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ENTOMON

Vol. 50

September 2025

No. 3

Contents

Articles

	Page
https://doi.org/10.33307/entomon.v50i3.1520	253
Six new additions to the Indian butterfly (Lepidoptera) fauna from Upper Siang, Arunachal Pradesh, India <i>Monsoon Jyoti Gogoi, Rajkamal Goswami, Seena Narayanan Karimbumkara and Agur Litin</i>	
https://doi.org/10.33307/entomon.v50i3.1521	261
Study of egg laying pattern and hatching line formation of <i>Culicoides peregrinus</i> Kieffer and <i>C. oxystoma</i> Kieffer (Diptera, Ceratopogonidae), vector of bluetongue virus <i>Paramita Banerjee, Ankita Sarkar and Abhijit Mazumdar</i>	
https://doi.org/10.33307/entomon.v50i3.1522	269
Aquatic beetles (Dytiscidae, Gyrinidae, Haliplidae, Hydrophilidae, Noteridae) of the Papikonda National Park from the Eastern Ghats, Andhra Pradesh, India <i>Shiva Shankar, Devadoss Kumar, Deepa Jaiswal, Karuthapandi Madasamy and Shrikant Jadhav</i>	

- <https://doi.org/10.33307/entomon.v50i3.1523>** 291
Molecular identification and phylogenetic analysis of spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera, Aleyrodidae) from different agroecological zones of Kerala, India
Drisya Pavithran, J.V. Siji, C.A. Jayaprakas and B.G. Sangeetha
- <https://doi.org/10.33307/entomon.v50i3.1524>** 299
Characterization and antibacterial properties of oak tasar and mulberry silkworm pupae oils
Kusumala Suman, Devika Srivastava and R. Venkatesh Kumar
- <https://doi.org/10.33307/entomon.v50i3.1525>** 307
Bioefficacy of certain insecticides in cabbage against lepidopteran pests
Ravi Singh, Sameer Kumar Singh, Kamal Ravi Sharma, Umesh Chandra and Pradeep Kumar Dalal
- <https://doi.org/10.33307/entomon.v50i3.1526>** 311
Effect of aqueous extracts of *Pithecellobium dulce* (Roxb.) Benth against *Pediculus humanus capitis* De Geer, 1778 (Psocodea, Pediculidae)
Susmita Gudulkar, Himanshu Dawda, Usha Mukundan and Somnath Kangralkar
- <https://doi.org/10.33307/entomon.v50i3.1527>** 317
High levels of endogenous polyamines indicate superiority of *Morus alba* L. varieties for improved silk production in *Bombyx mori* L.
Brinda Goda Lakshmi Didugu, Sayyad Sadiya Kousar, Degsew Bezabih Gebresilassie, Vaishnavi Ponugupati, Haroon Rashid MD, Madhavi Kasa and Anitha Mamillapalli
- <https://doi.org/10.33307/entomon.v50i3.1528>** 325
Toxic effects of pesticides on the green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Peterson) (Neuroptera, Chrysopidae)
N. Santhana Bharathi, T.P. Rabeesh and K. Sujatha

<https://doi.org/10.33307/entomon.v50i3.1529> 335

Evaluating wetland health using Odonata diversity and seasonal shifts in
Tulshi Reservoir, Maharashtra, India

Rahul S. Kamble, Gajanan K. Sontakke and Sagar A. Vhanalakar

<https://doi.org/10.33307/entomon.v50i3.1530> 347

Rediscovery of *Eristalinus (Merodonoides) multifarius* (Walker, 1852)
(Diptera, Syrphidae) from Kerala, India and its molecular phylogeny

*Aiswarya Joy, S.S. Anooj, Manju Mary Paul, B.Ramesha and
K.M. Sreekumar*

<https://doi.org/10.33307/entomon.v50i3.1531> 355

New state records and distributional range extensions of termite species in
India

M. Roy, J. Basak, B. Baraik and K. Rajmohana

Short Communications

<https://doi.org/10.33307/entomon.v50i3.1532> 363

Effect of host plants on the feeding potential of *Episyrphus balteatus*
(De Geer) preying on two different aphid species

Kimmi Kumari and Md. Equbal Ahmad

<https://doi.org/10.33307/entomon.v50i3.1533> 367

First record of *Leptus* Charles De Geer, 1778 (Acari, Erythraeidae) larval
parasitism on *Eyprepocnemis alacris alacris* Serville, 1838 (Orthoptera,
Acrididae) from Kerala, India

T. Deepthy and R.S.M. Shamsudeen

<https://doi.org/10.33307/entomon.v50i3.1534> 371

Studies on developmental biology of lesser grain borer, *Rhyzopertha*
dominica (F.) (Coleoptera, Bostrichidae) at different seasons on paddy

*V. Ramalakshmi, Deepayan Padhy, T. Mounika,
Manish Kumar Yadav and Goutam Hosamani*

<https://doi.org/10.33307/entomon.v50i3.1535>

375

First record of *Tachardiaephagus tachardiae* (Howard, 1896)
(Hymenoptera, Chalcidoidea, Encyrtidae) from an invasive Lac insect,
Kerria destructor Talukder and Das, 2020 (Hemiptera, Kerriidae)
Anil Kumar Dubey, A. Rameshkumar and S.R. Koteswar Rao

Author Index

379

Six new additions to the Indian butterfly (Lepidoptera) fauna from Upper Siang, Arunachal Pradesh, India

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ABSTRACT: This paper reports six new additions to the butterfly fauna of India, based on photographic evidence from the community forests of Simong Village in Upper Siang district of Arunachal Pradesh, India. The six species — *Horaga takanamii* Seki and Saito, 2004, *Dacalana vui* Saito and Inayoshi, 2022, *Euthalia zhaxidunzhui* Huang, 1998, *Athyma yui* Huang, 1998, *Stichophthalma neumogeni renqingduojiei* Huang, 1998 and *Stiboges elodinia* Fruhstorfer, 1914 — have not been previously reported from India. *Horaga takanamii* was previously known from Laos, while *D. vui* was known to be distributed in Northern Thailand (Nan Province) and Northern Vietnam and *S. elodinia* from Northern Thailand to China. *Euthalia zhaxidunzhui*, *A. yui* and *Stichophthalma neumogeni renqingduojiei* were earlier reported from Northern Myanmar and Metok county of the adjoining Tibet. These new reports highlight the importance of Siang valley as an important region of lepidopteran diversity in India.

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KEY WORDS: Community forest, distribution records, Eastern Himalayas, Metok county

INTRODUCTION

The Siang Valley, nestled in the Eastern Himalayas of India, is named after the Siang River which is known as *Yarlung Tsangpo* (or *Yarlung Zangbo*) in Tibet, where it flows for 1200kms. The river, also known as Brahmaputra in Assam, has been critical in shaping the ecological and cultural landscape of the region, which is bounded by the Mishmi Hills of Dibang Valley on its east and the Miri Hills of Ziro or Apatani Valley in the west. The northern part of the valley is bounded by the southeastern region of Tibet.

The predominantly mountainous valley spans a high elevational gradient ranging from 150m in the foothills to 5000 m, and is therefore extremely rich in biodiversity. The natural habitat ranges from alluvial wet grassland in the foothill plains to tropical evergreen forests between 150 – 1200m in the low elevation belt, sub-tropical and temperate broadleaf in mid-elevation belt (1200 – 2500m) and mixed conifer forests and alpine meadows at higher elevations between 2500 – 3500m and beyond.

Siang Valley experiences a tropical to alpine climate, with heavy monsoon rains between June and September, accounting for 75 per cent of the total

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annual rainfall, along with pre and post monsoon rains from February to May and in October. This results in an annual rainfall of 2500 – 3000 mm, which significantly influences vegetation and faunal distributions. Upper Siang supports a rich assemblage of flora and fauna, with high endemism due to its varied topography and altitudinal gradients (Singh and Singh, 2012; Datta–Roy *et al.*, 2018; Taram *et al.*, 2020; Sharma *et al.*, 2024). The forests here serve as critical habitat for several rare and range-restricted species, including high-altitude butterflies, highlighting the importance of biodiversity monitoring in the region.

Butterflies from Siang remain poorly studied (Evans, 1912; Sarkar, 2018). Several previous studies on butterflies and other flora and fauna from the adjacent Dibang valley have reported species similarities with Metok (variably called Medog or Motuo) region of Southeastern Tibet (Evans, 1915; Gogoi and Payra, 2019; Huang, 1998; Boruah *et al.*, 2022; Dodum, 2021). However, the presence of Metok species in Siang has not been confirmed so far despite both the regions being connected by the same river. This can be probably because the studies were opportunistic and lacked seasonal replicates in their survey design, leading to severe under-reporting of butterfly species from this region.

The first major butterfly survey in the Siang Valley was conducted during the infamous Abor military expedition, when H.W. Price and E.H. Sweet collected specimens from the valley and adjacent plains of Assam. Their records noted only 74 species, likely due to heavy rainfall during the collection period (Evans, 1912, 1932). More than a century later, in 2018, a team from the Wildlife Institute of India retraced the route of the British expedition and recorded 269 butterfly species during a 22-day survey; however, no formal species list was published (Sarkar, 2018). Since then, no systematic studies on the butterflies of Siang have appeared in peer-reviewed literature, leaving much of the valley's butterfly diversity—and its broader faunal composition—still poorly documented and understood.

MATERIALS AND METHODS

The butterfly records presented in this paper were collected during a camera trapping expedition aimed at documenting the biodiversity of Litin Community Conserved Area (henceforth, Litin CCA), situated within the Simong Community Forest (henceforth, Simong Forest), in Upper Siang district, Arunachal Pradesh, India. The local communities here, including the Adi and other indigenous groups, play a crucial role in conservation through traditional land stewardship practices. Located near Simong Village and the town of Yingkiong, Litin CCA spans an elevation of 1800 – 3800m, encompassing subtropical, temperate and alpine ecosystems. The area forms a key part of ATREE's long-term biodiversity monitoring and community-led conservation initiatives in the Siang Valley, contributing to the documentation and protection of the region's unique biota (ATREE's Siang Valley Biodiversity Conservation Programme). Opportunistic butterfly sampling was carried out alongside camera-trapping activities in Simong Forest (Fig. 7). The first survey was conducted on June 8–9, 2024, around Simong village, at elevations ranging from 1300 – 1500m, and included an additional survey at the same site on July 19, 2024. The second survey, from July 31 to August 3, 2024, focused on the higher elevation regions of the Simong Forest. This survey aimed to support the Litin clan in installing camera traps along the higher ridges of the Abor Hills, particularly in areas adjoining Mount Eko–Dumbin, which forms part of the Litin CCA. The total trail distances covered on foot during the first and second surveys were approximately 6 and 12km respectively. During the surveys, butterflies were photographed in their natural habitat. In some cases, individuals were gently handled to capture images of both the dorsal and ventral wing surfaces, after which they were released. Species identification and confirmation were carried out using standard references, including Huang (1998), Yago (2004), Saito and Inayoshi (2022), and Huang *et al.* (2024).

RESULTS AND DISCUSSION

During the survey, 90 butterfly species were recorded over a span of seven days. Among these,

the most notable observation was of *Horaga takanamii* Seki and Saito, 2004, previously known only from Laos - representing a new record for India. Additionally, a single individual each of *Dacalana vui* Saito & Inayoshi, 2022 and *Stiboges elodinia* Fruhstorfer, 1914, were also documented. The present study, which recorded three butterfly species previously known only from Metok County in southeastern Tibet, highlighting, the distribution of shared species and the habitat continuity with the Siang Valley. These finding reinforce the view that Metok falls within the Sino-Himalayan subregion of the Oriental (Indo-Malayalam) region, a transitional zone linking the Indian sub region to the west and south with the Chinese subregion to the east and north. Nearly all butterfly species recorded from Metok exhibit subspecific affinity with those from the Eastern Himalayas (Huang, 1998). A striking example is the Tibetan Jungle Queen, *Stichopthalma neumogeni renqingduojiei* Huang, a subspecies previously reported only from Metok. Other notable sightings, beyond the new records for India, included the

Bhutan Treebrown (*Lethe margaritae* Elwes, 1882), White-edged Woodbrown (*Lethe visrava* (Moore, [1866])), Columbine (*Stiboges nymphidia* Butler, 1876), Hockeystick Sailer (*Neptis nycteus* de Nicéville, 1890), and three species of Labyrinth – Yellow Labyrinth (*Neope armandii* (Oberthür, 1876)), Tailed Labyrinth (*Neope bhadra* (Moore, [1858])) and Veined Labyrinth (*Neope pulaha* (Moore, [1858])).

The taxonomic information, natural history, habitat preferences, behavioural traits, and ecological observations of newly recorded six butterfly species are provided with additional insights.

1. *Horaga takanamii* Seki and Saito, 2004 (Lycaenidae, Theclinae) (Figs. 1a, 1b) (suggested English common name: Litin Onyx)

Very little information is currently available on *Horaga takanamii* Seki and Saito. Previously known only from Laos, this species is allopatric to the Formosan Onyx, *Horaga rarasana* Sonan, 1936, which is endemic to Taiwan. Another species



Fig. 1a



Fig. 1b



Fig. 2a



Fig. 2b

Fig. 1a - Litin Onyx, *Horaga takanamii* Seki and Saito, 2004 – Underside wing; 1b - Litin Onyx – Female laying eggs on the host plant, *Symplocos* sp.

Fig. 2a - Tibetan Duke, *Euthalia zhaxidunzhui* Huang, 1998 – Upper side wing; 2b – Underside wing

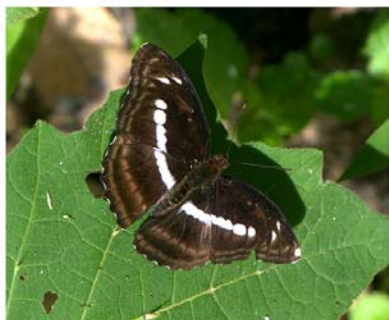


Fig. 3a



Fig. 3b



Fig. 4



Fig. 5

Fig. 3a - Tibetan Sergeant, *Athyma yui* Huang, 1998 – Upper side wing; 3b - Underside wing

Fig. 4 Tibetan Junglequeen, *Stichophthalma neumogeni renqingduojiei* Huang, 1998

Fig. 5 Narrow banded Royal, *Dacalana vui* Saito and Inayoshi, 2022

described from Laos, *H. uedai* Yago, 2004, was later synonymized with *H. takanamii* by Inayoshi (1996-2025) (<https://yutaka.it-n.jp/lyc4g/83185001.html>). *H. takanamii* is known from inland areas of the Asian subcontinent (Yago, 2004) and remains geographically isolated from *H. rarasana*, which is restricted to the high mountains of Taiwan.

The larval host plant of *H. takanamii* remains unknown in Laos but is speculated to be *Symplocos sumuntia* (Symplocaceae), similar to *H. rarasana* (Hsu and Yang, 1999), as one individual was observed ovipositing on a *Symplocos* species.

The identification of *H. takanamii* was based on key morphological traits described by Saito and Seki (2003):

- The postdiscal band on the underside is oblique in *H. takanamii*, whereas it is straight in *H. rarasana*.

- *H. takanamii* has a prominent marginal cell bar on the hindwing, which is absent in *H. rarasana*.
- The marginal band in *H. takanamii* is chocolate brown with metallic green scaling below vein 5, whereas in *H. rarasana*, metallic scaling extends up to vein 7.

This species is a new addition to the Indian butterfly fauna, based on the documentation of three individuals observed on 8–9 June 2024 in the Simong Forest. This finding extends the known range of the species by over 1,200km from Xam Neua, Laos, across the Myanmar-Yunnan Mountain ranges to the Eastern Himalayas. Common name Litin Onyx for *H. takanamii*, is proposed in honor of the *Litin* clan of the *Adi* community, who continue to protect their biodiversity-rich forests. Further molecular studies are warranted on this newly documented population from the Siang Valley to better understand its

evolutionary origin, dispersal, and biogeographic history.

2. Tibetan Duke, *Euthalia zhaxidunzhui* Huang, 1998 (Nymphalidae, Limenitidinae) (Figs. 2a, 2b)

Until the recent discovery, this butterfly species was previously known only from Metok, and Kachin State in North Myanmar (Huang *et al.*, 2024). The Northern Myanmar population was previously misidentified by as *Euthalia haradai* Yokochi, 1996 by Yokochi (2012). *Euthalia zhaxidunzhui* was considered a subspecies of *Euthalia strephon* species complex, and raised to species rank by Yokochi (2012).

The presence of this species in India is confirmed through photographic evidence recorded on 31, July 2024, from Simong Forest. Its potential distribution extends from Metok, Tibet, through eastern Arunachal Pradesh, to Kachin State, Myanmar, as illustrated in Huang *et al.* (2024).

3. Tibetan Sergeant, *Athyma yui* Huang, 1998 (Nymphalidae, Limenitidinae) (Figs. 3a, 3b)

Originally considered a subspecies of *Athyma selenophora*, this taxon was elevated to full species status by Koiwaya (2016). Its known distribution includes southeastern Tibet, northern Myanmar, Thailand, Laos, and Vietnam. The specimen recorded from the Siang Valley closely resembles individuals from Metok, particularly in exhibiting a narrower white band (Huang, 1998). In contrast,

specimens from Thailand show a broader band, and further validation is required to confirm its identity in that region.

This species represents a new record for India, confirmed through photographic documentation of an individual observed on 31 July 2024 in Simong Forest.

Tibetan Junglequeen, *Stichophthalma neumogeni renqingduojiei* Huang, 1998 (Nymphalidae, Morphinae) (Fig. 4)

Described from Metok (Huang, 1998), this subspecies is distinct from two related subspecies – one found in Sichuan, Shaanxi, Fujian, Jiangxi and another on Hainan Island, China. This butterfly is similar to *Stichophthalma nourmahal* (Westwood, 1851) (Chocolate Junglequeen) of the Eastern Himalayas but differs in having an extended blacktip on the upper forewing, a brighter chocolate colouration, and more distinct submarginal lines (Lang, 2010). This subspecies is new to India, confirmed by photographic evidence on July 31, 2024, and subsequent sightings from Simong Forest.

Narrow banded Royal, *Dacalana vui* Saito & Inayoshi, 2022 (Lycaenidae, Theclinae) (Fig. 5)

This recently described species is known from northern Thailand (Nan Province) and northern Vietnam. It was initially misidentified as *Dacalana inorthodoxa* Seki and Saito, 2006, and is considered rare, occurring in montane forests at moderate elevations. The species is readily

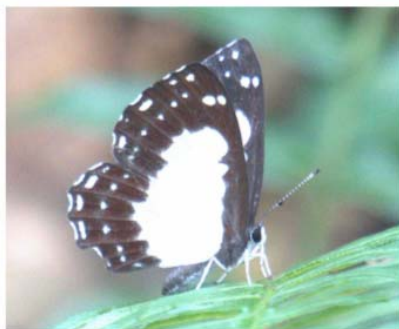


Fig. 6a



Fig. 6b

Fig. 6a - Mountain Columbine, *Stiboges elodinia* Fruhstorfer, 1914 – Underside wing; 6b - Upperside wing

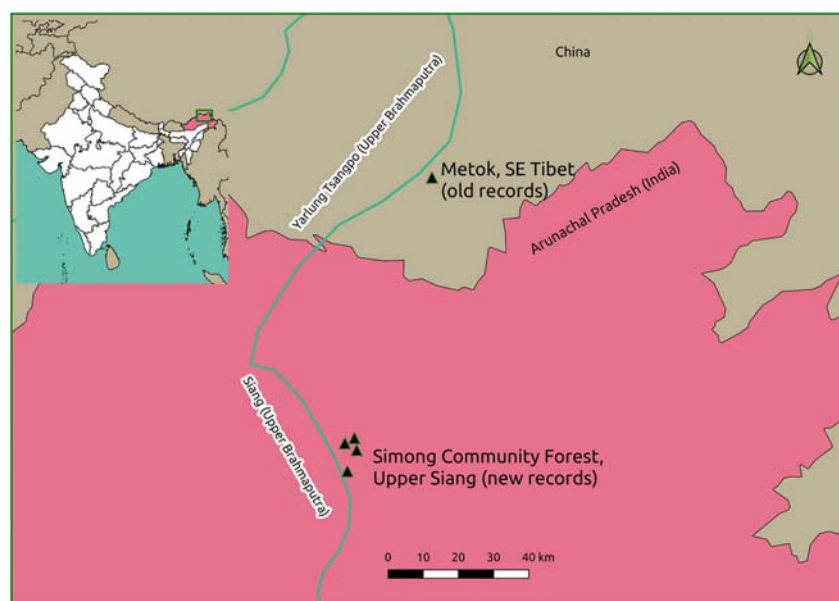


Fig. 7 Survey area in Simong, Arunachal Pradesh, and Metok County in Southeastern Tibet, where the butterflies were previously recorded.

distinguishable from all other known Indian *Dacalana* species by the presence of a narrow white band running across both wings.

This constitutes a new record for India, confirmed by photographic documentation of a single individual observed on 19 July 2024 in the Simong Forest, Upper Siang, Arunachal Pradesh. The sighting represents a significant range extension of the species from northern Thailand and northern Vietnam into the Eastern Himalayas.

Mountain Columbine, *Stiboges elodinia* Fruhstorfer, 1914 (Riodinidae, Nemeobiinae) (Figs. 6a, b)

A female individual of *Stiboges elodinia* was recorded on 31st July 2024, in Simong forest at an elevation of 2,200m. Prior to this observation, the species was known from northern Thailand, Laos, Vietnam, China, where it has been documented between elevations of 1,000 and 1,700m (<https://yutaka.it-n.jp/rio/8a330001.html>). It can be distinguished from *Stiboges nymphidia* by its more prominent circular white spots on the upper side of hindwings, a trait consistent across both sexes, along with a smoother wing border (Callaghan, 2009).

The present findings strongly suggest that additional butterfly species known from Metok County, southeastern Tibet, are likely to occur in the Siang Valley. The Brahmaputra River — which connects both Metok region of Tibet and the Siang Valley of Arunachal Pradesh — appears to play a critical biogeographic role, facilitating faunal continuity between southeastern Tibet and eastern Arunachal Pradesh.

The documentation of six previously unrecorded species within a short one-month survey underscores the striking lack of Lepidopteran surveys and conservation attention in the Indian Eastern Himalayas. Notably, *Stichophthalma neumogeni renqingduojiei*, previously thought to be localized in Metok, was found to be relatively common in Simong Forest, with an average of 20 individuals encountered daily — highlighting the absence of prior systematic surveys in the region. It is also plausible that *Horaga takanamii* could also occur in Northern Myanmar at elevations of 1400 – 1600m, like its range in Siang Valley. The same is likely true for *Dacalana vui*, which may inhabit adjacent areas of northern Myanmar.

In the same vein, *Calinaga aborica* Tytler, 1915

— commonly known as the Abor (or Dark) Freak and originally described from the Abor Hills — has often been considered a rare endemic species. However, our observations suggest otherwise. During surveys conducted in April–May, the species was recorded in large numbers across multiple locations including Damro, Yingku, and Kaying, with over 300 – 500 individuals observed per site, indicating its abundance in the region.

Despite its rich and unique biodiversity, the Siang Valley remains one of the least explored regions of the Eastern Himalayas. It is increasingly threatened by localised pressures such as timber extraction, hunting, and the expansion of cash crop plantations. Looming large-scale threats include the proposed Siang Dam project, which, paradoxically, would submerge Simong village — one of the oldest and most biodiverse settlements in the Siang Valley. Safeguarding the Siang Valley will require strengthening community conservation efforts alongside rigorous scientific documentation, before irreplaceable biodiversity is lost.

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Study of egg laying pattern and hatching line formation of *Culicoides peregrinus* Kieffer and *C. oxystoma* Kieffer (Diptera, Ceratopogonidae), vector of bluetongue virus

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ABSTRACT: Gravid *Culicoides* females play an effective role in the propagation of the midge population which is ultimately associated with the outbreak of the disease. The high abundance of the vectors *Culicoides peregrinus* Kieffer and *C. oxystoma* Kieffer (Diptera, Ceratopogonidae) has been recorded in West Bengal, followed by *C. fulvus* Sen and Das Gupta, *C. innoxius* Sen and Das Gupta and *C. anophelis* Edwards. The habitat of these vector species was reported from semi-aquatic muddy areas mixed with dung, but the species-specific micro-habitat remains unknown. The gravid females influenced by various cues like physicochemical parameter of habitat (i.e., pH, salinity, bacterial influence) to assess suitable oviposition site to optimize survival of progeny. To avoid competition, gravid female can express different oviposition strategies. The study focused on the timing and differences in egg laying pattern with initiation of hatching line in both species in laboratory condition. Oviposited eggs and the hatched eggs were examined by scanning electron microscope (SEM) to observe the changes in exochorion. The results revealed that both species showed crepuscular oviposition activity but *C. peregrinus* laid eggs in cluster and *C. oxystoma* oviposited singly. The arrangement of ansulae on egg surface and hatching pattern of eggs in both species was different. The egg laying and hatching pattern seems to be species specific which may be used as taxonomic marker. © 2025 Association for Advancement of Entomology

KEY WORDS: Ansulae, crepuscular observation, hatching line, SEM, exochorion, physicochemical parameter

INTRODUCTION

Culicoides sp. are the smallest haematophagous flies, with many species acting as biological vectors of pathogens of medical and veterinary importance (Meiswinkel *et al.*, 2004). They transmit pathogens such as bluetongue virus (BTV), epizootic haemorrhagic disease virus (EHDV), and African horse sickness virus (AHSV). The World Catalogue

on *Culicoides* documented 1347 extant species within this genus, based on adult morphology (Borkent and Dominiak, 2020). The relative abundance of *Culicoides* vectors within geographic areas would determine the likelihood of disease outbreaks. The gravid females are responsible for the propagation of the *Culicoides* midge population (Meiswinkel *et al.*, 2004). Commonly, crepuscular or nocturnal activity, i.e., peak activity of

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Culicoides species with low light intensity, was recorded by Mellor *et al.* (2000). Borkent (2014) reviewed the availability of information about immature and noted the need for more information for immature compared to adult midges. The midges *Culicoides peregrinus* Kieffer and *C. oxystoma* Kieffer showed high abundance in West Bengal followed by *C. fulvus* Sen and Das Gupta, *C. innoxius* Sen and Das Gupta, *C. actoni* Smith and *C. anophelis* Edwards (Harsha *et al.*, 2020; Mandal *et al.*, 2022; Kar *et al.*, 2022). The habitat of these vector species was reported from semi-aquatic muddy areas mixed with dung, but the species-specific habitat remains unknown (Uslu and Dik, 2010). However, the information about the suitable oviposition site selection parameters of these *Culicoides* spp. remains unknown. Despite the economic importance of the genus, descriptions of the eggs are available for only 47 of the extant species of *Culicoides* (Borkent, 2014). The outer surface of the eggs is covered with exochorion as a protective layer (Hinton, 1981; Harsha *et al.*, 2017a, b). Some small structures were noticed on the surface of *Culicoides* eggs by Hill (1947), Jobling (1953) and Becker (1961). Becker termed this structure as ansulae, which are likely to be involved in egg adhesion with the substrate. Kariya *et al.* (1989) first documented the grouping of these species based on their egg morphological similarities based on papillae and assumed that they shared similar habitats with similar morphological features of eggs. On the egg surface of *C. oxystoma*, papillae arranged in rows and the average diameter of each papilla was 1.25µm and in *C. peregrinus* papillae arranged randomly, measured 3.23µm. Previous studies have been recorded on the ultrastructure of the egg surface of some *Culicoides* spp. (Day *et al.*, 1997; Harsha *et al.*, 2017a, b). Mean length of *C. peregrinus* egg measured 489.36µm and mean width 51.28µm and in *C. oxystoma*, mean length measured 290µm and width 41µm. This study recorded the oviposition period and the egg laying pattern of the two vector species, *C. peregrinus* and *C. oxystoma*. This study aimed to determine the ultrastructural variation during hatching line formation and initiation of hatching line by light microscope and scanning electron microscope (SEM).

MATERIALS AND METHODS

Live adults were collected from the cattle shed using a UV LED light trap. Then *C. peregrinus* and *C. oxystoma* were kept in oviposition vials individually. These oviposition vials (n=100 for each species) were prepared with wet cotton and filter paper placed on them. Then vials were transferred to the Environmental Test Chamber (ETC) (at 26±1°C, RH 75%, 13L:11D) and observed up to 48-72 hrs at every 6-hr interval. To detect the egg-laying period, filter paper was changed every 6 hrs under a stereomicroscope (Olympus Magnus MS24). Eggs were counted on filter papers and kept in ETC for hatching. The egg laying time was considered as 0 hr, and few eggs were preserved for every 8 hr up to hatching. The eggs (n=10 for every hr) were prepared for scanning electron microscopy (SEM). After preservation in glutaraldehyde solution (2%) at a freezing temperature for 24 hr, and washed in PBS solution (0.2 M, pH 7.4). Eggs were treated with 1% osmium tetroxide, followed by ethanol dehydration and critical point drying. Specimens were then placed on stubs covered with sticky carbon tape coated with gold-palladium and examined for SEM (Model Sigma 300, Carl Zeiss) at the University Science Instrumentation Centre, The University of Burdwan (Turner and Mahowald, 1976; Abubekrov and Mullens, 2017). A few eggs were mounted on slides following Wirth and Marston (1968) to observe changes in egg from egg laying to hatching.

RESULTS AND DISCUSSION

The time of egg laying was recorded for both species. These both species have shown their activity at dawn and dusk period. Egg laying of *C. peregrinus* began around midnight, and this time was considered to be 0 hr. Within 48 hr, at every 6-hr interval, the laid eggs on filter paper were counted. This data demonstrates that the maximum egg-laying events recorded at night, and during the daytime, no egg-laying recorded. *Culicoides oxystoma* began laying eggs near dusk, and it continued until dawn. At the day time, no laid eggs were observed. The pattern of egg-laying was also observed in both species. *Culicoides peregrinus*



Fig. 1 Egg laying pattern of (a) *Culicoides peregrinus* and (b) *C. oxystoma*; (c) unfertilized egg (light appearance egg), fertilized egg (dark appearance egg) of *C. peregrinus*

commonly laid eggs in clutch (Fig. 1a). Each clutch consisted of 2-8 eggs. Sometimes, 10-12 eggs were also recorded. But *C. oxystoma* laid eggs individually (Fig. 1b). Just after oviposition, the eggs appeared light in appearance; then they turned dark (Fig. 1c). Noh *et al.* (2021) also observed this melanization of oviposited eggs in *Drosophila melanogaster*. The eggs from laid to hatching were mounted on slides for both species and the initiation of the hatching line, the appearance of the larval head during hatching and eggs after hatching were observed under the light microscope (Zeiss, Axioscope 5). The figures for eggs are presented in (Fig. 2, 3)

After that the eggs of every 8-hr interval examined in SEM and the terminology was followed by Harsha *et al.* (2017b) and Abubekrov and Mullens (2017). The external morphology of *C. peregrinus* eggs as shown in Fig. 4, is laterally compressed, dorsally curved and tapered at both ends at the time of oviposition. The range of length was 454-538 μm and width 42-57 μm . Eggs surface bear pillar-shaped ansulae and these were randomly arranged (Fig. 4a). The changes of ansulae every 8 hr are shown in Fig. 5. The ansulae became depressed (Fig. 5g, h) and the eggs became narrower (Fig. 4b). The hatching line was initiated from the mid of the eggs and it extended at anterior end (Fig. 4c, d).

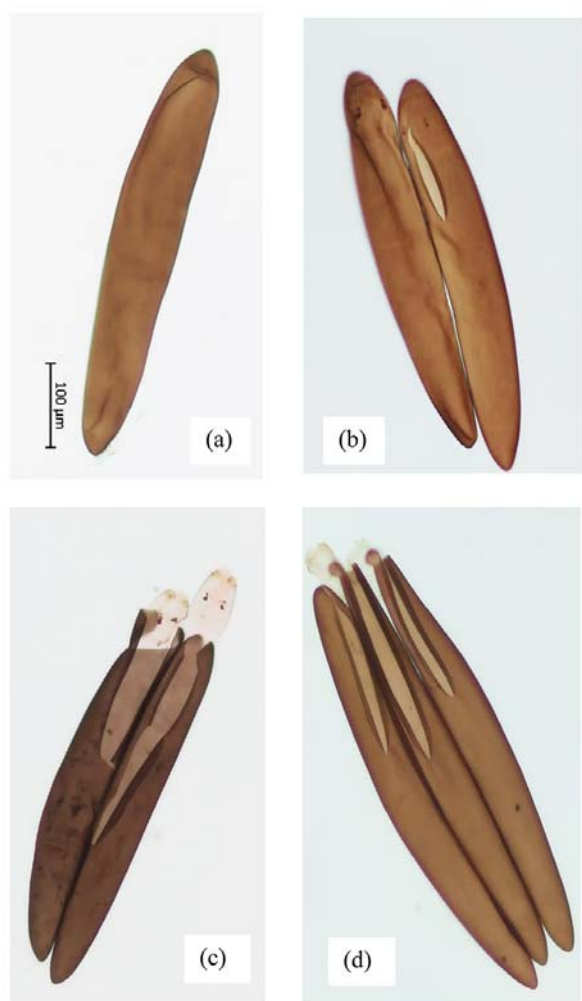


Fig. 2 *Culicoides peregrinus* eggs (a) oviposited egg (b) appearance of hatching line (c) protrusion of larva (d) eggs after hatching

The range of external length *C. oxystoma* egg was 262-354µm and width 34-62µm. The eggs are straight, gradually tapering posteriorly. The ansulae of the chorion on the egg surface were arranged in longitudinal rows (Fig. 4f). The ansulae were depressed with the progression of time (Fig. 6g). The hatching line of *C. oxystoma* eggs was initiated anterior portion of the eggs (Fig. 4g, h).

Monitoring of vector *Culicoides* midges is a method to consider population and management strategies in risk areas. *Culicoides* of medical and veterinary health importance require assessing the risk areas of disease outbreaks. According to Purse *et al.* (2015), these vectors generally have peak activity,

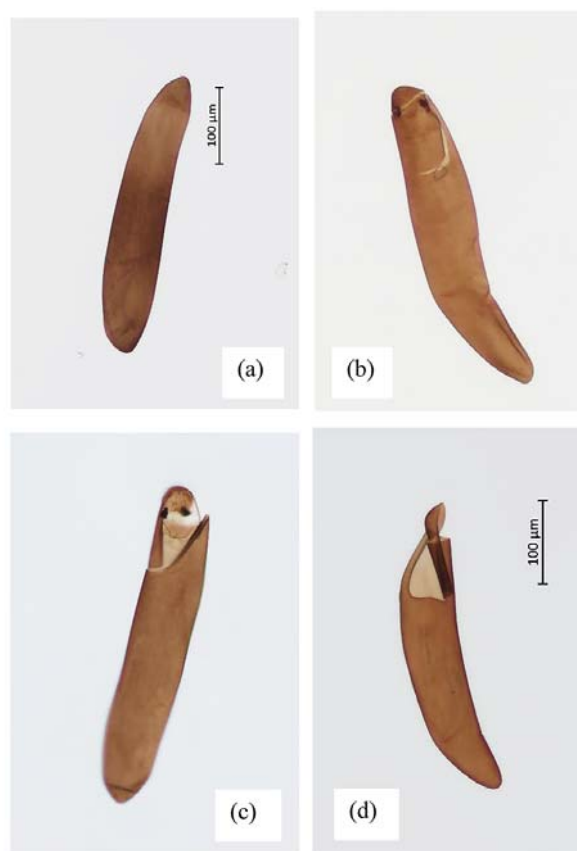


Fig. 3 *Culicoides oxystoma* eggs (a) oviposited egg (b) initiation of hatching line (c) larval protrusion (d) hatched egg

usually around dawn and dusk. The diel periodicity of several *Culicoides* species has been described worldwide, and it occurs during diurnal, nocturnal, and crepuscular periods depending of species. However, their periodic activity remains poorly known due to the absence of laboratory colonies of these vector species (Mellor *et al.*, 2000; Sanders *et al.*, 2012; Erram and Burkett - Cadena, 2021). The nocturnal activity of *C. peregrinus* was examined using an aspirator, while *C. oxystoma* and *C. fulvus* Sen and Das Gupta showed crepuscular activity (Bellis *et al.*, 2004; Kar *et al.*, 2022). Harsha *et al.* (2020) recorded similar activity periods for these *Culicoides* spp. This study showed that both species' active maximum egg-laying period was nocturnal. According to Mellor *et al.* (2000), it may be assumed that this nocturnal activity would adapt to the exposure of low risk and also benefit from low temperature with high

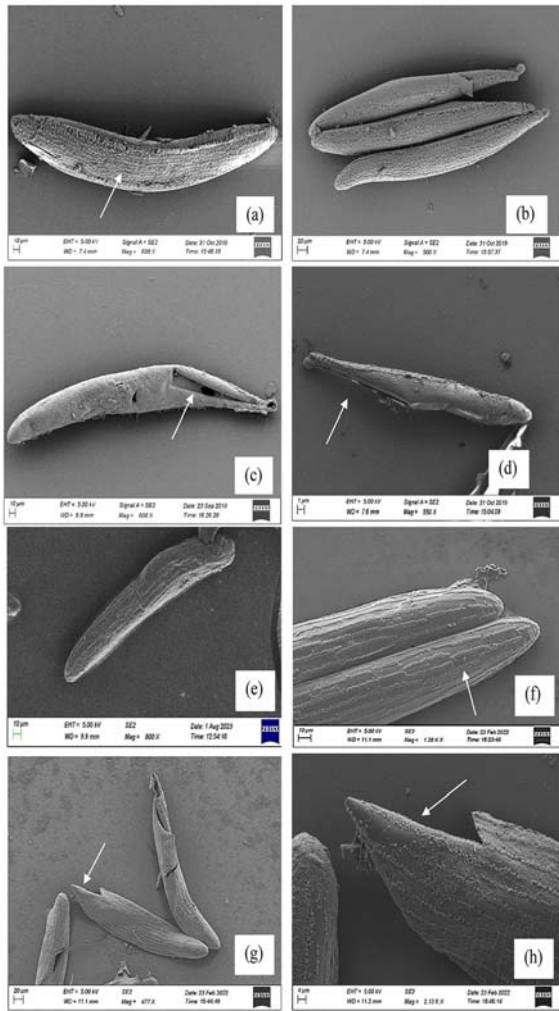


Fig. 4 Egg morphology with arrangement of ansulae and hatched eggs with hatching line of using SEM of *Culicoides peregrinus* (a-d) and *C. oxystoma* (e-h)

relative humidity of the habitat at night. The initiation of the hatching line for both species was different (Fig. 4). According to Pereira *et al.* (2006), the initiation of the hatching line was indicated by a small protrusion on the eggshell at the anterior pole, and hatching was completed at that point. The hatching occurs by the repetitive and synchronized movement of the larval head and the egg buster. These egg busters initiate small fissures in the chorion of the shell. Previously, some studies reported the ultrastructural morphology of chorionic tubercles (also referred to as hairlike papillae, ansulae) of eggs of *Culicoides* spp. The

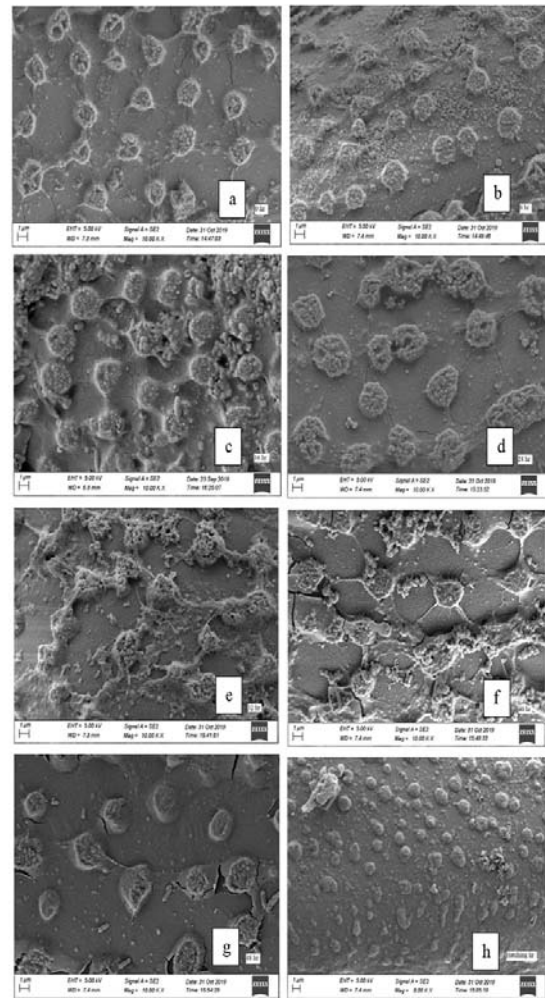


Fig. 5 Changes of ansulae of egg exochorion of *C. peregrinus* at 8 hr intervals from oviposited egg to hatched egg (a: 0 hour (oviposited eggs), b: 8hr, c: 16hr, d: 24hr, e: 32hr, f: 40hr, g: 48hr and h: hatched egg)

arrangement of ansulae is varied with the species of *Culicoides* such as these ansulae are arranged in rows in *C. circumscriptus* Kieffer, *C. gejjelensis* Dzhaferov, and *C. imicola* Kieffer (Day *et al.*, 1997); and in the egg of *C. arakawae* Arakawa, *C. oxystoma*, *C. punctatus* Latreille, *C. sumatrae* Macfie, and *C. actoni* Smith (Kariya *et al.*, 1989). These are randomly scattered over the egg surface of *C. brevitarsis* Kieffer (Campbell and Kettle, 1975), *C. maculatus* (Shiraki) (Kariya *et al.*, 1989), and *C. variipennis* (Coquillett) (Nunamaker *et al.*, 1987). The absence of ansulae was reported in the eggs of *C. molestus* (Skuse) and *C. brookmani*

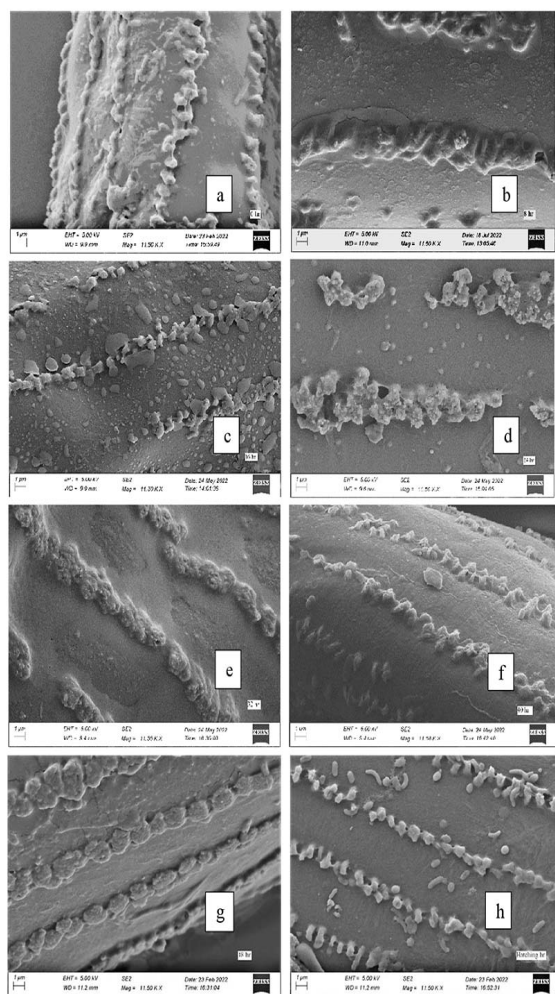


Fig. 6 Changes of ansulae of egg exochorion of *Culicoides oxystoma* at 8 hr intervals from oviposited egg to hatched egg (a: 0hr (oviposited eggs), b: 8hr, c: 16hr, d: 24hr, e: 32hr, f: 40hr, g: 48hr and h: hatched egg)

Wirth; it may be associated with their semi-aquatic habitat (Cribb and Chitra, 1998; Breidenbaugh and Mullens, 1999). Whereas species like *C. brevitarsis*, *C. imicola*, *C. circumscriptus* possess long ansulae that breed in semi-dried habitats (Campbell and Kettle, 1975; Kariya *et al.*, 1989; Day *et al.*, 1997). The correlation of ansulae with their habitat and the respiratory structure of mosquito species was studied (Linley *et al.*, 1993). The breeding habitat of both species of *Culicoides* was associated with muddy areas mixed with cow dung. This taxonomic information about the

immature stages will be useful in determining the oviposition sites, identifying immatures, and establishing management strategies against these vector species.

