biodiversity and Wildlife Conservation Lab, Department of Zoology, University of Lucknow, Lucknow 226007, Uttar Pradesh, India.
[3]DFO, Suhelwa Wildlife Sanctuary, Balrampur 271201, Uttar Pradesh, India.
[4]Department of Zoology, University of Lucknow, Lucknow 226007, Uttar Pradesh, India.
Email: jyotiantil5794@gmail.com

ABSTRACT: A first record of butterfly species *Kallima inachus* was reported in Uttar Pradesh. A four-day preliminary visit to Suhelwa Wildlife Sanctuary from the 6th of March 2022 to the 9th of March 2022 was undertaken and photographs and coordinates of butterfly were taken. The present record confirms the presence of *K. inachus* in Suhelwa Wildlife Sanctuary and is the first record of this species from the state of Uttar Pradesh. © 2024 Association for Advancement of Entomology

KEY WORDS: *Kallima inachus,* first record, Lepidoptera, Suhelwa

The orange oakleaf *Kallima inachus* (Boisduval, 1844) (Lepidoptera, Papilionoidea, Nymphalidae) is distributed from Pakistan (Tschikolovets and Pages, 2016), Nepal (Smith, 2006), along the Himalaya to Central China (Lewis, 1973) and Taiwan (Shirozu, 1960). Within India, the known distribution of the butterfly is from Jammu and Kashmir to Uttarakhand and Sikkim to N.E. India; Jharkhand, Eastern Ghats, Madhya Pradesh and Gujarat (Varshney and Smetacek, 2015). It is found in regions of heavy rainfall in the thick forests of mountainous (Wynter-Blyth, 1957). *K. inachus* is a reported to be on the wing from April to November from sea level to 1800 m (Kehimkar, 2016). The larval host plants belong to the Acanthaceae (*Strobilanthes* genus) and Urticaceae (*Girardinia diversifolia*) (Robinson *et al*., 2001). The unique topography of the area attributes to a mosaic of varied forest types, with deciduous forest dominated by Sal *Shorea robusta* interspersed with *Syzygium cumini*, *Terminalia tomentosa*, *Acacia catechu*, and grass species of *Vetiveria, Themeda, Imperata, Saccharum,* and *Arundo* (Bhargav *et al*., 2016).

A four-day preliminary visit to Suhelwa Wildlife Sanctuary from the 6th of March 2022 to the 9th of March 2022 was undertaken to study butterflies. *K. inachus* was photographed at near Hathiya Kunda naala of East Suhelwa Range, Suhelwa Wildlife Sanctuary, U.P., India elevation of 100m. The GPS Coordinates for the reported butterfly were N-27º47¹28.16¹¹ E-82º10¹50.99¹¹. The present record confirms the presence of *K. inachus* in Suhelwa Wildlife Sanctuary and is the first record of this species from the state of Uttar Pradesh. It also extends the known flying time from April to March.

There are two known subspecies along the Himalayas, *K. i. inachus* which is known from eastern Uttarakhand to N.E. India and *K. i.*

* Author for correspondence

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Fig.1: Upperside and underside of *Kallima inachus* from East Suhelwa range of Suhelwa Wildlife Sanctuary, Shravasti, Uttar Pradesh

Fig. 2: Underside of *Kallima inachus* from East Suhelwa range of Suhelwa Wildlife Sanctuary, Shravasti, Uttar Pradesh

Fig. 3: Landscape of East Suhelwa range of Suhelwa Wildlife Sanctuary, Shravasti, Uttar Pradesh where the record was found

huegeli from the western Himalaya. Since these are distinguished on the basis of the relative shade of the colours on the wings between seasonal forms, it follows that a series of specimens is required to correctly place the specimens to the subspecies level. Further observation will be required to clarify whether there is a breeding population within the sanctuary or whether the individual was a straggler from known habitats along the foothills of the Himalayas in Nepal.

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Ant-treehopper mutualism affecting biocontrol of *Parthenium hysterophorus* by Mexican beetle, *Zygogramma bicolorata* Pallister

Anand Harshana[1,2*] and Debjani Dey[1]

[1]*National Pusa Collection, Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India.*
[2]*Department of Entomology, College of Agriculture (RVSKVV), Indore 452001, Madhya Pradesh, India.*
Email: anandharshana@gmail.com

ABSTRACT: Infestation of a treehopper, *Gargara malabarica* Ananthasubramanian and Ananthakrishnan (Membracidae, Homoptera) on *Parthenium hysterophorus* L. and also a myrmicine ant, *Lophomyrmex quadrispinosus* (Jerdon) (Hymenoptera, Formicidae) tending these treehoppers is reported for the first time. The ant-treehopper association interferes with the biological control of *P. hysterophorus* by Mexican beetle, *Zygogramma bicolorata* Pallister (Coleoptera, Chrysomelidae). The *Parthenium* plants with ants supported significantly lesser number of grubs and adults of Mexican beetle compared to plants without ants. Mean number of Mexican beetle eggs was low on plants with ants, but it was non-significant. Further, the ants were observed to be disturbing the adult Mexican beetles from settling on the *Parthenium* plants, and this presumably led to the less Mexican beetle population.
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KEY WORDS: Weed, myrmicine ant, herbivory hindrance, mutualism

Parthenium hysterophorus L. (Asteraceae) is considered as one of the 'most invasive species in the world' by the Invasive Species Specialist Group of IUCN SSC (GISD, 2020). It is a common noxious weed in agricultural and urban areas of India. Mexican beetle, *Zygogramma bicolorata* Pallister was introduced from Mexico to India for biological control of *Parthenium* weed (Jayanth, 1987) and had established itself in many parts of India (Sushilkumar, 2009). Adults and grubs of Mexican beetle feed voraciously on *Parthenium* weed and satisfactorily reduce its density under natural field conditions. Other than Mexican beetle, other insect pests have been recorded on *Parthenium* plants. Among them, four species of treehoppers *viz*., *Coccosterphus minutus* (F.), *Oxyrhachis tarandus* (F.), *Telingana campbelli* Dist., and *Leptocentrus taurus* F. have been found feeding on *Parthenium* plants in India (Kumar *et al.,* 1979; Thangavelu, 1980). The treehopper, *Gargara malabarica* Ananthasubramanian and Ananthakrishnan (Membracidae, Homoptera) has been reported from the southern part of India feeding on Indian gooseberry, *Phyllanthus emblica* L. (Phyllanthaceae) (Ananthasubramanian and Ananthakrishnan, 1975). However it has not been

