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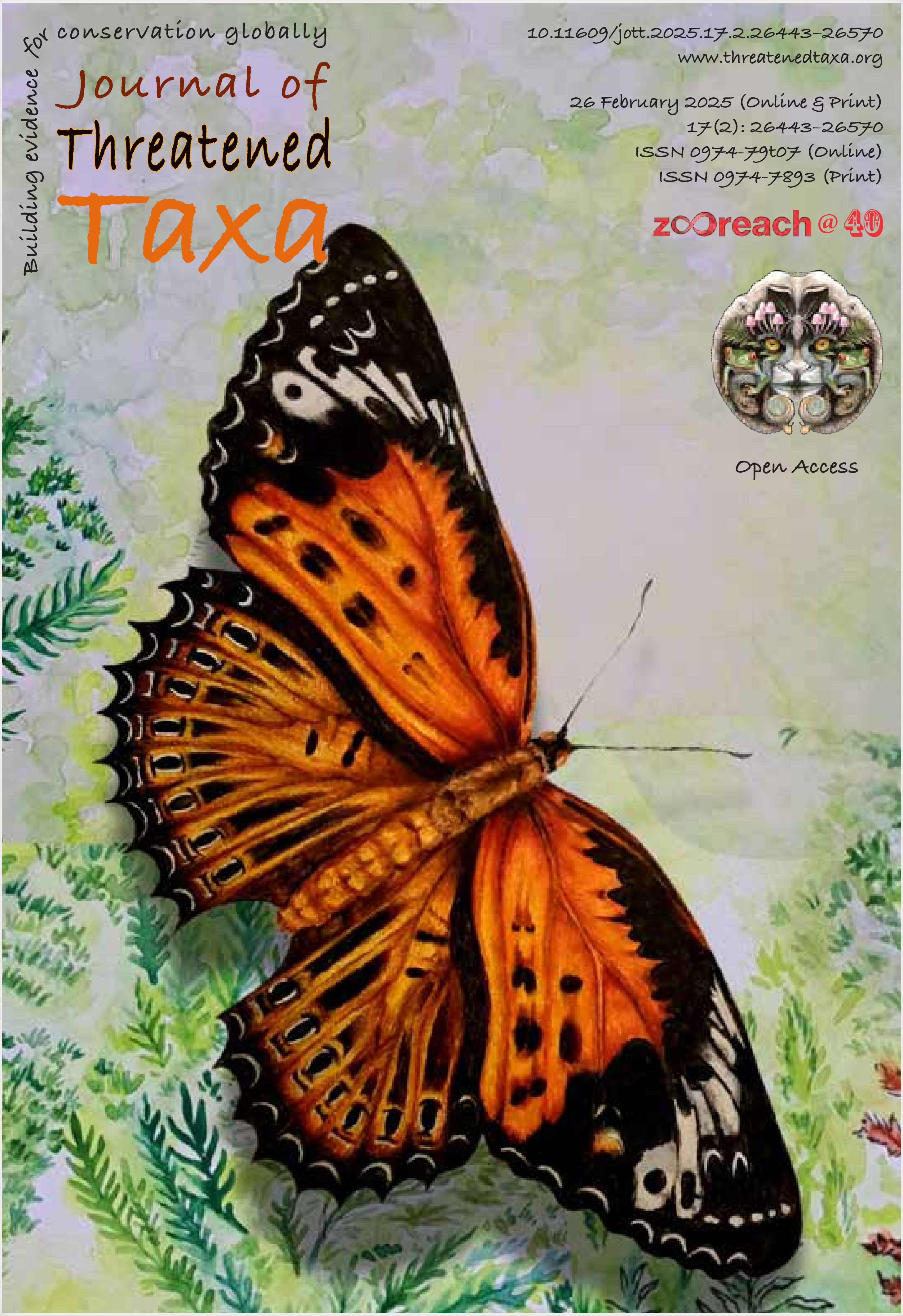

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**Cover: Tamil Lacewing *Cethosia nietneri* with colour pencils and watercolours for the background; detailing with fine liners by Elakshi Mahika Molur.**

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ARTICLE

# Culture and provisioning: the case of Human-Long-tailed Macaque *Macaca fascicularis* (Raffles, 1821) interactions in Sumile, Butuan City, Philippines

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**Abstract:** Understanding human-macaque interactions is crucial for species conservation and management. Hence, this study investigated the Human-Long-tailed Macaque *Macaca fascicularis* (Raffles 1821) interactions in Sumile, Butuan City from July 2022 to April 2023 through one-on-one interviews. A total of 271 randomly selected respondents were surveyed to determine their demographic and socioeconomic profiles. Their knowledge, attitudes, and perceptions of human-macaque interactions such as provisioning, regulation measures, and associated factors were also determined. Most of them were aware of the cultural importance of macaques (86.35%). The majority were not aware of the ecological (73.43%) and socioeconomic importance of macaques (52.03%), as well as RA 9147 or Wildlife Act (61.26%). Most residents provisioned macaques (99.26%). Macaque behaviors were household food foraging (94.84%), crop foraging (31.78%), trash consumption (69.37%), and trash dropping (30.63%). Most residents did nothing to food foraging in households (53.51%) or crop foraging (58.30%) while some resorted to throwing hard objects, hand clapping, or dog chasing. Educational attainment was the most common factor significantly associated with residents' knowledge. Length of residency and educational attainment were significantly associated with provisioning frequency while length of residency and occupation were associated with the prohibition of provisioning to avoid negative human-macaque interaction. This study implies that cultural factors influence rampant macaque provisioning. If uncontrolled, provisioning may lead to economic and health losses and negative attitudes toward macaques and conservation efforts. The top three human-macaque regulation measures suggested by most residents were government action, effective waste disposal, and increasing the food base in the forest. Researchers further recommend local conservation area establishment; culture-sensitive and controlled provisioning; and community-based conservation. This also entails educating the public on the adverse impacts of uncontrolled provisioning. Furthermore, periodic monitoring of macaque populations and conservation management strategies that balance ecological, socio-economic, and cultural considerations for human-macaque co-existence is necessary.

**Keywords:** Conservation, crop foraging, cultural belief, Elijan Park, foraging behavior, local communities, management, monkey feeding, perceptions, regulation measures.

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**Author contributions:** FHL, DSA & SLP conceptualized & designed the research, wrote the paper, reviewed, edited, and approved the submission of the final paper. FHL & DSA performed the surveys and field work activities and analysed the data.

**Filipino abstract:** See end of this article.

**Acknowledgements:** We express our gratitude to the local government officials of Sumile who allowed us to conduct the surveys in their communities. We are also grateful for the assistance and significant information from the Elijan Park caretakers and the local government head. Most of all, we highly appreciated the contribution of the mentors and the advisory committee of Fritche H. Lapore and Debbie S. Aseňas as this paper is part of their undergraduate thesis.

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## INTRODUCTION

Long-tailed Macaques *Macaca fascicularis* (Raffles, 1821) are found across southeastern Asia (Eudey et al. 2020) and are now classified as 'Endangered' due to habitat destruction and exploitation (Hansen et al. 2022). One of the 10 subspecies of *M. fascicularis* is found in the Philippines, which is *M. fascicularis philippinensis* (Grunstra et al. 2023), specifically known as the Philippine Long-tailed Macaque. Macaques often share space and food with humans which is an important aspect of the human-primate interface (Fuentes et al. 2007). The adaptability, flexibility, and synanthropic nature of Long-tailed Macaques enable them to inhabit diverse habitats including anthropogenic areas (Gumert et al. 2011), and consume various foods including human food (Sha & Hanya 2013). The macaque's adaptability to human-modified landscapes, their crop-foraging behavior, and the complex cultural and religious associations have made them particularly challenging for wildlife managers. The Long-tailed Macaques are often subject to culling despite being recently elevated to Endangered status on the IUCN Red list due to negative human-macaque interactions (Gamalo et al. 2023). The endemic Toque Macaque *Macaca sinica* in Sri Lanka is considered both endangered and nuisance species in certain habitats due to increased interactions with humans and crop foraging (Jayapali et al. 2023). In Nepal, negative Human-Rhesus Macaques *Macaca mulatta* interactions resulted from expanding monocultures, forest fragmentation, and habitat degradation (Koirala et al. 2021).

Long-tailed Macaques are frequently observed in roadsides, temples, towns, tourist sites, and agricultural areas (Muroyama & Eudey 2004; Lee & Priston 2005; Gumert et al. 2011). In these human-dominated areas, macaques are often provisioned by humans. Multiple factors contribute to macaque provisioning. Sengupta & Radhakrishna (2018) found that human attitudes significantly influence the degree of human-primate interaction. Their study highlighted the cyclical pattern where macaques are attracted to areas with food provided by humans, who in turn are motivated to feed them due to their behavior. Research in China and India showed that macaque provisioning was mainly due to concerns about wildlife food scarcity, desire for close observation, cultural factors, and religious beliefs (Zhao 2005; Sengupta & Radhakrishna 2018, 2020).

Macaque provisioning is prevalent in anthropogenic areas of the Philippines, including parks and human settlements adjacent to forests. Food provisioning was observed to be common in Puerto Princesa Subterranean River National Park in Palawan (Gamalo et al. 2019), and Subic Bay. In Butuan City, particularly in Barangay Sumile, food provisioning for the Philippine Long-tailed Macaques is frequently observed. These macaques inhabit Elijan Park in proximity to human settlement. Elijan Park is governed by a quasi-religious organization, the Knights of Rizal Agricultural Endeavor Foundation Incorporated (KRAEFI), and was established by tribal chieftain or Datu Santiago B. Ecleo, Sr. (Dominador Paglinawan pers. comm. 20.i.2022). The majority of residents in Sumile are affiliated with the KRAEFI organization. Most residents perceived macaque provisioning as an act of benevolence. KRAEFI members typically provide corn, bananas, and other human food items to macaques inside and outside the park boundaries. Elijan Park caretakers were also observed permitting tourists to provision the macaques.

Food provisioning can aid macaque survival (Kurita 2014). Macaques may consume human food during periods of natural food shortages to supplement their diet (McKinney 2010) which could similarly benefit macaques in Sumile during a food crisis. Food provisioning may also have negative consequences such as the attenuation of macaque natural survival instinct (Dubois & Fraser 2013), increased risks of human-macaque infectious disease transmission (Chapman et al. 2005), restriction of ecological functions, e.g., seed dispersal capabilities (Sengupta 2015), alterations in habitat use (Sengupta & Radhakrishna 2018), changes in macaque behavior (Hsu & Kao 2009) and abrupt increase in reproduction and population size (Sengupta & Radhakrishna 2020).

Macaques adapt to provisioning, and subsequently exhibit a preference for and actively seek human-provided food (Lee & Priston 2005). This often leads to an overabundance of macaques in human settlements, resulting in negative human-macaque interaction (Sengupta & Radhakrishna 2020) such as foraging in kitchens or refuse containers (Unwin & Smith 2010) and consuming crops (Agyei et al. 2019; Li & von Essen 2021). Crop and refuse foraging are linked to food provisioning issues in Puerto Princesa Subterranean River National Park, Palawan (Gamalo et al. 2019). Similarly, in Sumile, macaques forage in households, nearby farms, and refuse disposal sites. These are perceived as a consequence of macaque adaptation to provisioning.

Without regulation, provisioning in Sumile could lead to an overabundance of macaques and negative human-macaque interactions, fostering negative perceptions among residents (Muroyama & Eudey 2004; Matheson et al. 2006; Kuswanda & Hutapea 2023), and hindering conservation efforts. Identifying sociocultural predictors of human-macaque interactions is crucial for developing adaptive conservation plans for Long-tailed Macaques in Sumile (Humle & Hill 2016). Education programs, targeted behavior management, and consideration of cultural contexts are crucial components of such plans.

Therefore, the study aimed to assess the socioeconomic and cultural background of the respondents; knowledge of macaque significance and relevant legislation; perceptions and attitudes toward macaque provisioning and other human-macaque interactions, management measures, and conservation strategies; and associated socio-economic factors. This research aimed to prove if all Sumile residents engaged in provisioning and whether educational attainment, length of residency, and income negatively correlated with the provisioning frequency and positively correlated with its prohibition as a management measure alongside cultural factors. Studies like this are important, particularly in areas where sociocultural factors play a complex and important role in shaping human-macaque interactions. According to Priston & Mclennan (2012) and Dacks et al. (2019), it is essential to incorporate sociocultural indicators, alongside ecological ones for developing holistic conservation strategies in human-dominated landscapes where macaques reside. The findings will furnish critical information to aid policymakers in developing adaptive management measures and conservation interventions that respect cultural and socio-economic values. This research can guide information, education, and communication initiatives in Sumile, particularly concerning the ecological significance of macaques, protective laws, specific threats, and the importance of human-macaque co-existence. Additionally, it will provide baseline data for future studies on human-macaque interactions and related primate behavior.

## METHODS

### Study site

Sumile is located in Butuan City, Agusan del Norte, Mindanao Island, Philippines, at 8.826°N, 125.626°E with an elevation of 116.3 m (381.6 ft) (Image 2). It was declared as "barangay" on 30 May 30 1986, under Ordinance No. 450–85. It has a population of 2,271, which comprises 0.64% of Butuan's total population. Its population has increased by 585 individuals from 1,814 in 1990 to 2,399 in 2020.

One subspecies, *Macaca fascicularis philippensis* (Grunstra et al. 2023) is endemic to the Philippine archipelago and also inhabits Sumile, Butuan City, particularly in Elijan Park, known as the KRAEFI-Sumile Botanical & Zoological Garden (Image 1). Elijan Park is named after the first settlers in the area "Elijan" — individuals residing near the site since the 1960s (Dominador Paglinawan pers. comm. 20.i.2022). The macaque population is estimated at approximately 500 and is divided into three troops according to park caretakers. The Knights of Rizal Agricultural Endeavor Foundation, Inc. (KRAEFI) organization currently manages Elijan Park (Dominador Paglinawan pers. comm. 20.i.2022). Numerous tourists visit Elijan Park primarily due to the presence of macaques. The tourists do not pay entrance fees as the local government still complies with the necessary documents to officially designate the park as tourism destination (Dominador Paglinawan pers. comm. 20.i.2022).

Elijan Park was part of the extensive forest in Sumile during the 1970s (Dominador Paglinawan pers. comm. 20.i.2022). This park consists of primary and secondary forests with 28 species. The botanical garden is dominated by *Gmelina arborea*, followed by *Swietenia macrophylla, Artocarpus blancoi*, and *Shorea contorta* (Glenn Mary Daulat in litt. 20 June 2022). In 2016, the DENR-Caraga also established a dipterocarp (White Lauan) plantation in the area. Sumile has 10 Puroks. Puroks 1,2, & 3, being the closest to Elijan Park, were selected for this research.

**Image 1. KRAEFI-SUMILE Botanical & Zoological Garden (Elijan Park). © Fritche Lapore.**

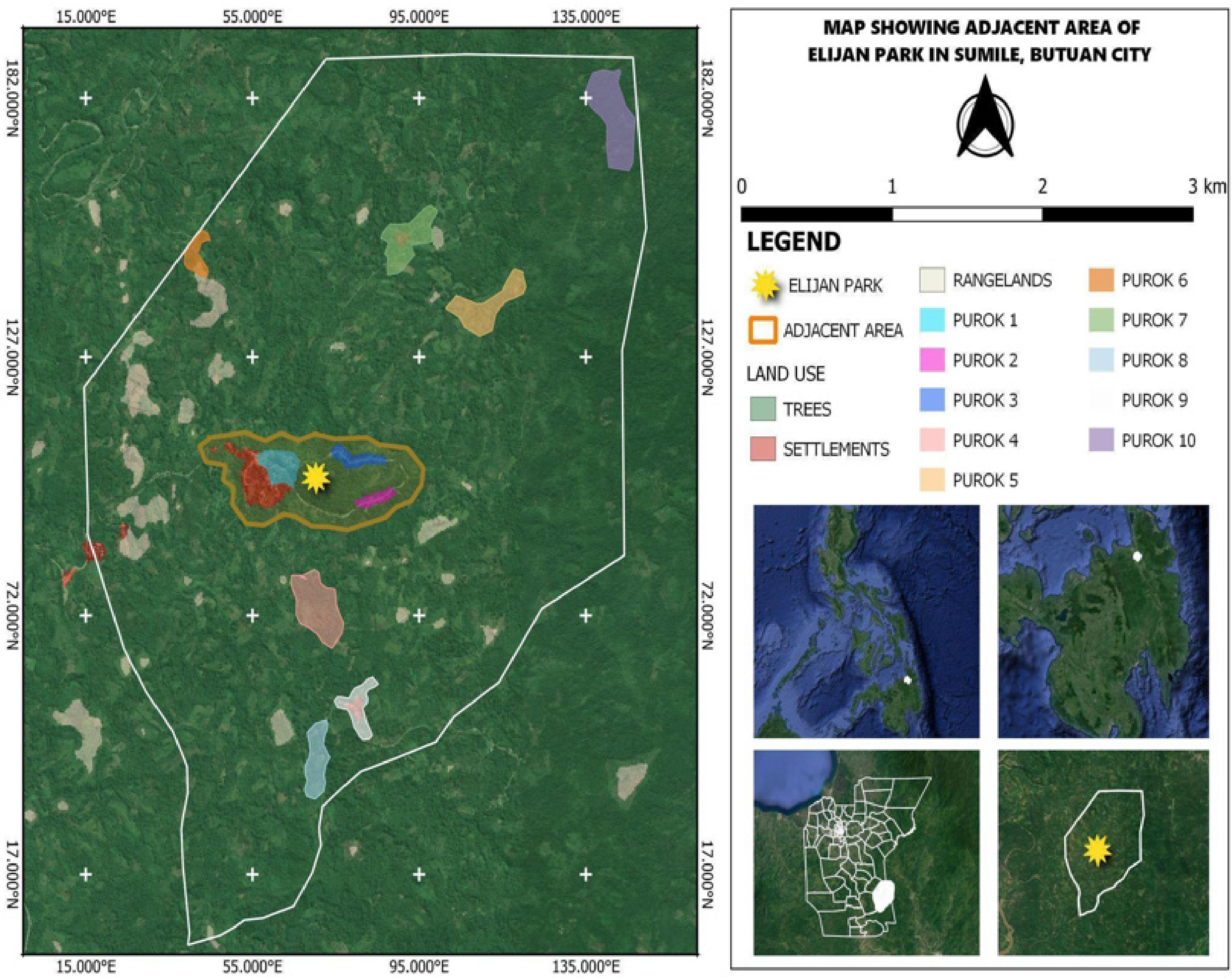


**Image 2. Map of Sumile showing Elijan Park and its adjacent areas (four smaller pictures on the lower right show the location of Elijan Park in the Philippines, Mindanao Island, Caraga, and Sumile perspectives). Source: Butuan City Local Government Unit.**

### Study design, questionnaire, and research ethics

The researchers secured research authorization from Sumile Local Government, KRAEFI, and the City Environment and Natural Resources Office (CENRO-Butuan) prior to initiating the study. A preliminary site visit was conducted for area familiarization, followed by a pilot test of the questionnaire with 15 respondents to gather feedback for revisions. The questions underwent review and validation by subject matter experts. Subsequently, the actual surveys were conducted in three Puroks (Purok 1,2 & 3) of Barangay Sumile, involving 271 randomly selected respondents: 82 in Purok 1, 96 in Purok 2, and 93 in Purok 3. Although the questionnaire was in English, it was translated into Visayan. During one-on-one interviews, respondents provided socio-demographic and economic information (first part of the questionnaire), including gender, civil status, age, length of residency, religion, ethnicity, household size, education, KRAEFI membership, occupation, and income (Appendix 1). The second part of the questionnaire assessed knowledge of macaque importance and conservation legislation and examined perceptions and attitudes toward provisioning, other human-macaque interactions, management, and conservation measures.

### Data analysis

Data were organized, coded, and analyzed utilizing descriptive and inferential statistics from jamovi version 2.3.28. Frequencies and percentages were calculated. The results are subsequently presented using tables, graphs, and qualitative descriptions. The chi-square test of association was used to determine the socioeconomic factors linked to knowledge (RA 9147 and importance of macaques — ecological, socio-economic, and cultural), attitudes (provisioning frequency, and deterrent actions to household food and crop foraging), and perceptions (potential management strategies such as the prohibition of provisioning, translocation, and dog patrolling) of the respondents. All reported statistical tests were conducted at a 95% confidence level.

## RESULTS

### Demographic and socio-economic profile of respondents

The majority of respondents were female (79.34%, n = 215) (Table 1) and married (80.07%, n = 217). The predominant age group was 26–45 years old (53%, n = 143), while the least represented age range was over 66 years (2%, n = 7). More than half of the participants had resided in the area for 16–35 years (57.94%, n = 157). Iglesia Filipina Independiente (IFI) was the dominant religious affiliation (83.13%, n = 225). Regarding ethnicity, only two respondents were indigenous: Higaonon (0.74%). The majority of the respondents were Bisaya (48.34%, n=131). Most households had 1–5 members (56.45%, n = 153). Nearly half of the respondents had attained Junior High School education without completion (44.28%, n = 120). In terms of organization affiliation, 87.82% of the respondents (n = 238) were members of the KRAEFI.

Nine occupations were identified in this study (Table 2), with housewives being the largest occupational group (60.53%, n = 164). The majority of respondents (83.76%, n = 227) had only one employed family member, and more than half of the respondents had no supplementary income sources (60.53%, n = 164). A small proportion of the remaining respondents earned from businesses (sari-sari store, ready-to-wear store, manicure/pedicure services) (3.69%, n = 10) and employment as government employees (8.49%, n = 18). The monthly income for most respondents (83.76%, n = 227) ranged from PHP1,000 to PHP5,000 per month. The primary sustenance came from farm produce such as vegetables, fruits, and root crops.

### Knowledge of respondents: macaque importance and related legislation

Approximately 26.57% (n = 72) of respondents considered macaques ecologically significant, while the majority (73.43%, n = 199) did not (Figure 1). Of those recognizing the ecological importance of macaques, 15.87% (n = 43) identified them as seed dispersers aiding tropical forest diversity. A few respondents (2.21%, n = 6) recognized macaques as important in maintaining forest balance while 1.11% (n = 3) linked them to preserving

**Table 1. Socio-demographic characteristics of the respondents in Sumile, Butuan City.**

| Socio-demographic characteristics | Frequency | Percentage |
|---|---|---|
| **Sex** | | |
| Female | 215 | 79.34 |
| Male | 56 | 20.66 |
| **Civil status** | | |
| Single | 51 | 18.82 |
| Married | 217 | 80.07 |
| Widowed | 3 | 1.11 |
| **Age** | | |
| 18–25 | 59 | 21.77 |
| 26–45 | 143 | 52.77 |
| 46–65 | 62 | 22.88 |
| 66 above | 7 | 2.58 |
| **Length of residency** | | |
| 1–5 | 8 | 2.95 |
| 6–15 | 34 | 12.54 |
| 16–25 | 74 | 27.31 |
| 26–35 | 83 | 30.63 |
| 36+ | 72 | 26.57 |
| **Religion** | | |
| Iglesia Filipina Independiente (IFI) | 225 | 83.03 |
| Roman Catholic | 37 | 13.65 |
| Born Again Christian | 9 | 3.32 |
| **Ethnicity** | | |
| Higaonon | 2 | 0.74 |
| Masbatenio | 73 | 26.94 |
| Leytenio | 11 | 4.05 |
| Bisaya | 131 | 48.34 |
| **Number of household members** | | |
| 1–5 | 153 | 56.45 |
| 6–10 | 114 | 42.07 |
| 11–15 | 4 | 1.48 |
| **Educational attainment** | | |
| Elementary Undergraduate | 22 | 8.12 |
| Elementary Graduate | 35 | 12.92 |
| Junior High School Undergraduate | 120 | 44.28 |
| Junior High School Graduate | 51 | 18.82 |
| Senior High School Undergraduate | 1 | 0.37 |
| Senior High School Graduate | 3 | 1.10 |
| College Undergraduate | 19 | 7.01 |
| College Graduate | 19 | 7.01 |
| Vocational | 1 | 0.37 |
| **KRAEFI membership** | | |
| Non-Member | 33 | 12.18 |
| Member | 238 | 87.82 |

forest diversity.

Most respondents (52.03%, n = 141) did not recognize macaques as socioeconomically important, whereas 47.97% (n = 130) did, primarily in relation to tourism. These respondents believed macaques could significantly boost Sumile's tourism revenue. Although Elijan Park has no mandatory entrance fee, visitors can make voluntary donations that can be utilized for park maintenance and community support. Majority of the respondents (86.35%; n = 234) regarded macaques as culturally important. Half of the respondents (51.29%, n = 139) viewed macaques as symbols of unity while 27.68% (n = 75) considered macaques sacred, and 7.38% (n = 20) believed that mistreating them could lead to negative karmic consequences.

More than half of the respondents (61.26%; n = 166) were unaware of the Wildlife Resources Conservation and Protection Act or Republic Act No. 9147, while 38.38% of the respondents (n = 104) were familiar with it. Among those aware of RA 9147, 17.71% (n = 48) identified it as wildlife protection legislation. Twenty-five respondents (9.23%) specifically mentioned macaque protection. Twenty-one respondents (7.75%) referred to it as providing wildlife handling guidelines, and 11 respondents (4.06%) recognized its role in wildlife conservation.

A chi-square test was employed to examine the association between socioeconomic factors and knowledge of macaque importance and RA 9147, with results in Table 3. There was a significant association between age ($X^2$ = 31.0, p = 0.011) and the knowledge of macaques' ecological importance. Educational attainment ($X^2$ = 30.0, p <0.01), income ($X^2$ = 13.2, p = 0.021), and occupation ($X^2$ = 46.6, p <0.01) were also significantly associated with this knowledge. Younger residents (18–45 years old), individuals with higher educational attainment, and those with higher monthly incomes were more likely to know the ecological significance of macaques. Local government officials and educators exhibited a higher level of knowledge concerning this information.

Significant associations with length of residency ($X^2$ = 14, p = 0.007), education ($X^2$ = 30.8, p <0.01), income ($X^2$ = 18.5, p = 0.002), and occupation ($X^2$ = 46.6, p <0.001) were also observed when respondents were asked about their knowledge of the socio-economic importance of macaques. Long-term residents, individuals who attained higher educational levels, and those with higher monthly incomes were more likely to be knowledgeable about the socioeconomic importance of macaques. Similarly, local government officials, teachers, and students were more likely to be knowledgeable about this information.

Additionally, the length of stay ($X^2$ = 17.2, p = 0.002) and education ($X^2$ = 25.2, p <0.001) were significantly linked to knowledge of macaque's cultural importance.

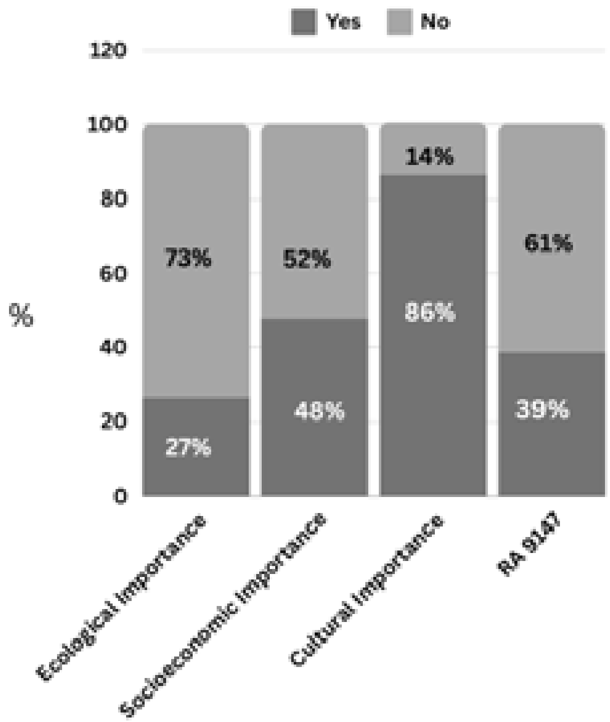


**Figure 1. Knowledge of respondents on the ecological, socio-economic, and cultural importance of macaques and RA 9147 or Wildlife Resources Conservation and Protection Act in Sumile, Butuan City.**

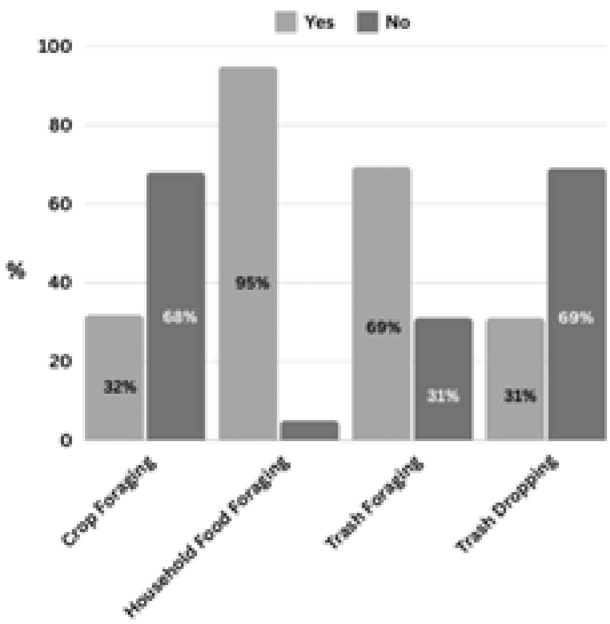


**Figure 2. Observed macaque behavior in Sumile, Butuan City.**

Long-term residents and individuals who attained higher educational levels demonstrated greater knowledge regarding the cultural significance of macaques. Age ($X^2$ = 31.0, p <0.01), length of residency in the village ($X^2$ = 20.4, p <0.01), educational attainment ($X^2$ = 44.6, p <0.01), income ($X^2$ = 26.3, p <0.01) and occupation ($X^2$ = 50.6, p <0.01) were significantly associated with knowledge of RA 9147. Individuals who were younger had a shorter-term residency, possessed higher educational attainment, and reported higher incomes demonstrated greater knowledge of RA 9147. Additionally, educators and students exhibited a higher level of familiarity with this legislation.

**Perceptions and attitudes of respondents toward human-macaque interactions and management**

**Macaque provisioning**

Nearly all respondents (99.26%, n = 269) engaged in macaque provisioning, while only two (0.74%) abstained due to perceived harm to macaques. A significant proportion of the respondents (71.59%, n = 194) provisioned macaques out of respect, 18.08% (n = 49) regarded macaque provisioning as a cultural practice, and 9.59% (n = 26) participated in macaque provisioning out of concern for macaque survival. Conversely, one respondent (0.36%) avoided provisioning to encourage natural foraging, and another respondent (0.36%) believed that provisioning does not provide macaques with a natural diet.

The five primary food items frequently provided to macaques in Sumile were Bananas *Musa acuminata*, Sweet Potatoes *Ipomoea batatas*, Corn *Zea mays*, Taro *Colocasia esculenta*, and biscuits. Bananas were the most common (35.42%, n = 96), followed by Sweet Potatoes (18.08%, n = 49), Corn (17.71%, n = 48), biscuits (17.71%, n = 48), and Taro (11.44%, n = 31). Additional foods included bread, vegetables (e.g., squash, eggplants, vegetable pear), root crops (cassava, other taro species, gabi, and ube), and fruits (cacao and jackfruit). Some respondents shared portions of their crop harvests with macaques.

More than half of the respondents 65.68% (n = 178) engaged in macaque provisioning during "community service" time in Elijan Park. Others (22.88%, n = 62) provisioned macaques upon encountering them within the village, during the recreational visits to Elijan Park (10.70%. n = 29), or deliberately prepared food for them (0.71%, n = 2). The majority of the respondents (60.15%, n = 163) provisioned macaques sometimes, while 38.38% (n = 104) always did, and 1.48% (n = 4) seldom provisioned macaques.

There was a significant association between age ($X^2$ = 25.2, p <0.001) and frequency of macaque provisioning. Other factors significantly associated included length of residency ($X^2$ = 42.3, p <0.001), education ($X^2$ = 73.6, p <0.001), and income ($X^2$ = 24.6, p = 0.006) (Table 3).

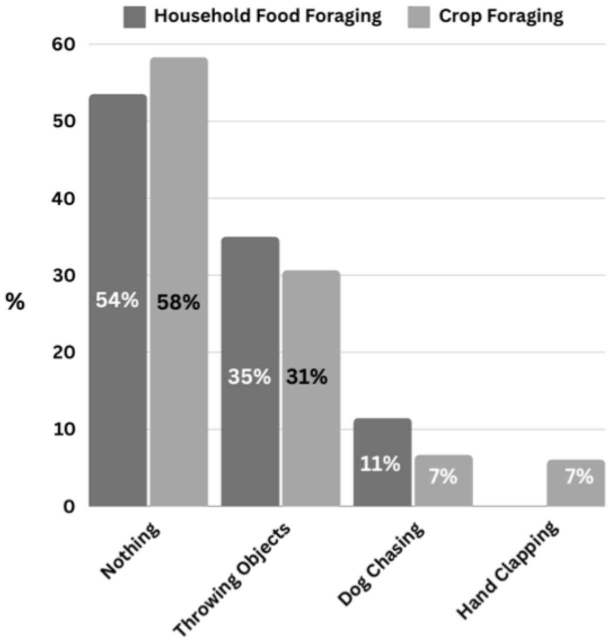


**Figure 3. Deterrent actions of the respondents to household food and crop foraging by macaques in Sumile, Butuan City.**

**Table 2. Socio-economic characteristics of the respondents in Sumile, Butuan City.**

| Socio-demographic characteristics | Frequency | Percentage |
|---|---|---|
| **Occupation of respondents** | | |
| Housewife | 164 | 60.53 |
| Farmer | 14 | 5.17 |
| Local government employees | 23 | 8.49 |
| Teacher | 18 | 6.64 |
| Engineer | 2 | 0.74 |
| Social Worker | 1 | 0.37 |
| Information technologist | 1 | 0.37 |
| Student | 38 | 14.02 |
| Businessman | 10 | 3.69 |
| **Monthly income** | | |
| 1,000–5,000 | 227 | 83.76 |
| 5,001–10,000 | 19 | 7.01 |
| 10,001–15,000 | 2 | 0.74 |
| 15,001–20,000 | 3 | 1.11 |
| 20,001–25,000 | 17 | 6.27 |
| 25,001–30,000 | 3 | 1.11 |

**Table 3. Factors associated with the knowledge, attitudes, and perceptions of residents towards human-macaque interactions and management strategies in Sumile, Butuan City.**

| Variables knowledge | Age | Length of residency | Educational attainment | Monthly income | Occupation |
|---|---|---|---|---|---|
| RA 9147 | $X^2$ (3, N = 271) = 31.0, **p <0.01** | $X^2$ (4, N = 271) = 20.4, **p <0.01** | $X^2$ (8, N = 271) = 44.6, **p <0.01** | $X^2$ (5, N = 271) = 26.3, **p <0.01** | $X^2$ (8, N = 271) = 50.6, **p <0.01** |
| Ecological importance of macaques | $X^2$ (3, N = 271) = 11.2, **p=0.011**. | $X^2$ (4, N = 271) = 6.03, p=0.197 | $X^2$ (8, N = 271) = 30.0, **p <0.01** | $X^2$ (5, N = 271) = 13.2, **p=0.021** | $X^2$ (8, N = 271) = 46.6, **p <0.01** |
| Socio-economic importance of macaques | $X^2$ (3, N = 271) = 5.92, p=0.153 | $X^2$ (4, N = 271) = 14, **p=0.007** | $X^2$ (8, N = 271) = 30.8, **p <0.001** | $X^2$ (5, N = 271) = 18.5, **p=0.002** | $X^2$ (8, N = 271) = 46.6, **p <0.001** |
| Cultural importance of macaques | $X^2$ (3, N = 271) = 5.92, p=0.115 | $X^2$ (4, N = 271) = 17.2, **p=0.002** | $X^2$ (8, N = 271) = 25.2, **p=0.001** | $X^2$ (5, N = 271) = 7.67, p=0.176 | $X^2$ (8, N = 271) = 10.9, p=0.208 |
| **Attitudes** | | | | | |
| Provisioning frequency | $X^2$ (6, N = 271) = 25.2, **p <0.001** | $X^2$ (8, N = 271) = 42.3, **p <0.001** | $X^2$ (16, N = 271) = 73.6, **p <0.001** | $X^2$ (10, N = 271) = 24.6, **p=0.006** | $X^2$ (16, N = 271) = 21.8, p=0.148 |
| Deterrent Action (Household Foraging) | $X^2$ (6, N = 271) = 25.5, **p <0.001** | $X^2$ (8, N = 271) = 36, **p <0.001** | $X^2$ (16, N = 271) = 80.7, **p <0.001** | $X^2$ (10, N = 271) = 38.1, **p <0.001** | $X^2$ (16, N = 271) = 45.7, **p <0.001** |
| Deterrent Action (Crop Foraging) | $X^2$ (9, N = 271) = 29.7, **p <0.001** | $X^2$ (12, N = 271) = 48.4, **p <0.001** | $X^2$ (24, N = 271) = 107, **p <0.001** | $X^2$ (15, N = 271) = 39.7, **p <0.001** | $X^2$ (24, N = 271) = 39.7, **p 0.015** |
| **Perceptions** | | | | | |
| Prohibit Provisioning | $X^2$ (3, N = 271) = 6.59, p=0.086 | $X^2$ (4, N = 271) = 23.3, **p <0.001** | $X^2$ (8, N = 271) = 12.4, P =0.136 | $X^2$ (5, N = 271) = 3.18, P=0.672 | $X^2$ (8, N = 271) = 31.3, **p <0.001** |
| Translocation of macaques | $X^2$ (3, N = 271) = 7.11, p=0.068 | $X^2$ (4, N = 271) = 4.95, p=0.292 | $X^2$ (8, N = 271) = 25, **P 0.002** | $X^2$ (5, N = 271) = 15.3, **P 0.009** | $X^2$ (8, N = 41.5) = 19.7, **p 0.012** |
| Dog patrolling | $X^2$ (3, N = 271) = 3.94, p=0.268 | $X^2$ (4, N = 271) = 35.3, **p <0.001** | $X^2$ (8, N = 271) = 25, **P 0.002** | $X^2$ (5, N = 271) = 16.7, **P 0.005** | $X^2$ (8, N = 41.5) = 28.7, **p <0.001** |

Individuals aged 25–45 years, long-term residents, those with lower educational attainment, and those with higher monthly incomes exhibited increased provisioning frequency.

### Other human-macaque interactions

Macaque behaviors in Sumile included crop foraging (31.78%), household food foraging (94.84%), trash foraging (69.37%), and trash dropping (30.63%) (Figure 2). Macaques were observed foraging most of their crops including Gabi *Xanthosoma sagittifolium*, Corn, Coconuts *Cocos nucifera*, Sweet Potatoes, Peanuts *Arachis hypogaea*, Chayotes *Sechium edule*, Bananas *Musa* spp., Cassava *Manihot esculenta*, and Eggplant *Solanum melongena*. All respondents (100%, n = 271) reported that macaques did not take non-food items from their households, and only one reported a monkey attack in 2013 near a stream in Sumile.

Most respondents did not intervene when macaques foraged household food (53.51%) and their crops (58.30%, n = 158). Deterrent actions in response to household food foraging included throwing hard objects e.g. slippers or stones (34.97%) and using a dog (11.44%) (Figure 3). Actions taken to deter crop foraging included throwing hard objects e.g. slippers or stones (30.62%), hand clapping (6.64%), and dog chasing (6.64%). Other preventive measures also involved closing doors and windows and installing nettings and fences.

Chi-square tests showed that age ($X^2$ = 25.5, p <0.001), length of residency ($X^2$ = 36, p<0.001), education ($X^2$ = 80.7, p <0.001), income ($X^2$ = 38.1, p=0.004), and occupation ($X^2$ = 45.7, p <0.001) were significantly associated with deterrent actions on household food foraging (Table 3). Younger residents (18–45 years old), long-term residents, respondents with college-level education, and those with higher incomes were more likely to ignore household food foraging. In contrast, older respondents (46–65 years old), short-term residents, individuals with lower educational attainment, and those with lower incomes demonstrated positive responses to throwing objects and allowing dogs to chase the macaques. Local government officials and teachers were more likely to disregard the food-foraging macaques, while students were more likely to throw objects and allow dogs to chase the macaques.

Similarly, for crop foraging deterrence, significant associations were found with age ($X^2$ = 25.2, p <0.001), length of residency ($X^2$ = 25.2, p <0.001), education ($X^2$ = 25.2, p <0.001), income ($X^2$ = 25.2, p <0.001) and occupation ($X^2$ = 25.2, p <0.001). Residents aged 26–45 years old, long-term residents (over 36 years), college graduates, and residents with higher incomes were more likely to ignore crop-foraging macaques. Older residents (46–65 years old), those with lower education levels, and those with lower incomes were more likely to throw objects to deter macaques. Local government

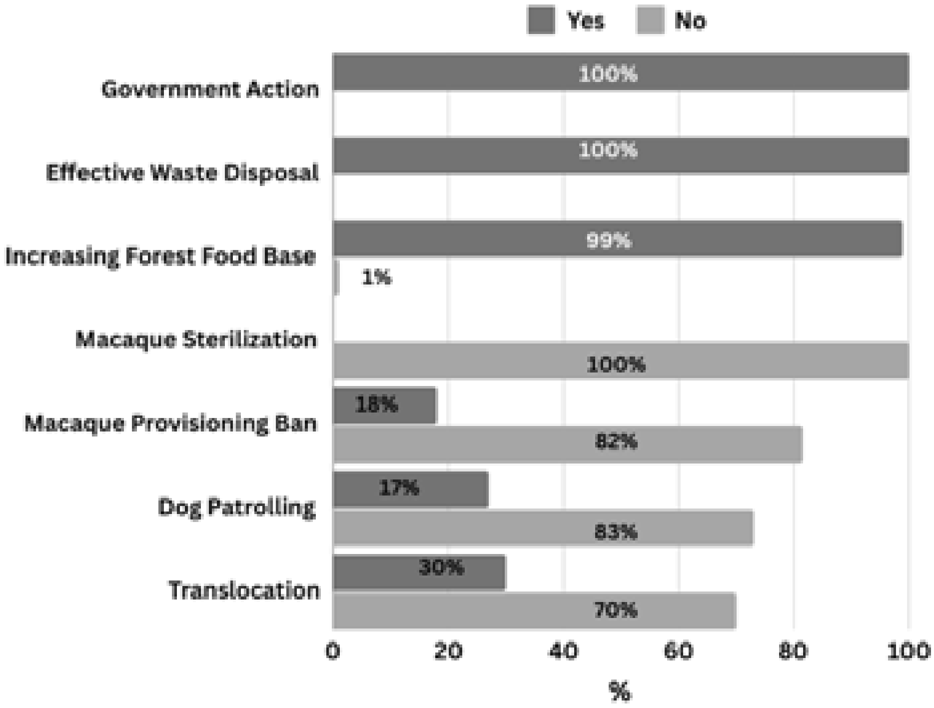


**Figure 4. Measures selected by the respondents to manage human-macaque interactions in Sumile, Butuan City.**

officials and teachers were more likely to disregard crop foraging, while housewives and students were more inclined to throw objects, use dogs, and hand clapping to deter macaques.

**Human-macaque interaction management measures**

All the respondents (100%) reported absence of management measures for negative human-macaque interactions in Sumile, allowing macaques to interact freely with humans. The three most suggested measures (Figure 4) were: (i) Government intervention to address negative human-macaque interactions (100%), with respondents emphasizing its importance for both macaques and human welfare. (ii) Effective waste disposal (100%), as macaques were seen foraging and dropping trash in the village. (iii) Increasing the food base in the forest (98.89%, n = 268), linked to observed household food foraging during food scarcity in Elijan Park. Respondents opposed measures such as sterilization (100%), culling of monkeys (100%), prohibition of monkey feeding (81.55%), patrolling by dogs (73%), and translocation (70%) due to cultural reasons.

The length of stay in the village ($X^2$ = 23.3, p <0.001) and occupation ($X^2$ = 31.3, p <0.001) are significantly associated with the respondents' perception of prohibiting macaque provisioning as a measure. Short-term residents, students, housewives, teachers, and local government officials were less likely to favor the prohibition of macaque provisioning. Perceptions of macaque translocation as a measure were significantly associated with education ($X^2$ = 25, p = 0.002), income ($X^2$ = 15.3, p = 0.009), and occupation ($X^2$ = 41.5, p = 0.012). Residents with lower education attainment and those with lower incomes were more in favor of translocation. Students, teachers, local government officials, and housewives were likely not in favor of translocation. Perceptions of dog patrolling as a management measure were significantly associated with the length of stay ($X^2$ = 35.3, p <0.001), education ($X^2$ = 25, p = 0.002), income ($X^2$ = 16.7, p = 0.005), and occupation ($X^2$ = 28.7, p <0.001). Short-term residents, individuals with lower educational attainment, and those with higher incomes were less likely to favor dog patrolling as a measure.

## DISCUSSION

The study indicates that the majority of Sumile residents engaged in macaque provisioning which could be attributed to their reverence for macaques, cultural beliefs, and concern for macaque survival. Most of the respondents were KRAEFI members who considered macaques sacred, reflecting traditional reverence for macaques common in southeastern Asia (Nahallage & Huffman 2013) including the Philippines.