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Aquatic beetles (Dytiscidae, Gyrinidae, Haliplidae, Hydrophilidae, Noteridae) of the Papikonda National Park from the Eastern Ghats, Andhra Pradesh, India

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ABSTRACT: The study documents 53 species of aquatic coleoptera belonging to 20 genera and 5 families (Dytiscidae, Gyrinidae, Haliplidae, Hydrophilidae and Noteridae) from Papikonda National Park, Andhra Pradesh, India for the first time. *Hydrobiomorpha spinicollis nordica* Mouchamps, 1959, *Lacconectus fulvescens* Motschulsky, 1855 and *Peschetius nilssoni* (Sheth, Ghate, Dahanukar and Hájek, 2021), are recorded for the first time from the state of Andhra Pradesh, India. Among the reported 53 species, five species, *Cybister rugulosus* (Redtenbacher, 1844), *L. regimbarti* Brancucci, 1986, *P. nilssoni* *P. toxophorus* Guignot, 1942 and *Gyrinus convexusculus* Macleay, 1871, have been noted as endemic to India. The most common and widespread species in the national park are *Hydroglyphus flammulatus* (Sharp, 1882), *Hydaticus discindens* Walker, 1858 and *Sternolophus acutipenis* (Nasserzadeh and Komarek, 2017). The study showed that temporary pools are holding more species than permanent water bodies. The zoogeographical distribution of all the species is also given.

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KEY WORDS: Faunistics, freshwater ecosystem, Papi hills, new records, endemic species, distributional records

INTRODUCTION

Beetles belong to the largest animal order Coleoptera, with more than 400,000 known species globally (Grove and Stork, 2001). The majority of the coleopterans are terrestrial, even though a great number are adapted to aquatic life. Aquatic beetles occur in almost all aquatic habitats except oceans. The diversity of aquatic beetles can be rich in lentic

habitats such as lakes, reservoirs, tanks, ponds and pools. Globally, more than 13000 species of aquatic beetles have been described, making them one of the most successful and adapted groups in the aquatic ecosystem (Short, 2017). So far, 786 species belonging to 137 genera, 17 families, and three suborders have been documented from India (Chandra *et al.*, 2017; Sheth *et al.*, 2018, 2020, 2021; Gupta *et al.*, 2024). Among these, the family

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Dytiscidae has the highest number of species, followed by Hydrophilidae, Gyrinidae, Noteridae, and Haliphilidae. Although diverse, state level documentation is still limited, with only 55 species of aquatic beetles recorded from Andhra Pradesh, India (Shankar *et al.*, 2023; Mukhopadhyay 2007; Mukhopadhyay and Ghosh, 2007; Ghosh and Nilsson, 2012) highlighting a considerable gap in regional biodiversity assessment.

MATERIALS AND METHODS

Papikonda National Park is located in the Eastern Ghats hill range of Papi Hills in the district of Alluri Sitaramaraju, Andhra Pradesh. It covers an area of about 1,012.86 km² and lies between 18°49'20"N to 19°18'14"N - 79°54'13"E to 83°23'35"E (Gujja *et al.*, 2006). The river Godavari passes through the national park, providing habitat for many aquatic fauna and flora (Rahmani *et al.*, 2016). The forest is moist deciduous to dry deciduous (Champion and Seth, 1968). The exploration and documentation of aquatic beetles from Papikonda National Park are limited, hence the present study aims to document the diversity of aquatic beetles in Papikonda National Park, Andhra Pradesh, India.

Aquatic beetles were collected from different habitats in Papikonda National Park, including temporary pools, ponds, streams and reservoirs (Figs. A, B, Table 1). An aquatic D-shaped net and common strainer were used to collect the aquatic beetles, which were subsequently preserved in 70 percent ethanol. The specimens were observed, dissected, and photographed with a Leica M205A (Germany). Combine ZP software used to stack several images to prepare the final image. Male genitalia were chosen for species identification. Original descriptions and reliable identification keys were followed to identify the species (Vazirani, 1968, 1984; Gustafson and Miller, 2015, 2017; Sheth *et al.*, 2018; Sheth *et al.*, 2021; Jaiswal *et al.*, 2021, 2022 a, b, 2023; Shankar *et al.*, 2023). Identified specimens were registered and deposited in the National Zoological Collections of the Zoological Survey of India, Freshwater Biology Regional Centre, Hyderabad, Telangana, India.

RESULTS AND DISCUSSION

A total of 53 species were recorded from the Papikonda National Park, Andhra Pradesh, India. The family Dytiscidae (30 species) comprises the highest number of species, followed by Hydrophilidae (13 species), Gyrinidae (6 species), Noteridae (3 species), and Haliphilidae (1 species). Three species namely, *Hydrobiomorpha spinicollis nordica* Mouchamps, 1959, *Lacconectus fulvescens* Motschulsky, 1855 and *Peschetius nilssoni* (Sheth, Ghate, Dahanukar and Hájek, 2021) are recorded for the first time from Andhra Pradesh and five species from the current collections, namely: *Cybister rugulosus* (Redtenbacher, 1844), *Lacconectus regimbarti* Brancucci, 1986, *Peschetius nilssoni* (Sheth, Ghate, Dahanukar and Hájek, 2021), *Peschetius toxophorus* Guignot, 1942 and *Gyrinus convexiusculus* Macleay, 1871 are noted as endemic species to India. The most common and widespread species in national park are *Hydroglyphus flammulatus* (Sharp, 1882), *Hydaticus discindens* Walker, 1858, *Sternolophus acutipenis* (Nasserzadeh and Komarek, 2017). Some species have been noted as rare, for example: *Laccophilus ceylonicus* Zimmermann, 1919, *Peschetius nilssoni* (Sheth, Ghate, Dahanukar and Hájek, 2021), *Hyphydrus renardi* Severin, 1890, *Hydaticus luczonicus* Aubé, 1838, *Cybister rugulosus* (Redtenbacher, 1844), *Dineutus (Cyclous) unidentatus* Aubé, 1838, *Coelostoma bhutanicum* Jayaswal, 1972.

Family: Dytiscidae Leach, 1815;

Subfamily: Copelatinae Branden, 1885

Genus: Copelatus Erichson, 1832

1. *Copelatus mysorensis* Vazirani, 1970 (Figs. 1, 54)

Material examined: 1 ♂, 2 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2459; 1 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2567; 1 ♂, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3288, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu, Telangana], Sri Lanka, Thailand) (Ghosh and Nilsson, 2012; Sheth *et al.*, 2018; Shankar *et al.*, 2023; Nilsson and Hájek, 2024a).

2. *Copelatus sociennus* Balfour-Browne, 1952 (Figs. 2, 55)

Material examined: 1 ♂, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3289; 1 ♂, 3 ♀, Pond near Gumpenagandi, 17°45'58.68"N, 81°46'31.44"E, 19.vii.2021, FBRC/ZSI/INS/3853, S. Shankar and Jaiswal, D leg.

Distribution: Oriental region (India [Andhra Pradesh, Telangana], Sri Lanka); Palaearctic Region (China, India [Uttarakhand], Nepal) (Ghosh and Nilsson, 2012; Sheth *et al.*, 2018; Shankar *et al.*, 2023; Nilsson and Hájek, 2023).

Genus: *Lacconectus* Motschulsky, 1855

3. *Lacconectus fulvescens* Motschulsky, 1855 (Figs. 3, 56)

Material examined: 5 ♂, 6 ♀, Stream near Kondavada, 17°28'1.56"N, 81°42'30.24"E, 5.xii.2021, FBRC/ZSI/INS/3833, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Assam, Manipur, Meghalaya, Odisha, Tamil Nadu, West Bengal], Indonesia, Malaysia, Myanmar); Palaearctic Region (India [Sikkim]) (Ghosh and Nilsson, 2012; Nilsson and Hájek, 2024a).

Remarks: Recorded for the first time from Andhra Pradesh.

4. *Lacconectus regimbarti* Brancucci, 1986 (Figs. 4, 57)

Material examined: 1 ♂, 1 ♀, Pond near Thurruru, 17°28'1.56"N, 81°42'30.24"E, 7.xii.2021, FBRC/ZSI/INS/3836, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Kerala, Tamil Nadu] (Ghosh and Nilsson,

2012; Nilsson and Hájek, 2024a)

Subfamily: Dytiscinae Leach, 1815

Genus: *Cybister* Curtis, 1827

5. *Cybister convexus* Sharp, 1882 (Figs. 5, 58)

Material examined: 1 ♂, Pond near Thurruru, 17°38'19.32"N, 81°44'16.8"E, 7.xii.2022, FBRC/ZSI/INS/3837, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Assam, Telangana, Manipur, Meghalaya, Uttar Pradesh, West Bengal]); Palaearctic Region (China, India [Sikkim]) (Ghosh and Nilsson, 2012; Shankar *et al.*, 2023; Nilsson and Hájek, 2024a).

6. *Cybister rugulosus* (Redtenbacher, 1844) (Figs. 6, 59)

Material examined: 1 ♂, Pond near Gumpenagandi, 17°45'58.68"N, 81°46'31.44"E, 19.vii.2021, FBRC/ZSI/INS/3957, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Jharkhand, Madhya Pradesh, Maharashtra, West Bengal]); Palaearctic Region (India [Jammu and Kashmir]) (Ghosh and Nilsson, 2012; Nilsson and Hájek, 2024a).

7. *Cybister tripunctatus lateralis* (Fabricius, 1798) (Figs. 7, 60)

Material examined: 1 ♂, 1 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.VII.2021, FBRC/ZSI/INS/2450; 2 ♂, 4 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.VII.2021, FBRC/ZSI/INS/2460; 1 ♀, Pond near GM Vallasa, 17°35'21.12"N, 81°38'56.04"E, 31.VII.2022, FBRC/ZSI/INS/3286, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, Bhutan, India [Andaman and Nicobar Islands, Andhra Pradesh, Assam, Delhi, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal], Myanmar, Sri Lanka); Palaearctic Region (Azerbaijan, Afghanistan, Arab

Emirates, Bhutan, China, Cyprus, India [Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand], Iran, Iraq, Japan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia, Syria, Tajikistan, Turkmenistan, Turkey, Uzbekistan) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2022a; Nilsson and Hájek, 2024a).

Genus: *Eretes* Castelnau, 1833

8. *Eretes griseus* (Fabricius, 1781) (Figs. 8, 61-61a)

Material examined: 1 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2458; 1 ♂, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2565; 1 ♂, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3287; 1 ♀, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3308, S. Shankar and Jaiswal, D leg.

Distribution: Afrotropical Region (Botswana, Kenya, Namibia, Sudan, South Africa, Zimbabwe); Nearctic Region (Mexico, USA); Neotropical Region (Ecuador, Peru, Puerto Rico, Venezuela); Oriental Region (Bangladesh, India [Andhra Pradesh, Assam, Bihar, Goa, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Punjab, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Myanmar, Thailand, Sri Lanka); Palaearctic Region (Afghanistan, Algeria, Canary Islands, Cyprus, Egypt, India [Himachal Pradesh, Sikkim, Uttarakhand, Iran, Kazakhstan, Qatar, Nepal, Pakistan [Quetta], Saudi Arabia, Tajikistan, Turkey, Turkmenistan, United Arab Emirate, Yemen) (Miller, 2002; Ghosh and Nilsson, 2012; Nilsson and Hájek, 2023).

Remarks: The genus *Eretes* Laporte, 1833 was revised by Miller (2002), who recognized four species worldwide. Among them, *Eretes griseus* (Fabricius, 1781) is reported from India. Prior to this revision, earlier authors had recorded two species, *E. sticticus* and *E. griseus*, from the country (Ghosh and Nilsson, 2012). However, Miller

(2002) confirmed that only a single species, *E. griseus*, occurs in India.

Genus: *Hydaticus* Leach, 1817

9. *Hydaticus discindens* Walker, 1858 (Figs. 9, 63)

Material examined: 1 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 19.xi.2019, FBRC/ZSI/INS/2079; 4 ♂, 2 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2456; 2 ♂, 5 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 4.xii.2021, FBRC/ZSI/INS/2735; 3 ♀, Pond near Thurruru, 17°28'1.56"N, 81°42'30.24"E, 7.xii.2021, FBRC/ZSI/INS/2657; 1 ♂, 1 ♀, Jalatarangini Waterfall, 17°37'13.08"N, 81°39'39.24"E, 1.viii.2022, FBRC/ZSI/INS/3295; 3 ♂, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3332, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Odisha, Tamil Nadu, Telangana], Sri Lanka); Palaearctic Region (Bhutan) (Ghosh and Nilsson, 2012; Wewalka, 2016; Nilsson and Hájek, 2023).

10. *Hydaticus luczonicus* Aubé, 1838 (Figs. 10, 64)

Material examined: 1 ♂, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3291, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Cambodia, Bhutan, India [Andaman and Nicobar Islands, Andhra Pradesh, Bihar, Delhi, Goa, Gujarat, Jharkhand, Karnataka, Madhya Pradesh, Maharashtra, Meghalaya, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal], Indonesia, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Vietnam); Palaearctic Region (India [Himachal Pradesh, Uttarakhand]) (Ghosh and Nilsson, 2012; Nilsson and Hájek, 2023).

11. *Hydaticus ricinus* Wewalka, 1979 (Figs. 11, 65)

Material examined: 1 ♂, Pond near, Kutrawada, 17°40'4.8"N, 81°44'12.12"E, 23.xi.2019, FBRC/

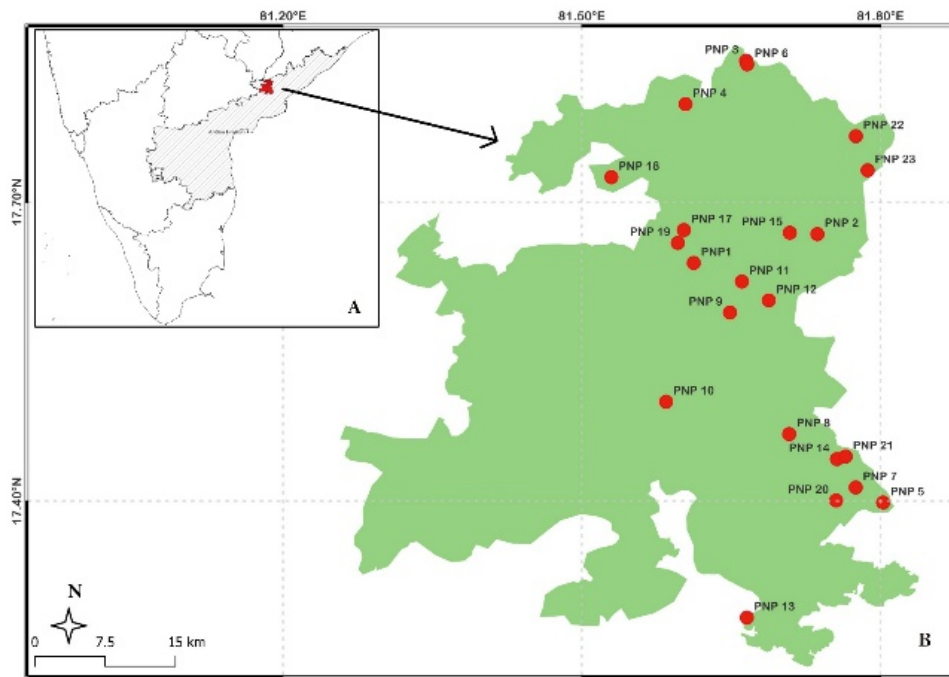


Fig. A - Papikonda National Park in Andhra Pradesh, India; B- Showing survey locations

ZSI/INS/2074; 3 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2463; 1 ♂, 1 ♀, Jalatarangini Waterfall, 17°37'13.08"N, 81°39'39.24"E, 1.viii.2022, FBRC/ZSI/INS/3294; 3 ♂, 7 ♀, Stream near Maredmali coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3331; 1 ♂, 4 ♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.VII.2021, FBRC/ZSI/INS/3825, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Brunei, Cambodia, India [Andhra Pradesh, Assam, Dadra and Nagar Haveli, Gujarat, Maharashtra, Odisha, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Indonesia, Laos, Malaysia, Myanmar, Singapore, Sri Lanka, Thailand, Vietnam); Palearctic Region (Afghanistan, Bhutan, China, India [Arunachal Pradesh, Himachal Pradesh, Uttarakhand], Nepal, Pakistan [Khyber Pakhtunkhwa, Sindh]) (Ghosh and Nilsson, 2012; Mustafa and Zubair, 2015; Jaiswal *et al.*, 2022a; Nilsson and Hájek, 2023).

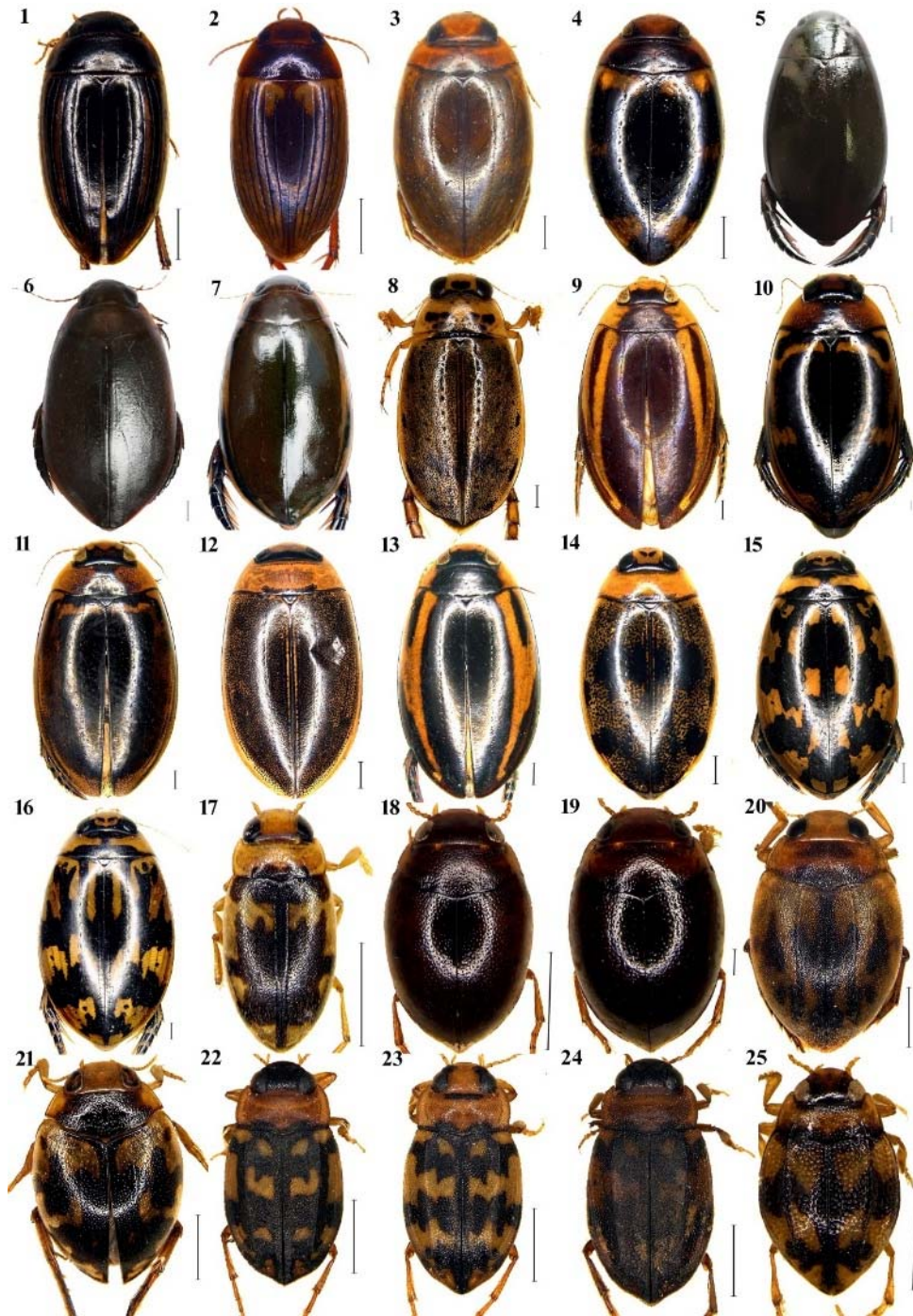
12. *Hydaticus bipunctatus* Wehncke, 1876. (Figs. 12, 62)

Material examined: 1 ♂, Stream near Maredmali coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3336, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Dadra and Nagar Haveli, Delhi, Goa, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Sikkim, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Indonesia, Laos, Myanmar, Philippines, Sri Lanka, Thailand, Vietnam); Palearctic Region (Bhutan, China, India [Assam, Arunachal Pradesh, Himachal Pradesh, Sikkim, Uttarakhand], Japan, Nepal, Oman, Taiwan) (Ghosh and Nilsson, 2012; Wewalka, 2020, Jaiswal *et al.*, 2022a, Nilsson and Hájek, 2023).

13. *Hydaticus vittatus vittatus* (Fabricius, 1775) (Figs. 13, 66)

Material examined: 1 ♂, 3 ♀, Pond near Chilakavidhi, 17°43'55.2"N, 81°47'13.92"E, 19.xi.2019, FBRC/ZSI/INS/2077; 2 ♀, Pond near Boduluru, 17°50'18.6"N, 81°39'58.32"E, 20.xi.2019, FBRC/ZSI/INS/2078, S. Shankar and



Figs. 1-25 General habitus of aquatic beetles collected from Papikonda National Park. (1). *Copelatus mysorensis*; (2). *C. sociennus*; (3). *Lacconectus fulvescens*; (4). *L. regimbarti*; (5). *Cybister convexus*; (6). *C. rugulosus*; (7). *C. tripunctatus lateralis*; (8). *Eretes griseus*; (9). *Hydaticus bipunctatus*; (10). *H. discindens*; (11). *H. luczonicus*; (12). *H. ricinus*; (13). *H. vittatus vittatus*; (14). *Rhantaticus congestus*; (15). *Sandracottus dejeanii*; (16). *S. festivus*; (17). *Hydroglyphus flammulatus*; (18). *Hydrovatus acuminatus*; (19). *H. seminarius*; (20). *Hyphydrus intermixtus*; (21). *H. renardi*; (22). *Peschetius nilssoni*; (23). *P. quadricostatus*; (24). *P. toxophorus*; (25). *Yola consanguinea*. (Scale= 1mm)

Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, Cambodia, India [Andaman and Nicobar Islands, Andhra Pradesh, Pradesh, Assam, Bihar, Gujarat, Jharkhand, Kerala, Lakshadweep, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Pondicherry, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal], Indonesia, Laos, Malaysia, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam); Palaearctic Region (Bhutan, China, India [Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand], Japan, South Korea, Nepal, Taiwan) (Ghosh and Nilsson, 2012; Wewalka, 2020; Nilsson and Hájek, 2023).

Genus: *Rhantaticus* Sharp, 1882

14. *Rhantaticus congestus* (Klug, 1832) (Figs. 14, 67)

Material examined: 2 ♂, 1 ♀, Pool near GM Vallasa, 17°35'5.64"N, 81°40'37.92"E, 19.vii.2021, FBRC/ZSI/INS/3841, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andhra Pradesh, Delhi, Jharkhand, Madhya Pradesh, Maharashtra, Manipur, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal], Philippines); Palaearctic Region (China, India [Arunachal Pradesh, Himachal Pradesh, Uttarakhand], Egypt, Japan, Nepal, Saudi Arabia, Taiwan) (Ghosh and Nilsson, 2012).

Genus: *Sandracottus* Sharp, 1882

15. *Sandracottus dejeanii* (Aube, 1838) (Figs. 15, 68)

Material examined: 1 ♀, Pond near, Kutrawada, 17°40'4.8"N, 81°44'12.12"E, 19.xi.2019, FBRC/ZSI/INS/2075; 1 ♂, 1 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2469, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Goa, Gujarat, Jharkhand, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Telangana, Tamil Nadu, Uttar Pradesh, West Bengal]); Palaearctic Region (India [Arunachal Pradesh, Himachal Pradesh, Sikkim], Iran,

Pakistan) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2022a).

16. *Sandracottus festivus* (Illiger, 1801) (Figs. 16, 69)

Material examined: 1 ♂, 4 ♀, Pond near, Kutrawada, 17°40'4.8"N, 81°44'12.12"E, 15.xi.2019, FBRC/ZSI/INS/2076, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Nagaland, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Indonesia, Myanmar, Vietnam); Palaearctic Region (China, India [Himachal Pradesh, Sikkim, Uttarakhand], Japan, South Korea) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2020, Nilsson and Hájek, 2023).

Subfamily: *Hydroporinae* Aubé, 1836

Genus: *Hydroglyphus* Motschulsky, 1853

17. *Hydroglyphus flammulatus* (Sharp, 1882) (Figs. 17, 70)

Material examined: 1 ♂, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2462; 6 ♂, 12 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2566; 1 ♂, 2 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/3056; 2 ♂, 6 ♀, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3290; 1 ♂, 5 ♀, Stream near Maredmali coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3333; 2 ♀, Polavaram, 17°16'58.08"N, 81°39'57.24"E, 29.iii.2023, FBRC/ZSI/INS/3715; 1 ♂, 1 ♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.vii.2021, FBRC/ZSI/INS/3826; 5 ♂, 3 ♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 13.vii.2021, FBRC/ZSI/INS/3852, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, Cambodia, India [Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Kerala, Madhya Pradesh, Maharashtra, Manipur, Odisha, Punjab, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh,

West Bengal], Myanmar, Indonesia, Sri Lanka, Thailand, Vietnam); Palaearctic Region (China, India [Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand], Iran, Japan, Nepal, Pakistan [Khyber Pakhtunkhwa, Sindh], South Korea, Taiwan) (Ghosh and Nilsson, 2012; Mustafa and Zubair, 2015; Nilsson and Hájek, 2023; 2024a).

Genus: *Hydrovatus* Motschulsky, 1853

18. *Hydrovatus acuminatus* Motschulsky, 1859 (Figs. 18, 71)

Material examined: 1 ♂, 1 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2468; 2 ♂, 3 ♀, Bupathipalem Reservoir, Rampachodavaram, 17°26'42.72"N, 81°45'59.04"E, 14.vii.2021, FBRC/ZSI/INS/2810; 1 ♂, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/3057, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Cambodia, India [Andaman and Nicobar Islands, Andhra Pradesh, Assam, Delhi, Jharkhand, Kerala, Odisha, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Indonesia, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Vietnam); Palaearctic Region (China, Egypt, Iran, Iraq, Israel, Japan, Libya, Nepal, Oman, Pakistan [Balochistan], Saudi Arabia, South Korea, Syria, Taiwan, Turkey, Yemen); Afrotropical Region (Chad, Comoros, Democratic Republic of the Congo, Ethiopia, Gambia, Ghana, Guinea Bissau, Kenya, Madagascar, Malawi, Mauretania, Mauritius, Mozambique, Nigeria, Senegal, Somalia, South Africa, Seychelles, Sudan, Tanzania, Uganda) (Biström, 1997, Ghosh and Nilsson, 2012, Nilsson and Hájek, 2024a).

19. *Hydrovatus seminarius* Motschulsky, 1859 (Figs. 19, 72)

Material examined: 6 ♂, 11 ♀, Pond near Peddur, 17°24'48.6"N, 81°46'30.72"E, 16.xii.2021, FBRC/ZSI/INS/2813; 2 ♀, Pond near Ijjaluru, 17°39'32.76"N, 81°35'47.4"E, 18.vii.2021, FBRC/ZSI/INS/3843, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andaman and Nicobar Islands, Andhra Pradesh, Assam, Gujarat, Jharkhand, Kerala, Odisha, Rajasthan, Tamil Nadu, West Bengal], Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand, Vietnam); Palaearctic Region (Bhutan, China, Japan, Nepal, Pakistan, Taiwan) (Ghosh and Nilsson, 2012).

Genus: *Hyphydrus* Illiger, 1802

20. *Hyphydrus intermixtus* (Walker, 1858) (Fig. 20, 73)

Material examined: 1 ♂, Stream near Pamuleru Vagu, 17°40'09.8"N, 81°42'32.4"E, 15.xii.2021, FBRC/ZSI/INS/3832, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Gujarat, Kerala, Maharashtra, Rajasthan, Tamil Nadu], Sri Lanka) (Ghosh and Nilsson, 2012)

21. *Hyphydrus renardi* Severin, 1890 (Figs. 21, 74)

Material examined: 2 ♂, 1 ♀, Pond near Chilakavidhi, 17°43'55.2"N, 81°47'13.92"E, 19.vii.2021, FBRC/ZSI/INS/3854, S. Shankar and Jaiswal, D leg.

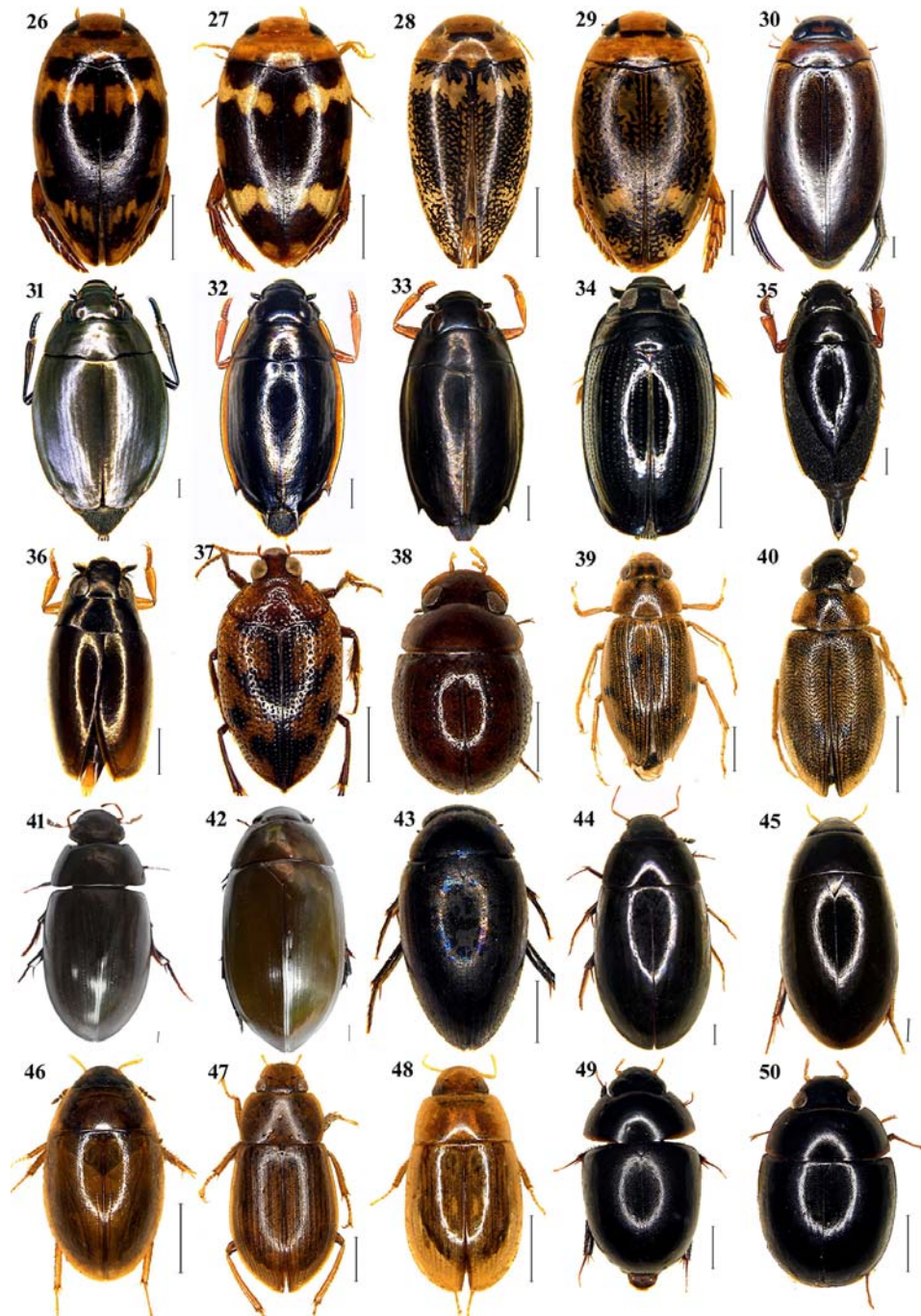
Distribution: Oriental Region (Bangladesh, Bhutan, India [Andhra Pradesh, Assam, Bihar, Gujarat, Jharkhand, Kerala, Madhya Pradesh, Maharashtra, Odisha, Rajasthan, Sikkim, Tamil Nadu, Telangana, Uttar Pradesh, Uttarakhand, West Bengal], Myanmar, Nepal, Pakistan, Sri Lanka); Palaearctic Region (India [Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand], Nepal, Pakistan) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2022a).

Genus: *Peschetius* Guignot, 1942

22. *Peschetius nilssoni* Sheth, Ghate, Dahanukar and Hájek, 2021 (Figs. 22, 75)

Material examined: 1 ♂, 1 ♀, Pond near Chilakavidhi, 17°43'55.2"N, 81°47'13.92"E, 19.vii.2021, FBRC/ZSI/INS/3856, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra



Figs. 26-50 General habitus of aquatic beetles collected from Papikonda National Park. (26). *Laccophilus anticatus anticatus*; (27). *L. ceylonicus*; (28). *L. parvulus parvulus*; (29). *L. sharpi*; (30). *Rhantus taprobanicus*; (31). *Dineutus (Cyclous) indicus*; (32). *D. (C.) spinosus*; (33). *D. (C.) unidentatus*; (34). *Gyrinus convexiusculus*; (35). *Patrus discifer*; (36). *Patrus limbatus*; (37). *Haliphus (Liaphlus) arrowi*; (38). *Amphiops mater*; (39). *Berosus indicus*; (40). *B. pulchellus*; (41). *Hydrobiomorpha spinicollis nordica*; (42). *Hydrophilus olivaceus*; (43). *Regimbartia attenuate*; (44). *Sternolophus acutipenis*; (45). *S. rufipes*; (46). *Enochrus esuriens*; (47). *Helochares anchoralis*; (48). *H. pallens*; (49). *Coelostoma bhutanicum*; (50). *C. horni* (Scale= 1mm)

Pradesh, Madhya Pradesh, Maharashtra, Rajasthan]) (Sheth *et al.*, 2021).

Remarks: Recorded for the first time from the state of Andhra Pradesh, Endemic to India.

23. *Peschetius quadricostatus* (Aube, 1838) (Figs. 23, 76)

Material examined: 1 ♂, 2 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2452; 2 ♂, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2470; 1 ♀, Pond near Thurruru, 17°28'1.56"N, 81°42'30.24"E, 7.xii.2021, FBRC/ZSI/INS/2660, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Goa, Gujarat, Jharkhand, Kerala, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, Telangana, Rajasthan Uttar Pradesh, West Bengal]); Palaearctic Region (India [Himachal Pradesh], Iran, Nepal) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2021; Nilsson and Hájek, 2024a).

24. *Peschetius toxophorus* Guignot, 1942 (Figs. 24, 77)

Material examined: 2 ♀, Bhupathipalem Reservoir,

Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2451, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Gujarat, Jharkhand, Karnataka, Kerala, Maharashtra, Odisha, Tamil Nadu, Telangana]) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2021; Sheth *et al.*, 2021; Nilsson and Hájek, 2024a).

Remarks: Species is endemic to India.

Genus: *Yola* Gozis, 1886

25. *Yola consanguinea* (Régimbart, 1892) (Figs. 25, 78)

Material examined: 1 ♂, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.vii.2021, FBRC/ZSI/INS/3828, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Gujarat, Jharkhand, Maharashtra, Odisha, Telangana]), Palaearctic Region (Nepal) (Ghosh and Nilsson, 2012; Nilsson and Hájek, 2024a).

Subfamily: Laccophilinae Gistel, 1856

Genus: *Laccophilus* Leach, 1815



Figs. 51-53 General habitus of aquatic beetles collected from Papikonda National Park. (51). *Canthydrus laetabilis*; (52). *C. luctuosus*; (53). *Neohydrocoptus subvittulus* (Scale= 1mm)

26. *Laccophilus anticatus anticatus* Sharp, 1890 (Figs. 26, 79)

Material examined: 1 ♂, 1 ♀, Pond near Peddur, 17°24'48.6"N, 81°46'30.72"E, 16.xii.2021, FBRC/ZSI/INS/3958, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andhra Pradesh, Assam, Bihar, Delhi, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Manipur, Odisha, Puducherry, Tamil Nadu, Tripura, Uttar Pradesh, West Bengal], Bangladesh, Indonesia, Sri Lanka); Palaearctic Region (India [Uttarakhand], Pakistan) (Ghosh and Nilsson, 2012; Nilsson and Hájek, 2024a).

27. *Laccophilus ceylonicus* Zimmermann, 1919 (Figs. 27, 80)

Material examined: 2 ♂, Pond near Chilakavidhi, 17°43'55.2"N, 81°47'13.92"E, 19.vii.2021, FBRC/ZSI/INS/3855, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andaman and Nicobar Islands, Andhra Pradesh, Karnataka, Kerala, Maharashtra, Nagaland, Odisha, Telangana, West Bengal], Myanmar, Sri Lanka) (Ghosh and Nilsson, 2012; Hájek and Brancucci, 2015).

28. *Laccophilus parvulus parvulus* Aubé, 1838 (Figs. 28, 81)

Material examined: 1 ♂, 1 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2455; 1 ♂, 3 ♀, Pond near Boduluru, 17°50'18.6"N, 81°39'58.32"E, 5.xii.2021, FBRC/ZSI/INS/2736, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Delhi, Goa, Gujarat, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Odisha, Puducherry, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, West Bengal], Indonesia, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Vietnam); Palaearctic Region (Bhutan, China, India [Arunachal Pradesh, Himachal Pradesh, Sikkim,

Uttarakhand], Nepal, Pakistan) (Ghosh and Nilsson, 2012; Nilsson and Hájek, 2023a).

29. *Laccophilus sharpi* Régimbart, 1889 (Figs. 29, 82)

Material examined: 2 ♂, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3292; 1 ♂, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3337; 1 ♂, 1 ♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.vii.2021, FBRC/ZSI/INS/3829; 1 ♀, Pond near Pamugandi, 17°24'1.44"N, 81°45'19.8"E, 17.vii.2021, FBRC/ZSI/INS/3851, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Haryana, Jharkhand, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Puducherry, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, West Bengal], Indonesia, Myanmar, Philippines, Sri Lanka, Vietnam); Palaearctic Region (China, Hong Kong, India [Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand], Iran, Iraq, Japan, Nepal, Pakistan [Balochistan], Saudi Arabia, South Korea, Taiwan, Yemen); Australian Region (Australia) (Ghosh and Nilsson, 2012; Jaiswal *et al.*, 2022a; Nilsson and Hájek, 2023).

Subfamily: Colymbetinae Erichson, 1837

Genus: *Rhantus* Dejean, 1833

30. *Rhantus taprobanicus* Sharp, 1890 (Figs. 30, 83)

Material examined: 1 ♂, Stream near Lakkavaram Bridge, 17°43'30.7"N, 81°31'47.5"E, 20.xii.2021, FBRC/ZSI/INS/3840, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Delhi, Gujarat, Jharkhand, Karnataka, Maharashtra, Manipur, Meghalaya, Puducherry, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Sri Lanka);

Palearctic Region (India [Himachal Pradesh, Jammu and Kashmir, Sikkim, Uttarakhand], Nepal, Pakistan) (Ghosh and Nilsson, 2012).

Family: Gyrinidae Latreille, 1810;

Subfamily: Gyrinae Latreille, 1810

Genus: *Dineutus* Macleay, 1825

Subgenus: *Dineutus (Cyclous)* Gustafson and Miller, 2017

31. *Dineutus (Cyclous) indicus* Aube, 1838 (Figs. 31, 84)

Material examined: 13♂, 20♀, Amruthadara Waterfall, 17°38'20.04"N, 81°36'45"E, 14.xi.2019, FBRC/ZSI/INS/2071; 4♀, Pond near, Kutrawada, 17°40'4.8"N, 81°44'12.12"E, 15.xi.2019, FBRC/ZSI/INS/2073; 1♂, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2461; 3♂, 8♀, Pond near Thurruru, 17°28'1.56"N, 81°42'30.24"E, 7.xii.2021, FBRC/ZSI/INS/2658; 1♂, 1♀, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3307.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Odisha, Puducherry, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal]); Palearctic Region (India [Himachal Pradesh, Jammu and Kashmir], Pakistan) (Vazirani, 1984; Jaiswal *et al.*, 2023).

32. *Dineutus (Cyclous) spinosus* Fabricius, 1781 (Figs. 32, 85)

Material examined: 1♂, 2♀, Pond near Tiger Camp, 17°40'19.56"N, 81°36'9.36"E, 20.ix.2021, FBRC/ZSI/INS/3834, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andhra Pradesh, Assam, Bihar, Manipur, Meghalaya, Odisha, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal], Malaysia, Myanmar, Thailand, Vietnam); Palearctic Region (Pakistan [Sindh]) (Vazirani, 1984; Mustafa and Zubair, 2015; Jaiswal *et al.*, 2023).

33. *Dineutus (Cyclous) unidentatus* Aubé, 1838 (Figs. 33, 86)

Material examined: 1♂, 1♀, Stream near Lakkavaram Bridge, 17°43'30.7"N, 81°31'47.5"E, 20.xii.2021, FBRC/ZSI/INS/3839, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Burma, India [Andhra Pradesh, Bihar, Madhya Pradesh, Kerala, Meghalaya, Odisha, Rajasthan, Tamil Nadu], Indonesia, Malaysia, Sri Lanka, Thailand) (Vazirani, 1984; Jaiswal *et al.*, 2023).

Genus: *Gyrinus* Geoffroy, 1762

34. *Gyrinus convexiusculus* Macleay, 1871 (Figs. 34, 87)

Material examined: 1♂, 1♀, Stream near Lakkavaram Bridge, 17°43'30.7"N, 81°31'47.5"E, 20.xii.2021, FBRC/ZSI/INS/3838, S. Shankar and Jaiswal, D leg.

Distribution: Australian (Australia); Oriental Region (India [Andhra Pradesh, Assam, Bihar, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Puducherry, Nagaland, Telangana, West Bengal]) (Vazirani, 1984; Jaiswal *et al.*, 2022a).

Genus: *Patrus* Aubé, 1838

35. *Patrus discifer* (Walker, 1859) (Figs. 35, 88)

Material examined: 9♂, 16♀, Stream near Pamuleru Vagu, 17°40'09.8"N, 81°42'32.4"E, 21.xi.2019, FBRC/ZSI/INS/2086, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Kerala, Madhya Pradesh, Maharashtra, Tamil Nadu, West Bengal], Sri Lanka) (Vazirani, 1984).