* Author for correspondence

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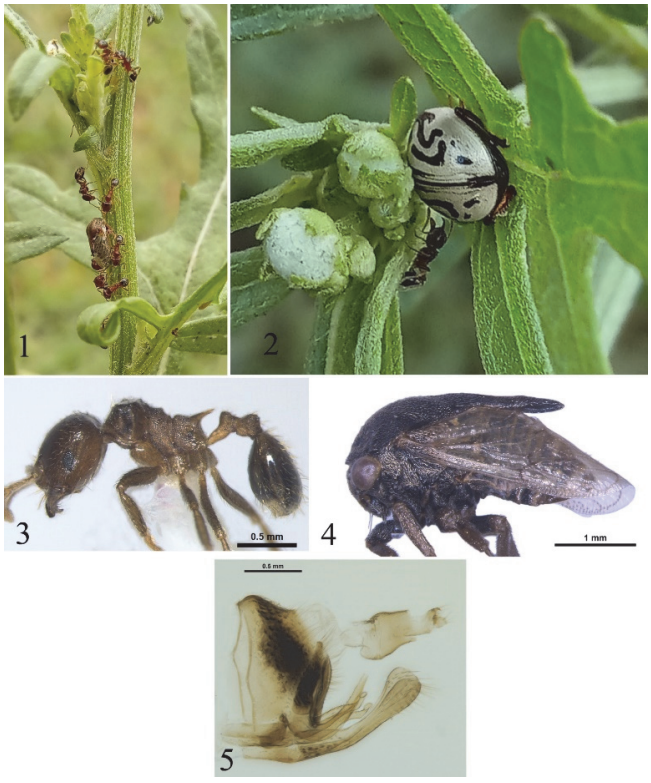
reported from Madhya Pradesh and its feeding on *Parthenium* plants. Ants are eusocial insects (Hymenoptera, Formicidae) that perform numerous ecological services like predation of a wide range of organisms, works as scavengers, help in nutrient cycling and enrichment of soils, myrmecochory, enhancing agricultural and horticultural productivity by biological control of herbivores (Way and Khoo, 1992; Choate and Drummond, 2011; Offenberg, 2015) and pathogens (Offenberg and Damgaard, 2019). *Lophomyrmex* Emery is a small, well-defined myrmicine ant genus represented by seven species in India. *Lophomyrmex quadrispinosus* (Jerdon) is the most widely distributed species of the genus in India (Bharti *et al.,* 2016). Workers of *Lophomyrmex* ants can be easily identified by the presence of 11 segmented antennae, masticatory margin of mandible with more than 8 teeth, anterior clypeal margin with a median anteriorly protruding point, pronotum with lateral irregular marginations or with anterolaterally directed dorsal teeth, propodeal spiracle well behind the margin of declivity in profile view, etc. These ants prey on small arthropods, act as scavengers of other organisms, harvest cotyledons from seeds, and they also respond strongly to bait like cooking oil, sugar water (Moffett, 1986).

Ant-treehopper mutualism is well known and widely reported phenomenon in nature. Their mutualism depends highly on the availability of honeydew (a sugar-rich nutritious liquid secreted by treehoppers). Ant tends treehoppers for their honeydew and in turn protect the treehoppers from their predators, parasitoids, and also the plant from other herbivores. The present study for the first time records the treehopper, *G. malabarica* from Madhya Pradesh, and *P. hysterophorus* as its new host record. The myrmicine ant, *L. quadrispinosus* was reported to be tending *G. malabarica* on *Parthenium* plants. The effect of this association, between treehopper and a myrmicine ant, on the population of biological control agent, *Z. bicolorata* of *Parthenium* plants has been statistically investigated here.

Observations were recorded on an area of 10×10 m^2 colonized by *Parthenium* weed along the bank of Morar river (26°14’06"N; 78°13’15"E), Gwalior (Madhya Pradesh). The effect of *Lophomyrmex* ants on the Mexican beetle population on *Parthenium* plants was studied. Five *Parthenium* plants were randomly selected in the study plot with the continuous presence of *Lophomyrmex* ant’s population tending treehoppers (*G. malabarica*) and another five *Parthenium* plants were selected which did not have the ant population. On each group of plants the presence of Mexican beetle population was recorded based on the number of eggs, grubs, and adults. Observations were recorded at three-day intervals from 11th August 2020 to 23rd August 2020. Further, a few specimens of ants and treehoppers on the *Parthenium* plants were collected. They were processed and mounted on card points and studied under the Leica S8AP0 stereo microscope. Photography was done using LEICA MC190 HD digital camera attached to the LEICA M205 C stereozoom automountage microscope. The identification of species was done based on available taxonomic literature (Ananthasubramanian, 1996; Sheela and Ghosh, 2008; Bharti and Kumar, 2012). All data recorded were subjected to student’s ‘t’ test after $\sqrt{x+0.5}$ transformations, at 5 per cent level of significance and n-1 degrees of freedom.

The treehopper was identified as *Gargara malabarica* Ananthasubramanian & Ananthakrishnan and the ant as *Lophomyrmex quadrispinosus* (Jerdon) (Hymenoptera: Formicidae). This is the first report of *G. malabarica* to be feeding on *Parthenium* plants, and also the myrmicine ant, *L. quadrispinosus* tending nymphs and adults of this treehopper. Analysis of the recorded data indicated that *Lophomyrmex* ants influenced the population of Mexican beetle on *Parthenium* plants (Table 1). *Parthenium* plants with *Lophomyrmex* ant population harbored significantly less mean number of Mexican beetle grubs (6/five plants) and adults (1.4/five plants) compared to plants without ants (23.6/five plants and 10.4/five plants, respectively) with ‘t’ values of 4.56 and 4.17, respectively. While the mean number of beetle eggs was lower too on plants with ant population but was non-significant (t value=2.73). The ants were observed to annoy the adult beetles by biting them and not letting them



Figs. 1-5: 1. *Lophomyrmex* ants tending treehopper (*G. malabarica*) on *Parthenium* plants; 2. *Lophomyrmex* ants annoying *Z. bicolorata* adults from staying on *Parthenium* plants; 3. *L. quadrispinosus* worker in profile view; 4. *G. malabarica* ♂; 5. Genital capsule of *G. malabarica* ♂

settle on *Parthenium* plants. This behavior of ants presumably led to the lower population of Mexican beetle and ultimately less damage to *Parthenium* plants with presence of ants.