The KRAEFI organization that governed Elijan Park encouraged weekly macaque provisioning in the park as "community service". More than half of the respondents also mentioned that most of their provisioning activity occurred during "community service" while others did so opportunistically around the village. The primary foods used for provisioning were bananas, sweet potatoes, corn, and biscuits, with some respondents preparing food specifically for macaques or sharing crop harvests.

This study did not include tourist interviews. Tourists were observed provisioning macaques when Elijan Park was open to the public, with KRAEFI and caretakers encouraging this behavior to habituate macaques to human presence and attract visitors. Frequent provisioning has resulted in the frequent observation of macaques in Elijan Park and adjacent communities, demonstrating their synanthropic behavior and adaptability to human environments and food (Gumert et al. 2011; Sha & Hanya 2013). Macaques foraging household food, crops, and refuse highlights their adaptation to human settlements in Sumile, especially during food scarcity in Elijan Park. Consistent with Suwannarong et al. (2023), cultural beliefs in Sumile prevented macaque killings, as harming them was believed to bring misfortune. The act of harming monkeys was perceived to incur misfortune. Consequently, most residents did not act against household food and crop foraging, using non-aggressive deterrents like dog chasing, throwing slippers or wood, and hand clapping.

The fear of spiritual retribution for killing macaques is the main reason for the taboo against hunting, trading, and consuming them in Sumile. Most residents fear spiritual consequences similar to those in Bali, Indonesia, where harming monkeys is believed to bring misfortune (Peterson & Riley 2017). In Sumile, locals cited instances of neighbors falling ill and dying after persecuting macaques. The residents' reverence for macaques helps protect the threatened Long-tailed Macaques from exploitation and harm.

The residents' affection and respect for macaques, demonstrated through provisioning, indicate a significant human-macaque relationship in Sumile. Many residents mistakenly view provisioning as a conservation measure, which may undermine long-term conservation efforts. Unregulated provisioning may lead to a substantial increase in the macaque population and adverse behavioral changes (Fa 1981; Newsome & Rodger 2008; Knight 2017; Sengupta & Radhakrishna 2020; Cui et al. 2021) in areas of sympatry (Dittus et al. 2019). If unchecked in Sumile, this could result in negative human-macaque interactions due to the growing macaque population's dependence on human food, similar to situations in western Sumatra, Indonesia (Ilham et al. 2017), and Hainan, China (Cui et al. 2021). Macaques in the Palawan Subterranean River National Park in the Philippines also exhibited problematic behaviors linked to widespread food provisioning (Gamalo et al. 2019). In Elijan Park, macaques displayed intraspecific conflict during provisioning. Despite prohibitions against harassing monkeys, some tourists disturb and provoke them, leading to macaque aggression, like Berman & Li's findings (2002).

Macaque adaptation to human food can reduce natural feeding and forest habitat use (O-Leary & Fa 1993; Sha & Hanya 2013; Sengupta et al. 2015; Sengupta & Radhakrishna 2018). Extensive provisioning and culturally influenced macaque tolerance in Sumile and Elijan Park may increase foraging on household food, crops, and waste. If unaddressed, these behaviors can escalate, leading to socio-economic and health issues, fostering hostility, and resulting in retaliatory actions against macaques. Negative attitudes toward macaques due to socioeconomic losses (Hill & Webber 2010) can undermine community support for conservation and human-macaque management, complicating human-wildlife interactions (Frank et al. 2019).

According to Pontzer (2023), macaques' dependence on human food and loss of natural foraging behavior can lead to health issues such as increased body size, higher stress, and alopecia in males (Maréchal et al. 2016). Physical contact during provisioning raises mutual disease transmission risks which is detrimental to macaque health and populations (Jones-Engel et al. 2005; Muehlenbein & Wallis 2014). For instance, provisioning by tourists and locals in Elijan Park and Sumile often follows bites, facilitating disease spread via fluid exchange.

In Sumile, food provisioning also leads to waste consumption and dispersion. Frequent provisioning habituates macaques to anthropogenic food sources in refuse areas (Bempah et al. 2021). The lack of proper waste receptacles exacerbates this behavior, potentially impacting human and primate health. Waste foraging can attract enteroparasites (Baloria et al. 2022), disease-carrying insects, and rodents, heightening disease transmission risks. Effective waste management and public awareness about provisioning risks and proper disposal practices can minimize refuse dispersion and reduce negative interactions.

The strong human-macaque connections in Sumile and the potential adverse effects of uncontrolled provisioning indicate a need to balance socio-cultural and ecological factors. Completely prohibiting provisioning may not be an optimal solution, as most residents did not support measures like prohibition, sterilization, translocation, and dog patrolling for managing human-macaque interactions. A bottom-up approach involving residents, KRAEFI leaders, local government officials, and other stakeholders in management planning is recommended. Decision-making should be culture-sensitive, participatory, and community-based to develop adaptive strategies for human-macaque coexistence.

The findings show that residents' educational level and occupation were significantly associated with knowledge of the ecological, socio-economic, and cultural importance of macaques, as well as RA 9147. More positive responses were seen from individuals with higher educational attainment, local government officials, teachers, and students. Education and length of stay in the village were also significantly linked to provisioning frequency. This underscores the need for comprehensive education and social media campaigns to raise public awareness of the ecological and health implications of uncontrolled macaque provisioning. In addition to local government officials, teachers, and students, it is crucial to educate local communities, KRAEFI officials, Elijan Park caretakers, and tourists on the conservation status of Long-tailed Macaques, their threats, behavior, ecological and socio-economic services, the importance of natural foraging behavior, conservation laws like RA 9147, and macaque-friendly management strategies. Engaging local religious leaders to include conservation messages in religious teachings and promote responsible macaque interaction is also recommended.

Results indicated that lower-income residents were more likely to throw objects and let dogs chase household food and crop-foraging macaques. This suggests that economically disadvantaged communities who are reliant on subsistence, may use deterrent tactics against macaques. A study on Buton Island, Indonesia, showed that lower-income communities employed violent control methods more frequently than wealthier ones, even when crop raiding was less severe (Hardwick et al. 2017). Although most Sumile residents did not act against foraging macaques and only a few used non-aggressive deterrents, this situation could change. Disadvantaged residents might develop negative attitudes if unregulated provisioning worsens macaque foraging behavior, potentially leading to conservation issues. Thus, a holistic approach combining education, coexistence incentives, macaque-friendly deterrents, and economic support is necessary. Wildlife managers, local governments, and communities need to collaborate on context-sensitive solutions to balance macaque conservation with community well-being (Koirala et al. 2021).

Macaque conservation should prioritize habitat restoration and natural food provision through science-based and community-driven establishment of local conservation areas. Multi-sectoral participation in the planning and implementation of local conservation areas should be encouraged. Volunteer programs for habitat restoration, observation, and education may be organized. Government officials and residents should develop culture-sensitive provisioning regulations alongside habitat rehabilitation and public education. In extreme cases, like during a food crisis, controlled provisioning supervised by wildlife professionals may be necessary. Proper waste management is also crucial to prevent macaques from consuming food remnants from refuse containers and other health issues. Instead of a total provisioning ban, a gradual reduction over time could encourage natural foraging behaviors.

Regular monitoring of the macaque population in Sumile is crucial to avoid overestimating their numbers in anthropogenic areas (Kyes et al. 2011), where they are often mistakenly seen as abundant (Eudey et al. 2020). Comprehensive and extensive research on macaque behavior, habitat preference, feeding patterns, and reproduction is needed to understand human-macaque-environment dynamics. One of the limitations of this study was that females (79.34%) and housewives (60.53%) constituted the majority of respondents, primarily due to their availability during the one-on-one interview process, as most of the husbands were at work during daytime hours. Therefore, it is recommended that future studies incorporate a balanced representation of male and female respondents. Additional studies on interactions with tourists in Elijan Park and farmers in the village will offer insights for managing human-wildlife interactions, coexistence strategies, and sustainable conservation efforts.

## CONCLUSIONS

The research findings indicate that rampant macaque provisioning in Sumile is influenced by cultural beliefs. The reverence for Philippine Long-tailed Macaques may contribute positively to the conservation of this threatened species. Uncontrolled provisioning in settlements and Elijan Park may potentially lead to population increase, zoonotic disease transmission, behavioral changes, and adverse human-macaque interactions, potentially undermining conservation and coexistence goals. The regulation of provisioning requires balancing cultural and scientific considerations. Culturally sensitive, participatory, and science-based planning and management strategies are recommended to balance the ecological, socioeconomic, and cultural aspects of human-macaque-environment interactions.

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**Appendix 1. Survey questionnaire.**

**Human and Philippine Long-tailed Macaque interaction in Sumile, Butuan City**

**Part 1: Sociodemographic and economic profile**

**Name of barangay/location: ____________________ Date of interview:_____________**
**1.1. Respondent no: __________ 1.2. Gender: ( ) Male ( ) Female ( ) LGBTQ+++**
**1.3. Age Group: ( ) 18–26years old**
**( ) 27–35 ( ) 36–44 ( ) 45–53 ( ) 54–62 ( ) 63 and above**

**1.4. How many years have you resided in the village? ________**
**1.5. Religion:**
**( ) Roman Catholic**
**( ) Iglesia ni Cristo**
**( ) Jehovah's Witness**
**( ) Mormon**
**( ) Protestant**
**( ) Other: ______________________________**
**( ) Muslim**
**( ) Seventh Day Adventist**
**( ) Baptist**
**( ) Born Again Cristian**
**( ) Rizalian**

**1.6. Civil Status: ( ) Single ( ) Married ( ) Widowed ( ) Separated ( ) Other: __**
**1.7. Number of Household m ember/s: __________________________________**
**1.8. Highest educational attainment:**
**( ) No formal schooling**
**( ) Elementary level**
**( ) Elementary graduate**
**( ) High school level**
**( ) High school graduate**
**( ) Vocational courses**
**( ) College level**
**( ) College graduate**
**( ) Post graduate level**

**1.9 Membership in organization (if any) and role**

**2.0. Family monthly income:**
**( )** 1,000 – 5,000/mo
**( )** 5,001 – 10,000/mo
**( )** 10,001 – 15,000/mo
**( )** 15,001 – 20,000/mo
**( )** 20,001- 25,000/mo
**( )** 25,001 – 30,000

**2.1. Tribal/Ethnic group (if any):**

**2.1. Occupation of respondents:**
**( ) Housewife ( ) Farmer ( ) Student ( ) Teacher**
**( ) Local government employee ( ) Social worker ( ) Self-employed**
**( ) Others, specify: _______________**

**Part 2: Knowledge of macaque importance and RA 9147**

| | Yes | No |
|---|---|---|
| Do you know about the **ecological importance** of macaques? | | |
| If yes, what do you know about the ecological importance of macaques? | | |
| Do you know about the **socio-economic importance** of macaques? | | |
| If yes, what do you know about the socio-economic importance of macaques? | | |
| Do you know about the cultural importance of macaques? | | |
| If yes, what do you know about the cultural importance of macaques? | | |
| Do you know about the **Wildlife Resources Conservation and Protection Act or Republic Act No. 9147**? | | |
| If yes, what do you know about **RA 9147**? | | |

**Part 3: Perceptions and attitudes toward human-macaque interactions and management measures**

| | **Yes** | **No** |
|---|---|---|
| Do macaques forage food in your household? | | |
| Aside from food, what else did macaques get from your households? | | |
| What did you do when macaques forage food in your households? | | |
| Do macaques forage your crops? | | |
| What crops did they forage? | | |
| What did you do when macaques forage your crops? | | |
| Have you observed the macaques foraging in the trash bin? | | |
| Have you observed the macaques dropping trash in the village? | | |
| Have you experienced hunting macaques? | | |
| If yes, when? ___________<br>How often?___________ How many were hunted? _______________<br>Purpose of hunting? ________________________________________ Where? _____________________ | | |
| Have you experienced trading macaques? | | |
| Yes  If yes, when? ___________<br>How often?___________ How many were sold? Price per piece _______________<br>Purpose of trading? ________________________________________<br>Who bought the macaques? ________________________________________________ | | |
| Have you eaten monkey meat? | | |
| If yes, when? __________________________________How often?__________<br>What does it taste like? _________________________ | | |
| Have you been attacked or bitten by a monkey? | | |
| If yes, when? _____________________ how often? ___________  where? _____________ | | |
| Have you experienced provisioning macaques? | | |
| | | |
| Do you think provisioning is right? | | |
| If yes, why? | | |
| What type of food did you provide to macaques? | | |
| When did you usually engage in provisioning? | | |
| How often did you provision macaques?<br><br>___Not al all  ___Seldom ___Sometimes ___Always | | |
| Please identify the observed behavior of macaques in Sumile. (Please check any of the following macaque behavior that you observe in Sumile.)<br>( ) Crop foraging<br>(  ) Household Food Foraging<br>(  ) Foraging Trash<br>(  ) Dropping Trash<br>(  ) Others, specify:__________________ | | |

| | | |
|---|---|---|
| Were there any management measures for human-macaque interactions implemented in the area? | | |
| If yes, can you name some? | | |
| What do you think are the measures to manage human-macaque interactions in Sumile? (Please check any of the following that you believe is correct)<br>( ) Sterilization of macaques<br>( ) Prohibition on provisioning<br>( ) Culling of macaques<br>( ) Translocation of macaques<br>( ) Patrolling by dogs<br>( ) Effective wastage disposal<br>( ) Government action<br>( ) Increasing food base in forests for macaques | | |
| Can you recommend other human-macaque management measures in Sumile? | | |

| | | | |
|---|---|---|---|
| Was there any macaque conservation activity conducted in the area? | | | |
| If yes, please specify the conservation activity: | | | |

Thank you for your participation!

**Abstrak:** Ang pag-unawa sa pakikipag-ugnayan ng tao at unggoy ay mahalaga para sa pangangalaga at pamamahala ng nasabing species. Samakatuwid, sinuri ng pag-aaral na ito ang mga pakikipag-ugnayan ng tao at unggoy sa Sumile, Butuan City mula Hulyo 2022 hanggang Abril 2023 sa pamamagitan ng one-on-one na panayam. May dalawang daan at pitompot isang mga respondente ang random na tinanong upang matukoy ang kanilang mga demograpikong at sosyoekonomikong katangian. Ang kanilang kaalaman, saloobin, at pang-unawa sa mga pakikipag-ugnayan ng tao at unggoy tulad ng pagpapakain sa mga unggoy, mga hakbang sa regulasyon, at mga kaugnay na kadahilanan ay tinukoy din. Karamihan sa kanila ay may kamalayan sa kultural na kahalagahan ng mga unggoy (86.35%). Gayunpaman, ang karamihan ay hindi alam ang ekolohikal (73.43%) at sosyoekonomikong kahalagahan ng mga ito (52.03%), gayundin ang RA 9147 o Wildlife Act (61.26%). Karamihan sa mga residente ay nagpakain ng mga unggoy (99.26%). Ang mga pag-uugali ng unggoy ay ang kumain ng pagkain sa bahay (94.84%), kumain ng pananim (31.78%), pagkain ng basura (69.37%), at pag-kalat ng basura (30.63%). Karamihan sa mga residente ay wala namang ginawa nang kumain ang mga unggoy ng pagkain sa mga sambahayan (53.51%) o nang kumain sila ng pananim (58.30%) habang ang ilan ay binato ang mga unggoy ng mga matigas na bagay, pumalakpak, o pinahabol nila ang unggoy sa aso. Ang antas ng edukasyon ang pinaka-karaniwang salik na may kaugnayan sa kaalaman ng mga residente. Ang haba ng paninirahan at edukasyon ay may kaugnayan sa dalas ng pagpapakain sa mga unggoy habang ang haba ng paninirahan at uri ng trabaho ay may kaugnayan sa pagbabawal ng pagpapakain bilang isang hakbang upang maiwasan ang negatibong pakikipag-ugnayan ng tao sa mga unggoy. Ipinahihiwatig ng pag-aaral na ito na ang mga pang kultura na kadahilanan ay nakakaimpluwensiya sa laganap na pagpapakain ng mga unggoy sa Sumile. Gayunman, kung hindi makontrol, ang pagpapakain ng mga unggoy ay maaaring humantong sa suliraning pang ekonomiya at kalusugan na maaring mag dudulot ng negatibong saloobin ng mga residente sa mga unggoy at sa mga pagsisikap para sa konserbasyon. Ang nangungunang tatlong hakbang sa regulasyon ng pakikipag-ugnayan ng tao sa unggoy na iminungkahi ng karamihan sa mga residente ay ang pagkilos ng pamahalaan, epektibong pag-aalis ng basura, at pagpaparami ng pagkain sa kagubatan. Inirerekomenda pa ng mga mananaliksik ang pagtatatag ng local conservation area; kontrolado na pagpapakain sa mga unggoy na may respeto sa kultura; at pamamaraan ng konserbasyon na nakabatay sa komunidad. Kasama rin dito ang pagtuturo sa publiko tungkol sa masamang epekto ng di-kinokontrol na pagpapakain sa mga unggoy. Bukod dito, kinakailangan ang regular na pagsubaybay sa populasyon ng mga unggoy at mga diskarte sa pagkonserba at pamamahala na nagbabalanse ng mga pang-ekolohikal, sosyo-ekonomiko at pangkultura na pagsasaalang-alang para sa pagpapanatili ng mapayang ugnayan ng tao at unggoy.

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# Noteworthy comments on birds for mega-diverse Myanmar

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**Abstract:** Myanmar, recognized for its rich biodiversity in South, Southeast, and East Asia, continues to unveil new avian taxa and record significant species range extensions. This study presents and analyzes bird observations from 2010 to 2023, emphasizing less accessible regions like the Hkakabo Razi Landscape, Shan States, Tanintharyi, and the Ayeyarwady delta. Utilizing audio-visual records, we document 13 bird species, including range extensions and novel sightings. Our findings highlight Myanmar's critical role in avian biodiversity, underscoring the importance of conservation efforts. This research contributes to narrowing the knowledge gap on bird species distributions within Myanmar, revealing a thriving community of bird enthusiasts and the potential for future discoveries.

**Keywords:** Biodiversity, distributional range extension, lack of information for conservation, new bird records.

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## INTRODUCTION

Myanmar is one of the most biodiverse countries in southern, southeastern, and eastern Asia (Suarez-Rubio et al. 2020; Bates et al. 2021). The country's avian diversity is regularly amended by the discovery of new bird taxa (Rappole et al. 2005, 2008; Renner et al. 2008, 2015, 2017). In addition, species recorded for the first time in Myanmar are frequent, underscoring Myanmar's role for new avian findings (Suarez-Rubio et al. 2016, 2023; Sai Sein Lin Oo et al. 2018, 2019a,b, 2020, 2022; Renner & Bates 2020; Myint Kyaw et al. 2021). Additionally, species' range extensions within Myanmar occur regularly (Rappole et al. 2005, 2008; Renner et al. 2008, 2009; Hla Naing et al. 2015; Thet Zaw Naing 2015; Suarez-Rubio et al. 2016, 2020; Zhang et al. 2017; Sai Sein Lin Oo et al. 2018, 2019a,b, 2020, 2022; Zöckler et al. 2020; Myint Kyaw et al. 2021). Particularly, the easy access to formerly restricted areas turned Myanmar into haven for bird enthusiasts worldwide during the democratic interlude from 2011 to 2021. Consequently, numerous records have emerged, encompassing new descriptions, range extensions, novel sightings of bird species, and observations in specific aspects of ecology and behaviour of birds.

While the compilation of recently added avian species in Myanmar is impressive (Thet Zaw Naing et al. 2020), there remains discoveries and particularly range extensions to be reported. In this context, we present and analyse a series of observations spanning from 2010 to 2023. Our focus lies on regions that have been relatively less accessible due to geographical constraints and administrative limitations. These observations are significant for Myanmar as they either introduce novel records at the species or subspecies level, or provide additional insights into behaviour and other ecological aspects, conservation biology related issues, or species' range distributions within Myanmar and beyond in southeastern Asia. Through this compilation, we aim at narrowing down the existing knowledge gap on bird species and their distributions within Myanmar. These insights reflect a lively community of birders with a profound interest in nature and birds, but also the potential for many more discoveries in the future.

## METHODS

The observations reported here are exclusively based on audio-visual records. All records originated after 2010 and are situated within Myanmar's territory (formerly Burma) but may have relevance for all of Southeast Asia. Each observation is accompanied by photographic evidence, dates, detailed localities including corresponding coordinates. When applicable, additional insights regarding behaviour and observed habitats are provided. Cases in which our species identification was uncertain or not entirely conclusive have been omitted from this report. Additional records, where there was the slightest doubt about the bird identification, were omitted until further information and confirmation is available.

Our surveys were not systematic, but rather focused on regions within Myanmar that have undergone varying degrees of exploration over the past two decades. Our survey focus on ad hoc reports from Myanmar; the areas can be summarized as: (1) the Hkakabo Razi Landscape (as defined by Suarez-Rubio et al. 2020; Bates et al. 2021), including Sagaing and Kachin north of Myitkyina, (2) the Shan States in eastern Myanmar, (3) the Tanintharyi region, and (4) Ayeyarwady delta area in southern Myanmar, extending north to Yangon metropolitan area. Regions more centrally located within Myanmar, such as the metropolitan areas of Mandalay and Bagan, as well as several protected areas (e.g., around Pyin Oo Lwin, Inle Lake, Chatthin Wildlife Sanctuary), have undergone consistent surveying efforts by others. As of now, our dataset lacks any novel records from these central parts of Myanmar.

Note: Some bird species face increased conservation concern since they are facing high hunting pressure or are overwhelmed by intense birdwatching and unethical bird photographers. To protect these species, we have not disclosed exact coordinates or village names. These data are available through the corresponding author on request only.

## RESULTS

We present findings concerning 13 species, each with interesting aspects (a summarized compilation of all records is provided in Table 1). For most species we documented notable range extensions within Myanmar. For all species, we provide accounts of any other notable findings in terms of breeding biology or ecology that we encountered.

Our results display a geographical bias within Myanmar, as our access was primarily concentrated on the above-mentioned regions. Consequently, we organize our reporting into distinct blocks, each corresponding to one area: (1) Northern Myanmar with a focus on the

Hkakabo Razi Landscape; (2) Shan States; (3) Tanintharyi (Tenasserim); and (4) the Ayeyarwady delta region. Otherwise we follow a systematic order within each of these three regions. Regarding taxonomy, we follow the IOC version 13.1 (Gill et al. 2023) for scientific names and adjust the English names as recommended.

1. Snow Partridge *Lerwa lerwa* (Image 1) has been found in the Hkakabo Razi Landscape before, but we extend the species range westwards by a new record from the western part of the Hkakabo Razi Landscape, i.e., Hponkan Razi Wildlife Sanctuary (Table 1). This constitutes a rather small-scale distributional range-extension within northern Myanmar (GBIF 2023l). The Putao museum holds a skin from the Tibet staff of the Hkakabo Razi National Park, which means that the specimen is from the higher elevations of the Tahaundam area.

2. Yellow Bittern *Ixobrychus sinensis* (Image 2) constitutes a new record for the Hkakabo Razi Landscape. *I. sinensis* has been found in Myanmar before, the closest record from Myanmar to our knowledge is from Hukaung Valley Wildlife Sanctuary (Tordorf et al. 2007; GBIF 2023m). The record from Hkakabo Razi Landscape is noteworthy (Table 1), because this enigmatic species has not yet been recorded in this area with high incidence of birders and ornithologists for the last nine decades. Moreover, the adequate habitat for the species is regionally abundant.

3. Common Buzzard *Buteo buteo* (Image 3) was found close to Putao between Machanbaw and Naung Mung (Table 1). While recorded before in Myanmar, these 37 records in Myanmar are sparse and occur mostly in the country's centre (GBIF 2023c). With our record, we add probably a vagrant individual to northern Myanmar. Common Buzzards are generally rare in southeastern Asia and have been recorded sparsely from Thailand, and especially from northern Myanmar (King et al. 2001). Birds recorded during two expeditions to northern Myanmar (Burma). While subspecies determination is difficult for many *Buteo* species, we assume we encountered *B. buteo vulpines* (Dickinson & Christidis 2014).

4. We found Black-naped Oriole *Oriolus chinensis* (Image 4) in the Hponkan Razi part of the Hkakabo Razi Landscape (Table 1), which constitutes a new record for northern Myanmar. *O. chinensis* has been found frequently in Myanmar and within adjacent Arunachal Pradesh (India) as well Yunnan (China), but not yet for Kachin (GBIF 2023k).

5. Crow-billed Drongo *Dicrurus annectens* is a rare bird throughout its range in continental southeastern Asia (GBIF 2023e). We have recorded the species in Putao (Table 1). Until today we found all other *Dicrurus* species, i.e., *leucophaeus, aeneus, remifer, hottentottus, paradiseus* in the Hkakabo Razi Landscape (Renner & Rappole 2011a; Renner et al. 2015), except for *D. annectens*. Nevertheless, we always hypothesized *D. annectens* to occur in the Hkakabo Razi Landscape (e.g., Renner et al. 2015) and can finally confirm the species with photo evidence (Image 5). *D. annectens* is uncommon in northern Myanmar (Tordorf et al. 2007) but common in other parts of southeastern Asia (Thailand, Laos, Vietnam, Malaysia, Singapore, Cambodia).

6. A Warbling White-eye *Zosterops japonicus* was photographed (Image 6) in Ma Khunkan village just north of Putao (Table 1). The species has been recorded once from Maymyo (Pyn Oo Lwin), Myanmar in the 1920s as a skin specimen, today stored in the St. Barbara Museum (GBIF 2023h), but never again from Myanmar. There are other sparse records of *Zosterops montanus* and *Z. japonicus* in Myanmar, and while *Z. palpebrosus* is reported as common throughout the country (Robson 2008), the photo (Image 6) clearly distinguishes *Z. japonicus* (Indian White-eye) as in the Hkakabo Razi Landscape from *Z. palbebrosus*. Hence, the record confirms presence of *Z. japonicus* in Myanmar (Table 1). Other records of the species from southeastern Asia are mainly as winter visitor or resident to Vietnam, Thailand, Laos, or Singapore.

7. We recorded several Golden-crested Myna *Ampeliceps coronatus* (Image 7) from Zeyerdam and Naung Mung (Table 1), which constitutes a range extension. The species is widespread in southeastern Asia, including the southern half of Myanmar (Craig & Feare 2020b; GBIF 2023g). *A. coronatus* has been recorded also from Namdapha National Park in Northeast India bordering to Myanmar, but so far not anywhere north of Indawgyi, Kachin (GBIF 2023g).

8. Daurian Starling *Agropsar sturninus* (Image 8) was recorded several times in the Hkakabo Razi Landscape (Table 1), which is a range extension to Hkakabo Razi Landscape. The record is interesting since several starlings and mynas have been recorded as new for Myanmar recently (e.g., Soe Naing et al. 2016), which is astonishing, since mynas and starlings are relatively large, charismatic and rather in open than in dense habitat. Daurian Starlings are widespread in eastern and southeastern Asia, including sparse records from Bangladesh and India, but have been hardly found in Myanmar so far. Several references, e.g., Craig & Feare (2020a), show the species further east of Myanmar only,

**Table 1. New and noteworthy records of birds in Myanmar. The sequence refers and cross-references to the species list in the text. Taxonomy follows IOC (Gill et al. 2023).**

| English Name | Species | Locality | What's new | Day | Month | Year | North | East |
|---|---|---|---|---|---|---|---|---|
| Snow Partridge | *Lerwa lerwa* | Hkakabo Razi National Park | Range extension | 20 | March | 2016 | 28.068 | 97.505 |
| Snow Partridge | *Lerwa lerwa* | Hkakabo Razi National Park | Range extension | 10 | January | 2004 | 28.068 | 97.505 |
| Snow Partridge | *Lerwa lerwa* | Hkakabo Razi National Park | Range extension | 1 | January | 2019 | n/a | n/a |
| Snow Partridge | *Lerwa lerwa* | Hkakabo Razi National Park, Madine | 2 specimens in Putao museum | 23 | August | 2004 | n/a | n/a |
| Snow Partridge | *Lerwa lerwa* | Near Mt. Phangram Razi, Hponkan Razi Wildlife Sanctuary | Range extension | 10 | May | 2015 | 27.607 | 96.918 |
| Yellow Bittern | *Ixobrychus sinensis* | Ngarwar field, Hkakabo Razi National Park | Range extension | 21 | July | 2017 | 27.758 | 97.818 |
| Common Buzzard | *Buteo buteo* | Between Machanbaw and Naung Mung | Range extension | 7 | April | 2018 | 27.281 | 97.704 |
| Black-naped Oriole | *Oriolus chinensis* | 3 in Myitsone (Ayeyarwady confluence) | Range extension | 4 | February | 2009 | 25.708 | 97.497 |
| Black-naped Oriole | *Oriolus chinensis* | 6 in Banbane | Range extension | 4 | February | 2009 | 25.716 | 97.860 |
| Black-naped Oriole | *Oriolus chinensis* | Warsardam, Hponkan Razi Wildlife Sanctuary | Range extension | 26 | May | 2016 | 27.514 | 97.195 |
| Crow-billed Drongo | *Dicrurus annectens* | Putao | Range extension | 15 | August | 2015 | 27.376 | 97.402 |
| Warbling White-eye | *Zosterops japonicus* | Ma Khunkan village | Range extension | 9 | February | 2018 | 27.643 | 98.244 |
| Golden-crested Myna | *Ampeliceps coronatus* | Naung Mung | Range extension | 28 | November | 2017 | 27.506 | 97.822 |
| Golden-crested Myna | *Ampeliceps coronatus* | several records Zyerdam, Hponkan Razi Wildlife Sanctuary | Range extension | 23 | November | 2013 | 27.579 | 97.098 |
| Daurian Starling | *Agropsar sturninus* | several records in Makhwunkan village, Hkakabo Razi Landscape | Range extension | 30 | April | 2012 | 27.644 | 98.244 |
| Pin-tailed Parrotfinch | *Erythrura rasine* | Machanbaw road to Madwe, Upper Mali Raing | Range extension | 27 | February | 2016 | 27.577 | 97.371 |
| Oriental Magpie | *Pica serica* | 1 in Hai Sheng, Laihka, Southern Shan; Paddy field, forest | Range extension | 31 | March | 2023 | 21.241 | 97.77 |
| Oriental Magpie | *Pica serica* | 1 in Lwae Sai, Namhsan, Northern Shan; Tea plantation, forest at pagoda | Range extension | 31 | May | 2005 | 22.867 | 97.229 |
| Oriental Magpie | *Pica serica* | 2 in Keng Tung village, Eastern Shan, Paddy field, forest | Range extension | 2 | August | 2016 | 21.398 | 99.627 |
| Oriental Magpie | *Pica serica* | 2 between Weng Kao and Wan Nawngnio, Southern Shan, in forest patches | Range extension | 5 | June | 2023 | 21.708 | 98.115 |
| Baikal Bush Warbler | *Locustella davidi* | A Ma Phya, Grassland, Ayeyarwady delta | Range extension | 16 | February | 2023 | 16.781 | 95.25 |
| Baikal Bush Warbler | *Locustella davidi* | Tat Seik, Grassland, Ayeyarwady delta | Range extension | 15 | February | 2023 | 16.763 | 95.257 |
| Baikal Bush Warbler | *Locustella davidi* | Tat Seik, Grassland, Ayeyarwady delta | Range extension | 20 | January | 2023 | 16.781 | 95.25 |
| Baikal Bush Warbler | *Locustella davidi* | Tat Seik, Grassland, Ayeyarwady delta | Range extension | 12 | March | 2023 | 16.781 | 95.25 |
| Blue-and-White Flycatcher | *Cyanoptila cyanomelana* | Kyauk Taung Camp, Tanintharyi | Range extension | 27 | March | 2012 | 17.029 | 96.098 |
| Rufous-bellied Swallow | *Cecropis badia* | 29 individuals at Ngawun Reserve Forest, Tanintharyi | Breeding confirmation | 3 | September | 2020 | 11.175 | 99.164 |
| Rufous-bellied Swallow | *Cecropis badia* | 5 females Forest river 27miles village Pyigyimandai, Tanintharyi | Breeding confirmation | 12 | May | 2012 | 11.188 | 99.159 |
| Rufous-bellied Swallow | *Cecropis badia* | 8 at Lenya Reserve Forest, Bokepyin, Tanintharyi | Breeding confirmation | 6 | April | 2022 | 11.244 | 99.184 |
| Rufous-bellied Swallow | *Cecropis badia* | documentation of the nest | Breeding confirmation | 20 | May | 2012 | 11.188 | 99.159 |
| Rufous-bellied Swallow | *Cecropis badia* | further 5 individuals at Ponekani Camp, Tanintharyi | Breeding confirmation | 15 | May | 2012 | 11.628 | 99.259 |

mainly in Thailand/Indonesia/Malaysia. The species is supposedly wintering and breeding in northeastern China (Craig & Feare 2020a); however, *A. sturninus* has been found all over southern Asia, too. The so far northernmost record in Myanmar was in Chanayethazan, Mandalay (GBIF 2023d). Note: several synonymies are found and these include Purple-backed Starling *Sturnus sturninus*.

9. Pin-tailed Parrotfinch *Erythrura prasina* (Image 9) from Machanbaw is a new record for northern Myanmar (Table 1), possibly for all northern half of Myanmar, since in the Shan-Thai border area the detailed occurrence remains imprecise (Payne 2020; GBIF 2023i). From distribution and plumage, we conclude that our individual photographed is from the subspecies *Erythrura prasina prasina*, occurring in Thailand and southern Myanmar (Tenasserim), Peninsular Malaysia, Cambodia, Laos, Vietnam, Sumatra, and Java. The species was recently recorded in China, specifically southern Yunnan (Sreekar et al. 2014), relatively close to our location, as well as in southern Bhutan (GBIF 2023i). We assume it is a female or immature male based on the plumage (Image 9: if the contrast of the photo is increased, red is visible and hence it should be considered an immature male; tail is relatively short and round, not pointed). The record is a substantial range extension towards north, while the species is considered resident in Tanintharyi and Thailand, Malaysia, Cambodia, Laos, and Viet Nam.

10. We recorded one Oriental Magpie *Pica serica* (Image 10, Table 1) in Lwae Sai Pagoda in a Tea plantation with forest patches wandering around the pagoda compound and perching on tree top; two individuals in Keng Tung village in paddy fields and some forest flying across from east to west direction (Image 10); one individual in Hai Sheng village in paddy field and some forest; and two individuals between Weng Kao and Wan Nawngnio villages in forest patches around primary school surrounded by paddy field. So far only two records of *P. serica* (Monywa and Maymyo; GBIF records 3243563386 and 3216275901) are known. An additional record from Kutkai in Northern Shan State is a specimen (GBIF record 1039546472) from 1958 (as *P. pica serica* stored in Yale Peabody Museum, 23.517 N 97.95 E). *P. serica* was recognised as a separate species following the revision of the *P. pica* species complex (Lee et al. 2003; Song et al. 2018), mtDNA phylogeny suggests that Eurasian Magpie comprises several potential species including Oriental (Korean) Magpie *P. serica*, Maghreb Magpie *P. mauritanica* and Asir Magpie *P. asirensis*. Oriental (Korean) Magpie should be split to address paraphyly with Nearctic species (Lee et al. 2003; Gill et al. 2023).

11. We recorded the Baikal Bush Warbler *Locustella davidi* (Image 11) from the Ayeyarwady delta area (Tat Seik and A Ma Phyar; Table 1). It is likely a rare winter visitor to the region. Previously Baikal Bush Warbler have been found in Myanmar, but mainly from central Kachin (Mohnyin and Wara Zup and Shan States (Dhan Ma Kan Wetland, Inle Lake, Pine Hill Resort in Shan (GBIF 2023a)).

12. We found Blue-and-White Flycatcher *Cyanoptila cyanomelana* (Image 12) twice in Kyauk Taung Camp, Tanintharyi (Table 1). This constitutes a range extension south, since so far the species has been found at Mount Popa (central Myanmar) and two at Hlawga Park just north of Yangon (GBIF 2023b).

13. Rufous-bellied Swallow *Cecropis badia*: Our documentation of the nest is likely the first proof of breeding attempt of *C. badia* in all of Myanmar (Image 13). We recorded five females at the river in the forest at Pyigyimandai, further five individuals at Ponekani Camp; 29 individuals at Ngawun Reserve Forest, and further eight at Lenya Reserve Forest (Table 1). The species has been recorded in the border area of Myanmar and Thailand for some time and is likely common in the area (Thet Zaw Naing et al. 2020; Kirwan & Turner 2021; GBIF 2023j).

## DISCUSSION

The quantification of bird species within specific territories is often challenging and offers typically a rough approximation rather than an exact number. However, despite this inherent imprecision, general estimates are possible. Myanmar is believed to host bird species ranging from around 1,022 to 1,216 species Naing (Thet Zaw Naing et al. 2020; BirdLife International 2023; GBIF 2023f).

Since the year 2000, noteworthy developments include the addition of several avian taxa to Myanmar's records. The endemic *Napothera naungmungensis*, two new subspecies *Alcippe cinereiceps hkakaboraziensis* and *Malacocincla abbotti kachinensis* (Renner et al. 2015), the taxa splitting of *Cyornis magnirostris* (Renner et al. 2009), *Tesia olivea olivea* (Renner et al. 2008), and first records of Rosy Starling *Pastor roseus* (Sai Sein Lin Oo et al. 2020), and White-throated Laughingthrush *Garrulax albogularis* (Sai Sein Lin Oo et al. 2019a) have added new taxa for Kachin alone. In addition, the Parasitic Jaeger *Stercorarius parasiticus* has been newly recorded in Shan (Sai Sein Lin Oo et al. 2022).


Image 1. Snow Partridge *Lerwa lerwa*, Nama Jason.


Image 2. Yellow Bittern *Ixobrychus sinensis*, Hkakabo Razi.


Image 3. Common Buzzard *Buteo buteo*, Putao.


Image 4. Black-naped Oriole *Oriolus chinensis*, Hponkan Razi.


Image 5. Crow-billed Drongo *Dicrurus annectens*, Putao.


Image 6. Warbling White-eye *Zosterops japonicus*, Hkakabo Razi.


Image 7. Golden-crested Myna *Ampeliceps coronatus*, Hponkan Razi.


Image 8. Daurian Starling *Agropsar sturninus*, Hkakabo Razi.

The Brahminy Starling *Sturnia pagodarum* (Soe Naing et al. 2016) and 42 other species have been designated as new records for Myanmar between 2005 and 2019 (Thet Zaw Naing et al. 2020). This accumulates to over 51 new recorded taxa since 2000 – an achievement that might be unparalleled globally and possible through the period of roughly 10 years open access to most parts of Myanmar. This achievement holds particular significance considering the scarcity of additions in the temperate regions, whereas in the Global South and the tropical regions, only selected geographic hotspots foster the description of new bird taxa (Renner et al. 2015), with the northern Andes (Colombia/Peru) and the eastern Himalayan, including northern Myanmar, serving as prominent examples (Renner & Rappole 2011b). In light of this rapid progress, we predict that a substantial part of undetected faunal diversity in Myanmar still is prone for discovery (Päckert et al. 2019).

From 2010 to 2023 we comment on 19 taxa, each with new aspects. Among these, three species stand out as new additions to Myanmar, with one of the three even new to southeastern Asia. While our findings have a strong focus on northern Kachin state, our additional two “hotspots”, namely Shan state and Tanintharyi Region, are very important landscapes because of the diverse ecosystems (Murray et al. 2020). Myanmar’s ecosystem diversity is highest along the Himalayan foothills in the north of Kachin state, where the Shan plateau meets the central dry zone; along the Rakhine range in the west; and much of the Tanintharyi lowlands. These


**Image 9. Pin-tailed Parrotfinch *Erythrura prasina*, Machan Baw.**


**Image 10. Oriental Magpie *Pica serica*, Keng Tung village, Eastern Shan.**

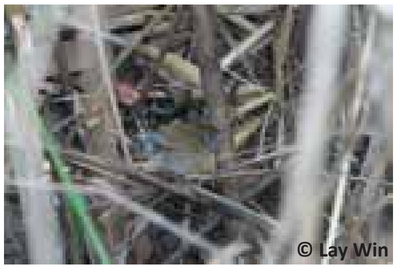


**Image 11. Baikal Bush Warbler *Locustella davidi,* Ayeyarwady delta area.**


**Image 12. Blue-and-White Flycatcher *Cyanoptila cyanomelana*, Putao.**


**Image 13. Rufous-bellied Swallow *Cecropis badia* on 12 May 2012. Payar Dan Cave near Kawthoung (Bokepyin Township), Tanintharyi.**

three habitats encompass a rich array of ecosystems and habitats, ranging from mountain conifer forests and montane temperate broadleaf forests to semi-evergreen forests, rainforests, limestone tropical evergreen forests, coastal wetlands, and mangroves. This ecological variety provides a favourable environment for many bird species. Given their irreplaceable nature, these areas warrant prioritized conservation efforts to ensure the protection of both known and potentially undiscovered species.

Among the 19 species considered, 13 were observed in northern Myanmar, predominantly within Kachin's northern parts. An additional six species were identified in the Shan and Tanintharyi Regions. Considering over 1,200 bird species existing within Myanmar (Thet Zaw Naing et al. 2020), 60 are globally threatened (IUCN Red List), 10 are country endemics, 873 land birds, 21 seabirds, 352 migratory (or visiting), and 158 water birds (BirdLife International 2023). Our records comprise approximately 1.6% of the known birds of Myanmar, but still each of our records is important, because each addition has its own unique habitat preferences and ecological requirements, allowing them to survive in various environments.

The presence of these bird species in a variety of ecosystems in Kachin, Shan states, Tanintharyi Region, and Ayeyarwady region highlights the importance of safeguarding and preserving diverse habitats for maintaining bird biodiversity. Different habitats provide essential resources like food, nesting sites, and shelter, supporting the survival of these bird species throughout their range. Conservation efforts should prioritize the protection of these ecosystems to ensure the continued existence of birds and their contributions to ecological resilience.

Last but not least, it is worth noting that particularly the far north of Myanmar hold potential for new species to be discovered, since not only birds, but also other vertebrates are continuously being added and new descriptions are not rare: species of bats (Soisook et al. 2017) and additions in bat diversity (Bates et al. 2021) are ongoing and several other mammal species such as the Leaf Deer (Rabinowitz et al. 1999) have been described relatively recently.

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အနှစ်ချုပ် -

မြန်မာနိုင်ငံသည် အရှေ့တောင်အာရှ၌သာမက တောင်အာရှနှင့် အရှေ့အာရှနိုင်ငံများ၌တွင်လည်း ဇီဝမျိုးစုံမျိုးကွဲ ကြွယ်ဝသော နိုင်ငံဖြစ်သည်။ ယခုထက်တိုင် မြန်မာနိုင်ငံအတွက် ငှက်မျိုးစိတ် မှတ်တမ်းသစ်များနှင့် မတူကွဲပြားသည့် စားကျက်နေရာဒေသသစ်များကို ရှာဖွေမှတ်တမ်းတင် ဖော်ထုတ်လျှက်ရှိပါသည်။ ဤလေ့လာမှုတွင် သွားရောက်လေ့လာရန် ခက်ခဲသော

နေရာဒေသများဖြစ်သည့် ခါကာဘိုရာဇီရေခဲတောင်ဒေသ၊ ရှမ်းကုန်းပြင်မြင့်ဒေသ၊ တနင်္သာရီတိုင်းဒေသကြီးနှင့် ဧရာဝတီမြစ်ဝကျွန်းပေါ်ဒေသများရှိ ထူးခြားငှက်မျိုးစိတ်များကို ၂၀၁၀ မှ ၂၀၂၃ ကာလအတွင်း အလေးထား ရှာဖွေစုဆောင်း မှတ်တမ်းတင်ထားခြင်း ဖြစ်သည်။ ငှက်မျိုးစိတ်ပေါင်း ၁၃ မျိုးကို အသံနှင့် ဓာတ်ပုံများကို အသုံးပြုကာ မှတ်တမ်းတင်ထားခြင်း ဖြစ်သည်။ ထိုမှတ်တမ်းတွင် မြန်မာနိုင်ငံ၏ အရေးကြီးသော ငှက်မျိုးစိတ်များ အခန်းကဏ္ဍနှင့် အလေးပေး ထိန်းသိမ်းရန်အတွက် ဖော်ပြထားခြင်း ဖြစ်သည်။ ဤလေ့လာမှုသည် မြန်မာနိုင်ငံအတွင်း ငှက်မျိုးစိတ်များ ကျင်လည်ကျက်စားမှုဆိုင်ရာ အသိပညာဗဟုသုတများကို ငှက်သုတေသီများနှင့် ငှက်ဝါသနာရှင်များအတွက် နောင်အနာဂတ်တွင် ဆက်လက်ဖော်ထုတ်နိုင်ရန် လမ်းကြောင်းပေးခြင်း ဖြစ်ပါသည်။

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ARTICLE

# Ultra-structure of antenna, eye, mouthparts and sensilla of *Cheilomenes sexmaculata* Fabricius, 1781 (Coccinellidae: Coleoptera)

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**Abstract:** *Cheilomenes sexmaculata* Fabricius, 1781 is a well-known ladybird beetle that feeds primarily on aphids, mealy bugs, phytophagous mites, scale insects, and other small insects and is an important bio-control agent of aphids. Evolution in the structure of mouthparts is known to be associated with the composition of food material and feeding behaviour. The sensilla present on mouthparts plays a pivotal role in feeding mechanisms. Feeding mechanism of ladybird beetles will be better understood if the fine structure of mouthparts, modifications in the structures, and presence of different sensilla on the mouthparts of *C. sexmaculata* are understood. Using scanning electron microscopy, the functional morphology of mouthparts of *C. sexmaculata* was studied and compared with the fine structures with those of other Lady Beetles. The labrum, mandibles, maxillae, labium, and hypopharynx make up the mouthpart of *C. sexmaculata.* This study includes a thorough description of each mouthpart and the different kinds of sensilla. In the current study, three types of sensilla chaetica, four types of sensilla basiconica, two types of sensilla styloconica, one type of sensilla placodea, one type of sensilla coeloconica, one type of sensilla campaniformia, and one type of cuticular pore using characters like the length, morphology, and distribution of the sensilla were studied. The structure of the mouthparts and the purposes of the sensilla on them is discussed with reference to other coleopterans. The results obtained in the current study will provide better insights into the functional morphology of lady beetles.