36. *Patrus limbatus* Régimbart, 1883 (Figs. 36, 89)

Material examined: 1♂, 3♀, Amruthadara Waterfall, 17°38'20.04"N, 81°36'45"E, 14.xi.2019, FBRC/ZSI/INS/2082; 14♂, 19♀, Stream near Maredmalli coffee plantation, 17°36'5.04"N,

Table 1. Sampling locations of aquatic beetles from Papikonda National Park (PNP), (District - Alluri Sitaramaraju, Andhra Pradesh) from the Eastern Ghats of India

Location	Sampling location	Latitude	Longitude
PNP1	Amruthadara Waterfall	17°38'20.04"N	81°36'45"E
PNP2	Pond near Kutrawada	17°40'4.8"N	81°44'12.12"E
PNP3	Bhupathipalem Reservoir, Rampachodavaram	17°50'31.56"N	81°39'54.36"E
PNP4	Pond near Koyalagugam	17°47'54.6"N	81°36'15.48"E
PNP5	Stream near Palakaluwa	17°23'53.88"N	81°48'9.72"E
PNP6	Pond near Boduluru	17°50'18.6"N	81°39'58.32"E
PNP7	Pond near Peddur	17°24'48.6"N	81°46'30.72"E
PNP8	Pond near Thurruru	17°28'1.56"N	81°42'30.24"E
PNP9	Pool near GM Vallasa	17°35'21.12"N	81°38'56.04"E
PNP10	Pond near Kintukuru Base Camp	17°29'58.56"N	81°35'5.28"E
PNP11	Jalatarangini Waterfall	17°37'13.08"N	81°39'39.24"E
PNP12	Stream near Maredmalli coffee plantation	17°36'5.04"N	81°41'16.44"E
PNP13	Polavaram	17°16'58.08"N	81°39'57.24"E
PNP14	Pond near Bhupathipalem Reservoir	17°26'31.3"N	81°45'23.2"E
PNP15	Stream near Pamuleru Vagu	17°40'09.8"N	81°42'32.4"E
PNP16	Stream near Kondavada	17°28'1.56"N	81°42'30.24"E
PNP17	Pond near Tiger Camp	17°40'19.56"N	81°36'9.36"E
PNP18	Stream Lakkavaram Bridge	17°43'30.7"N	81°31'47.5"E
PNP19	Pond near Ijjaluru	17°39'32.76"N	81°35'47.4"E
PNP20	Pond near Pamugandi	17°24'1.44"N	81°45'19.8"E
PNP21	Pool near Rampachodavaram	17°26'40.56"N	81°45'54.36"E
PNP22	Pond near Gumpenagandi	17°45'58.68"N	81°46'31.44"E
PNP23	Pond near Chilakavidhi	17°43'55.2"N	81°47'13.92"E

81°41'16.44"E, 13.xi.2019, FBRC/ZSI/INS/2085; 2 ♂, 5 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 13.vii.2021, FBRC/ZSI/INS/2529; 1 ♀, Palakaluwa, 17°23'53.88"N, 81°48'9.72"E, 4.xii.2021, FBRC/ZSI/INS/3824, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, Telangana, Uttar Pradesh],

Sri Lanka); Palaearctic Region (India [Himachal Pradesh] (Vazirani, 1984; Jaiswal *et al.*, 2022a).

Family: Haliplidae Aubé, 1836;

Subfamily: Haliplinae Aubé, 1836

Genus: *Haliplus* Latreille, 1802

37. *Haliplus* (*Liaphlus*) *arrowi* Guignot, 1936 (Figs. 37, 90)

Material examined: 1 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2466; 1 ♂, 1 ♀, Stream near Pamuleru Vagu, 17°40'09.8"N, 81°42'32.4"E, 15.xii.2021, FBRC/ZSI/INS/3835, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andhra Pradesh, Bihar, Maharashtra, Tamil Nadu, Uttar Pradesh, West Bengal], Laos, Myanmar); Palaearctic Region (Nepal, Pakistan) (Vazirani, 1984; Vondel, 1991; Sheth *et al.*, 2016).

Family: Hydrophilidae Latreille, 1802;

Subfamily: Hydrophilinae Latreille, 1802

Genus: *Amphiops* Erichson, 1843

38. *Amphiops mater* Sharp, 1873 (Figs. 38, 91)

Material examined: 4 ♂, 6 ♀, Pond near Chilakavidhi, 17°43'55.2"N, 81°47'13.92"E, 19.vii.2021, FBRC/ZSI/INS/3857, S. Shankar and Jaiswal, D leg.

Distribution: Australian (Australia); Oriental Region (Bangladesh, India [Andhra Pradesh, Delhi, Manipur, Sikkim, Telangana, West Bengal], Malaysia, Philippines, Sri Lanka, Vietnam); Palaearctic Region (China, India [Himachal Pradesh, Jammu and Kashmir], Iran, Japan, Nepal, South Korea) (Mukhopadhyay, 2015; PrzewoŹny, 2022).

Genus: *Berosus* Leach, 1817

39. *Berosus indicus* (Motschulsky, 1861) (Figs. 39, 92)

Material examined: 3 ♂, 7 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2449; 4 ♂, 1 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2564; 3 ♂, 2 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/3053; 1 ♂, 4 ♀, Polavaram, 17°16'58.08"N, 81°39'57.24"E, 29.iii.2023, FBRC/ZSI/INS/3713; 3 ♂, 2 ♀, Pond near Pamugandi, 17°24'1.44"N, 81°45'19.8"E,

17.vii.2021, FBRC/ZSI/INS/3847, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Bihar, Goa, Kerala, Jharkhand, Madhya Pradesh, Maharashtra, Manipur, Odisha, Punjab, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, West Bengal]); Palaearctic Region (India [Jammu and Kashmir, Himachal Pradesh, Sikkim], Pakistan [Khyber Pakhtunkhwa, Sanghar]). (Mukhopadhyay, 2015; Mustafa and Zubair, 2015; Jaiswal *et al.*, 2022a).

40. *Berosus pulchellus* Macleay, 1825 (Figs. 40, 93)

Material examined: 4 ♂, 8 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2563; 3 ♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/3054; 4 ♀, Pond near Kintukuru Base Camp, 17°29'58.56"N, 81°35'5.28"E, 30.vii.2022, FBRC/ZSI/INS/3293; 3 ♂, 9 ♀, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3334; 2 ♂, Polavaram, 17°16'58.08"N, 81°39'57.24"E, 29.iii.2023, FBRC/ZSI/INS/3714; 1 ♂, 1 ♀, Pond near Pamugandi, 17°24'1.44"N, 81°45'19.8"E, 17.vii.2021, FBRC/ZSI/INS/3848, S. Shankar and Jaiswal, D leg.

Distribution: Australian (Australia); Oriental Region (Bangladesh, India [Andhra Pradesh, Assam, Bihar, Delhi, Haryana, Kerala, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, Punjab, Odisha, Puducherry, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, West Bengal], Indonesia, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand, Vietnam); Palaearctic Region (China, Hong Kong, India [Jammu and Kashmir, Himachal Pradesh, Sikkim], Iran, Japan, Nepal, Taiwan) (Mukhopadhyay, 2015; PrzewoŹny, 2022; Jaiswal *et al.*, 2022a).

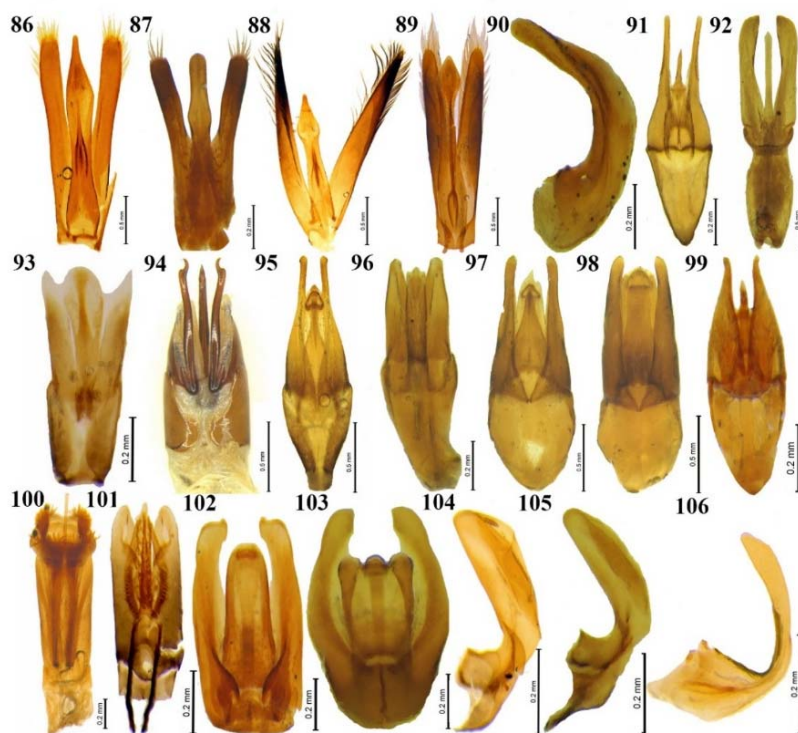
Genus: *Hydrobiomorpha* Blackburn, 1888

41. *Hydrobiomorpha spinicollis nordica* Mouchamps, 1959 (Figs. 41, 94)

Material examined: 2 ♂, 2 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E,



Figs. 54-85 Male genitalia of aquatic beetles collected from Papikonda National Park. (54). *Copelatus mysorensis*; (55). *C. sociennus*; (56). *Lacconectus fulvescens*; (57). *L. regimbarti*; (58). *Cybister convexus*; (59). *C. rugulosus* (60). *C. tripunctatus lateralis*; (61- 61a). *Eretes griseus*; (62). *Hydaticus bipunctatus*; (63). *H. discindens*; (64). *H. luczonicus*; (65). *H. ricinus*; (66). *H. vittatus*; (67). *Rhantaticus congestus*; (68). *Sandracottus dejeanii*; (69). *S. festivus*; (70). *Hydroglyphus flammulatus*; (71). *Hydrovatus acuminatus*; (72). *H. seminarius*; (73). *Hyphydrus intermixtus*; (74). *H. renardi*; (75). *Peschetius nilssoni*; (76). *P. quadricostatus*; (77). *P. toxophorus*; (78). *Yola consanguinea*; (79). *Laccophilus anticatus anticatus*; (80). *L. ceylonicus*; (81). *L. parvulus parvulus*; (82). *L. sharpi*; (83). *Rhantus taprobanicus*; (84). *Dineutus (Cyclous) indicus*; (85). *D. (C.) spinosus*



Figs. 86-106 Male genitalia of aquatic beetles collected from Papikonda National Park. (86). *Dineutus*; (*C.*) *unidentatus*; (87). *Gyrinus convexiusculus*; (88). *Patrus discifer*; (89). *Patrus limbatus*; (90). *Haliphus* (*Liaphlus*) *arrowi*; (91). *Amphiops mater*; (92). *Berosus indicus*; (93). *B. pulchellus*; (94). *Hydrobiomorpha spinicollis nordica*; (95). *Hydrophilus olivaceus*; (96). *Regimbartia attenuata*; (97). *Sternolophus acutipenis*; (98). *S. rufipes*; (99). *Enochrus esuriens* (100). *Helochares anchoralis*; (101). *H. pallens*; (102). *Coelostoma bhutanicum*; (103). *C. horni*; (104). *Canthydrus laetabilis*; (105). *C. luctuosus*; (106). *Neohydrocoptus subvittulus*

15.vii.2021, FBRC/ZSI/INS/2465; 1 ♀, Pond near Ijjaluru, 17°39'32.76"N, 81°35'47.4"E, 18.vii.2021, FBRC/ZSI/INS/3858, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Chhattisgarh, Maharashtra, Madhya Pradesh, Odisha], (Bhutan, Nepal); Palaearctic Region (India [Himachal Pradesh]) (Ghosh *et al.*, 2021; Sonali *et al.*, 2025).

Genus: *Hydrophilus* Geoffroy, 1762

42. *Hydrophilus olivaceus* Fabricius, 1781 (Figs. 42, 95)

Material examined: 1 ♂, 2 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E,

22.xi.2019, FBRC/ZSI/INS/2080, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Maharashtra, Tamil Nadu, Telangana, West Bengal]); Palaearctic Region (Tonkin) (Mukhopadhyay, 2015; Jaiswal *et al.*, 2022a).

Genus: *Regimbartia* Zaitzev, 1908

43. *Regimbartia attenuata* (Fabricius, 1801) (Figs. 43, 96)

Material examined: 1 ♂, Palakaluwa, 17°23'53.88"N, 81°48'9.72"E, 4.xii.2021, FBRC/ZSI/INS/3822; 1 ♂, 2 ♀, Pond near Ijjaluru, 17°39'32.76"N, 81°35'47.4"E, 18.vii.2021, FBRC/ZSI/INS/3844, S. Shankar and Jaiswal, D leg.

Distribution: Australian (Australia); Oriental Region (India [Andhra Pradesh, Maharashtra, Manipur, Meghalaya, Punjab, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, West Bengal], Indonesia, Philippines, Sri Lanka); Palaearctic Region (China, India [Jammu and Kashmir, Sikkim], Japan) (PrzewoŹny, 2022; Mukhopadhyay, 2015).

Genus: *Sternolophus* Solier, 1834

44. *Sternolophus acutipennis* (Nasserzadeh and Komarek, 2017) (Figs. 44, 97)

Material examined: 1 ♀, Pond near Tiger Camp, 17°40'19.56"N, 81°36'9.36"E, 22.xi.2019, FBRC/ZSI/INS/2088; 1 ♂, 1 ♀, Pond near Ijjaluru, 17°39'32.76"N, 81°35'47.4"E, 26.xi.2019, FBRC/ZSI/INS/2089; 2 ♂, 2 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2457; 1 ♂, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2568; 1 ♂, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 4.xii.2021, FBRC/ZSI/INS/2734; 1 ♀, Pond near Thurruru, 17°28'1.56"N, 81°42'30.24"E, 7.xii.2021, FBRC/ZSI/INS/2659; 1 ♂, 1 ♀, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3338, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Madhya Pradesh, Tamil Nadu], Indonesia, Myanmar, Thailand, Vietnam); Palaearctic Region (China) (Nasserzadeh and Komarek, 2017).

45. *Sternolophus rufipes* (Fabricius, 1792) (Figs. 45, 98)

Material examined: 1 ♂, 1 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2448; 1 ♂, 2 ♀, Pond near Koyalagudem, 17°47'54.6"N, 81°36'15.48"E, 15.vii.2021, FBRC/ZSI/INS/2464; 2 ♀, Stream near Maredmalli coffee plantation, 17°36'5.04"N, 81°41'16.44"E, 16.vii.2021, FBRC/ZSI/INS/3309; 1 ♂, Pond near Ijjaluru, 17°39'32.76"N, 81°35'47.4"E, 18.vii.2021, FBRC/ZSI/INS/3842, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bhutan, Cambodia,

India [Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Odisha, Punjab, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, West Bengal], Indonesia, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Vietnam); Palaearctic Region (China, Hong Kong, India [Himachal Pradesh, Jammu and Kashmir, Sikkim], Japan, Nepal, Pakistan [Khyber Pakhtunkhwa, Sindh], Taiwan, South Korea) (Mustafa and Zubair, 2015; Nasserzadeh and Komarek 2017; Shankar *et al.*, 2023).

Remarks: The species of genus *Sternolophus* share overlapping morphological characters, male genitalia and spine on metaventricle were used for distinguishing the species within the genus. *S. acutipennis* has a sharp carina in apical portion of male median lobe whereas in *S. rufipes* carina in apical portion absent, both paramere and median lobe almost equal.

Subfamily: Enochrinae Short and Fikáček, 2013

Genus: *Enochrus* Thomson, 1859

46. *Enochrus esuriens* (Walker, 1858) (Figs. 46, 99)

Material examined: 1 ♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2453; 1 ♂, 1 ♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.vii.2021, FBRC/ZSI/INS/3831, S. Shankar and Jaiswal, D leg.

Distribution: Australian (Australia); Oriental Region (India [Andhra Pradesh, Manipur, West Bengal], Indonesia, Myanmar, Philippines, Sri Lanka, Vietnam); Palaearctic Region (China, India [Himachal Pradesh, Jammu and Kashmir, Sikkim], Pakistan [Khyber Pakhtunkhwa, Sindh]) (Mukhopadhyay, 2015; Mustafa and Zubair, 2015; PrzewoŹny, 2022).

Subfamily: Acidocerinae (Zaitzev, 1908)

Genus: *Helochaers* Mulsant, 1844

47. *Helochaers anchoralis* Sharp, 1890 (Figs. 47, 100)

Material examined: 1 ♂, Palakaluwa, 17°23'53.88"N,

81°48'9.72"E, 4.xii.2021, FBRC/ZSI/INS/3823; 1♂, 3♀, Pond near Ijjaluru, 17°39'32.76"N, 81°35'47.4"E, 18.vii.2021, FBRC/ZSI/INS/3845, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andhra Pradesh, Assam, Bihar, Telangana, West Bengal]); Palaearctic Region (Pakistan [Khyber Pakhtunkhwa, Sindh]) (Mukhopadhyay, 2015; Mustafa and Zubair, 2015; PrzewoŹny, 2022).

48. *Helochares pallens* (MacLeay, 1825) (Figs. 48, 101)

Material examined: 1♂, 1♀, Bhupathipalem Reservoir, Rampachodavaram, 17°50'31.56"N, 81°39'54.36"E, 14.vii.2021, FBRC/ZSI/INS/2454; 3♂, 1♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.vii.2021, FBRC/ZSI/INS/3830, S. Shankar and Jaiswal, D leg.

Distribution: Afrotropical (Egypt); Oriental Region (India [Andhra Pradesh, Assam, Bihar, Telangana, West Bengal], Indonesia, Philippines); Palaearctic Region (China, Syria, Tonkin) (Mukhopadhyay, 2015; PrzewoŹny, 2022).

Genus: *Coelostoma* Brulle, 1835

49. *Coelostoma bhutanicum* Jayaswal, 1972 (Figs. 49, 102)

Material examined: 1♂, 2♀, Pond near Pamugandi, 17°24'1.44"N, 81°45'19.8"E, 17.vii.2021, FBRC/ZSI/INS/3849, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bhutan, India [Andhra Pradesh, Kerala, Telangana, Meghalaya, Rajasthan], Japan, Nepal); Palaearctic Region (China, Taiwan) (Sheth *et al.*, 2020; Jaiswal *et al.*, 2022a).

50. *Coelostoma horni* Regimbart, 1902 (Figs. 50, 103)

Material examined: 3♂, 1♀, Stream near Palakaluwa, 17°23'53.88"N, 81°48'9.72"E, 4.xii.2021, FBRC/ZSI/INS/2704; 1♀, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/3055, S. Shankar and Jaiswal, D leg.

Distribution: Afrotropical (South Africa); Oriental Region (Bhutan, India [Andaman Islands, Andhra Pradesh, Tamil Nadu, Telangana], Indonesia, Nepal, Sri Lanka, Thailand, Vietnam); Palaearctic Region (China, India [Sikkim], Oman, Saudi Arabia, Yemen) (PrzewoŹny, 2022; Jaiswal *et al.*, 2022a).

Family: Noteridae Thomson, 1860;

Subfamily: Noterinae Thomson, 1860

Genus: *Canthydrus* Sharp, 1882

51. *Canthydrus laetabilis* (Walker, 1858) (Figs. 51, 104)

Material examined: 1♂, Pool near Rampachodavaram, 17°26'40.56"N, 81°45'54.36"E, 14.vii.2021, FBRC/ZSI/INS/3846, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Bangladesh, India [Andhra Pradesh, Bihar, Gujarat, Maharashtra, Meghalaya, Telangana, West Bengal], Myanmar, Nepal, Sri Lanka); Palaearctic Region (India [Sikkim, Uttarakhand], Nepal, Pakistan) (Nilsson, 2011; Mukhopadhyay, 2015; Jaiswal *et al.*, 2022b; Nilsson and Hájek, 2024b).

52. *Canthydrus luctuosus* (Aubé, 1838) (Figs. 52, 105)

Material examined: 1♂, 4♀, Pond near Pamugandi, 17°24'1.44"N, 81°45'19.8"E, 17.vii.2021, FBRC/ZSI/INS/3850, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (Cambodia, India [Andhra Pradesh, Bihar, Maharashtra, Odisha], Indonesia, Sri Lanka, Vietnam); Palaearctic Region (Iran, Iraq, Saudi Arabia, Syria) (Nilsson, 2011; Mukhopadhyay, 2015; Nilsson and Hájek, 2024b).

Genus: *Neohydrocoptus* Satô, 1972

53. *Neohydrocoptus subvittulus* (Motschulsky, 1859) (Figs. 53, 106)

Material examined: 1♂, 1♀, Pond near Bhupathipalem Reservoir, 17°26'31.3"N, 81°45'23.2"E, 14.vii.2021, FBRC/ZSI/INS/3827, S. Shankar and Jaiswal, D leg.

Distribution: Oriental Region (India [Andaman and Nicobar Islands, Andhra Pradesh, Assam, Bihar, Odisha, West Bengal], Indonesia, Malaysia, Myanmar, Sri Lanka, Vietnam); Palearctic Region (China, Japan, Nepal, Pakistan) (Nilsson, 2011; Mukhopadhyay, 2015; Nilsson and Hájek, 2024b).

The study reports altogether 53 species for the Andhra Pradesh raising the number to 57 for the state. In this study 46 species were found in pools and ponds, 24 were found in both streams and ponds, 10 species occurred in reservoirs, and 6 species in streams in the Papikonda National Park. It appears that ponds and temporary pools hold a richer diversity compared with streams and it corroborates earlier studies (Kulkarni *et al.*, 2015; Sheth *et al.*, 2019). Studies on the aquatic beetle fauna of India's protected areas remain limited. Jaiswal *et al.* (2022a) documented 41 species from Kawal Tiger Reserve, Telangana, followed by 39 species from Satkosia Gorge Wildlife Sanctuary, Odisha, 35 from Sundarban Biosphere Reserve, West Bengal (Mitra, 2017), and 34 from Baisipalli Wildlife Sanctuary, Odisha. Other records include 21 species from Renuka Wildlife Sanctuary, Himachal Pradesh (Ghosh and Hedge, 2013b); 18 from Sandi Bird Sanctuary, Uttar Pradesh (Ghosh, 2011); and 13 from Suraha Tal Wildlife Sanctuary (Ghosh *et al.*, 2010). Dudhwa Tiger Reserve, Uttar Pradesh, reported 11 species (Ghosh and Hegde, 2013a), while Asan Wetland Conservation Reserve (Uttarakhand), Govind Sagar Wildlife Sanctuary (Himachal Pradesh), and Karnala Bird Sanctuary (Maharashtra) each reported nine (Mukhopadhyay and Ghosh, 2003; Ghosh, 2015; Ghosh and Hedge, 2015). In West Bengal, Buxa Tiger Reserve and Binsar Wildlife Sanctuary each yielded four species (Nandi *et al.*, 2004; Ghosh *et al.*, 2018; Ghosh *et al.*, 2011). The lowest diversity was reported from Keibul Lamjao National Park, Manipur, with only two species (Takhelmayum and Gupta, 2015). More recently, 42 species were recorded from Koundinya Wildlife Sanctuary, Andhra Pradesh (Shankar *et al.*, 2023). In contrast, the present study reports 53 species from Papikonda National Park, Eastern Ghats, Andhra Pradesh, representing the highest diversity recorded from any protected area in India to date. This study reveals that most of the non-

endemic aquatic beetles which are reported in this study have a wide range of distribution, with a geographical range that covers both Oriental and Palearctic Regions. However, the present study on the aquatic beetles of the Papikonda National Park is a primary study to comprehend the diversity of aquatic beetles, and it may be helpful in the future prospective to understand the diversity of aquatic beetles in the National Park as well as to help better management of the ecosystem.

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Molecular identification and phylogenetic analysis of spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera, Aleyrodidae) from different agroecological zones of Kerala, India

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ABSTRACT: This study represents the first investigation into the genetic diversity and phylogenetic analysis of *Aleurodicus dispersus* Russell, populations collected from various agroecological zones of Kerala. The analysis, based on the mitochondrial cytochrome oxidase I (mtCOI) gene, demonstrated a significant amount of genetic divergence among *A. dispersus* populations from different agroecological regions of Kerala. Samples of spiralling whitefly collected from the Palakkad plains were genetically more distinct than those collected from other agroecological zones. Isolates from coastal sandy and red loam soils exhibited 100 per cent similarity with accession no. OQ844129.1 available in the GenBank. This study facilitates rapid diagnosis of the diversity in spiralling whiteflies attacking plants of economic importance in Kerala. © 2025 Association for Advancement of Entomology

KEY WORDS: Genetic diversity, mitochondrial cytochrome oxidase I, rapid diagnosis, phylogenetic tree

INTRODUCTION

Aleurodicus dispersus Russell (Hemiptera, Aleyrodidae) is a highly polyphagous pest native to the Caribbean region and Central America (Russell, 1965), where it is known to inhabit a wide range of host plants without being considered a pest (Waterhouse and Norris, 1989). Globally, it is more commonly known as a 'spiralling whitefly' due to its characteristic egg-laying pattern, which forms a spiral shape (Kumashiro *et al.*, 1983). This pest exhibits a broad host range, affecting 481 plant species from 295 genera across 90 families of

vegetables, fruits, and ornamental trees (Srinivasa, 2000). In India, it was first documented on cassava in 1993 in Thiruvananthapuram, Kerala (Palaniswami *et al.*, 1995). Subsequently, it was reported from several parts of Kerala (David and Regu, 1995; Ranjith *et al.*, 1996), Tamil Nadu (David and Regu, 1995), Karnataka (Mani *et al.*, 2000), Andra Pradesh, Maharashtra (Charati *et al.*, 2003), Orissa (CABI/EPPO, 2006) and Mizoram (Boopathi, 2008). Since its first report in 1993, the species has spread throughout Southern and North-Eastern India, establishing itself as a significant pest of agricultural and horticultural crops (Boopathi and

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Karuppuchamy, 2013; Boopathi *et al.*, 2013a). Feeding damage was caused by the piercing and sucking of sap from foliage by immature and adult stages of whiteflies (Boopathi *et al.*, 2013b). High-density populations of this polyphagous pest can lead to pre mature leaf drop and the production of abundant honeydew, serving as a substrate for sooty mold growth, resulting in the abandonment of cassava fields by farmers (Akinlosotu *et al.*, 1993). Sooty mold formation blackens the leaves, reduces photosynthetic activity, and diminishes host vigour, often resulting in disfigurement (Kumashiro *et al.*, 1983; John *et al.*, 2007).

Mitochondrial DNA (mtDNA) has been extensively used in phylogenetic studies of animals, because it evolves much more rapidly than nuclear DNA, favouring the accumulation of nucleotide differences (i.e., polymorphisms) among closely related species (Brown *et al.*, 1979; Lunt *et al.*, 1996). Accumulation of these polymorphisms is primarily attributed to the loss or gain of restriction sites without a detectable change in genome size (Hebert *et al.*, 2003a). Sequence changes within the mitochondrial cytochrome oxidase I (COI) gene, make it an ideal candidate for use as a DNA barcode system (Hebert *et al.*, 2003b; Kress and Erickson, 2008). Generally, sequencing of the polymerase chain reaction fragment amplified from the COI gene is used for mitochondrial haplotype identification, which can then be associated with specific species (Shatters *et al.*, 2009). A molecular technique based on polymerase chain reaction is widely used in applied entomology (Harry *et al.*, 1998). Relatively little attention has been given to *A. dispersus*, including genetic studies particularly in India (Callejas *et al.*, 2005; Boopathi *et al.*, 2014). Despite the economic importance of *A. dispersus*, understanding of the level and patterns of genetic variability within its populations remains limited. Therefore, this study employed a molecular technique focusing on the mitochondrial genome to investigate the genetic variability of *A. dispersus* populations in Kerala.

MATERIALS AND METHODS

Surveys were conducted in 13 different

agroecological zones of Kerala, where cassava is grown (Table 1) and samples of whiteflies were collected using an aspirator and preserved in 90 per cent ethanol. Adult whiteflies were identified based on the taxonomic description (Russell, 1965). Total genomic DNA was obtained from spiralling whiteflies using the acetyl trimethyl ammonium bromide method (CTAB) adopted by Gawel and Jarrett (1991). The DNA barcode region was amplified from all whitefly species using 101M of universal DNA primers LCO1490 (50-GGTCAACAAATCATAAAGATATTGG-30) and HCO 2198 (50-TAAACTTCAGGGTGACCAAA AAATCA-30) for the amplification of mitochondrial cytochrome c oxidase subunit I -COI (Folmer *et al.*, 1994). PCR was conducted in a thermal cycler (AB-Applied Biosystems, Veriti 96 wells) with the following cycles; Initial denaturation at 94°C for 5 min. followed by 35 cycles (45 sec at 94°C, 45 sec at 47°C, and 45 sec at 72°C) with a final extension period of 72°C for 10 min. PCR products were tested on one per cent agarose gel. DNA ladders (1.0 kb plus) were used to measure the size of the obtained DNA fragments. The electrophoresis was followed by documentation under ultraviolet light and photographed by a Gel Documentation System (BioRad XR+).

DNA Sequences were edited to the spiralling whitefly species-specific COI sequence by eliminating the vector and universal primer sequence. DNA sequences were compared with those in the NCBI databases using the Basic Local Alignment Search Tool (<http://www.ncbi.nlm.nih.gov/BLAST>). From the aligned sequences phylogenetic tree was constructed using MEGA 11 software (Tamura *et al.*, 2013). The Mt (COX-1) gene sequences have been deposited in the NCBI Gen Bank, public databases.

RESULTS AND DISCUSSION

Initially, the mtDNA COI universal primers LCO1490 and HCO2198 were employed to amplify the COI region of thirteen *A. dispersus* populations collected from various geographic regions of Kerala. The COI gene (COX1) is one of 3 coding protein genes in the mitochondrial genome. The

Table 1. Locations of survey in Kerala

No	Zone	Place (Code)	Location
1	Onattukara	Kayamkulam (KYM)	9.1504°N; 76.3223°E
2	Coastal sandy	Chavakkad (CHA)	9.2243°N; 76.2145°E
3	Southern midlands	Sreekariyam (SKM)	8.3247°N; 76.5500°E
4	Central midlands	Perumbavoor (PBR)	10.0651°N; 76.2838°E
5	Northern midlands	Payyanur (PNR)	12.0550°N; 75.1137°E
6	Malappuram type	Kozhikode (KZH)	11.1536°N; 75.4649°E
7	Malayoram	Konni (KON)	9.1337°N; 76.5057°E
8	Palakkad plains	Palakkad (PKD)	10.4714°N; 76.3916°E
9	Red loam	Neyyatinkara (NYT)	8.2412°N; 77.0512°E
10	Chittoor black soil	Chittur (CTR)	10.3650°N; 76.4125°E
11	Kuttanad	Champakulam (CBK)	9.2439°N; 76.2437°E
12	Riverbank alluvium	Muvattupuzha (MVP)	9.5922°N; 76.3442°E
13	High ranges	SulthanBathery (SRY)	11.4805°N; 76.0017°E

amplification of the COX-1 gene of spiralling whitefly isolate was carried out and the amplicon product size was 658 bp. Gel images of PCR amplification (Fig.1). All the sequences obtained were aligned using BioEdit software. BLAST analysis of spiralling whitefly isolates showed 100 per cent similarity to *A. dispersus* sequences available in the GenBank. The sequence data generated were deposited in the GenBank nucleotide database (NCBI). The simple matching, Neighbour joining dendrogram of *A. dispersus* populations from 13 agroecological zones of Kerala revealed two major clusters with a few out-group individuals (Fig. 2). The cluster analysis revealed that *A. dispersus* populations are generally distributed independently across the sampled localities. However, certain clusters were evident, joining populations according to the regions. This pattern suggests that population differentiation has already occurred, likely influenced by regional factors. Heterogeneous grouping of the population may be attributed to variations in nutritional resources and behavioural traits. Additionally, the observed differentiation could be a result of multiple introductions of *A. dispersus* into different regions of India (Boopathi *et al.*, 2014) from diverse sources

rather than a single founding population. COI sequence analysis has proven to be a good molecular marker for intra- and interspecific variation in whitefly species (Thao and Baumann, 2004; Boykin *et al.*, 2007; Shoorcheh *et al.*, 2008; Chu *et al.*, 2012; Henri *et al.*, 2013).

The spiralling whitefly isolates from various agroecological regions of Kerala were matched with existing sequences in the GenBank database, revealing 100 per cent similarity with *A. dispersus* sequences (Table 2). All isolates showed 100 per

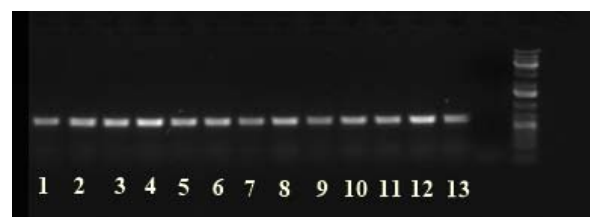


Fig.1 Gel images of PCR amplification of insect DNA *Aleurodicus dispersus* from different agro-ecological zones of Kerala (lane 1- CHA, lane 2- SKM, lane 3- PBR, lane 4- PNR, lane 5- KZH, lane 6- KON, lane 7- PKD, lane 8 - NYT, lane 9- CTR, lane 10- CBK, lane 11 - MVP, lane 12- SBY, lane 13- KYM), NTC- Non template control, L: 100 bp molecular weight marker

cent similarity with *Aleurodicus dispersus* sequences, confirming their molecular identity. The matching sequences correspond to various genes, including cytochrome c oxidase subunit I (COX1) and cytochrome oxidase subunit I (COI), and were retrieved from different isolates and clones. Despite the geographic spread, there is no apparent sequence variation among the isolates. Some isolates (e.g. CHA, NYT) match the same sequence (OQ844129.1) indicating possible genetic similarity or shared origin. The isolates from KYM, SKM, PBR, PNR, KZH, KON, PKD, CTR, CBK, MVP, and SRY regions were matched with existing sequences, confirming their identity as *A. dispersus*. This high degree of sequence identity

was observed across various genetic markers, including the cytochrome c oxidase subunit I (COX1) and cytochrome oxidase subunit I (COI) genes.

The uniformity in sequences suggests a recent common ancestry among the isolates, implying a high level of gene flow and genetic exchange within the population. Furthermore, the absence of significant genetic variation among the isolates implies a lack of geographic structuring, suggesting that the spiralling whitefly population is genetically homogeneous across the sampled regions. These findings have important implications for understanding the ecology, behaviour, and



Fig. 2 Phylogenetic tree of *Aleurodicus dispersus* mitochondrial cytochrome oxidase (COX-1) sequences from 13 different agroecological zones of Kerala

Table 2. Identification of *Aleurodicus dispersus* from different agroecological zones of Kerala

Isolates with acc No.	Identification – <i>A. dispersus</i>	Acc No. (reference)	Similarity
KYM - OP968041.1	mitochondrion, partial genome	KR063274.1	100%
MVP - PP133062.1			
CHA - OQ028271.1	Isolate; Pundibari cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	OQ844129.1	100%
NYT - PP133051.1			
SKM - OQ061321.1	Isolate; ADC cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	OP303313.1	100%
PBR - OQ073870.1	Isolate; ADB cytochrome oxidase subunit I(CO1) gene, partial cds; mitochondrial.	MK543950	100%
PNR - OQ073874.1	Isolate; CHI cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial	OR672939.1	100%
KZH - ON150855.1	Isolate; 411NdubWalg cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	MN022652.1	100%
KON - ON150772.1			
PKD - ON171232.1	Clone; WFG1KT1 cytochrome oxidase subunit I (coxI) gene, partial cds; mitochondrial	KY574540.1	100%
SRY - OP641807.1			
CTR - ON171233.1	Isolate; 131_KtnKab cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	MN022643.1	100%
CBK - PP133087.1	Clone; WFG1AL1 cytochrome oxidase subunit I (coxI) gene, partial cds; mitochondrial	KY574539.1	100%

management of this economically important pest species. However, more advanced molecular tools have recently been used to understand the population dynamics of whiteflies. For example, *Elfekih et al.* (2018) reported that the genetic mixing between populations of the same invasive species within the *B. tabaci* complex from various geographical localities worldwide using a dense array of genome-wide SNPs.

Earlier studies indicated that this species was first reported in 1993 on cassava (Palaniswami *et al.*, 1995), which means that this species had 30 years to adapt to the environment on the Indian subcontinent. Therefore, it is speculated that the populations were pre-diverged and from multiple sources. The very low level of heterozygosity indicates little gene flow between host-specific spiralling whiteflies. Low gene flow might be the result of restricted outcrossing between populations. Similar results are reported in earlier studies on whitefly populations from distant geographic

locations in India (Boopathi *et al.*, 2014) and 18 districts in Tamil Nadu, India (Boopathi *et al.*, 2017). The large-scale spread from neighbouring countries such as Sri Lanka (Ranjith *et al.*, 1996) and Myanmar (Boopathi, 2008) may have further contributed to genetic variations and changes in allele frequencies across regions.

The primer sequences for around 10 SSR markers are publicly available for studying the genome of the spiralling whitefly (Ma *et al.*, 2011). SSR markers are used in the analysis of genetic divergence and geographical distribution of *A. dispersus* (Boopathi *et al.*, 2014, 2017b). Earlier, the SSR markers were utilized for studying the genetic diversity of *B. tabaci* differentiating the variants and determining the genetic relationships of closely related populations (Gawel and Jarrett, 1991; De Barro *et al.*, 2003; Palaniswami, 2004; De Barro, 2005; Delatte *et al.*, 2006; Tsagkarakou *et al.*, 2007; Gauthier *et al.*, 2008; Palaniswami and Henneberry, 2011; Diaz *et al.*, 2015). Elfekih *et al.*

(2018) have developed a useful molecular diagnostic toolkit for detecting invasive *B. tabaci* via sequence characterization of mtDNA haplotypes.

Furthermore, molecular protocols also play a crucial role in studying predator communities that prey on whiteflies (Lundgren *et al.*, 2013). Through molecular gut content analysis, the predators linked to a specific whitefly species can be identified, that provide valuable insights for developing effective biological control programs to managing these pests (Kheirodin *et al.*, 2020). Additionally, it has been observed that endosymbiotic bacteria found in *A. dispersus* contribute to the protection of their host insects against natural enemies such as predators and pathogens, and may even be involved in mechanisms of insecticide resistance (Zindel *et al.*, 2011; Drisy *et al.*, 2022). The present study highlights the potential of molecular approaches in understanding *A. dispersus* ecology, in accurate species identification and genetic diversity. Future research could therefore focus on utilizing molecular gut content analysis to identify key natural enemies of *A. dispersus* across different agroecological zones of Kerala, similarly molecular characterization of endosymbionts in geographically distinct populations could reveal their role in shaping host adaptation, survival, and resistance mechanisms.

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Characterization and antibacterial properties of oak tasar and mulberry silkworm pupae oils

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ABSTRACT: Current study provides a valuable insight into physical, thermal, and antibacterial studies of oak tasar pupae oil (OTPO) and mulberry silkworm pupae oil (MSPO). It was observed that both OTPO and MSPO showed comparable melting ranges which were -105°C to -70°C for OTPO and -96°C to -70°C for MSPO. However, this also shows that OTPO solidifies at lower temperatures than MSPO. The viscosity of OTPO was observed as 26.16 cP, whereas, for MSPO it was 25.60 cP. The colour of the OTPO appeared as brown and yellow for MSPO which corresponds to the values 18Y + 2.1 R and 14Y + 1.0 R displayed through the tintometer, respectively. The specific gravity values OTPO and MSPO were 0.863 ± 0.0016 and 0.910 ± 0.001 respectively. The antibacterial activity of the oil samples was evaluated against *Streptococcus pyogenes* (MTCC733) and *Salmonella typhi* (MTCC442). Both OTPO and MSPO emphasized significant bactericidal abilities against the selected bacterial strains. OTPO exhibited higher antibacterial activity on gram-negative *S. typhi* of 15.33 ± 1.15 mm at 70 µL. Whereas, MSPO exhibited a maximum zone of inhibition of 13 ± 1 mm at 70 µL on gram-positive *S. pyogenes*. Further, at 20 µL, MSPO showed an inhibition of 9 ± 0 mm against *S. pyogenes*, while OTPO showed no activity against any of the strains. Furthermore, at 50 µL, the zone of inhibition of 9.67 ± 0.58 mm was observed for both OTPO and MSPO against *S. pyogenes*. Although, at the same concentration 12.67 ± 1.15 mm zone of inhibition was measured for OTPO and no activity for MSPO was obtained for *S. typhi*. The results showed significant antibacterial activity, for OTPO, which exhibited a maximum inhibition zone of 15.33 ± 1.15 mm against *S. typhi*.

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KEY WORDS: *Antheraea proylei*, *Bombyx mori*, specific gravity, viscosity, *Streptococcus pyogenes*, *Salmonella typhi*, inhibition

INTRODUCTION

The physical characteristics of insect oils, such as viscosity, density and melting point, are influenced by their fatty acid composition (Chantakun *et al.*, 2024). For instance, oils with a higher proportion of Saturated fatty acids (SFAs) tend to have a higher melting point, making them solid at room temperature, while those rich in Polyunsaturated fatty acids (PUFAs) are typically liquid. Insect oils

also exhibit unique thermal properties, which have been studied for potential applications in the food and cosmetic industries (Kim *et al.*, 2022). Beyond their nutritional and physical properties, insect oils have been found to possess significant antibacterial activity. The antimicrobial properties of insect-derived oils are attributed to the presence of bioactive compounds such as fatty acids, terpenes, and phenolics. These compounds can disrupt bacterial cell membranes, inhibit enzyme activity,

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or interfere with bacterial communication systems, thereby exerting antibacterial effects (Yan *et al.*, 2021). For example, research on the antibacterial properties of black soldier fly larvae oil demonstrated its effectiveness against Gram-positive bacteria, suggesting its potential use in food preservation and medicinal applications (Sprangers *et al.*, 2017).

Traditionally, silkworm pupae are considered a massive co-product of the sericulture textile industry. The depletion of resources with the rising global population is an alarming issue. Amid increasing food demands silkworm pupae emerged as a valuable alternative dietary supplement with high nutrition and therapeutic value (Fig. 1). In the previous study (Suman and Kumar, 2024), the authors extracted a fine amount of the pupae oils through the soxhlet method. Further, also reported the presence of conjugated linoleic acid (Ω -6 fatty acids) and other bioactive components in both oak tasar pupae oil (OTPO) and mulberry silkworm pupae oil (MSPO) through Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared (FTIR) analysis. Therefore, in the current study, aim to explore the potential physical, thermal and anti-bacterial benefits of OTPO and MSPO..

MATERIALS AND METHODS

The Indian oak tasar pupae enclosed were procured from the local seri-farmers allied to Central Tasar Research and Training Institute, RSRS Bhimtal, Uttarakhand, India. Further, pure Mysore variety of mulberry silkworm pupae encased in cocoon were purchased from the local cocoon market with the help of Central Silk Board, Bengaluru, Karnataka, India. Both the samples were freshly procured and subjected to the experiment. The pupae of both samples were taken out from the cocoons by cutting the cocoons. The pupae were air dried at 70°C in a hot air oven until the moisture was eliminated. After drying, the dried pupae were ground to a fine powder using an electric blender. The blended pupae powder (100g) was subjected for oil extraction using Soxhlet apparatus as suggested by Thirupathaiah *et al.* (2016) and

Suman and Kumar (2024). The recovered oils were kept in separate jars and labelled as OTPO for oak tasar pupae oil and MSPO for mulberry silkworm pupae oil. Both oil samples were kept in a refrigerator for further analysis.