Table 1. Effect of *Lophomyrmex* ant's population on Mexican beetle population

No.	Date	Plants with ants			Plants without ants		
		Numbers/ five plants			Numbers/ five plants		
		Egg	Grub	Adult	Egg	Grub	Adult
1	11/8/2020	6	1	1	7	17	10
2	14/8/2020	12	6	2	89	10	7
3	17/8/2020	13	4	1	59	13	15
4	20/8/2020	28	8	1	40	33	16
5	23/8/2020	6	11	2	29	45	4
Mean		13	6	1.4	44.4	23.6	10.4
't' value		Eggs (2.73), Grubs (4.56), Adults (4.17)					

't' value calculated based on the √x+0.5 transformed values and 't' table value at 4 df- 2.78

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Studies on the insect pests of brinjal in Hoshiarpur District of Punjab, India

Amrit Deep Kaur, Sajad Ahmad Bhat and Kamlpred Kaur*

Department of Zoology, School of Agriculture and Natural Sciences, CT University, Ludhiana - 142024, Punjab, India.
**Department of Zoology, Punjabi University, Patiala 147002, Punjab, India.*
Email: amritdeepkaurgch@gmail.com; Sajadbio6@gmail.com: kamattiwana3300@gmail.com

ABSTRACT: Seventeen insect pests belonging to five orders were recorded from selected transects. Hemipterans were found to be very common and most abundant with seven insect pests. Neuroptera the least abundant with one pest was found during the experimental period, with orders like Lepidoptera, Orthoptera, and Coleoptera with three insect pests each. Wind speed and bright sunshine showed a negative correlation (r = - 0.126) and (r = - 0 .778) during 2021 and (r = -0.73 and r = -0.41) during 2022 respectively. Rainfall, humidity, and evaporation have a positive correlation (r = 0.368, r = 0.551, and r = 0.297) in 2021 and (r = .31, r = 0.89, and r = 0.81) during 2022 respectively. At maximum temperature (38.4°C) during April and May pest population was minimum. Rainfall and relative humidity favored the pest population. © 2024 Association for Advancement of Entomology

KEY WORDS: Abundance, temperature, rainfall, humidity, wind speed

The occurrence of most insect pests is dependent on certain weather conditions: Temperature, humidity, rainfall, and drought. Weather and temperature data are beneficial in predicting pests' life cycles. This article deals with the impact of weather conditions and climatic factors on the diversity of insect pests of brinjal. The study was carried out for two cropping periods in the Doaba region of Punjab Dist. Hoshiarpur. Brinjal was sown in the field for two kharif seasons [April to September 2021 and April to September 2022]. The meteorological data was obtained from the regional campus of Punjab Agriculture University. The site was visited twice a week and observations were taken for every season. Preserving and pinning of insects were done for identification of insects. The insects belonging to different orders along with their habitat (crop plant) were collected and identified. Relative abundance was calculated. The weekly data on weather conditions during the period of study was recorded. Randomly five plants were observed twice a week. Different species of insect pests were collected by hand picking, pitfall traps, colored traps, and insect collecting nets are used. After collecting the insect pests in seventy percent alcohol in glass vials for small and soft-bodied insects. Pinning was done for large-size insects. The preserved specimens were identified in the agriculture department at CT University with the help of keys (Zettler *et al.*, 2016; Schell *et al.,* 2007). The data collected were subjected to statistical analysis using ANOVA and correlation.

Seventeen insect pests belonging to five orders were recorded from selected transects.

* Author for correspondence

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Hemipterans were found to be very common and most abundant insect pests. Hemipteran appeared to be the most crowded order with seven insect pests. Neuroptera to be least abundant with one pest was found to be during the study period. Hemipteran mainly sucks the sap from all parts of plants such as leaves, stems, and flowers. Both nymphs and adults are destructive when hemipteran sucks the sap causing leaves to turn yellow and progress by clinking, curling, and destruction. Leaves and growing tips of plants resulted in stunted growth and poor fruit formation.

Caterpillars bore into tender shoots and in developing fruits, resulting in the drying of the tip. Larvae make holes and deposit excreta in them. Due to this stems of plants wither and wilt. Coleopteran grubs and adults both feed on the upper surface of leaves by scrapping the leaf tissues and only veins remains intact . It causes severe damage to the leaves. Orthopterans both nymphs and adults eating the leaves of plants and causes damage to newer tender parts of plant. Neuroptetans either act as minor pests, both nymphs and adults attack the upper surface of leaves and suck sap from it, causing a yellowing of leaves in patches (Table1).

During the growing season 2021, the correlation analysis results clearly showed that the pest population fluctuation in Brinjal depends upon weather parameters. From the month, April to September in May and June temperature increases up to August, increased minimum temperature positively correlated with pest population (r =0.656) and maximum temperature is negatively correlated with pest population (r = - 0.059). Relative humidity (r = 0.489 and r = 0.551) morning and evening respectively, evaporation (r =0.297), and rainfall (r =0.368) were positively associated with the pest population. Increase in relative humidity, evaporation, and rainfall the pest population increases. Sunshine (r = -0.778), wind speed (r = 0.126), and maximum temperature (r = -0.059) showed a negative correlation with the pest population.

During the growing season of 2022, it is observed that *Epilachna* and *Aphis gossypii* cause a maximum attack on the brinjal crop. During August month *cordius janus* abruptly disappeared with the end of the rainy season but *Phenacoccus soleni* attacked brinjal crops severely and caused the destruction of plants. Attack of *Epilachna* continued from vegetative to reproductive phase of plant. Maximum infestation of aphids occurs during August and September. High density and diversity were observed during the July and August months of the year 2022. *Aphis gossypii* (Aphids) infestation was high during September and became the cause of major crop damage. High density and diversity of insect pests were observed during July and August when relative humidity and rainfall were high but temperature is low as compared to May and June, Insect pest infestation positively correlated with minimum temperature and negatively correlated with maximum temperature (as temperature increases the pest population decreases. Relative humidity (r = 0.896) and rainfall (r = 0.313) have a positive correlation with the insect population. With the increase in humidity and rainfall pest population increases, wind speed (r = -0.731), sunshine (r = -0.413), and evaporation (r = -0.811) have a negative correlation on pest population.

Phenacococcus soleni infestation was higher in brinjal from July to September and became the cause of major damage to the brinjal crop followed by *cordius janus*. *Epilachana* acts as a major pest that attacks crops from the growing to the maturation phase. Other insect pest acts as minor pests but the density and diversity of insect pests are high during July to September due to low temperature, high rainfall, and humidity. Pest density and diversity were low from mid-April to July's first week due to high temperatures and sunshine.

It can be concluded that *the Phenacococcus soleni* attack was maximum during July, August, and September of Kharif 2021, and the maximum *Aphis gossypii* population in Kharif was recorded during August and September 2022. Epilachana acts as a continuous pest from the vegetative to the reproductive stage. Correlation analysis results clearly showed that pest population fluctuation in Brinjal depends upon weather parameters during

Table 1. Diversity of insect pests on brinjal (Kharif Crop) during period of 2021-22.