**Keywords:** Functional morphology, labrum, labium, ladybird beetles, mandible, maxilla, scanning electron microscopy, sensilla.

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**Author contributions:** PGG, MSP involved in the designing experiments, conduct of experiments, data analysis and manuscript writing. All authors have read and approved the final manuscript.

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## INTRODUCTION

One environmentally friendly way to combat the threat of pests is through the use of biological control agents (Ayer et al. 1976). The biological control of aphids, mealy bugs, phytophagous mites, and scale insects is attributed to some species of lady beetles (Samways et al. 1997; Sarwar & Saqib 2010; Li et al. 2012). In most coleopterans, mouthparts have evolved for chewing solid food material (Belhoucine et al. 2013). The members of the Coccinellidae family are equipped with chewing mouthparts (Ayer et al. 1976). Several mouthparts, including the mandible, maxilla, labium, and palpi, are significant in feeding behavior (Seo & Youn 2000; Wang et al. 2000; Luo et al. 2006; Wei et al. 2015; Hao et al. 2019). In ladybird beetles, the feeding process is quite complicated, and the maxillary palpi and labial palpi play a crucial role in contact reception and the chewing process (Hao et al. 2019). Most of these structures are generally classified as the labio-maxillary complex and may show differences in type and feature depending on their feeding mechanism. Sensilla, or sensory structures are present in the insect body and are home to neurons responsible for smell, taste, sound, touch, proprioception, and perception of temperature, humidity, and space (Ruschioni et al. 2019). The variation in feeding behavior is mainly influenced by sensilla presented on mouthparts (Brożek & Bourgoin 2013). In addition to aiding in identifying food resources, these sensilla are crucial for determining the precise microhabitat and assessing the quality of food (Ruschioni et al. 2019). Similarly, mouthparts and antennal sensilla help in finding mating partners and in finding suitable oviposition sites on the host plants (Li et al. 2022). In this context, a detailed study of mouthparts is important for understanding the feeding mechanism of bio-control agents and for developing effective management strategies against pests (Rani & Madhavendra 1995, 2005; Hao et al. 2019). Numerous studies have been carried out to comprehend the functional morphology of the mouthparts of beetles earlier (Eilers et al. 2012; Wilhelmi & Krenn 2012; Belhoucine et al. 2013; Moon 2015; Chen et al. 2016).

Very few have described the ultra-structure of the mouthparts of the predatory lady beetles (Thornham et al. 2007; Hao et al. 2019). *C. sexmaculata* Fabricius, 1781 is a member of Coccinellidae and is well known for predating on aphids and small insects (Yilda et al. 2017). Understanding the ultrastructure of mouthparts will provide better insight into the feeding mechanism and will be helpful in designing and implementing an effective biological control program. To the best of the knowledge, no previous attempts have been made to elucidate the fine structure of mouthparts of *C. sexmaculata* using scanning electron microscopy. In this study, the functional morphology of mouthparts, especially the fine morphology of labrum, labium, mandibles, maxillae, eye, antenna, and various sensilla of *C. sexmaculata,* was studied using the scanning electron microscope. Additionally, the importance of the structure of mouthparts and sensilla in the feeding habit of *C. sexmaculata* were also discussed.

## MATERIAL AND METHODS

### Collection sites

*Cheilomenes sexmaculata* adults were collected from Alapalli (19.430 °N, 80.047 °E) and Gadchiroli (20.158 °N, 79.998 °E) areas of central India, eastern Vidarbha region of Maharashtra. The Gadchiroli district's forest includes reserved and protected forest areas measuring 218,529.27 ha excluding 882.22 ha Zudpi jungle (https://mahaforest.gov.in). Forest cover mainly consists of dry deciduous miscellaneous forests.

### Sample collection

The specimens were collected by net and handpicking and then kept in the laboratory at 4°C after being preserved in 70% ethanol. Voucher specimens are deposited in the Government Science College specimen voucher repository (Accession Number GSC/Zoo/2021/Coleoptera-6).

### Sample preparation for scanning electron microscopy

The mouthparts of *Cheilomenes sexmaculata* were dissected using a stereo microscope (Stemi 509, Carl Zeiss, Germany) using fine needles and then cleaned using 70% ethanol. After that, the samples were dehydrated for 30 min each in ethanol grades of 80%, 90%, & 95%; for 45 min in 100% ethanol; and subsequently dried in a desiccator. The dried mouthparts and whole head were glued to aluminium stubs using conducting carbon tape and coated with gold/palladium (40/60) using a high-resolution sputter coater (Quorum, United Kingdom) for a thickness of about 10 nm. The samples were subsequently examined with a Tescan Wega 3 scanning electron microscope (Tescan, Czech Republic) operated at 20 kV using an SE detector. Mouthparts of five males and five females were observed initially under a stereo microscope (Stemi 509 stereomicroscope equipped with camera and imaging software, Zeiss, Germany) and no clear sex-dependent morphological differences were found in the mouthparts. The description of sensilla was followed as per the nomenclature given by Altner & Prillinger (1980), and Brożek & Bourgoin (2013). Using Tescan Wega threes software, the length, diameter, and distribution of each type of sensilla from 10 adult male and 10 adult female specimens of *Cheilomenes sexmaculata* were measured. Each type of sensilla's length, base, and diameter are measured from 146 microphotographs of various mouthparts, and the results are tabulated as mean ± SD (Tescan Electron Microscope Software, Wega 3, Tescan Czech Republic) (Table 1).

## RESULTS

Mouthparts in *C. sexmaculata* consist of the mandibles, maxillae, labium, labrum, and hypopharynx. Only the labrum and the maxillary palp can be seen on the front of the head when observed dorsally. From the ventral view, part of the mandibles appears below the labrum. The labium is positioned between the left and right maxillae in the central part (Image 1). There is a non-sclerotized hypopharynx on the labium's inner wall. The eyes and antennae are visible from the dorsal side.

### Morphology of the labrum.

The anterior border of the anteclypeus has an oval-shaped labrum attached to it. Sensilla chaetica 3 (Sch3) are found arranged in a row close to the frontal margin on the dorsal surface of the labrum, while sensilla chaetica 2 (Sch2) are arranged on the left and right margins (Images 2). Sensilla basiconica 1 and 2 (Sb1, Sb2) and several cuticular pores (cp) are present at the margin (Image 2). Sensilla coeloconica (sco) is observed on the ventral surface of the labrum, close to a row of sensilla chaetica 3 (Image 2). Campaniform sensilla (Sca) are also observed on the ventral surface. The ventral surface of the labrum was concave (Image 2) and covered with different cuticular, spiny (Image 2), and palmate processes (Image 2).

### Morphology of mandibles

Left and right mandibles are found attached to the head with dorsal and ventral articulations (Image 3). The inner margin of the mandible is divided into two main regions: the molar region and the incisor region (Image 3). The incisor region possesses pointed teeth apically. The mandible's dorsal view revealed a rough cuticle (Image 3), a lot of cuticular pores (Cp) strewn across the surface, and sensilla chaetica 1 (Sch1) near the condyle (Image 3). The ventral surface of the mandible also appears rough, and palmate cuticular processes are seen close to the ventral condyle; no other sensilla are noticed (Image 3). Petaloid cuticular processes covered the upper portion of the dorsal condyle (Image 3). Single tubercle forms the molar region (Image 3). On mandibles, a prostheca with short, comb-like setae extends along the incisor margin up to the base of the incisor projection (Image 3). The prostheca has a smooth surface with low cuticular pores (Cp). The articulating surface is hemispheric with a smooth surface ventrally and oval with cuticular processes dorsally (Image 3). Around the ventral condyles, the cuticle is smooth and has many cuticular pores (Cp) (Image 3). On the margin of the ventral region, there is an additional cluster of spiny cuticular processes.

### Morphology of maxillae

The paired maxillae are found placed in longitudinal clefts on either side of the labium (Image 1) and show the usual parts: cardo, stipites, galea, lacinia, and maxillary palps (Image 1 & 4). The cuticle of the semicircular cardines (Image 4) has Cp on it. The stipites are flat plates with sensilla chaetica 1 and 2 (sch1, sch2) on the surface (Image 4). The maxilla includes the inner lacinia and outer galea, the two distal lobes (Image 4). The galea is a spoon-like structure, and it is covered with sensilla basiconica 3, 4 (Sb3, Sb4) and cuticular pores (Cp) (Images 4A,B,J,K). A triangular depression is present on the top of the galea (Images 4J,K). Brush-like lacinia is located below the galea, and sensilla basiconica 3 (Sb3) is present at the edge and on the whole apical area of lacinia and sensilla basiconica 4 (Sb4) on the ventral side (Image 4). In *C. sexmaculata*, the maxillary palpi are well developed and comprise four articles that widen gradually; the final article has a somewhat triangular shape (Image 4). Maxillary palpi surfaces are scaly, with cuticular pores (Cp), sensilla chaetica 2 (Sch2), and sensilla basiconica 1 and 2 (sb1 and sb2) dispersed across each segment (Image 4). In the middle region, two main types of sensilla styloconica (Sty1 and Sty2) and sensilla placodea 1 (Sp1) at the margin of the sensory field of the maxillary palp are present (Image 4I).

### Morphology of labium

In general, the labium of an insect mouthpart is composed of the prementum, postmentum, glossae, paraglossae, and labial palpi. In *C. sexmaculata,* the paraglossa and glossa formed a combined structure named ligula (Image 5). The prementum is wider and

**Table 1. Morphology measurements and distribution of sensilla on mouthpart of *Cheilomenes sexmaculata*.**

| Type | Shape | Socket | Surface | Pore | Length (µm) with average and standard deviation | Basal diameter (µm) with average and standard deviation | Distribution |
|---|---|---|---|---|---|---|---|
| **Sch1** | Hair, peg | Concave | Grooved | No | 34.02 (6.83) | 3.7 (0.75) | lig, mx, mp, antenna, lm |
| **Sch2** | Hair | Concave | Grooved | No | 62.2 (15.99) | 4.47 (0.93) | lm, lig, prm, mp, mx, lp |
| **Sch3** | Hair, peg | Concave | Grooved | No | 348.54 (137.15) | 13.2 (7.45) | lm, lig, mp |
| **Sb1** | Peg | Concave | Smooth | No | 6.44 (2.25) | 2.1 (0.73) | lm, lp, prm, md, mp, mx, gal, lig, |
| **Sb2** | Peg | Concave | Smooth | No | 15.64 (4.32) | 3.27 (1.17) | lm, md, mp, gal, lac, lig, mp, lp |
| **Sb3** | Hair, peg | Concave | Smooth | No | 34.94 (9.06) | 5.41 (0.45) | lm, md, mx, lac, lig |
| **Sb4** | Hair, peg | Concave | Smooth | No | 67.65 (7.00) | 4.55 (0.76) | gal, lac |
| **Sty1** | Conical | Convex | Grooved | Tp | 3.12 (0.52) | 1.61 (0.27) | lp, mp |
| **Sty2** | Cylindrical | Convex | Grooved | Tp | 2.64 (0.35) | 1.99 (0.38) | mp |
| **Sco** | Round | | | | | | lm |
| **Sca** | Round | | | | | | lm |
| **Cp** | Round | | | | | | lm, lig, mx |

Sch 1–3—sensilla chaetica I–III | Sb 1–4—sensilla basiconica I–IV | Sty 1–2—sensilla styloconica I–II | Sco—sensilla coeloconica | Sca—sensilla campaniformia | Cp—cuticular pore | Tp—terminal pore | mp—maxillary palp | gal—galea | lac—lacinia | lb—labium | lm—labrum | lp—labial palp | md—mandible | mx—maxillae.

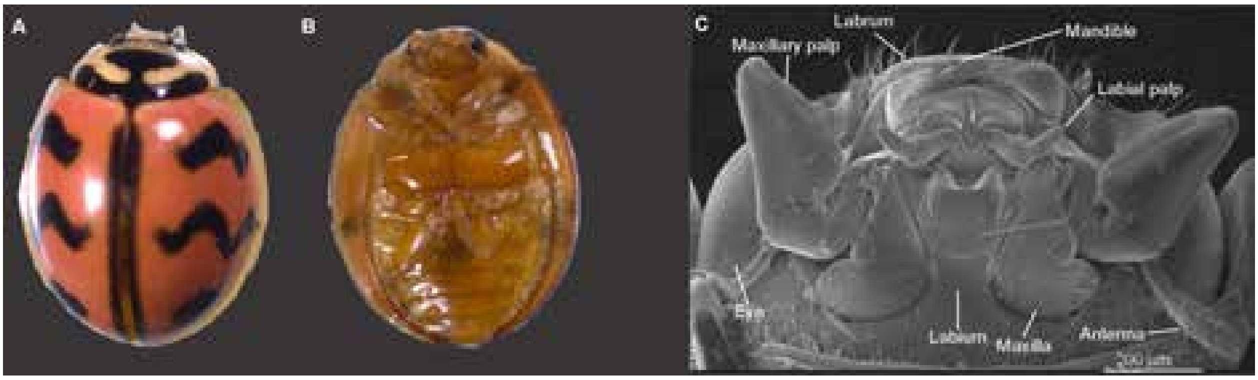


**Image 1. Stereo microscope images of adult *Cheilomenes sexmaculata* and scanning electron micrographs of its mouthparts: A—dorsal view | B—ventral view | C—SEM ventral view of head showing the position and morphology of labrum, mandibles, maxillae, maxillary palpi, labium, and labial palpi. © Mandar Painganka & Prakash Ghagargunde.**

elevated at the upmost end while a little narrow and without elevation at the bottom (Image 5). The cuticle of the postmentum is smooth with numerous cuticular pores (Cp) and sensilla chaetica 2 (Sch2) (Images 1 & 5). Two symmetrical labial palpi are present on the ligula, which are inserted into the prementum (Image 5). Sensilla basiconica 2 (Sb2), spiny and palmate processes cover the terminal portion of the ligula (Images 5), while sensilla chaetica 1 and 2 (Sch1, 2) and cuticular pores (Cp) are present in the proximal part (Image 5). The labial palp is elongated and shows three segments. The basal segment is short, bearing almost no sensillum (Image 5). With a reticulated surface, the elongated middle segment progressively widens from base to top and bears sensilla chaetica 1 (Sch1), sensilla basiconica 1 and 3 (Sb1, Sb3) (Image 5). With a single circular sensory field at the distal end, the final segment has a bullet-like shape (Image 5). In this sensory field, there are- 18 sensilla styloconica 1 (Sty1), but no other sensilla or cuticular pore is observed. (Image 5).

### Types of sensilla

The mouthparts of *C. sexmaculata* exhibit 13 different subtypes of sensilla in total: these are three types of sensilla chaetica, four types of sensilla basiconica, two types of sensilla styloconica, one type of sensilla placodea, one type of sensilla coeloconica, one type of sensilla campaniformia, and one type of cuticular pore

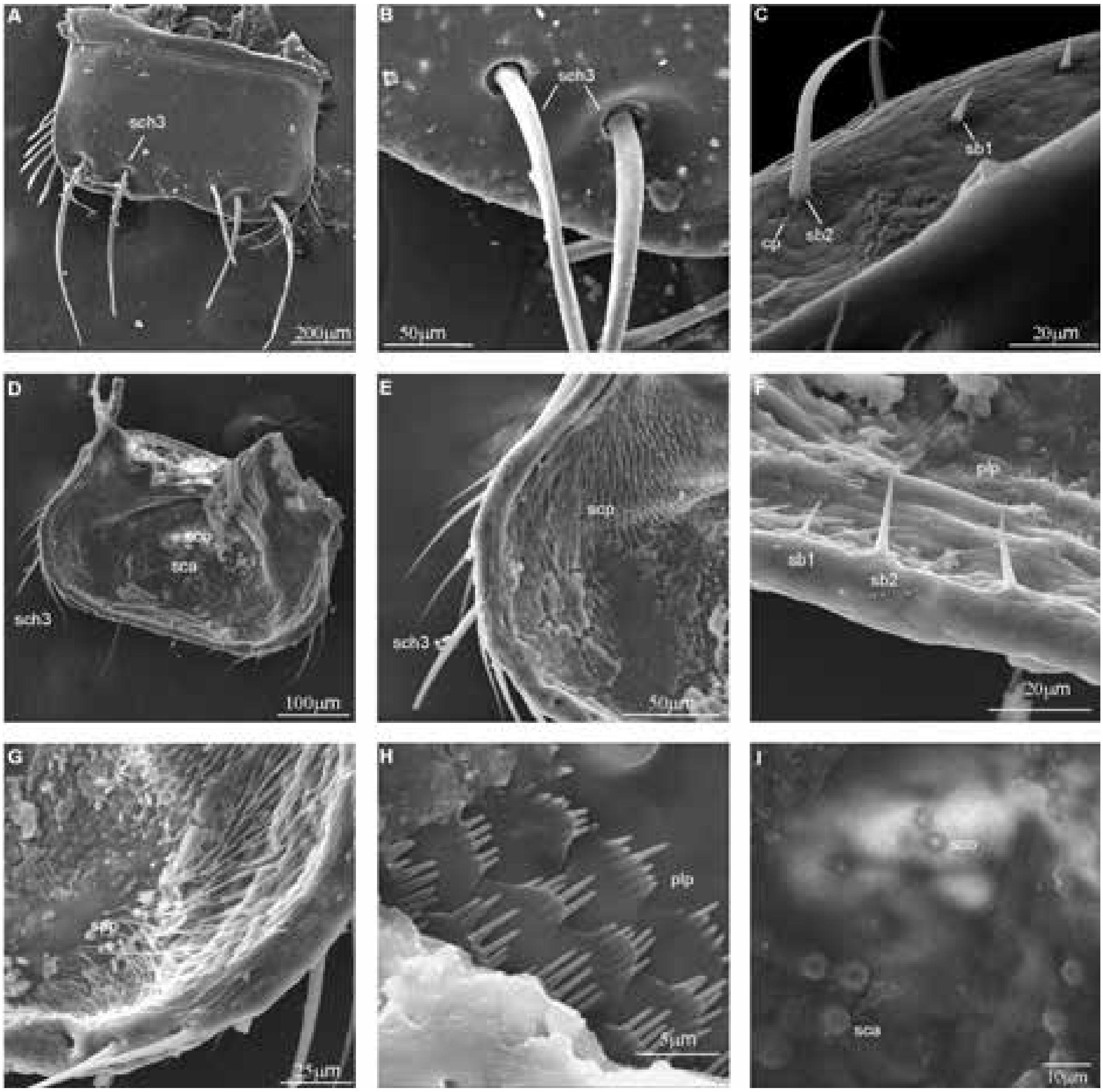


**Image 2. Scanning electron micrographs of the labrum of *Cheilomenes sexmaculata*: A—dorsal view of labrum | B—sensilla chaetica3 (sch3) | C—cuticular pores (cp), sensilla basiconica1 and 2 (sb1,2) at margin | D—ventral view of labrum | E—scaly process on epipharynx | F—sensilla basiconica1,2 (sb1,2) at margin and palmate process on epipharynx | G—spiny process on epipharynx | H—palmate processes | I—sensilla campaniformia (sca), sensilla coeloconica (sco) on epipharynx. © Mandar Painganka & Prakash Ghagargunde.**

(Table 1).

Based on length and diameter, three types of sensilla chaetica, sensilla chaetica 1 (Sch1) , sensilla chaetica 2 (Sch2), and sensilla chaetica 3 (Sch3) are observed on the mouthpart surfaces of *C. sexmaculata*. Sensilla chaetica 1 (Sch1) are short, robust sensilla that resemble hair or pegs and are placed into a round, concave socket. They are present on the mandibles, stipites of maxilla, ligula, prementum, and labial palp of labium (Images 3,4,5, I,K). Sensilla chaetica 2 (Sch2) has an external morphology resembling a peg. They are distributed on the lateral margin of the labrum, mandibles, stipites of the maxilla, ligula, and prementum of the labium (Images 3,4,5,D,I). Sensilla chaetica 3 (Sch3) resembles sensilla chaetica 1 and 2 (Sch1, Sch2) in terms of their morphology and resembles hair or pegs. Sensilla chaetica 3 (Sch3) tapers toward the apical end from a broad base. They are dispersed throughout the ligula of the labium and the outer front margin of the labrum (Images 2 & 5).

Four types of sensilla basiconica, sensilla basiconica

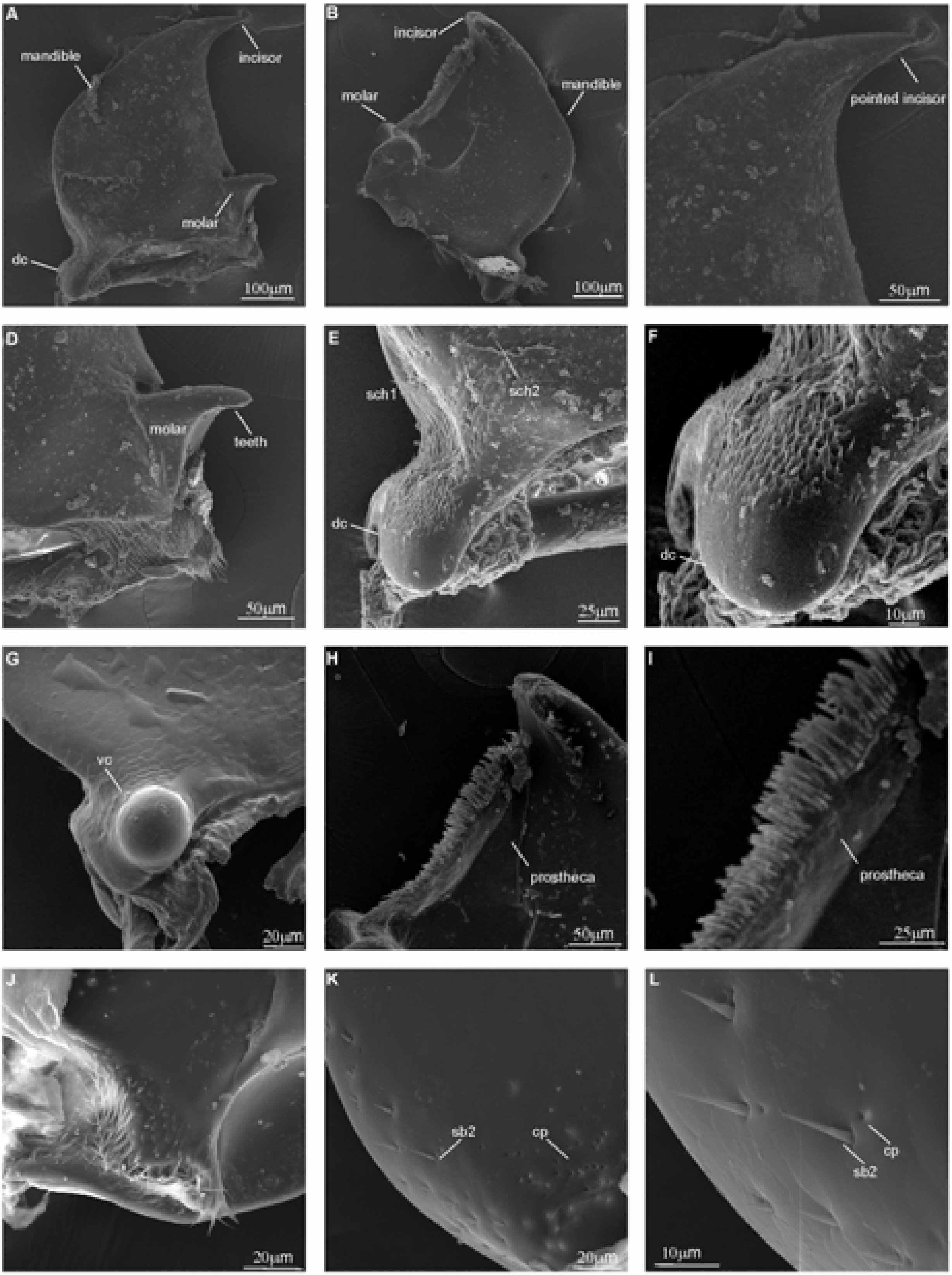


**Image 3. Scanning electron micrographs of the mandible of *Cheilomenes sexmaculata*: A—dorsal view | B—ventral view with dorsal and ventral teeth (Dt, Vt) | C—pointed incisor | D—molar teeth | E—dorsal condyle | F—close view of dorsal condyle | G—ventral condyle | H—prostheca | I—setae of prostheca | J—spiny processes close to molar teeth | K—sensilla basiconica 2 (Sb2) and cuticular pores (cp) on mandible | L—sensilla basiconica 2 and cuticular pores. © Mandar Paingankar & Prakash Ghagargunde.**

1 (Sb1), sensilla basiconica 2 (Sb2), sensilla basiconica 3 (Sb3), and sensilla basiconica 4 (Sb4) are observed on different mouthparts of *C. sexmaculata*. Sensilla basiconica 1 (Sb1) is inserted into a round, concave socket and is short, straight, thick at the base, and sharp at the tip. Sensilla basiconica 1 (Sb1) has a smooth surface. They are found distributed on the side margin of the labrum, maxillary palp, and labial palp (Images 2,4). Sensilla basiconica 2 (Sb2) is relatively straight and longer than sensilla basiconica 1 (Sb1), has a smooth surface, and is thick at the base while sharp at the tip (Images 2,3). These are distributed on the ventral margin of the labrum and mandibles. Placed in concave sockets, sensilla basiconica 3 (Sb3) is stronger than sensilla basiconica 1 and 2 (Sb1, Sb2). Sensilla basiconica 3 (Sb3) has a smooth exterior devoid of pores. Sensilla basiconica 4 (sb4) was found to be stronger and longer than the other, hair- and peg-like sensilla with a smooth surface bearing no pores, and they are only present on the galea and lacinia of the maxilla (Images 4).

Sensilla styloconica 1 (Sty1) is a conical structure with a petal-shaped convex socket. They are present on the central part of the maxillary palpi and labial palpi (Images 4,5). The cylindrical sensilla styloconica 2 (Sty2) is placed into a convex socket. These sensilla's tips are flat, and their upper surfaces are smooth. They are visible on the central region of the maxillary palpi (Image 4).

At the margin of the sensory field of maxillary palp, spiny, triangular, non-sensory, dentiform cuticular projections are distributed among these circular, slightly convex, rugose sensilla placodea 1 (sp1) found embedded (Images 4H–I).

Sensilla coeloconica (Sco) are round structures with bumps in the center that cannot be distinguished clearly. They are present on the ventral surface of the labrum (Image 2).

Sensilla campaniformia (Sca) are round with a circular depression at the center and observed only on the ventral surface of the labrum (Image 2). The labrum, mandibles, maxilla, and labium all have small, round, pores called cuticular pores (Cp) (Images 2,3,4,5).

**Morphology of antenna and eye**

The antennae of *C. sexmaculata* are made up of a scape, pedicel, and flagellum. There are nine flagellomeres in the flagellum; the first flagellomere has sensilla chaetica 1 and 2 (Sch1, Sch2), while the scape has sensilla chaetica 3 (Sch3). Sensilla basiconica 1 and 2 (Sb1, Sb2) are present on the tip of the ninth flagellomere and lower part of scape (Image 6). *C. sexmaculata* has compound eyes made up of ommatidia. The surface of the ommatidia is smooth; only a few randomly distributed interommatidial hairs are present (Image 6).

## DISCUSSION

Information about the feeding ecology of several insect species has been well documented in the literature; very few studies have been targeted to describe the mouthparts at the ultra-microscopic level (Belhoucine et al. 2013; Moon 2015; Chen et al. 2016; Nel et al. 2018; Hao et al. 2019). Ample literature is available on the mouthpart structures and feeding mechanisms of ground beetles, coccinellid beetles, and other coleopterans (Forsythe 1982, 1983; Ricci & Stella 1988; Pradhan 1938; Samways et al. 1997; Moon 2008; Karolyi et al. 2016), very few studies are available on predatory lady beetles. Lady beetles are known as potent bio-control agents, therefore, understanding the ultrastructure of mouthpart morphology will provide better insights into the feeding mechanism of *C. sexmaculata*. The better understanding of the ultrastructure of mouthparts will be helpful in designing and implementing an effective biological control program (Karolyi et al. 2016). One significant aphidophagous predator in Asia is the ladybird *Cheilomenes sexmaculata* (F.) (Agarwala & Yasuda 2000). Aphids such as *Aphis craccivora* (Koch) (Agarwala et al. 2001), *Aphis gossypii* (Glover), *Rhopalosiphum maidis* (Fitch), *Myzus persicae* (Sulzer), *Uroleucon compositae* (Theobald), *Lipaphis erysimi* (Kaltenbach), and *Aphis nerii* (Boyer de Fonscolombe) have all been observed to be among its prey (Omkar & Bind 2004). In high-temperature agroecosystems and greenhouses, its voracity and heat endurance may make it an effective biological control agent (Wang et al. 2013). In the current study, using scanning electron microscopy,the morphology of mouthparts of *C. sexmaculata* was described. This may be the first attempt to comprehensively describe the fine structures and various sensilla present on its mouthparts.

The scanning electron microscopic studies showed typical chewing types of mouthparts of *C. sexmaculata*. The ultrastructure of mouthparts of *C. sexmaculata* showed a resemblance with other ladybird beetles such as C. *transversguttata and H. variegate* (Hao et al. 2019). Unlike other zoophagous species, *C. sexmaculata* shares a mandibular morphology with polyphagous species, which are versatile, non-specialized, and lack particular adaptations for crushing or capturing prey (Forsythe 1983). Unlike *C. sexmaculata*, zoophagous species,

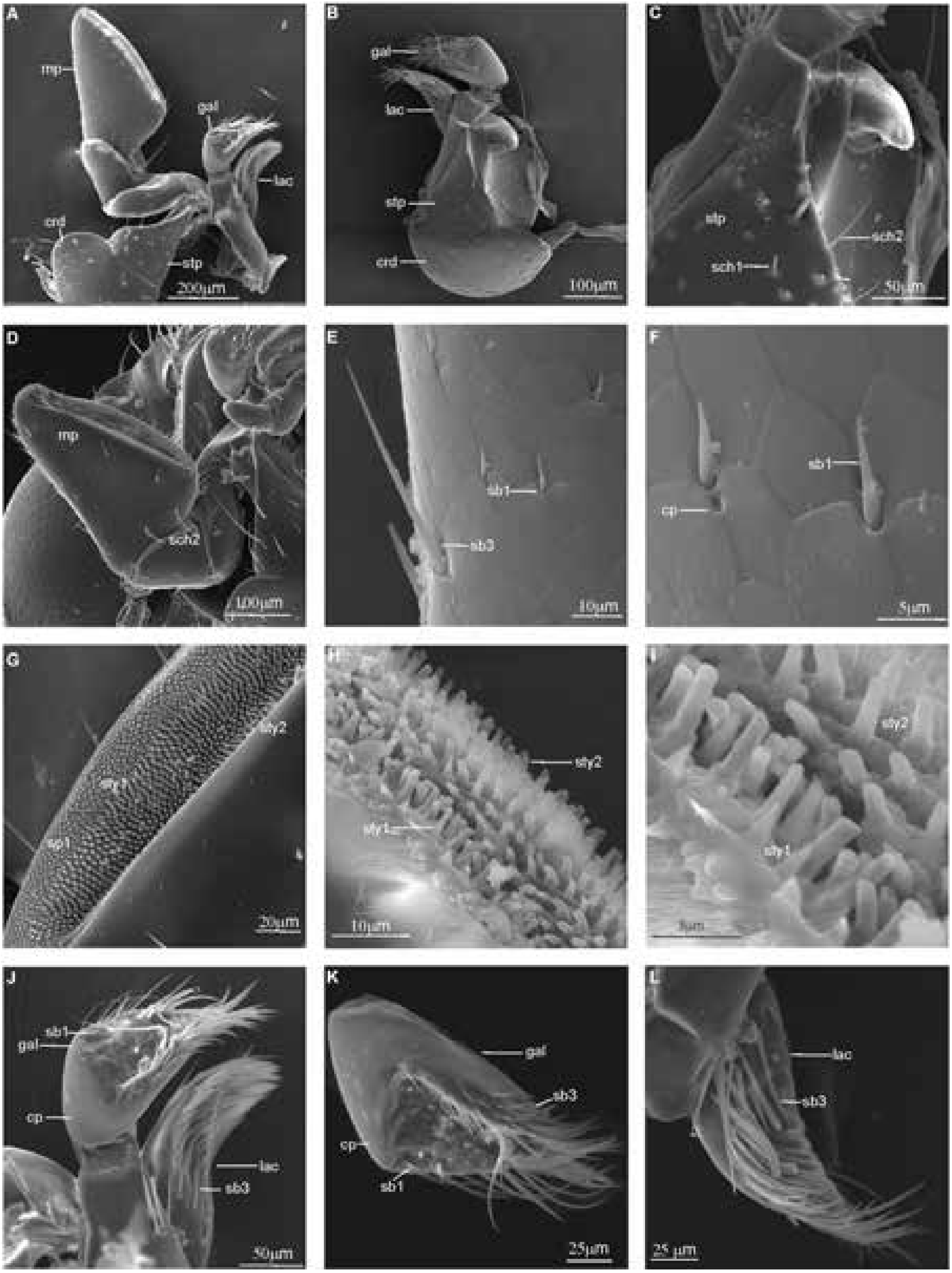


**Image 4. Scanning electron micrographs of the maxilla of *Cheilomenes sexmaculata*: A—ventral view | B—galea, lacinia, stipes and cardo | C—stipes | D—maxillary palp | E—sensilla basiconica1 and 3 (sb1,3) on maxillary palp | F—sensilla basiconica 1 (sb1) and cuticular pore (cp) close view | G—sensory field of maxillary palp | H—sensory field on maxillalry palp | I—sensilla styloconica 1 and 2 (sty1,2) | J—galea, lacinia with sensilla basiconica 1 and 3 (sb1,3) | K—galea showing sensilla basiconica 1, 3 (sb1,3) and cuticular pores (Cp) | L—lacinia showing sensilla basiconica 3, 4 (sb3,4). © Mandar Paingankar & Prakash Ghagargunde.**

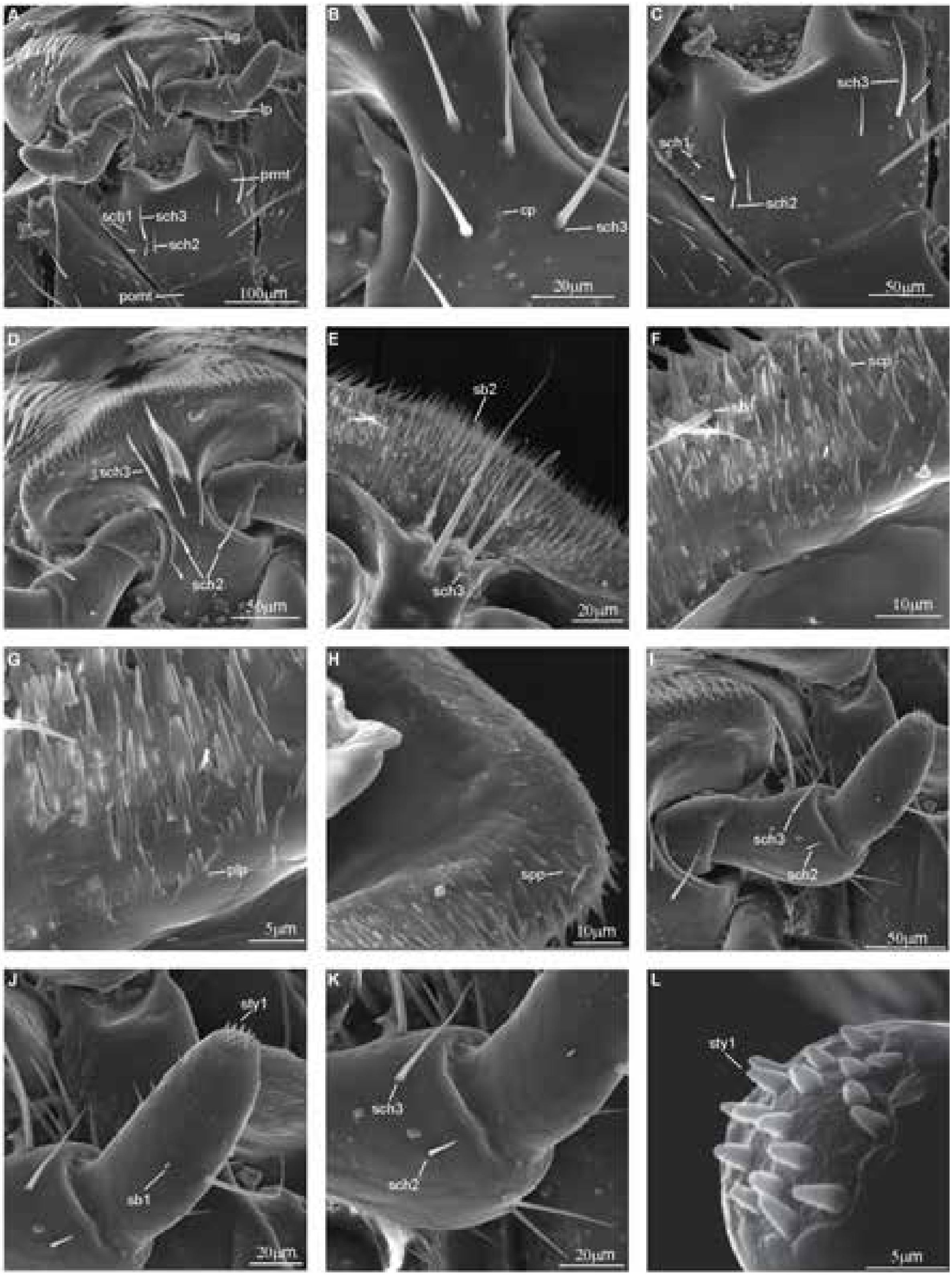


**Image 5. Scanning electron micrographs of the labium of *Cheilomenes sexmaculata*: A—ventral view showing ligula, labial palp, prementum, postmentum | B—sensilla chaetica 3 (sch3) and cuticular pore (cp) on ligula | C—sensilla chaetica 1, 2, 3 (sch1,2,3) on prementum | D—sensilla chaetica 2, 3 (sch2,3) on ligula close view | E—sensilla chaetica 3 (sch3) and sensilla basiconica 2 (sb2) on ligula | F—scaly and palmate processes | G—palmate processes | H—scaly processes | I—sensilla chaetica 2, 3 (sch2,3) on labial palp | J—sensilla styloconica 1 (sty1) on last segment of labial palp | K—sensilla chaetica 2, 3 (sch2,3) on middle segment of labial palp | L—sensilla styloconica 1 (sty1) and sensilla placodea 1 (sp1) at sensory field on top of last segment of labial palp. © Mandar Paingankar & Prakash Ghagargunde.**

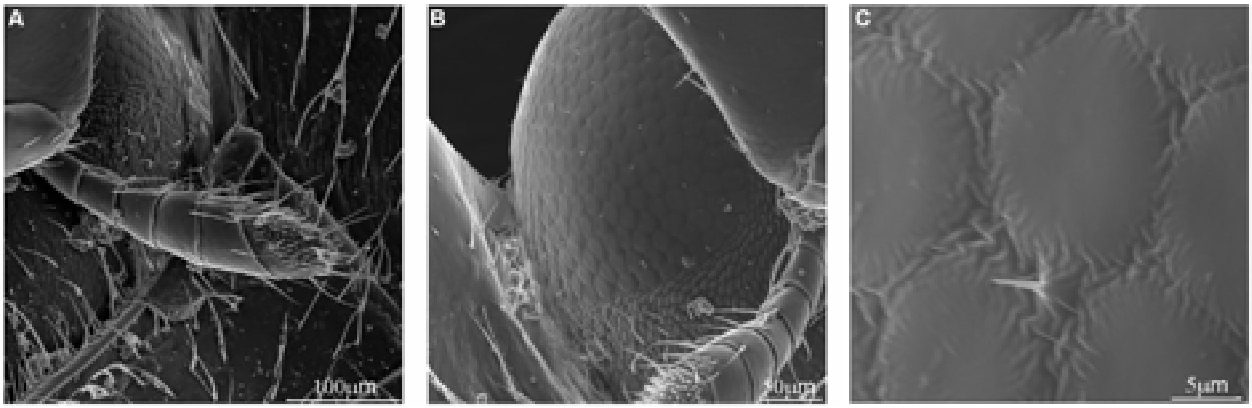


**Image 6. Ultrastructure of the antenna and eye of *Cheilomenes sexmaculata*: A—antenna showing flagellum, scape, and sensilla | B—compound eye | C—hexagonal ommatidia with interommatidial hairs. © Mandar Paingankar & Prakash Ghagargunde.**

such as tiger beetles, have mandibles that are trigonal in dorso-ventral aspect (Ball et al. 2011). Phytophagous species ingest plant juices by scraping leaf surfaces with multidentate mandibles, whereas insectivorous species use unidentate or bifid incisors of mandibles for piercing the prey. During the scarcity of natural prey, few insectivorous species feed on pollen as an alternative source (Samways et al. 1997). It was observed that *C. sexmaculata's* mandibular morphology resembles that of insectivorous beetles like *Coccinella transversoguttata* (Hao et al. 2019). Gut content analysis of *C. sexmaculata* showed the presence of traces of plant material in a few specimens. These observations suggest that when there is limited availability of prey, *C. sexmaculata* might be feeding on plant material. *C. sexmaculata* has a setal row on its prostheca. In scavenger carabids like *Carabus, Calosoma, Cychrus,* and *Scarites*, a brush of dense setae was present on the ventral surface of each mandible (Evans & Forsythe 1985), but in *C. sexmaculata* it is present between the incisor and molar region. In the submolar region of some mandibulate Carabidae, a tuft of mesally projecting setae and, more posteriorly, a soft flap-like structure was observed (Evans & Forsythe 1985)

A long incisor tooth with two or three terebral teeth has been documented in the majority of Cicindelidae taxa (Ball et. al. 2011). Smooth dorsal surface of the mandible with sch1, Cp, bifid incisor, ventral teeth smaller than dorsal teeth, and molars formed by single triangular and sharp teeth are observed in *C. sexmaculata,* which is significantly different from other zoophagous species and indicate the special adaptations for crushing and capturing prey (Forsythe 1983). In this study, two types of sensilla styloconica (Sty1 and Sty2) were found: one type of sensilla placodea (sp1) at the distal end of the maxillary palp and sensilla styloconica (sty1) at the tip of the labial palps, indicating the role of sensilla in gustatory, mechanical, and contact reception while searching for aphids. The base of sensilla styloconica (sty2) was bigger in size as compared to sty1 in *C. sexmaculata,* which indicates that these sensilla might be olfactory in nature.

In the current study, one type of sensilla placodea, one type of sensilla coeloconica, one type of sensilla campaniformia, three types of sensilla chaetica, four types of sensilla basiconica, two types of sensilla styloconica, and one type of cuticular pore are identified on the various mouthparts of *C. sexmaculata*. Interestingly*, t*he mouthparts of *C. sexmaculata* show similarities with some polyphagous and mixed feeder beetles. The results obtained in this study provide baseline information on the ultrastructure of mouthparts and sensilla of *C. sexmaculata* and provide the basis for further investigations on the role of sensilla and feeding mechanisms in the Coccinelidae family. To comprehend the feeding mechanism and the evolution of the remarkably varied feeding habits in the Coccinelidae, more research into the structural features of mouthparts and their functional significance is necessary.

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ARTICLE

# Morphological characterization and ecological insights of *Pseudonapaeus* cf. *candelaris* (L. Pfeiffer, 1846) in the Pir Panjal Range of western Himalaya

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**Abstract:** *Pseudonapaeus* cf. *candelaris* is a terrestrial snail species belonging to the Enidae family, specifically inhabiting the Pir Panjal Range of the western Himalaya. This species displays a unique shell morphology with prominent features including deep furrows, a smooth outer surface, cylindrical form, gradually tapering apex, spiral bands and axial ribs. This study examines the morphometric characteristics of shell and distinct structural patterns observed in specimens from various sites within the region. By documenting these morphological attributes, the research enhances our taxonomic understanding of *P.* cf. *candelaris*, addressing aspects of its classification and ecological role. The findings present critical information for conservation efforts aimed at protecting this regionally significant, endemic snail species.