Melting point determination by DSC: The melting point of OTPO and MSPO was determined by differential scanning calorimetry (DSC) (PerkinElmer DSC 4000). For DSC analysis, pure indium was used to calibrate the equipment, and an empty open aluminium pan was used to obtain the baseline with the sample. 3.5 ± 0.5 mg of OTPO and MSPO were weighed into open aluminium pans and placed in the equipment's sample chamber. The constant temperature was set for five distinct temperatures (100, 110, 120, 130 and 140°C), and nitrogen was used as the purge gas flowed at ~ 20 mL min⁻¹. Once the experiment was completed, the data was obtained in the form of graphs for analyses (Pradauil *et al.*, 2011).

Viscosity determination: The dynamic viscosity (μ) of OTPO and MSPO was determined by a Brookfield viscometer (USA) equipped with a thermo-container and programmable temperature controller. The oil samples were kept in the beaker and the viscometer spindle was emerged into the oil samples. Further, the motor was switched on and the spindle was allowed to rotate until a steady reading was obtained (Sahasrabudhe *et al.*, 2017).

Colour determination: The colour determination of OTPO and MSPO was done using a Lovibond tintometer. In the Lovibond method color is expressed as red and yellow components, (ISI, 1984; IS 548 (Part 1)-1964).

$$\text{Colour reading} = (a Y + 5 b R)$$

Where:

a = sum of the various yellow slides (Y) used

b = sum-total of the various red (R) slides used

Y + 5R = mode of expressing the colour of light-colored oils

Specific gravity: Specific gravity was determined as the weight of a specific volume of the OTPO and MSPO divided by the weight of a volume equivalent to the water (reference material) at room

temperature. For the specific gravity measurement firstly the weight of an empty specific bottle was recorded using a digital weight machine then the specific gravity bottle was filled with OTPO and MSPO separately and accurate weights of both the samples were recorded. After that the distilled water (DW) was filled in a clean specific gravity bottle and weight was taken (FSSAI, 2021). The specific gravities of OTPO and MSPO were determined as -

$$\text{Specific Gravity (at given temperature)} = \frac{A(T) - B}{C - B}$$

$$\text{Specific Gravity (at given temperature)} = \frac{A(M) - B}{C - B}$$

Where,

A (T) = Weight of the SG bottle containing OTPO.

A (M) = Weight of the SG bottle containing MSPO.

B= Weight of the empty SG bottle.

C = Weight of the SG bottle containing DW.

Antibacterial analysis: The antibacterial activity of the pupae oil was evaluated using agar well diffusion method against bacterial strains namely *Streptococcus pyogenes* (MTCC733) and *Salmonella typhi* (MTCC442) procured from Microbial Type Culture Collection (MTCC) Chandigarh, India. Mueller Hinton Agar (MHA) was used as a culture medium which was prepared by adding 38g of MHA in 1L of water separately. After that medium was sterilized using autoclave at 121°C and at 15 psi for 15 minutes. After sterilization, MHA plates were prepared by pouring 30mL of culture medium into sterile glass petri plates and the plates were allowed to solidify. The antibacterial efficacy of OTPO and MSPO was tested at different serial dilutions. For the serial dilution's preparation, 20, 50 and 70µL of both the oil samples were dissolved in 1 ml of hexane. Ten mg of ciprofloxacin was served as the positive control. Different dilutions of ciprofloxacin were prepared by dissolving 200, 500 and 700µg in to one ml of Hexane. The final volume of prepared serial dilutions of OTPO and MSPO (20, 50 and 70µL) and ciprofloxacin (100, 200, 300 and 400µg) was increased to 80µl by adding Hexane. Further, using a sterile cotton swab, the bacterial inoculum

was uniformly spread on agar plate. Ten minutes after spreading, wells were punched using sterile tips and each well was filled with 20µL of samples at desired concentration. The samples were allowed to disperse into the culture medium and then the MH agar plates were sealed and incubated at 37°C for 24 hours. After 24 hours of incubation, the plates were examined for the zone of inhibition which was measured in millimeters. The experiment was performed in triplicates. Hexane was used as the negative control against the bacterial strains tested (Wiegand *et al.*, 2008).

RESULTS AND DISCUSSIONS

Melting point determination by DSC: The melting point of OTPO and MSPO was determined and presented in the form calorimetric graph (Fig. 2). It was observed that both OTPO and MSPO showed comparable melting ranges which were -105°C to -70°C for OTPO and -96°C to -70°C for MSPO. However, this also shows that OTPO solidifies at lower temperatures than MSPO. The peak temperature for OTPO was -80.81°C whereas for MSPO was -79.73°C this minor difference in the peak temperatures indicates that melting OTPO is a little earlier than MSPO. MSPO exhibits a somewhat more prominent melting transition or a greater absorption of heat during the phase change, as seen by its higher peak height (1.8521mW) compared to OTPO (1.7066mW). Indicating a higher total heat absorption during melting, OTPO's larger area (81.2411mJ) and higher enthalpy change (22.8971 J g⁻¹) raise the possibility that it contains more solid components



Fig. 1 Schematic figure showing extraction of nutritious pupae oil from oak and mulberry silkworm pupae waste after the reeling process

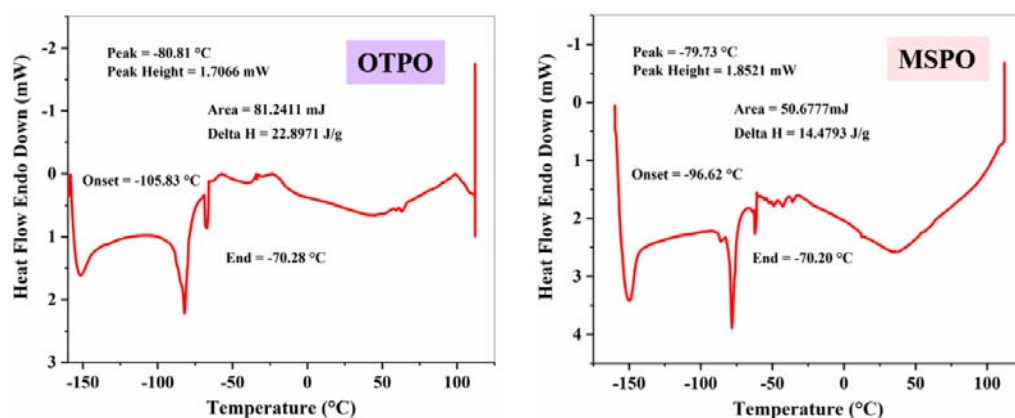


Fig. 2 Differential Scanning Calorimetric (DSC) thermograms of OTPO and MSPO showing their specific melting transitions

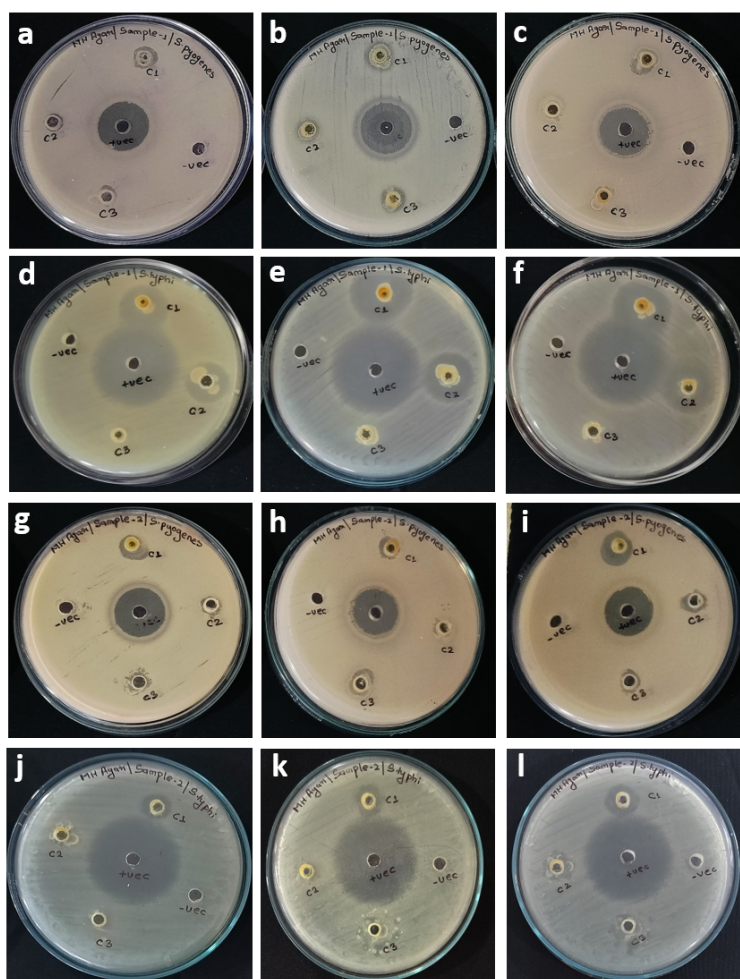


Fig. 3 Determination of antibacterial activity using Agar well diffusion assay carried on *S. pyogenes* and *S. typhi* with Oak tasar pupae oil (a–f) and Mulberry silkworm pupae oil (g–h) at different concentrations (20, 50 and 70 μ L) labelled as C1, C2, and C3 respectively on plates. Standard drug Ciprofloxacin (+VEC) and hexane (-VEC) solvent used as a positive and negative control respectively against the tested bacterial strains for comparison

Table 1. Determination of physical characteristics of OTPO and MSPO

Parameters	Mean $\pm$ SD		Range for edible oils	References
	OTPO	MSPO		
Viscosity	26.16cP	25.60cP	~ 30-100cP	Diamante and Lan, 2014
Colour	Brownish yellow (18Y + 2.1 R)	Yellow (14Y + 1.0 R)	Light yellow to brown	McNicholas, 1935
Specific gravity	0.8639 $\pm$ 0.0016	0.9105 $\pm$ 0.001	~ 0.91-0.93	Ichu and Nwakanma, 2019

or a more complex mixture of fatty acids (or other components) that require more energy to melt. MSPO, in contrast, requires less energy to transform from solid to liquid than OTPO because of its smaller area (50.6777mJ) and lower enthalpy change (14.4793 J g⁻¹). Differential scanning calorimeter is a useful instrument for the determination of thermal-driven behavior including the melting point, oxidative stability, crystallization, and thermal phase transition of oils and fats especially food-grade oils such as fish oil (Tolstorebrov *et al.*, 2014), coconut oil (Srivastava *et al.*, 2017), passion fruit oil (Pardaul *et al.*, 2011), Sunflower oil (Rudakov *et al.*, 2021). Each peak in the DSC graph is associated with a detailed comprehensive physical or chemical process (Qi *et al.*, 2016). Such as the coordinates of the maxima of peaks on the abscissa axis and their areas are considerably correlated with the concentrations of basic fatty acids and triacylglycerols (Rudakov *et al.*, 2021). In the current study, DSC analysis revealed that OTPO showed a high melting point as compared with MSPO which has a low melting point. This clearly indicate that OTPO remains solid at room temperature which might be due to the presence of high concentrations of saturated fatty acids.

Viscosity determination: The viscosity of OTPO was observed as 26.16cP whereas, for MSPO it was 25.60cP (Table 1). In food processing, viscosity measurement has become a crucial and essential part of many quality control processes. It is an attribute influencing oil flow (Cullen *et al.*, 2000). The viscosity of OTPO and MSPO was reported marginally less than the eri pupae oil (Ravinder *et al.*, 2016). However, the results of the current study

were accorded with the obtained outcomes of Ferdousi *et al.* (2013) for MSPO.

Colour determination: The colour of the OTPO appeared as brown and yellow for MSPO which corresponds to the values 18Y + 2.1 R and 14Y + 1.0 R displayed through the tintometer, respectively (Table 1). The detailed measurement of the colour of fats and oils is a common and crucial criterion for the manufacturing of oils, fats, margarine, and mayonnaise (Brühl *et al.*, 2021). The Lovibond tintometer was used to determine the colour of the pupae oils. Colour is expressed as red and yellow components in the Lovibond approach. Usually, 84 Lovibond interference filters in the device are categorized into eleven groups, which include neutral, yellow, red, and blue filters, and completely refined oil maybe 0.8 (red) or 8.0 (yellow) (Zhang *et al.*, 2018). In this study, MSPO appeared yellow which is consistent with Srivastava *et al.*, (2023).

Specific gravity: Specific gravity rises when the quantity of aromatic compounds in the oil increases, whereas specific gravity falls when the amount of saturated compounds increases (Yahaya *et al.*, 2012; Abu-Ellella *et al.*, 2015). The specific gravity values OTPO and MSPO were 0.863 $\pm$ 0.0016 and 0.910 $\pm$ 0.001 respectively (Table 1). The specific gravity of OTPO was found to be similar to the value of tropical tasar pupae oil in a previous study done by Srivastava *et al.* (2024). Similarly, the value of OTPO was nearly equal to the specific gravity value of olive oil which ranges from 0.915 to 0.928 (Ichu and Nwakanma, 2019). Further, the specific gravity value of MSPO was found to be similar to the value of jojoba oil (0.863) (Gad *et al.*, 2021).

Table 2. Interpretation of antibacterial activity of OTPO and MSPO against gram-positive *S. pyogenes* and gram-negative *S. typhi*

Organisms	Zone of inhibition (mm)					
	20 μ L		50 μ L		70 μ L	
<i>S. pyogenes</i>	OTPO	MSPO	OTPO	MSPO	OTPO	MSPO
	Nil	9 $\pm$ 0	9.67 $\pm$ 0.58	9.67 $\pm$ 0.58	12.67 $\pm$ 0.58	13 $\pm$ 1
<i>S. typhi</i>	Nil	Nil	12.67 $\pm$ 1.15	Nil	15.33 $\pm$ 1.15	12.27 $\pm$ 0.46

Antibacterial activity: The antibacterial activity of OTPO and MSPO was observed through the agar well diffusion method (Fig. 3, Table 2). Both OTPO and MSPO emphasized significant bactericidal abilities against the selected bacterial strains. OTPO exhibited higher antibacterial activity on gram-negative *S. typhi* of 15.33 $\pm$ 1.15mm at 70 μ L. Whereas, MSPO exhibited a maximum zone of inhibition of 13 $\pm$ 1mm at 70 μ L on gram-positive *S. pyogenes*. Further, at 20 μ L, MSPO showed an inhibition of 9 $\pm$ 0mm against *S. pyogenes*, while OTPO showed no activity against any of the strains. Furthermore, at 50 μ L, the zone of inhibition of 9.67 $\pm$ 0.58mm was observed for both OTPO and MSPO against *S. pyogenes*. Although, at the same concentration 12.67 $\pm$ 1.15mm zone of inhibition was measured for OTPO and no activity for MSPO was obtained for *S. typhi*. Further, no intrinsic toxicity was observed by Hexane. Ciprofloxacin was used as a control to compare the obtained results.

Earlier, Suman and Kumar (2024) demonstrated a potential antibacterial activity of OTPO against *Escherichia coli*, *Enterobacter cloacae*, and *S. typhi* at different dilutions while MSPO was found to be ineffective against the tested bacterial strains using LB media. However, in the current study authors used MHA nutrient media and both OTPO and MSPO emphasized significant bactericidal abilities against the selected bacterial strains. Therefore, use of different nutrient media may also play a vital role in the evaluation. Dev *et al.*, 2017 found the antibacterial properties of *B. mori* pupae oil against *S. sciuri*. Additionally, Saviane *et al.* (2021) obtained equivalent outcomes when deploying *B. mori* pupae oil against Gram-positive

Bacillus subtilis and *S. aureus*. Likewise, Tasar pupae oil (TPO) also exhibited an antibacterial ability against *S. aureus* and *E. coli*. (Ramappa *et al.*, 2023 and Srivastava *et al.*, 2023). The results of the study reveal an effective antibacterial potential of OTPO and MSPO oils attributing to the inclusion of several bioactive components, especially MUFA and PUFA.

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Bioefficacy of certain insecticides in cabbage against lepidopteran pests

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ABSTRACT: Among the insecticides evaluated for bioefficacy against lepidopteran pests (diamond back moth, *Plutella xylostella*, leaf webber, *Crociodolomia binotalis* Zell and tobacco caterpillar, *Spodoptera litura* Fab), chlorantraniliprole 18.5 SC @10g a.i ha⁻¹ was most effective. Incidence of diamond back moth was 0.72±0.11 and 0.47±0.07 larvae plant⁻¹, respectively after first and second spray, leaf webber 0.62±0.10 and 0.54±0.08 larvae plant⁻¹ respectively, after first and second spray and tobacco caterpillar 0.91±0.07 and 0.65±0.08 larvae plant⁻¹, respectively, after first and second spray. Flubendiamide 20 WG @25g a.i ha⁻¹ was next best and novaluron 10 EC @75g a.i ha⁻¹ which was least effective. The maximum yield of 264.54 q ha⁻¹ was obtained from chlorantraniliprole. while the minimum yield of 215.60 q ha⁻¹ was recorded in novaluron. The highest cost-benefit ratio of 1:33.23 was recorded in chlorantraniliprole, while the lowest ratio of 1:6.69 in novaluron.

KEY WORDS: Diamond back moth, leaf webber, tobacco caterpillar, yield, incremental cost-benefit ratio, chlorantraniliprole

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is a short-duration, nutrient-rich vegetable crop capable of producing high-quality food per unit area and time, making it an important component of modern agriculture. In India, cabbage is cultivated over 397 thousand hectares, with an annual production of 9.21 million metric tons and an average productivity of 23.19 MT ha⁻¹. Major cabbage-growing states include West Bengal, Uttar Pradesh, Odisha, Bihar, Assam, Maharashtra, and Karnataka, with Uttar Pradesh alone contributing 348.94 thousand MT annually (Sharma *et al.*, 2022). Cabbage production

in India is severely constrained by insect pest infestations, with around 37 species reported. Among these, the diamondback moth (*Plutella xylostella* L.), cabbage leaf webber (*Crociodolomia binotalis* Zell.), tobacco caterpillar (*Spodoptera litura* Fab.), cabbage semilooper (*Trichoplusia ni* Hübner), cabbage butterfly (*Pieris brassicae* L.), flea beetles (*Phyllotreta cruciferae* Goeze), aphid (*Brevicoryne brassicae* Linn.), and mustard sawfly (*Athalia lugens proxima* Klug) are the most destructive. These pests can cause yield losses ranging from 80–100 per cent in nurseries to 10–25 per cent in field crops (Lal *et al.*, 2020). The tobacco caterpillar is an economically important

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polyphagous pest with a broad host range, attacking plants from nearly 120 families, including cultivated crops, weeds, vegetables, fruits, and ornamentals, and is distributed worldwide (Dodiya *et al.*, 2023). In India, the diamondback moth is a particularly serious pest of cabbage, causing annual marketable yield losses of 50–80 per cent (Kumar *et al.*, 2025). Similarly, the cabbage leaf webber inflicts severe damage, with yield losses reported between 37.2 and 81.8 per cent (Kumbar *et al.*, 2022). Bio-efficacy evaluations of insecticides are essential for identifying the most suitable options for pest control. Such studies help determine optimal application strategies, support resistance management, and facilitate the integration of chemical control with biological and cultural practices within an Integrated Pest Management (IPM) framework. Therefore, the present study was undertaken to evaluate the bio-efficacy of selected insecticides against major insect pests of cabbage under field conditions.

MATERIALS AND METHODS

The bio-efficacy of selected insecticides (Table 1) against lepidopteran borers was evaluated at the Students' Instructional Farm (SIF) using a Randomized Block Design (RBD) with eight treatments (Table 1) and three replications. Each plot measured 1.8×2.7 m, with a one metre field border. The spacing was maintained at 45 cm between rows and 45 cm between plants, with a 0.5 m gap between plots. Observations were recorded on the larval population of *Spodoptera litura*, *Crociodolomia binotalis* and *Plutella xylostella*, expressed as the number of larvae per plant. Pre-treatment counts were taken one day before spraying, followed by counts at 3, 7 and 10 days after each application. The mean pest population after each spray was computed from these observations.

The recommended volume of spray solution was applied to each plot (except untreated control plots). The required amount of formulation was calculated using the formula:

$$\frac{\text{Amount of formulation (kg or L ha}^{-1}\text{)} = \text{Dose recommended in a.i./ha (kg or g/ha)}}{\text{Concentration of toxicant in insecticidal formulation \%}} \times 100$$

Concentration of toxicant in insecticidal formulation %

Where:

a.i. = active ingredient

Dose recommended in a.i. ha⁻¹ = the amount of pure active ingredient needed per hectare

Concentration of toxicant (%) = percentage of active ingredient in the insecticidal formulation

The spray mixture for each treatment was prepared by mixing the required quantity of insecticide formulation in water to achieve a spray volume equivalent to 500 L ha⁻¹. For each treatment, the spray solution was prepared fresh before application and applied to the respective plots using a knapsack hand sprayer fitted with a cone-type nozzle. Spraying was carried out in the morning hours under suitable wind conditions to minimize spray drift to adjacent plots and reduce loss of spray fluid due to high wind velocity. All necessary protective measures were followed during application. After spraying, the sprayer was thoroughly cleaned and washed. In each treatment, head yield was recorded separately. The plot yields were converted to yield per hectare and subjected to statistical analysis to determine the significance of differences among treatments. The Incremental Cost–Benefit Ratio (ICBR) was calculated using the formula:

$$\text{ICBR} = \frac{\text{Additional return from treatment (Rs/ha)}}{\text{Additional total cost of treatment (Rs./ha.)}}$$

RESULTS AND DISCUSSION

Diamondback moth (*P. xylostella*): Among all treatments, chlorantraniliprole (18.5% SC) proved most effective, recording the lowest larval population (0.72 ± 0.11 and 0.47 ± 0.07 larvae plant⁻¹) and the highest per cent efficacy after the first and second sprays (75.77 and 85.01% respectively). This was followed by cyantraniliprole (10.26% OD) (0.97 ± 0.09 and 0.68 ± 0.04 larvae plant⁻¹ after the first and second sprays respectively and per cent efficacy after the first and second sprays as 67.41 and 78.28% respectively). These results are consistent with Sawant and Patil (2018), who reported maximum larval reduction of *P. xylostella* in chlorantraniliprole-treated plots (91.30% with 1.02 larvae plant⁻¹). Beena and Selvi (2022) similarly noted that diamide insecticides such as chlorantraniliprole, cyantraniliprole and

Table1. Bio-efficacy of certain insecticides against caterpillar pests in cabbage (mean larval population of plant⁻¹)

Treatments/ dose ha ⁻¹	DBM				Leaf webber				<i>Spodoptera littoralis</i>			
	1 spray		2 spray		1 spray		2 spray		1 spray		2 spray	
	Mean*	PROC	Mean*	PROC	Mean*	PROC	Mean*	PROC	Mean*	PROC	Mean*	PROC
Emamectin benzoate 5SG@10g	1.31±0.12 (1.33)	57.26	0.90±0.03 (1.18)	71.01	1.10±0.11 (1.26)	64.79	0.86±0.07 (1.16)	73.26	1.16±0.17 (1.28)	65.51	1.08±0.07 (1.31)	69.73
Indoxacarb 14.5SC@40g	1.53±0.16 (1.39)	52.25	1.26±0.06 (1.32)	59.69	1.58±0.08 (1.39)	54.31	1.01±0.16 (1.23)	68.56	1.42±0.13 (1.38)	57.71	1.39±0.06 (1.42)	60.83
Chlorantraniliprole 18.5 SC@10g	0.72±0.11 (1.10)	75.77	0.47±0.07 (0.98)	85.01	0.62±0.10 (1.06)	80.12	0.54±0.08 (1.02)	83.26	0.91±0.07 (1.18)	72.97	0.65±0.08 (1.13)	81.84
Flubendiamide 20WG@25g	1.28±0.04 (1.26)	63.58	0.79±0.08 (1.13)	74.61	0.83±0.15 (1.15)	73.38	0.72±0.03 (1.10)	77.71	1.00±0.08 (1.22)	70.15	0.84±0.07 (1.21)	76.44
Cyantraniliprole 10.26% OD@60g	0.97±0.09 (1.21)	67.41	0.68±0.04 (1.08)	78.28	1.05±0.12 (1.24)	66.60	0.78±0.10 (1.13)	75.81	1.08±0.17 (1.25)	67.83	0.99±0.08 (1.28)	72.22
Novaluron 10EC@75g	1.82±0.05 (1.49)	42.07	1.47±0.11 (1.40)	55.02	1.71±0.10 (1.48)	45.47	1.13±0.07 (1.27)	65.04	1.72±0.19 (1.49)	48.59	1.50±0.15 (1.47)	57.87
Spinosad 2.5SC@17.5g	1.47±0.15 (1.37)	54.18	1.03±0.10 (1.24)	66.84	1.27±0.02 (1.33)	59.50	0.89±0.09 (1.18)	72.29	1.28±0.14 (1.33)	61.69	1.26±0.10 (1.37)	64.73
Control	2.99±0.06 (1.87)	-	3.12±0.12 (1.90)	-	3.13±0.08 (1.90)	-	3.22±0.06 (1.93)	-	3.35±0.10 (1.96)	-	3.56±0.12 (2.04)	-
SEm±	(0.02)	-	(0.02)	-	(0.02)	-	(0.02)	-	(0.05)	-	(0.02)	-
CD at 5%	(0.07)	-	(0.06)	-	(0.08)	-	(0.07)	-	(0.14)	-	(0.06)	-

Figures in the parenthesis are "x + 0.5 transformed values, NS= Non- significant, DBS= A Day before spray, DAS= Days after spray, PROC= Percent reduction over control, *Mean of three replications

flubendiamide achieved over 95 per cent population reduction compared with the untreated control (Table 1).

Leaf webber (*C. binotalis*): The lowest mean larval population of *C. binotalis* was recorded in plots treated with chlorantraniliprole (18.5% SC) after the first and second sprays (0.62 ± 0.10 and 0.54 ± 0.08 larvae plant⁻¹; efficacy as 80.12 and 83.26%), followed by flubendiamide (20% WG) (0.83 ± 0.15 and 0.72 ± 0.03 larvae plant⁻¹; efficacy as 73.38 and 77.71%) after the first and second sprays, respectively. These findings align with Karuppaiah and Srivastava (2013) and Jemimah *et al.* (2021), both of whom highlighted the superior efficacy of chlorantraniliprole against leaf webber infestation (Table 1).

Tobacco caterpillar (*S. litura*): Chlorantraniliprole again recorded the lowest larval counts after the first and second sprays (0.91 ± 0.07 and 0.65 ± 0.08 larvae plant⁻¹; efficacy as 72.97 and 81.84%). Flubendiamide followed closely (1.00 ± 0.08 and 0.84 ± 0.07 larvae plant⁻¹; efficacy as 70.15 and 76.44%). Similar results were reported by Smriti *et al.* (2019), who found chlorantraniliprole highly toxic to third instar larvae of *S. litura*, and by Nayaka *et al.* (2019), who recorded the lowest larval population (0.38 and 0.29 larvae plant⁻¹) in treated plots (Table 1).

Effect of different insecticides on yield:

Cabbage yield varied between 215.60 and 264.54 q ha⁻¹ in treated plots. The highest yield was obtained with chlorantraniliprole (264.54 q ha⁻¹), which was significantly superior to all other treatments, followed by flubendiamide (259.13 q ha⁻¹) and cyantraniliprole (254.30 q ha⁻¹). The lowest yield among treatments was recorded for novaluron (215.60 q ha⁻¹), while the untreated control produced 201.59 q ha⁻¹. Sawant and Patil (2018) reported maximum yield (238.15 q ha⁻¹) with chlorantraniliprole, representing a 129.23 per cent increase over the control, followed by flubendiamide. Gill *et al.* (2010) also documented the highest yields from flubendiamide treated plots (Table 2).

Economics of management: The highest benefit–cost ratio (BCR) was achieved with chlorantraniliprole (1:33.23), followed by flubendiamide (1:27.62), emamectin benzoate 5 SG (1:23.77), indoxacarb 14.5 SC (1:9.97), cyantraniliprole (1:8.11), spinosad 2.5 SC (1:7.03), and novaluron (1:6.69). Sawant and Patil (2018) also found the highest incremental cost–benefit ratio for chlorantraniliprole (1:16.40), followed by flubendiamide (1:14.98) (Table 2).

Bioefficacy of insecticides evaluated against lepidopteran pests in cabbage, revealed that chlorantraniliprole 18.5 SC @10g a.i ha⁻¹ was most effective, followed by flubendiamide 20 WG @25g

Table 2. Economics (ha⁻¹) of certain insecticides used for management of lepidopteran borers in cabbage

Treatments/ dose ha ⁻¹	Cost of spray	Yield (q)	Yield over Control (q)	Total return	Net return	ICBR	Rank
T1- Emamectin benzoate 5 SG @10g	2088	231.31	29.72	51712.8	49624.8	23.77	III
T2-Indoxacarb 14.5 SC@40g	2900	219.88	18.29	31824.6	28924.6	9.97	IV
T3-Chlorantraniliprole 18.5 SC@10g	3200	264.54	62.95	109533	106333	33.23	I
T4- Flubendiamide 20 WG@25g	3498	259.13	57.54	100119.6	96621.6	27.62	II
T5-Cyantraniliprole 10.26% OD@60g	10068	254.3	52.71	91715.4	81647.4	8.11	V
T6- Novaluron 10 EC@75g	3170	215.6	14.01	24377.4	21207.4	6.69	VII
T7- Spinosad 2.5 SC@17.5g	5336	226.23	24.64	42873.6	37537.6	7.03	VI
T8- Control	-	201.59	-	-	-	-	

Cost of produce @Rs.1050/q during Rabi 2023-24; ICBR -Incremental cost benefit ratio

a.i ha⁻¹. These may useful in the Integrated Pest Management (IPM) strategies that combine chemical, biological, and cultural controls, promoting environmentally friendly and safe agricultural practices.

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Effect of aqueous extracts of *Pithecellobium dulce* (Roxb.) Benth against *Pediculus humanus capitis* De Geer, 1778 (Procodea, Pediculidae)

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ABSTRACT: The study explores a safe and natural approach to louse management through the use of extracts derived from *Pithecellobium dulce* (Roxb.) Benth., commonly known as Manila tamarind. The efficacy of the aqueous extracts was assessed based on their ability to kill lice and their eggs, employing the filter paper diffusion method. The extracts from leaf, fruit, bark and stem of the plant revealed a mean mortality of 66.7, 40.0, 26.7 and 53.3 per cent respectively against nymphs and adults. The extracts were effective on nits, on the tenth day. The average emergence of nymphs, from the nits treated with leaf, fruit, bark and stem extracts were 06.7, 73.3, 66.7 and 20.0 per cent respectively. The study revealed that the aqueous extract of the leaf of *P. dulce* exhibited remarkable antilice and ovicidal properties.

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KEY WORDS: Manila tamarind, pediculicide, saponin, anti-lice

INTRODUCTION

The relationship between parasites and their hosts has fascinated scientists for centuries, with some of the most complex interactions occurring between ectoparasites and mammals. Among these, lice classified under the order Phthiraptera and suborder Anoplura, stand out due to their specialized adaptations. They are also known as sucking lice, and these ectoparasites sustain themselves by feeding on the blood of placental mammals, including humans, for survival (Durden and Musser, 1994). Having identified over 530 species, lice are highly host-specific, often evolving to thrive on particular mammalian hosts (Durden and Musser, 1994; Amanzougaghene *et al.*, 2020). Lice

infestations lead to severe itching. This irritation can result in symptoms such as pruritus and eczematous changes (Mumcuoglu *et al.*, 2021; Muršič *et al.*, 2018; Guenther, 2015). Individuals with head lice infestations commonly report itching due to the irritative effects of lice saliva (Mumcuoglu *et al.*, 2021).

Lice infestation is not only a health problem, but also a social problem, particularly affecting children, who may experience anxiety, depression, and social isolation due to the stigma associated with these parasites (Babazadeh *et al.*, 2023; Banafshi and Khatony, 2024). Lice infestation occurs primarily through close physical contact or sharing personal items such as hats, combs, towels, etc. (Firoozfar

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et al., 2019). Although effective, these chemical treatments often have drawbacks, including side effects such as skin irritation and the development of resistance among lice populations (Ghavami *et al.*, 2023). Additionally, it may not effectively kill lice eggs, necessitating repeated treatments (Wismer, 2016). Synthetic pediculicides typically work by killing lice by disrupting the functioning of their nervous system (Nolt *et al.*, 2022), but growing concerns about chemical resistance necessitate research into safer alternative methods to control lice infestations. Bioinsecticides are highly effective, safe, and ecologically acceptable. These properties make bioinsecticides a promising alternative for managing head lice infestations, as they reduce the risk of environmental pollution and adverse health effects associated with synthetic chemicals (Anila and Hashim, 2023). *Pithecellobium dulce* (Roxb.) Benth, commonly known as “Manila tamarind,” is rich in secondary metabolites such as saponins, flavonoids tannins, phenols and terpenoids (Srinivas *et al.*, 2018; Murugesan *et al.*, 2019). These secondary metabolites work together to effectively combat lice by disrupting their cellular structure and metabolic processes (Katekhaye and Kale, 2012; Kumar *et al.*, 2013; Kondabolu *et al.*, 2023). The study explores a safe and natural approach to lice management through the use of extracts derived from *P. dulce*. The efficacy of the extracts was assessed based on their ability to kill lice and their eggs, employing the filter paper diffusion method.

MATERIALS AND METHODS

Collection and authentication of the plant

material: The leaves, fruits, bark, and stems of *P. dulce* were collected from Ghatkopar, Mumbai, India. These plant materials were identified and taxonomically authenticated by the Blatter Herbarium at St. Xavier’s College in Mumbai, Maharashtra, India. The collected specimen corresponded to specimen number JP-3229, originally cataloged by J. Pallithanam in the Blatter Herbarium collection (Kumaran, 2018).

Preparation of aqueous plant extract: The various parts were collected, shade dried, and finely powdered using a laboratory grinder. The powdered

material was subsequently sieved through a 180µm mesh to ensure uniform particle size. To prepare a five per cent aqueous decoction, 5g of plant powder was refluxed in 100 mL of distilled water at 90°C for one hour (Ennaifer *et al.*, 2018; Rudraswamy *et al.*, 2021). Following the refluxing process, the extract was allowed to cool at ambient temperature. The cooled decoction was subsequently filtered by using Whatman filter paper no. 1 to remove any residue. The filtrate was then dried in a hot air oven at 70-75°C until completely desiccated. The reconstituted extract obtained from this process was utilized for further analysis in the study.

Specimens of *Pediculus humanus capitis*, comprising of adults, nymphs and nits, were collected from children aged 8 to 12 years and transferred into sterile plastic containers. The container lids were loosely secured with parafilm to permit ventilation, and samples were promptly taken to the research laboratory for the study. Testing for lice and ovicidal effects *in vitro* began within one hour of collection. Using a stereo zoom microscope (MICRON OPTIK-PRIME), preliminary examination was conducted to identify and separate adult lice and nymphs for further study (Samuel *et al.*, 2009; Vijayalakshmi *et al.*, 2010; Sherwani *et al.*, 2013).

Antilice activity: To study the pediculocidal activity of aqueous extracts obtained from different parts, a filter paper diffusion methodology was employed (Sherwani *et al.*, 2013). The plant extracts were dissolved in distilled water to prepare two concentrations (2 and 5%). Preliminary results indicated no significant difference in efficacy between two concentrations, so only the data for the two per cent concentration are presented here. Test subjects, consisting of adult lice and nymphs at a ratio of 3.6/1.4, were classified into five groups with five lice per group. Each group was carefully positioned on Whatman No. 1 filter paper at the base of a petri dish (55mm diameter, 10mm height), which remained uncovered. Consecutively, 0.5mL of the respective aqueous extract was applied directly onto the lice in each Petri dish, allowing the extract to spread evenly in a thin layer (Samuel *et al.*, 2009; Sherwani *et al.*, 2013; Candy *et al.*,

2018). The petri dishes were subsequently moved to a dark and humid chamber maintained at $26 \pm 0.5^\circ\text{C}$ with 70 ± 1 per cent relative humidity. The study was conducted by forming groups (Group I-V). Group I, treated with 0.5mL of distilled water, served as the control, while Groups II through V received the 0.5mL of plant extracts. Observations were carried out at one-hour intervals using a stereo zoom microscope. After five hours, the petri dishes were re-examined under the stereo zoom microscope, and the lice showing no movement were confirmed as dead (Yingklang *et al.*, 2022). All the experiments were performed in triplicate and repeated twice.

Ovicidal Activity: Head louse eggs were examined using a stereo zoom microscope and categorized into early stages, characterized by the presence of a yolk cell, or late stages, identified by the appearance of eye spots. This classification was performed prior to the conduct of the tests. The ovicidal activity was assessed by positioning five brown, oval-shaped eggs with intact operculum on Whatman filter paper no. 1, placed at the base of each petri dish (55mm diameter, 10mm height). A volume of 0.5mL of each test solution and control was carefully applied to the eggs. The dishes were subsequently incubated in a dark chamber maintained at $26 \pm 0.5^\circ\text{C}$ for 14 days. To ensure adequate moisture, 0.1mL of distilled water was added every 48 hours. The hatching process was monitored microscopically, and the percentage of emergence was observed. Each experimental treatment was performed in triplicate and repeated twice to ensure reliability (Samuel *et al.*, 2009; Yingklang *et al.*, 2022).

One-way analysis of variance (ANOVA) was performed between treatment and control groups to determine the significance difference in the mortality/nymph emergence. Tukey's test was performed to determine the difference between the mean percentage mortality/ nymph emergence. Statistical significance was established at the $p < 0.05$.

RESULTS AND DISCUSSION

The aqueous extracts of different parts of *P. dulce*

were effective against nymph and adults of *P. humanus capitis*. The average mortality of adults was 66.7 per cent with the leaf extract, followed by stem extract by 53.3 per cent, fruit extract by 40.0 per cent and bark extracts by 26.7 per cent. Leaf extracts showed significant mortality at all-time points followed by stem extracts (Fig. 4) compared to control groups which are treated with distilled water (showed no mortality during 5 hours assessment). The experimental images showing mortality of lice after treatment with aqueous extracts from different parts of *P. dulce* are presented as leaf - Fig. 1C, fruit - Fig. 1D, bark - Fig. 1E, and stem - Fig. 1F.

Evaluation of aqueous extracts of different parts of *P. dulce* against nits of *P. humanus capitis*, revealed their effectiveness in preventing the emergence/ hatchability. The average emergence of nymphs on day 10 was very low upon treatment with leaf extract (6.7%) and significantly superior compared to control group which has shown an emergence of 93 per cent, indicating its potent ovicidal efficacy. Others extracts from stem, fruit and bark have shown very milder effects on emergence level by 20, 73.3 and 66.7 respectively (Table 1). There was normal developmental progression of hatched egg in control group in distilled water (Figs. 2A – F), while the developmental progression of egg treated with the different aqueous extracts of *P. dulce* were significant (Fig. 3A-D). The results indicated that

Table 1. Percentage of Nymph emergence at different intervals (0-10 days) after application of aqueous extract of *P. dulce* leaf, bark, fruit, stem and water

Percentage of nymph emergence			
Part	Day 0-5	Day 6-10	Total at Day 10
Fruit	33.33 $\pm$ 11.54	40.00 $\pm$ 20.00	73.33 $\pm$ 31.54
Leaf	6.66** $\pm$ 11.54	0.00** $\pm$ 0.00	6.66** $\pm$ 11.54
Bark	26.66 $\pm$ 11.54	40.00 $\pm$ 0.00	66.66 $\pm$ 11.54
Stem	0.00 $\pm$ 0.00	20.00 $\pm$ 20.00	20.00 $\pm$ 20.00
Water	20.00 $\pm$ 0.00	73.33 $\pm$ 11.54	93.33 $\pm$ 11.54

Values in column followed by ** are significant at p value < 0.05 as per Turkey's test; [N=5 (Experiment repeated=3)]

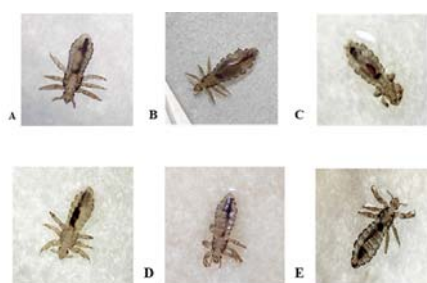


Fig. 1 A. Nymph and B. Adult stages of human lice showing no mortality in the control group. Fig. 1C-F. Antilice activity resulting in lice mortality in - C. leaf extract, D. fruit extract, E. bark extract and F. stem extract

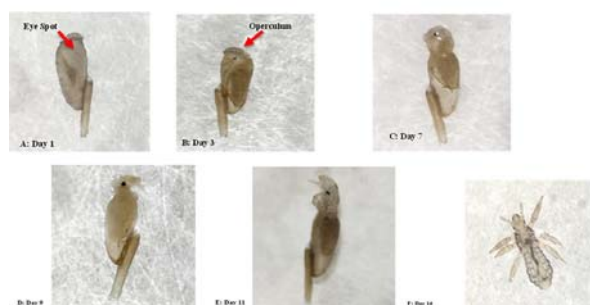


Fig. 2 Developmental progression of hatched eggs (A to F) in control groups (Distilled Water)

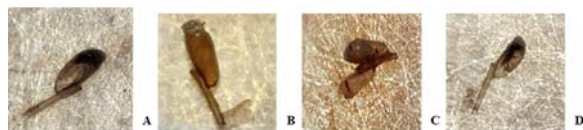


Fig. 3 Ovicidal activity showing un-hatched eggs in the treatments - A. leaf extract, B. fruit extract, C. bark extract and D. stem extract

the aqueous extracts derived from different parts of *P. dulce* exhibited notable activities against lice and their eggs. This bioactivity is likely due to a variety of secondary metabolites present, such as saponins, phenols, flavonoids, terpenoids, and tannins (Katekhaye and Kale, 2012; Kumar *et al.*, 2013; Kondabolu *et al.*, 2023) which may work together to effectively act as antilice agents. Saponins are heterosides, sourced from plants and consist of one or more sugar units, demonstrate considerable potential as pesticides. When applied as an active substance against hatched lice, saponins affect the cell protoplasm, which is a liquid crystalline framework. This interaction results in the solidification of the protoplasm, thereby creating holes in the cell membrane. As a result, the cell fluids escape, ultimately leading to death of the lice (Diaz *et al.*, 2015). Furthermore, tannins and phenolic compounds could serve a vital role in the effectiveness of the extract. Tannins are capable of penetrating the outer membrane of lice,

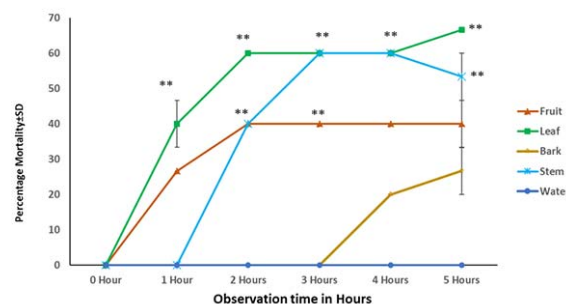


Fig. 4 Percent of head lice mortality at different time intervals (0-5 Hour) after application of aqueous extract of *P. dulce* leaf, bark, fruit stem and water. **significant at $p < 0.05$ as per Turkey's test

compromising cellular structure and obstructing nutrient uptake, which can lead to starvation and death. Their phenolic compound might cause tissue shrinkage and block proteins on the lice's surface, disrupting essential functions. Additionally, phenolic compounds may enhance these impacts by interfering with metabolic processes, reducing nutrient absorption, and inducing overall dysfunction (Samiasih *et al.*, 2023). Together, these compounds could target both the structural and metabolic systems of lice, enhancing the extract's anti-lice efficacy. Unlike synthetic pediculicides like permethrin, which can leave residues contributing to resistance (Samuel *et al.*, 2009), natural extracts from *P. dulce* offer a safer, sustainable solution with diverse modes of action that minimize resistance risk.