No	Name	Order/ Family	Damage
1	Hadda beetle *Epilachna varivestis* (F)	Coleoptera/ Coccinellidae	Grubs and adults make leaves during April to September
2	Pumpkin beetle *Aulcophora frontalis* (Augustae)	Coleoptera/ Chrysomelidae	Adults feed on foliage and flowers during April to May
3	*Aphis gosspyii* (Glover)	Hemiptera/ Aphididae	Sucks the sap during September
4	Blister beetle *Hycleus phaleratus* (Pallas)	Coleoptera/ Meliodae	Grubs and adults feed on growing tips, chew and bore into stems, feed on fruits and flowers during May -September.
5	*Melanoplus bibittatus* (Stal)	Orthoptera/ Acrididae	Adults feed on leaves during July - September.
6	Cow bug *Oxyrachis tarandus* (Rafinesque)	Hemiptera/ Membracidae	Adults and nymphs suck the sap from leaves and stems during August - September
7	Mealy bug *Phenacoccus solani* (Ferris)	Hemiptera/ Pseudococcidae	Adults and nymphs suck the sap from leaves and stems during July to October
8	White fly *Bemisia tabci* (Genn)	Hemiptera/ Aleyroidide	Nymphs and adults suck cell sap from lower surface of leaves and growing tips during July to September
9	Red pumpkin bug *Cordius janus* (F)	Hemiptera/ Dinidoridae	Adults suck the sap during July to September
10	Dock bug *Coreus marginatus* (L)	Hemiptera/ Coreidae	Adults suck the sap from leaves and stems during August to September.
11	Leaf roller *Antoba eublemma olivacea* (Walker)	Lepidoptera/ Noctuidae	Caterpillars feed leaves by rolling leaves from tip towards inside during July to September
12	Shoot and fruit borer *Leucinodes orbanalis* (Guenee)	Lepidoptera/ Pyralidae	Caterpillars bores into tender shoots resulting in drying of tip. Larvae attacks the fruits during July to September
13	*Eretmocera impactella* (Walker)	Lepidoptera/ Scythridiae	Feed on leaves during July to September
14	Lacewing bug *Urentius hystricellus* (Richter)	Neuroptera/ Tingidae	Both Nymphs and adults suck sap from leaves and cause yellowing of leaves in patches, during July to September
15	Jassids *Amrasca biguttula biguttula*	Hemiptera/ Cicadellidae	Feeding on plant sap during July to September
16	Grasshopper *Gamphocerippus rufus* (L)	Orthoptera/ Acridiae	Adults feed on leaves during April to September
17	Grasshopper *Attactomorha crenulata* (F)	Orthoptera/ Pyrgomorphidae	Adults feed on leaves during April to September

both growing seasons 2021 and 2022. Insect pests have a negative correlation with a maximum temperature (r = -0.05) the year 2021 and (r = -0.78) the year 2022 and positive correlation with relative humidity (r = 0.551) during the year 2021 and (r = 0.313) during the year 2022. It is observed during both growing seasons with an increase in rainfall of 283.5 mm in the year 2021 and 10 mm during the year 2022. In July and August, the density and diversity of all pests increased but at the high temperature during May and June, maximum temperature (38°C) and maximum sunshine (6.5)

Table 2. Relative abundance of insect pests of brinjal (Kharif Crop) during April to September 2021-2022

Insect	year	April	May	June	July	August	September
Epilachna varivestis	2021	8.63 ± 1.60[a]	36.25± 5.23[c]	19.37 ± 3.1[b]	24.75± 3.45[b]	37± 5.17[c]	3± 1.13 [a]
	2022	2.37±0.53[a]	2.75±.25[a]	2.75±0.45[a]	9±1.25[c]	3.75± .81[b]	3.63 ± .59[a]
Autoba olivacea	2021	NF*	NF	NF	NF	NF	NF
	2022	1.50±0.5[a]	1.75±0.36[a]	1.75±0.36[a]	0±0[a]	7.75 ± 1.66[d]	3.75 ± .52[a]
Melanoplus bibittatus	2021	NF	NF	NF	NF	NF	NF
	2022	0± 0[a]	0±0[a]	0±0[a]	9.15±1.34[c]	3.12± .78[b]	2.25 ± .25[a]
Austroasca viridis	2021	6.875 ± .39 [a]	1.125± .22 [a]	3.125 ± .63 [a]	8.75± 2.12 [b]	5.375± .53 [a]	2.37± .56 [a]
	2022	NF	NF	NF	NF	NF	NF
Gamphocerippus rufus	2021	2.13± .51 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]	1.75± .25 [a]	5.37± 1.42 [a]
	2022	3.87±0.74[b]	3.75±0.83[b]	2.50±0.59[b]	2±0.26[a]	2.6 ± .46[a]	1.37 ± .32[a]
Urentius hystricellus	2021	0 ± 0.00 [a]	3 ± .65 [a]	3.75± 1.04 [a]	2.5± .56 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]
	2022	NF	NF	NF	NF	NF	NF
Oxyrachis tarandus	2021	0 ± 0.00 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]	6.5± 2.04 [b]	4± .77 [a]	7± .77 [a]
	2022	0±0[a]	0±0[a]	1±0.44[a]	1.87±0 .22[a]	0 ± 0[a]	1.12 ± .35[a]
Aulcophora frontalis	2021	0 ± 0.00 [a]	0 ± 0.00 [a]	2± .46 [a]	3.375± .56 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]
	2022	0±0[a]	0±0[a]	0±0[a]	3.12±0.78[b]	3.75 ± .67[b]	0 ± 0[a]
Phenacoccus soleni	2021	0 ± 0.00 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]	19.63± 3.28 [b]	161.15± 9.15 [a]	161.15± 9.15 [c]
	2022	0±0[a]	0±0[a]	0±0[a]	7.87 ± 2.53[c]	1.75 ± .36[a]	0 ± 0[a]
Cordius janus	2021	0 ± 0.00 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]	38.87 ± 11.60 [f]	79.63 ± 1.87 [a]	0 ± 0.00 [a]
	2022	0±0[a]	0±0[a]	0±0[a]	2 ± .614[b]	1 ± .35[a]	0 ± 0[a]
Attactomorpha crenulata	2021	3± .37 [a]	0 ± 0.00 [a]	0 ± 0.00 [a]	3±.37 [a]	6.25± .72 [a]	0 ± 0.00 [a]
	2022	NF	NF	NF	NF	NF	NF
Eretmocera impactells	2021	NF	NF	NF	NF	NF	NF
	2022	0±0[a]	0±0[a]	2.87±0.44[a]	2 ± 1.37[c]	0 ± 0[a]	0 ± 0[a]
Blister Beetle	2021	NF	NF	NF	NF	NF	NF
	2022	0± 0	1.75±0.36[a]	2±0.42[a]	11.12 ± 1.63[d]	11 ± 1.63[d]	8 ± .87[b]
Aphids	2021	NF	NF	NF	NF	NF	NF
	2022	0±0[a]	0±0[a]	0±0[a]	0 ± 0[a]	21.62 ± 2.40[c]	110.62 ± 17.17[f]
Leucinodes orbonalis	2021	NF	NF	NF	NF	NF	NF
	2022	0±0[a]	0±0[a]	1.12±0.35[a]	3.37 ± .71[b]	3 ± .59[a]	4.25 ± .65[b]
Bemisia tabaci	2021	NF	NF	NF	NF	NF	NF
	2022	0±0[a]	0±0[a]	0.75±0.31[a]	3 ± 1.37[c]	3 ± .32[a]	2 ± .1[a]
Gonocerus (Dock Bug)	2021	NF	NF	NF	NF	NF	NF
	2022	0±0[a]	0±0[a]	1.12±0.75[b]	3.37 ± 1.37[c]	3 ± .1[a]	4.25 ± .87[b]