**Keywords:** Biodiversity, endemic, gastropods, high altitude, molluscs, shell morphology, snail, terrestrial.

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**Competing interests:** The authors declare no competing interests.

**Author details:** Hilal Ahmed, a senior research fellow at the Department of Zoology, University of Kashmir has been engaged in fisheries and molluscan studies since 2019. Dr. Imtiaz Ahmed, a professor at the Department of Zoology, University of Kashmir focuses his teaching and research efforts on fish diversity, nutrition, physiology and molluscan biodiversity since 2006. Dr. Neelavar Ananthram Aravind, a professor at ATREE, Bengaluru brings 25 years of expertise in land and freshwater molluscs in India, focusing on ecology, systematics, biogeography, and conservation. His research employs molecular, spatial and citizen science tools to address pertinent questions in molluscan studies.

**Author contributions:** HA led the entire field sampling, data collection and preparation of the manuscript. IA and Dr. NAA gave study conceptualization, design, manuscript review, editing and supervision. Their significant contributions were crucial for the improvement of the overall quality of the manuscript.

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## INTRODUCTION

The family Enidae comprises a group of pulmonate gastropod molluscs that primarily inhabit terrestrial environments. Woodward (1903) was the first to describe these land snails and they are well known for their air breathing mechanism. Enidae, a taxonomic family belonging to the phylum Mollusca, exhibits notable diversification within this phylum, encompassing approximately 66 genera and 300 species (Molluscabase 2024). The family Enidae is native to India, comprises approximately 29 species. These species are classified into three genera: *Mirus* Albers, 1850 (consisting of five species), *Serina* Gredler, 1898 (consisting of five species), and *Pseudonapaeus* Westerlund, 1887 (consisting of 19 species). Among these species, 21 are found exclusively in India (Kobelt & Mollendorff 1903; Gude 1914; Ray 1951; Solem 1979; Ramakrishna et al. 2010; Tripathy et al. 2018). With the progress of technology, numerous taxa have undergone taxonomic redescriptions. The genus *Pupinidius* Moellendorff belongs to the family Enidae and has undergone comprehensive examination, resulting in the redescriptions of 14 out of the 16 known species and subspecies (Wu & Zheng 2009). In a study conducted by Alonso et al. (2006), it was reported that five previously unidentified species belonging to the family Enidae of land snails were identified from La Gomera, located in the Canary Islands. The findings of Wu & Wu (2009, 2013) improves the understanding about the characteristics of this family, which still needs to be clarified in terms of its subdivision or make-up of subgroups. The taxonomic classification of the family Enidae has undergone thorough scrutiny and revision throughout its history. It has also been reported that taxonomy of this species delineated several subfamilies, including Pseudonapaeinae, Chondrulopsininae, Merdigerinae, Buliminusinae, Andronakiinae, Retowskiinae, Eninae, and Multidentulini (Schileyko 1998). Furthermore, the following taxonomy by Bouchet et al. (2017) tried to simplify things by narrowing the classification down to two subfamilies: Eninae and Buliminusinae. Bank & Neubert (2016) made improvements to the existing classification system by dividing the Eninae into four distinct tribes, namely: Enini, Multidentulini, Merdigera, and Pseudonapaeini. In addition to taxonomic factors, Enidae snails have notable versatility in adapting to a wide range of terrestrial environments, including forests, grasslands, and cold deserts (Ożgo 2014; Zhukov et al. 2019). The presence of adaptation in this species is responsible for the intricate morphological and behavioural characteristics identified within the taxonomic group. Additionally, their evolution of a functional lung distinguishes them from their aquatic relatives and allows them to thrive on land (Lodi & Koene 2016; Nandy et al. 2022). The family Enidae is a compelling illustration of evolutionary flexibility among gastropod molluscs, including around 20% of the overall population of land snails (Alonso et al. 2006).

The taxonomic classification of the genus *Pseudonapaeus*, as first proposed by Westerlund (1887), occupies a notable position within the Eninae subfamily of the Enidae. According to Tripathy et al. (2022), *Pseudonapaeus* encompasses 136 recognized species and exhibits a broad geographical distribution spanning central, southern, southeastern Asia, and Japan. The distribution of this genus in India is primarily limited to the western Himalaya, comprising 18 species (Ahmed et al. 2023). The taxonomic complexity surrounding this genus has played a significant role in advancing our knowledge of terrestrial gastropods. The taxonomy of *Pseudonapaeus* has faced difficulties, particularly in classification and species identification (Feruza 2017). The complexities occur due to the varied physical traits of different species, posing challenges in accurately defining separate taxonomic groups. The taxonomic classification of this group includes multiple species, each of which poses distinct difficulties in accurate identification. The challenges arise from nuanced variations in shell form, coloration, and size, underscoring the importance for taxonomists to demonstrate meticulousness in discerning distinguishing characteristics. The *Pseudonapaeus* presents a captivating subject for scholarly investigation, providing valuable perspectives on the difficulties related to taxonomy, classification, and species identification within the Enidae family and the broader realm of terrestrial gastropods.

The Pir Panjal Range, a significant mountain range located in the northern section of the Indian subcontinent, has a crucial role in shaping the distribution patterns of the Enidae family and the genus *Pseudonapaeus* within the area. *Pseudonapaeus* cf. *candelaris*, a unique species of the genus *Pseudonapaeus*, demonstrates a significant distribution within the specified geographic area. The existing literature over the last two centuries emphasizes the difficulties encountered in taxonomy and the ever-evolving nature of scientific investigation. The progress in technology and methods has made it easier to learn more about *Pseudonapaeus* cf. *candelaris* and the Enidae family, which is an essential contribution to the field of malacology. The extensive body of literature on gastropod studies offers a significant basis for present and future research, underscoring the enduring significance of investigating these land-dwelling molluscs within shifting scientific frameworks.

The scholarly discourse surrounding the distribution patterns and ecological preferences of the *Pseudonapaeus* cf. *candelaris* species in the western Himalaya has undergone significant development, owing to the outstanding contributions made by numerous workers. Huge Cuming, Esq. initially gathered the specimens, which were subsequently documented by Pfeiffer (1846). Pfeiffer's knowledge of the species' environment was limited, whereas Reeve asserted that it originated from regions including Europe and Asia (Reeve 1849). Specimens of the species were gathered by Thomas Thomson from Takht-i-Suleiman in Srinagar, India, during the exploration conducted in 1847–48 (Woodward 1856; Benson 1857). The occurrence of the species in the higher slopes of Kashmir was further investigated in subsequent research conducted by Hanley & Theobald (1876). Later on, Nevill (1878) expanded the geographical range of this family included Tandali from Himachal Pradesh, India. Theobald (1878) expanded upon the existing studies by investigating the elevated regions in Kashmir. Gude (1914) conducted an extensive study encompassing Fort Lockhart in Pakistan as a habitat for the subject under examination.

Conversely, Ramakrishna et al. (2010) identified Kashmir as the location of occurrence based on significant literature sources. Various geographical areas, such as Chandak from the Poonch District of the Pir Panjal Range in India (Ahmed et al. 2023), exemplify the inclusive scope of these investigations. The body of literature, which spans over a century, encompasses diverse research endeavours that have significantly enhanced our comprehension of the distribution and ecology of *Pseudonapaeus* cf. *candelaris* in the western Himalaya.

There is a significant knowledge deficit on the variety and ecological aspects of land molluscs, specifically *Pseudonapaeus* cf. *candelaris*, in the vicinity of the Takht-i-Suleiman type locality situated on the eastern flank of the Pir Panjal Range. Even with the proximity of the species, existing literature indicates a significant gap in detailed research about the ecology and diversity of land molluscs within this geographic region. Significantly, the research conducted by Ahmed et al. (2023) underscores the necessity for further investigation and the dearth of periodic evaluations of species by checklists. This study notably draws attention to the inadequate focus on terrestrial molluscs such as *Pseudonapaeus* cf. *candelaris* and their ecological significance. The research holds great importance, mainly due to its proximity to the type locality. It presents a distinct chance to acquire knowledge regarding habitat preferences, population dynamics, and prospective conservation strategies for this species. In addition, the need for more consideration of the ecological aspects of land molluscs in the region gives rise to apprehensions regarding the potential ramifications for the agricultural sector at the local level. It is imperative to undertake a targeted research endeavour that examines the ecology of *Pseudonapaeus* cf. *candelaris* and other terrestrial molluscs in the Pir Panjal Range. Addressing this study would make a valuable contribution to the scientific comprehension of the species while also carrying practical consequences for promoting local agricultural sustainability and preserving biodiversity.

It is imperative to adopt a multidimensional approach to effectively address the research gap in precisely describing pupillid shell features. Using ultrastructure images of the shell surfaces gives us a high-resolution picture that lets us look at many different morphological features, like the structure of the sutures, the structure of the tips, and the structure of the shell openings. The utilization of this sophisticated imaging technology overcomes the constraints inherent in traditional microscopy methods. Integrating molecular identification techniques, particularly DNA barcoding, can enhance taxonomy classifications with additional precision. The primary objective of molecular analysis will be to investigate genetic markers, enhancing species identification's precision. Putting these methods together and linking morphological and molecular approaches creates a strong foundation for fully understanding the features of the pupillid shell. The comprehensive nature of this technique addresses the deficiency in morphological analysis and establishes a foundation for the following research endeavours, encompassing ecological investigations. The objective is to enhance our comprehension of these species, aiding in the evaluation of damage, development of conservation measures and implementation of management methods that are well-informed and grounded in a more comprehensive and precise understanding of pupillid land snails. The present study significantly enhances our comprehension of terrestrial snails' biodiversity and ecological processes within the demanding topographies of the Pir Panjal Range and the wider Himalayan region besides provide us significant opportunities for enhancing the understanding of the complex interplay between these gastropods and their alpine habitats.

## MATERIAL AND METHODS

### Field sampling

From April 2021 to March 2023, the specimens of *Pseudonapaeus* cf. *candelaris* were meticulously collected in the Rajouri and Poonch Districts of the Pir Panjal Range (Figure 1). Monthly surveys, following an active visual search strategy. Specimens underwent photographic documentation and precise shell measurements in the laboratory using a Leica M205 stereo microscope and Mitutoyo digital calliper. Identification was confirmed through morphological examination and comparison with type specimens. Preserved at the ATREE Biodiversity Laboratory, this research significantly enhances the understanding of terrestrial mollusc taxonomy in the region.

### Morphological analysis

The collected individuals were morphologically examined for standard conchological characteristics such as shell shape, size, aperture shape, width, architecture on the shell, etc. The shell morphology was compared with earlier descriptions (Pfeiffer 1846; Benson 1857). The photos of the syntype from the Natural History Museum London and other voucher specimens (ZMA. MOLL.34612 at Naturalis Biodiversity Center) were compared for identification.

## RESULTS

### Systematic position

Order: Gastropoda
Subclass: Heterobranchia
Order: Stylommatophora
Family: Enidae B.B. Woodward, 1903 (1880)
Subfamily: Eninae B.B. Woodward, 1903 (1880)
Genus: *Pseudonapaeus* Westerlund, 1887
*Pseudonapaeus* cf. *candelaris*

### Shell morphology

The shell of this sinistral gastropod exhibits a cylindrical morphology characterized by a sleek outer surface and faintly pigmented bands. On average, it is

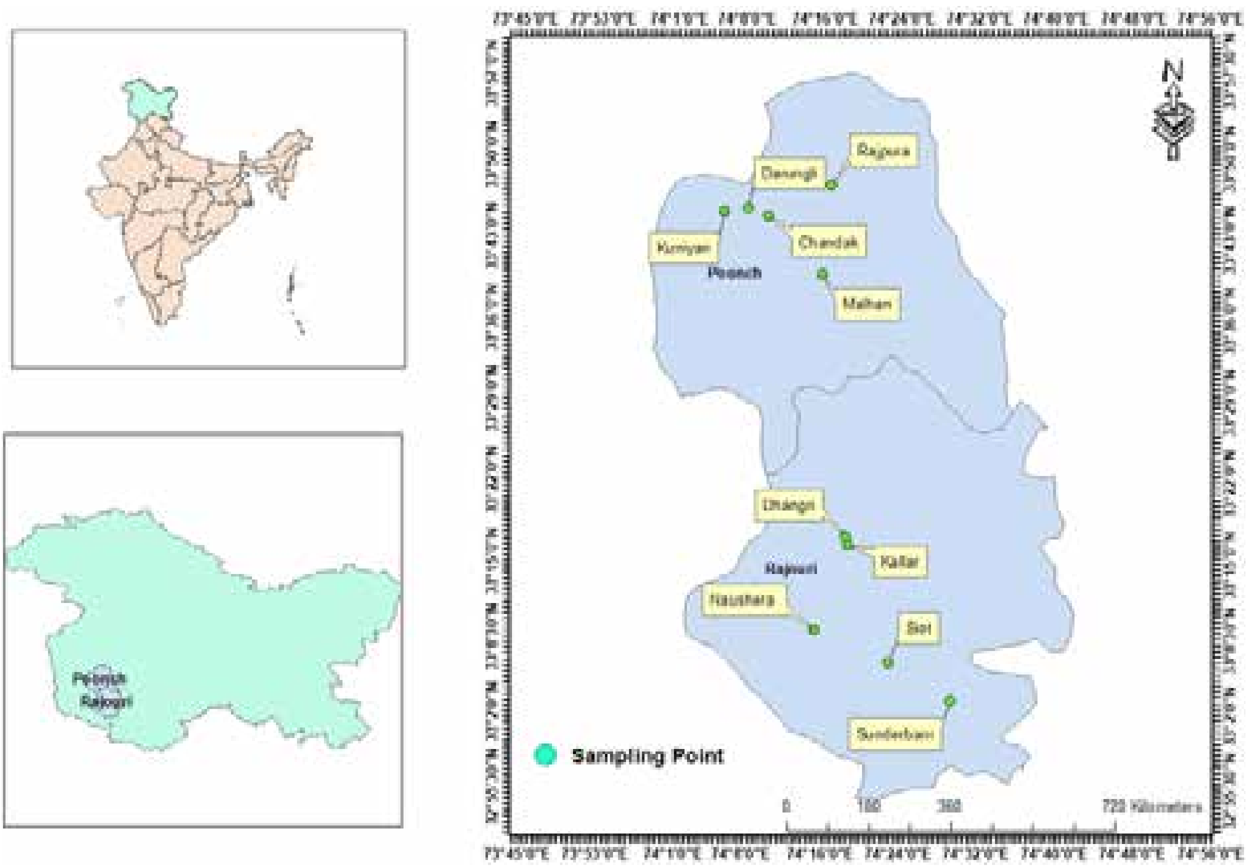


**Figure 1. Map showing the sampling locations in Poonch and Rajouri Districts in Jammu & Kashmir (J&K), India. The inset maps show the location of the J&K, Poonch and Rajouri districts.**

25.2 mm in length and has a diameter of 5.8 mm (Image 1). Deep grooves and a top that gradually narrows to a sharp point distinguish the shell. It displays slightly slanted stripes over an off-white base colour. The terminal one exhibits a series of nine flat bends, gradually decreasing in obliquity, culminating in a rounded base. The semioval aperture has an interior characteristic of bright white coloration. A peristome with enlarged white folds encircles the opening, harmoniously connecting them with a delicate callus along the borders. Significantly, the columellar region exhibits a pronounced dilation, contributing to the complexities inherent in the morphological characteristics of this snail shell. On examining the ultrastructure images of the shell (Image 1a), the surface is smooth with a slowly obliquely slight band without horizontal streaks, the groove is not too wide and the shell tip is concentrated bluntly without a sharp tip.

**Ecological habitat**

By methodically collecting 79 specimens, it was possible to ensure a complete representation of the species' range across various environments. A rigorous assessment was carried out to examine the species' ecological intricacies, focusing on its habits and environment. The study on *Pseudonapaeus* cf. *candelaris* in the Pir Panjal Range of the western Himalaya has provided a detailed understanding of species preferences for several environmental and habitat factors in the terrestrial ecosystem. The species exhibited a consistent occurrence in environments defined by soils with good drainage, particularly red clay, located beneath a thick layer of fallen leaves and within regions with abundant vegetation that get limited direct sunlight (Image 2b). The altitude distribution of *P.* cf. *candelaris*, which spans from 900 to 1,500 m, has been identified as a crucial element that highlights its ability to adapt to specific elevational ranges within the Pir Panjal Range (Image 2a). The soil study indicated a pH range of 6.5–7.8, with a distinct inclination towards habitats with a substantial calcium content of 7.9–9.8 mg/g. This preference is particularly evident in locations that experience semi-arid climatic conditions. Observing dead specimens within the sandy mud and loamy soil indicates the possibility of these locations serving as potential hibernation places during high temperatures and aridity. Field observations showed a notable increase in specimen abundance from August to mid-September. This time frame accounted for approximately 75% of the studied population. The ecological study provides a complete overview of the essential climatic and ecological elements that play a crucial role in regulating the distribution patterns of *P.* cf. *candelaris*. These parameters, identified as critical determinants, include humidity, altitude, soil type, and temperature.


**Image 1. The shell image of *Pseudonapaeus* cf. *candelaris* collected from the Pir Panjal area: a—during the present study | b—the syntype with catalogue number 20180423 from the Natural History Museum, London. Scale 1 cm. © Hilal Ahmed.**

**Table 1. Comparative morphological study of different morphologically similar land snails of the Enidae family distributed in northern India.**

| | Characters | *Pseudonapaeus purii* | *P. domina* | *P. arcuatus* | *P. vibex* | *Serina nevilliana* | *Pseudonapaeus* cf. *candelaris* |
|---|---|---|---|---|---|---|---|
| 1 | Shell shape and size | Cylindrical, 15 mm x 5.5 mm | Cylindrical, 23 mm x 9 mm | Variable, 12.5–21 mm x 5-8 mm | Cylindrical, 15.1 mm x 5.0 mm | Cylindrical, similar to *P. purii,* 11.8 x 4.3 mm | Cylindrical, elongated, 25.2 mm x 5.8 mm |
| 2 | Coloration | Chalky-white coating on last four whorls; original horny-brown on uppermost whorls | Shell with reddish-brown oblique stripes | Dark horny-yellow, faint white lines on body whorls | Dark horny-yellow, with characteristic hyaline white lines | Grayish-white to off-white, similar to *P.* cf. *candelaris* | Off-white with slightly slanted stripes; bright white aperture interior |
| 3 | Surface texture | Distinct longitudinal plications, corrugated similar to *Serina nevilliana* | Smooth, slight striations on outer surface | Smooth with faint transverse lines | Smooth with hyaline white lines | Smooth with faint grooves | Smooth with faintly pigmented bands; deep grooves; tip blunt without sharp point |
| 4 | Aperture | Oblong-ovate, pale brownish interior, edentate | Semi-oval, slightly oblique | Variable, 5 mm x 4 mm to 7.5 mm x 6 mm | Rounded, variable diameter | Semi-oval, similar to *P.* cf. *candelaris* | Semi-oval, bright white interior; peristome with thick white folds; distinct columellar dilation |
| 5 | Whorls | Four distinct whorls, last one with chalky-white coat | Five to six, with reddish-brown markings on final whorls | Five to six, smooth, regular in structure | Four to five, with hyaline lines | Five to six, faintly pigmented | Nine slightly oblique bends on final whorl, decreasing in obliquity towards the rounded base |
| 6 | Habitat | High-altitude mostly under leaf litter (>3,000 m) | Western Himalaya, deep clay soil | Swagni Maidan, sloping meadow (<3,000 m) | Chakrata and Deoban, high-altitudes (2,424–3,030 m) | High-altitude regions, semi-arid conditions | Well-drained red clay at altitudinal range 900–1,500 m |
| 7 | Endemism | Yes, rediscovered in 2018, Great Himalayan National Park | Yes, western Himalaya | Yes, found in lesser Himalayan region | Yes, prevalent in middle Himalayan region | Endemic to some Himalayan regions | Yes, widespread across western Himalaya |
| | References | Tripathy et al. 2022; Ray 1951 | Benson 1857; Gude 1914 | Reeve 1849; Gude 1914; Rensch 1955 | Rensch 1955; Gude 1914 | Theobald 1881; Gude 1914 | Present study |


**Image 2. a—The typical habitat of *Pseudonapaeus* cf. *candelaris* in the Pir Panjal region of Jammu & Kashmir | b—the microhabitat with a living individual. © Hilal Ahmed.**

## DISCUSSION

The rediscovery of *Pseudonapaeus* cf. *candelaris*, a species belonging to the Enidae family and native to the Pir Panjal Range in the western Himalaya, represents a significant achievement in malacological research. This species has been challenging to find since it was documented 170 years ago at Takht-i-Suleiman in Srinagar, Kashmir (Woodward 1856). Many scholars from different periods have been interested in studying this species, including Reeve (1849), Woodward (1856), Hanley & Theobald (1876), Nevill (1878), Theobald (1878), Gude (1914), Ramakrishna et al. (2010), and Tripathy et al. (2018). Old records do not always have solid proof; they often rely on lists or collections of historical writings (Gude 1914; Ramakrishna et al. 2010; Tripathy et al. 2018) that do not give essential details like reference numbers and pictures, or drawings that are needed to confirm the specimens that were reported. Reeve's (1849) early claim that the species was limited to Asia and Europe, which was later withdrawn, introduces a level of intricacy to the historical account. The problem worsens because specimens still need to be correctly identified as *Zootecus* sp. even though they have apparent physical features that make them easy to spot. The persistent uncertainty and lack of modern reports highlight the rarity of *P.* cf. *candelaris* in the Pir Panjal Range, as emphasized in recent studies (Ahmed et al. 2023). The clear connection of the species with its original location in Kashmir is an undeniable aspect of its importance, both in the past and present.

The shells of *Pseudonapaeus* cf. *candelaris* in the Pir Panjal range exhibit unique traits specific to the species. Typically, these shells have a cylindrical shape with a pointed tip that resembles a candle. The oval-shaped aperture and the spire height, which is around half of the shell's overall height, are distinctive characteristics of the species. Certain shells exhibit chromatic differences and development lines, indicating the possibility of variety within the population. *P.* cf. *candelaris* can be distinguished from similar species like *P. domina* and *P. arctuatus* based on its distinctive physical characteristics (Table 1). These include a sinistral shell, a smooth surface, a thick aperture shell without horizontal streaks and 3–4 middle whorls that are almost the same width. According to historical records by Nevill (1878) and Theobald (1878), many sinistral shells were recorded, while dextral shells were less commonly seen. The lack of visual evidence and preserved specimens in historical literature raises doubts about the occurrence of dextral shells. This scepticism is particularly relevant considering the extensive surveys conducted by multiple expeditions and authors over the past two years (Ahmed et al. 2023). Theobald (1878) also talks about how the sinistral and dextral shells are very different in size and suggests that the dextral shells might be *Pseudonapaeus domina* with close-type locations. Discernible distinguishing characteristics have yet to be found between the two.

This study provides a comprehensive analysis of shell morphology for *Pseudonapaeus* cf. *candelaris* in the Pir Panjal range of the northwestern Himalaya. In addition, it includes a succinct ecological investigation specific to this species. By combining traditional taxonomic methods with ecological assessment, the understanding of this mollusc species is significantly improved in this ecologically crucial region. Future research should prioritize an in-depth exploration of the ecological implications of *P.* cf. *candelaris* and its role within the Pir Panjal ecosystem.

This research underscores the need for extensive biodiversity studies in the western Himalayas, inspired by the rediscovery of *P.* cf. *candelaris*—a species initially documented by scientists over 170 years ago during several expeditions. This rediscovery emphasizes the pressing need to investigate and preserve the region's rich biodiversity.

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COMMUNICATION

# Diet and nutrient balance of wild Asian Elephants *Elephas maximus* in Nepal

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**Abstract:** We estimated the nutritional content of major wild (n = 22) and agricultural crop plants (n = 3) consumed by Asian Elephants *Elephas maximus* in Nepal during the wet and dry seasons, respectively. We then used nutritional geometry to explore the macronutrient balance of these plant species, as well as the overall diet of elephants in both the dry and wet seasons. Furthermore, we compared the diet of the Nepal elephants with the previously published diet of Indian population of elephants. We found that despite intraspecific and seasonal variation, the overall diet of elephants was relatively stable in protein (P) intake relative to non-protein macronutrients (fat + carbohydrate; non-protein (NP)), and neutral detergent fibre (NDF) between the wet (16% crude protein (CP): 26.7 % NP: 57.3% NDF; and, 10.4% CP: 13.7% NP: 75.7% NDF) in dry season, which suggests protein intake prioritization in support of previous work on captive elephants. Furthermore, the diet of Indian population of elephants (wet season: 16.0%P: 22.5%NP: 61.4%NDF and dry season: 11.1%P: 18.0 %NP: 70.7 %NDF) showed a similar pattern to the Nepal elephants, suggesting active regulation of macronutrient and NDF intake across populations despite differences in food consumed as part of their diets. Importantly, NDF intake in addition to non-protein macronutrients is likely necessary for elephants to stabilize their protein intake balance; thus, it is important to consider a multidimensional nutritional perspective in elephant conservation planning. The study has concluded that in a well-managed seasonal habitat, elephants can regulate their preferred macronutrient and NDF intake from available natural food plants without resorting to agricultural crop depredation.

**Keywords:** Crop, depredation, Elephantidae, macronutrient balance, Mammalia, NDF, nutritional geometry, right angled mixture triangle.

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**Author contributions:** RKK—conceptualized the project, Written the ms, data analysis. SC—analysed, reviewed.

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## INTRODUCTION

The diet and nutritional demands of wildlife are crucial aspects to consider when formulating conservation and management strategies. Foraging, however, is a complex process, involving the inter play of physiological and behavioural factors with an animal's multidimensional nutritional environment (Raubenheimer et al. 2009; Simpson & Raubenheimer 2012). The Asian Elephant *Elephas maximus* is a species of conservation concern often involved in food-related human-elephant interactions (Desai 1991) through crop depredation (Santiapillai & Jackson 1990; Nyhus & Sumianto 2000). Recently, studies of captive Asian Elephants suggest that they regulate their intake of multiple nutrients, with protein (P) intake more tightly regulated relative to non-protein macronutrients (fat + carbohydrate; NP) and fibre (NDF; (Koirala 2018). This type of nutrient regulation, however, has not been explored in wild elephant populations.

In this paper, we estimated the proximate nutritional content of plants consumed by migratory and resident elephants during the wet and dry seasons in the far eastern region and central region of Chitwan National Park (CNP) and Parsa National Park (PNP) of Nepal. The consumed plants were identified through feeding sign survey and micro-histological analysis of dung, and also based on a previous study (Koirala et al. 2016). We used nutritional geometry (i.e., right-angled mixture triangle (RMT); Raubenheimer 2011) to explore the proportions of macronutrients and fibre in the seasonal food plants and diets of elephants, including both natural and agriculture crop plants, to evaluate evidence for nutrient intake regulation similar to previous studies. Furthermore, we used published literature on the diet of wild elephants in India (Das et al. 2014) to evaluate if they showed similar dietary patterns to the Nepal elephants, which might suggest active regulation of macronutrient and NDF intake across populations despite consuming different diets.

## MATERIALS AND METHODS

The CNP (952.632 km$^2$) and PNP (637.37 km$^2$) are two of Nepal's protected areas, and are situated in the south-central region of the country (Koirala et al. 2016). On the other hand, Nepal gets migratory elephants from northern Bengal of India (Koirala et al. 2015) in the forests of the eastern district of Jhapa. We collected food plant species of elephants' diets for nutritional analysis based on our previous study on food preference, which identified 57 plant species (12 grasses, five shrubs, two climbers, one herb, and 37 tree species) consumed by elephants (Koirala et al. 2016 ). However, only the most preferred species (n = 22) and three agriculture crop plants were collected for proximate nutrient analysis (Table 1). Plant and crop samples were collected during the late rainy ("wet") season (August/September 2013) and summer "dry" season (March/April 2014) from elephant habitat, where habitat was determined by the presence of elephant foraging signs and direct observation. The wet season collection period coincided with the beginning of crop raiding time in late monsoon season with a peak in pre-winter, while April/May was the beginning of crop raiding in the dry season. After collection, samples were air-dried and kept in paper bags for transport to the laboratory. The proximate nutritional estimates were analysed in the laboratory of Nepal Agricultural Research Centre and Nepal Environmental and Scientific Services, Kathmandu, Nepal, following standard methods (AOAC 2012) for crude protein (Kjeldahl; CP), ether extract for lipid (Soxhlet extraction; EE), fiber (digestion method; NDF), and ash. Non-structural carbohydrate (NSC) was calculated by difference. To correct for indigestible waxes and lipids in plants consumed, we subtracted 1% from EE to estimate crude fat (CF) following Rothman et al. (2012). Proportional data were transformed using a "logit" transformation to approximate normality before running the stat test.

We used the Right angled mixture triangle (RMT) to investigate the proportion of macronutrients and NDF in the food plants and seasonal diet of elephants. Following Koirala et al. (2016), we plotted NP and NDF on the x- and y-axis of the RMT, respectively, while CP was represented on the implicit axis (z) which varies inversely with distance from the origin (Raubenheimer 2011). Macronutrients and NDF were expressed as a percentage of the sum of each (i.e., non-structural carbohydrate + neutral detergent fibre + lipid + protein) on a dry matter basis. NDF was included in the analysis, because elephants derive energy from fibre through hindgut fermentation (Anguita et al. 2006). We estimated the seasonal mixture space provided by the plant foods by forming minimum convex polygons around food points for each season.

We determined the overall macronutrient balance of seasonal diets by weighing the nutritional estimates for each plant species by the relative utilisation percentage determined as described by Koirala et al. (2016), which is the product of the frequency of occurrence and rank score of each plant in the micro-histological analysis (Holechek & Gross 1982) and feeding-sign survey respectively (Koirala et al. 2016). We also used RMT analysis to compare the balance of CP, NP, and NDF (Koirala 2018) in the diet of elephants in our study area with existing data on the available diets of wild elephants in India (Das et al. 2014).

Independent sample t- test was performed to see the seasonal difference in the nutrient dry matter/ balance in the plants. Pearson correlation was performed to see the relationship between utilisation and availability of protein and NDF in the diet. All tests were done using Excel and IBM SPSS statistical package version 22.

## RESULTS

The nutrient contents of the leaves of plants consumed by elephants didn't vary with species and season (Table 1). The highest estimates for CP (*Lagerstroemia parviflora*; 25.97%), NSC (*Litsea monopetala*; 31.85%), and NDF (*Saccharum bengalensis*; 88.62%) were found in the wet season. The average percent dry matter CP content of food plants was 12% in the wet season and 11 % in the dry season ($t_{37}$ = 0.372, p = 0.712). The average NDF content was 55.9% (wet season) and 66% (dry season) ($t_{37}$ = -1.556, p = 0.128), and average EE content was 1.7 % and 1.2% ($t_{37}$ = 1.427, p = 0.162) in wet and dry seasons, respectively.

The proportion of P: NP: NDF in plants was variable between seasons (Figure 1). For example, the protein balance of most frequently consumed plants *Spatholobus parviflorus* and *Mallotus philippensis* was higher during the wet season. In both seasons, however, most of the dominant plant species consumed were similar, for example, *Spatholobus parviflorus*, *Mallotus philippensis* (Koirala et al. 2016). In the case of agricultural crops, in the wet season paddy was 12.34 % P: 18.31% NP: 69.35% NDF, and in the dry season 9.55% P: 15.42% NP: 75.03% NDF (Figure 1).

The estimated seasonal diets of elephants was (Figure 2): 16 % CP: 26.7 % NP: 57.3% NDF in wet season; and, 10.4% CP: 13.7% NP: 75.7 NDF in dry season.

## DISCUSSION

The utilisation pattern of food plants showed that browse forms the major diet of elephants in the dry season in both PWR and CNP. While the wet season diet was slightly dominated by grass in PWR and browse in CNP (Koirala et al. 2016). The nutritional content (Table 1) of plant species was stable between seasons.

The combined dry season diet was greater in NDF than the wet season diet. The combined wet season diet was greater in protein and non-protein than the dry season diet. However, both summer and winter diets were somewhat similar in nutrient balance. NDF balance was the highest difference of 12% (±2.09 SE) while non-protein showed a difference of 11.6% (±2.04 SE) and protein at the least difference of 6% (±1.05 SE).

During the wet season, the protein content of food plants like *Acacia catechu, Litsea monopetala,* and *Lagerstroemia parviflora* was high to compensate for the deficiency of protein from *Saccharum bengalensis, Saccharum spontaneum*, and *Phragmites karka*, suggesting that these foods were complementary to each other (Figure 1A).

Similarly, in dry season, the protein balance of diet of highly utilised browse and agricultural crops like paddy and wheat were similar. Although there was less protein in *Spatholobus parviflorus,* a highly preferred plant in the dry season, so elephants may be using plants species like *Phragmatus karka, Acacia catechu, Litsea monopetala*, and *Ficus semicordata* to slightly increase protein content to balance the deficit of protein from *Spatholobus parviflorus, Cymbopogon* sp., *Saccharum spontaneum*, and *Saccharum bengalensis* (Figure 1B).

Moreover, there was no significant relationship between utilisation and availability of protein (r=-0.146, p=0.418 and NDF (r = -0.188, r = 0.293) in the weighed diet. The preference of plants is different irrespective of their presence and frequencies in the diet. The utilisation of these plants varies with season and localities (Koirala et al. 2016*)*. Subsequently, the combined macronutrient balance of dry and wet season food plants was almost similar. The balance of different macronutrients showed no significant seasonal difference ($t_{30}$ = 1.030, p = 0.311) protein; (t=$_{30}$ 0.760, p = 0.453) and non-protein $t_{30}$=-2.039, p= 0.050 NDF. This gives an indication that although elephants utilised many types of food plants with different nutritional content, the animals compose their diet to achieve a preferred macronutrient intake target (Raubenheimer 2011; Coogan 2014).

At the time of this study in both of these study periods, crops act as a complementary food source to replace low protein grasses. The lower protein in grasses like *Cymbopogon* sp., *Saccharum spontaneum*, *S. bengalensis,* and *Digitaria* spp. in in the dry season and higher accessibility and protein content in crops may lead to crop raiding. This is consistent with the assertion that the nutritional composition of crops could

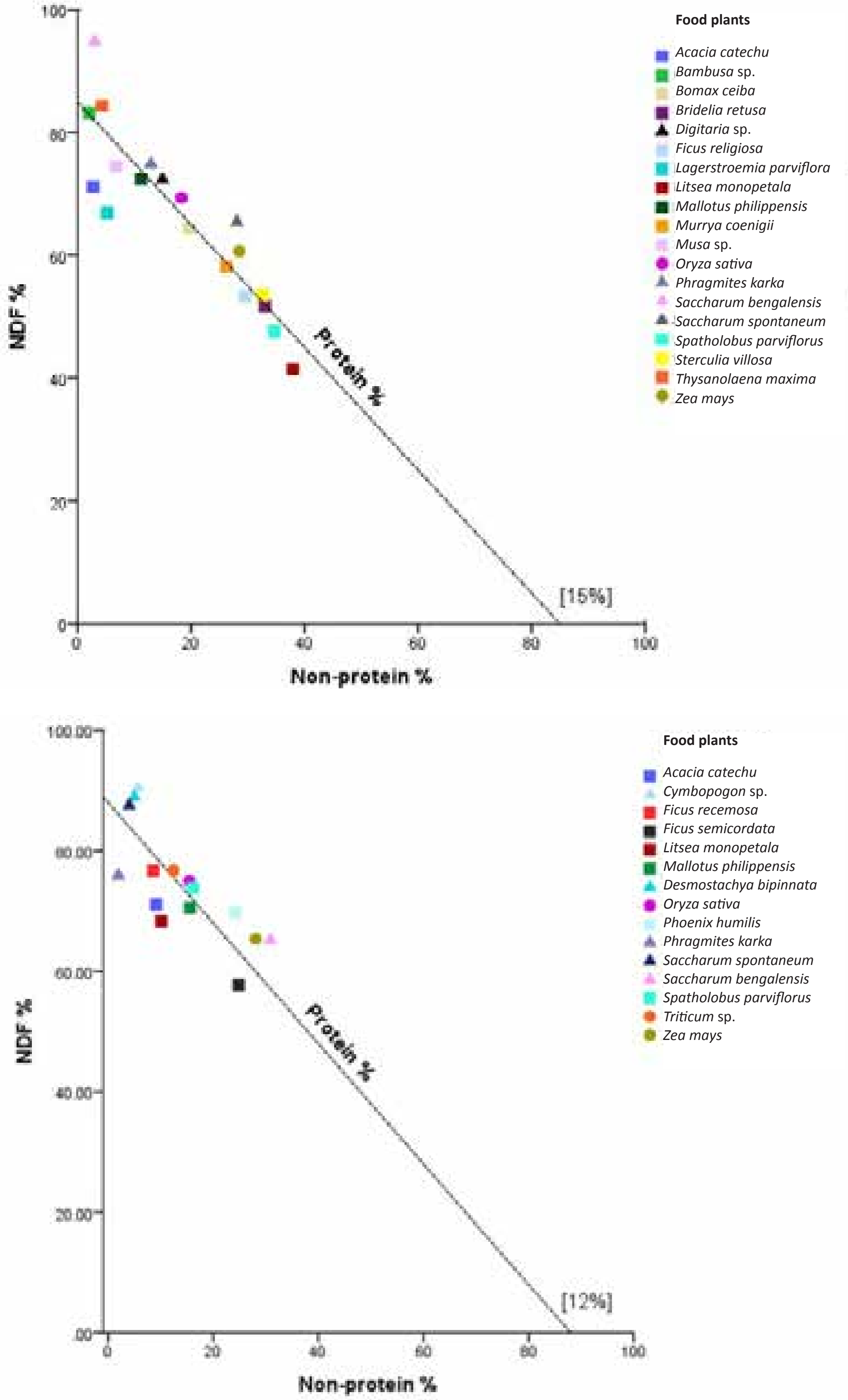


**Figure 1. RMT showing the balance of crude protein, non–protein macronutrients (NSC+ crude fat), and NDF in plants consumed by elephants in both the seasons: A—wet season | B—dry season. Browse species are shown as squares, grasses as triangles, and agriculture crops as circles.**

**Table 1. Nutritional composition (percent dry matter) of major plants consumed by Asian elephants, including crude protein (CP), ether extract (EE), ash, neutral detergent fibre (NDF), acid detergent lignin (ADL), and non-structural carbohydrates (NSC).**

| Plant species | CP | (EE) | Ash | NDF | ADF | ADL | NSC |
|---|---|---|---|---|---|---|---|
| Wet season | | | | | | | |
| *Lagerstroemia parviflora* | 25.97 | 1.20 | 5.82 | 62.32 | 52.14 | 24.32 | 4.69 |
| *Ficus religiosa* | 13.99 | 1.40 | 16.90 | 43.86 | 35.79 | 12.98 | 23.85 |
| *Thysanolaena maxima* | 10.20 | 0.87 | 8.67 | 76.35 | 57.30 | 12.90 | 3.91 |
| *Musa* sp. | 15.89 | 6.25 | 12.62 | 63.65 | 59.89 | 11.58 | 1.59 |
| *Saccharum bengalensis* | 2.07 | 0.80 | 5.73 | 88.62 | 62.22 | 32.58 | 2.77 |
| *Saccharum spontaneum* | 5.76 | 0.80 | 11.00 | 57.67 | 49.15 | 39.30 | 24.76 |
| *Sterculia villosa* | 12.80 | 1.00 | 6.40 | 49.60 | 46.50 | 19.50 | 31.20 |
| *Bomax ceiba* | 14.04 | 0.86 | 11.10 | 56.57 | 41.36 | 19.09 | 18.29 |
| *Acacia catechu* | 24.15 | 1.30 | 6.35 | 65.94 | 43.65 | 12.40 | 2.26 |
| *Digitaria ciliaris* | 10.80 | 0.86 | 14.04 | 61.55 | 43.13 | 9.07 | 12.75 |
| *Paspalum scrobiculatum L* | 5.00 | 3.00 | 7.18 | 7.77 | 2.50 | 2.05 | 4.71 |
| *Murrya coenigii* | 13.70 | 0.90 | 6.60 | 54.32 | 41.22 | 14.25 | 24.48 |
| *Bridelia retusa* | 13.75 | 1.20 | 8.36 | 46.95 | 41.04 | 16.74 | 29.74 |
| *Mallotus philippensis* | 14.35 | 2.30 | 11.25 | 63.54 | 53.98 | 25.62 | 8.56 |
| *Spatholobus parviflorus* | 14.69 | 2.85 | 11.70 | 41.99 | 35.02 | 11.18 | 28.77 |
| *Phragmites karka* | 6.60 | 0.60 | 10.36 | 80.99 | 64.97 | 40.88 | 1.45 |
| *Saccharum spontaneum* | 6.10 | 0.65 | 6.32 | 82.45 | 66.02 | 13.20 | 4.48 |
| *Bambusa* sp. | 12.50 | 2.00 | 13.80 | 70.91 | 51.85 | 17.52 | 0.79 |
| *Litsea manopetala* | 17.44 | 3.22 | 10.22 | 37.27 | 28.22 | 13.12 | 31.85 |
| *Saccharum bengalensis* | 3.56 | 1.87 | 7.31 | 60.84 | 1.31 | 6.62 | 27.42 |
| *Desmostachya bipinnata* | 12.17 | 1.78 | 9.59 | 55.95 | 43.86 | 17.75 | 14.42 |
| Paddy | 11.25 | 0.60 | 8.20 | 63.25 | 52.35 | 24.30 | 16.70 |
| Maize | 9.69 | 0.90 | 10.00 | 54.00 | 42.00 | 34.00 | 25.41 |
| Dry season | | | | | | | |
| *Spatholobus parviflorus (bark)* | 6.50 | 0.34 | 15.03 | 54.73 | 48.19 | 21.43 | 23.40 |
| *Saccharum spontaneum* | 7.56 | 1.25 | 9.92 | 77.97 | 54.13 | 11.95 | 3.30 |
| *Saccharum bengalensis* | 7.94 | 1.0 | 8.88 | 77.46 | 50.47 | 7.1 | 4.72 |
| *Acacia catechu* | 18.50 | 1.40 | 5.30 | 66.65 | 59.89 | 11.58 | 8.15 |
| *Cymbopogon* sp. | 3.55 | 0.50 | 8.36 | 82.45 | 81.48 | 12.92 | 5.14 |
| *Spatholobus parviflorus* | 8.70 | 0.34 | 12.03 | 64.73 | 47.11 | 21.46 | 14.20 |
| *Mallotus philippensis* | 12.35 | 3.78 | 10.40 | 62.50 | 51.78 | 22.72 | 10.97 |
| *Ficus semicordata* | 15.56 | 0.73 | 10.99 | 51.35 | 47.16 | 19.90 | 21.37 |
| *Ficus racemosa* | 12.61 | 1.20 | 13.53 | 65.54 | 49.97 | 29.08 | 7.12 |
| *Phoenix humilis* | 6.00 | 1.50 | 0.96 | 64.23 | 53.12 | 19.20 | 27.31 |
| *Phragmites karka* | 20.56 | 1.31 | 6.98 | 70.68 | 34.79 | 7.48 | 0.47 |
| *Litsea monopetala* | 20 | 2.56 | 6.56 | 63.10 | 49.34 | 27.85 | 8.82 |
| Paddy | 8.53 | 0.7 | 10 | 67 | * | * | 13.77 |
| Maize | 5.76 | 0.80 | 11.00 | 57.67 | 49.15 | 39.30 | 24.76 |
| Wheat | 9.75 | 0.6 | 9.5 | 69 | * | * | 11.15 |
| Unidentified | 10.66 | 1.26 | 9.39 | 65.73 | 51.89 | 17.81 | 12.95 |

* Indicates analysis was not done.