Several studies have demonstrated the effectiveness of plant-based extracts such as *Melia azedarach* L. (Carpinella *et al.*, 2007), *Callistemon viminalis* essential oil (Yingklang *et al.*, 2022), and *Pongamia pinnata* petroleum ether extract (Samuel *et al.*, 2009) in treating lice infestations through significant, dose and concentration-dependent anti-lice and ovicidal properties. The results of the present study emphasize the effectiveness of extracts in controlling lice outbreaks and reducing egg emergence. Among the various extracts evaluated, the leaf extract exhibited the highest efficacy in eliminating lice and inhibiting egg hatching. The stem extract also demonstrated notable effectiveness against lice and in preventing

eggs from hatching, while the fruit and bark extracts were less potent but still surpassed the control group that received distilled water. These findings indicate that *P. dulce* leaf extract, presents a strong natural alternative for synthetic treatments.

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High levels of endogenous polyamines indicate superiority of *Morus alba* L. varieties for improved silk production in *Bombyx mori* L.

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ABSTRACT: Mulberry (*Morus alba* L.) is an essential plant for sericulture. The composition of mulberry leaf determines the growth of *Bombyx mori* L. Polyamines (PA) were shown to be vital for growth, development, and stress tolerance in various plants. However, their presence and role in mulberry have not been studied yet. Experiments to examine the polyamines (PA) in four distinct cultivars of *M. alba* leaves by quantitative and qualitative analysis, revealed that PAs were found to be present in all four mulberry cultivars, and the cultivar V1 showed significantly higher PA levels when compared to the others. Thin-layer chromatography revealed several PA bands with different strengths among leaf varieties. The common polyamines found in all four cultivars were putrescine, spermidine, and spermine. The antioxidant potential of the V1 mulberry cultivar was significantly higher when compared to the others. V1 fed larvae of *B. mori* showed significant enhancement in the larval weight, cocoon weight, and length of the filament reeled. This study illustrates that higher endogenous PA levels of the V1 mulberry cultivar led to higher production of silk. © 2025 Association for Advancement of Entomology

KEYWORDS: Mulberry, silkworm, filament length, spermidine, antioxidants, silk production

INTRODUCTION

Morus alba L. is a commercially important plant utilized in sericulture, and pharmaceutical industries (Wang *et al.*, 2012; Budiman *et al.*, 2017). Its leaves serve as the primary food source for silkworms, *B. mori*, making mulberry cultivation essential for silk production. The nutritional quality and availability of its leaves play a crucial role in the growth of silkworms and the overall efficiency

of silk production in sericulture (Lalfelpui *et al.*, 2014). Mulberry cultivation faces significant challenges due to different abiotic and biotic stresses. Drought, salinity, temperature fluctuations, and pest infestations are major factors that effects its cultivation. Understanding the mechanisms underlying stress resistance in mulberry is essential for developing resilient varieties that can thrive under adverse conditions. Biochemical parameters and PAs have emerged as key players in plant stress

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responses, offering valuable insights into the physiological adaptations and defence mechanisms against different stress (Shao *et al.*, 2022; Tyagi *et al.*, 2023). Biochemical parameters such as antioxidants, enzymes, and osmolytes play roles in reducing the effects of stress by eliminating reactive oxygen species (ROS), and maintaining osmotic balance (Mehla *et al.*, 2017). PAs have been implicated in various stress responses, including regulation of gene expression, membrane stability, and ROS scavenging (Liu *et al.*, 2015).

PAs, putrescine (Put), spermidine (Spd), and spermine (Spm) are essential biogenic amines found abundantly in plants (Lenis *et al.*, 2017). They regulate diverse cellular functions, including DNA stabilization, protein synthesis, and stress tolerance, making them vital for, development, adaptation, and growth (Liu *et al.*, 2007). Put and Spd are the two vital PAs involved in cellular stress resistance. Put serves as a precursor for Spd and Spm synthesis. Spd, a three amino group PA, is particularly crucial for stress response, aiding in cellular mechanisms (Shao *et al.*, 2022). Cadaverine (Cad) has been correlated with root growth promotion and osmotic stress reduction in rice (Cassan *et al.*, 2009). Cad plays important physiological roles in plant growth, differentiation, and stress tolerance induced by biotic and abiotic factors (Jancewicz *et al.*, 2016; Ozmen *et al.*, 2023). Exogenous supplementation of Spd showed enhancement in growth and commercial properties of silk produced by *B. mori* (Kasa *et al.*, 2023; Yerra *et al.*, 2017). This study aimed to examine the levels of PAs and antioxidants in mulberry leaves from four different varieties to evaluate their impact on the growth of *B. mori* larvae.

MATERIALS AND METHODS

Mulberry leaf samples of Victory1 (V1), S13, Kosen (K), and Berhampur (B) cultivars were collected from a sericulture field located in Anandapuram, Visakhapatnam, Andhra Pradesh, India (Fig. S1). The varieties were selected based on the reported data with regard to their growth and stress tolerance characteristics (Table 1). Identification of leaves was done in the presence of a sericulture field officer, Government of Andhra Pradesh. Collected

samples were used for qualitative and quantitative analysis of PAs, antioxidant, and feeding experiments. Fresh leaves (100 mg) of different varieties were homogenised with 99 per cent ethanol, centrifuged, and the supernatant was used for estimation of antioxidants. Fresh leaves (100 mg) were weighed, and homogenised with 5 per cent Perchloric acid (PCA), and the supernatant was used for the estimation of PAs.

Quantification of PAs in different mulberry leaves: Derivatization of the PAs with dansyl chloride was carried out as described previously, with some modification (Rajan *et al.*, 2022b). Proline was added to stop the reaction and to remove unbound dansyl chloride. Dansylated PAs were extracted and used for quantitative and qualitative analysis. The benzene extracted dansylated PAs were diluted with DMSO, and fluorescent intensity was measured with the excitation at 350nm and emission at 495nm wavelengths using BioTek synergy H1 microplate reader. A standard curve was plotted using the Spd of different concentrations. The free PAs of samples were expressed in $\mu\text{g mg}^{-1}$. The experiment was performed in biological triplicates.

Qualitative PA analysis by thin layer chromatography (TLC): Dansylated PAs from the different leaf cultivars and standard containing known dansylated PAs; mixture of ornithine (Orn; RM057-25G), Put (RM5438-5G), Spd (RM443-5G), and Spm (RM7506-5G) were separated by loading on activated silica TLC plates. The loaded silica plate was placed in the glass chromatographic tank containing saturated ethyl acetate and n-hexane solvent (Lima *et al.*, 2023). Once the solvent front reached the desired distance, the TLC plates were removed from the tank and air-dried. The dried TLC plates were visualised under UV light. The intensity of the selected bands among different leaf cultivars was measured using Bio-Rad Image Lab 6.0 software.

Estimation of antioxidants: Antioxidants were tested by the TLC dot blot method following the protocol of (Huang *et al.*, 2005). Different concentrations of ascorbic acid and quercetin were used as standards. Ethanolic extracts of all the leaf

varieties were blotted on the activated TLC plate. Subsequently, 2% of 10 ml DPPH was blotted onto the sample. The change in colour from purple pink to yellow was observed which indicates the inhibition of DPPH radical by antioxidants present in the leaf sample.

Feeding different mulberry leaves to *B. mori*: *B. mori* larvae (CSR2 X CSR4) were procured from a sericulture farm located in Narsipatnam town, Anakapalle district, Andhra Pradesh, India. The larvae were divided into four groups of 10 larvae each ($n = 10$). Fresh, healthy, and mature leaves of four different varieties (V1, K, S13, and B) were fed to the *B. mori* larvae from day 1 to day 7 of the 5th instar stage. Feed was given to the worm three times a day. The leaves were evenly spread over the larvae in thin layers to ensure that all larvae could feed equally. Weights of the larvae (day 7) and cocoons were recorded from each group to assess the impact of the leaf variety on growth.

Economic parameter of the silk fibre: Cocoons from various groups were collected and submerged in hot water (65–75°C). A reeling device, Epprouvette, was used to extract the mono-cocoon fibre. The total fibre length was measured in meters, while its weight was recorded in grams (g). The filament denier was then calculated using the formula.

$$\text{Denier} = \frac{\text{weight of the filament}}{\text{length of the filament}} \times 9000$$

Statistical analysis was conducted using one-way ANOVA, and Student's t-test was performed with GraphPad software 10.2.3. among the different varieties and between two varieties of the mulberry leaves. The values superscripted with * are significant ($p < 0.05$) and with ** are highly significant ($p < 0.01$). The commercially important characters, namely length of silk filament, denier, cocoon weight, and larval weight, were correlated with PA content of different mulberry cultivars using a Pearson correlation matrix with pandas.DataFrame.corr function.

RESULTS

PAs show differences in abundance among the different mulberry varieties: Quantification of

PAs in different mulberry leaves: The quantity of PAs in cells is crucial for stress response because elevated levels have been shown to impact various cellular functions. Total free PA levels were checked for 100mg of leaf sample from each cultivar. Statistically elevated PA levels ($p < 0.01$) were observed in V1 ($1.08 \pm 0.13 \mu\text{g}$) followed by B ($0.52 \pm 0.048 \mu\text{g}$), S13 ($0.35 \pm 0.087 \mu\text{g}$), and K ($0.30 \pm 0.059 \mu\text{g}$), respectively. The pairwise comparison demonstrated that the S13, B, and K varieties showed significantly lower PA levels ($p < 0.05$) when compared to V1 (Fig. 1a, Table 2).

PAs show qualitative differences among the different mulberry varieties: Different PAs have specific affinities or activities that make them more suited for various cellular processes. Dansylated PAs were separated on TLC to check the differences in the number and their abundance among the selected varieties (Fig. 1b). The R_f values of the separated bands from samples and standards were calculated. R_f values of Band 1 (0.31), Band 2 (0.38), Band 3 (0.53) and Band 4 (0.61)

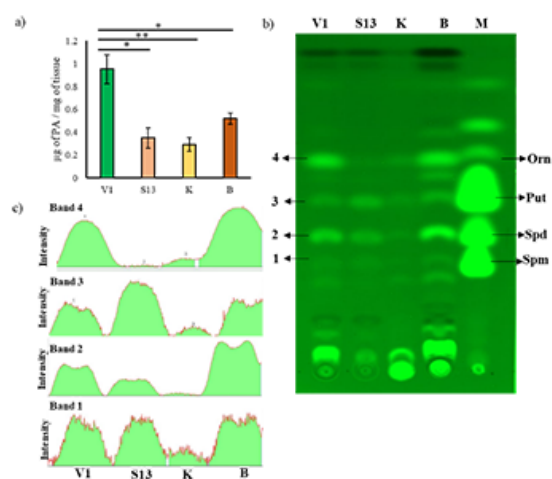


Fig. 1 Dansylated PAs profile in various mulberry cultivars. (a) Free PAs in 1mg of leaf samples. * Indicates significance difference ($p < 0.05$) and ** indicates highly significant difference ($p < 0.01$). (b) Separation of dansylated PAs from leaf samples along with dansylated standard PA mix on silica TLC. 'M' represents mixture of commercially available amines (Spm, Spd, Put, Ornithine), and numbers on the right side represent four selected bands. (c) Quantification of the selected bands was carried out by densitometry analysis using Bio-Rad Image Lab software

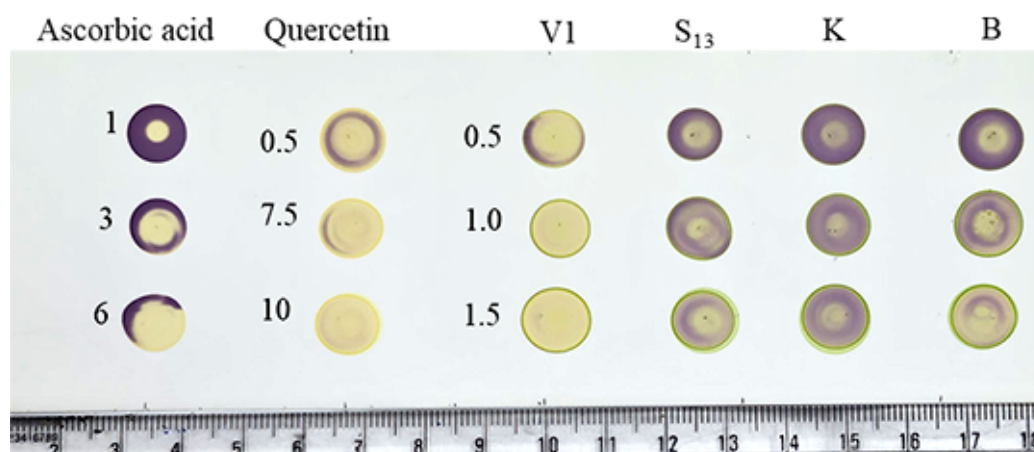


Fig. 2 Free radical scavenging activity of different mulberry cultivars by dot blot on TLC plate (n=3). The numbers given against standards (ascorbic acid and quercetin) represent concentration (μg). The numbers given against the mulberry cultivars represent the leaf weight (mg)

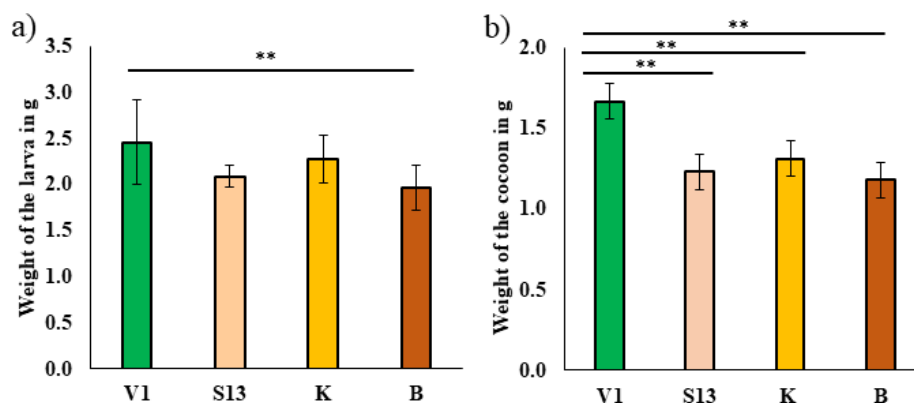


Fig. 3 Growth parameters of *B. mori* larvae fed with different varieties of mulberry leaves (a) Larval weight on day 7 (n=8) (b) Cocoon weight on day 15 (n=8)

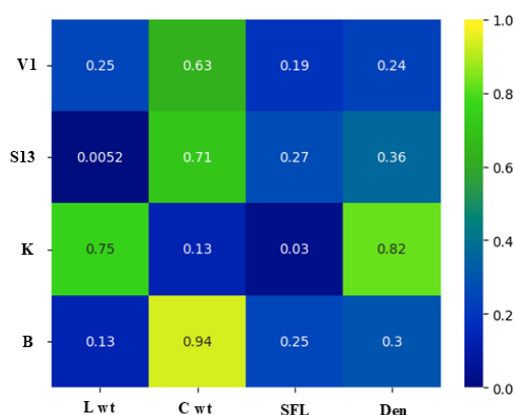


Fig. 4 Heat map representing correlation analysis of larval weight (L wt), cocoon weight (C wt), silk filament length (SFL), and denier (Den) against PA levels of different varieties of mulberry leaves. The colour intensity varies from blue to yellow, where blue signifies weak correlation and yellow signifies strong correlation

correspond to Spm (0.31), Spd (0.38), Put (0.53), and Orn (0.61) respectively. PA bands similar to Orn, Put, Spd, and Spm standards were detected with different intensities in all the varieties. Densitometric analysis showed high intensity of Orn in B, followed by V1, K, and S13. Put intensity was more in S13, followed by B, V1, and K. A highly intense Spd band was recorded in B, followed by V1, S13, and K. Spm recorded no significant difference among V1, S13, B, and K, however, it showed the least intensity in K variety (Fig. 1c).

Retention factor (Rf) values of dansylated marker bands and selected dansylated PA bands from mulberry leaves Spm (0.31), Spd (0.38), Put (0.53), Orn (0.61), Band 1 (0.31), Band 2 (0.38), Band 3(0.53) and Band 4 (0.61).

Table 1. High-yielding mulberry varieties used in the study

Variety	Region cultivated	Developed by	Characteristics
Victory-1 (V1)	South India, irrigated	India	Superior variety with high protein content
S-13	South India, rainfed	India	Drought resistant
Berhampore (B)	Irrigated regions of Eastern and Northeastern India	Philippines	Resistant to powdery mildew
Kosen (K)	Hills of Eastern and Northeastern India	Japan	Moderately tolerant to leaf spot disease

Table 2. Comparative analysis of polyamine levels among the different varieties of mulberry leaves (ANOVA) and pairwise comparison (t - test)

Variety	Mean	SD	F-test	t-test (p value)	
V1	1.08	0.13	52.69 p-value 0.000**	-	
S13	0.35	0.087		V1 vs S13	0.013*
K	0.30	0.059		V1 vs k	0.008*
B	0.52	0.048		V1 vs B	0.014*

** indicates ($p < 0.01$), and * indicates ($p < 0.05$) significant difference

Mulberry cultivars with high PA levels show elevated antioxidant potential: Antioxidants play a crucial role in scavenging and eliminating free radicals and are directly related with enhancing stress resistance in mulberry. The level of oxidative stress was checked with the help of DPPH dot blot. The results of the DPPH scavenging activity of mulberry leaves among four varieties showed notable differences in the antioxidant levels. Maximum scavenging activity was observed in V1, followed by S13, B, and K, respectively (Fig. 2). Increased PAs, Spd, and Put were known to play a role in the decrease of oxidative stress in mulberry leaves.

Mulberry cultivar with high PA levels shows enhanced growth parameters of silkworm: PA levels were shown to be directly related to the growth of the organisms by several studies. To assess the effect of PA levels in mulberry leaves on the larval weight of the *B. mori*, day 1 of 5th instar larvae were divided into four groups and fed with different varieties of leaves. Larval weights

were recorded on day 7, at the end of the feeding stage. A comparative analysis of larval weights between groups showed high significance with the highest larval weight in V1 (2.433 ± 0.31 g), followed by K (2.272 ± 0.26 g), S13 (2.092 ± 0.13 g), and B (1.972 ± 0.25 g), respectively. The pairwise comparison demonstrated that the S13 and B varieties had significantly ($p < 0.05$) decreased larval weight compared to the V1 varieties, and the K variety did not show a significant difference in larval weight when compared to the V1 (Fig. 3a, Table 3).

The larvae from different groups were allowed to form cocoons, and their weights were recorded on day 15. A comparative analysis of cocoon weights between the group showed highly significant difference ($p < 0.01$) with the highest cocoon weight in V1 (1.66 ± 0.21 g) followed by K (1.31 ± 0.36 g), S13 (1.23 ± 0.25 g) and B (1.18 ± 0.18 g) respectively. The pairwise comparison demonstrated that the S13, K, and B varieties had significantly ($p < 0.01$) decreased cocoon weight compared to the V1 variety (Fig. 3b, Table 4).

Table 3. Comparative analysis of larval weights on day 7 of the 5th instar among the different varieties (ANOVA) and pairwise comparison (t-test)

Variety	Mean	SD	F-test	t-test (p value)	
V1	2.461	0.46	2.58 p-value 0.089	-	
S13	2.092	0.13		V1 vs S13	0.051
K	2.272	0.26		V1 vs k	0.134
B	1.972	0.25		V1 vs B	0.006**

** indicates ($p < 0.01$) significant difference

Table 4. Comparative analysis of cocoon weights (g) among the different varieties (ANOVA) and pairwise comparison (t- test)

Variety	Mean	SD	F-test	t-test (p value)	
V1	1.66	0.21	5.65 p-value 0.003**		
S13	1.23	0.25		V1 vs S13	0.001**
K	1.31	0.36		V1 vs k	0.006**
B	1.18	0.18		V1 vs B	0.001**

** indicates highly significant difference ($p < 0.01$)

Mulberry cultivars with high PA levels show enhanced economic parameters of silk fibre:

Economic parameters of silk fibre were analysed by the length of the filament and denier. The length of the silk filament refers to the total continuous fibre length that can be reeled from a single cocoon. Intergroup comparison showed that the length of silk filament reeled was significantly higher in the V1 fed group ($p < 0.01$). The filament denier varies over the reelable filament's length. The quality of the yarn largely depends on denier. Silk is finer when the denier is less. The silk filament produced by the V1 and K variety fed silkworms exhibited lower denier values (Table 5).

Correlation analysis between PAs of mulberry cultivars and economic parameters

Correlation heat map analysis was carried out

Table 5. Comparative analysis of silk filament length (m) and denier among the different varieties (ANOVA) and pairwise comparison (t-test)

Variety	Mean	SD	F-test	t-test (p value)	
V1	814.45	58.18	30.45 p-value 0.000**		
S13	584.50	30.31		V1 vs S13	0.0001**
K	687.45	27.58		V1 vs k	0.0025**
B	657.13	46.87		V1 vs B	0.0012**

Denier

Variety	Mean	SD	F-test	t-test (p value)	
V1	3.158	0.222	8.109 p-value 0.000**		
S13	3.202	0.167		V1 vs S13	0.354
K	3.117	0.381		V1 vs K	0.419
B	3.845	0.359		V1 vs B	0.0015**

**indicates highly significant difference ($p < 0.01$)

between PAs and economic parameters (larval weight, cocoon weight, silk filament length, and denier). A positive correlation was observed between PA content and economic parameters. PAs showed a strong correlation with larval weight in K (0.75), cocoon weight in B (0.94), silk filament length in S13 (0.27), and lowest denier in V1 (0.24) mulberry varieties. PAs in V1 variety made a comprehensive contribution to every factor, making it the superior variety (Fig. 4)

DISCUSSION

PAs, known for their role in cellular homeostasis and stress adaptation, are essential for enhancing plant resilience to both abiotic and biotic stressors. Increased PA levels indicate a specialized adaptive mechanisms that help to mitigate stress-induced damage and optimize growth under adverse conditions (Sagar *et al.*, 2021). Previous studies have confirmed the presence of PAs in mulberry leaves (Hamana *et al.*, 1984). However, this study is the first to provide both quantitative and qualitative analyses of PAs in mulberry leaves.

Mulberry plants, the primary food source for *B. mori* in sericulture, hold significant economic importance. Several studies have demonstrated a direct relationship between PA content and cellular growth (Sagar *et al.*, 2021). Additionally, previous research has shown that Spd supplementation enhances silk production in mulberry silkworms (Lattala *et al.*, 2014). In this study, the V1 mulberry cultivar exhibited the highest PA levels. Earlier findings suggest that mulberry varieties with greater antioxidant potential, along with higher levels of Put, Spd, and Spm, demonstrate greater stress resistance (Chen *et al.*, 2023).

Qualitative analysis of PAs and antioxidants highlights the strong capacity of the V1 cultivar to regulate stress responses and withstand environmental challenges. Previous research has also emphasized the role of PAs in the development of *B. mori* larvae (Rajan *et al.*, 2022a). Our findings reveal that larvae fed on V1 leaves, which have high PA content, exhibited greater larval weight. Economic parameter analysis further showed that the V1-fed group produced longer

filament length with a lower denier. Among the cultivars studied, V1 exhibited the highest antioxidant activity, followed by B, S13, and K. A positive correlation between PAs and economic parameters of silk was observed in the present study. Attempts should be made to enhance the mulberry leaf components for providing a nutritionally enriched diet to silkworms. Such a diet can help the silkworms to grow better and also provide stress tolerance to them, which eventually helps in the enhancement of silk production. Higher levels of PAs and antioxidants in the V1 mulberry variety could have helped in enhanced larval and cocoon weights of the silkworm *B. mori*. The study is the first of its kind correlating PA content with the growth of the silkworm, *B. mori*.

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Toxic effects of pesticides on the green lacewing, *Chrysoperla zastrowi sillemi* (Esben-Peterson) (Neuroptera, Chrysopidae)

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ABSTRACT: The study evaluated the toxic effects of quinalphos 25% EC, deltamethrin 2.8% EC, bifenthrin 8% SC, thiamethoxam 25% WG, thiacloprid 21.7% SC, thiamethoxam 12.6% + λ -cyhalothrin 9.5% SC, propargite 57% EC, hexythiazox 5.45% EC, fenpyroximate 5% EC, and spiromesifen 22.9% SC, on various life stages of *Chrysoperla zastrowi sillemi* (Esben-Petersen) under laboratory conditions, including eggs, larvae (1st, 2nd and 3rd instars) and adults. The results showed that quinalphos, deltamethrin and spiromesifen had significantly high ovicidal activity percentages of 56, 43.3 and 42.7 respectively, on *C. z. sillemi* eggs. The toxicity of the pesticides on *C. z. sillemi* larvae gradually increased over time after application and decreased with the increasing life stage of the larvae. Quinalphos and deltamethrin exhibited 100 per cent mortality across all larval stages of *C. z. sillemi*. Similarly, quinalphos and deltamethrin caused 100 per cent adult mortality after 24 hours of application, followed by thiamethoxam + λ -cyhalothrin and thiacloprid, (which showed 92 and 60% mortality, respectively, after 96 hours). However, pesticides such as bifenthrin, thiamethoxam, hexythiazox, fenpyroximate, and propargite did not exhibit significant acute toxicity on eggs, larvae, and adults of *C. z. sillemi*. Furthermore, the pesticide-treated adults were evaluated to determine sub-lethal toxicity effects on the pre-oviposition period, post-oviposition period, fecundity, fertility, adult emergence, and adult longevity. The findings indicated that thiamethoxam + λ -cyhalothrin, spiromesifen, thiacloprid, and propargite-treated adults experienced reduced longevity, fecundity and fertility. © 2025 Association for Advancement of Entomology

KEY WORDS: Acute toxicity, sub-lethal toxicity, reduced longevity, fertility, fecundity

INTRODUCTION

Common lacewings belong to the order Neuroptera, consisting of soft-bodied insects with biting mouthparts and two pairs of similar membranous wings. Lacewings have a long history of biological control and are naturally occurring in all cropping

systems throughout the year. They play a vital role as bio-control agents against various crop pests (Pappas *et al.*, 2011). Lacewing larvae are predators, while adults feed on pollen and nectar (de Oliveira *et al.*, 2016). The *Chrysoperla carnea* group comprises about 20 very similar-looking species of green lacewings. Among them,

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Chrysoperla zastrowi sillemi, a sub species of *Chrysoperla* is the most widely distributed lacewing species on Earth (Henry *et al.*, 2013). *C. z. sillemi*, extends its range across central and northern Africa, the Middle East, western Asia, and the Indian subcontinent (Henry *et al.*, 2010). *C. z. sillemi* is an important natural predator of various soft-bodied insects such as aphids, coccids, mealybugs, thrips, psyllids, whiteflies, and eggs and larvae of many lepidopteran pests and mites occurring on various crops (Senior and McEwen, 2001).

Naturally, *C. z. sillemi* is a voracious predator against red spider mites (RSM), *Oligonychus coffeae* (Acari, Tetranychidae), and tea thrips, *Scirtothrips bispinosus* (Thysanoptera, Thripidae) in the South Indian tea ecosystem. In order to keep pest populations below the economic threshold level (ETL), planters have traditionally relied on conventional methods, utilizing synthetic pesticides approved by the Tea Board of India under the Plant Protection Code (PPC v.16, February 2024) (<https://www.teaboard.gov.in/TEABOARDPAGE/ODY=>). Intensive pesticide use has adverse effects, including non-specificity, the development of insecticide resistance in pests, accumulation and persistence of pesticide compounds in the environment, and non-target effects on beneficial insects. The use of synthetic pesticides also negatively affects beneficial insects in the agroecosystem (Ono *et al.*, 2017). The lethal and sub-lethal impacts of pesticides on natural enemies must be evaluated for a better understanding. Sub-lethal effects, which do not kill insects but cause small changes affecting development and reproduction, must be considered, as they impact the demographic parameters of insects (Desneux *et al.*, 2006). To gain a better understanding of the toxic effects of pesticides on *C. z. sillemi*, conducted an evaluation of ten approved and commonly used pesticides on various life stages of *C. z. sillemi* including eggs, larvae (1st, 2nd and 3rd instars), pupae and adults under laboratory conditions.

MATERIALS AND METHODS

Experimental conditions: The experiments were

conducted at the Division of Entomology, UPASI TRF Tea Research Institute (10°16'09.6" N: 76°58'02.6" E), Valparai, Tamil Nadu, India. All experiments took place in a controlled environment with conditions set at 25±2°C, 75±5 per cent relative humidity and a photoperiod of 16:8 (Light: Dark).

Mass rearing of the study insect: Newly hatched *C. z. sillemi* larvae were transferred into a plastic container (15cm × 10cm) and fed with the eggs of *Corcyra cephalonica* (Lepidoptera, Pyralidae) until they pupated. The pupae were then carefully transferred to another plastic container (25cm × 10cm) and monitored until they emerged as adults. Once the adults emerged, they were separated and transferred to new plastic containers (25cm × 10cm). The adults were fed an artificial diet containing ground tea pollen and a 30 per cent sugar solution on cotton wool. For the toxicity study, each life stage, including eggs, 3rd instar larvae, pupae, and adults, was randomly obtained from the mass-rearing unit.

Pesticides: The study focused on commonly used pesticides in tea plantations. The selected pesticides were quinalphos (25% EC), thiamethoxam (25% WG), deltamethrin (2.8% EC), thiamethoxam (12.6%) + λ-cyhalothrin (9.5% SC), propargite (57% EC), hexythiazox (5.45% EC), fenpyroximate (5% EC), spiromesifen and bifenthrin (8% SC). These pesticides were directly purchased from the certified pesticide vendor.

Effect of pesticides on eggs of *C. z. sillemi*: The required number of *C. z. sillemi* eggs (<12 hours old) were randomly collected from the laboratory mass-rearing unit and exposed to the selected pesticides by spraying using a Potter tower adjusted to a pressure of 15 lb.pol"2. Subsequently, the treated eggs were individually transferred to Eppendorf tubes (2.0 ml) with small holes made on the cap for aeration. These Eppendorf tubes were then kept under the previously described conditions. The bioassay was conducted in a completely randomized design (CRD), consisting of eleven treatments with three replications, each treatment comprising fifty eggs. Larvae from treated eggs were fed with *C. cephalonica* eggs. The incubation period, larval hatching percentage, 1st instar larvae

mortality, and 1st instar duration were observed and recorded.

Effect of pesticides on larvae of *C. z. sillemi*:

The required number of 1st, 2nd, and 3rd instar larvae (<12 hours old) were randomly collected from the mass-rearing unit and exposed to the pesticides as previously described. The treated larvae were individually transferred to Eppendorf tubes (2.0 ml) and kept under the same conditions. The bioassay was conducted in a completely randomized design (CRD), consisting of eleven treatments with three replications, each treatment containing forty larvae. The larval mortality rate, third instar duration, number of larvae reaching the pupal stage, total pupal period, and number of emerged adults were observed and recorded, with larval mortality being recorded every 24 hours up to 96 hours.

Effect of pesticides on pupae of *C. z. sillemi*:

The required number of pupae (<12 hours old) were randomly collected from the laboratory mass-rearing unit and exposed to the pesticides as described earlier. Following the spraying, the treated pupae were individually placed in glass vials (10 cm height × 2cm diameter) covered with cotton cloth. The bioassay was carried out in a completely randomized design (CRD), with eleven treatments and three replications. Each treatment included forty pupae. Pupicidal percentage, pupal duration, and adult emergence percentage were monitored and recorded throughout the study.

Effect of pesticides on adults of *C. z. sillemi*:

The necessary number of newly hatched adults (<12 hours old) were randomly selected from the laboratory mass-rearing unit and exposed to the pesticides as described earlier. After spraying, couples were formed and introduced into plastic containers (25cm × 10cm) and fed accordingly. The bioassay was conducted in a completely randomized design with seven treatments and three replications, each treatment containing forty adults. Adult mortality was observed every 24 hours up to 96 hours. To assess adult reproductive characteristics, at least three pairs of insects were separately kept in plastic bottles and fed as previously described. The pre-oviposition period, post-oviposition period, fecundity, fertility, adult emergence, and adult

longevity were observed and recorded throughout the study.

Statistical analysis: All collected data were pooled and subjected to Analysis of Variance (ANOVA), with means separated by Duncan's Multiple Range Test (DMRT). All statistical analyses were performed using IBM SPSS v23.0 software.

RESULTS AND DISCUSSION

Pesticide toxicity on eggs of *C. z. sillemi*:

Among the tested pesticides, quinalphos showed significantly higher ovicidal action (56%) followed by deltamethrin and spiromesifen (43.3%) and (42.7%) respectively, against *C. z. sillemi* eggs (Table 1). However, thiacloprid, propargite, thiamethoxam, hexythiazox, and fenpyroximate showed no harm to eggs, yielding results similar to the control. The egg incubation period and 1st instar larval duration were not significantly affected by any of the evaluated pesticides. Cumulative mortality at the end of the 1st instar larvae stage was significantly high in deltamethrin and quinalphos (62.5 and 61.5% respectively). Similarly, spiromesifen, thiamethoxam + λ -cyhalothrin, and thiacloprid also produced notable mortality in 1st instar larvae.

Pesticide toxicity on larvae of *C. z. sillemi*:

The evaluated pesticides caused significant mortality in the 1st, 2nd and third instars of *C. z. sillemi*. After 24 hours of applications, all pesticides showed lower mortality on all three instars, gradually increasing, with the highest observed after 96 hours of application. The 1st and 2nd instars were highly susceptible to the pesticides, quinalphos and deltamethrin, causing 100 per cent mortality even after 24 hours of application (Tables 2 and 3). Both pesticides were found to be more toxic to both instars. Additionally, thiamethoxam + λ -cyhalothrin also exhibited higher mortality (70 and 66.5% on the 1st and 2nd instars, respectively). Similarly, thiacloprid and spiromesifen also moderately affected both the 1st and 2nd instars of *C. z. sillemi*.

In the third instar, quinalphos and deltamethrin exhibited 100 per cent mortality after 48 and 72 hours of application, respectively followed by

Table 1. Toxic effects of commonly used pesticides on the eggs of *C. z. sillemi*

Treatments	Ovicidal % ¹	Incubation (hrs)	Hatching % ²	Larval period ³ (hours)	Accumulated mortality ⁴
Quinalphos	56.0 ± 9.2 ^d	100.8 ± 8.6	44.0 ± 5.29 ^a	97.6 ± 13.2	61.5 ± 6.4 ^d
Deltamethrin	43.3 ± 6.1 ^c	92.8 ± 6.9	56.7 ± 3.53 ^b	93.6 ± 7.2	62.5 ± 3.9 ^d
Bifenthrin	20.0 ± 5.3 ^b	96.0 ± 8.3	80.0 ± 3.06 ^c	99.2 ± 8.4	10.1 ± 3.1 ^a
Thiacloprid	4.0 ± 5.3 ^a	91.2 ± 8.7	96.0 ± 3.51 ^d	96.8 ± 4.9	32.8 ± 6.8 ^b
Thiamethoxam	8.6 ± 6.4 ^a	96.8 ± 2.8	91.3 ± 3.71 ^d	99.2 ± 7.7	13.1 ± 1.4 ^a
Thiamethoxam+ λ-cyhalothrin	8.7 ± 6.4 ^a	94.4 ± 8.4	91.3 ± 3.6 ^d	94.4 ± 1.4	35.9 ± 2.4 ^b ^c
Propargite	6.0 ± 2.0 ^a	93.6 ± 6.3	94.0 ± 1.15 ^d	96.8 ± 3.7	11.3 ± 1.1 ^a
Hexythiazox	8.0 ± 6.0 ^a	91.2 ± 4.8	92.0 ± 3.46 ^d	96.0 ± 7.2	9.5 ± 3.5 ^a
Fenpyroximate	2.7 ± 3.1 ^a	92.0 ± 9.6	97.3 ± 1.76 ^d	95.2 ± 2.8	8.2 ± 2.1 ^a
Spiromesifen	42.7 ± 5.0 ^c	98.4 ± 2.4	58.3 ± 2.91 ^b	96.8 ± 3.7	42.7 ± 7.7 ^c
Untreated control	2.7 ± 3.1 ^a	93.6 ± 6.3	97.3 ± 1.76 ^d	92.8 ± 2.8	9.6 ± 3.3 ^a

¹Mean ovicidal percent at the end of the incubation period; ²Mean larval hatching percent from the treated eggs; ³larval period of 1st instar; ⁴Mean mortality at the end of the 1st instar; Mean±SD (n=3) followed by the same alphabet in the vertical column are not significantly different at 5% level ($p < 0.05$) as per DMRT.

Table 2. Toxic effects of commonly used pesticides on 1st instar larvae of *C. z. sillemi*

Treatments	Mortality %				Developmental duration ¹ (in hours)
	24 HAT	48 HAT	72 HAT	96 HAT	
Quinalphos	100 ± 0 ^j	100 ± 0 ⁱ	100 ± 0 ^j	100 ± 0 ^h	NA
Deltamethrin	100 ± 0 ^j	100 ± 0 ⁱ	100 ± 0 ^j	100 ± 0 ^h	NA
Bifenthrin	8.8 ± 0.87 ^e	11.5 ± 1.05 ^d	16.6 ± 0.78 ^e	24.7 ± 0.78 ^d	104.5 ± 0.96 ^{bc}
Thiacloprid	38.7 ± 0.66 ^h	42.5 ± 0.66 ^g	44.4 ± 0.90 ^h	54.7 ± 0.81 ^f	107.3 ± 0.90 ^d
Thiamethoxam	16.7 ± 0.06 ^f	20.6 ± 1.11 ^e	21.9 ± 1.62 ^f	24.4 ± 1.01 ^d	105.4 ± 0.85 ^c
Thiamethoxam+ λ-cyhalothrin	55.9 ± 0.5 ⁱ	60.1 ± 1.48 ^h	63.8 ± 1.48 ⁱ	70.0 ± 0.40 ^g	109.8 ± 0.35 ^e
Propargite	5.2 ± 0.32 ^d	7.6 ± 0.40 ^c	9.0 ± 0.44 ^d	9.9 ± 0.55 ^c	103.7 ± 0.44 ^{ab}
Hexythiazox	3.3 ± 0.38 ^c	5.2 ± 0.32 ^b	7.6 ± 0.35 ^c	8.5 ± 0.45 ^b	103.2 ± 0.96 ^a
Fenpyroximate	2.4 ± 0.40 ^b	4.1 ± 0.15 ^b	5.5 ± 0.21 ^b	7.2 ± 0.40 ^b	102.9 ± 0.38 ^a
Spiromesifen	32.3 ± 0.79 ^g	36.6 ± 0.92 ^f	42.7 ± 1.00 ^g	51.4 ± 0.96 ^e	104.8 ± 0.30 ^{bc}
Untreated control	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	104.1 ± 0.50 ^{ab}

¹Developmental duration of 1st instar larvae; Mean ±SD (n=3) followed by the same letter in the column is not significant at the 5% level ($p < 0.05$) as per DMRT; HAT = Hours after treatment; NA = Not Applicable.

thiamethoxam + lambda-cyhalothrin and thiacloprid (61.7 and 29.6%, respectively). However, insecticides including propargite, hexythiazox, fenpyroximate and bifenthrin were found to be less toxic to the 1st, 2nd and 3rd instars of *C. z. sillemi* (Table 4).

Pesticide toxicity on pupae of *C. z. sillemi*: The evaluated pesticides were found to be non-harmful to the pupae of *C. z. sillemi*. Among the pesticides, quinalphos alone caused a maximum of 5 per cent of pupicidal activity, which is not a promising result, and other pesticides also showed nil mortality on pupae of *C. z. sillemi*.

Pesticide toxicity on adults of *C. z. sillemi*: In adults, the pesticides quinalphos and deltamethrin exhibited 100 per cent mortality after 24 hours of application. This was followed by thiamethoxam + lambda-cyhalothrin and thiacloprid, which showed 92.0 and 60 per cent mortality, respectively, after 96 hours of application. Other treatments such as thiamethoxam and fenpyroximate, also showed

approximately 30.6 and 26.6 per cent mortality, respectively after 96 hours of applications. However, pesticides like bifenthrin, propargite, hexythiazox and spiromesifen were less toxic to the adults (Table 5).

Sublethal effects on the reproductive parameters of pesticide-treated adults were assessed. No adults survived after the application of quinalphos and deltamethrin, so both treatments were removed from the biological parameters study. The pre-oviposition and post-oviposition periods of treated females were similar to those of untreated females. Male and female longevity ranged from 23 to 36 days and 31 to 46 days, respectively (Table 6). The shortest male and female longevity was observed in thiacloprid and spiromesifen, with 23.6 and 31.3 days, respectively. The pesticides evaluated had a significant impact on male longevity. Similarly, spiromesifen, thiamethoxam + λ -cyhalothrin, thiacloprid, and propargite-treated females showed lower longevity compared to others. Fecundity among pesticide-treated females ranged from 268.3

Table 3. Toxicity of commonly used pesticides against the 2nd instar larvae of *C. z. sillemi*

Treatments	Mortality %				Developmental duration ¹ (in hours)
	24 HAT	48 HAT	72 HAT	96 HAT	
Quinalphos	100 $\pm$ 0 ^h	100 $\pm$ 0 ⁱ	100 $\pm$ 0 ⁱ	100 $\pm$ 0 ^h	NA
Deltamethrin	100 $\pm$ 0 ^h	100 $\pm$ 0 ⁱ	100 $\pm$ 0 ⁱ	100 $\pm$ 0 ^h	NA
Bifenthrin	3.2 $\pm$ 0.26 ^c	6.4 $\pm$ 0.40 ^d	8.3 $\pm$ 0.26 ^d	10.9 $\pm$ 0.40 ^c	112.8 $\pm$ 0.30 ^b
Thiacloprid	25.6 $\pm$ 1.00 ^f	31.1 $\pm$ 0.55 ^g	33.4 $\pm$ 0.66 ^g	41.0 $\pm$ 0.49 ^f	114.7 $\pm$ 0.46 ^c
Thiamethoxam	8.1 $\pm$ 0.35 ^d	11.0 $\pm$ 0.36 ^e	12.7 $\pm$ 0.67 ^e	16.1 $\pm$ 1.18 ^d	111.9 $\pm$ 0.65 ^{ab}
Thiamethoxam+ λ -cyhalothrin	50.9 $\pm$ 0.64 ^g	55.6 $\pm$ 0.92 ^h	63.2 $\pm$ 0.44 ^h	66.5 $\pm$ 1.02 ^g	117.5 $\pm$ 0.52 ^e
Propargite	2.8 $\pm$ 0.67 ^c	7.1 $\pm$ 0.98 ^d	8.2 $\pm$ 0.51 ^d	9.9 $\pm$ 0.21 ^c	112.7 $\pm$ 0.64 ^b
Hexythiazox	1.3 $\pm$ 0.31 ^b	3.3 $\pm$ 0.31 ^b	5.8 $\pm$ 0.56 ^b	8.7 $\pm$ 0.25 ^b	111.2 $\pm$ 0.45 ^a
Fenpyroximate	2.2 $\pm$ 0.15 ^c	5.5 $\pm$ 0.32 ^c	7.2 $\pm$ 0.26 ^c	7.8 $\pm$ 0.40 ^b	112.1 $\pm$ 0.49 ^{ab}
Spiromesifen	22.4 $\pm$ 1.11 ^e	26.2 $\pm$ 0.57 ^f	31.0 $\pm$ 0.42 ^f	34.5 $\pm$ 1.10 ^e	115.9 $\pm$ 0.67 ^d
Untreated control	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	114.0 $\pm$ 0.50 ^c

¹Developmental duration of 2nd instar larvae; Mean $\pm$ SD ($n=3$) followed by the same letter in the column is not significant at the 5% level ($p<0.05$) as per DMRT; HAT = Hours after treatment; NA = Not Applicable.