Values are Mean ± SE. Figures followed with different super scripts indicate significant difference (P< 0.05) by using Duncan multiple range test. Values are Mean ± SE. *NF= Not Found; Figures followed with different super scripts indicate significant difference (P< 0.05) by using Duncan multiple range test

showed a negative correlation (r = -0.78 and r = -0.41) respectively with insect pest population. Regarding crop production, changes in weather or climatic factors act as major factors. The pest population is favored by high relative humidity and rainfall from July to September a proactive and scientific approach will be required to deal with high pest populations during these months. Therefore, there is a great need for planning and formulation of strategies to prevent loss of crop yield during these months. During the experimental study of two years (2021-2022), *Mylabris pustulata* was observed very active and its population peaked during August and September. The study was supported by Bibha Kumari *et al.* (2022) with the same observations that the highest number of insect species was noticed in September and the lowest in May. *Mylabris flexuosa* were captured during mating in August and September (rainy season).

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Description of a new species of potter wasp (Hymenoptera, Vespidae, Eumeninae) from northeast India

E. Benny Femi[1*], A. P. Ranjith[1, 2] and D.R. Priyadarsanan[1]

[1]*Ashoka Trust for Research in Ecology and the Environment (ATREE), Royal Enclave, Srirampura, Jakkur Post, Bangaluru 560064, Karnataka, India.*
[2]*Integrative Insect Ecology Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.*
Email: femibenny94@gmail.com; ridhuranjith@gmail.com; priyan@atree.org

ABSTRACT: *Pseumenes* Giordani Soika, 1935 is a small genus of potter wasps occurring in Oriental, Australian and Palearctic Regions. Only one species, *Pseumenes depressus* (de Saussure, 1855) is known so far from India. A new species, *Pseumenes siangensis* **sp. nov.** from Arunachal Pradesh, is described. The morphological affinities of the new species are discussed. The new species is compared with the closely related *P. depressus* as well as *P. laboriosus.* Since *P. depressus* is similar to *P. laboriosus*, comparisons were made between *P. laboriosus* and *P. siangensis* **sp. nov**. The apical teeth of the propodeum are medium sized and blunt in *P. siangensis* **sp. nov**. (long and sharp in *P. laboriosus*); the posterior part of the first tergite is densely punctate in the middle *P. siangensis* **sp. nov**. (almost impunctate in *P. laboriosus*). The clypeus without a median black spot in *P. siangensis* **sp. nov.** (with median black spot in *P. laboriosus*).
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KEY WORDS: Eastern Himalayas, new description, taxonomy, morphological affinities

The potter wasps, belonging to the subfamily Eumeninae, stand out as the most diverse group within the family Vespidae, with around 3,795 species across 205 genera (Selis 2017; Kumar *et al*., 2019; Li *et al*., 2019; Lien *et al*., 2020). These wasps are cosmopolitan in distribution and are mostly known for their solitary or occasionally sub-social lifestyle (Pannure *et al*., 2016). They vary in size and shape, from small and stout to large and elongate, with their metasoma varying from sessile to distinctly petiolate. The Indian potter wasp fauna is represented by 193 species under 48 genera (Gawas *et al*., 2020). Despite their diversity, the study of potter wasps in India remains limited (Pannure *et al*., 2016; Kumar *et al*., 2019). The genus *Pseumenes*, described by Giordani Soika in 1935 includes a small group of solitary wasps with eight species within the Oriental Region, as documented by Giordani Soika (1935, 1941), Vecht (1963), Selis (2017) and Lien *et al*. (2020). Till now only one species, *P. depressus* (de Saussure, 1855) has been reported from India. Here a new species, *Pseumenes siangensis* **sp. nov.,** collected from Arunachal Pradesh, India in the eastern Himalayas is described with illustration. The new species is compared with the closely related *P. depressus* as well as *P. laboriosus.*

The lone specimen was collected with a sweep net from Upper Siang, Arunachal Pradesh. It was

* Author for correspondence

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treated with hexamethyldisilazane (Heraty and Hawks, 1998) and later dried and pinned. Images were taken with Keyence VHX-6000 digital microscope. Morphological terminology and cuticular sculpture are adapted from Carpenter and Cumming (1985) and Yamane (1990). Type specimen is deposited in the collections of the ATREE Insect Museum, Bengaluru, India (AIMB/Hy/Vs300001) and will be subsequently transferred to the National repository of ICAR-National Bureau of Agricultural Insect Resources (ICAR-NBAIR).

Terms and measurements: T1–T6: Tergite 1–Tergite 6; S1–S6: Sternite 1– Sternite 6; IOD: Inter-ocellar distance, the distance between the two posterior ocelli; OOD: Ocello-ocular distance, the minimum distance between the posterior ocellus and eye.

Order Hymenoptera Linnaeus, 1758;
Family Vespidae Latreille, 1802;
Subfamily Eumeninae (Leach, 1815);
Genus *Pseumenes* Giordani Soika, 1935

Pseumenes Giordani Soika, 1935, 57: 145, subgenus of *Pareumenes* de Saussure. Type species: *Eumenes eximius* Smith, 1861, by original designation.

Diagnosis: See Lien *et al*. (2020); **Distribution:** Oriental Region

***Pseumenes siangensis* sp. nov.** (Figs. 1–3)

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Type material. Holotype female, India: Arunachal Pradesh, Arpung River, Upper Siang, 16°2412 N, 74°2242 E, 615m a.s.l., 17.x.2022, coll. Ranjith A.P. (AIMB).