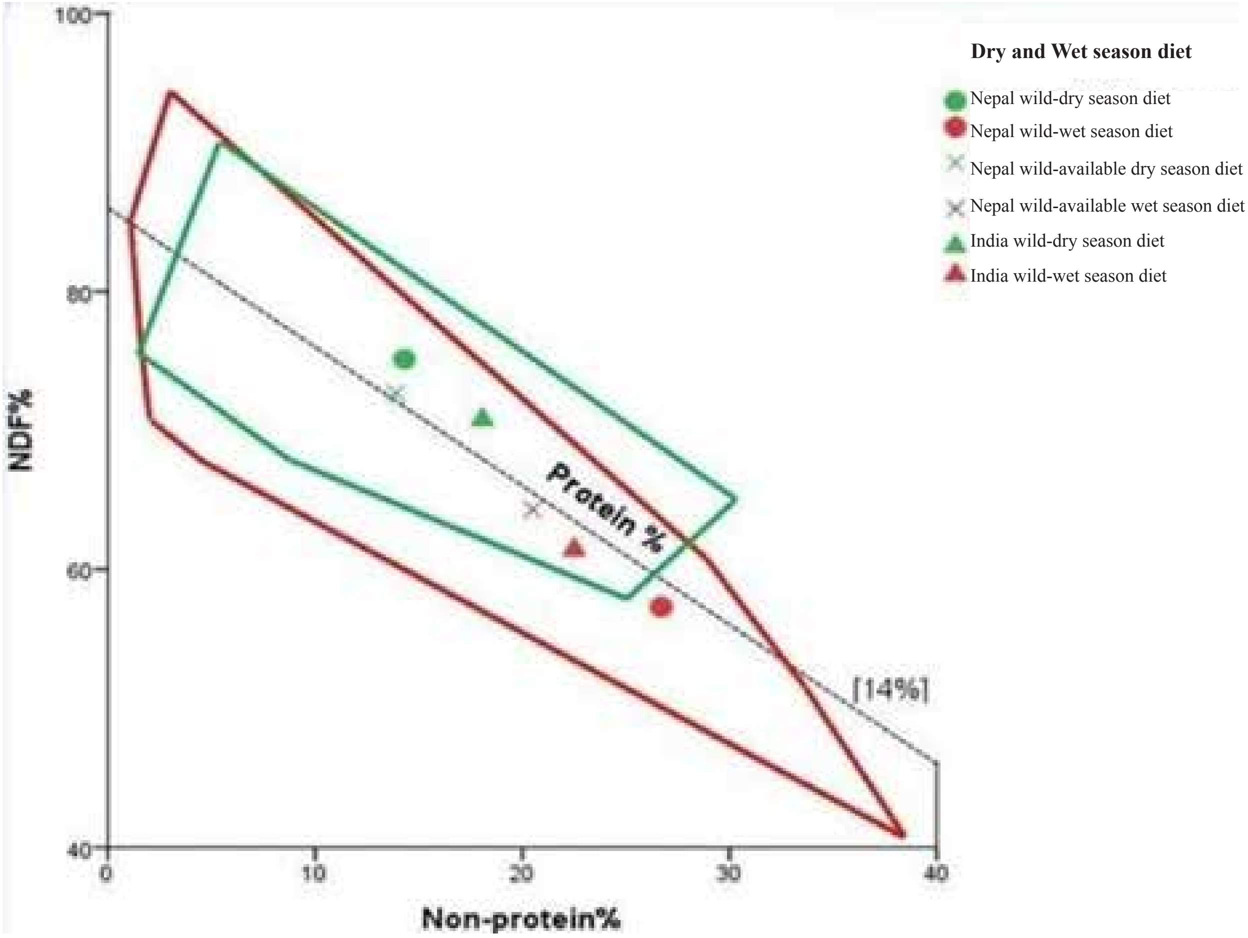


**Figure 2. An enlarged view estimated diet composition of combined food in Right-angled mixture triangles (RMT; Raubenheimer 2011). Solid green circle (Dry season), and solid red circle, (wet season) diet weighed by utilisation percentage (Koirala et al. 2016). The crops and the leaves of all plants combined weighed by the seasonal availability in the environment (solid cross symbols), wet season (Red symbol), and dry season (Green symbol). The red and green solid triangles represent the wet and dry season diet of Asian Elephants (India population) respectively. Crude protein is represented on the implicit axis which varies inversely with distance from the origin. For reference, the dashed grey line indicates 14% protein content. The estimated seasonal nutrient space provided by food plants is shown by green polygon dry season and red polygon wet season around the food points.**

be related to the crop raiding behaviour of elephants (Sukumar & Gadgil 1988; Sukumar 1989, 1991, 2006).

Our previous study has found that there was a negative relationship between utilisation and availability. As such there could be a reasonable selection of foods. The present study attempted to validate our hypothesis that the elephants are selectively feeding with a null hypothesis of feeding proportional to availability through the pattern of use seen in some of the highly preferred food plants like *Mallotus philippensis*, *Bambusa* sp., *Bombax ceiba*, *Spatholobus perviflorus*, and *Thysanolaena maxima*. The difference shown in the availability and utilisation in dry and wet season diet showed that there is selective mode of feeding. The availability of plant foods and their utilisation determines the preference. The preference based on availability and usage may be primary information in relation to conservation of probable food plants in the habitat. However for the long term population sustainability of elephants, utilisation information based on nutritional content of plants is vital for the conservation and management of habitat for elephants. Further, the geometric analysis of food plants has revealed that besides the relationship between utilisation and macro nutrient content, the balance of nutrients of different diets plays a vital role in food selection. The ratio focused selection was located by this study as the diagonal clustering of expected dry and wet seasonal diet points, together with similar seasonal diet points of Indian wild elephants. The significant relationship of utilisation and macronutrient balance of highly preferred browse, grasses, and crops at least in these periods of the year have revealed that crop raiding can be seen as part of protein makeup of elephants due to lower protein in grasses and some browse. In nutrient space, crops have been found to be occupying a place in between browse and grass. Thus, the elephants move away from their natural habitat to seek an alternative source of fodder with a high nutritive value such as crops.

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COMMUNICATION



# Avian diversity in wetlands of southwestern Kerala of India during COVID

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**Abstract:** The COVID-19 pandemic, known as the COVIDian era, has impacted ecosystems worldwide, including wetlands, which are essential habitats for avian biodiversity. The study on bird diversity in the wetlands of Kollam District, southwestern part of Kerala, employed a combination of field surveys and observational methods to assess the avian communities across various habitats. The survey areas were selected based on their significance as wetland ecosystems and included Polachira, Pozhikkara, and associated wetlands within Kollam District. The study documented 98 bird species across 41 families and 15 orders. Passeriformes was the most diverse order with 37 species, followed by Pelecaniformes and Charadriiformes with 13 species each. Apodiformes, Podicipediformes, and Psittaciformes each had a single recorded species. Ardeidae was the most abundant family with 10 species, followed by Rallidae (six species), Sturnidae, and Scolopacidae (five species each). Among the bird species recorded, 16 were migratory, 76 were resident, and six were local migrants. The study assessed bird diversity across Polachira, Pozhikkara, and associated wetlands using various indices, including Simpson, Simpson's λ, Simpson's D, Margalef, Berger-Parker, Shannon-Wiener, and Pielou. Shannon-Wiener diversity index at Polachira is 4.46, Pozhikkara 4.47 and associated wetlands is 4.45, which suggests that the overall avian diversity is comparable among these wetlands. Pozhikkara appears to have slightly higher species richness (Margalef's index 14.64) and slightly lower dominance (Berger-Parker index 35.90) compared to the other two sites. This study elucidates the effects of pandemic-related disruptions on bird populations, highlighting the resilience and vulnerabilities of avifauna in wetland ecosystems.

**Keywords:** Avian diversity, Berger-Parker, biodiversity indices, COVIDian era, Margalef, Pielou, Polachira, Pozhikkara, Shannon-Wiener.

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**Author details:** Dr. Vijayakumari Sudhakaran Bindu, of the Department of Zoology, MSM College, Kayamkulam (University of Kerala), is a leading expert on the avian, butterfly, and fish diversity of the region, where she is actively engaged in research. Her primary research interests include biodiversity, chemical ecology, and environmental DNA, and she has made significant contributions to field ecology and ecotoxicology. Ms. S. Sajitha, an independent researcher and MSc Zoology graduate from the same department, is an avid bird watcher in the Kollam district.

**Author contributions:** The authors contributed equally in the preparation of the manuscript.

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## INTRODUCTION

The onset of the COVID-19 pandemic in early 2020 introduced unprecedented challenges to both human society and natural ecosystems, potentially influencing bird populations and their habitats in complex ways (Warrington et al. 2022).

While wetlands in India are recognized for their ecological significance, there is a notable gap in understanding the dynamics of wetland bird populations, particularly in the context of the COVID-19 pandemic, termed the COVIDian era (Madhok & Gulati 2022). The impact of the pandemic on wetland ecosystems and avian biodiversity remains understudied, despite its potential to induce both direct and indirect effects on bird populations. Investigating the diversity of wetland birds in the Kollam District during the COVIDian era can elucidate how anthropogenic disturbances and environmental changes interact to shape avian communities in these critical habitats.

The COVID-19 pandemic has influenced the diversity and distribution of wetland birds in Kollam District, with potential alterations in species composition and abundance. The reduction in human disturbances, such as tourism and industrial activities, during lockdown periods, may have provided temporary respite for certain bird species, leading to shifts in their population dynamics (Cooke et al. 2021; Friedrich et al. 2021). Conversely, changes in land use patterns and habitat degradation may have posed challenges to wetland bird conservation efforts, affecting the overall resilience of avian communities. This study is to enhance the understanding of the dynamics of wetland bird populations in the context of the COVID-19 pandemic, contributing valuable insights to conservation and management efforts in wetlands and beyond. This study aimed to investigate the impact of the COVID-19 pandemic on avian diversity within the diverse wetland ecosystems of Kollam District, southwestern Kerala, India. The study sites, Polachira, Pozhikkara, and associated wetlands within the district, were specifically chosen to represent a range of wetland types. This diversity allowed for a comprehensive assessment of avian responses to potential pandemic-related disruptions across different wetland ecosystems.

## MATERIAL AND METHODS

### Study area

The study area encompassed Kollam District, located along the southwestern coast of Kerala, India, bordering the Arabian Sea. Within the district, this study specifically focused on the avian diversity of Polachira, Pozhikkara, and associated wetland ecosystems. The location map of the study area is depicted in Figure 1.

### Polachira

Polachira (8.83 °N, 76.70 °E) holds a diverse range of aquatic habitats, including marshes and shallow water bodies spanning over 600 ha. Polachira is primarily composed of paddy fields, characterized as 'Moonupoovu Nilam,' allowing farmers to cultivate rice thrice annually. Soil is highly fertile promising substantial yield. A significant challenge faced by farmers is the perpetual waterlogging of the fields. Polachira's rich biodiversity, particularly the abundance of fish and mussels, attracts numerous avian fauna including migratory birds. Its proximity to urban centres makes it an accessible location for studying wetland bird diversity.

### Pozhikkara

Pozhikkara (8.81 °N, 76.65 °E) is a small town on the western border of the Paravur in Kollam District, flanked by the Arabian Sea on one side and the backwaters on the other side. Pozhikkara is a heritage site in Kerala blessed with estuaries, backwaters and a sublime beach. The ecology of this region is unique. It has flora and fauna that adapt to both saltwater and freshwater. This coastal wetland presents a unique opportunity to examine the avian fauna associated with estuarine and coastal habitats, offering insights into the ecological significance of these transitional ecosystems.

### Associated wetlands

In addition to Polachira and Pozhikkara, the study also includes associated wetlands (8.82 °N, 76.67 °E) in the form of paddy fields and marshy areas between Polachira and Pozhikkara. These diverse wetlands provide habitat for a variety of bird species, contributing to the overall richness of avian biodiversity in the region.

### Methods

The study on bird diversity in the wetlands employed a combination of field surveys and observational methods to assess the avian communities. Field observations were conducted from May 2020 to April 2021. Bird species richness, abundance, and distribution patterns were documented using standardized bird-watching protocols and ecological surveys. Field surveys were conducted monthly over 12 months to investigate bird

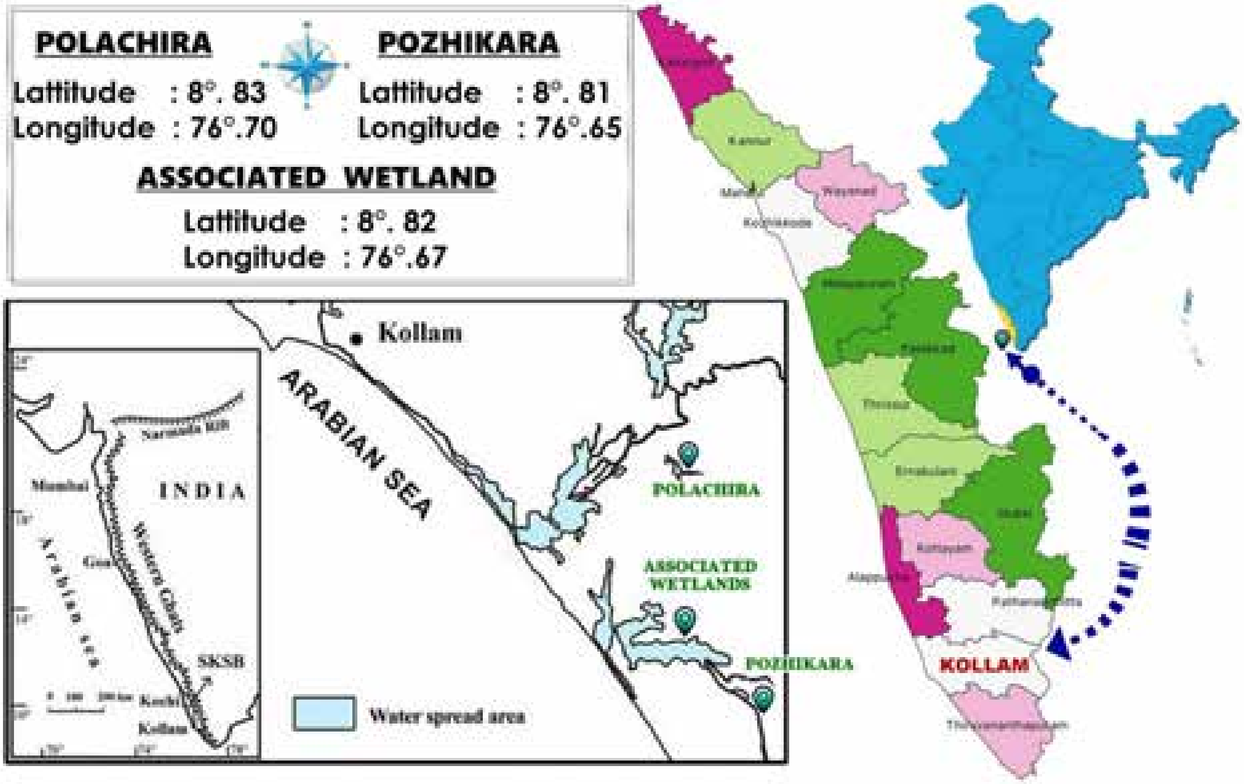


**Figure 1.** ***Sphaeroma taborans*** **sp. nov. (ZSI/WGRC/1.R.-INV.28482) holotype: A—dorsal view | B—antennule | C—antenna | D—epistome | E—right mandible | F—maxilla.**

diversity within each wetland. Surveys were conducted during morning (0600–1000 h) and evening (1600–1900 h) (Byju et al. 2023) to capture diurnal and nocturnal avian activity, with particular attention being paid to dawn and dusk periods when bird activity is typically high. Field researchers and trained ornithologists conducted systematic surveys of wetland habitats, utilizing binoculars, telescopes, and field notebooks to record bird sightings. Transect surveys along pre-established routes ensured comprehensive coverage of the diverse wetland habitats and microenvironments within the study area (Buckland et al. 2008). Bird surveys were conducted along established transects within the wetlands. Three- line transects were established within each of the three wetlands. Each transect was 500 × 100 $m^2$. Line transect methodology was employed, with observers walking along predetermined paths and recording all bird species detected within a specified distance of 50 m on either side. Along each transect, three sampling points were designated at 150-m intervals. At each sampling point, a point count method was employed. Observers remained stationary for a 30-min period, recording all bird species seen or heard (Nadeau et al. 2008) within a 50-m radius. A 5-min settling period was allowed at each point to minimize disturbance to bird activity.

Avian diversity indices at different sites were calculated using the Shannon- Wiener index (Shannon & Weaver 1949), Berger-Parker index (Berger & Parker 1970), Pielou index (Pielou 1969) Margalef index and Simpson index D (Margalef 1958).

I. Shannon- Wiener Diversity Index:

The Shannon Diversity Index is represented as H', where pi denotes the relative abundance of each group of organisms.

$H' = -\sum pi1n(pi)$

II. Berger-Parker Index:

The Berger-Parker Index, denoted as d, is calculated as Nmax divided by N, where Nmax represents the number of individuals in the most abundant group, and N represents the total number of individuals.

III. Margalef Index:

The Margalef Index, denoted as dMa, is computed as (S–1) divided by the natural logarithm of N, where S stands for the species number and N indicates the total number of individuals.

IV. Simpson Index:

The Simpson Index, represented as λ, is calculated as the sum of the squared proportions of individuals, where pi represents the proportion of individuals belonging to each species.

$\lambda=\sum pi^2$

V. Gini-Simpson index:

The Gini-Simpson Index, labeled as D, is derived as 1 minus the Simpson Index (λ), where λ denotes the Simpson Index.

VI. Pielou index:

The Pielou Index, represented as E', is calculated as the sum of pi multiplied by the logarithm of pi, divided by the logarithm of the total number of species (R), where pi represents the relative abundance of each species.

## RESULTS

In the present study, 98 species of birds belonging to 41 families and 15 orders were recorded. Order Passeriformes dominated with 37 species followed by Pelecaniformes and Charadriiformes with 13 species each. The orders Apodiformes, Podicipediformes, and Psittaciformes exhibited the lowest species richness, each represented by a single species. Family Ardeidae dominated with 10 species followed by Rallidae with six species and Sturnidae and Scolopacidae with five species each. Among 98 recorded avifauna, 16 were migratory, 76 were residents and six were local migrants. Order Charadriiiformes dominated with eight migratory species followed by Passeriformes with four species, Coraciiformes with two species. The lowest number of migratory bird species were reported from orders Ciconiiformes and Palecaniformes with one species each. Order Passeriformes dominate with 33 resident bird species followed by Pelecaniformes, Gruiformes, Coraciiformes, Piciformes, Ciconiiformes, Anseriformes, Cuculiformes, Accipitriformes, Podicipediformes, Psittaciformes, Suliformes, and Apodiformes. Six species of local migrants were observed of which order Pelecaniformes dominated with four bird species. According to the IUCN Red List (2024), the Asian Woolly-necked Stork was the only 'Near Threatened' species, while all other bird species observed in the study were categorized as 'Least Concern'. Residential status, IUCN status, order, and family-wise distribution of bird species are depicted in Table 1.

### Biodiversity indices

The diversity indices such as Simpson index, Simpson index —λ, Simpson index —D, Margalef index, Berger-Parker index, Shannon- Wiener index and Pielou index in the Polachira, Pozhikka associated wetlands are shown in Table 2.

The Berger-Parker index, which measures the dominance of the most abundant species, is consistently

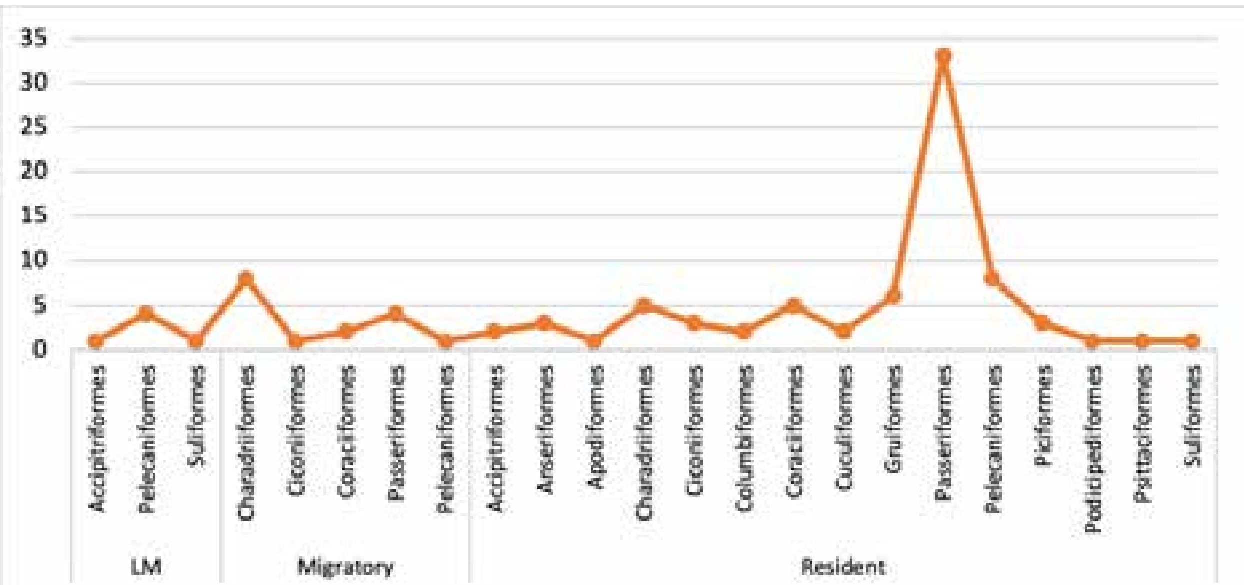


**Figure 2. Residential status: order-wise distribution of birds.**

**Table 1. Residential status, IUCN status, order, and family-wise distribution of bird species.**

| | Common name | Scientific name | Order | Family | Residential status | IUCN Red List status |
|---|---|---|---|---|---|---|
| 1 | Brahminy Kite | *Haliastur indus* | Accipitriformes | Accipitridae | LM | LC |
| 2 | Black Kite | *Milvus migrans* | Accipitriformes | Accipitridae | R | LC |
| 3 | Western Marsh Harrier | *Circus aeruginosus* | Accipitriformes | Accipitridae | R | LC |
| 4 | Lesser Whistling-Duck | *Dendrocygna javanica* | Anseriformes | Anatidae | R | LC |
| 5 | Indian Spot-billed Duck | *Anas poecilorhyncha* | Anseriformes | Anatidae | R | LC |
| 6 | Asian Palm Swift | *Cypsiurus balasiensis* | Apodiformes | Apodidae | R | LC |
| 7 | Indian Swiftlet | *Aerodramus unicolor* | Apodiformes | Apodidae | R | LC |
| 8 | Red Wattled Lapwing | *Vanellus indicus* | Charadriiformes | Charadriidae | R | LC |
| 9 | Kentish Plover | *Charadrius alexandrines* | Charadriiformes | Charadriidae | M | LC |
| 10 | Tibetan Sand-Plover | *Charadrius mongolus* | Charadriiformes | Charadriidae | M | LC |
| 11 | Pacific Golden-Plover | *Pluvialis fulva* | Charadriiformes | Charadriidae | M | LC |
| 12 | Bronze- winged Jacana | *Metopidius indicus* | Charadriiformes | Jacanidae | R | LC |
| 13 | Pheasant-tailed Jacana | *Hydrophasianus chirurgus* | Charadriiformes | Jacanidae | R | LC |
| 14 | Brown-headed Gull | *Chroicocephalus brunnicephalus* | Charadriiformes | Laridae | R | LC |
| 15 | Black-winged Stilt | *Himantopus himantopus* | Charadriiformes | Recurvirostridae | R | LC |
| 16 | Green Sandpiper | *Tringa ochropus* | Charadriiformes | Scolopacidae | M | LC |
| 17 | Common Sandpiper | *Actitis hypoleucos* | Charadriiformes | Scolopacidae | M | LC |
| 18 | Common Greenshank | *Tringa nebularia* | Charadriiformes | Scolopacidae | M | LC |
| 19 | Temminck's Stint | *Calidris temminckii* | Charadriiformes | Scolopacidae | M | LC |
| 20 | Common Snipe | *Gallinago gallinago* | Charadriiformes | Scolopacidae | M | LC |
| 21 | Painted Stork | *Mycteria leucocephala* | Ciconiiformes | Ciconiidae | R | LC |
| 22 | Asian Woolly-necked Stork | *Ciconia episcopus* | Ciconiiformes | Ciconiidae | R | NT |
| 23 | White Stork | *Ciconia ciconia* | Ciconiiformes | Ciconiidae | M | LC |
| 24 | Asian Openbill | *Anastomus oscitans* | Ciconiiformes | Ciconiidae | R | LC |
| 25 | Yellow-footed Green-Pigeon | *Treron phoenicopterus* | Columbiformes | Columbidae | R | LC |
| 26 | Spotted Dove | *Spilopelia chinensis* | Columbiformes | Columbidae | R | LC |
| 27 | Common Kingfisher | *Alcedo atthis* | Coraciiformes | Alcedinidae | R | LC |
| 28 | Pied Kingfisher | *Ceryle rudis* | Coraciiformes | Alcedinidae | R | LC |
| 29 | Indian Roller | *Coracias benghalensis* | Coraciiformes | Coraciidae | R | LC |
| 30 | White-throated Kingfisher | *Halcyon smyrnensis* | Coraciiformes | Halcyonidae | R | LC |
| 31 | Chestnut-headed Bee eater | *Merops leschenaultia* | Coraciiformes | Meropidae | R | LC |
| 32 | Blue-tailed Bee-eater | *Merops philippinus* | Coraciiformes | Meropidae | M | LC |
| 33 | Asian Green Bee-eater | *Merops orientalis* | Coraciiformes | Meropidae | M | LC |
| 34 | Greater Coucal | *Centropus sinensis* | Cuculiformes | Cuculidae | R | LC |
| 35 | Asian Koel | *Eudynamys scolopaceus* | Cuculiformes | Cuculidae | R | LC |
| 36 | Common Moorhen | *Gallinula chloropus* | Gruiformes | Rallidae | R | LC |
| 37 | White- breasted Waterhen | *Amaurornis phoenicurus* | Gruiformes | Rallidae | R | LC |
| 38 | Western Swamphen. | *Porphyrio porphyrio* | Gruiformes | Rallidae | R | LC |
| 39 | Grey-headed Swamphen | *Porphyrio poliocephalus* | Gruiformes | Rallidae | R | LC |
| 40 | Eurasian Coot | *Fulica atra* | Gruiformes | Rallidae | R | LC |
| 41 | Watercock | *Gallicrex cinerea* | Gruiformes | Rallidae | R | LC |
| 42 | Blyth's Reed Warbler | *Acrocephalus dumetorum* | Passeriformes | Acrocephalidae | M | LC |
| 43 | Clamorous Reed Warbler | *Acrocephalus stentoreus* | Passeriformes | Acrocephalidae | R | LC |

| | Common name | Scientific name | Order | Family | Residential status | IUCN Red List status |
|---|---|---|---|---|---|---|
| 44 | Ashy Woodswallow | *Artamus fuscus* | Passeriformes | Artamidae | R | LC |
| 45 | Common Tailorbird | *Orthotomus sutorius* | Passeriformes | Cisticolidae | R | LC |
| 46 | Zitting Cisticola | *Cisticola juncidis* | Passeriformes | Cisticolidae | R | LC |
| 47 | Plain Prinia | *Prinia inornata* | Passeriformes | Cisticolidae | R | LC |
| 48 | Ashy Prinia | *Prinia socialis* | Passeriformes | Cisticolidae | R | LC |
| 49 | House Crow | *Corvus splendens* | Passeriformes | Corvidae | R | LC |
| 50 | Indian Jungle Crow | *Corvus culminatus* | Passeriformes | Corvidae | R | LC |
| 51 | Rufous Treepie | *Dendrocitta vagabunda* | Passeriformes | Corvidae | R | LC |
| 52 | Large-billed Crow | *Corvus macrorhynchos* | Passeriformes | Corvidae | R | LC |
| 53 | Pale-billed Flowerpecker | *Dicaeum erythrorhynchos* | Passeriformes | Dicaeidae | R | LC |
| 54 | Black Drongo | *Dicrurus macrocercus* | Passeriformes | Dicruridae | R | LC |
| 55 | Black/Ashy Drongo | *Dicrurus adsimilis* | Passeriformes | Dicruridae | R | LC |
| 56 | White-rumped Munia | *Lonchura striata* | Passeriformes | Estrildidae | R | LC |
| 57 | Tricolored Munia | *Lonchura malacca* | Passeriformes | Estrildidae | R | LC |
| 58 | Scaly-breasted Munia | *Lonchura punctulata* | Passeriformes | Estrildidae | R | LC |
| 59 | Barn Swallow | *Hirundo rustica* | Passeriformes | Hirundinidae | LM | LC |
| 60 | Red-rumped Swallow | *Cecropis daurica* | Passeriformes | Hirundinidae | M | LC |
| 61 | Brown Shrike | *Lanius cristatus* | Passeriformes | Laniidae | R | LC |
| 62 | Jungle Babbler | *Argya striata* | Passeriformes | Leiothrichidae | R | LC |
| 63 | Yellow-billed Babbler | *Argya affinis* | Passeriformes | Leiothrichidae | R | LC |
| 64 | Western Yellow Wagtail | *Motacilla flava* | Passeriformes | Motacillidae | M | LC |
| 65 | Paddyfield Pipit | *Anthus rufulus* | Passeriformes | Motacillidae | R | LC |
| 66 | Oriental Magpie-robin | *Copsychus saularis* | Passeriformes | Muscicapidae | R | LC |
| 67 | Purple-rumped Sunbird | *Leptocoma zeylonica* | Passeriformes | Nectariniidae | R | LC |
| 68 | Black hooded Oriole | *Oriolus xanthornus* | Passeriformes | Oriolidae | R | LC |
| 69 | Indian Golden Oriole | *Oriolus kundoo* | Passeriformes | Oriolidae | R | LC |
| 70 | Green Warbler | *Phylloscopus nitidus* | Passeriformes | Phylloscopidae | M | LC |
| 71 | Baya Weaver | *Ploceus philippinus* | Passeriformes | Ploceidae | R | LC |
| 72 | Red-whiskered Bulbul | *Pycnonotus jocosus* | Passeriformes | Pycnonotidae | R | LC |
| 73 | Red-vented Bulbul | *Pycnonotus cafer* | Passeriformes | Pycnonotidae | R | LC |
| 74 | Common Myna | *Acridotheres tristis* | Passeriformes | Sturnidae | R | LC |
| 75 | Jungle Myna | *Acridotheres fuscus* | Passeriformes | Sturnidae | R | LC |
| 76 | Chestnut-tailed Starling | *Sturnia malabarica* | Passeriformes | Sturnidae | R | LC |
| 77 | Common/Jungle Myna | *Acridotheres tristis* | Passeriformes | Sturnidae | R | LC |
| 78 | Malabar Starling | *Sturnia blythii* | Passeriformes | Sturnidae | R | LC |
| 79 | Cattle Egret | *Bubulcus ibis* | Pelecaniformes | Ardeidae | LM | LC |
| 80 | Large Egret | *Ardea alba* | Pelecaniformes | Ardeidae | LM | LC |
| 81 | Little Egret | *Egretta garzetta* | Pelecaniformes | Ardeidae | R | LC |
| 82 | Median Egret | *Ardea intermedia* | Pelecaniformes | Ardeidae | R | LC |
| 83 | Purple Heron | *Ardea purpurea* | Pelecaniformes | Ardeidae | R | LC |
| 84 | Indian Pond Heron | *Ardeola grayii* | Pelecaniformes | Ardeidae | R | LC |
| 85 | Night Heron | *Nycticorax nycticorax* | Pelecaniformes | Ardeidae | LM | LC |
| 86 | Medium Egret | *Ardea intermedia* | Pelecaniformes | Ardeidae | R | LC |
| 87 | White Egret sp. | *Ardea alba modesta* | Pelecaniformes | Ardeidae | R | LC |
| 88 | Grey Heron | *Ardea cinerea* | Pelecaniformes | Ardeidae | R | LC |

| | Common name | Scientific name | Order | Family | Residential status | IUCN Red List status |
|---|---|---|---|---|---|---|
| 89 | Oriental Darter | *Anhinga melanogaster* | Pelecaniformes | Phalacrocoracidae | R | LC |
| 90 | Oriental White Ibis | *Threskiornis melanocephalus* | Pelecaniformes | Threskiornithidae | LM | LC |
| 91 | Glossy Ibis | *Plegadis falcinellus* | Pelecaniformes | Threskiornithidae | M | LC |
| 92 | White-cheeked Barbet | *Psilopogon viridis* | Piciformes | Megalaimidae | R | LC |
| 93 | Black-rumped Flameback Woodpecker | *Dinopium benghalense* | Piciformes | Picidae | R | LC |
| 94 | Common Flameback Woodpecker | *Dinopium javanense* | Piciformes | Picidae | R | |
| 95 | Little Grebe | *Tachybaptus ruficollis* | Podicipediformes | Podicipedidae | R | LC |
| 96 | Rose-ringed Parakeet | *Psittacula krameri* | Psittaciformes | Psittaculidae | R | LC |
| 97 | Little Cormorant | *Phalacrocorax niger* | Suliformes | Phalacrocoracidae | R | LC |
| 98 | Indian Cormorant | *Phalacrocorax fuscicollis* | Suliformes | Phalacrocoracidae | LM | LC |

LM—Local Migrant | M—Migrant | R—Resident | LC—Least Concern | NT—Near Threatened.

high across Polachira, Pozhikkara, and associated wetlands. The Margalef index, which primarily reflects species richness, shows relatively high values across all sites. Simpson's index λ and Pielou's evenness index both indicate relatively high evenness within the avian communities. The Shannon-Wiener index, a comprehensive measure of diversity, shows very similar values across all three sites (4.46, 4.47, and 4.45). This suggests that the overall avian diversity is comparable among these wetlands.

## DISCUSSION

Increases in human population facilitate urbanization globally which in turn leads to changes in the structure and ecology of landscape ultimately leading to biodiversity loss due to anthropogenic threats (McKinney 2006; Rocha & Fellowes 2018). Populations of birds, which are one of the most common wild fauna in the urban area, are facing threats and have been declining as a result of the rapid urbanization-expansion process (Bolwig et al. 2006; Strohbach 2009; Evans et al. 2011; Gatesire et al. 2014). In the present study, 98 species of birds belonging to 41 families and 15 orders from Pozhikkara, Polachira, and associated wetlands were recorded. Twenty-four species of birds in 11 families and nine orders were reported from wetlands in Kollam, mainly Kandachira and nearby paddy fields (Anoop et al. 2017). From large stretches of wetlands in the form of paddy fields, ponds and canals in Chadayamangalam and Nilamel in Kollam District 14 species of birds belonging to five families were identified (Lekshmy 2014). The Berger-Parker index, which measures the dominance, in the present study suggests that a few species such as *Anas poecilorhyncha*, *Ardea intermedia*, *Fulica atra*, *Porphyrio poliocephalus*, *Dendrocygna javanica*, *Hirundo rustica*, *Egretta garzetta* dominate the avian communities in these wetlands. Simpson's index λ (0.01) and Pielou's evenness index (0.97) suggests that some species such as *Anas poecilorhyncha*, *Ardea intermedia* may be more abundant than *Vanellus indicus* and *Corvus culminatus*, the distribution of individuals among species is relatively equitable. The Margalef index indicates a considerable number of bird species present in each wetland. The Shannon-Wiener index (Polachira 4.46, Pozhikkara 4.47, and associated wetlands 4.45) suggests that the overall avian diversity is comparable among these wetlands of study. Species richness showed similar values among the three study sites, with minimum variations and associated wetland had slightly higher dominance (based on Berger-Parker index 37.20) compared to the other two sites. Overall, all three wetland sites exhibit relatively high species richness, moderate dominance, and high evenness in their avian communities. The similar values of the Shannon-Wiener index (4.45–4.47) across all sites suggest that the overall avian diversity is comparable among these three wetlands. Laseetha et al. (2023) reported 86 bird species in Polachira and associated wetlands. The study recorded a higher bird diversity of 98 species across Pozhikkara, Polachira, and associated wetlands. This apparent increase in species richness, particularly during a period of restricted human activity (lockdown), may suggest increased human encroachment on these natural habitats.

The COVID-19 pandemic brought about unexpected changes in humans such as social distancing, remote work, and lockdowns (Zellmer et al. 2020) which, throughout the world, led to a marked pollution

**Table 2. Biodiversity indices.**

| Wetland | Berger-Parker index | Margalef index | Simpson index λ | Simpson index D | Shannon-Wiener index | Pielou index |
|---|---|---|---|---|---|---|
| Polachira | 34.70 | 14.52 | 0.01 | 0.99 | 4.46 | 0.97 |
| Pozhikkara | 35.90 | 14.64 | 0.01 | 0.99 | 4.47 | 0.97 |
| Associated Wetlands | 37.20 | 14.67 | 0.01 | 0.99 | 4.45 | 0.97 |

reduction in the air (Venter et al. 2020) and noise (Lecocqm et al. 2020), which created an environment potentially benefiting bird communication, foraging, and breeding success and improved air quality had positive impacts on bird health and foraging opportunities. Noise pollution negatively affects bird abundance and distribution (McClure et al. 2013; Shannon et al. 2015), as it interferes with mating signals and defense mechanisms (Slabbekoorn 2013). Water bodies polluted with biological wastes, which in turn affect biological oxygen demand, can cause significant damage to the abundance of aquatic invertebrates, zooplankton and fish (Schirmel et al. 2016), and can also affect bird populations (Klemetsen & Knudsen 2013; Mallin et al. 2016). The analysis of water quality parameters such as suspended particulate matter (SPM) concentration in Asthamudi Lake in Kollam District using Landsat 8 OLI image shows that the concentration of SPM values in lockdown (mean SPM 8.01 mg/l) is lower than that of pre-lockdown (10.03 mg/l) and last five-year average (9.1 mg/l) (Aswathy et al. 2021). Noise pollution significantly impacts avian abundance and occupancy by disrupting critical acoustic communication pathways (Shannon et al. 2015). Elevated noise levels can mask crucial vocalizations, such as mating calls and alarm calls, hindering successful reproduction and increasing vulnerability to predation (Slabbekoorn 2013). Air quality is also attributed for increase in avifauna diversity. Lockdowns led to significant reductions in PM 2.5 and other pollutants in megacity of Delhi (Mahato et al. 2020) and 22 cities covering different regions of India (Sharma et al. 2020). Air Quality Index (AQI) temporal variability in Kollam demonstrated improved air quality during the lockdown period (median: 49, range: 30–105) compared with both the pre-lockdown (median: 89, range: 48–205) and post-lockdown (median: 75, range: 47–124) periods (Thomas et al. 2023). The exposure to particulate matter can negatively impact species diversity (Sanderfoot & Holloway 2017; Liang et al. 2020). The observed increase in bird diversity in this study may be partly attributed to the improved air quality conditions experienced during the lockdown period.

Decreased human activity due to lockdowns and travel restrictions have led to reduced disturbance to bird habitats as restricted movement of humans helped the unrestricted movement of wild animals and birds during the pandemic period (Sahagun 2020). Diversity is an important ecological indicator for estimating the health and quality of ecosystems and birds play crucial roles in natural activities such as pest control, pollination and seed dispersal (Jaman et al. 1999). Anthropogenic activities such as poisoning, hunting, trapping, killing and destroying the habitat of birds compel birds to change their habitats due to scarcity of food and shelter (Rajia et al. 2015; Shome et al. 2020). A survey conducted by Lekshmy (2014) in the Nilamel and Chadayamangalam regions in Kollam and post COVID avian survey of Laseetha et al. (2023) in the Polachira Wetland reported a total of 14 and 86 bird species respectively. In the present study, conducted during the COVID-19 lockdown period, documented an extensive avian diversity in the Kollam region. This notable increase in bird diversity, observed during the time of reduced anthropogenic activity, suggests the relationship between human disturbance and local avifauna.

## CONCLUSION

This study provides valuable insights into the avian diversity within the Polachira, Pozhikkara, and associated wetlands of Kollam District during the COVID-19 pandemic. The study reveals a consistent pattern of high species richness and moderate evenness across all study sites. The study suggests a moderate level of dominance by a few species, the overall avian diversity remains remarkably similar among these wetlands. These findings emphasize the importance of these wetland ecosystems in supporting diverse avian communities within Kollam District.

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COMMUNICATION



# Checklist on the ichthyofaunal resources and conservation status of Dikhu River, Nagaland, India

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**Abstract:** Dikhu River constitutes one of the major perennial rivers of Nagaland and due to its pristine beauty aids as a tourism spot. From the present study, ichthyofaunal diversity accounted for a total of 28 fish species belonging to 6 orders, 13 families, and 3 subfamilies. From the investigation, order Cypriniformes (67.9%) was found to be the most dominant, followed by Siluriformes (14.3%) and Anabantiformes (7.1%) while order Beloniformes, Synbranchiformes and Anguilliformes was found to be the least common with 10.7% in total. The IUCN red list of threatened species shows 71.4% are Least Concern, 7.1% Near Threatened, 3.6% Vulnerable, 3.6% Endangered, 10.7% Not Accessed and 3.6% Data Deficient. IUCN population status data shows that 57.1% are unknown, 10.7% stable, 10.7% not accessed, and 21.4% with decreasing population trends. Diversity indices (Shannon, Simpson, and Evenness) indices ranged 2.497–2.912, 0.892–0.936, and 0.820–0.908, respectively. Relative abundance determinations ranged from a high of 15.55% for *Devario aequipinnatus* to the lowest values for *Tariqilabeo latius* and *Botia rostrata* at 0.08%. Anthropogenic pressure on the Dikhu River has resulted in habitat modification and fragmentation, posing a hazard to fish diversity. Fish abundance was found to be highest in the post-monsoon season and lowest in winter. These findings are significant for academic purposes and support conservation strategies for local fish resources.

**Keywords:** Assessment, biodiversity, diversity index, fish species, fisheries management, ichthyofauna, Nagaland fisheries, population status, riverine habitat, threatened species.

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**Author details:** Metevinu Kechu is a research scholar and is actively engaged in ichthyofaunal exploration and documentation in Nagaland. Pranay Punj Pankaj is engaged in teaching fisheries as well as research and development activities on the subject in the region.

**Author contributions:** MK surveyed the Dikhu River of Nagaland and confirmation of the identity of the species. PPP supervised the work and interpreted the taxonomic information gathered by the Mk.

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## INTRODUCTION

India has been identified as one of the mega biodiversity countries in the world (Mittermeier & Mitemeir 1997) and the northeastern part of India with its unique topography has been considered a biodiversity hotspot in the world (Groombridge & Jenkins 1998). The region's rich biodiversity is attributed to its tectonic placement (Kottelat 1989) and is considered a freshwater fish biodiversity hotspot in the world (Kottelat et al. 1996).

The state of Nagaland, located in the northeastern part of India, is known for its rich biodiversity, including a diverse range of fish species. Nagaland is bounded by beautiful hilly terrain and riverine system and shares boundaries with Assam (northern and western part), Arunachal Pradesh (northeastern part), Myanmar (eastern part), and Manipur (southern) (Kosygin & Vishwanath 1998). The three principal drainages of Nagaland consist of Brahmaputra and Barack of Indian origin and Chindwin drainage of Indo-Burma origin. The Dikhu River, flowing through Nagaland, serves as a prime location to study the ichthyofaunal diversity in the region. This river is characterized by its running waters and hilly terrain, making it an ideal habitat for various fish species. Furthermore, little information exists on the biology of these fish species, emphasizing the need for a comprehensive study of their diversity. The study of fish diversity in the Dikhu River would provide valuable insights into the ecological dynamics and conservation efforts in the region.

Biodiversity hotspots under freshwater ecosystems, are increasingly under threat, making their conservation a critical concern. Major factors such as habitat destruction, invasive species, overexploitation, and the impacts of climate change are driving the rapid decline of species populations. Studies have highlighted that approximately 24% of freshwater species are facing a high risk of extinction due to threats including pollution, dam construction, water extraction, agricultural practices, and the introduction of invasive species (Sayer et al. 2025). In the northeastern region of India, studies have provided valuable insights into the conservation status of freshwater fish species, illustrating both the rich diversity and the significant threats they face (Vishwanath 2017). A report presented at COP 28 in the United Arab Emirates further revealed that 25% of freshwater fish species worldwide are at risk of extinction, with climate change directly impacting at least 17% of these species (IUCN 2023). These findings underscore the urgent need for targeted conservation efforts to safeguard freshwater biodiversity.

The Shannon Index, Simpson Index, and Jacquard's Evenness Index are commonly used to measure biodiversity and community structure in ecological studies. Biodiversity is often astonishingly altered or overused to define the population of a community. It is a measure of the number of species that make up a biological community and is considered one of the most important aspects of community organization or structure (Jewel et al. 2018). Information about ichthyofaunal diversity is scarce in this region and only a few notable works have been done by Hora (1936), Menon (1954), Acharjee et al. (2012), and a few studies have been done on the biodiversity status in Dikhu River individually by Ezung et al. (2022) and Konyak & Limatemjen (2022). The use of these diversity indices would allow researchers to assess the species richness, evenness, and dominance of fish populations in the Dikhu River. By quantifying the diversity indices, researchers can determine the overall health and stability of the fish community in the river. Understanding the fish diversity in the Dikhu River is crucial for several reasons. Firstly, it aids in the conservation and management of fish populations. This information helps in formulating policies for sustainable fisheries management. Though it is considered a significant river in the state, there has been scarce information regarding its status and its habitat ecology and hence this present study is an attempt to identify the current fish species diversity in this river.

## MATERIALS AND METHOD

### Study area

Dikhu River is one of the tributaries of the Brahmaputra River and six stations were selected for sampling (Table 1). The study was conducted for a period of 12 months from March 2019 to February 2020. The locations of the study sites were taken using GPS (Garmin etrex-10) (Image 1). It originates from the Naruto Hill, Zunheboto, and later confluences towards the Brahmaputra River from Naginimora, Assam. It then channels through most of the Zunheboto and Mokokchung districts of Nagaland, covering a total distance of 170 km. The two major tributaries of the Dikhu River are Yangyu in Tuensang district and Nanung (Langpangkong range) in Mokokchung district (Ao et al. 2008).