Table 4. Toxicity of commonly used pesticides against the third instar larvae of *C. z. sillemi*

Treatments	Mortality %				Developmental duration ¹ (in hours)
	24 HAT	48 HAT	72 HAT	96 HAT	
Quinalphos	85.8 ± 0.46 ^d	100 ± 0 ^g	100 ± 0 ⁱ	100 ± 0 ^g	NA
Deltamethrin	86.6 ± 0.90 ^d	95.8 ± 3.82 ^f	100 ± 0 ⁱ	100 ± 0 ^g	NA
Bifenthrin	2.3 ± 0.31 ^a	3.3 ± 0.21 ^b	5.3 ± 0.15 ^e	8.1 ± 0.4 ^c	118.4 ± 1.04 ^{ns}
Thiacloprid	13.2 ± 0.4 ^b	15.4 ± 1.11 ^d	22.3 ± 0.92 ^g	29.6 ± 0.05 ^e	120.8 ± 0.99 ^{ns}
Thiamethoxam	0.6 ± 0.1 ^a	2.2 ± 0.49 ^{ab}	3.8 ± 0.36 ^d	6.8 ± 0.3 ^{bc}	118.4 ± 0.66 ^{ns}
Thiamethoxam + λ-cyhalothrin	43.0 ± 0.55 ^c	46.9 ± 0.53 ^e	55.4 ± 0.91 ^h	61.7 ± 0.77 ^f	117.6 ± 1.21 ^{ns}
Propargite	1.1 ± 0.15 ^a	1.4 ± 0.38 ^{ab}	2.9 ± 0.61 ^{cd}	5.8 ± 0.62 ^{bc}	116.8 ± 0.49 ^{ns}
Hexythiazox	1.0 ± 0.25 ^a	1.7 ± 0.1 ^{ab}	2.5 ± 0.36 ^c	3.2 ± 0.57 ^{ab}	117.6 ± 0.69 ^{ns}
Fenpyroximate	0 ± 0 ^a	0.5 ± 0.06 ^b	1.5 ± 0.1 ^b	4.2 ± 0.29 ^{abc}	119.2 ± 0.57 ^{ns}
Spiromesifen	8.5 ± 0.45 ^b	9.9 ± 0.61 ^c	12.0 ± 1.04 ^f	14.2 ± 0.71 ^d	117.6 ± 0.72 ^{ns}
Untreated control	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	0 ± 0 ^a	117.6 ± 1.48 ^{ns}

¹Developmental duration of third instar larvae; Mean ± SD ($n=3$) followed by the same letter in the column is not significant at the 5% level ($p<0.05$) as per DMRT; HAT = Hours after treatment; NA = Not Applicable; ns = Not significant.

to 377.6. The maximum and minimum fecundity were observed in untreated females and thiacloprid-treated females, respectively. The treatments with hexythiazox, bifenthrin, thiamethoxam, and fenpyroximate-treated females showed fecundity rates similar to untreated females. In terms of fertility, untreated females and thiamethoxam-treated females had the highest fertility percentages at 90.9 and 83.7, respectively (Table 6). The lowest fertility percentage was observed in thiamethoxam + λ-cyhalothrin, spiromesifen, thiacloprid, and propargite-treated females. The adult emergence percentage of thiamethoxam and fenpyroximate-treated females was comparable to untreated females, while in other treatments, the adult emergence percentage was similar across the board.

Pesticides from various chemical groups and modes of action were tested for their toxic effects on *C. z. sillemi*. Among the evaluated pesticides, quinalphos, deltamethrin, spiromesifen, thiacloprid, and thiamethoxam + λ-cyhalothrin were found to

be extremely harmful to third instar larvae, adults, and eggs of *C. z. sillemi*, but harmless to the pupal stage. The same product used at the same dosage showed a range of effects from harmless to harmful, depending on the developmental stages of the natural enemy (Souza *et al.*, 2014). Certain synthetic pesticides cause acute toxicity to various life stages of Chrysopids. In addition to mortality, they can have sublethal physiological effects such as inhibition of development, decreased adult longevity, low immunity, reduced fecundity and fertility, and behavioural effects on mobility and predation rates. Similar observations have been reported in various Chrysopids, including *C. externa* (Desneux *et al.*, 2007), *C. johnsoni* (Amarasekare and Shearer, 2013), *C. carnea* (Garzon *et al.*, 2015), *Ceraeochrysa cubana* (Rugno *et al.*, 2018) and *C. sinica* (Shan *et al.*, 2020).

In the present study, quinalphos was found to be more toxic against larvae and adults of *C. z. sillemi*, with moderate toxicity observed on eggs. Chakraborty *et al.* (2011) reported that quinalphos

caused 89 per cent mortality in eggs and 70 per cent mortality in larvae of *C. z. sillemi*. Similar findings were reported by Darwish and Farghal (1990) and Srinivasan and Babu (2000) in *C. carnea*. Additionally, the eggs, larvae, and adults of *C. z. sillemi* were highly susceptible to deltamethrin and thiamethoxam + λ -cyhalothrin, which are synthetic pyrethroids known for their insecticidal properties. The residual toxicity of deltamethrin was also observed in *C. carnea* by Bigler and Waldburger (1994) and Giolo *et al.* (2009). Larval stages of predators may be more susceptible to pesticides than adults due to their contact with treated surface, as noted by Medina *et al.* 2004. Rugno *et al.* (2018) found that thiamethoxam + λ -cyhalothrin significantly reduced larval survival rates in *Ceraeochrysa cubana* without affecting the total developmental time of surviving larvae.

Among the neonicotinoids tested, thiamethoxam was mildly harmful to adults but harmless to eggs, larvae, and pupae of *C. z. sillemi*. Rugno *et al.*

(2018) reported extreme lethal and sublethal toxicity of thiamethoxam in *C. cubana*, highlighting potential side effects on lacewings that feed on floral nectar. Spiromesifen was found to be extremely harmful to all stages of *C. z. sillemi* except the pupal stage. Limited studies exist on the toxic effects of spiromesifen on green lacewings, but it has been shown to have lethal and sub-lethal effects on other natural enemies, such as *Neoseiulus californicus* (Mollaloo *et al.*, 2016), *Eretmocerus mundus*, *Orius laevigatus* (Bielza *et al.*, 2009), *Serangium parcesetosum*, and *Oxyopes javanus* (Nazli and Butt, 2020).

Propargite, hexythiazox, and fenpyroximate did not show acute lethal effects on *C. z. sillemi*, with propargite exhibiting sub-lethal toxicity in adults, propargite treated females showed low fertility (67.8%) and low adult emergence (69.9%). Rezaei *et al.* (2006) found that propargite was slightly harmful (Class 2) to *C. carnea* according to the International Organization for Biological Control (IOBC) system, reducing fecundity and survival

Table 5. Acute toxicity of commonly used pesticides on adults of *C. z. sillemi*

Chemical	Adult mortality %			
	24 HAT	48 HAT	72 HAT	96 HAT
Quinalphos	100 $\pm$ 0 ^e	100 $\pm$ 0 ^g	100 $\pm$ 0 ^g	100 $\pm$ 0 ⁱ
Deltamethrin	100 $\pm$ 0 ^e	100 $\pm$ 0 ^g	100 $\pm$ 0 ^g	100 $\pm$ 0 ⁱ
Bifenthrin	4 $\pm$ 4 ^a	8 $\pm$ 4 ^{bc}	8 $\pm$ 4 ^c	9.33 $\pm$ 2.31 ^c
Thiacloprid	41.33 $\pm$ 6.11 ^c	45.33 $\pm$ 4.62 ^e	52 $\pm$ 4 ^e	60 $\pm$ 4 ^g
Thiamethoxam	16 $\pm$ 4 ^b	20 $\pm$ 4 ^d	24 $\pm$ 4 ^d	30.67 $\pm$ 2.31 ^f
Thiamethoxam+ λ -cyhalothrin	80 $\pm$ 8 ^d	84 $\pm$ 4 ^f	90.67 $\pm$ 4.62 ^f	92 $\pm$ 4 ^h
Propargite	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	1.33 $\pm$ 2.31 ^{ab}	4 $\pm$ 0 ^b
Hexythiazox	4 $\pm$ 4 ^a	5.33 $\pm$ 2.3 ^{ab}	6.67 $\pm$ 2.31 ^{bc}	9.33 $\pm$ 0 ^c
Fenpyroximate	12 $\pm$ 4 ^b	22.67 $\pm$ 6.11 ^d	24 $\pm$ 4 ^d	26.67 $\pm$ 2.31 ^e
Spiromesifen	12 $\pm$ 4 ^b	12 $\pm$ 4 ^c	12 $\pm$ 4 ^c	16 $\pm$ 0 ^d
Untreated control	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a	0 $\pm$ 0 ^a

HAT=Hours after treatment; Mean $\pm$ SD ($n=3$) followed by the same alphabet in the vertical column are not significantly different at 5% level ($p<0.05$) as per DMRT.

Table 6. Sub lethal toxicity effects of commonly used pesticides on adults of *C. z. sillemi*

Treatments	Pre-Oviposition period	Post-Oviposition period	Male longevity	Female longevity	Fecundity	Fertility % ¹	Adult emerging % ²
Bifenthrin	3.5±0.1	2.8±0.4	36.6±2.3 ^d	42.7±3.5 ^{cd}	355.0±13.6 ^b	79.9±12.5 ^c	74.2±9.8 ^a
Thiacloprid	3.3±0.2	2.7±0.3	23.6±2.5 ^a	33.6±2.1 ^{ab}	268.3±19.0 ^a	67.5±1.4 ^{ab}	64.3±4.4 ^a
Thiamethoxam	3.7±0.2	2.9±0.2	35.0±3.6 ^{cd}	44.0±4.4 ^{cd}	358.7±27.1 ^b	83.7±2.8 ^{cd}	91.2±3.2 ^b
Thiamethoxam+ λ-cyhalothrin	3.9±0.3	2.0±0.9	30.3±5.1 ^{bc}	31.6±3.1 ^a	274.7±10.7 ^a	64.7±2.2 ^a	71.1±0.9 ^a
Propargite	3.4±0.2	2.6±0.3	36.3±2.5 ^d	39.7±3.2 ^{bc}	286.3±20.7 ^a	67.8±5.7 ^{ab}	69.9±0.3 ^a
Hexythiazox	3.3±0.3	2.8±0.3	34.0±1.0 ^{cd}	45.6±5.5 ^{cd}	353.3±25.3 ^b	79.3±4.7 ^c	74.5±9.7 ^a
Fenpyroximate	3.4±0.3	2.5±0.6	36.0±5.4 ^d	46.3±8.1 ^{cd}	360.3±21.8 ^b	78.3±6.8 ^{bc}	91.2±3.4 ^b
Spiromesifen	3.5±0.5	3.0±0.4	28.7±2.5 ^{ab}	31.3±1.5 ^a	269.3±07.6 ^a	66.6±6.4 ^a	65.4±4.3 ^a
Untreated control	3.5±0.1	2.7±0.5	44.6±3.5 ^e	49.3±3.2 ^d	377.6±17.9 ^b	90.9±4.1 ^d	90.6±3.7 ^b

¹Mean fertility percent of pesticide treated females; ²Mean adult emerging percent calculated from the fertility percent; Mean±SD (n=3) followed by the same alphabet in the vertical column are not significantly different at 5% level (p<0.05) as per DMRT.

rates. Abd El-Baset *et al.* (2023) stated that hexythiazox was harmless to 2nd instar larvae of *C. carnea*.

Pathan *et al.* (2010) observed higher intrinsic rates, fecundity, adult longevity, doubling time, and predatory potential in insecticide-resistant populations compared to laboratory-susceptible populations. Quinalphos, deltamethrin, spiromesifen, thiacloprid, and thiamethoxam + λ-cyhalothrin were found to cause acute toxicity to *C. z. sillemi*. In addition to acute toxicity, some pesticides produce sub-lethal effects that impact life table parameters. Further laboratory studies are needed to understand these sub-lethal effects. Field studies are also necessary to fully comprehend the selectivity of the tested pesticides on *C. z. sillemi*, especially those that were toxic in the laboratory.

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Evaluating wetland health using Odonata diversity and seasonal shifts in Tulshi Reservoir, Maharashtra, India

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ABSTRACT: The study assesses species composition and seasonal variation of odonates at Tulshi Reservoir in Kolhapur district, northern Western Ghats, Maharashtra. Field surveys were carried out during pre-monsoon, monsoon, and post-monsoon seasons through direct observations and photographic documentation. A total of 32 species belonging to two suborders (Anisoptera and Zygoptera) and four families (Libellulidae, Gomphidae, Platycnemididae, and Coenagrionidae) were recorded, with Libellulidae being the most dominant. Species richness peaked in the post-monsoon season, likely due to improved water quality and enhanced aquatic vegetation. Generalist taxa such as *Trithemis aurora* (Burmeister, 1839) and *Pseudagrion decorum* (Rambur, 1842) occurred throughout the year, whereas certain species, including *Amphiallagma parvum* (Selys, 1876), appeared only in specific seasons. These findings emphasize the ecological importance of Tulshi Reservoir in sustaining odonate diversity and support the use of these insects as effective indicators of wetland health.

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KEY WORDS: Freshwater ecosystem, species composition, richness, seasonal variation, Western Ghats

INTRODUCTION

Freshwater ecosystems, though covering a small fraction of the Earth's surface, support exceptionally rich biodiversity and deliver essential ecological services. Among aquatic insects, dragonflies and damselflies (Order- Odonata) are widely recognized as bioindicators because of their biphasic life cycle, habitat specificity, and sensitivity to environmental changes (Choudhary and Ahi, 2015; Kulkarni and Subramanian, 2015). Larvae respond to water quality variables such as dissolved oxygen, pH, and nutrient levels, while adults reflect riparian and

terrestrial habitat conditions (Husband, 2022). Their visibility, ecological role, and ease of identification make them effective tools for monitoring freshwater health.

Worldwide, insect communities are undergoing significant changes, with Odonata frequently highlighted in biodiversity assessments for their responsiveness to environmental pressures (Van Klink *et al.*, 2020; Jähnig *et al.*, 2021). In India, freshwater habitats are increasingly threatened by urbanization, agricultural intensification, and climate variability. The Western Ghats, a global biodiversity

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hotspot, supports diverse odonate assemblages, including many endemic species (Koparde *et al.*, 2015; Vincy *et al.*, 2016). Artificial wetlands such as reservoirs, despite being human-made, can provide important refuges and dispersal corridors for aquatic fauna in modified landscapes (Deacon *et al.*, 2019).

Seasonal changes strongly influence odonate abundance and species composition, with patterns linked to rainfall, temperature, and vegetation dynamics (Pattanayak *et al.*, 2019; Dutta Saha, 2022; Aghade and Saraf, 2023; Olambe *et al.*, 2024). However, data on reservoir habitats in Kolhapur district remain scarce. This study documents the diversity and seasonal variation of Odonata at Tulshi Reservoir, providing baseline information for conservation planning and highlighting the role of artificial wetlands in sustaining freshwater biodiversity in the Western Ghats.

MATERIALS AND METHODS

The study was conducted at the Tulshi Reservoir, located in the Kolhapur district of Maharashtra state, India, in the northern Western Ghats. The reservoir lies at approximately 16.2°N ; 74.2°E, at an approximate elevation of 700m above sea level. The study site is a man-made freshwater reservoir covering a catchment area of 34.12 km² (135 ha), surrounded by a varied landscape of farmland, semi-deciduous forest patches, and rural villages. The area experiences a tropical monsoon climate, with distinct pre-monsoon (February to May), monsoon (June to September), and post-monsoon (October to January) seasons. The reservoir receives inflow from local streams and retains water throughout the year creating a stable aquatic environment suitable for both lentic and riparian biodiversity (Fig. 1).

From January 2022 to December 2023, odonate surveys were conducted across the region's three seasons: pre-monsoon, monsoon, and post-monsoon. Sampling was carried out fortnightly during two daily intervals, from 7.30 am to 11.30 am and 4.00 pm to 6.00 pm, under favourable weather conditions. These timeframes were selected to align with peak

adult odonate activity, thereby maximizing the accuracy of species detection and identification.

Dragonflies and damselflies were identified primarily through visual observation using 10×42 binoculars and high-resolution digital photography (Nikon D5600 DSLR with a 70–300mm lens). Where required for species-level confirmation, representative specimens were captured using insect sweep nets and handled carefully to avoid damage. Captured individuals were placed briefly in insect collection tubes, examined in the field under hand lenses, and released at the point of capture.

Identification and Taxonomic Validation: Odonate species were identified using a combination of morphological keys, field guides, and regional checklists specific to South Asia and India. Primary identification references included Fraser (1931, 1933, 1934, 1936), Subramanian and Sivaramakrishnan (2007), Subramanian *et al.* (2018), Kalkman *et al.* (2020), Nair *et al.* (2021) and Subramanian and Babu (2024).

Diversity Analysis: Diversity indices, including species richness, Shannon–Wiener, Simpson's dominance, and evenness, were calculated using PAST software (version 4.03).

Dragonfly Biotic Index (DBI): The ecological quality of the study sites was assessed using the Dragonfly Biotic Index (DBI) (Clark and Samways, 1996; Samways and Steytler, 1996). Field surveys were undertaken to record dragonfly species, which were identified using standard field guides and taxonomic keys (Fraser, 1933–1936; Subramanian, 2005). Each species was assigned a DBI score ranging from 0 to 9, derived from three attributes: (i) Geographical distribution (0–3), with widespread species scoring 0–1, moderately restricted 2, and endemic or highly restricted 3; (ii) Habitat Sensitivity (0–3), with tolerant or generalist species scoring 0–1, moderately sensitive 2, and habitat specialists 3; and (iii) Threat Status (0–3), based on IUCN Red List categories, where Not Threatened = 0, Near Threatened = 1, Vulnerable = 2, and Endangered/Critically Endangered = 3. The DBI of each species was obtained by summing these values:

$DBI_{(species)} = \text{Distribution} + \text{Sensitivity} + \text{Threat Status}$

Two indices were then calculated for each site: (a) Total DBI, the sum of DBI scores for all recorded species, and (b) Mean DBI, calculated as:

$$DBI_{(mean)} = \frac{DBI_{(Total)}}{\text{Number of Species}}$$

Interpretation followed Clark and Samways (1996): low DBI values indicate habitats dominated by widespread, tolerant taxa and thus ecological degradation, while high DBI values reflect the presence of restricted, sensitive, or threatened species, signifying healthier, less disturbed ecosystems (Subramanian *et al.*, 2008).

RESULTS AND DISCUSSION

A total of 32 species of Odonata were recorded at Tulshi Reservoir, representing two suborders, Anisoptera (dragonflies) and Zygoptera (damselflies), distributed across four families: Libellulidae, Gomphidae, Platycnemididae, and Coenagrionidae. The suborder Anisoptera accounted for 17 species, with Libellulidae being the most dominant family, comprising 15 species (Fig. 3). Notable species such as *Trithemis aurora*

(Burmeister, 1839), *Trithemis kirbyi* (Selys, 1891), *Rhodothemis rufa* (Rambur, 1842), *Brachythemis contaminata* (Fabricius, 1793), and *Lathrecista asiatica* (Fabricius, 1798) were observed consistently throughout the year, indicating their adaptability to changing seasonal conditions. Several species, including *Pantala flavescens* (Fabricius, 1798), *Hydrobasileus croceus* (Brauer, 1867), *Trithemis pallidinervis* (Kirby, 1889), *Diplacodes trivialis* (Rambur, 1842), *Orthetrum sabina* (Drury, 1770), *Macrodiplax cora* (Kaup, 1867), and *Urothemis signata* (Rambur, 1842), were exclusive to the monsoon and post-monsoon seasons, suggesting a strong seasonal emergence pattern. The family Gomphidae was represented by a single species, *Ictinogomphus rapax* (Rambur, 1842), *Paragomphus lineatus* (Selys, 1850) recorded only during the monsoon and post-monsoon periods (Fig. 3).

A total of 17 species were evaluated, with DBI values ranging between 2 and 4 (Table 3). The majority of species, such as *Trithemis aurora*, *T. kirbyi*, *Diplacodes trivialis*, and *Crocothemis servilia*, scored 2, indicating their widespread distribution and generalist nature. Species including *Orthetrum pruinatum*, *Rhodothemis rufa*, *Urothemis signata*, *Macrodiplax cora*, *Tholymis*

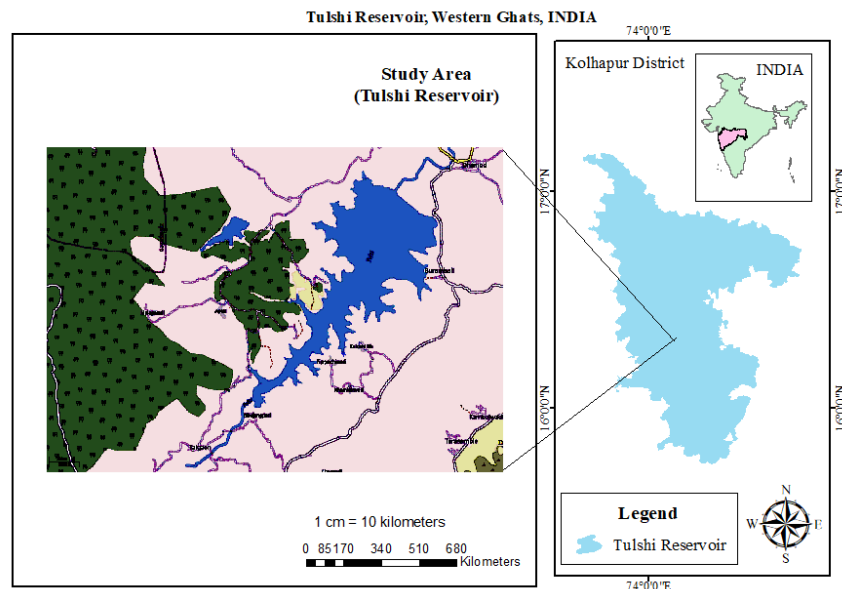


Fig. 1 Study area: Tulshi Reservoir, Maharashtra, India

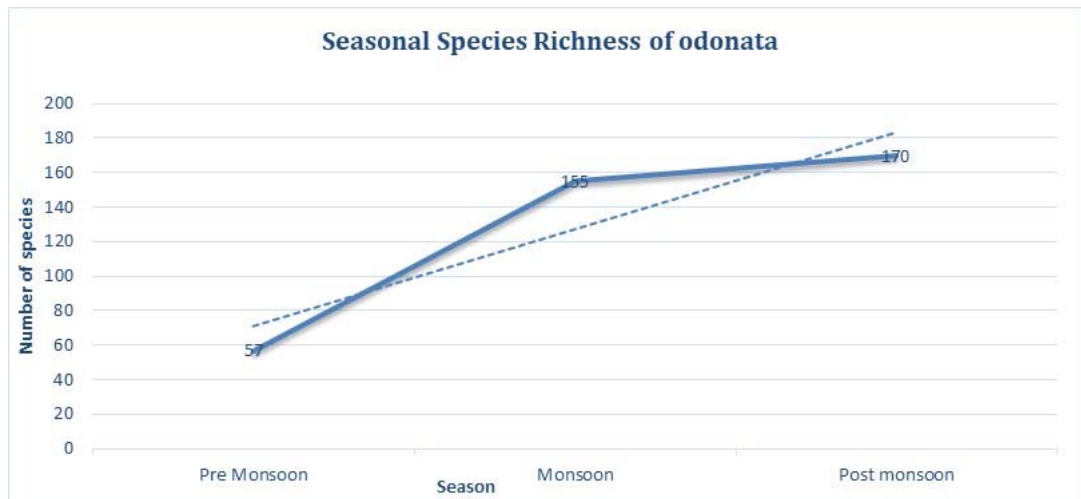


Fig. 2 Seasonal species richness of Odonata

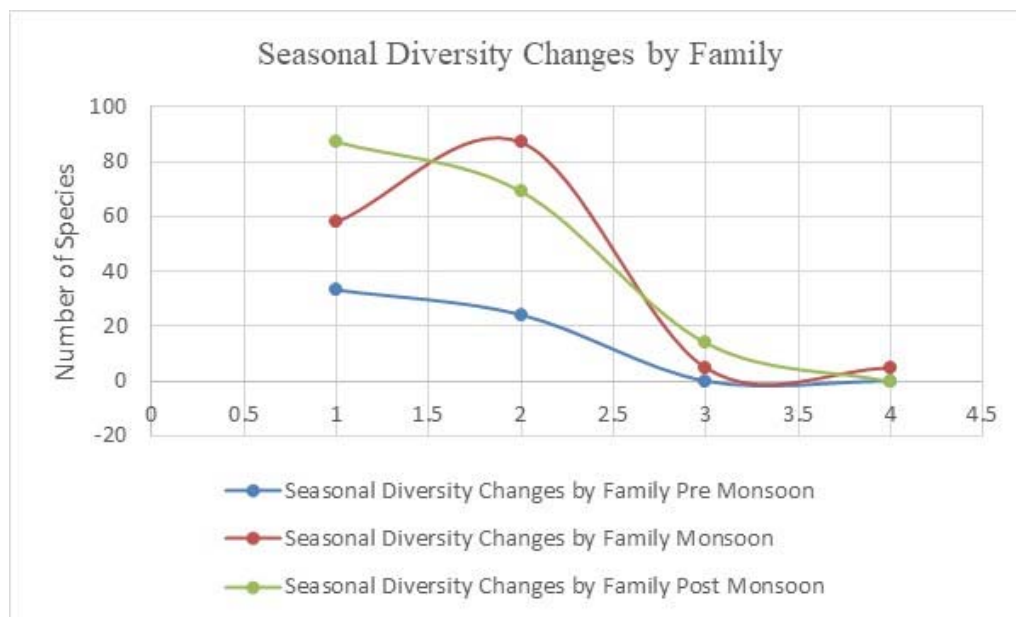


Fig. 3 Familywise seasonal diversity changes

tillarga, *Lathrecista asiatica*, and *Paragomphus lineatus* showed slightly higher DBI values (3), reflecting moderate habitat sensitivity and restricted distribution. The highest DBI score (4) was recorded for *Hydrobasileus croceus* and *Ictinogomphus rapax*, suggesting their greater ecological significance as sensitive taxa with

relatively limited distribution. The absence of threatened or endangered species kept threat scores uniformly low (0), highlighting the dominance of common and regionally distributed taxa in the assemblage. Overall, the DBI analysis indicates that the Tulshi Reservoir supports a moderately diverse Odonate community with species of both generalist

Table 1. Distribution of Odonata species at Tulshi reservoir during different periods, indicating presences (+) and absences ()

Common Name	Suborder/Family/Scientific Name	Pre	M	Post
Suborder—Anisoptera/ Family-Libellulidae				
Crimson Marsh Glider	<i>Trithemis aurora</i> (Burmeister, 1839)	+	+	+
Kirby's Drop wing	<i>T. kirbyi</i> (Selys, 1891)	+	+	+
Green Marsh Hawk	<i>Orthetrum sabina</i> (Drury, 1770)	-	+	+
Crimson-tailed Marsh Hawk	<i>O. pruinatum</i> (Burmeister, 1839)	+	+	+
Ground Skimmer	<i>Diplacodes trivialis</i> (Rambur, 1842)	-	+	+
Scarlet Skimmer	<i>Crocothemis servilia</i> (Drury, 1773)	+	+	+
Wandering Glider	<i>Pantala flavescens</i> (Fabricius, 1798)	-	+	+
Long-legged Marsh Glider	<i>T. pallidinervis</i> (Kirby, 1889)	-	+	+
Rufous Marsh Glider	<i>Rhodothemis rufa</i> (Rambur, 1842)	+	+	+
Greater Crimson Glider	<i>Urothemis signata</i> (Rambur, 1842)	-	+	+
Coastal Glider	<i>Macrodiplax cora</i> (Kaup, 1867)	-	+	+
Amber-winged Marsh Glider	<i>Hydrobasileus croceus</i> (Brauer, 1867)	-	+	+
Coral-tailed Cloudwing	<i>Tholymis tillarga</i> (Fabricius, 1798)	+	-	+
Asiatic Blood Tail	<i>Lathrecista asiatica</i> (Fabricius, 1798)	+	+	+
Ditch Jewel	<i>Brachythemis contaminata</i> (Fabricius, 1793)	+	+	+
Family-Gomphidae				
Common Clubtail	<i>Ictinogomphus rapax</i> (Rambur, 1842)	-	+	+
Lined Cocktail	<i>Paragomphus lineatus</i> (Selys, 1850)	-	+	+
Suborder—ZYGOPTERA				
Family-Coenagrionidae				
Red-tipped Dartlet	<i>Argiocnemis rubescens</i> (Selys, 1877)	-	+	+
Olive Marsh Dart	<i>Ceriagrion olivaceum</i> (Laidlaw, 1914)	-	+	+
Pygmy Dartlet	<i>Argiocnemis pygmaea</i> (Rambur, 1842)	+	-	+
Variable Dartlet	<i>Argiocnemis femina</i> (Brauer, 1868)	+	-	+
Aurora Bluetail	<i>Ischnura aurora</i> (Brauer, 1865)	-	+	+
Senegal Golden Dartlet	<i>Ischnura senegalensis</i> (Rambur, 1842)	+	+	+
Coromandel Marsh Dart	<i>Ceriagrion coromandelianum</i> (Fabricius, 1798)	+	+	+
Orange Marsh Dart	<i>Ceriagrion cerinorubellum</i> (Brauer, 1865)	+	+	+
Saffron-faced Blue Dart	<i>Pseudagrion rubriceps</i> (Selys, 1876)	+	+	+
Blue Grass Dart	<i>Pseudagrion microcephalum</i> (Rambur, 1842)	+	+	+
Azure Dartlet	<i>Amphiagrion parvum</i> (Selys, 1876)	-	+	+
Abor Marsh Dart	<i>Mortonagrion aborense</i> (Laidlaw, 1914)	-	+	+
Three-lined Dartlet	<i>Pseudagrion decorum</i> (Rambur, 1842)	+	+	+
Western Golden Dart	<i>Ichnura rubilio</i> (Selys, 1876)	-	+	-
Family: Platycnemididae				
Blue Bush Dart	<i>Copera vittata</i> (Selys, 1863)	-	+	-

Pre - Pre-monsoon; M - Monsoon; Post - Post-monsoon

and sensitive ecological categories.

The suborder Zygoptera included 15 species, belonging to the Coenagrionidae and Platycnemididae families (Fig. 3). This group showed a marked increase in species richness during the monsoon and post-monsoon season (Fig. 2). Species such as *Pseudagrion decorum* (Rambur, 1842), *Pseudagrion rubriceps* (Selys, 1876), *Ceriagrion cerinorubellum* (Brauer, 1865), *Pseudagrion decorum* (Rambur, 1842), and *Ischnura senegalensis* (Rambur, 1842) were present in all three seasons, indicating wide ecological tolerance (Table 1). In contrast, species like *Amphiallagma parvum* (Selys, 1876), *Mortonagrion aborens* (Laidlaw, 1914), *Ischnura aurora* (Brauer, 1865), and *Ceriagrion olivaceum* (Laidlaw, 1914) were recorded in the monsoon and post-monsoon periods, possibly due to favorable habitat conditions such as increased aquatic vegetation and stable water levels. Additionally, *Ichnura rubilio* (Brauer, 1865) and *Copera vittata* (Selys, 1863) species were exclusively observed during monsoon season (Table 1).

The post-monsoon season showed the highest odonate diversity, indicating rich ecological conditions at the reservoir (Fig. 2). Stable water levels and dense aquatic vegetation likely supported adult emergence and activity. The monsoon season was also important, offering suitable conditions for breeding and larval development. High humidity,

rainfall, and flooded habitats favoured early life stages. These findings highlight the role of odonates as reliable indicators of wetland health (Table 1).

The analysis of seasonal variation in odonate diversity at Tulshi Reservoir demonstrated clear differences among the three sampling periods (Table 2). The number of individuals increased steadily from 57 in the pre-monsoon to 155 during the monsoon and reached a maximum of 170 in the post-monsoon. Indices of species richness showed notable variation, with Margalef's index rising from 4.223 in pre-monsoon to 5.578 in monsoon, followed by a slight decline in post-monsoon (5.147). Menhinick's index followed a decreasing trend (1.967–1.681), reflecting the effect of higher abundance on richness values. Diversity indices such as Shannon–Wiener (H_2') and Brillouin indicated a gradual increase from pre-monsoon (2.143 and 1.896, respectively) to post-monsoon (2.367 and 2.211), suggesting enhanced heterogeneity in species composition with seasonal progression. Simpson's index (1–D) remained relatively stable across seasons (0.735–0.7408), pointing to a balanced community structure throughout. Dominance (D) showed a slight reduction from 0.265 to 0.2592, indicating lower single-species dominance in later seasons. Evenness (e^H/S) was highest in the pre-monsoon (0.406), declined during the monsoon (0.3207), and marginally improved in the post-monsoon (0.3439), showing that individuals were more evenly distributed before the rains. These results highlight that monsoon conditions favoured species richness and abundance, while pre-monsoon conditions supported more equitable distribution among species.

The present study documented 32 odonate species from Tulshi Reservoir, encompassing two suborders and four families, with marked variation across seasons. The overall patterns demonstrate the influence of hydrological fluctuations and habitat heterogeneity on community composition, trends that are consistent with observations from other parts of the Western Ghats and peninsular India (Gaikwad, 2021; Nair *et al.*, 2021; Vijayakumaran *et al.*, 2025).

Table 2. Seasonal variation in abundance and diversity indices of odonates assemblages at Tulshi Reservoir, Western Ghats, India

No.	Diversity indices	Pre-monsoon	Monsoon	Post-monsoon
1.	Individuals	57	155	170
2.	Dominance_D	0.265	0.2599	0.2592
3.	Margalef	4.223	5.578	5.147
4.	Simpson_1-D	0.735	0.7401	0.7408
5.	Evenness_ e^H/S	0.406	0.3207	0.3439
6.	Menhinick	1.967	1.874	1.681
7.	Brillouin	1.896	2.189	2.211
8.	Shannon_H	2.143	2.359	2.367

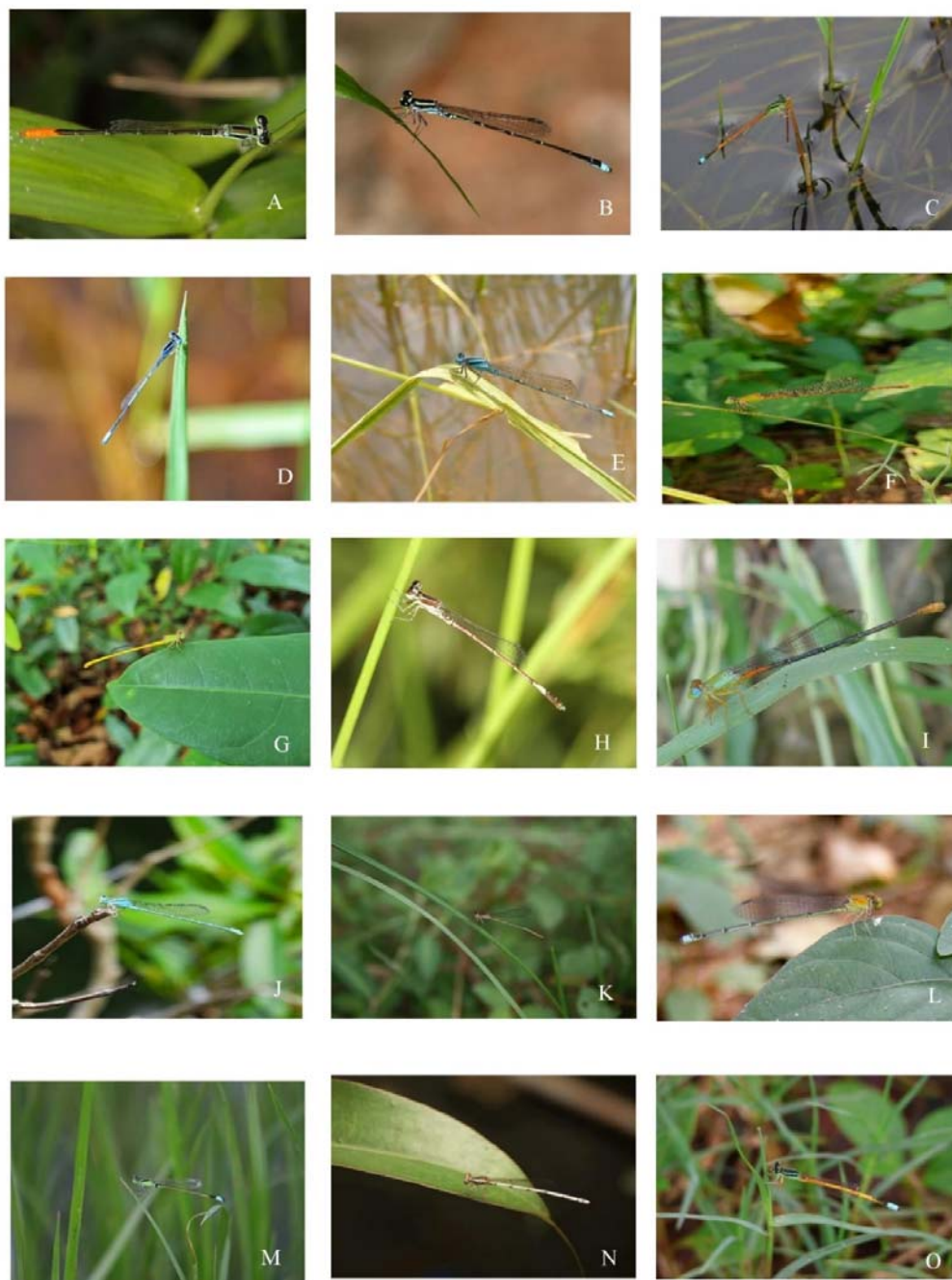


Fig. 4 A to O: Representative damselfly species (Zygoptera) recorded at Tulshi Reservoir, Western Ghats, India. A- *Agriocnemis femina*; B- *Argiocnemis rubescens*; C- *Ischnura aurora*; D- *Amphiallagma parvum*; E- *Pseudagrion microcephalum*; F- *Ceriagrion olivaceum*; G- *Ceriagrion coromandelianum*; H- *Mortonagrion aborens*; I- *Ceriagrion cerinorubellum*; J- *Pseudagrion decorum*; K- *Agriocnemis pygmaea*; L- *Pseudagrion rubriceps*; M- *Ischnura senegalensis*; N- *Copea vittata*; O- *Ichnura rubilio*

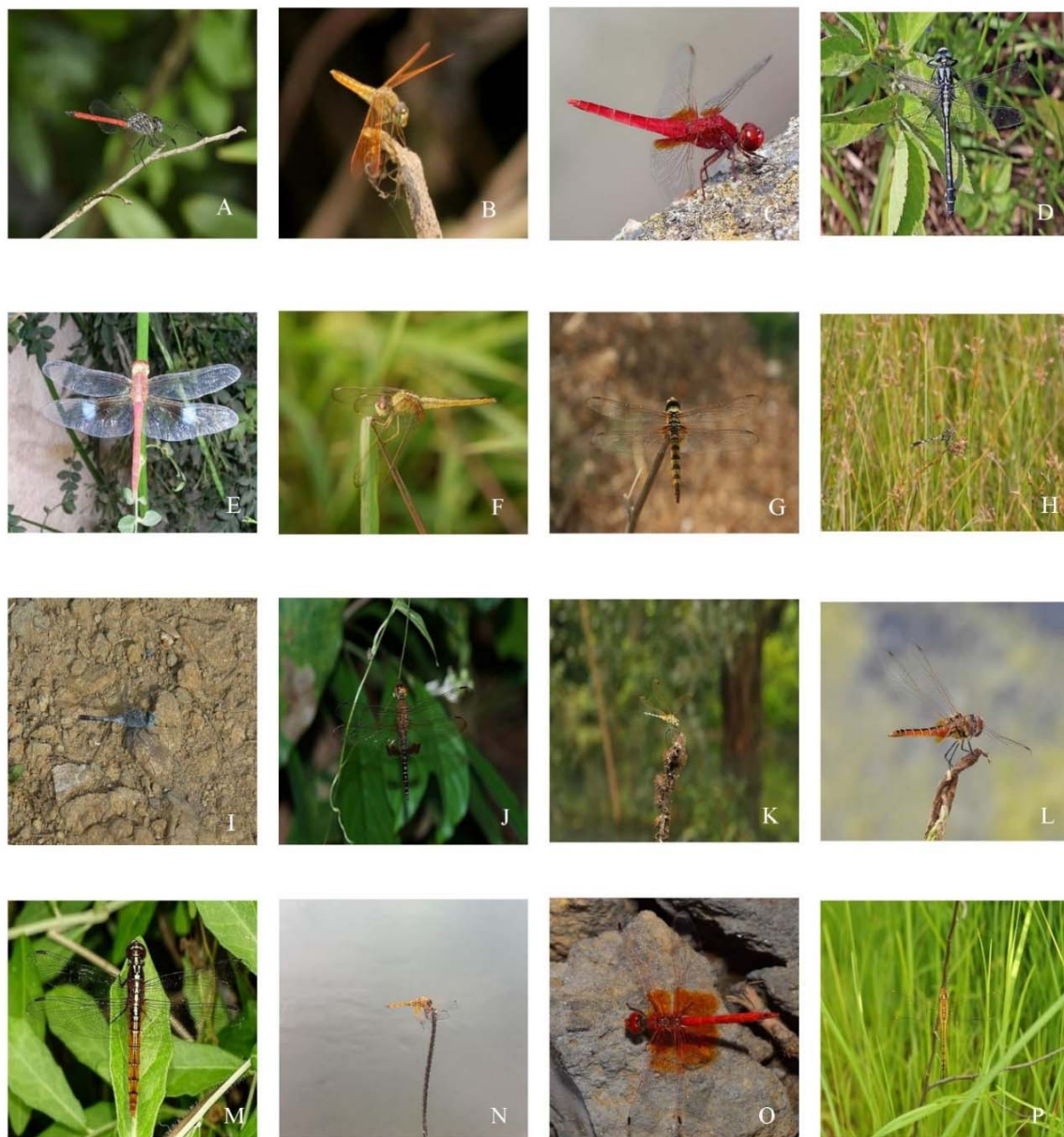


Fig. 5 A to P: Representative Dragonfly species (*Anisoptera*) recorded at Tulshi Reservoir, Western Ghats, India. A- *Lathrecista asiatica*; B- *Brachythemis contaminata*; C- *Crocothemis servilia*; D- *Ictinogomphus rapax*; E- *Tholymis tillarga*; F- *Orthetrum pruinatum*; G- *Urothemis signata*; H- *Orthetrum sabina*; I- *Diplacodes trivialis*; J- *Hydrobasileus croceus*; K- *Trithemis pallidinervis*; L- *Macrodiplax cora*; M- *Rhodothemis rufa*; N- *Trithemis aurora*; O- *Trithemis kirbyi*; P- *Pantala flavescens*

Table 3. Dragonfly Biotic Index (DBI) scores of the species recorded from the Tulshi Reservoir, Maharashtra, India

Name of the species	S	G	T	DBI
<i>Trithemis aurora</i> (Burmeister, 1839)	1	1	0	2
<i>T. kirbyi</i> (Selys, 1891)	1	1	0	2
<i>T. pallidinervis</i> (Kirby, 1889)	1	1	0	2
<i>Orthetrum sabina</i> (Drury, 1770)	0	2	0	2
<i>O. pruinsum</i> (Burmeister, 1839)	1	2	0	3
<i>Diplacodes trivialis</i> (Rambur, 1842)	0	2	0	2
<i>Crocothemis servilia</i> (Drury, 1773)	0	2	0	2
<i>Pantala flavescens</i> (Fabricius, 1798)	0	3	0	3
<i>Rhodothemis rufa</i> (Rambur, 1842)	2	1	0	3
<i>Urothemis signata</i> (Rambur, 1842)	2	1	0	3
<i>Macrodiplax cora</i> (Kaup, 1867)	1	2	0	3
<i>Hydrobasileus croceus</i> (Brauer, 1867)	2	2	0	4
<i>Tholymis tillarga</i> (Fabricius, 1798)	1	2	0	3
<i>Lathrecista asiatica</i> (Fabricius, 1798)	1	2	0	3
<i>Brachythemis contaminata</i> (Fab, 1793)	0	2	0	2
<i>Ictinogomphus rapax</i> (Rambur, 1842)	2	2	0	4
<i>Paragomphus lineatus</i> (Selys, 1850)	2	1	0	3

S - Habitat Sensitivity; G - Geographical; T. Threat

Within Anisoptera, the family Libellulidae was dominant, contributing 15 species. The prevalence of Libellulidae has also been reported in Kerala (Nair *et al.*, 2021), the Bavali River catchments (Vijayakumaran *et al.*, 2025), and Thattekkad wetlands (Varghese *et al.*, 2014), where their ecological plasticity allows them to thrive under fluctuating hydrological regimes. Widespread species such as *Trithemis aurora* (Burmeister, 1839), *Brachythemis contaminata* (Fabricius, 1793), and *Orthetrum sabina* (Drury, 1770) were observed in all three seasons, reflecting their tolerance to changing conditions, a pattern also highlighted in Kolhapur wetlands by Gaikwad (2021). Conversely, post-monsoon exclusives like *Pantala flavescens* (Fabricius, 1798) and *Macrodiplax cora* (Kaup, 1867) indicate synchronization with seasonal emergence windows, echoing findings from high-altitude habitats of Munnar, where post-monsoon stability facilitated

odonate diversity (Krishnanunni *et al.*, 2024).