Diagnosis: This new species differs from all other Oriental species of *Pseumenes* in having the following combination of characters: punctuation in the petiole deep, distinct and coarse (Figs. 2C, E); yellow markings on mesoscutum not arcuate (Fig. 1F); two submedial yellow spots on petiole absent (Figs. 1A, 2C, E); inner eye margin with discontinuous yellow longitudinal band (Fig. 1B); T2 not medially emarginate and with only apical band and no oval spot (Fig. 3A); only one yellow patch in mesopleuron (Fig. 2A); sub tegular yellow spot small (Fig. 1F); T5 without any yellow markings (Figs. 2D, 3A, C); anterior width of third submarginal cell 5.0 × that of second submarginal cell (Figs. 3B, D).

Description: Holotype, female, length of body from head to apex of last tergite 30.2 mm; fore wing length 19.3mm.

Head: Head in anterior view longer than wide, about 1.2 × as long as wide. Ocelli rather flat than raised, the posterior pair 1.1 × far apart than the distance from the eyes (OOD), the anterior ocellus 1.4 × wider than the posterior ocelli, IOD: 0.51mm (Fig. 1C). Frons densely covered with coarse punctures except apical 1/4th area (Fig. 1B). Vertex without fovea, with coarser and denser punctures than on frons; punctures on gena smaller and sparser than on vertex, space between punctures smooth (Figs. 1C, D); area behind ocellus triangle not prominently swollen. Distance from posterior ocelli to apical margin of vertex about 1.5 × distance from posterior ocelli to inner eye margin (Fig. 1C). Gena in lateral view 1.2 × as wide as eye at ocular sinus, sharply tapering in lower third (Fig. 1D). Occipital carina present only laterally, indistinct dorsally (Fig. 1C). Inner eye margins almost parallel, weakly converging below (Fig. 1B). Clypeus with a small flattened area just above apical margin, disc of clypeus in lateral view gradually convex from base to near apical margin, then slightly bent backwards, in frontal view 0.9 ×as long as wide, with basal margin slightly convex medially, almost touching antennal sockets; apical margin emarginate medially, forming blunt tooth on each lateral side, width of emargination much less than 0.2 × width of clypeus between inner eye margins (Figs. 1B, E). Clypeus sparsely punctured, each puncture bearing a medium length seta, area between punctures without micro punctures. Mandible smooth, broad, with five prominent teeth, first and second teeth from base truncated, third one triangular and fourth and fifth pointed (Figs. 1D, E). Antenna with 12 segments, antennal scape long, about 4.4 × as long as its maximum width; flagellomere I 1.8 × as long as wide, flagellomeres II–V, VII and IX slightly longer than wide, VI, VIII

Fig. 1 *Psuemenes siangensis* **sp. nov**., holotype, female A) habitus, lateral view; B) head, anterior view; C) head, dorsal view; D) head, lateral view; E) head, ventral view; F) mesosoma, dorsal view.

Fig. 2 *Psuemenes siangensis* **sp. nov**., holotype, female A) mesosoma, lateral view; B) propodeum, dorsal view; C) T1, lateral view; D) metasoma, lateral view; E) T1, dorsal view; F) T1, ventral view.

as long as wide, terminal flagellomere bullet shaped, longer than wide (Figs. 1D, E).

Mesosoma: 1.3 × longer than wide in dorsal view. Pronotal carina strongly raised through out, reaching ventral corner of pronotum (Fig. 2A). Apical margin of pronotum with punctures similar to those on frons, shallow punctures on basal region (Fig. 1F). Mesoscutum in lateral view strongly convex, in dorsal view as long as wide between tegulae with a short mid longitudinal groove anteriorly, without any trace of prescutal furrows near apical margin, lateral impressed longitudinal lines near lateral sides also indistinct, uniformly covered with strong coarse punctures except lateral side of apical margin (Fig. 2A). Tegula with sparse, small punctures (Fig. 1F). Punctures on scutellum similar to those near lateral side of apical margin of mesoscutum (Figs. 1F, 2B). Disc of scutellum nearly flat, in lateral view at same level of apical margin of mesoscutum. Metanotum slightly convex, sloping down to apical margin. Punctures on metanotum larger than those on scutellum, distinct in apical half (Fig. 2B). Mesepisternum with punctures on upper dorsal part very coarse, space between punctures strongly raised to form reticulation, with strong and large punctures postero-dorsally, several shallow and small punctures antero-ventrally. Metapleuron largely smooth, apical part with several long strong striae, and basal part with several short strong striae, with some shallow and very sparse punctures (Fig. 2A). Propodeum short and broad dorsally, moderately convex, deeply excavated medially, with the excavation wide, less than one-third width of propodeum, excavation with edges raised like two parallel and sharp carinae, basal triangular area with longitudinal basal fovea, with median carina runs from fovea to apical margin, several obliquely ribbed flank on both sides of median carina, lateral side shallowly emarginate at apex, apical teeth of propodeum medium sized, blunt (Fig. 2B). Most of mesosoma with short setae, setae on propodeum longer, with coarse and dense punctures on each side of excavated area medially, lateral surface of excavated area obliquely rugose, with several shallow punctures in between rugose area.

Wings: Fore wing vein 1-M 1.4 × as long as vein 1-SR; second submarginal cell sessile anteriorly, 1.6 × as long as third cell diagonally (Figs. 3B, D).

Metasoma: T1 long and slender, 0.7 × less than that of mesosoma; parallel sided in basal two-third, inflated in apical third, in dorsal view nearly 2.4 × as long as wide, lateral tubercles inconspicuous, in lateral view gradually convex from base, then slightly depressed and gradually convex near apical margin, distinctly narrower than T2, covered with medium punctures, sparse in basal 1/4th, gradually denser towards apex (Figs. 2C, E); T2 in dorsal view 1.2 × wider than long, in lateral view regularly convex from base to apical margin, without raised lamellae at apical margin, not medially emarginate, punctures at lateral sides of T2 similar but sparser than those on apical part of T1 with an impunctate area in basolateral side, punctures at dorsal part fainter (Figs. 2D, 3A). S1 parallel in basal two-thirds, enlarged rather abruptly in apical third with several distinct and short transverse striae in middle of narrowed basal region, remaining surface towards apex without striate, with strong punctures (Fig. 2F). S2 in lateral view gradually and slightly convex from base to apical margin. Punctures on T3–T6 much smaller and weaker than those on T2 (Figs. 3A, C); T5–T6 with minute punctures; punctures on S2– S4 sparse basally, punctures on S5 and S6 very sparse, S6 with minute punctures (Fig. 2D).

Fig. 3 *Psuemenes siangensis* **sp. nov**., holotype, female A) metasoma, dorsal view; B, D) wings, dorsal view; C) metasoma, distal tergites.