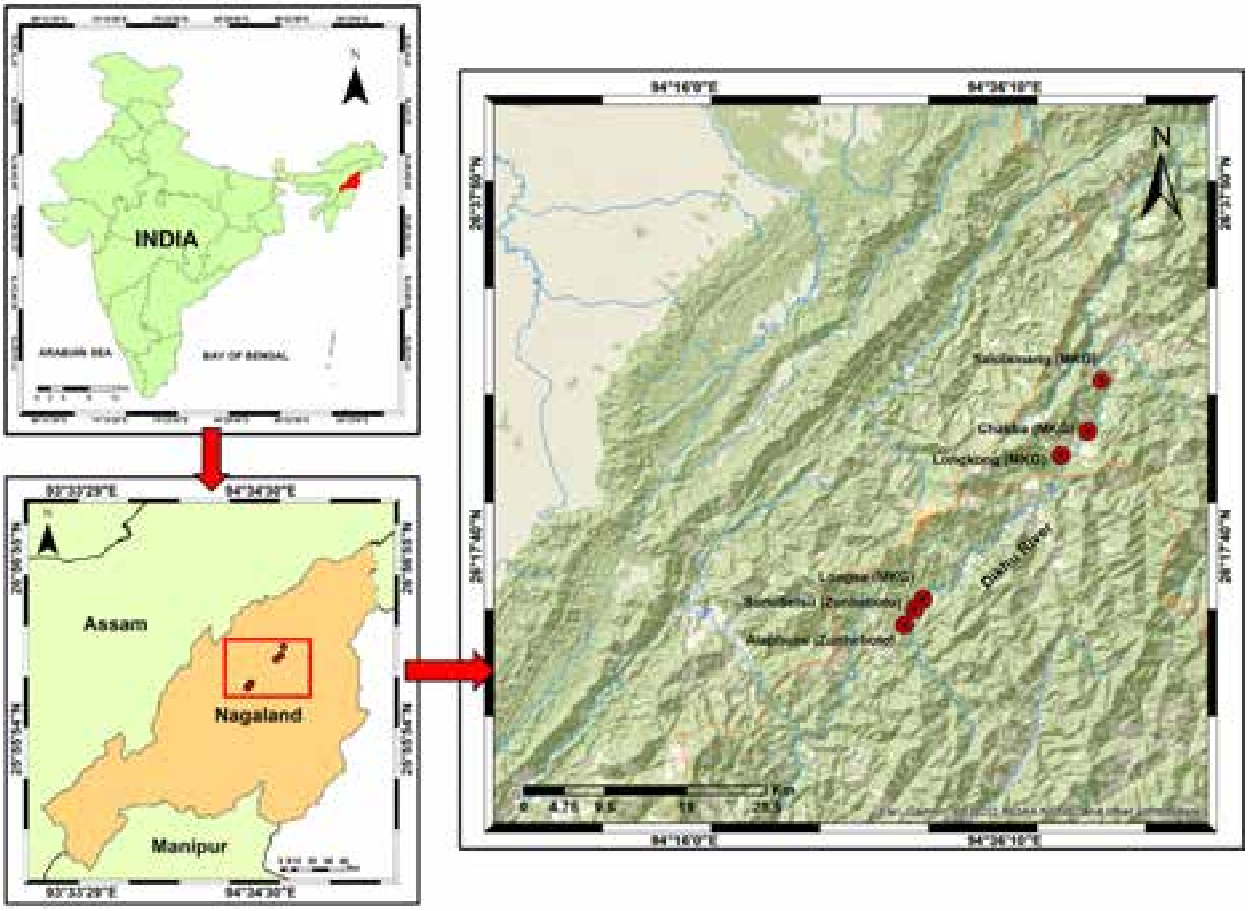


Image 1. Geospatial mapping of study site distribution along the Dikhu River, Nagaland.

## Fish sampling

Fish samples were collected from various sampling sites along the course of the river with gears such as cast nets, gill nets, scoop nets, hooks and lines, and locally available indigenous traps. Sampling collection was done on a monthly basis. Collected fish samples were photographed with a Canon EOS 3000D camera and measured using a vernier caliper and graduated ruler (graduations in mm) while excess fishes were released back into the river then a few samples were preserved in 10% formalin solution for further identification. Samples were identified with the help of key identification characters given by Talwar & Jhingran (1991), Menon (1999), Vishwanath et al. (2007), and Jayaram (2010), and the latest nomenclature was based in accordance with the Catalog of Fishes (Fricke et al. 2025). Fish classification was conducted following the guidelines outlined by Nelson et al. (2016).

## Species diversity

Shannon diversity index, (Shannon & Weaver 1949)

Table 1. Sampling sites of Dikhu River, Nagaland.

| Stations | Geographical coordinates | Altitude |
|---|---|---|
| Longsa (MKG) | 26.244° N 94.517° E | 2230 ft or 679.7 m |
| Longkong (MKG) | 26.380° N 94.662° E | 1390 ft or 423 m |
| Chakba (MKG) | 26.403° N 94.690° E | 1292 ft or 393.8 m |
| Alaphumi (Zunheboto) | 26.219° N 94.497° E | 2383 ft or 726.3 m |
| Salulamang (MKG) | 26.452° N 94.704° E | 1164 ft or 354.7 m |
| SumiSetsü (Zunheboto) | 26.234° N 94.508° E | 2271 ft or 692.2 m |

$H' = - \Sigma \; pi \ln (pi)$,

Where H' is the Shannon Diversity Index and pi is the number of individuals in the $i^{th}$ species as a proportion of the total population. ln (pi) is the natural log of pi

The formula used for calculating Simpson's index (Simpson 1949) is:

$D = 1- (\Sigma \; n*(n-1)/N*(N-1))$

Where n is the number of individuals of a specific species. N is the total number of individuals of all species.

Pielou's Evenness Index (J) is calculated using the Shannon Diversity Index (H) and ln(S) is the natural logarithm of the total number of species (S) in the community. The formula for Pielou's Evenness Index (Pielou 1966) is:

$J' = H'/H'max = H' / \ln(S)$

The relative abundance (percentage composition) of fish species across the six sampling stations was calculated using the following formula.

Relative Abundance (%) = (Number of individuals of a species × 100) / Total number of species

Statistical analysis: For data conversion and analysis, Microsoft Excel was used.

## RESULTS

### Fish assemblage

Fish species composition of Dikhu River showed the presence of 28 fish species belonging to six orders 13 families and three subfamilies (Images 2 & 3). Table 2 provides a detailed checklist of fish species, organized according to their IUCN conservation status (IUCN 2024) and documented population trends. Among the order of fish species, Cypriniformes were observed as the most dominant group with 67.9% followed by Siluriformes (14.3%) and Anabantiformes (7.1%) while the least common order group belonged to Beloniformes, Synbranchiformes, and Anguilliformes with combined (10.7%) in total (Figure 1). Cyprinidae dominated among the families represented by 14 species and among the genus group *Garra*, *Opsarius,* and *Danio* were the most common with three species each. Based on the IUCN red list of threatened species category 71.4% was represented under Least Concern (LC), 7.1% under Near Threatened (NT), 3.6% under Vulnerable (VU), 3.6% under Endangered (EN), 10.7% under Not Assessed (NA) and 3.6% under Data Deficient (DD) status (Figure 2). The population trend of fish species as per IUCN also showed 57.1% as unknown, 10.7% as stable, 10.7% as not accessed, and 21.4% as decreasing population trends (Figure 3). Assessing the species according to its economic value were categorized as 46.4% under food and ornamental, 35.7% under ornamental, 10.7% under food, and 7.1% under food and sport category (Figure 4).

### Relative abundance

The relative abundance of small indigenous fish *Devario aequipinnatus* (15.55%), *Opsarius bendelisis* (11.39%), *Garra naganensis* (9.52%), and *Amblyceps apangi* (7.73%) were found to be high in the river under study indicating its abundance and dominance. Species under different conservation categories like *Neolissochilus hexagonolepis* (NT) showed relatively high RA with values of 4.31%, which indicates the stability of their population despite natural and anthropogenic threats in the sampling sites. While others under the threatened category like *Tor putitora* (EN), *Anguilla bengalensis* (NT), and *Botia rostrata* (VU) indicated decreasing trends with RA values of 0.62%, 0.16%, and 0.08 %. Other species with fairly high RA were *Schistura savona* (6.61%), *Psilorhynchus homaloptera* (5.91%), *Pterocryptis indica* (5.71%) and *Pethia conchonius* (5.25%). The lowest RA was recorded in *Tariqilabeo latius* and *Botia rostrata* with values of 0.08% each. It has been observed that the highest number of catches was recorded during the post-monsoon followed by the pre-monsoon season compared to the other seasons (Figure 5).

### Diversity index

Diversity was highest (H' = 2.912, 1-D = 0.936) in post-monsoon season and lowest in monsoon season (H' = 2.497, 1-D = 0.892), and the values of evenness index (J') were recorded highest (J' = 0.908) in pre-monsoon season and lowest in monsoon season (J' = 0.82). The mean value and standard deviation of species found in each season, Shannon diversity (H'), Simpson's index (1-D), and Pielou's evenness (J') indices were recorded as, 23.75 ± 2.217, 2.716± 0.194, 0.915± 0.022 and 0.864± 0.044 (Table 3). The study concluded that the Dikhu River supports rich fish diversity, while there is a notable shift in the fish community structure.

## DISCUSSION

The spatial distribution of fish species in the Dikhu River is influenced by a complex interplay of ecological factors, including abiotic conditions, biotic interactions, and evolutionary adaptations within the habitat (Pelicice et al. 2015; Bose et al. 2019; Satpathy et al. 2021; Alam et al. 2024). Among these factors, the dominance of Cypriniformes in the fish assemblages of the river is particularly notable. This pattern mirrors findings from other studies across northeastern Indian rivers, indicating a widespread dominance of this order (Taro et al. 2022; Ahmed et al. 2023; Chetry et al. 2023; Singh et al. 2024). The success of Cypriniformes in these ecosystems can be attributed to their evolutionary adaptations, including

**Table 2. Comprehensive overview of fish catch composition of Dikhu River, Nagaland: conservation status, population trends, and economic significance.**

| | Systematic position | Local name (Ao) | Common name | IUCN status | Population trends | Economic value | Specimen number |
|---|---|---|---|---|---|---|---|
| **Order: Anguilliformes<br>Family: Anguillidae** | | | | | | | |
| 1 | *Anguilla bengalensis* (Gray, 1831) | Angulang | Indian Mottled Eel | NT | Unknown | Fd | NUFM 1390 |
| **Order: Cypriniformes<br>Family: Cyprinidae<br>Subfamily: Barbinae** | | | | | | | |
| 2 | *Neolissochilus hexagonolepis* (McClelland, 1839) | Seben | Katli or Chocolate mahseer | NT | Decreasing | Fd, Sp | NUFM 1279 |
| 3 | *Tor putitora* (Hamilton, 1822) | Tzünger | Golden mahseer | EN | Decreasing | Fd, Sp | NUFM 1285 |
| 4 | *Pethia conchonius* (Hamilton, 1822) | Tzünger | Rosy barb | LC | Unknown | Fd, Or | NUFM 1289 |
| **Subfamily: Danioninae** | | | | | | | |
| 5 | *Opsarius bendelisis* (Hamilton, 1807) | Tawa | Indian hill trout | LC | Stable | Fd, Or | NUFM 1310 |
| 6 | *Opsarius tileo* (Hamilton, 1822) | Tawa | Tileo baril | LC | Unknown | Fd, Or | NUFM 1316 |
| 7 | *Opsarius barna* (Hamilton, 1822) | Tawa | Barna Baril | LC | Stable | Fd, Or | NUFM 1317 |
| 8 | *Danio dangila* (Hamilton, 1822) | Zer | Dangila Danio | LC | Decreasing | Fd, Or | NUFM 1325 |
| 9 | *Danio rerio* (Hamilton, 1822) | Zer | Zebra fish | LC | Decreasing | Fd, Or | NUFM 1329 |
| 10 | *Danio assamila* (Kullander, 2015) | Zer | Not accessed | Not accessed | Not accessed | Fd, Or | NUFM 1332 |
| 11 | *Devario aequipinnatus* (McClelland, 1839) | Zer | Giant danio | LC | Unknown | Fd, Or | NUFM 1319 |
| **Subfamily: Labeoninae** | | | | | | | |
| 12 | *Tariqilabeo latius* (Hamilton, 1822) | Anget | GangeticLatia | LC | Unknown | Fd | NUFM 1295 |
| 13 | *Garra lissorhynchus* (McClelland, 1842) | Anget | Khasi garra | LC | Unknown | Fd, Or | NUFM 1296 |
| 14 | *Garra birostris* (Nebeshwar & Vishwanath, 2013) | Anget | Not accessed | Not accessed | Not accessed | Fd, Or | NUFM 1302 |
| 15 | *Garra naganensis* (Hora, 1921) | Anget | Naga garra | LC | Unknown | Fd, Or | NUFM 1304 |
| **Family: Psilorhynchidae** | | | | | | | |
| 16 | *Psilorhynchus homaloptera* (Hora & Mukherji, 1935) | Mernngo | Homaloptera minnow | LC | Unknown | Or | NUFM 1347 |
| 17 | *Psilorhynchus arunachalensis* (Nebeshwar, Bagra & Das, 2007) | Mernngo | Not accessed | DD | Unknown | Or | NUFM 1353 |
| **Family: Botiidae** | | | | | | | |
| 18 | *Botia rostrata* (Günther, 1868) | Nga-medaktsü | Gangetic loach | VU | Decreasing | Or | NUFM 1356 |
| **Family: Nemacheilidae** | | | | | | | |
| 19 | *Paracanthocobitis botia* (Hamilton, 1822) | Sangsert | Mottled loach | LC | Decreasing | Or | NUFM 1335 |
| 20 | *Schistura savona* (Hamilton, 1822) | Retong | *Half-banded Loach* | LC | Unknown | Or | NUFM 1341 |
| **Order: Siluriformes<br>Family: Siluridae** | | | | | | | |
| 21 | *Pterocryptis indica* (Datta, Barman & Jayaram, 1987) | Lorng | Siluras Catfish | DD | Unknown | Fd | NUFM 1360 |
| **Family: Bagridae** | | | | | | | |
| 22 | *Olyra longicaudata* (McClelland, 1842) | Nenak | Torrent Catfish | LC | Unknown | Or | NUFM 1357 |
| **Family: Amblycipitidae** | | | | | | | |
| 23 | *Amblyceps apangi* (Nath & Dey, 1989) | Nenak | Indian Torrent Catfish | LC | Unknown | Or | NUFM 1368 |
| **Family: Sisoridae** | | | | | | | |
| 24 | *Glyptothorax indicus* (Talwar, 1991) | Jangmu | Catfish | LC | Unknown | Or | NUFM 1366 |
| **Order: Anabantiformes<br>Family: Channidae** | | | | | | | |
| 25 | *Channa melanostigma* (Geetakumari & Vishwanath, 2011) | Alopungo | snakehead | Not accessed | Not accessed | Fd, Or | NUFM 1374 |

| | Systematic position | Local name (Ao) | Common name | IUCN status | Population trends | Economic value | Specimen number |
|---|---|---|---|---|---|---|---|
| **Family: Badidae** | | | | | | | |
| 26 | *Badis badis* (Hamilton, 1822) | Akngo | Badis | LC | Unknown | Or | NUFM 1379 |
| **Order: Beloniformes**<br>**Family: Belonidae** | | | | | | | |
| 27 | *Xenentodon cancila* (Hamilton, 1822) | Jokli | Freshwater Garfish | LC | Unknown | Or | NUFM 1385 |
| **Order: Synbranchiformes**<br>**Family: Mastacembelidae** | | | | | | | |
| 28 | *Mastacembelus armatus* (Lacepède, 1800) | Merü | Spiny Eel | LC | Stable | Fd, Or | NUFM 1388 |

DD—Data Deficient | EN—Endangered | LC—Least Concern | NT—Near Threatened | VU—Vulnerable | Fd—Food | Sp—Sport | Or—Ornamental.

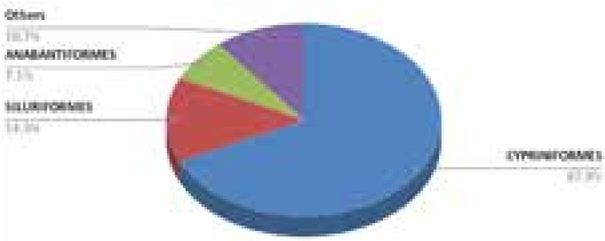


Figure 1. Distribution of fish species by order group.

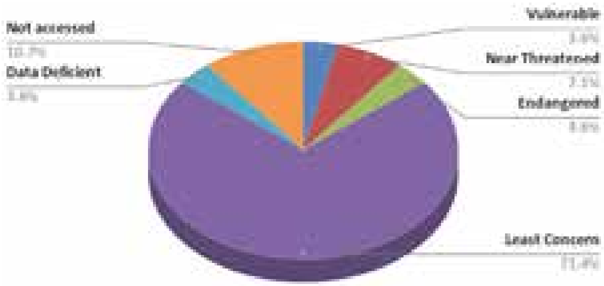


Figure 2. Distribution via IUCN conservation status.

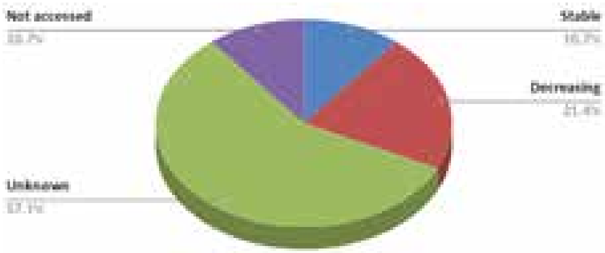


Figure 3. Distribution by population trend.


Figure 4. Distribution by economic value.

the ability to thrive in a range of water conditions, their varied feeding strategies, and reproductive behaviors, which have enabled them to outcompete other species in these freshwater habitats. Furthermore, their efficient use of resources has positioned Cypriniformes as central components of the ecological dynamics in these rivers (Mondal & Bhat 2020). Similar patterns of Cypriniformes dominance have been reported in studies conducted in Nagaland, with most species classified by the IUCN as either 'Least Concern' or 'Data Deficient' (Tsurunla et al. 2024), emphasizing the need for more focused studies on the region's ichthyofauna. The present study also identified a significant proportion (21.4%) of species experiencing a declining population trend, which is likely attributable to a combination of natural environmental pressures and anthropogenic influences (Nel et al. 2009; Kechu et al. 2021).

Relative abundance (RA) is an important metric in ichthyological studies as it reflects the proportional representation of species within a community. This measure is crucial for understanding species dominance, interspecies competition, and the overall health and stability of aquatic ecosystems (Hubbell 2005). In this study, *Devario aequipinnatus* and *Opsarius bendelisis* were found to have the highest RA, with values similar to those reported by Valentina et al. (2015) in Karbi Anglong district, Assam. Additionally, the post-monsoon season was marked by the highest number of catches, consistent with findings by Ali et al. (2004), who observed that receding water levels during this period tend to concentrate fish in shallower areas, thereby increasing catch rates.

The Shannon Diversity Index is a key tool for assessing the health of aquatic ecosystems. Values below 1

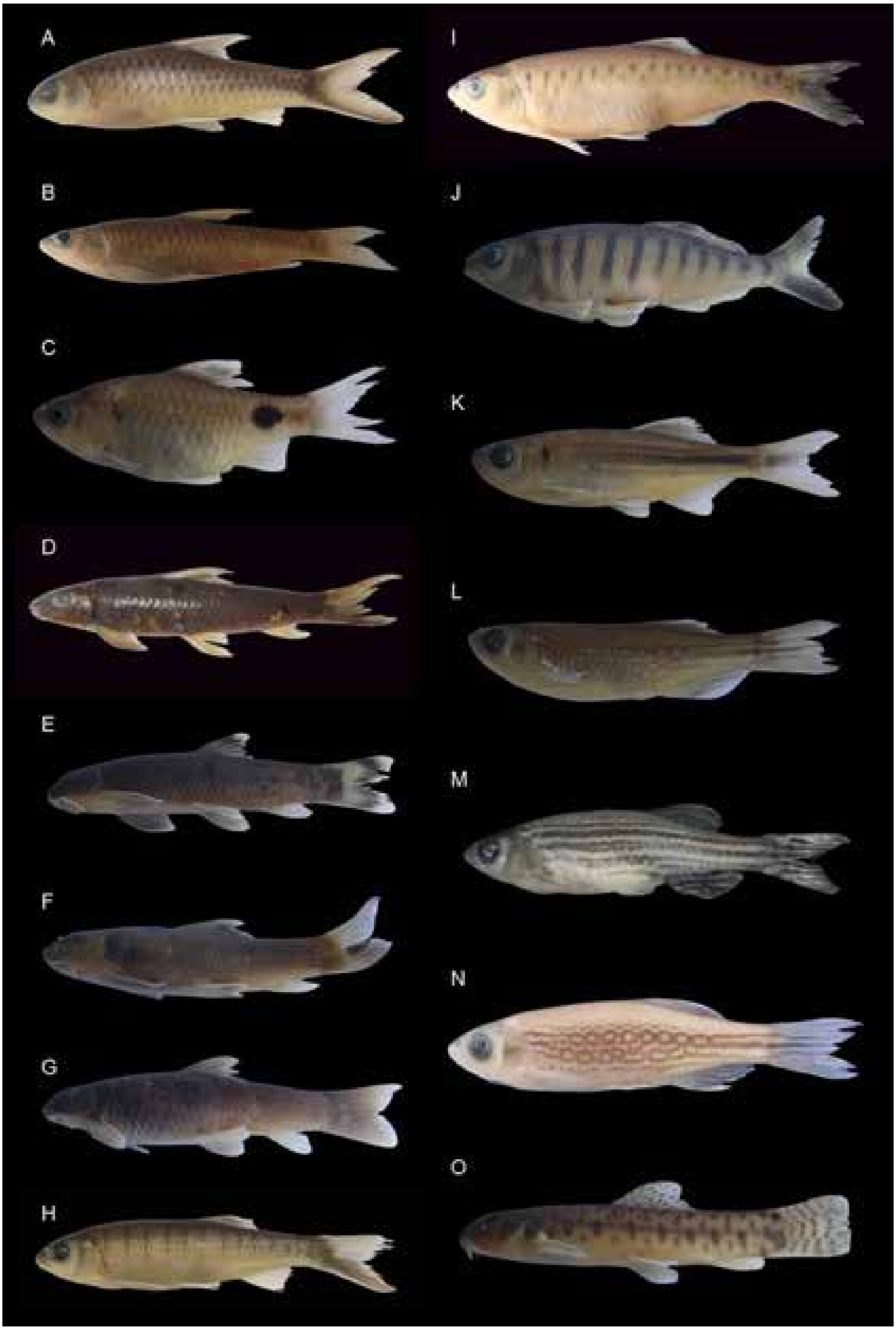


**Image 2. Fish species recorded in Dikhu River, Nagaland: A—*Neolissochilus hexagonolepis* | B—*Tor putitora* | C—*Pethia conchonius* | D—*Tariqilabeo latius* | E—*Garra lissorhynchus* | F—*Garra birostris* | G—*Garra naganensis* | H—*Opsarius bendelisis* | I—*Opsarius tileo* | J—*Opsarius barna* | K—*Devario aequipinnatus* | L—*Danio dangila* | M—*Danio rerio* | N—*Danio assamila* | O—*Paracanthocobitis botia*. © Metevinu Kechu.**

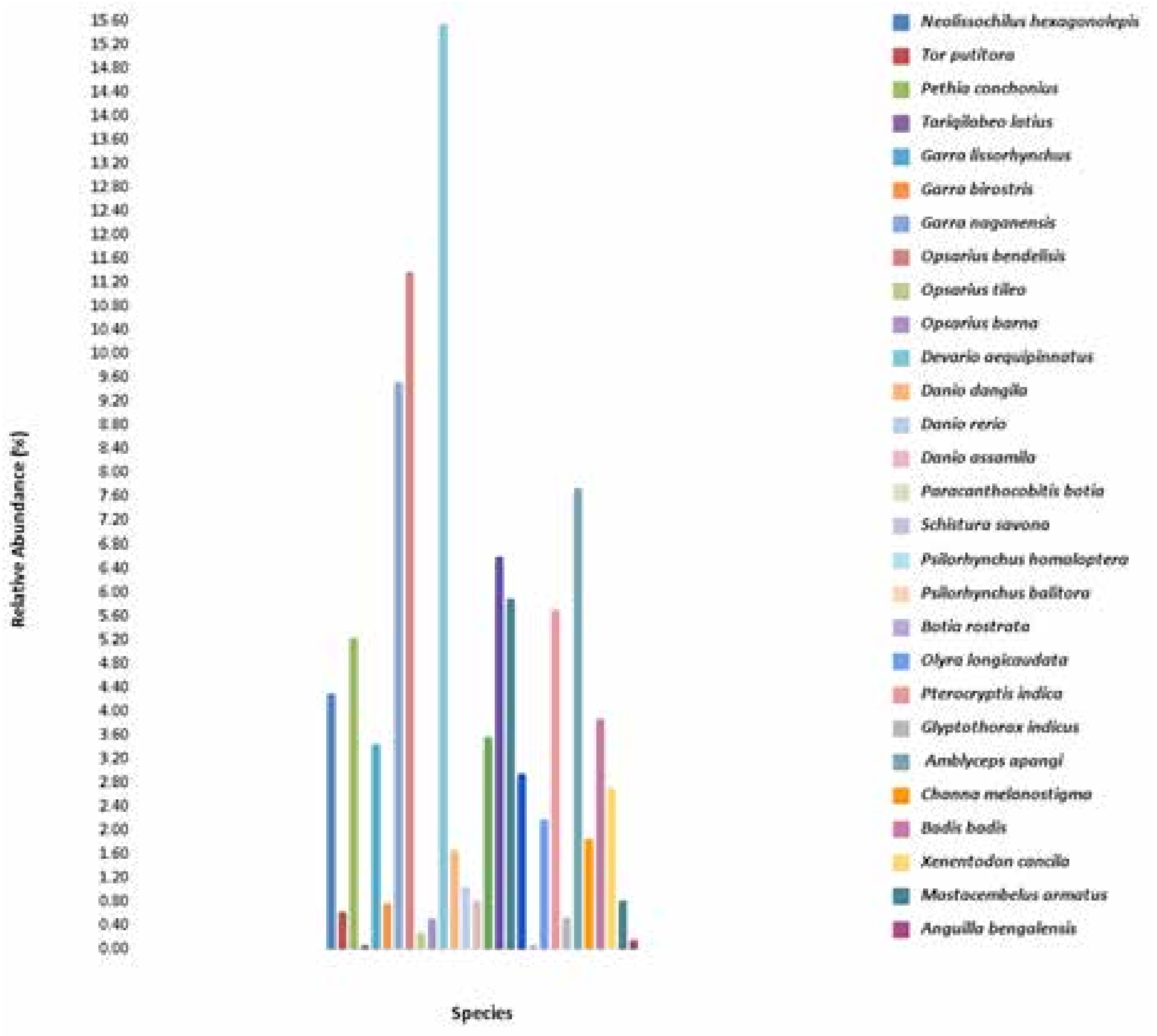


**Figure 5. Relative abundance of fish species.**

generally indicate high pollution and stress, values between 1 and 2 suggest moderate stability and values above 3 reflect a healthy, stable environment conducive to species survival (Stub et al. 1970). The findings from this study align with those of Dey & Sarma (2018), who reported the highest fish diversity during the post-monsoon season in the Manas River. Similarly, Satpathy et al. (2021) recorded a Shannon diversity value of 2.76 for the Subansiri River, suggesting moderate ecosystem health. Simpson's dominance index, which emphasizes the abundance of species over richness, also contributes to understanding the evenness of fish communities (Islam & Yasmin 2018). In this study, the highest evenness (J') was recorded during the pre-monsoon season, while the lowest value occurred during the monsoon. The average evenness across all seasons was 0.864 ± 0.044, indicating a fairly balanced distribution of species. The post-monsoon season consistently displayed the highest species richness and diversity, likely due to the influx of water from various sources, which brings additional species into the river system and enhances community diversity. Additionally, there is a rise in species richness due to the migration of fish from larger rivers for breeding and spawning. These seasonal dynamics underscore the critical role of water-level fluctuations in shaping fish community composition, with the post-monsoon period supporting the highest levels of biodiversity across the study area.

During field surveys, interviews with local villagers revealed a noticeable decline in fish diversity, primarily attributed to the use of destructive fishing practices. The increasing availability and affordability of such tools have

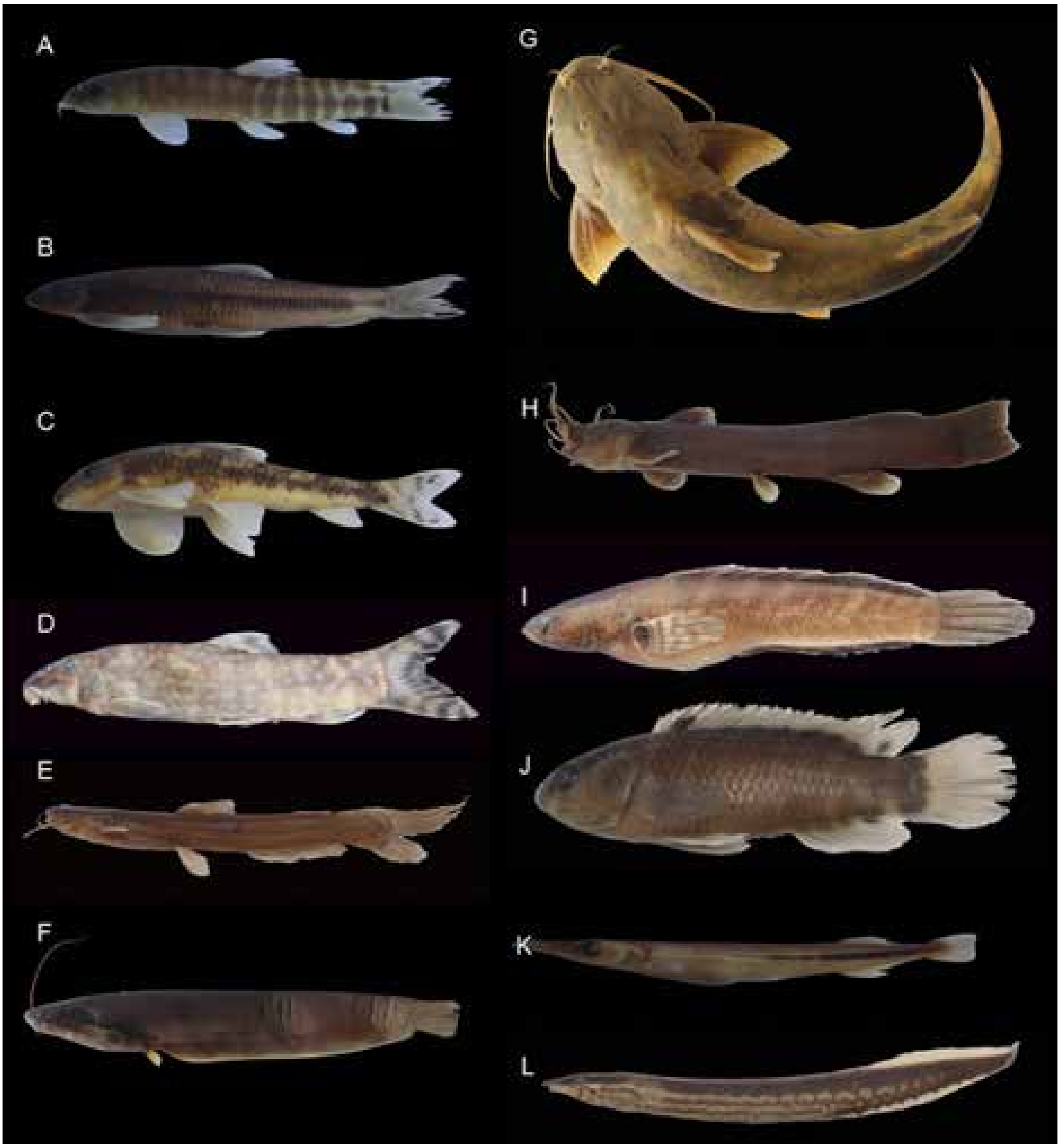


**Image 3. Fish species recorded in Dikhu River, Nagaland: A—*Schistura savona* | B—*Psilorhynchus homaloptera* | C—*Psilorhynchus balitora* | D—*Botia rostrate* | E—*Olyra longicaudata* | F—*Pterocryptis indica* | G—*Glyptothorax indicus* | H—*Amblyceps apangi* | I—*Channa melanostigma* | J—*Badis badis* | K—*Xenentodon cancila* | L—*Mastacembelus armatus*. © Metevinu Kechu.**

exacerbated the problem, leading not only to a reduction in fish populations but also negatively affecting the reproductive success of species, thereby causing long-term ecological harm. These observations are consistent with research suggesting that anthropogenic activities, such as overfishing and the use of harmful fishing techniques, are major contributors to fish population declines in river systems across Nagaland (Kechu et al. 2021).

The lack of comprehensive taxonomic surveys, genetic studies, and an understanding of the impacts of environmental changes poses significant challenges to the conservation of freshwater species in northeastern India. These gaps hinder accurate species identification

**Table 3. Diversity indices distribution in four seasons in Dikhu River.**

| Season | Total individuals | Total species found in each season | Shannon diversity index H' | Simpson's dominance index (D) | Evenness (J) |
|---|---|---|---|---|---|
| Pre-monsoon | 798 | 25 | 2.842 | 0.930 | 0.908 |
| Monsoon | 407 | 21 | 2.497 | 0.892 | 0.820 |
| Post-monsoon | 891 | 26 | 2.912 | 0.936 | 0.894 |
| Winter | 477 | 23 | 2.613 | 0.901 | 0.833 |
| Total | 2573 | | | | |
| Total no. of fishes | 28 | | | | |
| Mean ± SD | | 23.75 ± 2.217 | 2.716± 0.194 | 0.915± 0.022 | 0.864± 0.044 |

and impede effective conservation planning, particularly for rare or endemic species. Integrating traditional ecological knowledge from local communities can provide valuable insights into fish migration patterns, breeding cycles, and habitat usage, complementing modern scientific research and enhancing conservation efforts (Albuquerque et al. 2021). There is an urgent need for more holistic and integrated conservation strategies, including habitat restoration, the promotion of sustainable fishing practices, and community-based conservation programs. Regular ecological monitoring and biodiversity assessments are crucial for safeguarding the aquatic ecosystems of the Dikhu River and for meeting both local and global biodiversity conservation goals.

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OPEN ACCESS

COMMUNICATION

# A study on the diversity of butterflies in selected landscapes of the Indian Institute of Technology, Guwahati campus, Assam, India

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**Abstract:** A study has been carried out to find out the diversity of butterflies at the Indian Institute of Technology, Guwahati campus, Assam India from September 2019 to March 2022. In the present study, a total of 82 species with a total of 1,378 individuals of butterflies belonging to six families, namely, Papilionidae, Pieridae, Lycaenidae, Nymphalidae, Hesperiidae, and Riodinidae have been recorded. During the survey, the maximum number of butterflies were observed in the old E-type site and D-type site and its adjoining areas, where there are abundant flowering, host, and nectar-collecting plants and wildflowers, and a minimum number of butterflies were listed from old and new guest house site and transit campsites. Among four study years, 2020 had the highest genera and species number followed by the year 2021. From the present study it can be concluded that despite urbanization, there is a good diversity of butterflies. Therefore, the implementation of appropriate and effective conservation methods is of utmost importance in order to protect the diversity.

**Keywords:** Conservation ecology, diversity, ecological indicator, flowering plants, Kamrup District, Lepidoptera, northeastern India, seasonal variation, species richness, urbanization.

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**Author contributions:** UD—contributed to the study design, conceptualisation, supervision, draft writing, review editing, data collection, spotting of the species and photography. SD—contributed to data collection, survey work, photography, draft preparation, figure and table preparation and bibliographic study. DM—contributed to table and figure preparation and some part of survey work. The first draft of the manuscript was written by Uma Dutta and edited by Sonali Dey. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## INTRODUCTION

Butterflies act as an ecological indicator of environmental variation and are highly sensitive to disturbances and changes in habitat (Nally & Fleishman 2004). In the field of conservation ecology, butterflies are considered an umbrella species (Betrus et al. 2005). It is worth mentioning that butterfly diversity indirectly indicates plant diversity because both butterfly adults and caterpillars are highly reliable on specific host plants (Padhye et al. 2006).

In India, 1,379 butterfly species, from six different families, viz., Papilionidae, Pieridae, Lycaenidae, Riodinidae, Nymphalidae, and Hesperiidae, with 74 endemic species were observed. Among these, 1,143 species (82.9%) were identified as Oriental elements, 206 species (14.9%) as Palearctic elements, and 23 species (1.7%) as Afrotropical elements. Over two-thirds of the species were documented in the northeastern states of India (Das et al. 2023). Most of the species of order Lepidoptera indicates meta population which are exclusively phytophagous in nature (Menken et al. 2010). Northeastern India comprises eight states, viz., Arunachal Pradesh, Assam, Manipur, Mizoram, Nagaland, Sikkim, and Tripura are one the richest biodiversity areas which supports a rich butterfly fauna (Bora & Meitei 2014). A huge variety of flowering plants, suitable habitats, topography and climates are ideal for butterfly distribution, diversity and abundance. Eastern Himalayan part as well as northeastern region of India comprises 58% of butterflies found in the Indian subcontinent and Myanmar (Evans 1932). Evans (1932) reported that about 962 species and subspecies of butterflies belonging to five taxonomic families are found in northeastern India alone.

Limited research has been carried out on the butterflies of Assam. A total of 70 species of butterflies belonging to 45 genera were documented from the Regional Research Laboratory Campus, Jorhat, Assam (Bhuyan et al. 2005). In various parts of Guwahati city, a number of studies were conducted to find out the number of butterfly species. A total of 72 species have been reported from Assam State Zoo-Cum-Botanical Garden, Guwahati (Ali & Basistha 2000). Saikia et al. (2015) provided an excellent documentation of about 18 species of butterflies from Jalukbari and Gauhati university campus, Guwahati. A survey in Nambor–Doigrung Wildlife Sanctuary, Assam, identified 224 butterfly species across 137 genera and five families, with Nymphalidae being the most dominant. A study surveyed butterfly diversity in Dangori Reserve Forest, Upper Assam, documenting 121 species across six families, with Nymphalidae being the most dominant. Significant findings include the recording of rare species such as *Tanaecia julii* and *Lethe chandica*, along with endemic species like *Arhopala ganesa* and *Mycalesis mineus* (Boruah & Das 2017). A study was conducted in Panbari Forest, Kaziranga, upper Assam, that presented a checklist of 137 skipper butterfly species (Hesperiidae) including species such as Purple Lancer *Salanoemia fuscicornis*, Red-vein Lancer *Pyroneura niasana burmana*, Pied Flat *Celaenorrhinus moreana*, and various species of *Choaspes*, *Potanthus*, and *Halpe* (Gogoi 2013).

Under this contemplated background, the present study was carried out to identify and estimate the butterfly diversity in IIT, Guwahati campus.

## MATERIALS AND METHODS

### Study Area

The present study was conducted on the IIT Guwahati campus in Assam, renowned as one of the most beautiful campuses in India. Located at 26.187 N and 91.691 E, the campus lies on the northern banks of the Brahmaputra River, connected to northern Guwahati's Amingaon town, and is approximately 20 km from the city center. Spanning 700 ac (2.8 km$^2$), the campus features undulating terrain, hillocks, and a variety of landscapes, including evergreen, semi-evergreen, and deciduous vegetation, as well as shrubs, grasslands, and wetlands interspersed with lakes.

The campus's diverse vegetation, host plants, food plants, and nectar-rich flowers support a rich variety of reptiles, birds, mammals, insects, and, notably, many vibrant moth and butterfly species. Study sites included urban habitat areas, hilly terrains, lakes, wetlands, and specific locations such as the guest house, administrative block, and serpentile lake. The relatively undisturbed and tranquil environment of residential and non-residential areas further contributes to butterfly richness. Detailed descriptions of the selected study sites are provided in Table 1 and Image 1.

### Survey Method

The study was conducted across various sites at IIT from September 2019 to March 2022 using the Pollard Walk method. Surveys were performed twice yearly at each site, between 0900 h and 1700 h on sunny days. Observers walked fixed transects, recording butterflies within 3–5 m. Unidentified butterflies were caught, identified using field methods and references,

**Table 1. Types of habitats present in different study sites of IIT campus of Guwahati, Assam.**

| | Study site | Name of the study site | Habitats |
|---|---|---|---|
| 1 | Site 1 | D type and its adjoining area | Flowering plants, urban habitat, children park |
| 2 | Site 2 | New E type | Vegetation, different plants |
| 3 | Site 3 | Old E type | Urban habitat, lots of plantation, flowering plants, children park |
| 4 | Site 4 | F type and its adjoining area | Urban habitat, good management of naturally growing flowering plants |
| 5 | Site 5 | Old and new guest house site | Urban habitat, flowering plants, vegetation |
| 6 | Site 6 | Manas hostel site | Vegetation, small water body, flowering plants |
| 7 | Site 7 | Hill top | Dense vegetation, different flowering plants and fruit trees |
| 8 | Site 8 | Serpentile lake and its adjoining area | Grass beds, vegetation and plants, road side plantation |
| 9 | Site 9 | Transit camp site | Wild flowering plants, vegetation |
| 10 | Site 10 | Admin site and its adjoining area | Two lakes, vegetation |

and released (Yasmin et al. 2023). Identification was primarily done on-site, with photographs used for challenging cases. Data on date, location, and weather were recorded. The best time for observation and photography was early mornings, especially after rain showers, when flowering plants and nectar sources attracted the highest butterfly activity.

The identifications were done with the help of Evans (1932), and Kehimkar (2008). The WPA, 1972 status of butterflies was obtained from the database available at https://vindhyabachao.org/wildlife_guidelines/schedule_species_insects.pdf

Relative abundance is calculated by the formula:

$$\text{Species relative abundance} = \frac{\text{Species abundance}}{\text{Total abundance}} \times 100$$

## RESULTS

During the survey period from September 2019 to March 2022, a total of 82 species with a total of 1,378 individuals of butterfly belonging to six families and 57 different genera were recorded from different sites of IIT, Guwahati campus. Checklist of butterfly species and their abundance in different study sites are shown in Table 2. The study analyzed the composition of butterfly families over four years (2019–2022). Nymphalidae emerged as the most dominant family, with the highest species count and abundance each year, followed by Papilionidae, Lycaenidae, Pieridae, Hesperiidae, and Riodinidae. Each family displayed variations in the number of genera, species, and individuals annually, with the details summarized in Table 3 and Figure 1. Overall, Nymphalidae consistently led in diversity and population.

The majority of butterfly species were observed on the old E-type site and the D-type site with its adjoining areas, which are rich in flowering plants, host plants, and nectar-collecting wildflowers. In 2019, out of 77 species, 19 were recorded at the old E-type site, while 14 were found at the D-type site and nearby areas. In 2020, out of 83 species, both sites recorded 17 species each. Similarly, in 2021 and 2022, out of 79 and 71 species respectively, 20 and 15 species were found at the old E-type site, while 14 and 16 species were recorded at the D-type site and its surroundings (Table 4). Figure 2 illustrates butterfly abundance over four years, showing that 2020 had the highest number of genera and species, followed by 2021.

Table 5 presents the relative abundance of butterfly species, while Table 6 and Figure 3 highlight the relative abundance of different families. The study found that in 2019, *Junonia atlites* had the highest relative abundance (3%), whereas *Telicota linna* had the lowest (0.09%). In 2020, 2021, and 2022, *Papilio polytes* recorded the highest relative abundance at 2.25%, 2.72%, and 3.35%, respectively. Conversely, *Rapala tara*, *Sarangesa desahara*, and *Abisara neophron* had the lowest relative abundance (0.15%) in 2020, while *Appias galba* (0.18%) and *Orsotriaena medus* (0.12%) showed the lowest relative abundance in 2021 and 2022, respectively. Across all four years, the family Nymphalidae consistently exhibited the highest relative abundance, while Riodinidae had the lowest (Figure 2).

During this survey, 15 butterfly species with protected status under the Schedule II (Part H with serial numbers) of The Wild Life (Protection) Amendment Act, 2022, were recorded. These include *Papilio slateri, Graphium sarpedon sarpedon, Graphium eurypylus, Graphium aristeus anticrates, Cepora nadina nadina, Artipe eryx, Poritia hewitsoni, Spindasis lohita, Neptis magadha khasiana, Tanaecia lepidea, Charaxes bernardus, Melanitis zitenius, Ragadia crisilda, Parthenos sylvia gambrisius* and *Lethe insana* (Table 2).