The Zygoptera, represented by Coenagrionidae and Platycnemididae, showed high richness during post-monsoon. This agrees with studies from Kerala and Tamil Nadu, which documented zygopteran proliferation in habitats with abundant macrophytes and stable water levels (Varghese *et al.*, 2014; Kannagi *et al.*, 2016; Nair *et al.*, 2021). At Tulshi, *Pseudagrion decorum* (Rambur, 1842) and *Ceriagrion cerinorubellum* (Brauer, 1865) occurred consistently across seasons, suggesting broad ecological tolerance, while species such as *Amphiallagma parvum* (Selys, 1876) and *Mortonagrion aborensense* (Laidlaw, 1914) were restricted to post-monsoon, likely benefiting from dense aquatic vegetation. Similar habitat-linked associations were reported by Pawar *et al.* (2024) in organic paddy fields of Satara, where vegetation complexity promoted greater odonate diversity.

The seasonal variation in diversity indices further reinforces these ecological trends. The Shannon–Wiener and Brillouin indices showed gradual increases from pre-monsoon to post-monsoon, reflecting growing heterogeneity, while Simpson’s index remained stable, indicating structural consistency in the community. These trends are comparable to those observed by Pawar *et al.* (2024), who noted stable diversity but fluctuating evenness across cropping cycles, and by Patil and Chougale (2025) and Kolhapur and Gaikwad (2021) in Rankala Lake, where richness was strongly linked to rainfall. The decline in evenness during monsoon at Tulshi suggests dominance by a few abundant species, a phenomenon also observed in deciduous forests of Tamil Nadu (Kannagi *et al.*, 2016). In contrast, the relatively high evenness during pre-monsoon resembles patterns from Sangli, where low water levels constrained species richness but supported more equitable distribution (More, 2024).

Globally, shifts in family ratios, particularly the resilience of Libellulidae compared to other groups, have been associated with environmental change and habitat disturbance (Šigutová *et al.*, 2024). The findings at Tulshi, where libellulidae dominate and

zygopterans exhibit seasonal restriction, align with these broader trends, underscoring the sensitivity of odonate assemblages to both local hydrological cycles and wider climatic influences. The findings from Tulshi Reservoir underline the importance of seasonal hydrological cycles and habitat heterogeneity in maintaining odonate diversity. The dominance of adaptable Libellulidae alongside the seasonal exclusivity of several Zygoptera reflects both resilience and sensitivity within the community. Such patterns not only confirm the role of odonates as bioindicators of freshwater ecosystem health but also stress the need for habitat management that conserves aquatic vegetation, water quality, and seasonal flow regimes. Similar concerns have been highlighted in studies from Kerala, Satara, and the Bavali River catchments, where odonate assemblages responded strongly to environmental changes (Nair *et al.*, 2021; Pawar *et al.*, 2024; Vijayakumaran *et al.*, 2025). In the context of increasing anthropogenic pressures and climate variability in the Western Ghats, protecting reservoirs like Tulshi is critical for sustaining both odonate diversity and the ecological services they support, including mosquito regulation and food-web stability. Long-term monitoring and community-based conservation programs will be essential to ensure the persistence of these keystone insect groups.

A total of 32 odonate species were documented from Tulshi Reservoir, showing distinct seasonal differences in richness, abundance, and community structure. Libellulidae dominated the assemblage, while Zygoptera showed stronger seasonal variation. Species richness peaked during monsoon and post-monsoon, whereas evenness was greater in the pre-monsoon period. These findings confirm the value of odonates as sensitive indicators of freshwater ecosystems and emphasize the need for habitat conservation and long-term monitoring in the Western Ghats.

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Rediscovery of *Eristalinus (Merodonoides) multifarius* (Walker, 1852) (Diptera, Syrphidae) from Kerala, India and its molecular phylogeny

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ABSTRACT: The hoverfly species *Eristalinus (Merodonoides) multifarius* (Walker, 1852) is rediscovered from Kerala after 45 years. The total number of species of hoverflies in Kerala has been updated to 60, based on the previously omitted first record of the species. Male of the species have been redescribed along with illustrations of male genitalia. The species have been molecular characterised for the mtCO1 gene for the first time. Phylogenetic analysis of the members of the tribe *Eristalini* showed close affinity of *E. (Merodonoides) multifarius* with other members of *Eristalinus* Rondani followed by *Mallota* Meigen. © 2025 Association for Advancement of Entomology

KEY WORDS: Hoverfly species, redescription, male genitalia, molecular characterisation, mtCO1, phylogenetic analysis, *Eristalini*

INTRODUCTION

The hoverflies (Diptera, Syrphidae) are bristle-less, small to medium-sized vividly coloured flies. The primary characteristic is the presence of spurious or false vein between the third and fourth veins of the wings. Many of them mimic bees and wasps. Syrphids play a crucial role in pollination and serve as an important pollinator of many plants (Larson *et al.*, 2001). In India, about 366 species of syrphids

in 77 genera under 13 tribes and four subfamilies have been recorded (Sengupta *et al.*, 2024). In Kerala, 59 species of syrphids were reported (Sankar *et al.*, 2024). The tribe *Eristalini* is represented by 12 species of four genera in Kerala (Sankar *et al.*, 2024). Many species under the tribe *Eristalini* are gaining importance as an effective pollination agent especially in polyhouses due to the ease of mass multiplication, owing to their saprophytic character (Hodgkiss, 2019; Dunn *et al.*,

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2020). There is only very less information on the tribe Eristalini from Kerala. This study reports the rediscovery of an Eristalini from Kerala along with its redescription. The phylogenetic analysis to find the genetic relationship of the species with other members of the tribe is also presented.

MATERIALS AND METHODS

Surveys were conducted in various localities of Thenmala, Kollam district (9°00'25.2"N; 76°59'04.9"E) and Rajakkad, Idukki district (9°58'01.0"N; 77°05'38.2"E) in Kerala; which included open grass land, semievergreen forest patches, and roadside vegetation. The collections were carried out using aerial nets and preserved the specimens in 99 per cent ethanol. Collected flies were later relaxed overnight using a mixture of ethyl alcohol, ethyl acetate and distilled water (1:1:1) and were pinned with entomological pins (No. 1) and dry preserved. The taxonomic identification of specimens and redescription followed Brunetti (1923). The photographs of dorsal, lateral, and ventral habitus, facial profile and male genitalia were captured using Leica M80 stereozoom microscope mounted with Leica MC 170 HD camera. Multiple images of each part of the specimens were taken and combined to a stacked image using Combine ZM software. The stacked images were touched/ digitally optimized using Adobe Photoshop CS6. Terminology used in redescription follows Brunetti (1923).

Preparation of male genitalia: The terminal abdominal segments of the specimen were dissected out and soaked overnight in 10 per cent KOH solution. It was washed with distilled water and then soaked in glacial acetic acid for one to two minutes, then cleaned with distilled water. The digested abdominal parts were transferred to a microscopic slide containing a drop of glycerine using forceps. By observing under stereo zoom binocular microscope, the male genitalia were extracted from abdomen using a No. 0 entomological pin and the attaching tissues were cleaned by using a No. 0 entomological pin with hooked tip.

DNA isolation: DNA was isolated using the 'DNA

Sure Tissue Mini Kit' (Genetix). Total DNA from legs of one side was isolated. The dissected legs were crushed using the pre-autoclaved micro pestle. Standard protocols given in 'DNA Sure Tissue Mini Kit' were immediately followed.

PCR amplification: The mitochondrial DNA cytochrome oxidase subunit-I (CO-I) region of total DNA was amplified using the SimpliAmp Thermal cycler of Thermo Fisher Scientific. The universal primer designed by Foler *et al.*, 1994, was used. The name and 5'-3' sequence of the forward and reverse primers are given below;

LCO1490

GGTCAACAAATCATAAAGATATTGG

HCO2198

TAAACTTCAGGGTGACCAAAAAATCA

The primers were diluted to 10µM using DNAase and RNAase-free molecular water. The PCR master mix used was the EmeraldAmp GT PCR Master mix (TAKARA). The master mix included an optimised buffer, PCR enzyme, dNTP mixture, gel loading dye (emerald green), and a density reagent in a 2x premix format. Gradient PCR was carried out for standardising the annealing temperature. The best annealing temperature for this reaction was recorded as 51°C.

For PCR amplification of mitochondrial cytochrome oxidase subunit I locus, a reaction mixture of 40µL was prepared. The composition of the reaction mixture was as follows: 20µL PCR master mix, 2µL template DNA, 1µL forward, 1µL reverse primer, and 16µL molecular water. The PCR product obtained was stored at 4°C.

DNA sequencing: Sanger sequencing of PCR product was done by sending PCR product to Eurofin Genomics India Pvt. Ltd., Bengaluru. For each sample, bidirectional sequencing was done. Data were received in FASTA, ab1, and pdf formats.

Bioinformatic analysis: The forward and reverse sequence were trimmed and contig was generated using bioedit software. The sequence was submitted to NCBI through Bankit portal and accession number was obtained. Molecular studies were done

to understand the phylogenetic affinity of the species, for which genetic sequences for mtCO1 gene of representative genera under the tribe Eristalini was retrieved from NCBI. They were aligned and the phylogenetic tree was constructed in MEGA 11, by Neighbour joining method with Jukes- Cantor model and a bootstrap value of 1000.

RESULTS AND DISCUSSION

Eristalinus (Merodonoides) multifarius (Walker, 1852), is rediscovered after 45 years from Kerala. The male specimens of the species have been redescribed along with illustrations of male genitalia.

Systematic account

Eristalinus (Merodonoides) multifarius (Walker, 1852)

Eristalis multifarius Walker, 1852

Merodonoides circularis Curran, 1931

M. minutus Hull, 1944

M. czernyi Hull, 1944

Eristalis (Merodonoides) kandyensis Keiser, 1958

Eristalis yamunanagarensis Awtar Singh, Sodhi & Gupta, 1986

Pseudomeromacrus setipenitus Li, 1994

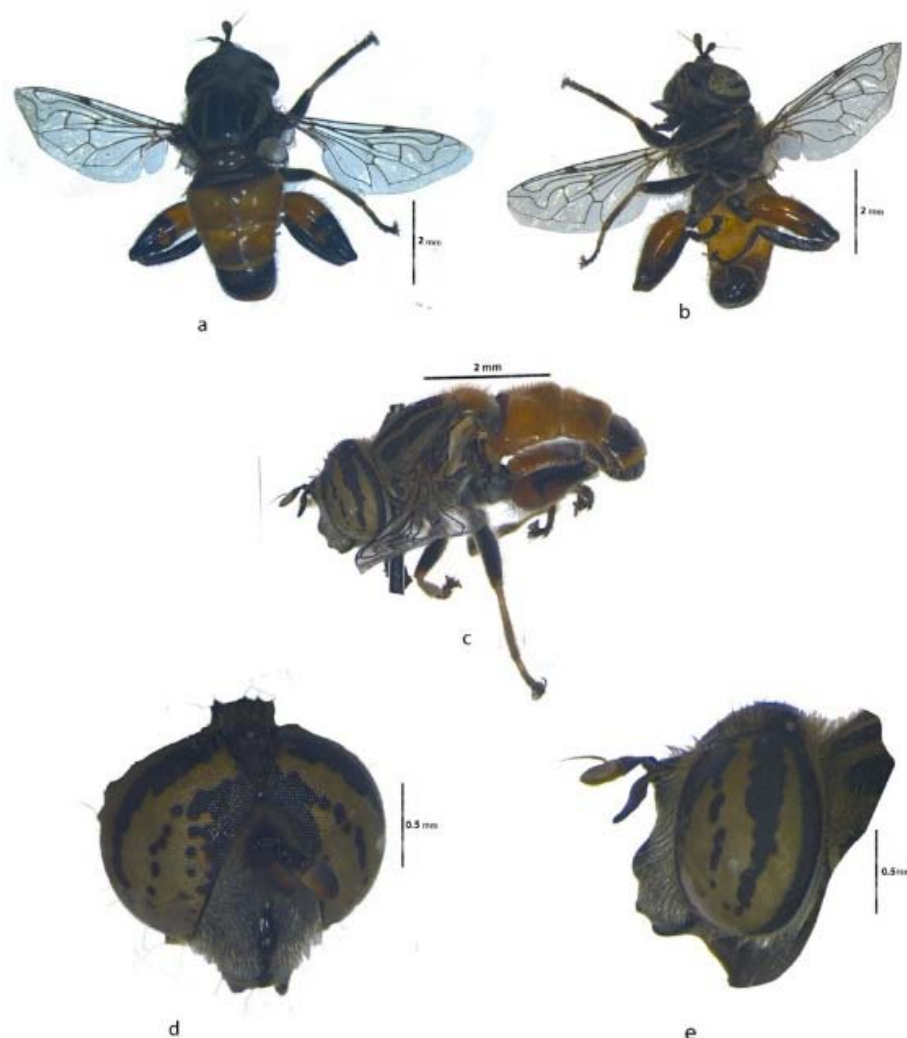


Fig. 1 *Eristalinus (Merodonoides) multifarius* (Walker); a-c. Habitus a. dorsal, b. ventral, c. lateral, d & e. head d. frontal, e. lateral

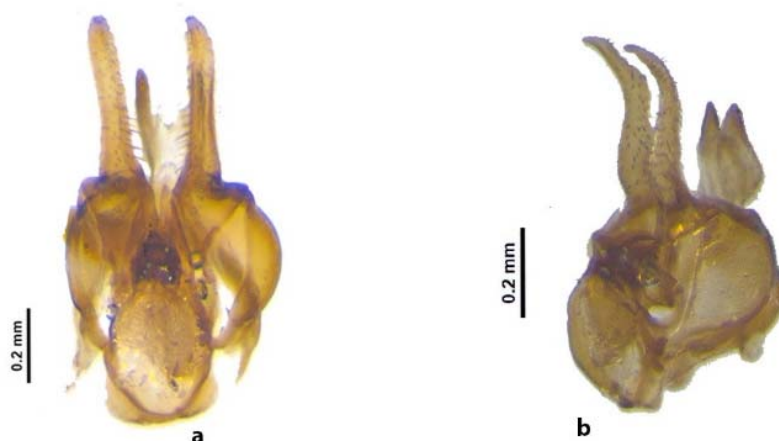


Fig. 2 Male genitalia of *Eristalinus (Merodonoides) multifarius* (Walker): (a) Ventral view: surstyli is more elongate and narrower; rows of fine bristles are present at the inner edge; (b) Lateral view: basoventral lobe is elongated and sickle shaped; cercus broad and narrowed towards the tip

Description:

Size: 9 mm in length

Male:

Head: Eyes light reddish-brown, bare, featuring two distinct black stripes on the disc, alongside dark inner and hind borders; frons and face are moderately shining black, primarily covered with greyish-white dust, leaving the vertex broadly black and a transverse space across the middle of the frons; face gradually widens and is somewhat prominent, with a short, narrow median shining black stripe that points at the upper end; lower part of the head is bare, with only whitish dust and shining black stripes on each side; occiput is dusted with ash-grey and has whitish pubescence on the lower part (Fig. 1d, e). **Thorax:** entirely ash-grey, with four shining black stripes in the usual pattern, positioned well away from the anterior and hind margins, and the outer stripes are pointed behind; hind corners of the dorsum form a narrow shining black ridge with stiff black hairs, and the pleurae are ash-grey; scutellum is entirely orange, with yellowish pubescence covering the whole thoracic dorsum and scutellum, while the pleurae have whitish pubescence (Fig. 1a, c). **Abdomen:** 1st segment is pale grey with whitish pubescence; 2nd segment is

mainly orange, with a narrowly black anterior margin (except at the corners) and a transverse blackish band near the hind margin (not reaching the sides). 3rd segment is predominantly orange, featuring a large semicircular blackish spot on the hinder half that is clear of the hind margin and corners, not extending to the anterior margin. 4th segment is entirely black, except for narrowly orange anterior corners, while 5th segment is wholly blackish. 2nd, 3rd, and 4th segments have pale yellow extreme hind margins and a fringe of pale-yellow hairs; pubescence on the dorsum and sides is whitish, while the venter is mainly orange in the basal half and black in the apical half (Fig. 1a). **Legs:** coxae and anterior legs are black, with the tips of the femora and the basal half of the fore tibiae being yellowish; middle tibiae mostly yellowish but brownish at the apical part, and the anterior tarsi are blackish-brown, often orange at the base, particularly in the middle pair; hind femora are significantly thickened, brownish-orange with broadly black tips, featuring an incomplete black subapical ring on the outer side; hind tibiae and tarsi are black or blackish-brown, and the pubescence of the legs is whitish, with orange-yellow on the underside of the hind tarsi (Fig. 1a, b, c). **Wings:** very pale grey and vitreous, with the 2nd longitudinal vein distinctly curved downward in a loop near the

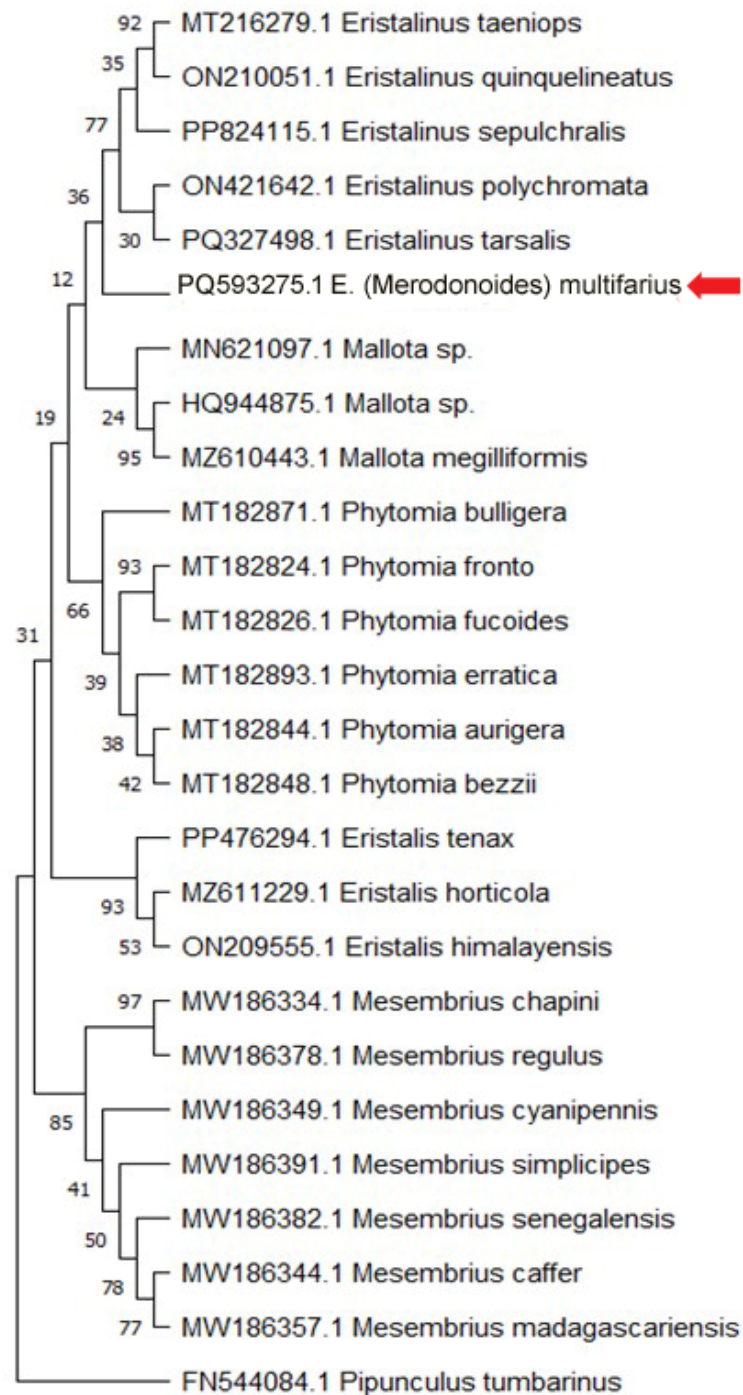


Fig. 3 The phylogenetic tree presenting the relationship of the *Eristalinus (Merodonoides) multifarius* (Walker) with other species of tribe *Eristalini*, constructed in MEGA 11, by neighbour joining method, with a booter trap value of 1000

tip; 3rd vein is deeply looped, and the anterior cross-vein is longer than usual, narrowing the basal part of the submarginal cell; squamae are grey with brown margins; halteres are orange.

Male genitalia: *Ventral view:* surstyli is more elongate and narrower; rows of fine bristles are present at the inner edge (Fig. 2a). *Lateral view:* basiventral lobe is elongated and appears to be sickle shaped; cercus broad and narrowed towards the tip (Fig. 2b).

Material examined: 3, labelled 'India: Kerala: 1 ♂, 1 ♀ Rajakkad (9°58'01.0"N; 77°05'38.2"E) 28.i.2024; 1 ♂, Thenmala (9°00'25.2"N; 76°59'04.9"E) 19.ii.2024 'Aerial net', 28.i.2024. Coll. Aiswarya Joy.

Distribution in India: Gujarat, Haryana, Karnataka, Meghalaya, Assam, Madhya Pradesh, Rajasthan, Tamil Nadu, Kerala (Ghorpade, 2014, 2019).

The DNA sequence generated for the species using mtCO1 marker furnished below is the first representation of molecular characterization of the species.

ATCATAAAGATATTGGAACATTATATTTTG
TATTTGGTACATGAGCCGGAATAGTAGG
AACTTCCCTTAGTATCCTAATTCGAGCTG
AATTAG GTCATCCTGGTGCATTAATTGGA
GATGATCAAATTTATAATGTTATTGT AACA
GCTCATGCTTTTGTAATAATTTTCTTTATA
GTAATACCTATTATAATTGGAGGATTTGG
AAATTGATTAGTTCCTTTAATATTAGGAG
CACCTGATATAGCTTTTCCTCGAATAAATA
ATATAAGTTTTTGATTACTTCCTCCTTCTT
TAACCTTTATTATTAGTAAGAAGTATAG
TAGAAAATGGGGCTGGGACTGGATGAAC
AGTTTATCCCCCTTTATCAAGAAATATTGC
TCACGGTGGGGCTTCAGTTGATTTAGCTA
TTTTTCCCT TCATTTATCCGGTATATCTT
CAATTTTAGGAGCAGTAAATTTTATTAC
AACAGTAATTAATATACGTTTCGACAGG
AATTACATATGATCGAATACCTTTATTTGT
TTGATCAGTTGCTATTACAGCATTA
TTACTTTTATTATCATTACCAGTACTAGCCG
GAGCTATTACAATATTATTAACAGATC

GAAATCTTAATACTTCTTTTTTTTGACCC
TGCAGGAGGAGGTGACCCTATTTTATA
TCAACATTTATTCTGA

GenBank accession number of *E. (Merodonoides) multifarius*: PQ593275.

E. (Merodonoides) multifarius was described as *Eristalis multifarius* Walker 1854 from the type female in the British Museum from India and another female from the lower ranges of North Khasi Hills, Assam. The types were deposited in British Museum from India. Brunetti, 1923 had then suggested to place it outside *Eristalis* Latreille owing to characters like the greatly thickened hind femora and the dipped second vein. Later the genus *Merodonoides* was erected by Curran, 1931 based on characters *viz.*, thickened hind femora and distinctive eye pattern with four conspicuous brown stripes.

The males of the species was described by Singh *et al.*, 1986, as a new species *Eristalis yamunanagarensis* Awtar Singh, Sodhi and Gupta, 1986, which was later synonymized with *E. (Merodonoides) multifarius* (Ghorpade, 2014). The most recent synonymization of the species was done by Thomson, 2003, wherein he synonymised *Pseudomeromaerus setiprinitus* Li, 1994; which was described as a new genus species from China. The colour plate of the male specimen of *E. (Merodonoides) multifarius* along with the male genitalia had been illustrated here.

E. (Merodonoides) multifarius is a wide spread species distributed across a wide range in the Indian subcontinent, Southeast Asia, and southern China, with records from diverse ecological regions like the Western Ghats, Eastern Droogs, and the Indochinese Peninsula (Ghorpade, 2019). *E. (Merodonoides) multifarius* had been reported from Gujarat, Haryana, Karnataka, Meghalaya, Madhya Pradesh, Rajasthan, Tamil Nadu, (Ghorpade, 2014), other than its type locality, Assam. Though the species occur wide spread in India, it was reported from Kerala only from a single female specimen collected by A.N.T. Joseph in 1979 from Parambikulam, Palghat district (Datta and Chakraborti (1986). Subsequent workers who

had prepared the checklist of hoverflies of India (Mitra *et al.*, 2015; Sengupta *et al.*, 2024) and Kerala (Sankar *et al.*, 2024) have omitted the record and have presented that the species as not recorded in Kerala. The recent checklist of hoverflies of Kerala by Sankar *et al.* (2024) had omitted the aforesaid study and had presented the total number of syrphids reported from Kerala as 59, whereas it should have been 60. The record of the species from Thenmala, Kollam district and Rajakad, Idukki district, furnished here, presents the rediscovery of the species from Kerala after 45 years, ever since it was first collected by A.N.T. Joseph in 1979 and also the first confirmed report of males of the species from, Kerala.

The mitochondrial cytochrome-c oxidase subunit I (*mtCO1*) gene has proven to be a powerful tool for species identification, primarily due to its strong phylogenetic signal, which enables clear discrimination among species (Hebert *et al.*, 2003; Hebert and Gregory, 2005). Previous studies on hoverflies have demonstrated the utility of this approach, successfully identifying and distinguishing various species (Pérez-Bañón *et al.*, 2003; Ståhls *et al.*, 2009; Gibson *et al.*, 2011). In this study also the *mtCO1* marker was found to be very reliable. The DNA sequence of *E. (Merodonoides) multifarius* for *mtCO1* marker furnished here is the first ever sequence for the species and the sequence submitted to the NCBI GenBank database will help in understanding the phylogenetic affinities of the subgenus *Merodonoides*. The subgenus closely resembles the *Eristalinus* Rondani 1845 on overall similarity, but shares a few characters similar to that of *Mallota* Meigen, 1822 like the swollen hind femur and colouration. Phylogenetic studies are necessary to find out the actual affinity of the subgenus with its related genera.

Merodonoides is a poorly studied taxa phylogenetically, with not even a single DNA sequence of the subgenus available in the GenBank previously. The molecular characterisation and the phylogenetic studies presented here will aid in addressing the gaps regarding phylogenetic affinities within the genus *Eristalinus*. The phylogenetic analysis of *E. (Merodonoides) multifarius* with

other members in tribe *Eristalini* is presented (Fig. 3). The phylogenetic tree revealed that the subgenus *Merodonoides* was closely related to *Eristalinus* Rondani. *E. (Merodonoides) multifarius* was found basal to all other members of *Eristalinus*. The genus *Mallota* Meigen, appeared as basal to *Eristalinus*. *Eristalis* Latreille was found be basal to the clade which consisted of *Eristalinus*, *Mallota* and *Phytomia* Guérin-Méneville. *Mesembrius* Rondani was found basal to all other genera analysed. The outgroup *Pipunculus tumbarinus* Kehlmaier got separated out in a different branch outside of main tree which confirmed the validity of the generated phylogenetic tree.

E. (Merodonoides) multifarius shares habitat almost similar to that of most other *Eristalinus* and possess both morphological and genetic similarity with other members of *Eristalinus*, but it is an uncommon species in Kerala. The phylogenetic study reveals that *Mallota* is the next closely related taxa to the subgenus *Merodonoides*, after the clade which encompasses other *Eristalini*. Further studies are needed on the distribution and biology of this uncommon member of *Eristalini*, for any kind of exploration of the species as a pollinating agent locally.

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New state records and distributional range extensions of termite species in India

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ABSTRACT: The present study documents new distributional records of thirteen termite species from four Indian states. Taxonomic studies revealed five species as new addition to the termite fauna of both Arunachal Pradesh and Odisha, while two species are added to the termite fauna of Tamil Nadu and one species to West Bengal. The Indian endemic genus *Ampoulitermes* Mathur and Thapa, 1962 and genus *Pericapritermes* Silvestri, 1914 are reported for the first time from Tamil Nadu and Odisha respectively. These findings not only contribute to the understanding of termite diversity in India but also highlight the significance of continued taxonomic surveys in underexplored regions.

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KEY WORDS: Diversity, first report, *Ampoulitermes*, *Pericapritermes*, Indian states

INTRODUCTION

Termites (Order Blattodea) constitute one of the most fascinating groups of social insects due to their ubiquitous distribution in the tropics, superb architectural ability, cryptobiotic nature of life and enormous economic importance. They are ecosystem engineers (Jones *et al.* 1994) and are among the most dominant macroinvertebrates (Bignell and Eggleton, 2000), arthropod decomposers in tropical soils as well as they also play a central role in nutrient fluxes. Their nesting activities promote soil aeration, improve the absorption and storage of water in the soil, and facilitate carbon flux (Sileshi *et al.*, 2010). As per the revised classification by Hellemans *et al.* 2024, worldwide there are more than 3000 species of living and fossil termites classified into 13 families and 330 genera. However, only 321 species are

known from India classified under 53 genera and 8 families (Baraik *et al.*, in press). The family Termitidae is the most dominant among termites worldwide. About 70 per cent of termite species belongs to single family Termitidae, comprising 238 genera. In India, Termitidae is represented by 208 species across 35 genera, classified under seven subfamilies, also accounting for 71 per cent of the country's termite diversity (Baraik *et al.*, in press). Among the Indian states, Kerala harbors the highest termite diversity with 94 recorded species (Abhirami and Prasad, 2024), followed by Karnataka (76 species), Tamil Nadu (64 species), and West Bengal (62 species) (Baraik *et al.*, in press). The present study reports the first state records of thirteen termite species, documenting their newly observed distributions in the Indian states of Arunachal Pradesh, Odisha, Tamil Nadu, and West Bengal.

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MATERIALS AND METHODS

The present study is based on termite specimens preserved in the National Zoological Collection, Isoptera Section, Zoological Survey of India (ZSI), Kolkata. The examined material comprises specimens collected during various faunal surveys conducted between 1971–1973, 1989, 2014, and 2019–2024 across different states of India by ZSI team, including the authors of this paper. Several specimens within these collections had remained unidentified, and this study was undertaken to examine, identify, and document these previously unidentified materials, along with freshly collected specimens.

Measurements of attributes of termite specimens were made under a Leica EZ4 microscope with specimens kept in absolute alcohol. Images were captured using a Leica M205A stereo microscope fitted with a Leica DFC500 camera and processed using the extended focus software LAS Version 3.6. Species identification and description follow Roonwal and Chhotani (1989) and Chhotani (1997). All the specimens are deposited in Zoological Survey of India, Kolkata.

RESULTS AND DISCUSSION

A total of 13 termite species representing nine genera were documented in the present study as detailed in the systematic account. Of these, twelve species belong to the family Termitidae, distributed across four subfamilies: Macrotermitinae, Microcerotermitinae, Mirocapritermitinae, and Nasutitermitinae. The family Heterotermitidae is represented only by a single species. All thirteen species are detailed in the systematic account and represent new distributional records for the states of Arunachal Pradesh, Odisha, Tamil Nadu and West Bengal.

Systematic Account

Family 1: Heterotermitidae Froggatt, 1897;

Genus *Heterotermes* Froggatt, 1897

1. *Heterotermes indicola* (Wasmann, 1902) (Fig. 1)

Leucotermes indicola Wasmann, 1902: Zool. Jb.

(Syst.), 17(1): 118–119. S, W. Lectotype: S (designated O.B.Chhotani), in Nat. Hist. Mus., Maastricht.

Material examined: Odisha, Sundargarh, 25 Km SE of Panposh Railway station, 10.xii.1971, 3 soldiers and 6 workers, Coll: P.K. Maiti.

Distribution: Afghanistan, Bangladesh, Iran, Nepal, Pakistan (Krishna *et al.*, 2013), India: Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Maharashtra, Punjab, Rajasthan, Uttar Pradesh (Krishna *et al.*, 2013), Odisha (New Record).

Family 2: Termitidae Latreille, 1802;

Subfamily Macrotermitinae Kemner, 1934;

Genus *Macrotermes* Holmgren, 1909

2. *Macrotermes gilvus* (Hagen, 1858) (Fig. 2)

Termes gilvus Hagen, 1858: Linn. Ent., 12: 148–151, Pl. 3 (Fig. 14). Im., Q, S maj., S min. Syntypes: In Coll. Selys Longchamps, Brussels, Mus. Vienna, Mus. Berlin.

Material examined: Arunachal Pradesh, Upper Siang, Jengging and around, 26.ii.2024, 1 soldier and 7 workers, Coll: Dilip Mondal; Arunachal Pradesh, Dibang Valley, Etalin, in and around Inspection bungalow, 26.ii.2024, 1 soldier and 10 workers, Coll: Rahul Mondal.

Distribution: Bangladesh, Indonesia (Krishna *et al.*, 2013), India: Assam, Meghalaya, Tripura (Krishna *et al.*, 2013) and Arunachal Pradesh (New Record).

Genus *Microtermes* Wasmann, 1902

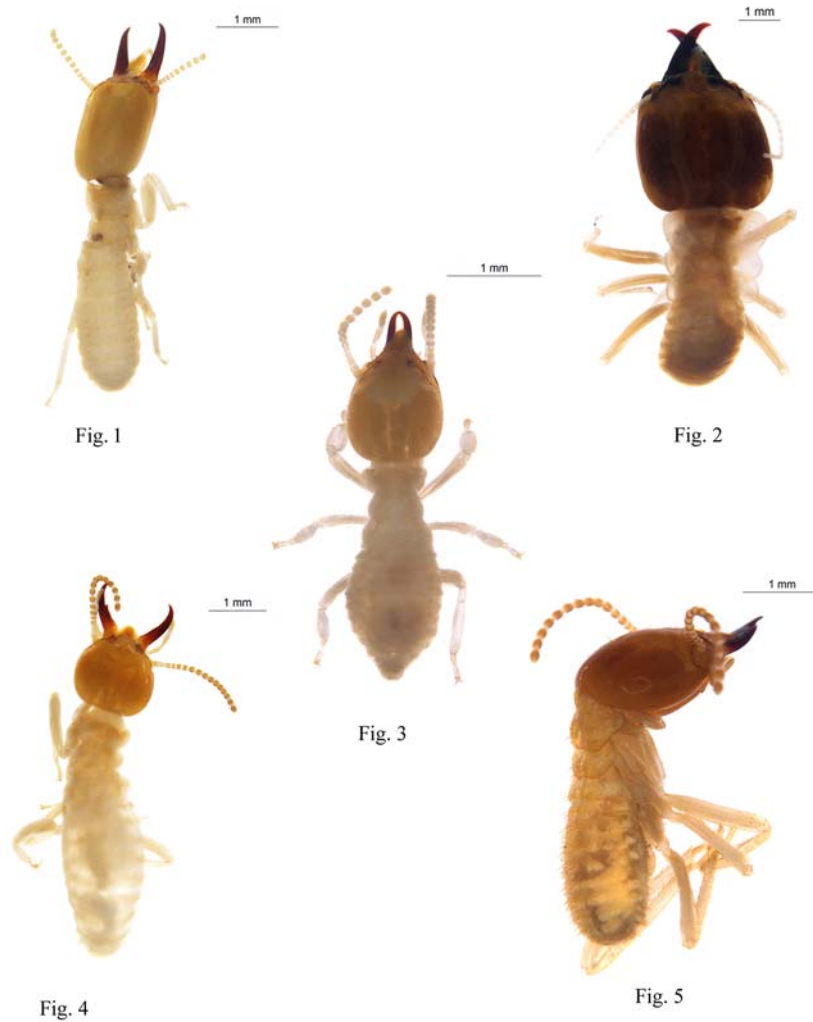
3. *Microtermes incertoides* Holmgren, 1913 (Fig. 3)

Microtermes incertoides Holmgren, 1913: J. Bombay nat. Hist. Soc., 22 (1): 113–114. S and W.

Material examined: Arunachal Pradesh, East Kameng, Upper Dekoria, Seijusa, 29.x.2014, 4 soldiers and 10 workers, Coll: N. Shah.

Distribution: India: Andhra Pradesh, Daman,

PLATE- I

Fig. 1 *Heterotermes indicola* (Wasmann, 1902)Fig. 2 *Macrotermes gilvus* (Hagen, 1858)Fig. 3 *Microtermes incertoides* Holmgren, 1913Fig. 4 *Odontotermes gurdaspurensis* Holmgren and Holmgren, 1917Fig. 5 *Odontotermes kapuri* Roonwal and Chhotani, 1962

Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Tamil Nadu, Uttar Pradesh (Krishna *et al.* 2013) and Arunachal Pradesh (New Record).

Genus *Odontotermes* Holmgren, 1910

4. *Odontotermes gurdaspurensis* Holmgren and Holmgren, 1917 (Fig. 4)

Odontotermes gurdaspurensis Holmgren and Holmgren, 1917, Mem. Dept. agric. Sci., 5: 149-150.

Material examined: Odisha, Sambhalpur, Sonapali village, 23.xii.1971, 6 soldiers and 7 workers, Coll: P.K. Maiti; Odisha, Rampur forest, 26.xii.1971, 8 soldiers and 40 workers, Coll: P.K. Maiti; Odisha, Hathisilpara, 1km west of forest quarters, 30.xii.1971, 1 soldier and 15 workers, Coll: P.K. Maiti; Odisha, Sundargarh, Sabulia Khasra forest, 01.v.1973, 2 soldiers and 50 workers, Coll: P.K. Maiti.

Distribution: Pakistan (Krishna *et al.*, 2013),

India: Gujarat, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Punjab, Haryana, Rajasthan, Uttarakhand, Uttar Pradesh, West Bengal (Krishna *et al.*, 2013) and Odisha (New Record).

5. *Odontotermes kapuri* Roonwal and Chhotani, 1962 (Fig. 5)

Odontotermes kapuri Roonwal and Chhotani, 1962: Proc. Natn. Inst. Sci. India, (B) 28 (4): 296, 352-356. S and W.

Material examined: Arunachal Pradesh, Dibang Valley, Etalin village, 27.ii.2024, 2 soldiers, Coll: Ravi Kushwaha.

Distribution: Bhutan (Krishna *et al.*, 2013), India: Manipur, Meghalaya, Tripura (Krishna *et al.*, 2013) and Arunachal Pradesh (New Record).

6. *Odontotermes vaishno* Bose, 1975 (Fig. 6)

Odontotermes vaishno Bose, 1975: Oriental Insects, 9 (2): 161-164. S and W.

Material examined: West Bengal, Jhargram, Binpur, Lalgargh Forest, 05.iv.2024, 2 soldiers and 1 worker, Coll: B. Baraik.

Distribution: India: Andhra Pradesh (Ranjith *et al.*, 2025), Haryana (Kumar *et al.*, 2023), Karnataka, Kerala (Krishna *et al.*, 2013) and West Bengal (New Record).

Subfamily Microcerotermitinae Holmgren, 1910

Genus *Microcerotermes* Silvestri, 1901

7. *Microcerotermes cameroni* Snyder, 1934 (Fig. 7)

Microcerotermes cameroni Snyder, 1934: Indian For. Rec., 20 (11): 21-22. Im and S. Holotype. Im in BM.

Material examined: Odisha, Sundargarh, Champajharan forest, 10.xii.1971, 5 soldiers and 25 workers, Coll: P.K. Maiti; Odisha, Sundargarh, 10.xii.1971, 5 soldiers and 30 workers, Coll: P.K. Maiti; Odisha, Kaira village, 15.xii.1971, 3 soldiers and 12 workers, Coll: P.K. Maiti; Odisha, Bolangir,

12 kms south west of Bolangir, 31.xii.1971, Coll: P.K. Maiti; Odisha, Sundargarh, Balijun village, 11.i.1972, 2 soldiers and 40 workers, Coll: P.K. Maiti

Distribution: India: Andhra Pradesh, Daman, Gujarat, Karnataka, Kerala, Madhya Pradesh, Sikkim, Tamil Nadu, West Bengal (Krishna *et al.*, 2013) and Odisha (New Record).

8. *Microcerotermes fletcheri* Holmgren and Holmgren, 1917 (Fig. 8)

Microcerotermes fletcheri Holmgren and Holmgren, 1917: Mem. Dep. Agric. India (Ent.), 5 (2): 170-171. S and W.

Material examined: Odisha, Balukh and Konark Wildlife sanctuary, 20.ix.2023, 6 soldiers and 14 workers, Coll: Tamal Mandal.

Distribution: India: Karnataka, Kerala, Tamil Nadu, Uttar Pradesh (Krishna *et al.*, 2013) and Odisha (New Record).

Subfamily Mirocapritermitinae Kemner, 1934 Genus *Pericapritermes* Silvestri, 1934

9. *Pericapritermes butenzorgi* (Holmgren, 1914) (Fig. 9)

Capritermes (*Capritermes*) *buitenzorgi* Holmgren, 1914: Zool. jb. (Syst.) 36 (2-3): 280 S. and W.

Material examined: Arunachal Pradesh, East Kameng, Rhino Camp, Seijusa, 30.x.2014, 2 soldiers and 8 workers, Coll: N. Shah.

Distribution: Indonesia, Malaysia (Krishna *et al.*, 2013), India: Andaman Islands (Maiti and Chakraborty, 1994; Krishna *et al.*, 2013) and Arunachal Pradesh (New Record).

10. *Pericapritermes tetraphilus* (Silvestri, 1922) (Fig. 10)

Capritermes (*Pericapritermes*) *tetraphilus* Silvestri, 1922: Rec. Indian Mus., 24 (4): 543-545.

Material examined: Odisha, Balukh and Konark Wildlife sanctuary, 20.ix.2023, 1 soldier Coll: Tamal Mandal.