Colour: Black; body covered with majorly golden setae, following parts yellow: clypeus except apical flattened area, ocular sinus, two irregular spots on inner margin of eye, large spot between antennal toruli and narrowly extending to anterior ocellus, antennal scape beneath (Fig. 1B), two lateral spots on occiput extending from gena, two discontinuous bands on pronotum (Fig. 1F), subcircular spot on mesopleuron (Fig. 2A), two submedial longitudinal band on mesoscutum (Fig. 1F), tegula except medially, parategula, two lateral spots on scutellum, fore femur, tibiae dorsally, apical bands on T1–T4 (Figs. 3A, C), small spot on lateral side of S2 (Fig. 2D) yellow. Small flattened area at apical margin of clypeus (Fig. 1B), antennal scape ventrally, vertex, occiput except laterally, basal margin of pronotum, mesoscutum except submedial bands, setae on mesoscutum, metanotum, metapleuron, medial excavation and two antero-lateral spots of propodeum, pair of small lateral spots near apical margin of T1, T5–T6 (Figs. 3A, C), S1–S6 except pair of small spots on lateral side of S2 black. Flagellomeres beneath ferruginous (Fig. 1E). Propodeal valvulae light brown (Fig. 2B). T2–T6 with subapical row of long golden setae (Fig. 2D). Wings infuscate, veins dark brown (Figs. 3B, D).

Male: Unknown; **Biology:** Unknown; **Distribution:** India (Arunachal Pradesh).

Etymology: The specific epithet '*siangensis*' is derived from the Siang Valley in Arunachal Pradesh, India, where the specimen was collected. The name honors the collection locality and highlights the biological richness and ecological significance of this region.

Remarks: This species comes close to *P. depressus* (de Saussure, 1855), but can be distinguished from the latter by having punctuation

in the petiole distinct and coarse (apical part sparsely and strongly punctate medially in *P. depressus*); yellow markings on mesoscutum not arcuate (strongly arcuate almost touching tegula in *P. depressus*); two submedial yellow spots on petiole absent (present in *P. depressus*); inner eye margin with discontinuous yellow longitudinal band (continuous in *P. depressus*); T2 not medially emarginate and with only apical band and no oval spot antero-laterally (medially emarginate and with both apical band and oval spot in *P. depressus*); only one yellow patch in mesopleuron (two in *P. depressus*); subtegular yellow spot small sized (longer in *P. depressus*); T5 without any yellow markings (with yellow markings in *P. depressus*); anterior width of third submarginal cell 5.0 × that of second submarginal cell (2.3 × in *P. depressus*). Since *P. depressus* is similar to *P. laboriosus*, comparisons were made between *P. laboriosus* and *P. siangensis* **sp. nov**. The apical teeth of the propodeum are medium sized and blunt in *P. siangensis* **sp. nov**. (long and sharp in *P. laboriosus*); the posterior part of the first tergite is densely punctate in the middle *P. siangensis* **sp. nov**. (almost impunctate in *P. laboriosus*). The clypeus without a median black spot in *P. siangensis* **sp. nov.** (with median black spot in *P. laboriosus*).

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First report of mealybug, *Crisicoccus hirsutus* (Newstead) on cocoa (Hemiptera, Peudococcidae) from India

Jayalaxmi Narayan Hegde*, Indhusri Chavan, G. A. Kavya Yadav and R. Darshan

Department of Entomology, College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga 577201, Karnataka, India
Email: jnh@uahs.edu.in

ABSTRACT: During the survey, five species of mealybugs *viz*., *Pseudococcus longispinus* (Targioni Tozzetti), *Planococcus citri* (Risso), *Rastrococcus* sp., *Planococcus lilacinus* Cockerell and *Crisicoccus hirsutus* (Newstead) were found damaging cocoa crop. Among these, *C. hirsutus* collected from cocoa plantation of Sagara taluk of Shivamogga District, Karnataka, is a new record from India. © 2024 Association for Advancement of Entomology

KEY WORDS: Pests, survey, new record

Cocoa (*Theobroma cacao* L.) is native of Amazon region of South America. It is an important plantation crop grown for Chocolates around the world. In India, cocoa is being cultivated in the states of Kerala, Karnataka, Andhra Pradesh and Tamil Nadu in an area of 1,03,376 ha with total production of 27,072 MT. In Karnataka, it is cultivated in an area of 14,216 ha with a production of 3,719.10 MT and productivity of 525 kg ha^{-1} (DCCD, 2020-21). Cocoa crop has several constraints for attaining its maximum yield potential which include the problems of pest and diseases, nutritional imbalance, water stress *etc*. Among these, problem of pests would bring about loss in yield to the greater extent. Though over 150 different insects are known to feed on cocoa, only 2 per cent are of economic importance. Mirid bugs (*Helopeltis antonii* Signoret, *H. bradyii* Waterhouse), cocoa pod borer and mealybug are most significant and widely occurring insect pests of cocoa. Mealybugs are generally not only major pest themselves, but are well known vector for viruses that are known to transmit cocoa swollen shoot virus (Strickland, 1951; Andres *et al*., 2017)

Mealybugs (Hemiptera, Pseudococcidae) are one of the destructive insect pests and damage a wide range of horticultural and agricultural crops such as cocoa, coffee, guava, Solanum spp. and citrus (Bodenheimer, 1951). Many species of mealybugs have become serious invasive pests when introduced into new areas beyond their native (or natural) distribution (Miller *et al*., 2002). About ten species of mealybugs are known to attack cocoa crop (Campbell, 1983). Mealybugs are hard to kill pests of trees and very difficult to manage them (Dhawan *et al*., 2009). They reduce the fruit quality by sucking at the base of pods, tender twigs, shoots, stalks of tender pods, calyx and tender leaves. This leads to weakening of stalk base, uncharacteristic marks on pods and drying of tender pods. In addition, it also causes the accumulation of honeydew and sooty mould growth on leaves and pods (Lower,

* Author for correspondence

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1968; Sforza *et al*., 2003).

Periodical surveys were undertaken to study the species diversity of mealybugs in cocoa ecosystem in different places of malnad region during 2017-18 and 2018-2019. The incidence of mealybugs on cocoa was typically observed as cluster of cotton like masses on trunks, stems, chupons (basal shoots), leaves, flowers and pods. The mealybugs were collected carefully with the help of paint brush. Collected mealybugs were preserved in 2 ml tubes containing ethanol (75%). Each sample was given a unique number to associate it with the relevant collection data.

There were more than one species of mealybugs affecting cocoa crop. Species diversity of mealybugs varied with the location, and five species of mealybugs *viz*., *Pseudococcus longispinus* (Targioni Tozzetti), *Planococcus citri* (Risso), *Rastrococcus* sp., *Planococcus lilacinus* Cockerell and *Crisicoccus hirsutus* (Newstead) were recorded from different locations damaging difeertn parts of cocoa. Among these, *Crisicoccus hirsutus* (Newstead) was the newly identified species which was collected from Sagara taluk of Shivamogga District of Karnataka. *Crisicoccus hirsutus* (Newstead) is the new record of mealybug species from cocoa from India.