**Image 1. Different study sites of IIT Campus, Guwahati, Assam, India.**

**Table 2. Checklist of butterfly species and their abundance in different study areas.**

| | Common name | Scientific Name | Year 2019 | 2020 | 2021 | 2022 | Site | Status | Conservation status, 2022 |
|---|---|---|---|---|---|---|---|---|---|
| **Family: Papilionidae** | | | | | | | | | |
| 1 | Common Mormon | *Papilio polytes* | 4+ | 4+ | 4+ | 3+ | Site 3, site 7, site 9 | Very common | |
| 2 | Great Mormon | *Papilio memnon agenor* | 3+ | 4+ | 4+ | 3+ | All sites | Common | |
| 3 | Common lime | *Papilio demoleus* | 3+ | 3+ | 2+ | 2+ | Site 1 site 2, site 5 | Very common | |
| 4 | Common mime | *Papilio clytia clytia* | 3+ | 4+ | 4+ | 3+ | Site 1, site 3, site 6, site 10 | Common | |
| 5 | Blue-striped Mime | *Papilio slateri* | – | 3+ | 3+ | 2+ | Site 2, site 7 | Not rare | Schedule II (Part H; No. 250) |
| 6 | Red Helen | *Papilio helenus* | 2+ | 3+ | 3+ | + | Site 1, site 8 | Not rare | |
| 7 | Yellow Helen | *Papilio nephelus* | 4+ | 4+ | 4+ | 3+ | Site 3, site 4, site 8, | Not rare | |
| 8 | Common Bluebottle | *Graphium sarpedon sarpedon* | + | + | 2+ | + | Site 6, site 10 | Common | Schedule II (Part H; No. 255) |
| 9 | Fivebar Swordtail | *Graphium antiphates pompilius* | + | 2+ | 3+ | 3+ | Site 4, Site5 | Not rare | |
| 10 | Fourbar Swordtail | *Graphium agetes aestes* | + | 2+ | – | – | Site 1, Site 7 | Rare | |
| 11 | Common Jay | *Graphium doson* | 2+ | 4+ | 3+ | 2+ | Site 1, site 2, site 3 | Not rare | |
| 12 | Great Jay | *Graphium eurypylus cheronus* | 2+ | 3+ | 2+ | – | Site 1, site 3 | Not rare | Schedule II (Part H; No. 264) |
| 13 | Great Zebra | *Graphium xenocles xenocles* | 2+ | 2+ | 1+ | 1+ | site 2, site 6 | Not rare | |
| 14 | Chain Swordtail | *Graphium aristeus anticrates* | 2+ | 3+ | 2+ | – | Site 5, site 6 | Not rare | Schedule II (Part H; No. 252) |
| 15 | Common Rose | *Atrophaneuraaristolochiae* | + | 2+ | + | + | Site 3, Site 7 | Rare | |
| 16 | Common Birdwing | *Troides Helena cerberus* | 2+ | 3+ | 3+ | 2+ | Site 1, site 3 | Not rare | |
| 17 | White Dragontail | *Lamproptera curius curius* | 3+ | 3+ | 2+ | 2+ | Site 5, site 6 | Not rare | |
| 18 | Great Windmill | *Byasa dasarada dasarada* | 2+ | 3+ | 2+ | 2+ | Site 2, site 3 | Not rare | |
| 19 | Common Banded Awl | *Hasora chromus* | 3+ | 4+ | 3+ | 2+ | Site 1, site 7 | Common | |
| 20 | Yellow Gorgon | *Meandrusa payeni evan* | 3+ | 3+ | 2+ | 2+ | Site 3, Site 5 | Not rare | |
| **Family: Pieridae** | | | | | | | | | |
| 1 | Common grass yellow | *Eurema hacabe* | 3+ | 4+ | 3+ | 2+ | Site 3, site 7, site 10 | Very common | |
| 2 | Great orangetip | *Hebomoia glaucippe* | 3+ | 3+ | 2+ | 2+ | Site 1, site 3 | Common | |
| 3 | Common Emigrant | *Catopsilia Pomona* | 3+ | 4+ | 3+ | 3+ | Site 7, site 9 | Very common | |
| 4 | Mottled emigrant | *Catopsilia pyranthe* | 2+ | 3+ | 2+ | 2+ | Site 3, site 7 | Very common | |
| 5 | Orange Albatross | *Appias galba* | + | + | + | – | Site 4 | Rare | |
| 6 | Striped Albatross | *Appias olferna* | 2+ | 2+ | + | 2+ | Site 4 | Common | |
| 7 | Spot Puffin | *Appias lalage* | 2+ | + | – | + | site 3, Site 5 | Not rare | |
| 8 | Lesser Gull | *Cepora nadina nadina* | 2+ | 3+ | 3+ | 2+ | Site 1, Site 3 | Not rare | Schedule II (Part H; No. 286) |
| 9 | Redspot Jezebel | *Delias descombesi* | 3+ | 4+ | 4+ | 3+ | Site 1, site 5, site 7 | Common | |
| **Family: Lycaenidae** | | | | | | | | | |
| 1 | Lesser grass blue | *Zizina otis* | 3+ | 4+ | 3+ | 3+ | Site 3, Site 5, site 10 | Not rare | |
| 2 | Forget me not | *Catochrysops strabo* | 3+ | 4+ | 3+ | 2+ | Site 2, site 6, site 8 | Common | |
| 3 | Zebra blue | *Leptotes plinius* | 3+ | 3+ | 2+ | 2+ | Site 1 site 3 | Common | |
| 4 | Yamfly | *Loxura atymnus* | 2+ | 2+ | 2+ | + | Site 1, site 3 | Not rare | |
| 5 | Assam Flash | *Rapala tara* | – | + | 2+ | 2+ | Site 5 | Rare | |

| | Common name | Scientific Name | Year 2019 | 2020 | 2021 | 2022 | Site | Status | Conservation status, 2022 |
|---|---|---|---|---|---|---|---|---|---|
| 6 | Green Flash | *Artipe eryx* | 2+ | 2+ | + | + | Site 7, site 8 | Not rare | Schedule II (Part H, No. 39) |
| 7 | Dingy Lineblue | *Petrelaea dana* | 3+ | 2+ | 2+ | + | Site 4, site 5 | Common | |
| 8 | Common Tit | *Hypolycaena erylus himavantus* | 2+ | + | + | – | Site 6 | Common | |
| 9 | Common Gem | *Poritia hewitsoni* | 2+ | 3+ | 2+ | – | Site1, site 9 | Not rare | Schedule II (Part H, No. 23) |
| 10 | Common Lineblue | *Prosotas nora nora* | 4+ | 4+ | 3+ | 2+ | site 3, Site 7 | common | |
| 11 | Long-banded Silverline | *Spindasis lohita* | 3+ | 3+ | 2+ | 3+ | Site 2, Site 3 | Not rare | Schedule II (Part H, No. 53) |
| **Family: Nymphalidae** | | | | | | | | | |
| 1 | Leopard lacewing | *Cethosia cyane* | 3+ | 2+ | 2+ | + | Site 6, site 7 | Not rare | |
| 2 | Peacock pansy | *Junonia almana* | + | 2+ | + | – | Site 1, site 3 | Not rare | |
| 3 | Lemon pansy | *Junonia lemonias* | 4+ | 3+ | 2+ | 2+ | Site 5, site 8, site 10 | Very common | |
| 4 | Grey pansy | *Junonia atlites* | 4+ | 3+ | 3+ | 3+ | Site 2, site 10 | Very common | |
| 5 | Yellow pansy | *Junonia hiertia* | 3+ | 4+ | 3+ | 2+ | Site 7, site 5 | Common | |
| 6 | Chocolate Soldier | *Junonia iphita* | 2+ | 3+ | 2+ | 2+ | Site 3, site 6 | Common | |
| 7 | Great eggfly | *Hypolimnasbolina* | 3+ | 3+ | 3+ | 2+ | Site 1, site 3, site 7 | Very common | |
| 8 | Plain Tiger | *Danaus chrysippus* | 3+ | 3+ | 2+ | 2+ | Site 5, site 9 | Common | |
| 9 | Common four ring | *Ypthima huebneri* | 3+ | 4+ | 4+ | 3+ | Site 3, site 6 | Common | |
| 10 | Common fivering | *Ypthima baldus* | 2+ | 2+ | – | + | Site 6 | Common | |
| 11 | Common palmfly | *Elymnias hypermnestra* | – | + | + | – | Site 5 | Rare | |
| 12 | Common Bushbrown | *Mycalesis persius* | 4+ | 4+ | 3+ | 3+ | Site 1, site 10 | Very common | |
| 13 | Blue tiger | *Tirumala limniace* | 3+ | 3+ | 2+ | 2+ | Site 3, site 5 | Common | |
| 14 | Dark Blue Tiger | *Tirumala septentrionis* | – | + | 2+ | + | Site 2, site 4 | Not rare | |
| 15 | Medus Brown | *Orsotriaena medus* | 2+ | + | + | + | Site 1, site 7 | Rare | |
| 16 | Common Indian crow | *Euploea core* | 4+ | 3+ | 2+ | 2+ | Site 5, Site 9 | Common | |
| 17 | Common sailor | *Neptis hylas astola* | 3+ | 2+ | 3+ | 2+ | Site 1, site 3, Site 10 | Common | |
| 18 | Spotted Sailor | *Neptis magadha khasiana* | + | 2+ | + | 2+ | Site 3, site 5 | Rare | Schedule II (Part H; No. 216) |
| 19 | Grey count | *Tanaecia lepidea* | 2+ | 4+ | 3+ | 3+ | Site 1, site 4 | Common | Schedule II (Part H; No. 165) |
| 20 | Plain Earl | *Tanaecia jahnu* | + | 2+ | + | + | Site 3, Site 6 | Rare | |
| 21 | Colour sergeant female | *Athyma nefte* | 3+ | 2+ | + | 2+ | Site 5, site 6 | Rare | |
| 22 | Tawny Rajah | *Charaxes bernardus* | 2+ | 4+ | 3+ | 3+ | Site 7, site 10 | Common | Schedule II (Part H; No. 223) |
| 23 | Tawny coster | *Acraea terpsicore* | 2+ | 4+ | 3+ | 2+ | Site 3, site 5 | Common | |
| 24 | Common Evening Brown | *Melanitis leda* | 3+ | 2+ | 2+ | + | Site 6, site 10 | Common | |
| 25 | Great Evening Brown | *Melanitis zitenius* | + | 2+ | 2+ | 2+ | Site 1, site 3 | Rare | Schedule II (Part H; No. 160) |
| 26 | Plain Tiger | *Danaus chrysippus* | 3+ | 3+ | 2+ | + | Site 3, Site 8 | Very common | |
| 27 | Glassy Tiger | *Parantica aglea* | + | 2+ | 4+ | 2+ | Site 3, site 7 | Common | |
| 28 | Vagrant | *Vagrans egista* | 2+ | + | – | 2+ | Site 6 | Not rare | |
| 29 | Common Sergeant | *Athyma perius* | 2+ | 3+ | 2+ | 2+ | Site 8 | Common | |
| 30 | Striped Ringlet | *Ragadia crisilda* | – | 2+ | 2+ | 2+ | Site 2, site 6 | Not rare | Schedule II (Part H; No. 218) |
| 31 | Clipper | *Parthenos sylvia gambrisius* | 2+ | 2+ | + | – | Site 2, Site 5 | Rare | Schedule II (Part H; No. 135) |
| 32 | Common Forester | *Lethe insana* | 3+ | + | + | - | Site 1, site 5, site 7 | Not rare | Schedule II (Part H; No. 141) |

| | Common name | Scientific Name | Year | | | | Site | Status | Conservation status, 2022 |
|---|---|---|---|---|---|---|---|---|---|
| | | | 2019 | 2020 | 2021 | 2022 | | | |
| 33 | Great Nawab | *Polyura eudamippus eudamippus* | + | 2 + | + | 2+ | site 1, Site 3 | Rare | |
| 34 | Rustic | *Cupha erymanthis lotis* | 2+ | 3+ | 3+ | 2+ | Site 6 | Not rare | |
| **Family: Hesperiidae** | | | | | | | | | |
| 1 | Common small flat | *Sarangesa desahara* | – | + | 2+ | + | Site 2, site 5 | rare | |
| 2 | Common snow flat | *Tagiades japetus atticus* | 4+ | 4+ | 3+ | 2+ | Site 3, site 10 | Common | |
| 3 | Paint Brush Swift | *Baoris farri* | 3+ | 3+ | 2+ | 2+ | Site 1, Site 7 | common | |
| 4 | Great Swift | *Pelopidas assamensis* | + | 2+ | + | – | Site 3, site 4 | Not rare | |
| 5 | Linna Palm Dart | *Telicota linna* | + | 2+ | 2+ | – | Site 3, site 7 | Common | |
| **Family: Riodinidae** | | | | | | | | | |
| 1 | Punchinello | *Zemeros flegyas indicus* | 3+ | 4+ | 3+ | 2+ | Site 3, site 10 | Common | |
| 2 | Double Banded Judy | *Abisara bifasciata* | 3+ | 2+ | 2+ | 2+ | Site 1, site 3 | Not rare | |
| 3 | Tailed Judy | *Abisara neophron* | 2+ | + | + | - | Site 2, site 10 | Not rare | |

N.B. On the basis of abundance, Butterfly species were included under classes: 4+ (highly abundant, more than 25 sightings), 3+(moderately abundant, 16–25 sighting), 2+(abundant, 6–15 sighting); +(present, 1–5 sighting), -(absent).

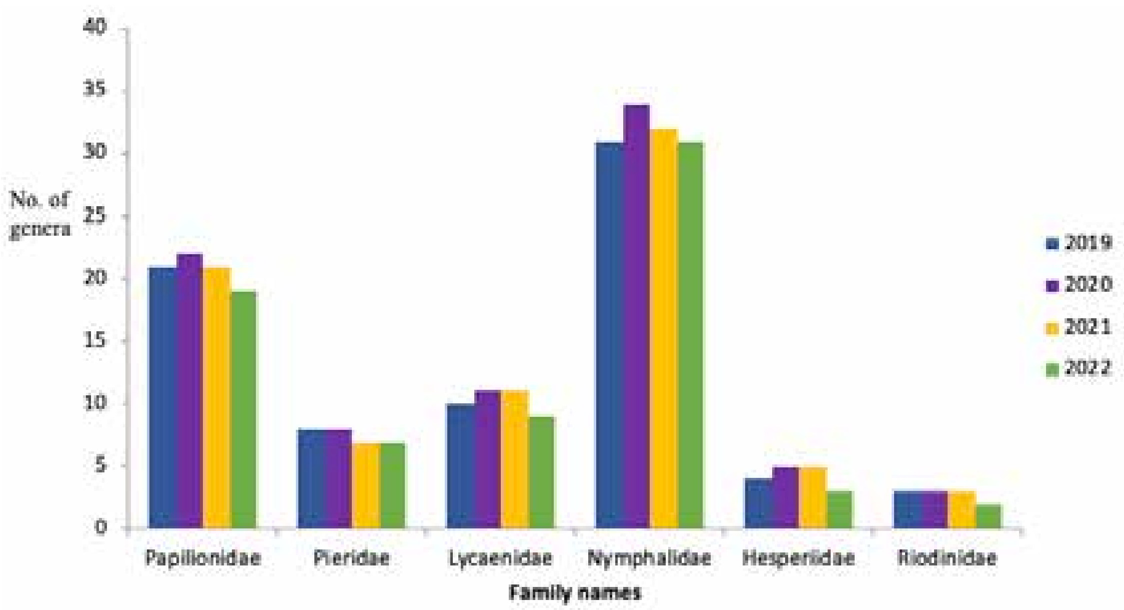



**Figure 1. Bar diagram showing the number of genera of different butterfly families in four successive years.**

## DISCUSSION

During the survey, a total of 82 species with about 1,378 individuals of butterfly belonging to six families (Papilionidae, Pieridae, Lycaenidae, Nymphalidae, Hesperiidae and Riodinidae) and 57 different genera were recorded in the study area.

Similar studies were reported by Gogoi et al. (2023) in Soraipung Range of Dehing Patkai National Park where they recorded a total of 92 butterfly species from five families, among which 13 species were classified as protected under different schedules of the Indian Wildlife (Protection) Act, 1972 but according to Wild Life (Protection) Amendment Act, 2022, nine species among the list of 13 species are now classified as protected under Schedule I and II.

In the present investigation, a maximum number of butterflies were recorded in the year 2020 (57 genera

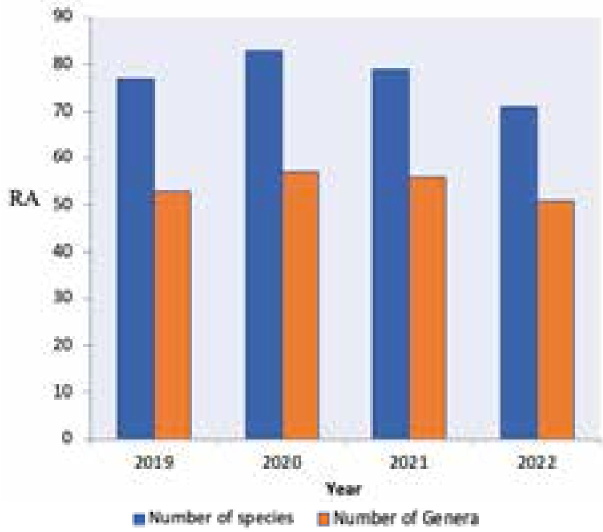



**Figure 2. Bar diagram showing abundance of butterflies in four successive years across the study area.**

and 63 species) and 2021 (56 genera and 79 species). This might have happened because of less human interference, disturbances and environmental pollution occurred due to COVID-19 pandemic during the year 2020 and 2021. Comparatively, a lower number of butterflies were documented in the year 2022 (51 genera and 71 species) and 2019 (53 genera and 77 species). Lower number of butterflies in 2019 may be due to restoration of day-to-day human activities in these areas.

In the present study, Nymphalidae family had the highest number and percentage of species of butterflies in all four years of study period compared with the other families. The result of the present survey is in close consortium with the findings of Ali & Basistha (2000). They documented 72 identified species of butterflies belonging to five families with the highest number of species of the Nymphalidae family from Assam State Zoo-cum-Botanical Garden, Guwahati, Assam. Furthermore, the survey of Bohra & Purkayastha (2021) of the urban landscape, of Guwahati, Assam, India, listed 249 species of butterflies belonging to six families. The Nymphalidae family was represented as dominant during the survey period. Adaptation and proper landscape management could be the reason for the high diversity of the family Nymphalidae. Another reason for the rich diversity of the family Nymphalidae might be due to their strong active flying capability and their polyphagous nature

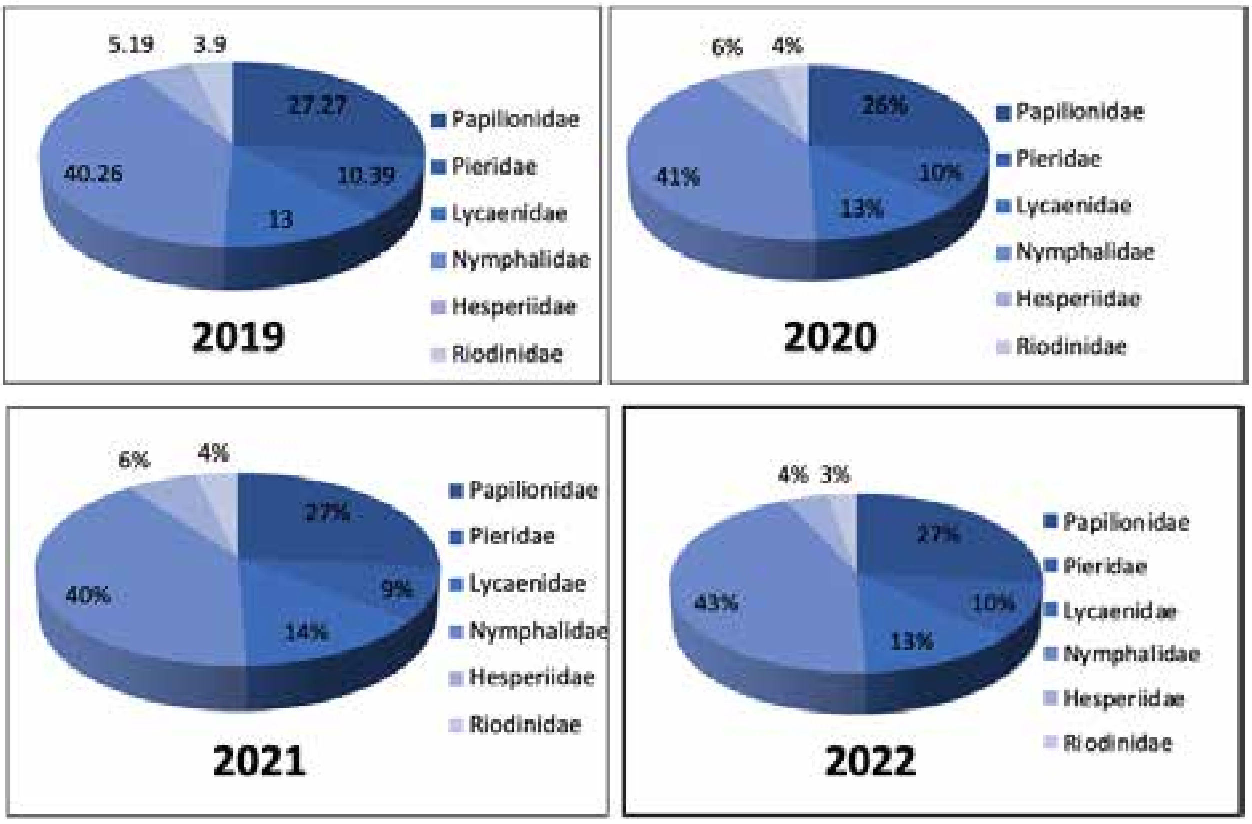



**Figure 3. Pie chart showing the relative abundance of six butterfly families in different years.**

**Table 3. Number of genera and species of five families of butterfly.**

| Family | Year | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2019 | | | | 2020 | | | | 2021 | | | | 2022 | | | |
| | No. of Genera | % | No. of Species | % | No. of Genera | % | No. of Species | % | No. of Genera | % | No. of Species | % | No. of genera | % | No. of Species | % |
| Papilionidae | 9 | 16.98 | 21 | 27.27 | 9 | 15.79 | 22 | 26.51 | 9 | 16.07 | 21 | 26.58 | 9 | 17.65 | 19 | 26.76 |
| Pieridae | 6 | 11.32 | 8 | 10.39 | 6 | 10.53 | 8 | 9.64 | 6 | 10.71 | 7 | 8.86 | 6 | 11.76 | 7 | 9.86 |
| Lycaenidae | 10 | 18.87 | 10 | 12.99 | 11 | 19.30 | 11 | 13.25 | 11 | 19.64 | 11 | 13.92 | 9 | 17.64 | 9 | 12.68 |
| Nymphalidae | 22 | 41.51 | 31 | 40.26 | 24 | 42.11 | 34 | 40.96 | 23 | 41.07 | 32 | 40.51 | 22 | 43.14 | 31 | 43.66 |
| Hesperiidae | 4 | 7.55 | 4 | 5.19 | 5 | 8.77 | 5 | 6.02 | 5 | 8.93 | 5 | 6.33 | 3 | 5.88 | 3 | 4.23 |
| Riodinidae | 2 | 3.77 | 3 | 3.90 | 2 | 3.51 | 3 | 3.61 | 2 | 3.57 | 3 | 3.80 | 2 | 3.92 | 2 | 2.82 |
| | Total = 53 | | Total = 77 | | Total = 57 | | Total = 81 | | Total = 56 | | Total = 79 | | Total = 51 | | Total = 71 | |

**Table 4. Number of butterfly species in different sites of study area.**

| Sites | Name of the site | 2019 | 2020 | 2021 | 2022 |
|---|---|---|---|---|---|
| Site 1 | D-type and its adjoining area | 14 | 17 | 14 | 16 |
| Site 2 | New E-type | 7 | 5 | 2 | 1 |
| Site 3 | Old E-type | 19 | 17 | 20 | 15 |
| Site 4 | F type and its adjoining area | 8 | 12 | 15 | 12 |
| Site 5 | Old and new guest house | 1 | 2 | 3 | 1 |
| Site 6 | Manas hostel site | 9 | 11 | 8 | 5 |
| Site 7 | Hill top | 11 | 13 | 9 | 7 |
| Site 8 | Serpentile lake and its adjoining area | 4 | 2 | 3 | 5 |
| Site 9 | Transit camp site | 2 | 1 | 1 | 3 |
| Site 10 | Admin site and its adjoining area | 2 | 3 | 4 | 6 |

which facilitates them to cover large areas and utilize a variety of host plants (Eswaran & Pramod 2005; Janz 2005; Padhye et al. 2006).

Faunal diversity is dependent upon the habitat types, food resources and food quality. The diversity and distribution of butterfly species are also influenced by sufficient larval and adult plant resources (Ramesh et al. 2010). In the present study, the highest relative abundances of different species of butterfly family were found in old E-type and hill top sites. Even though the old E-type site is an urbanized area and has human interference, the residents of this area have transformed the environment of the place in such a way that it has become an attractive and favourable place for butterflies. In agreement with the result of the present study, different earlier studies have shown that butterfly diversity in disturbed habitats is more than in undisturbed areas (Spitzer et al.1993; Hamer et al. 1997). Hill top is the least disturbed area and the occurrence of sufficient host plants make it more favourable for butterflies. *Junonia atlites* was found to have the highest relative abundance in 2019 and in the years 2020, 2021 and 2022. *Papilio polytes* showed highest relative abundance. Both *Junonia atlites* and *Papilio polytes* are common and most frequently observed butterflies and this may happen due to their adaptation power and the presence of a large number of host plants.

Adult butterflies generally prefer forest areas with medium altitude and larvae prefer ecotones with an abundance of food plants with large leaves (Piccini et al. 2022). Therefore, to conserve this beautiful creation or Nature’s jewels, the suitable environment for the butterflies should be maintained as well as enhanced. Therefore, to restore growth of butterfly population, enough plantation should be carried out in and around the IIT Guwahati campus area. Prevention of human interventions and disturbances and also deforestation for the purpose of clearing land for buildings in the hilly arears and lake sides, by the management, will be a huge step towards the conservation of these amazing insects.

The findings of this study suggest that despite ongoing urbanization within the IIT Guwahati campus in Assam, the area still supports a thriving diversity of butterflies. During the study period, a total of 82 butterfly species, comprising 1,378 individuals from six families and 57 genera, were documented. Among these families, Nymphalidae exhibited the highest species count and percentage, followed by Papilionidae, Lycaenidae, Pieridae, Hesperiidae, and Riodinidae, in descending order of abundance (Nymphalidae > Papilionidae > Lycaenidae > Pieridae > Hesperiidae > Riodinidae).

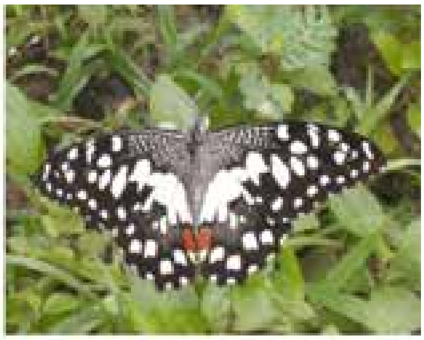
**Table 5. Numerical abundance and relative abundance (RA) of individual butterflies across the study area.**

| | Common name | Family | 2019 | | 2020 | | 2021 | | 2022 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | RA (%) | No. | RA (%) | No. | RA (%) | No. | RA (%) |
| 1 | Common Mormon | Papilionidae | 27 | 2.45 | 31 | 2.25 | 30 | 2.72 | 28 | 3.35 |
| 2 | Great Mormon | | 24 | 2.18 | 28 | 2.03 | 26 | 2.36 | 23 | 2.75 |
| 3 | Common lime | | 19 | 1.73 | 21 | 1.52 | 15 | 1.36 | 13 | 1.55 |
| 4 | Common mime | | 22 | 2 | 29 | 2.1 | 27 | 2.45 | 24 | 2.89 |
| 5 | Blue-striped Mime | | - | - | 18 | 1.31 | 16 | 1.45 | 13 | 1.55 |
| 6 | Red Helen | | 7 | 0.64 | 16 | 1.16 | 17 | 1.54 | 5 | 0.6 |
| 7 | Yellow Helen | | 27 | 2.45 | 29 | 2.1 | 28 | 2.54 | 23 | 2.75 |
| 8 | Common Bluebottle | | 2 | 0.18 | 5 | 0.36 | 7 | 0.64 | 2 | 0.24 |
| 9 | Fivebar Swordtail | | 5 | 0.45 | 9 | 0.65 | 16 | 1.45 | 19 | 2.27 |
| 10 | Fourbar Swordtail | | 3 | 0.27 | 7 | 0.51 | - | - | - | - |
| 11 | Common Jay | | 14 | 1.27 | 26 | 1.89 | 23 | 2.09 | 16 | 1.91 |
| 12 | Great Jay | | 9 | 0.82 | 18 | 1.31 | 11 | 1 | - | - |
| 13 | Great Zebra | | 11 | 1 | 15 | 1.09 | 12 | 1.09 | 9 | 1.08 |
| 14 | Chain Swordtail | | 7 | 0.64 | 15 | 1.09 | 6 | 0.54 | - | - |
| 15 | Common Rose | | 3 | 0.27 | 9 | 0.65 | 5 | 0.45 | 3 | 0.36 |
| 16 | Common Birdwing | | 12 | 1.09 | 17 | 1.23 | 19 | 1.72 | 15 | 1.8 |
| 17 | White Dragontail | | 17 | 1.55 | 20 | 1.45 | 14 | 1.27 | 12 | 1.43 |
| 18 | Great Windmill | | 8 | 0.73 | 18 | 1.31 | 10 | 0.91 | 11 | 1.31 |
| 19 | Common Banded Awl | | 21 | 1.91 | 27 | 1.96 | 24 | 2.18 | 13 | 1.55 |
| 20 | Yellow Gorgon | | 21 | 1.91 | 19 | 1.38 | 13 | 1.18 | 10 | 1.19 |
| 21 | Common grass yellow | Pieridae | 21 | 1.91 | 29 | 2.1 | 26 | 2.36 | 15 | 1.8 |
| 22 | Great orangetip | | 19 | 1.73 | 21 | 1.52 | 15 | 1.36 | 11 | 1.31 |
| 23 | Common Emigrant | | 25 | 2.27 | 27 | 1.96 | 23 | 2.09 | 22 | 2.63 |
| 24 | Mottled emigrant | | 13 | 1.18 | 18 | 1.31 | 15 | 1.36 | 14 | 1.67 |
| 25 | Orange Albatross | | 8 | 0.73 | 5 | 0.36 | 2 | 0.18 | - | - |
| 26 | Striped Albatross | | 12 | 1.09 | 13 | 0.94 | 11 | 1 | 14 | 1.67 |
| 27 | Spot Puffin | | 6 | 0.55 | 4 | 0.29 | - | - | 2 | 0.24 |
| 28 | Lesser Gull | | 11 | 1 | 16 | 1.16 | 17 | 1.54 | 12 | 1.43 |
| 29 | Redspot Jezebel | | 19 | 1.72 | 26 | 1.89 | 29 | 2.63 | 21 | 2.51 |
| 30 | Lesser grass blue | Lycaenidae | 24 | 2.18 | 28 | 2.03 | 24 | 2.18 | 20 | 2.39 |
| 31 | Forget me not | | 25 | 2.27 | 31 | 2.25 | 21 | 1.91 | 14 | 1.67 |
| 32 | Zebra blue | | 17 | 1.55 | 22 | 1.6 | 11 | 1 | 9 | 1.08 |
| 33 | Yamfly | | 5 | 0.45 | 7 | 0.51 | 4 | 0.36 | 3 | 0.36 |
| 34 | Assam Flash | | - | - | 2 | 0.15 | 7 | 0.64 | 5 | 0.6 |
| 35 | Green Flash | | 8 | 0.73 | 5 | 0.36 | 3 | 0.27 | 2 | 0.24 |
| 36 | Dingy Lineblue | | 17 | 1.55 | 13 | 0.94 | 12 | 1.09 | 5 | 0.6 |
| 37 | Common Tit | | 7 | 0.64 | 4 | 0.29 | 4 | 0.36 | 3 | 0.36 |
| 38 | Common Gem | | 8 | 0.73 | 16 | 1.16 | 6 | 0.54 | - | - |
| 39 | Common Lineblue | | 27 | 2.45 | 30 | 2.18 | 25 | 2.27 | 14 | 1.67 |
| 40 | Long-banded Silverline | | 17 | 1.55 | 23 | 1.67 | 13 | 1.18 | 27 | 3.23 |

| | Common name | Family | 2019 | | 2020 | | 2021 | | 2022 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | RA (%) | No. | RA (%) | No. | RA (%) | No. | RA (%) |
| 41 | Leopard lacewing | Nymphalidae | 21 | 1.91 | 15 | 1.09 | 10 | 0.91 | 5 | 0.6 |
| 42 | Peacock pancy | | 3 | 0.27 | 8 | 0.58 | 4 | 0.36 | - | - |
| 43 | Lemon pancy | | 29 | 2.64 | 22 | 1.6 | 14 | 1.27 | 9 | 1.08 |
| 44 | Grey pancy | | 33 | 3 | 25 | 1.81 | 25 | 2.27 | 24 | 2.87 |
| 45 | Yellow pansy | | 24 | 2.18 | 30 | 2.18 | 21 | 1.91 | 15 | 1.79 |
| 46 | Chocolate Soldier | | 8 | 0.73 | 19 | 1.38 | 16 | 1.45 | 14 | 1.67 |
| 47 | Great eggfly | | 16 | 1.45 | 22 | 1.6 | 19 | 1.72 | 14 | 1.67 |
| 48 | Danaid eggfly | | 16 | 1.45 | 18 | 1.31 | 11 | 1 | 8 | 0.96 |
| 49 | Common fouring | | 23 | 2.09 | 28 | 2.03 | 28 | 2.54 | 25 | 2.99 |
| 50 | Common fivering | | 7 | 0.64 | 6 | 0.44 | - | - | 2 | 0.24 |
| 51 | Blue striped palmfly | | - | - | 5 | 0.36 | 3 | 0.27 | - | - |
| 52 | Common Bushbrown | | 27 | 2.45 | 31 | 2.25 | 29 | 2.63 | 23 | 2.75 |
| 53 | Blue tiger | | 20 | 1.82 | 24 | 1.74 | 13 | 1.18 | 14 | 1.67 |
| 54 | Dark Blue Tiger | | - | - | 3 | 0.22 | 8 | 0.73 | 5 | 0.6 |
| 55 | Pointed Palmfly | | 9 | 0.82 | 7 | 0.51 | 3 | 0.27 | 1 | 0.12 |
| 56 | Common Indian crow | | 27 | 2.45 | 21 | 1.52 | 13 | 1.18 | 14 | 1.67 |
| 57 | Common sailor | | 20 | 1.82 | 14 | 1.02 | 19 | 1.72 | 12 | 1.43 |
| 58 | Spotted Sailor | | 5 | 0.45 | 11 | 0.79 | 4 | 0.36 | 9 | 1.08 |
| 59 | Grey count | | 13 | 1.18 | 27 | 1.96 | 22 | 2 | 19 | 2.27 |
| 60 | Plain Earl | | 2 | 0.18 | 6 | 0.44 | 3 | 0.27 | 3 | 0.36 |
| 61 | Perak Lascar | | 23 | 2.09 | 12 | 0.87 | 5 | 0.45 | 11 | 1.31 |
| 62 | Tawny Rajah | | 13 | 1.18 | 28 | 2.03 | 20 | 1.81 | 14 | 1.67 |
| 63 | Tawny coster | | 15 | 1.36 | 29 | 2.10 | 25 | 2.27 | 15 | 1.79 |
| 64 | Common EveningBrown | | 21 | 1.91 | 9 | 0.65 | 10 | 0.91 | 4 | 0.48 |
| 65 | Great EveningBrown | | 2 | 0.18 | 11 | 0.79 | 8 | 0.73 | 6 | 0.72 |
| 66 | Plain Tiger | | 18 | 1.64 | 17 | 1.23 | 9 | 0.82 | 5 | 0.6 |
| 67 | Glassy Tiger | | 5 | 0.45 | 14 | 1.02 | 27 | 2.45 | 13 | 1.55 |
| 68 | Vagrant | | 7 | 0.64 | 3 | 0.22 | - | - | 9 | 1.08 |
| 69 | Common Sergeant | | 13 | 1.18 | 24 | 1.74 | 14 | 1.27 | 12 | 1.43 |
| 70 | Striped Ringlet | | - | - | 6 | 0.44 | 10 | 0.91 | 8 | 0.96 |
| 71 | Clipper | | 11 | 1 | 8 | 0.58 | 4 | 0.36 | - | - |
| 72 | Common Forester | | 14 | 1.27 | 23 | 1.67 | 9 | 0.82 | 4 | 0.48 |
| 73 | Great Nawab | | 2 | 0.18 | 8 | 0.58 | 5 | 0.45 | 9 | 1.08 |
| 74 | Rustic | | 12 | 1.09 | 21 | 1.52 | 19 | 1.72 | 13 | 1.55 |
| 75 | Common small flat | Hesperiidae | - | - | 2 | 0.15 | 7 | 0.64 | 4 | 0.48 |
| 76 | Common snow flat | | 26 | 2.36 | 27 | 1.96 | 23 | 2.09 | 15 | 1.79 |
| 77 | Paint Brush Swift | | 20 | 1.82 | 18 | 1.31 | 8 | 0.73 | 7 | 0.84 |
| 78 | Great Swift | | 3 | 0.27 | 9 | 0.65 | 4 | 0.36 | - | - |
| 79 | Linna Palm Dart | | 1 | 0.09 | 6 | 0.44 | 3 | 0.27 | - | - |
| 80 | Punchinello | Riodinidae | 21 | 1.91 | 30 | 2.18 | 22 | 2 | 19 | 2.27 |
| 81 | Plum Judy | | 17 | 1.55 | 12 | 0.87 | 13 | 1.18 | 9 | 1.08 |
| 82 | Tailed Judy | | 8 | 0.73 | 2 | 0.15 | 3 | 0.27 | - | - |


*Papilio polytes* Common Mormon


*Papilio clytia clytia* Common Mime


*Papilio demoleus* Common Lime


*Eurema hacabe* Common Grass Yellow


*Hebomia glaucippe* Great Orange Tip


*Tagiades japetus atticus* Common Snow Flat


*Catopsilia Pomona* Common Emigrant


*Catopsilia pyranthe* Mottled Emigrant


*Pieris canidia* Indian Cabbage White


*Appias olferna* Striped Albatross


*Zemeros flegyas indicus* Punchinello


*Zizina otis* Lesser Glass Blue

**Image 2. Photographs of some of the species of butterfly observed in the IIT Guwahati campus.**

*Catochrysops strabo* Forget-me-not

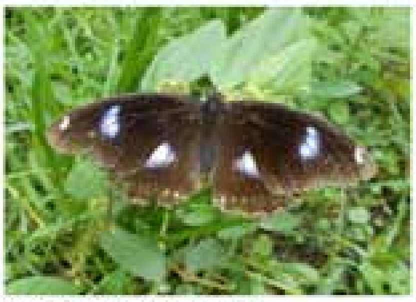
*Leptotes plinius* Zebra Blue

*Cethosia cyane* Leopard Lacewing

*Junonia almana* Peacock Pancy

*Junonia lemonias* Lemon Pancy

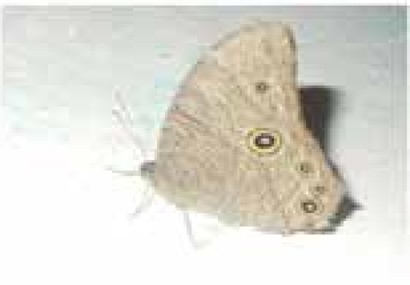
*Junonia atlites* Grey Pancy



*Hypolimnas bolina* Great Eggfly

*Danaus chrysippus* Plain Tiger

*Ypthima huebneri* Common four ring



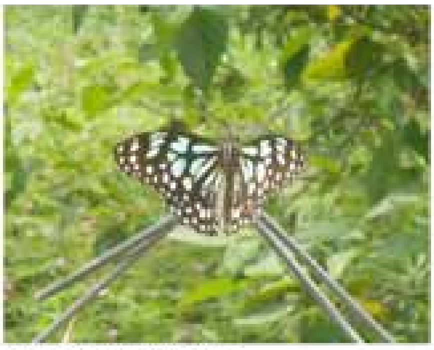
*Ypthima baldus* Common Five-ring

*Elymnias hypermnestra* Common Palmfly

*Mycalesis perseus* Common Bushbrown

***Appias galba*** **Orange Albatross**

**Image 2 cont.. Photographs of some of the species of butterfly observed in the IIT Guwahati campus.**



*Tirumala limniace* Blue Tiger

*Euploia core* Common Crow

*Neptis hylas* Common Sailor

*Sarangesadesahara* Common Small Flat

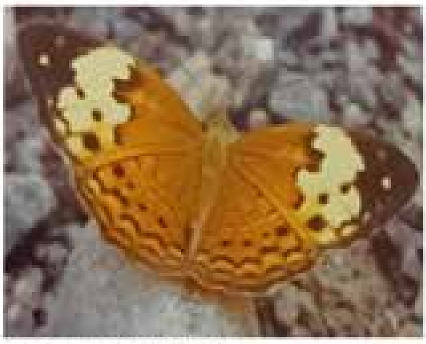
*Tanaecia lepidea* Grey Count

*Athyma nefte* Colour Sergeant female

*Acraea terpsicore* Tawny Castor

*Ariadne ariadne* Angled Castor

*Neptis ananta* Yellow Sailor



*Cupha erymanthislotis* Rustic

*Polyura eudamippus* The Great Nawab

*Charaxes bernardus* Tawny Rajah

**Image 2 cont.. Photographs of some of the species of butterfly observed in the IIT Guwahati campus.**
**© All the photographs are credited by Dr. Uma Dutta except *Neptis hylas* Common Sailor, *Tirumala limniace* Blue Tiger and *Junonia almana* Peacock Pancy, which are taken by Sonali Dey.**

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COMMUNICATION

# *Sphaeroma taborans* sp. nov., a new species of wood-boring isopod (Crustacea: Isopoda: Sphaeromatidae) from Munroe Island, Ashtamudi Estuary, Kerala, India

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**Abstract:** *Sphaeroma taborans* sp. nov. is described from Munroe Island, part of Ashtamudi Estuary, Kerala, India. This wood-boring species can be distinguished from all other congeners by its dorsal surface with three longitudinal bands between eyes, a prominent blackish tubercle on the anterior band, and scattered weak tubercles. Epistome subtriangular and nontruncated. Pleon and pleotelson bearing appressed hair layers. Pleotelsonic dorsal surface with six stout tubercles in a transverse row near the anterior followed by three pairs of median prominent blunt tubercles. Apex curved triangle, slightly curved upwards. Uropodal exopod with 4–5 external teeth and an acute apex thorn-like setae along lateral margins with acute apex extending beyond pleotelsonic apex.

**Keywords:** Ashtamudi Lake, epistome, holotype, mangrove ecosystems, paratype, pereopod, pleopod, pleotelson, Ramsar wetland, Sphaeromatidae, uropodal exopod, taxonomic description, tubercle.

**Malayalam:** ഫെറോമാ താബോറൻസ് എന്ന പുതിയ ഇനം തടിതുരപ്പൻ ഐസോപോഡ് സ്പീഷീസിനെ ഇന്ത്യയുടെ തെക്കുപടിഞ്ഞാറൻ സംസ്ഥാനമായ കേരളത്തിലെ അഷ്ടമുടി കായലിന്റെ ഭാഗമായ മൺറോ ഐലൻഡിൽ നിന്നും കണ്ടെത്തി. തലയുടെ ഭാഗത്തായി രണ്ടു കണ്ണുകൾക്കുമിടയിലായി കാണപ്പെടുന്ന മൂന്ന് ബാൻഡുകളും അതിലെ മുന്നിലുള്ള ബാൻഡിലായി കാണുന്ന കറുത്ത വൃത്താകൃതിയിലുള്ളതുമായ ഒരു ട്യൂബർക്കിളിനോടൊപ്പം ചിതറി കിടക്കുന്നഏതാനും ചെറിയ ട്യൂബർകിളുകളുമാണ് ഈ പുതിയ സ്പീഷിസിനെ മറ്റു സ്പീഷീസുകളിൽ നിന്നും വ്യത്യസ്തമാക്കുന്നത്. ഉപത്രികോണാകൃതിയിലുള്ളതും മുകളിലേക്ക് ചെറുതായി വളഞ്ഞിരിക്കുന്നതുമായ എപ്പിസ്റ്റോം, പ്ലിയോണിലും പ്ലിയോടെൽസണിലും അമർന്നുകിടക്കുന്ന രോമപാളികൾ, തിരശ്ചീനമായ ആറ് ട്യൂബർക്കിളുകളുള്ള പ്ലിയോടെൽസോണിക് ഡോർസൽ ഉപരിതലം, തുടർന്ന് മൂന്ന് ജോഡി മൂർച്ചയുള്ള ട്യൂബർക്കിളുകൾ, ത്രികോണാകൃതിയിലുള്ളതും അല്പം മുകളിലായി വളഞ്ഞിരിക്കുന്നതുമായ ശരീരത്തിന്റെ താഴത്തെ അഗ്രം, കൂർത്ത അഗ്രമുള്ളതും ഇരു വശങ്ങളിലും മുള്ളു പോലെയുള്ള കുറ്റിരോമങ്ങളുടെ ഘടനയോടുകൂടിയതും പ്ലിയോടെൽസന്റെ അഗ്രത്തിന് അപ്പുറത്തേക്ക് നീണ്ടുകിടക്കുന്നതും നാലു മുതൽ അഞ്ചു വരെയുള്ള ബാഹ്യ പല്ലുകളുമുള്ള യൂറോപ്പോഡൽ എക്സോപോഡ് എന്നിവയൊക്കെയാണ് ഫെറോമാ താബോറൻസിന്റെ സവിശേഷതകൾ.