PLATE- II

Fig. 6 *Odontotermes vaishno* Bose, 1975Fig. 7 *Microcerotermes cameroni* Snyder, 1934Fig. 8 *Microcerotermes fletcheri* Holmgren and Holmgren, 1917Fig. 9 *Pericapritermes buitenzorgi* (Holmgren, 1914)

Distribution: Bangladesh, Burma, China (Krishna *et al.*, 2013), India: Assam, Madhya Pradesh, Manipur, Sikkim, West Bengal (Krishna *et al.*, 2013) and Odisha (New Record).

Subfamily Nasutitermitinae Hare, 1937

Genus *Ampoulitermes* Mathur and Thapa, 1962

11. *Ampoulitermes wynaadensis* Mathur and Thapa, 1962 (Fig. 11)

Ampoulitermes wynaadensis Mathur and Thapa,

1962: Indian Forester, 88: 50-52. S and W.

Material examined: Tamil Nadu, Fern hill wild life sanctuary, 25.viii.2019, 3 soldiers and 6 workers, Coll: Dinesh, K; Tamil Nadu, Courtallam forest, 26.viii.2019, 2 soldiers and 5 workers, Coll: Dinesh, K. P.

Distribution: India: Kerala (Krishna *et al.*, 2013) and Tamil Nadu (New Record).

Genus *Hospitalitermes* Holmgren, 1912

12. *Hospitalitermes jepsoni* (Snyder, 1934) (Fig. 12)

PLATE- III



Fig. 10



Fig. 11



Fig. 12



Fig. 13

Fig. 10 *Pericapritermes tetraphilus* (Silvestri, 1922)

Fig. 11 *Ampoulitermes wynaadensis* Mathur and Thapa, 1962

Fig. 12 *Hospitalitermes jepsoni* (Snyder, 1934)

Fig. 13 *Nasutitermes emersoni* Snyder, 1934

Nasutitermes (*Hospitalitermes*) *jepsoni* Snyder, 1934: Indian For. Rec., 20 (11): 16.

Material examined: Tamil Nadu, Chinnar, 14.iii.2020, 4 soldiers and 4 workers, Coll: Dinesh, K.P.

Distribution: India: Assam (Krishna *et al.*, 2013) and Tamil Nadu (New Record).

Genus: *Nasutitermes* Dudley, 1890

13. *Nasutitermes emersoni* Snyder, 1934 (Fig. 13)

Nasutitermes emersoni Snyder, 1934: Indian For. Rec., 20 (11): 11-12.

Material examined: Arunachal Pradesh, Upper Siang, Jengging and around, 26.ii.2024, 4 soldiers and 4 workers, Coll: Dilip Mondal; Arunachal Pradesh, West Siang, Nyork village, 19.iii.2024, 6 soldiers and 3 workers, Coll: Dilip Mondal.

Distribution: Bangladesh (Krishna *et al.*, 2013), India: Bihar, Sikkim, Tripura (Krishna *et al.*, 2013) and Arunachal Pradesh (New Record).

The present study adds five termite species each to the known fauna of Arunachal Pradesh and Odisha, two species to Tamil Nadu, and one species to West Bengal. Among the four states, Tamil Nadu possesses the richest termite fauna, with 64 species

belonging to 27 genera, followed by West Bengal with 62 species across 25 genera, Arunachal Pradesh with 37 species in 17 genera, and Odisha with 30 species in 15 genera. Beyond representing the actual diversity, these numbers may largely reflect the extent of taxonomic exploration, as certain regions have been studied more extensively than others over time. For instance, despite Odisha's diverse physiographic features—ranging from luxuriant, dense forests to dry deciduous forests occurring in parts of the Chota Nagpur Plateau—the recorded termite fauna appears to be underrepresented.

Heterotermes indicola, a species with broad ecological tolerance, inhabits a wide range of habitats including arid and semi-arid zones, forested regions, and agricultural landscapes. Although widely distributed across India, the record from Odisha marks its first documentation from eastern India. Historically, this species was responsible for the complete destruction of the township of Shri Hargovindapur in Gurdaspur, Punjab, reducing it to a bombed-out ghost town (Roonwal, 1955). It is known from both the Oriental and Palearctic regions (Krishna, 2013). In the present study, *H. indicola* and *Odontotermes gurdaspurensis* Holmgren and Holmgren, 1917 are reported for the first time from the Eastern Ghats region of Odisha (Basak *et al.*, 2025).

The genus *Ampoulitermes*, which is endemic to India and previously known only from Kerala, is represented by two species, *A. wayanadensis* and *A. zacharia*, both restricted to the Western Ghats of Kerala. The present study reports *A. wayanadensis* from the Western Ghat region of Tamil Nadu, indicating a range extension. However, the species remains endemic to the Western Ghats as a whole. The open foraging species, *Hospitalitermes jepsoni* which was previously known only from the tropical forests of Assam, now documented from the Western Ghats of Tamil Nadu, indicating its extremely wide distributional range; evidence of its occurrence in intervening areas are yet to be discovered. The Indian endemic species *Microcerotermes cameroni*, is hereby reported from the Eastern Ghats region of

Odisha, for the first time. Another endemic species, *M. fletcheri*, is recorded from Konark Balukhand Wildlife Sanctuary, an area characterized by saline and sandy soils due to its coastal location along the Bay of Bengal. The soil dwelling species, *Pericapritermes butenzorgi* (Holmgren, 1914), previously known in India only from Andaman Islands (Maiti and Chakraborti, 1994), is now recorded for the first time from the mainland. Species like *O. vaishno* have wide distribution, exhibiting broad ecological tolerance. They occupy diverse habitats ranging from semi-arid zones and forested regions to agricultural landscapes, while *O. kapuri* is known only from north east India.

The present study thus highlights the scope for further exploration and documentation of termites, given the wide range of climatic conditions and diverse habitats found across India. Such comprehensive taxonomic studies provide essential baseline data not only for taxonomic and biodiversity research but also for ecological studies, as termites play a vital role in modifying soil conditions, which in turn influence the composition and distribution of both flora and fauna.

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Effect of host plants on the feeding potential of *Episyrphus balteatus* (De Geer) preying on two different aphid species

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ABSTRACT: In the study, effect of three host plants (*Cajanus cajan* (L.), *Lablab purpureus* (L.) and *Vicia faba* L.) on the feeding potential of syrphid fly *Episyrphus balteatus* (De Geer) on *Aphis craccivora* Koch. were studied under laboratory condition. It was observed that feeding potential was highly influenced by host plants. Maximum consumption of aphid by a single larva of *E. balteatus* was observed on *V. faba* (261 ± 6.58 aphids) followed by *C. cajan* (244.2 ± 8.38 aphids). Minimum consumption (222.2 ± 5.97 aphids) was recorded on *L. purpureus*. The feeding potential of *E. balteatus* was also observed on different aphid on *C. cajan*. The maximum larval consumption (558.8 ± 11.95 aphids) was recorded on *A. gossypii* as compared to *A. craccivora* on *C. cajan*. © 2025 Association for Advancement of Entomology

KEY WORDS: *Aphis craccivora*, syrphid, *Cajanus cajan*, *A. gossypii*

Aphids are small sap sucking plant bugs. They are serious pests of agricultural and horticulture crops. They reproduce parthenogenetically and increase their number rapidly above economically threshold level (Ahmad and Parween, 2009; Ahmad *et al.*, 2024). Their sap sucking behaviour causes retardation in growth and development of the plant. They also secrete honey dew which develops black sooty mould and retard the process of photosynthesis. They are also responsible for transmission of several plant viruses (Singh and Singh, 2016). Syrphids are important predators of several aphid species (Chambers *et al.*, 1983; Radhakrishnan and Muraleedharan, 1993; Tenhumberg, 1995). In India, 48 species of syrphids were reported on 72 species of aphids infesting more than 141 food plants (Ahmad and Kumari,

2024). *Episyrphus balteatus* (Diptera, Syrphidae) is a predominant species worldwide (Sadeghi, 2008) and it was reported preying on 57 species of aphids, infesting 107 species of plants from 20 states of union territories in India (Singh and Ahmad, 2025). Adults are good pollinating agent and larvae of are very important syrphid predator of aphids and other homopteran insects (Sadeghi and Gilbert, 2000; Routray and Dey, 2016). Sadeghi *et al.* (2014) reported that *E. balteatus* is a generalists species in their preference for prey. Bombosch (1963) was the first to propose model of predator feeding potential as a means of assessing the impact of syrphids. Tamaki *et al.* (1974) and Chambers and Adams (1986) have expanded this strategy, although these models continue to rely on the relationship between predatory potential in the field

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and laboratory. Samuel *et al.* (2005) studied the feeding potential of *E. balteatus* (De Geer) on *Lipaphis erysimi* (Kaltenbach) at variable host density. Varshney and Bisht (2016) also used *L. erysimi* for the feeding experiment of *E. balteatus*. Similarly, Muralibaskaran *et al.* (2009) observed feeding efficiency of *E. balteatus* and other syrphid species on *A. gossypii*. In Bihar, *E. balteatus* was recorded on several species of aphids on different host plants with moderate to high intensity of predation (Kumar *et al.*, 2015; Kumar and Ahmad, 2017; Ahmad *et al.*, 2020; Parween *et al.*, 2023). Hence, due to the wide host range, the feeding potential of *E. balteatus* on *A. craccivora* and *A. gossypii* on different host plants was investigated to examine their efficacy for possible use in biological control programme.

Adults were collected from the field and brought to the laboratory where they were maintained in rearing cages (25cm x 25cm x 25cm) for mating and egg-laying. The collected eggs were kept into glass jars. After hatching, freshly emerged larvae were again kept into separate glass jars. For this experiment, mass culture of *A. gossypii* on *C. cajan* and *A. craccivora* on *C. cajan*, *Lablab purpureus* and *Vicia faba* were done. Each newly emerged larva was provided with a host plant twig infested with 150 aphids on daily basis until the complete larval developmental period. The unconsumed and dead aphids were also counted every day before providing fresh aphids for evaluating predatory potential.

The feeding potential of larval stages of *E. balteatus* was studied on *A. craccivora* on different host plants. Maximum aphid consumption by 1st instar (48.2±3.39 aphids), 2nd instar (85.2±2.40 aphids) and 3rd instar (127.6±5.05 aphids) larvae was observed when reared on *V. faba* as compared to *C. cajan* (1st instar 36.6±1.13 aphids, 2nd instar 82.4±6.49 aphids and 3rd instar 125.2±1.2 aphids). However, the lowest consumption was recorded (1st instar 26.0±3.0 aphids, 2nd instar 74.8±4.88 aphids and 3rd instar 121.4±3.62 aphids) on *L. purpureus* (Table 1). The difference in the consumption by 1st instar larvae between three host plants was observed significant

by ANOVA ($F = 17$, $P \leq 0.05$). However, there was no significant difference between 2nd instar ($F = 1.2$, $P \leq 0.05$) and the 3rd instar ($F = 0.7$, $P \leq 0.05$). Total consumption of aphids during larval period was observed maximum (261±6.58 aphids) on *V. faba* and minimum on *L. purpureus* (222.2±5.97 aphids) (Table 1). The difference in larval consumption for three host plants is found significant by ANOVA ($F = 7.6$, $P \leq 0.05$). Thus, on the basis of above investigation, it can be concluded that *V. faba* is more suitable host plant as compared to other host plant.

The feeding potential of *E. balteatus* was also observed on different aphids (*A. craccivora* and *A. gossypii*) on *C. cajan* plant. Maximum consumption of aphids by 1st instar (51.6±5.49 aphids), 2nd instar (229.6±9.25 aphids) and 3rd instar (277.6±7.19 aphids) larvae of *E. balteatus* and total consumption (558.8±11.95 aphids) was observed on *A. gossypii* as compared to *A. craccivora* when reared on *C. cajan* (Table 1). This difference in feeding potential was found highly significant between *A. craccivora* and *A. gossypii* consumption by each larval instars (1st instar, $t = 2.68$; 2nd instar, $t = 13.04$; 3rd instar, $t = 20.92$) and total consumption ($t = 20.61$ at 5%).

It is concluded that *E. balteatus* larvae preferred *A. gossypii* than *A. craccivora*. The consumption efficiency of *E. balteatus* varied according to different aphid species. Muralibaskaran *et al.* (2009) reported less larval consumption of *E. balteatus* on *A. gossypii* (1st instar 52.8±10.45, 2nd instar 112.4±25.01 and 3rd instar 245.6±52.14 aphids and total consumption was 410.0±87.60 aphids. Hindayna *et al.* (2001) demonstrated that *E. balteatus* larvae had the greatest predatory capacity on *A. gossypii*. Hong and Hung (2010) also reported more feeding capability of *E. balteatus* on *A. gossypii*. Many workers observed that the consumption efficiency of *E. balteatus* varied according to different aphid species. Total consumption of aphids by *E. balteatus* larvae was 411.8±57.88 on *Lipaphis erysimi* (Varshney and Bisht, 2016). Faheem *et al.* (2019) reported the total consumption as 398.37±9.45 by *E. balteatus* larvae on *Schizaphis graminum*. Similarly, Singh

Table 1. Predatory efficiency of larvae of *E. balteatus* on *A. craccivora* on different hosts and *A. gossypii* on *C. cajan* (mean $\pm$ S.E)

Larval instars	<i>Aphis craccivora</i>		<i>A. gossypii</i>	
	<i>C. cajan</i>	<i>L. purpureus</i>	<i>V. faba</i>	<i>C. cajan</i>
1st instar	36.6 $\pm$ 1.13	26.0 $\pm$ 3.0	48.2 $\pm$ 3.39	51.6 $\pm$ 5.49
2nd instar	82.4 $\pm$ 6.49	74.8 $\pm$ 4.88	85.2 $\pm$ 2.40	229.6 $\pm$ 9.25
3rd instar	125.2 $\pm$ 1.2	121.4 $\pm$ 3.62	127.6 $\pm$ 5.05	277.6 $\pm$ 7.19
Total	244.2 $\pm$ 8.38	222.2 $\pm$ 5.97	261 $\pm$ 6.58	558.8 $\pm$ 11.95

et al. (2020) reported total consumption as 393.6 $\pm$ 3.44 by the larvae on *Brevicoryne brassicae*. Routray and Dey (2016) recorded gross consumption of 523.33 $\pm$ 25.374 on *A. craccivora*. In the present investigation, the total consumption by larval stages of *E. balteatus* was 244.2 $\pm$ 8.38 on *A. craccivora* and 558.8 $\pm$ 11.95 on *A. gossypii* reared on *C. cajan* which is highest as compare to other aphid species.

It was also observed that feeding potential was greatly influenced by host plants. The maximum consumption of aphid by *E. balteatus* larvae observed on *V. faba*. Thus, on the basis of above information, *A. gossypii* and *V. faba* are more suitable aphid and host plants respectively for this syrphid predator.

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First record of *Leptus* Charles De Geer, 1778 (Acari, Erythraeidae) larval parasitism on *Eyprepocnemis alacris alacris* Serville, 1838 (Orthoptera, Acrididae) from Kerala, India

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ABSTRACT: *Eyprepocnemis alacris alacris* is a species of short-horned grasshopper commonly found in grasslands, forests, and open habitats across Kerala. *Leptus* larvae, which are six-legged parasitic mites, typically inhabit soil and leaf litter. For the first time, a severe infestation of *Leptus* larvae on *E. a. alacris* was recorded in Aralam Wildlife Sanctuary, Kerala. *Leptus* larvae were collected from the host's abdomen, antennae and hind wings. These larvae penetrate the host's cuticle, creating deep puncture wounds, particularly in the abdominal region, to feed on body fluids. The heavy infestation suggests that the larvae exploit the grasshopper not only for nourishment but also as a means of dispersal.

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KEY WORDS: Ectoparasite, mites, infestation, dispersal

Interactions between insects and ectoparasitic mites are widespread, influencing host ecology, behaviour, and population dynamics. *Leptus* Charles De Geer, 1778 (Acari, Erythraeidae) larvae are six-legged parasitic mites infecting a variety of arthropod hosts, including, though rarely, grasshoppers. *Eyprepocnemis alacris alacris* (Serville, 1838) (Orthoptera, Acrididae), is native to the Western Ghats of India, particularly in Kerala.

Field surveys were conducted in Aralam Wildlife Sanctuary, Kerala, India (11.9536° N; 75.8284° E), to assess the prevalence and distribution of short-horned grasshoppers. Sampling was conducted

between May 2022 and December 2024, for 10 days each month, between 09:00 AM and 12:00 PM. Grasshoppers were collected using standard sweep netting techniques across various microhabitats within the sanctuary. During summer, grasshoppers were more conspicuous due to reduced vegetation density. A close examination of collected specimens in the field revealed one short-horned grasshopper *specimen* with small, bright orange-red larval mites attached to the intersegmental membranes of the thorax and abdomen, particularly at the coxa–sternum junctions, and occasionally near the spiracles. The specimen also showed some external abnormalities and

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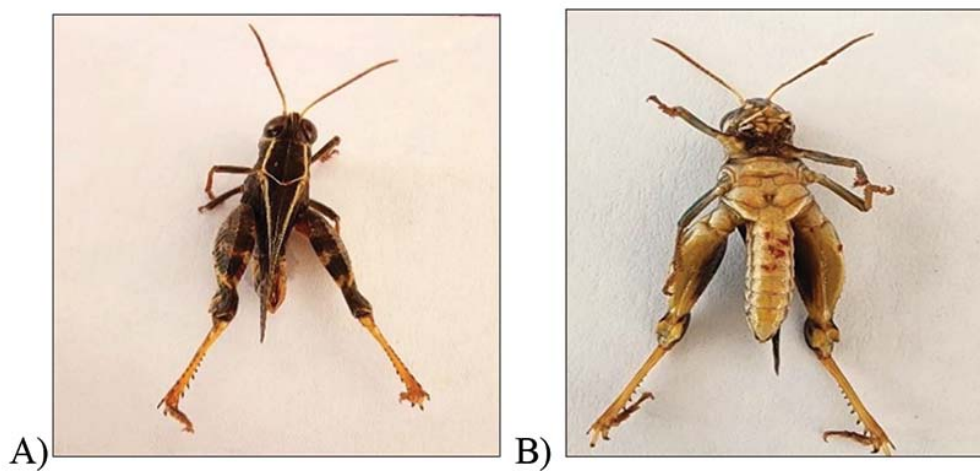


Fig. 1 Parasitised host body (Dorsal A & Ventral B) showing antennoal, leg and abdominal infection



Fig. 2 Collected larvae from the host

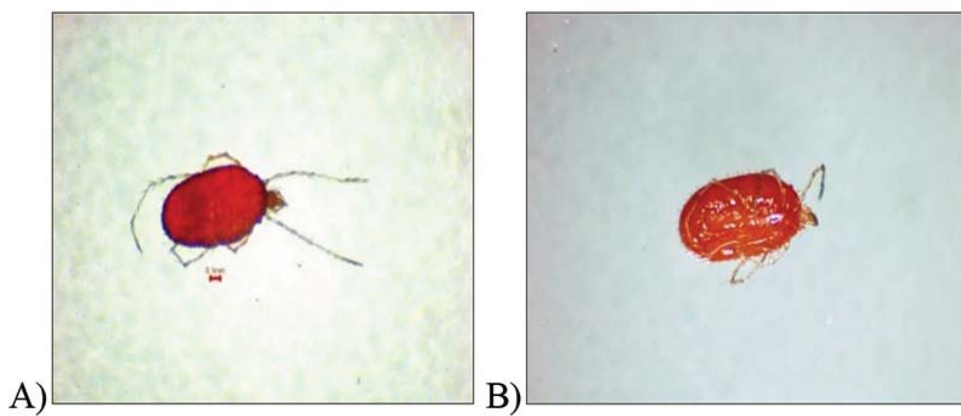


Fig. 3 Stereomicrographs of *Leptus* larvae (A: Dorsal, B: Ventral view) from the host body

sluggish movement. This individual was separated from the sweep net collection and kept in a separate bottle for detailed laboratory examination.

Infected sample were preserved in ethanol (70%) and subjected to detailed laboratory analysis to identify the parasite and assess its effects. Larvae were gently extracted using fine forceps and mounted on slides in lactic acid (50%). The slides were briefly warmed over a 40W electric lamp until lactic acid fumes appeared, facilitating better clearing of the specimens. Microscopic examination was performed using a Magnus Trinocular Stereo Microscope, and images were captured via a connected computer system. Identification of the host was based on morphological characteristics using standard taxonomic keys and expert consultation. The parasite was confirmed as six-legged larval mites belonging to the genus *Leptus*, identifiable by their distinct morphology under magnification (Figs. 1, 2, 3).

Host (*Eyprepocnemis alacris alacris*): Nymphs and adults occur among long coarse grass with bushes. This species considerably causes damage to paddy, maize, cucumber, oat, cowpea and fodder-like grasses. Both adults and nymphs are found from April, May to November. The trend of the population is a biennial generation (Shishodia, 2000). It is distributed in the states of Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Haryana, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Punjab, Tamil Nadu and West Bengal.

Parasite: Species of *Leptus* is characterised by gnathosoma with elongated, chelate cheliseræ; palps 5 segmented with terminal sensory setae; and pretarsal, claws or hooked setae for attachments. Larva is bright orange red with soft cuticle, sensory setae present on idiosoma, muscular pharynx adapted for fluid feeding and the absence of reproductive organs. In India, *Leptus* mites are typically found in various habitats like tropical and subtropical forests, open grasslands, savannahs,

farmlands and fields. They live in leaf litter and soil where they can find their hosts and where conditions are suitable for their survival. It is distributed in the states of Maharashtra, Karnataka, Kerala, Tamil Nadu, West Bengal, Uttar Pradesh, Himachal Pradesh and Punjab.

This is the first record of *Leptus* larval parasitism on the short-horned grasshopper *E. alacris alacris*. The finding broadens the known host range of *Leptus* mites and provides new insight into their ecological interaction with Acrididae.

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Studies on developmental biology of lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae) at different seasons on paddy

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ABSTRACT: Developmental biology of *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae) was conducted using the paddy variety RNR-15048 across three seasons: the rainy season, winter season and summer season. The results indicated that the highest and lowest fecundity was observed during the rainy season and summer seasons 200-220 eggs and 46-57 eggs per female, respectively. The longest egg period was found in winter with a mean of 7.80 ± 0.77 days, while the shortest duration was observed with a mean of 4.53 ± 1.33 days in summer. The grub and pupal period were longest in winter with an average of 75.33 ± 7.00 days, and shortest in summer with an average 35.23 ± 1.74 days. The data showed that adult male lesser grain borers take about 69-74 days for egg laying to adult emergence in the winter, and 31-37 days in summer, while adult females require long time of 81-93 days in winter, and 33-39 days in summer.

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KEY WORDS: Adult longevity, fecundity, life cycle, seasonal effect, sex ratio

Rice (*Oryza sativa* L.) is a vital cereal crop worldwide, serving as a staple food for more than half of the global population. Its significance is emphasized by the economic and qualitative aspects of rice production, distinguishing it from other cereals. Unlike other grains, rice is typically consumed as whole grains. However, when stored for over three months, rice becomes highly susceptible to insect infestations, leading to both quantitative and qualitative losses (Manandhar *et al.*, 2018). In India, these losses range from 5 to 25 per cent due to storage insects (Prakash and Rao, 1995). *Rhyzopertha dominica* (F.) (Coleoptera,

Bostrichidae) is one of the most significant pests affecting stored cereals, including rice, the adult female lays eggs outside the grain kernel, and the newly hatched larvae feed voraciously inside, developing within the grain until they emerge as adults (Sola *et al.*, 2017). Notably, *R. dominica* causes damage at both grub and adults stages. Adult beetles exhibit migratory behaviour between storage facilities, leading to substantial losses in both quantity and quality of grain and its derivatives (Arthur *et al.*, 2012; Ramalakshmi *et al.*, 2024). Moreover, infestation by *R. dominica* diminishes essential amino acid levels in wheat, maize, and

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sorghum, as well as the germination capacity and vigour of seeds (Jood *et al.*, 1996). Infested grains become vulnerable to further damage by secondary pests and fungi, amplifying the overall detriment to stored grains (Subramanyam *et al.*, 1996). Common strategies to minimize insect infestation during storage include altering the abiotic components of storage, such as grain moisture content, temperature conditions, and the type of storage structure, to prevent insect development. Given the pest's importance, laboratory studies were conducted to understand its biology better.

The biology of *R. dominica* was studied using the paddy variety RNR-15048, sourced from the seed production unit of Centurion University of Technology and Management, during the rainy season (July-October 2022), winter season (November 2022-February 2023), and summer season (March-June 2023) at the Entomology laboratory, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management. Grains were disinfected (Kumawat, 2007) and equilibrate moisture content

in grains (Solomon, 1951). This pre-conditioned seed material was then used for the biology studies. Ten pairs of 1- to 2-day old *R. dominica* adults were introduced into Petri dishes (10 cm diameter, 1.5 cm depth), covered with black paper (21 cm × 15 cm), and containing 50 paddy grains for egg-laying. Eggs were collected and transferred to glass vials (9.5 cm long × 2.5 cm diameter) containing 10 grams of grains. Each vial was inoculated with 30 eggs, with ten replications maintained to observe the total developmental period from egg to adult and to assess the sex ratio. Additionally, 50 eggs were placed in separate Petri dishes, with five dishes serving as replications. These setups were provided with a few sound grains to study the incubation, larval, and pupal periods, as well as adult longevity, pre-oviposition, oviposition, and post-oviposition periods, and fecundity.

The findings revealed significant seasonal variations in developmental periods, fecundity, and longevity (Table 1). The egg period ranged from 5-8 days (mean 6.70 ± 0.94 days) in the rainy season, 7-9 days (mean 7.80 ± 0.77 days) in winter, and 3-7

Table 1. Developmental period and biological attributes of *R. dominica* on rice (Var. RNR-15048)

Life Stages and attributes	Developmental period (days) and fecundity (no) during different seasons					
	Rainy season		Winter season		Summer season	
	Range	Mean±SD	Range	Mean ±SD	Range	Mean ±SD
Egg period (n=60)	5-8	6.70±0.94	7-9	7.80±0.77	3-7	4.53±1.33
Grub and pupal period (n=60)	31-45	40.2±4.05	62-83	75.33±7.00	32-37	35.23±1.74
Total development period -male (n=30)	37-42	40.06±1.63	69-74	71.16±1.86	31-37	34.16±1.63
Total development period –Female (n=30)	39-46	42.16±2.29	81-93	87.70±4.49	33-39	37.07±1.93
Sex ratio		1.27:1		1.08:1		0.72:1
Pre-oviposition period (n=30)	4-6	4.77±0.80	5-8	6.37±1.14	6-13	9.1±1.98
Oviposition period (n=30)	22-28	24.73±2.06	15-24	22.23±3.18	10-18	12.5±2.24
Fecundity (eggs/female) (n=30)	200-220	211.16±6.28	89-134	113.03± 16.01	46 -57	52.3±2.97
Post-oviposition period (n=30)	7-9	8.03±0.87	6-7	6.50±0.50	4-7	5.33±0.78
Longevity of Adult Male (n=30)	25-38	35.20±3.96	33-41	38.03±2.81	13-27	19.63±5.6
Longevity of Adult Female (n=30)	35-41	38.50±1.66	37-45	39.26±1.94	16-29	24.63±3.31

Note: Rainy season (July-Oct, 2022; mean min temp-17.4°C; mean max temp-29.0°C; Avg. RH -87.89%); Winter season (Nov, 2022-Feb, 2023; mean min temp- 7.3°C; mean max temp-28.9°C; Avg. RH -75.51%); Summer season (March-June, 2023; mean min temp- 15.63°C; mean max temp-36.05°C; Av.RH-69.2%); n= Number of individuals observed

days (mean 4.53 ± 1.33 days) in summer, indicating the shortest duration during summer. The grub and pupal period also varied considerably, being 31-45 days (mean 40.20 ± 4.05 days) in the rainy season, 62-83 days (mean 75.33 ± 7.00 days) in winter, and 32-37 days (mean 35.23 ± 1.74 days) in summer.

From egg-laying to adult emergence, males required 37-42 days (mean 40.06 ± 1.63 days) in the rainy season, 69-74 days (mean 71.16 ± 1.86 days) in winter, and 31-37 days (mean 34.16 ± 1.63 days) in summer. Females took a slightly longer duration, ranging from 39-46 days (mean 42.16 ± 2.29 days) in the rainy season, 81-93 days (mean 87.70 ± 4.49 days) in winter, and 33-39 days (mean 37.07 ± 1.93 days) in summer. Notably, the sex ratio (female: male) varied across seasons, being 1.27:1 in the rainy season, 1.08:1 in winter, and 0.72:1 in summer, indicating a male-biased emergence during summer.

The pre-oviposition period was shortest in the rainy season (4-6 days, mean 4.77 ± 0.80 days) and longest in summer (6-13 days, mean 9.10 ± 1.98 days). Similarly, the oviposition period was longest during the rainy season (22-28 days, mean 24.73 ± 2.06 days), followed by winter (15-24 days, mean 22.23 ± 3.18 days) and summer (10-18 days, mean 12.50 ± 2.24 days). Correspondingly, fecundity was highest in the rainy season (200-220 eggs, mean 211.16 ± 6.28 eggs per female) and lowest in summer (46-57 eggs, mean 52.30 ± 2.97 eggs per female). Post-oviposition periods varied across seasons, being longest in the rainy season (7-9 days, mean 8.03 ± 0.87 days) and shortest in summer (4-7 days, mean 5.33 ± 0.78 days).

Adult longevity also showed seasonal differences. Males lived 33-41 days (mean 38.03 ± 2.81 days) in winter, 25-38 days (mean 35.20 ± 3.96 days) in the rainy season, and 13-27 days (mean 19.63 ± 5.60 days) in summer. Females exhibited longer lifespans than males in all seasons, with 37-45 days (mean 39.26 ± 1.94 days) in winter, 35-41 days (mean 38.50 ± 1.66 days) in the rainy season, and 16-29 days (mean 24.63 ± 3.31 days) in summer.

These findings align with earlier studies. Kumawat (2007) reported that fecundity peaked at moderate temperatures ($30 \pm 10^\circ\text{C}$) with relative humidity

($75 \pm 5\%$), similar to the present study's observations during the rainy season. Conversely, fecundity was reduced under unfavourable conditions, such as higher temperatures and lower humidity, consistent with the observed trends in summer. Win *et al.* (2020) demonstrated significant seasonal differences in fecundity and developmental durations, with higher egg counts and shorter developmental periods during the rainy season. Kumar *et al.* (2017) also reported comparable developmental trends, highlighting the influence of temperature and humidity on insect biology. Ajaykumara *et al.* (2018) noted seasonal impacts on the life cycle of *R. dominica*, with shorter durations in summer and kharif seasons. The present study corroborates these findings, emphasizing the influence of seasonal variations on the life history traits of *R. dominica*.

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**First record of *Tachardiaephagus tachardiae*
(Howard, 1896) (Hymenoptera, Chalcidoidea, Encyrtidae)
from an invasive Lac insect, *Kerria destructor*
Talukder and Das, 2020 (Hemiptera, Kerriidae)**

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ABSTRACT: An invasive species of Lac insect, *Kerria destructor* Talukder and Das, 2020 was described from West Bengal, India. Since then, there is no record of any natural enemies on the insect. Here, reporting an encyrtid parasitoid, *Tachardiaephagus tachardiae* (Howard, 1896) as natural enemy of the invasive lac insect. *Annona squamosa* and *Peltophorum pterocarpum* are reported as new host plants for this pest with its geographical extension in Andhra Pradesh. Intraspecific variation was noted in the number of anal ring setae of *K. destructor*. © 2025 Association for Advancement of Entomology

KEY WORDS: Parasitoid, new host plants, geographical extension, intra-specific variation

Invasive species quickly spread in a new environment due to their high reproductive potential and lack of competitors or natural enemies. The Parties of Convention on Biological Diversity (CBD) prepared Strategic Action Plan recognizing threat from Invasive Alien Species to biodiversity. The National Biodiversity Authority (NBA) has enlisted 157 Invasive Alien Species of animals from India which includes 59 terrestrial fauna and 98 marine fauna (Sandilyan *et al.*, 2019). Majority of them are insects belonging to the order Hemiptera followed by Lepidoptera, Diptera and Hymenoptera. The Lac Insect, *Kerria destructor* was described by Talukder and Das in 2020 from West Bengal, reporting mortality of road side rain tree (*Albizia saman* (Jacq)) in urban areas of West

Bengal. Severe infestation of lac resulting in defoliation and death of rain tree in various districts is reported as serious threat to biodiversity, and the rain tree has great potential for CO₂ sequestration (Talukder and Das, 2020). *Albizia saman* is also known to be infested with six species of *Kerria* (Hemiptera, Kerriidae) viz., *K. chinensis* (Mahdihassan), *Kerria destructor* Talukder and Das, *K. fici* (Green), *K. lacca* (Kerr), *K. maduraiensis* Ahmad and Ramamurthy and *K. pusana* (Misra). The family Kerriidae includes 27 species worldwide including 23 species from India. The identification of lac insect is based on the slide mounted globose adult female involving complex morphological characters. *Kerria destructor* differ from related *Kerria* spp. in having circular brachial

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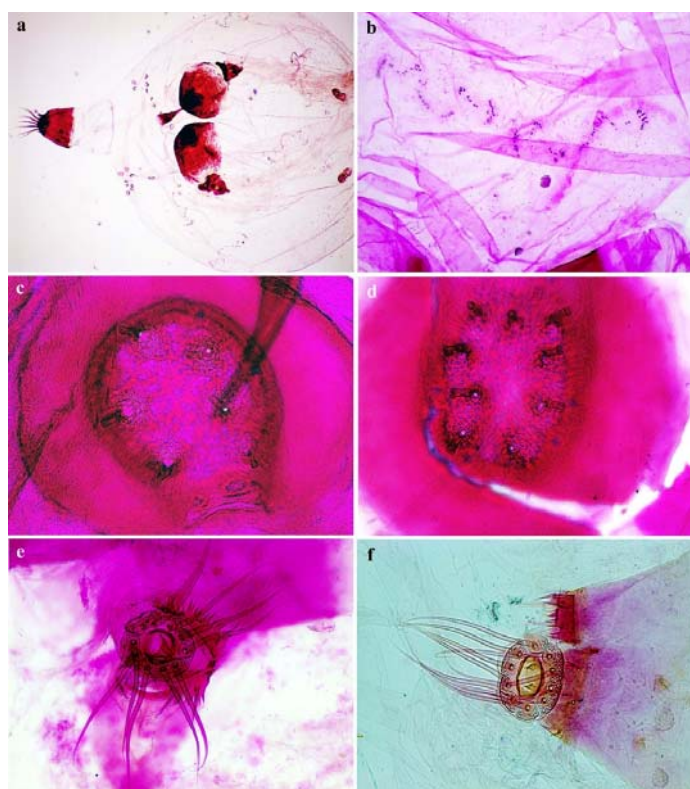


Fig. 1. *Kerria destructor*, female: **a**- adult female, **b**- marginal duct clusters, **c**- brachial plate with six dimples, **d**- brachial plate with nine dimples, **e**- anal ring with 10 setae, **f**- anal ring with 11 setae, intraspecific variation

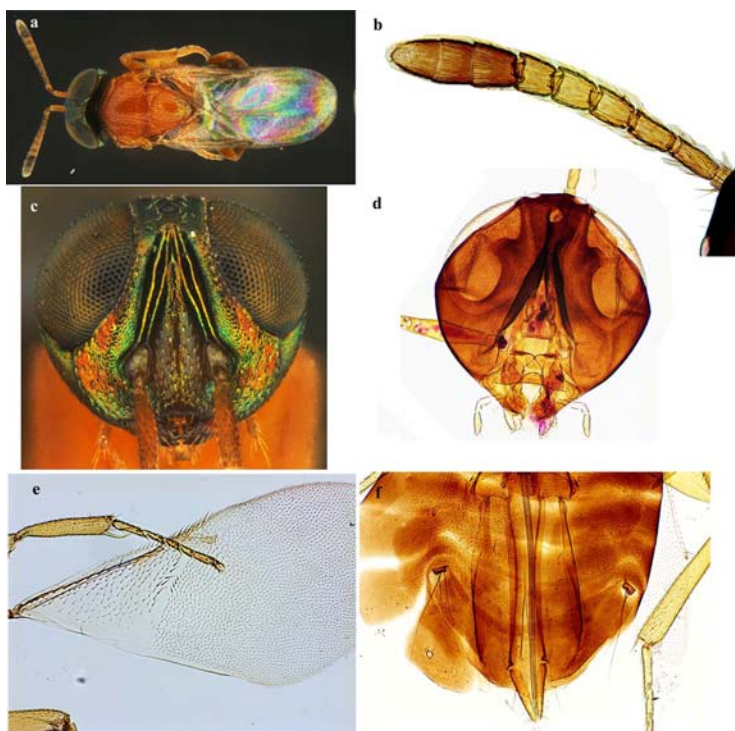


Fig. 2 *Tachardiaephagus tachardiae*: **a**. habitus, female; **b**. antenna; **c**. head, frontal view; **d**. head, ventral view; **e**. fore wing; **f**. ovipositor

plate with 6-9 dimples and in lacking quinquelocular pores near mouth parts. Intraspecific variation was noticed in the number of setae on anal ring being 10 or 11 in a female.

Lac insects are beneficial Hemiptera pests of tree species, and are host specific, mainly *Butea monosperma*, *Schleichera oleosa* and *Ziziphus mauritiana*. They are commercially cultivated in Central to Central-East Indian states Chhattisgarh, Madhya Pradesh, Odisha, Jharkhand and West Bengal. Of these, Jharkhand produces nearly 71 per cent of the total Lac produced in the country (Yogi *et al.*, 2014). However, they spread in to non-commercial crops outside the range of agricultural fields is of concern and great loss of avenue trees. Recently, Lac insect encrustation were noticed on twigs of *Annona squamosa* in Guntur Dist. of Andhra Pradesh, *Albizia saman* in Khammam Dist. and *Peltophorum pterocarpum* in Hanamkonda Dist. of Telangana States. So far, *K. destructor* was known from the *A. saman* only, and restricted to West Bengal. Here, *Annona squamosa* and *Peltophorum pterocarpum* are reported as new host plants for this pest, with its extended distribution range in Andhra Pradesh and Telangana. A newspaper reported death of over 2000 rain trees around Kolkata (Anonymous in 2015 in Talukder and Das, 2020), and also the invasive lac species is severely affecting rain trees in rural areas. The spread of *K. destructor* in Andhra Pradesh and Telangana is of concern specially for the fruit plant, *A. squamosa*, and an ornamental tree, *P. pterocarpum*.

The Lac insect encrusted twigs were cut and placed in plastic tubes for parasitoid emergence, later filled with alcohol, and sent to the Zoological Survey of India, Kolkata by Prof. S.R. Koteswar Rao for identification. A few female lac insects and emerged parasitoids were mounted on glass slides following standard mounting methods. The Lac insects were identified using the key of Ahmead *et al.* (2013) and Talukder and Das (2020).

Material examined. *K. destructor*: INDIA: Telangana, Chennur, Manchiryal, 34 ♀ on *Albizia saman*, 06.iv.2025, S.R.K. Rao; Manchiryal,

Chennur, 25 ♀ on *Annona squamosa*, 21.x.2024, S.R.K. Rao; Telangana, Hanamkonda Dist., 12 ♀ on *Peltophorum pterocarpum*, 25.i.2025, S. R. K. Rao; Khammam, 11 ♀ on *Albizia saman*, 5.xi.2024, S.R.K. Rao.

Majority of Encyrtidae are primary parasitoid of larvae of Hemiptera. *Tachardiaephagus tachardiae* is known to parasitise *K. lacca*, however it is the first time the parasitoid is recorded from the invasive Lac insect, *K. destructor* from Andhra Pradesh and Telangana.

Tachardiaephagus tachardiae (Howard, 1896) (Hymenoptera, Chalcidoidea, Encyrtidae)

Encyrtus tachardiae Howard, in Howard & Ashmead, 1896: 641, male. Sri Lanka, Pundaluoya (USNM)

Tachardiaephagus thoracicus Ashmead, 1904: 303, 390, female. Synonymy by Ferrière, 1928: 171.

Lissencyrtus troupi Cameron, 1913; *Lissencyrtus troupi* Cameron: Mahdihassan, 1925; *Tachardiaephagus flavipodus* Kaul & Agarwal, 1986.

Tachardiaephagus hyderabadensis Kaul & Agarwal, 1986.

Tachardiaephagus kashmirensis Kaul & Agarwal, 1986.

Adult female parasitoid is metallic green with orange yellow mesosoma; antennae and legs yellowish, antennal club brown and scrobes long, impressed deeply and reaching nearly three quarters distance from toruli to anterior ocellus, inter-torular prominence triangular, separated from frontovertex. Males are similar to female, except dark brown flagellomeres (personal observation by Rameshkumar).

Parasitoid: INDIA: Telangana, Chennur, Manchiryal, 1 ♀, 06.iv.2025, ex *K. destructor* on *Albizia saman*, Coll. S.R.K. Rao (Deposited in NZC, ZSI, Kolkata).

Remarks. The record of the parasitoid

T. tachardiae from *K. destructor* indicates that it could be a promising biocontrol agent to minimize pest population in avenue host tree.

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AUTHOR INDEX

- | | |
|-----------------------------------|-----------------------------------|
| Abhijit Mazumdar, 261 | Monsoon Jyoti Gogoi, 253 |
| Agur Litin, 253 | Mounika T., 371 |
| Aiswarya Joy, 347 | Paramita Banerjee, 261 |
| Anil Kumar Dubey, 375 | Pradeep Kumar Dalal, 307 |
| Anitha Mamillapalli, 317 | Rabeesh T.P., 325 |
| Ankita Sarkar, 261 | Rahul S. Kamble, 335 |
| Anooj S.S., 347 | Rajkamal Goswami, 253 |
| Baraik B., 355 | Rajmohana K., 355 |
| Basak J., 355 | Ramalakshmi V., 371 |
| Brinda Goda Lakshmi Didugu, 317 | Ramesha B., 347 |
| Deepa Jaiswal, 269 | Rameshkumar A., 375 |
| Deepayan Padhy, 371 | Ravi Singh, 307 |
| Deepthy T., 367 | Roy M., 355 |
| Degsew Bezabih Gebresilassie, 317 | Sagar A. Vhanalakar, 335 |
| Devadoss Kumar, 269 | Sameer Kumar Singh, 307 |
| Devika Srivastava, 299 | Sangeetha B.G., 291 |
| Drisya Pavithran, 291 | Santhana Bharathi N., 325 |
| Equbal Ahmad Md., 363 | Sayyad Sadiya Kousar, 317 |
| Gajanan K. Sontakke, 335 | Seena Narayanan Karimbumkara, 253 |
| Goutam Hosamani, 371 | Shamsudeen R.S.M., 367 |
| Haroon Rashid MD, 317 | Shiva Shankar, 269 |
| Himanshu Dawda, 311 | Shrikant Jadhav, 269 |
| Jayaprakas C.A., 291 | Siji J.V., 291 |
| Kamal Ravi Sharma, 307 | Somnath Kangralkar, 311 |
| Karuthapandi Madasamy, 269 | Sreekumar K.M., 347 |
| Kimmi Kumari, 363 | Sujatha K., 325 |
| Koteswar Rao S.R., 375 | Susmita Gudulkar, 311 |
| Kusumala Suman, 299 | Umesh Chandra, 307 |
| Madhavi Kasa, 317 | Usha Mukundan, 311 |
| Manish Kumar Yadav, 371 | Vaishnavi Ponugupati, 317 |
| Manju Mary Paul, 347 | Venkatesh Kumar R., 299 |

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