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OBITUARY

Dr. Jayaprakas C.A.
(1961 – 2024)

It is with deep sorrow that we announce the passing of Dr. Jayaprakas C.A., a renowned Scientist (retired as Principal Scientist from ICAR - Central Tuber Crops Research Institute, Thiruvananthapuram on 31 May 2023) and a cherished member of our community. Dr. Jayaprakas passed away on 14 September 2024 at the age of 63, in his residence at Thiruvananthapuram.

Dr. Jayaprakas joined Agricultural Research Service as Scientist in Agricultural Entomology in 1989 and subsequently joined the ICAR - Central Tuber Crops Research Institute, Thiruvananthapuram. He dedicated his life to the pursuit of knowledge and discovery, making significant contributions in the field of ecofriendly pest management strategies. He was very popular among farmers for his innovations and farmer-friendly technologies, most famously cassava-based biopesticides (Nanma, Menma, Shreya), for which he received patents.

Dr. Jayaprakas was not only known for his professional achievements but also for his active involvement in various social services activities. He was involved in blood donation for more than 30 years. After retirement, he was serving as visiting

professor in Mahatma Gandhi University, Kottayam, Kerala. He was nominated as General Council Member of Kerala Agricultural University, Thrissur. He was recipient of several awards, including the Best Scientist Award (2015) from the Government of Kerala, Fellow of the Kerala Science Academy (2013), Karma Shreshta Award (2013), Swadeshi Innovation Award (2012), Bhodananda Research Foundation award for team work (2011), and the Eminent Scientist Award (2011). He also served as President, Indian Society for Root Crops (ISRC), Joint Secretary, Association of Advancement of Entomology (AAE), Executive Council Member of AAE, Executive Council Member, Central University of Kerala, Expert Committee Member, Programme Advisory Committee (PAC), Technology Development Programme (TDP) of DST, Government of India. He was recognised as a Ph. D guide both at the University of Kerala and Kannur University, where he supervised 12 Ph. D students. He also published several research papers, books and many other articles.

Dr. Jayaprakas, a native of Eramangalam, Ponnani, Malappuram, is survived by his wife, Dr. Bindu, T., (Family Health Centre, Pallichal, Thiruvananthapuram), and his daughter, Dr. Radhika Jayaprakas. His hard work and innovative ideas inspired many new generations of scientists, including me, and will definitely motivate many more in the future.

Dr. Harish E.R., ARS
Senior Scientist (Agricultural Entomology)
ICAR - Central Tuber Crops Research Institute,
Thiruvananthapuram 695 017

AUTHOR INDEX

Abesh Chakraborty, 355
Abhinav Saxena, 417
Abhisubesh V., 425
Alyas Ali, 447
Amar Singh, 343
Amita Kanaujia, 453
Amrit Deep Kaur, 459
Anand Harshana 455
Aneha K., 425
Anindita Das, 373
Annu Kumari, 355
Anurudha Chakraborty, 355
Arka Mukherjee, 295
Arun P.R., 309
Ashiq P.P, 309
Atanu Naskar, 295
Benny Femi E., 465
Bhagyashree Bora, 425
Chaithra Shree J., 309
Darshan R., 471
Debdeep Pramanik, 295
Debjani Dey, 455
Debjani Ghosh, 295
Deen Mohd Bhat, 445
Dhriti Banerjee, 295
Diksha Rabha, 327
Dip Jyoti Haloi, 327
Geetha I., 425
Gokul Krishna Dhanapati, 435
Harminder Pal Singh, 399
Hemaladkshmi P., 425
Inayat Ullah Lone, 445
Indhusri Chavan, 471
Innifa Hasan, 327
Jayalaxmi Narayan Hegde, 471
Jayita Sengupta, 295
Jeevith S., 309
Jibi Lukose, 425
Jyoti Antil, 453
Kakhuangailiu Gangmei, 425
Kamlpred Kaur, 459
Kartik Omanakuttan,335
Kavya Yadav G.A., 471
Kavyamol P.M., 363
Koustav Mukherjee, 295
Kuldeep Singh Barwal1, 399
Madhu Rana, 343
Mahender Singh Thakur, 343
Manoj Kumar Arya, 335
Mathivanana A., 425
Mayoushree Gogoi, 355
Mohd Ali, 447
Nivedita Pattanaik, 399
Oishik Kar, 295
Paul Pop, 399
Poopathi S., 425
Pramod P., 309
Prashant Tripathi, 453
Priyadarsanan D.R., 465
Puneet Pandey, 399
Rajinder Kaur, 417
Randeep Singh, 399
Ranjith A.P., 465
Sahadiya Mandodan, 425
Saikat Sarkar, 295
Sajad Ahmad Bhat, 459
Sajad Ahmad Khan, 445
Sangeeta Biswas, 355
Sanjana Goswami, 335
Sanjay Sondhi, 399
Semmaran M., 453
Shabbir Hussain, 447
Shilpa K.. Satheesan, 381
Shreevihar Santhosh, 381
Sri Sowmiya M., 309
Suprakash Pal, 435
Susanta Kumar Chakraborty, 373
Swapna Rani K., 435
Vineesh P.J., 363
Vineetha V.P., 363

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Department of Agricultural Entomology, Kerala Agricultural University,
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3. Professor G. Madhavan Nair, Former Head, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram, 695522, Kerala, India.
4. **Dr. C.A. Jayaprakas, Former Principal Scientist and Head, Division of Crop Protection, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram 695017, Kerala, India.**
5. Dr. T. Sivakumar, Subject Matter Specialist (Entomology), KVK, ICAR- Central Plantaion Crops Research Institute, Kayamkulam 690533, Kerala, India.
6. Dr. T. Santhoshkumar, Assistant Professor, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram, 695522, Kerala, India.
7. Dr. S. Poopathi , Scientist-G (Director Grade), Head of Microbiology and Molecular Biology, Vector Control Research Centre, Indian Council of Medical Research, Pondicherry 605 006, India.
8. Dr. Ambili Paul, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram, 695522, Kerala, India.
9. Dr. E. R. Harish, Scientist, Division of Crop Protection, ICAR – Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, 695017, Kerala, India.
10. Dr. M. S. Palaniswami, Chief Editor, ENTOMON, AAE.
11. Dr. K. M. Sreekumar, Professor and Head, Department of Agricultural Entomology, Kerala Agricultural University, College of Agriculture, Padnekkad, Nileswaram, Kasaragod, Kerala, India.
12. Dr. C. Bijoy, Assistant Professor and Research Supervisor, Department of Zoology, Christ College (Autonomous), Irinjalakuda, Thrissur 680 125, Kerala, India.

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