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**Author contributions:** AMS and DB carried out fieldwork, identification, and manuscript preparation, BA reviewed the manuscript.

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## INTRODUCTION

The *Sphaeroma* Bosc, 1801, is a widely distributed genus worldwide in tropical and subtropical waters. Seven species of *Sphaeroma* have been identified from the Indian waters: (*Sphaeroma annandalei* Stebbing, 1911, *S. travancorensis* Pillai, 1955, *S. globicauda* Dana, 1851, *S. terebrans* Bate, 1866, *S. triste* Heller, 1865, *S. tuberculatum* Purusotham & Rao, 1971, and *S. walkeri* Stebbing, 1905). These wood-boring isopods inhabit the trunks and prop roots of mangrove plants, thus affecting their structure and stability. Moreover, sphaeromatid isopods reduce the root production and growth rate which leads to altering the nutrient supply (Thiri & Yang 2022). Previous investigations revealed that the burrowing activity of *Sphaeroma* isopods also has harmful effects on manmade coastal wooden structures such as jetties (Dodge-Wan & Nagarajan 2020).

Munroe Island is a critically vulnerable ecosystem in Kerala, south India (Rafeeque et al. 2023). This Island is between the Kallada River and the Ashtamudi Estuary (Arya et al. 2023). Specimens collected from the Peringalam Station in Munroe Island proved to be a new species of *Sphaeroma* genus and are described here as *Sphaeroma taborans* sp. nov. This isopod builds burrows in substrates such as dead wood and decaying roots of mangroves. It is generally supposed that the burrow serves as a shelter for the *S. taborans* sp. nov. rather than as a food resource.

## MATERIALS AND METHODS

Specimens for this study were collected from Munroe Island, part of Ashtamudi Estuary, Kerala (Image 1). Specimens were preserved in the field in 70% ethanol and dissected in diluted glycerine. Followed by the examination using a dissecting microscope (Weswox SZM105). Drawings were made using a standard camera lucida mounted on a compound microscope (Olympus CX33), and all images were processed with Photoshop CS5. Terminology of the morphological characters follows that of Bate, 1866; Harrison & Holdich 1984; Khalaji-Pirbalouty & Waegele 2010). The holotype (ZSI/WGRC/1.R.-INV.28482) was deposited in the Zoological Survey of India Kozhikode, Kerala, India. Paratypes (SSCDZ/Iso/01/2024 and SSCDZ/Iso/02/2024) were deposited in the Department Museum, Zoology Research Centre, St. Stephen's College, Pathanapuram.


**Image 1. The type locality of *Sphaeroma taborans* sp. nov. in Munroe Island, India.**

**RESULTS**

**Systematics**

Suborder Sphaeromatidea Wägele, 1989
Superfamily Sphaeromatoidea Latreille, 1825
Family Sphaeromatidae Latreille, 1825
Genus *Sphaeroma* Bosc, 1801

***Sphaeroma taborans* sp. nov.**

(Figures 1A–F, 2A–C, 3A–D)

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**Type Locality:** Munroe Island, 8.996 $^0$N, 76.596 $^0$E, Asthamudi Estuary, Kerala, India.

**Material examined:** Holotype: Female (8 mm), India, Kerala, Munroe Is, 8.996 $^0$N, 76.596 $^0$E, 7 September 2023, Perungalam stn. 5 m (ZSI/WGRC/1.R.-INV.28482). Paratypes: 1 female (8 mm) (SSCDZ/Iso/01/2024), 1 male (10 mm) (SSCDZ/Iso/02/2024) same data as holotype.

**Description of female:** Body about two times as long as highest width.

Head (Figure 1) dorsal surface with three longitudinal bands between eyes and a prominent blackish tubercle on the anterior band and scattered weak tubercles on the surface. Eyes bulbous.

Pereonites (Figure 1; Table 1) 1–4 with weak tubercles, 5–7 with a transverse row of continuous prominent, conical, setigerous tubercles; 6–7 with two transverse rows of thorn-like structure laterally.

Antennule (Figure 1) first peduncle article bearing

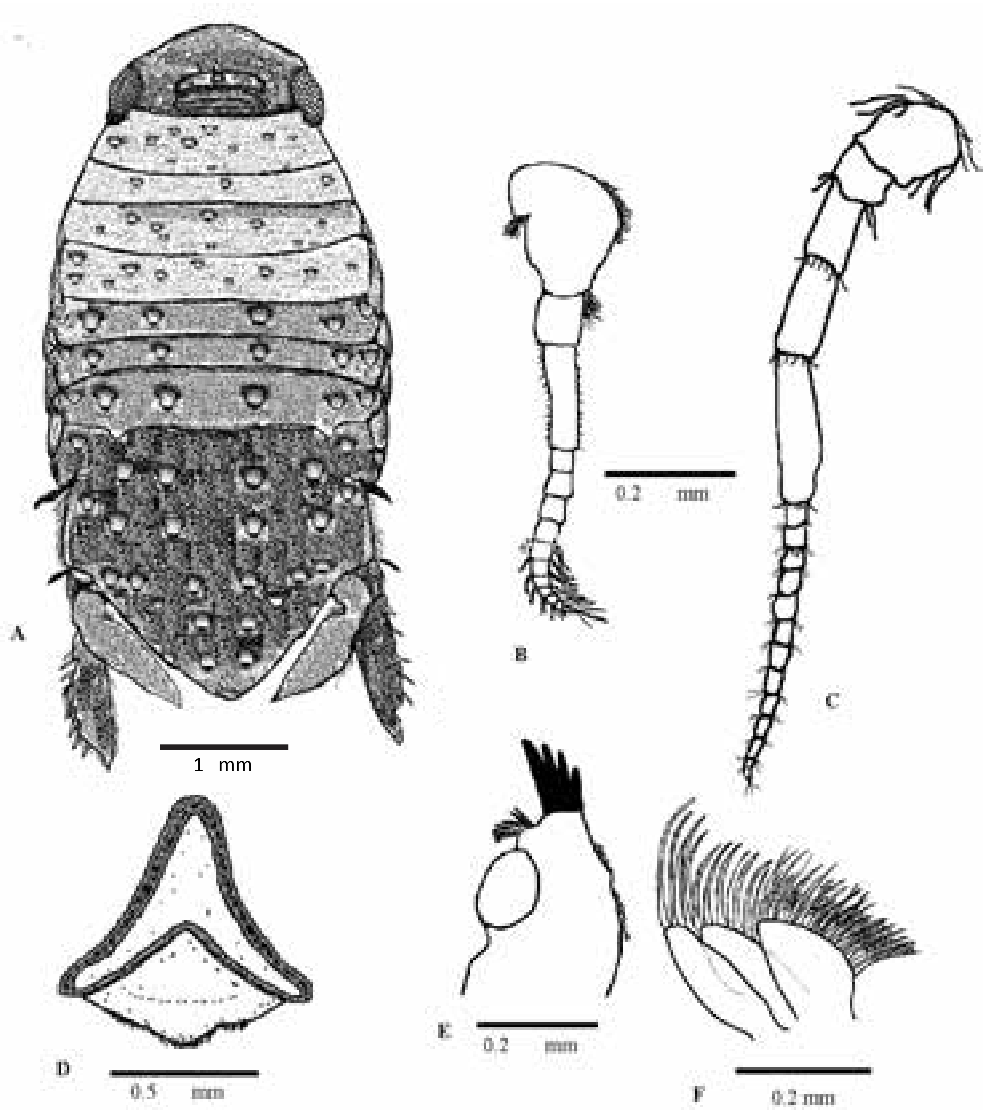


**Figure 1. *Sphaeroma taborans* sp. nov. (ZSI/WGRC/1.R.-INV.28482) holotype: A — dorsal view | B — antennule | C — antenna | D — epistome | E — right mandible | F — maxilla.**

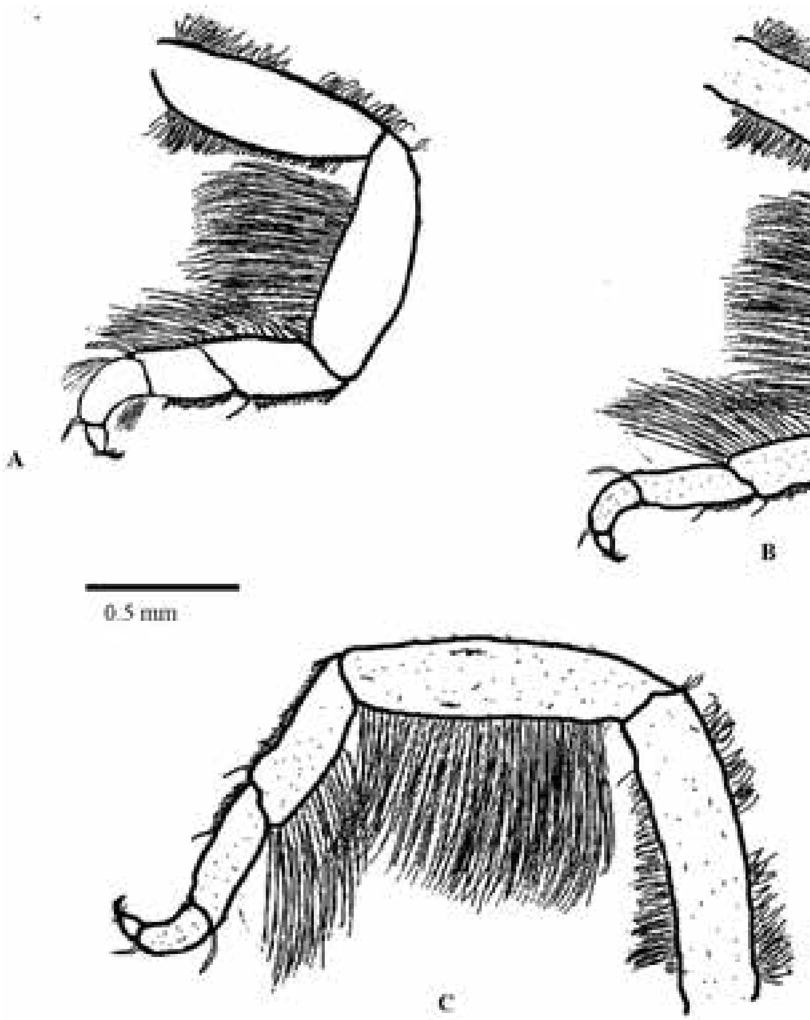


**Figure 2. *Sphaeroma taborans* sp. nov., holotype: A–C—pereopods 1–3.**

weak tubercles and short setae, with some short plumose setae on the upper surface; article two with some short plumose setae on dorsal and ventral margins, article three slender, 1.7 times as long as article two; flagellum with 11 articles, articles 4–9 each bearing single aesthetasc.

Antenna (Figure 1) peduncle article one setose; articles 2–4 each with long setae on posterior end; article five about 0.84 times as long as article four; flagellum with 15 articles.

Epistome (Figure 1) granulate, subtriangular apex, lateral margin concave and sublinear.

Left mandible (Figure 1) incisor with four cusps; incisor processes are blackish, indicating a high degree of sclerotization; molar processes are almost oval; reduced lacinia mobilis.

Maxillule lateral lobe apex without robust setae.

Maxilla (Figure 1) lateral and middle endites with long, slender, curved spines; mesial endite with dense internal plumose setae.

Maxilliped endite wide distally, with plumose setae, fine simple setae in distal margin, mesial margin bears one hook, ventral surface with a row of long robust plumose setae.

Pereopod 1 (Figure 2) basis about 2.5 times as long as greatest width; ischium and merus inner lateral margin with dense long setae; carpus inner lateral margin with fine setae; propodus superior margin setose; dactylus inferior margin with fine setae, fused to a blackish claw.

Pereopod 2 (Figure 2) ischium lateral margins with long comb setae; merus inner margin with long comb setae; carpus inferior end with one long fine setae; propodus inferior margin with long setae; dactylus fused to a blackish claw.

Pereopod 3 (Figure 2) is similar to pereopod 2.

Pereopod 4 (Figure 2) ischium superior margin with biserrate setae on a medial angle; merus and carpus inferior margin with fine setae; propodus inferior margin

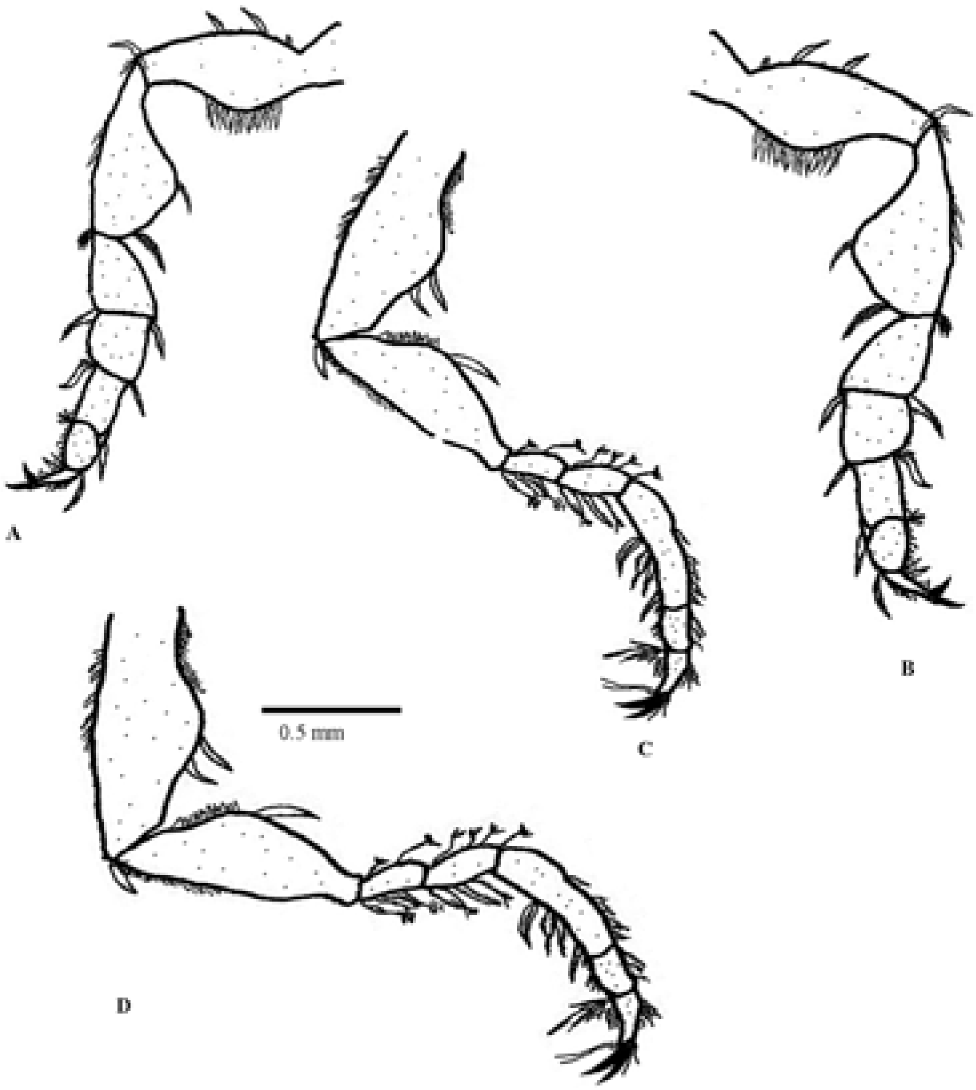


**Figure 3.** ***Sphaeroma taborans*** **sp. nov., holotype: A–D—pereopods 4–7**

with three long robust setae; dactylus with fine setae fused to blackish claws.

Pereopod 5 (Figure 3) is similar to pereopod 4.

Pereopod 6 (Figure 3) basis about 3.2 times as long as greatest width, superior margin with several fine and two robust setae; ischium superior margin with long and small serrate setae distally; merus superior distal margin with fine setae, inferior margin with biserrate seta and a long robust seta; carpus subequal in length to merus, inferior margin with two palmate setae, distal margin with simple setae; propodus superodistal corner with four slender setae; dactylus with fine setae fused to blackish claws.

Pereopod 7 (Figure 3) is similar to 6.

Pleon (Figure 1) with a transverse row of prominent tubercles at the middorsal surface and weak dense tubercles and two transverse rows of a tuft of setae over two sutures at lateral sides, posterior margin with two pointed extensions; posterolateral end with a long thorn-like structure.

Pleopod 1 subtriangular endopod, subequal in length to apically truncate exopod; bearing coupling hooks.

Pleopod 2 with rami as in pleopod 1; appendix masculina absent; bearing coupling hooks.

Pleopod 3 subtriangular, and broad endopod, subequal in length to apically truncate exopod; bearing coupling hooks.

Pleopod 4 endopod with an internal indentation.

Pleopod 5 endopod with rounded apex.

Pleotelson (Figure 1; Table 1) bearing weak dense appressed hair layers over surface, pleotelsonic medial lobe rounded triangle, lateral lobes weak and blunt;

**Table 1. Comparison among the congeners in the genus *Sphaeroma*.**

| Characteristics | Pereon segments | Pleotelson | Uropod rami | Type locality |
|---|---|---|---|---|
| *S. walkerii* | 2–4 each bearing two irregular transverse rows of low tubercles; 5–7 with a transverse row of prominent tubercles. | Dorsal surface granulated with scattered tubercles, either side of midline bearing a longitudinal row of five prominent tubercles, broadly rounded posteriorly with upturned and crenulated borders. | Uropodal exopod slightly longer than endopod with 5–6 external teeth and an acute apex; endopod margins fringed with dense simple setae, narrowly rounded apex, dorsal surface bearing 2–3 prominent tubercles, and an oblique ridge on the basal region. | Jokkenpiddi Paar, Sri Lanka |
| *S. annandaelei* | 1–7 posterior margin with a fringe of small setules; 2–7 with coxal plate sutures clearly visible; 4–7 dorsally with transverse ridges. | Dorsal surface with two pairs of prominent blunt tubercles followed by a single median prominent tubercle, Posterior margin curved upwards with rounded apex. | Uropodal rami subequal, extending well beyond pleotelsonic apex; endopod margins fringed with numerous simple marginal setae; exopod lateral margin clearly serrate, with 4–5 prominent and proximally further tiny teeth. | Port Canning, western India. |
| *S. terebrans* | 2–7 with a transverse ridge; 5–7 with four equidistant posterior submarginal tubercles each carrying a bunch of long stiff hairs; 4, 6, and 7 each bear four large tubercles. | Dorsal surface with four stout tubercles in a transverse row near the anterior margin, apex subtriangular, narrowly rounded, slightly curved upwards with minute apical hairs. | Uropodal exopod bears five prominent teeth at the outer edges, endopod apically acute, extending just beyond pleotelsonic apex, margins straight bearing fringes of long setae, and dorsal surface with a pronounced proximal tuft of long setae. | Hinchinbrook Island, Australia |
| *S. travancorensis* | Peraeon segments with simple ridges. | Pleotelsonic apex narrow, dorsally tuberculated. | Uropodal exopod serrate, with prominent and tiny teeth. | Kerala Coast, India |
| *S. taborans* sp. nov. | 1–4 weak tubercles, 5–7 transverse rows of prominent tubercles; 6–7 two transverse rows of thorn-like structures. | Dorsal surface with six stout tubercles in a transverse row near the anterior followed by three pairs of median prominent blunt tubercles. Apex curved triangle, slightly curved upwards with appressed hairs. | Uropodal exopod longer than endopod with 4–5 external teeth and an acute apex; endopod surface with dense hairs, thorn-like setae along lateral margins with acute apex extending beyond pleotelsonic apex. | Munroe Island, India |

medial lobe surpassing the apex of lateral lobes; five pairs of internal pouches. Pleotelson apex is very short and not reaching to the distal end of the uropod and endopod.

Uropod rami (Figure 1; Table 1) are perfectly knife-shaped and noticeably shorter than endopod, both bear blackish spots, appressed setae on the surface, and long thorn-like setae along lateral and distal margins; endopod laterally serrated and distal margin slightly pointed.

**Diagnosis of an adult female**. Pleon and pleotelson with appressed hair layers, pleotelsonic dorsal surface with six stout tubercles in a transverse row near the anterior followed by three pairs of median prominent setigerous tubercles. Apex curved triangle, slightly curved upwards bearing a prominent blackish tubercle on the anterior band on the head; epistome subtriangular; flagellum of antennule and antenna with 11 and 15 articles respectively; long thorn-like structures at the lateral margins of 6–7 pereonites and posterolateral end of pleon; knife-shaped uropodal exopod and endopod longer than pleotelsonic apex, both bears blackish spots.

**Male.** Apart from sexual characteristics similar to females, the body size is slightly larger than in female and pleotelson is longer than female.

## Etymology

This specific epithet 'taborans' refers to the Mount Tabor Ashram, the Headquarters of the Institution where this significant research was conducted at the Zoology Research Centre, St. Stephen's College, Pathanapuram.

**Distribution:** Known only from Munroe Island, Ashtamudi Estuary, Kerala, India.

**Ecological notes**

The specimens of *Sphaeroma taborans* sp. nov. were collected from the dead wood in the mangrove areas of Munroe Island.

## Remarks

*Sphaeroma taborans* can be recognized by the dorsal surface with three longitudinal bands between eyes a prominent blackish tubercle on the anterior band and scattered weak tubercles. Epistome with subtriangular apex. Pleon and pleotelson bearing appressed hair layers. Pleotelsonic apex curved triangle, slightly curved upwards with appressed hairs and surpassing the apex of lateral lobes. The new species *S. taborans* differs from *S. walkerii* Stebbing, 1905 (known from Jokkenpiddi Paar, Sri Lanka). The latter has crenulated pleotelsonic

**Key to the species of the genus *Sphaeroma* (females)**

1. Epistome with triangular apex ............................................................................................................. 2
- Epistome with subtriangular apex ........................................................................................................ 3

2. Pleotelsonic apex broadly rounded posteriorly with upturned and crenulated borders; Uropodal exopod with 5–6 external teeth and an acute apex; lacinia mobilis with two cusps ............................. ***S. walkerii***
- Pleotelsonic posterior margin curved upwards with rounded apex; lacinia mobilis absent; Uropodal exopod lateral margin serrate, with 4 –5 prominent and proximally further tiny teeth ...... ***S. annandaelei***

3. Pleotelsonic apex subtriangular, narrowly rounded, slightly curved upwards with minute apical hairs; Uropodal exopod with five prominent teeth at outer edges, endopod apically acute, extending just beyond pleotelsonic apex, dorsal surface with a pronounced proximal tuft of long setae; ......... ***S. terebrans***
- Pleotelsonic apex narrow, dorsally tuberculated; Peraeon segments with simple ridges; Uropodal exopod serrate, with prominent and tiny teeth; ..................... ***S. travancorensis.***
- Pleotelsonic apex curved triangle, slightly curved upwards with appressed hairs; Uropodal exopod with 4–5 external teeth and an acute apex; endopod surface with dense hairs, thorn-like setae along lateral margins with acute apex extending beyond pleotelsonic apex; three longitudinal bands between eyes and a prominent blackish tubercle on the anterior band ....................... ***S. taborans* sp. nov.**

borders, triangular epistomal apex, cusped lacinia mobilis, and distinct surface ornamentation. *S. taborans* is similar to *S. terebrans* Spence Bate, 1866 in having an equal number of antennular segments, and prominent teeth at the outer edges of the exopod. *S. taborans* differs from *S. terebrans* in having distinct pleotelsonic apex and surface ornamentation. Investigation of *S. taborans* showed that this species is more similar to *S. annandalei* Stebbing, 1911 (known from Port Canning, western India) and *S. travancorensis* Pillai, 1954 (known from Kerala Coast, southern India). *S. taborans* differs from *S. annandalei* and *S. travancorensis* in having the following distinct features such as surface ornamentation, three longitudinal bands between the eyes, a prominent blackish tubercle on the anterior band; pleotelsonic apex with appressed hairs; pleon with a long thorn-like structure at the posterolateral end; Antennal flagellum with 15 articles; Epistome with subtriangular apex; Uropodal exopod with 4–5 external teeth, endopod surface with dense hairs, thorn-like setae along lateral margins.

## DISCUSSION

Although the *Sphaeroma* is one of the largest and most widely distributed genera in the Sphaeromatidae family (Khalaji-Pirbalouty & Gagnon 2023), the taxonomic information on the genus is scanty in Kerala. According to Roy (2019), only seven species of *Sphaeroma* were reported in the Indian waters. The present study increases the *Sphaeroma* record to eight species with the description of *Sphaeroma taborans* sp. nov. The discovery of this new species after 61 years is indicative of the imperative to discover the isopod diversity from this region, particularly from the Indian mangrove estuaries. Hence, a comprehensive sampling at various estuarine locales, and mangrove ecosystems of India would expose more undescribed species of these sphaeromatid isopods.

*Sphaeroma taborans* sp. nov. is closely related to *S. travancorensis*, which was originally described from the Thiruvananthapuram District (formerly known as Travancore), Kerala, India. It is morphologically distinct, with notable differences including a curved triangular pleotelsonic apex, a uropodal exopod featuring 4–5 external teeth, and an acute apex. Consequently, *S. travancorensis* is considered a valid species.

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COMMUNICATION

# A report on Conidae (Gastropoda) from the Karnataka coast – distribution and shell morphometry

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**Abstract:** Conidae are a diverse group of predatory marine gastropods known for their highly potent venom, which may hold potential for biomedical applications. This study presents findings from a survey of Conidae species inhabiting the coastal shorelines of Karnataka. Shell measurements and morphometric analyses were conducted on four species: *Conus biliosus*, *C. inscriptus*, *C. milneedwardsi*, and *Conasprella dictator*. Molecular phylogenetic analysis of *C. biliosus* was performed using the partial mitochondrial cytochrome oxidase subunit I (COI) gene sequence.

**Keywords:** Cone snails, *Conasprella*, *Conus*, Cytochrome oxidase subunit I (COI), marine biodiversity, shell morphometrics, venomous molluscs.

**Kannada:** ಕೋನಿಡೆ ಎಂಬುದು ಪರಭಕ್ಷಕ ಸಮುದ್ರದ ಗ್ಯಾಸ್ಟ್ರೋಪಾಡ್‌ಗಳ ವೈವಿಧ್ಯಮಯ ಗುಂಪಾಗಿದ್ದು, ಅಮಗಳ ಅತ್ಯಂತ ಪ್ರಬಲವಾದ ವಿಷಕ್ಕೆ ಹೆಸರುವಾಸಿಯಾಗಿದೆ, ಇದು ಜೈವಿಕ ವೈದ್ಯಕೀಯ ಅನ್ವಯಗಳಿಗೆ ಸಂಭಾವ್ಯತೆಯನ್ನು ಹೊಂದಿರಬಹುದು. ಈ ಅಧ್ಯಯನಮ ಕರ್ನಾಟಕದ ಕರಾವಳಿ ತೀರಗಳಲ್ಲಿ ವಾಸಿಸುವ ಕೋನಿಡೆ ಪ್ರಭೇದಗಳ ಸಮೀಕ್ಷೆಯ ಫಲಿತಾಂಶಗಳನ್ನು ಪ್ರಸ್ತುತಪಡಿಸುತ್ತದೆ. ಶಂಖದ ಮಾಪನ ಮತ್ತು ಮಾರ್ಫೊಮೆಟ್ರಿಕ್ ವಿಶ್ಲೇಷಣೆಗಳನ್ನು ನಾಲ್ಕು ಪ್ರಭೇದಗಳದ ಕೊನಸ್ ಬಿಲಿಯೊಸಸ್, ಕೊನಸ್ ಇನ್ಸ್ಕ್ರಿಪ್ಟಸ್, ಕೊನಸ್ ಮಿಲ್ನೀಡ್ವರ್ಡಿ ಮತ್ತು ಕೊನಾಸ್ಪ್ರೆಲ್ಲಾ ಡಿಕ್ಟೇಟರ್ ಮೇಲೆ ನಡೆಸಲಾಯಿತು. ಭಾಗಶಃ ಮೈಟೊಕಾಂಡ್ರಿಯದ ಸೈಟೋಕ್ರೋಮ್ ಆಕ್ಸಿಡೇಸ್ ಸಬ್ಯೂನಿಟ್ I (COI) ಅನುವಂಶಿಕ ಧಾತು ಅನುಕ್ರಮವನ್ನು ಬಳಸಿಕೊಂಡು ಸಿ. ಬಿಲಿಯೊಸಸ್‌ನ ಆಣ್ವಿಕ ಫೈಲೋಜೆನೆಟಿಕ್ ವಿಶ್ಲೇಷಣೆಯನ್ನು ನಡೆಸಲಾಯಿತು.

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**Data availability:** The data used in this work are available publicly as given in the tables and supplemental.

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**Author contributions:** BSC and MSM planned the work. BSC collected the data. BSC and RSPR analysed the data. BSC drafted the paper and RSPR revised the paper. All authors contributed intellectually, and edited/reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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NITTE (Deemed to be University)

## INTRODUCTION

The family Conidae Fleming, 1822 (Dutertre & Lewis 2023) is a widely-distributed species-rich group of marine gastropod molluscs (Rockel et al. 1995). Cone shells are found in all tropical and subtropical oceans, with the Indo-West-Pacific region having the greatest species diversity (Filmer 2001). Studies on the taxonomy and distribution of Conidae in India date back to the latter half of the 19$^{th}$ century (Kohn 1978), and recently 76 of the 93 species known from India were reported from the collections of the Zoological Survey of India (Venkitesan et al. 2019). A total of 77 species of Conidae were documented from Indian waters (Kohn 1978). Regionally, 60 species were documented from Tamil Nadu (Franklin et al. 2009), 84 from the Gulf of Mannar and Lakshadweep Islands (Edward et al. 2022), and 46 from the Kerala coast (Ravinesh et al. 2022).

More than 50 species of cone shells have been identified by various researchers from the Andaman & Nicobar Islands (Rao 2003; Venkataraman et al. 2004; Franklin et al. 2013; Franklin & Apte 2021). A total of 78 cone snail species have been documented from the Lakshadweep archipelago (Smith 1894; Hornell 1921; Nagabhushanam & Rao 1972; Appukuttan et al. 1989; Rao & Rao 1991; Ravinesh & Kumar 2015; Edward et al. 2022). More recently, Ravinesh et al. (2018) recorded 49 species from Lakshadweep, including four newly reported species, three of which had not been previously recorded in India.

Until now, there have been no specific reports of *Conus* from the coast of Karnataka, India. This study presents the findings of a Conidae survey conducted in the year 2022–23 across several coastal regions of Karnataka. Field observations documented the regional distribution of four Conidae species, and shell morphometric analyses were carried out. Only one species was observed alive, and its molecular phylogenetic analysis was performed using the partial mitochondrial cytochrome oxidase subunit I (COI) gene sequence.

## MATERIALS AND METHODS

### Sample collection

Field surveys were conducted on accessible beaches across three coastal districts of Karnataka: Dakshina Kannada, Udupi, and Uttara Kannada (Image 1A). Transect and trawl net surveys were carried out along the shorelines in various types of coastal marine habitats, including intertidal and subtidal sandy bottoms, shallow sandy areas, rocky shorelines, and algae-covered rocks. The frequency of each species in these habitats was recorded (Image 1B). The specimens were collected using the handpicking method. Live specimens of *Conus biliosus* (n = 2) were observed and collected exclusively from rocky shorelines and algae-covered rocks in Karwar, Uttara Kannada District. The identification of collected specimens was based on the shell morphology descriptions (Rocket et al. 1995). Foot tissue from the live specimen was preserved in 90% ethanol for molecular analysis.

### Morphometric analysis

The collected shells were covered with algae, so for easier identification and measurement, they were cleaned using a mixture of commercial liquid bleach (“Rin”), containing nonylphenol ethoxylate, EDTA and sodium xylene sulfonate in water. To preserve the specimens, the surface of the shell was polished with mineral oil. Shell measurements were taken using Vernier Callipers (Kohn & Riggs 1975). The following variables were recorded: weight (W, in grams), shell length (SL), maximum diameter (MD), height of maximum diameter (HMD), aperture height (AH), aperture length (AL), aperture width (AW), height of penultimate whorl (HPW), and spire height (SH) (Fig. S1). All linear measurements were recorded in millimetres (mm).

### DNA extraction and PCR amplification

The foot tissue of *C. biliosus* was used as the source of genetic material and DNA was isolated from 40 mg of tissue using the CTAB method (Doyle & Doyle 1987) yielding approximately 500 ng/µl in a total volume of 60 µl. The mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using universal primers dgLCO: GGTCAACAAATCATAAAGAYATYGG and dgHCO: TAAACTTCAGGGTGACCAAARAAYCA (Folmer et al. 1994). Additionally, 12S1: GGCTTGGCGGTGTTTTAGAC and 12S3: GTGCACGTTTCAGAGCCCTA (Simon et al. 1991), and 16Sar: CGCCTGTTTACCAAAAACAT and 16Sbr: CCGGTCTGAACTCAGATCACGT (Palumbi 1996) primers were used to amplify 12S rRNA and 16S rRNA genes. PCR reactions were conducted in a total volume of 30µl, containing 3µl DNA, 1.5µl of each primer, 13.5µl of Takara master mix, 1.5 µl $MgCl_2$, and 9µl of PCR gradient water. The protocol began with initial amplification reaction that denatured at 94°C for 4 minutes, followed by 35 cycle denaturation for 40 seconds, annealing at 51°C for 40 seconds and extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was

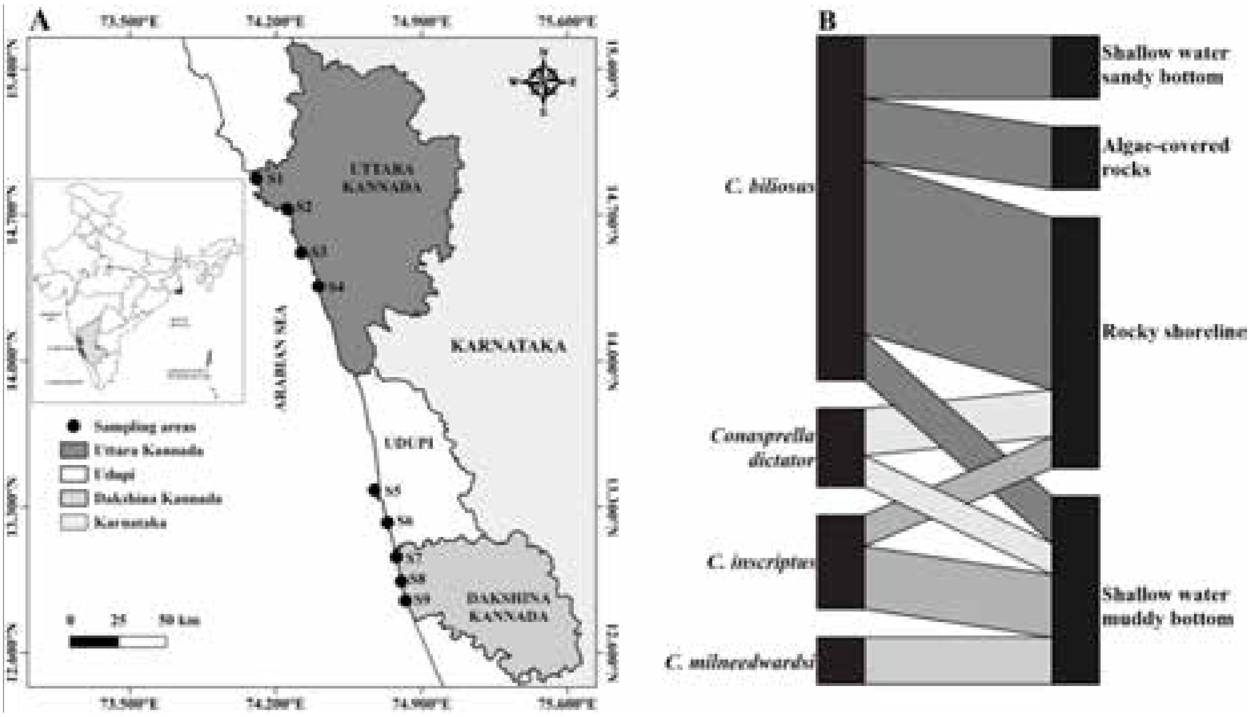


**Image 1. Sampling sites (S1–S9) of Conidae in the Karnataka coast. (B) Natural habitat of cone snails collected.**

included (Laxmilatha et al. 2021). The genomic DNA concentration and yield of PCR products were quantified using a Nano Drop spectrophotometer (Thermo Fisher Scientific Pvt. Ltd.) and assessed qualitatively using 0.8% agarose gel electrophoresis for DNA and 2% acrylamide gel electrophoresis for PCR products.

### Phylogenetic analysis

The amplified PCR products were sequenced using the Sanger sequencing method (Barcode Biosciences). The resulting sequences were compared against the NCBI nr database using BLAST, and the top hits corresponding to *C. biliosus* were downloaded. Additionally, sequences of closely related species (*C. shikamai*) and other COI sequences were retrieved for use as an outgroup. Multiple sequence alignment was performed using MUSCLE, and a phylogenetic tree was constructed using the maximum likelihood (ML) method with the Kimura 2-parameter (K2P) model in MEGA7 software (Kumar et al. 2016). Bootstrap analysis was conducted with 1,000 replicates to assess the tree's robustness.

### Data/Statistical analysis:

The morphometric measurements were recorded in a Microsoft Excel spreadsheet and summarized as mean (±standard deviation) along with minimum-maximum values. A Spearman's rank correlation coefficient matrix of the morphometric variables was generated using the R *corrplot* package (Wei & Simko 2021), and scatter plots of morphometric variables (Raup 1961; Kohn & Riggs 1975) were created in Microsoft Excel. Principal component analysis (PCA) was then performed using the R *ggplot2* package (Wickham 2016).

## RESULTS

### Distribution

Four species were recorded: *Conus biliosus*, *C. inscriptus*, *C. milneedwardsi*, and *Conasprella dictator;* only *C. biliosus* was sampled alive. Altogether, 27 shells, including two live specimens were collected. *Conus milneedwardsi* is listed under Schedule I of the Indian Wildlife (Protection) Act, 1972, Part G: Mollusca (Ravinesh et al. 2019; Samuel et al. 2021).

Based on the sampling data (Image 2A–D), *Conus biliosus*, *C. inscriptus*, and *Conasprella dictator* were observed in the Uttara Kannada and Dakshina Kannada districts, while *C. milneedwardsi* was recorded only in the Udupi District. Sampling of cone shells included several marine habitats. *Conus biliosus* was found in shallow water sandy bottoms and rocky habitats (Image 1B, 2N–Q) with live specimens collected from algae-covered rocks. *Conasprella dictator* and *Conus inscriptus* shells were found in rocky shoreline areas and shallow muddy bottoms (Image 1B), In contrast, *C. milneedwardsi* shells were collected by trawling in shallow muddy bottoms.

**Family: Conidae Fleming, 1822**
**Genus: *Conus* Linnaeus, 1758**
***Conus biliosus*** (Röding, 1798)

Method of collection: Handpicking.

Condition: Live specimens (n = 2) and shells (n = 11).

Habitat: Rocky shore and algae-covered rocks.

Description (Image 2A, 2E–M): Shell length approximately ranges from 20 mm to 40 mm, the body is covered with low, wavy spiral ridges that run from shell base to shoulder and spiral growth ridges are frequently visible, but in some shells they are smooth. We observed this species with different shades (Image 2E–M) of orange, brown, brown-black, and pale brown (juvenile).

***Conus inscriptus*** Reeve, 1843

Method of collection: Trawl bycatch.

Condition: Shells (n = 6).

Habitat: Shallow to subtidal sandy bottom.

Description (Image 2B): Shell length approximately ranges 40–55 mm. Shells are solid with a monotonous finish in a pale brown colour. Anteriorly, spiral grooves present – grooves are wide and contain spiral threads. In the sub-shoulder, bands are usually less noticeable than anterior bands.

***Conus milneedwardsi*** Jousseaume, 1894

Method of collection: Trawl bycatch.

Condition: Shells (n = 3).

Habitat: Subtidal sandy bottoms.

Description (Image 2C): Shell length approximately ranges 86–136 mm. Shells are reddish-white or brownish-white with prominent reticulated patches and spiral bands.

**Genus: *Conasprella* Thiele, 1929**
***Conasprella dictator*** (Melvill, 1898)

Method of collection: Handpicking and trawl bycatch.

Condition: Shells (n = 5).

Habitat: Shallow sandy bottoms.

Description (Image 2D): Shell length approximately ranges 25–35 mm. Shell is brownish-cream color, surrounded by distinct, dark-brown to reddish-brown bands, which are particularly prominent on the body whorl.

**Morphometric analysis**

The morphometric data for *C. biliosus* (n = 13), *Conasprella dictator* (n = 5), *C. inscriptus* (n = 6), and *C. milneedwardsi* (n = 3) is given in Table 1.

The Spearman's rank correlation coefficient matrix of the morphometric variables indicated that all variables were positively correlated (Figure 1A), with correlation coefficients ranging from $\rho = 0.38$ to $\rho = 0.99$. The scatter plots of morphometric variables against shell length are shown in Figure 1B–I. The variables such as HMD ($R^2$=0.994) and AL ($R^2$ = 0.987) exhibited excellent predictability in relation to shell length. Some variables, such as AW and HPW appeared to be more species-specific as *C. milneedwardsi* samples deviated from the general trend. HPW demonstrated the lowest predictability with respect to shell length among and within species.

A PCA (Figure 2) biplot of morphometric measurements explained 75.6% of the variance in PC1 and 15.5% in PC2. The samples from all four species formed distinct clusters, though the *Conasprella dictator* cluster overlapped with that of *C. biliosus*. As indicated by the lower variability of morphometric measurements (Table 1), *Conasprella dictator* was less spread compared to the other species in the PCA biplot. The variables projected onto the biplot revealed that SL, MD, and

**Table 1. The morphometric measurements of the collected cone shells.**

| Variables | *C. biliosus* (n = 13) Mean (±SD) Min–Max | *Conasprella dictator* (n = 5) Mean (±SD) Min–Max | *C. inscriptus* (n = 6) Mean (±SD) Min–Max | *C. milneedwardsi* (n = 3) Mean (±SD) Min–Max |
|---|---|---|---|---|
| W | 4.2 (2.3)<br>1.3–8.4 | 2.1 (0.7)<br>1.4–3.3 | 10.7 (3.6)<br>6.8–15.3 | 41.1 (21.8)<br>17.7–60.8 |
| SL | 28.2 (7.2)<br>19.1–41.2 | 29.1 (4.2)<br>25–35.5 | 48.8 (5.5)<br>41.9–54.6 | 114.7 (25.8)<br>86–136 |
| MD | 17.2 (3.7)<br>11.2–22.6 | 13.6 (1.9)<br>12.1–16.9 | 24.2 (3.4)<br>20.3–27.8 | 38.0 (12.5)<br>24–48 |
| HMD | 19.8 (6.0)<br>12.7–32.3 | 20.1 (2.8)<br>17.8–24.7 | 36.3 (4.9)<br>29.2–41.2 | 72.4 (16.0)<br>54.1–84 |
| AH | 25.5 (6.4)<br>16.7–35.5 | 23.1 (2.5)<br>21–27 | 39.3 (4.8)<br>34.8–45.8 | 76.7 (14.4)<br>60.1–86 |
| AL | 23.0 (6.3)<br>14.1–32 | 21.8 (2.6)<br>19.7–26 | 39.4 (5.6)<br>33–45.3 | 74.7 (16.3)<br>55.9–85.2 |
| AW | 3.2 (1.0)<br>1.8–4.9 | 3.0 (1.1)<br>1.9–4.4 | 5.0 (0.7)<br>4.1–5.8 | 4.2 (0.4)<br>3.8–4.5 |
| HPW | 1.4 (0.4)<br>0.7–2.3 | 1.3 (0.4)<br>0.6–1.9 | 3.7 (1.4)<br>2.2–6.0 | 2.2 (0.2)<br>2.0–2.3 |
| SH | 3.5 (1.1)<br>2.5–6.1 | 13.8 (6.3)<br>6.7–20.0 | 8.7 (1.0)<br>7.2–9.9 | 31.7 (4.1)<br>27.2–35 |

Abbreviations: W—Weight | SL—Shell length | MD—Maximum diameter | HMD—Height of maximum diameter | AH—Aperture height | AL—Aperture length | AW—Aperture width | HPW—Height of penultimate whorl | SH—Spire height. The measurements/values for W are in grams, and all others are in millimeter.

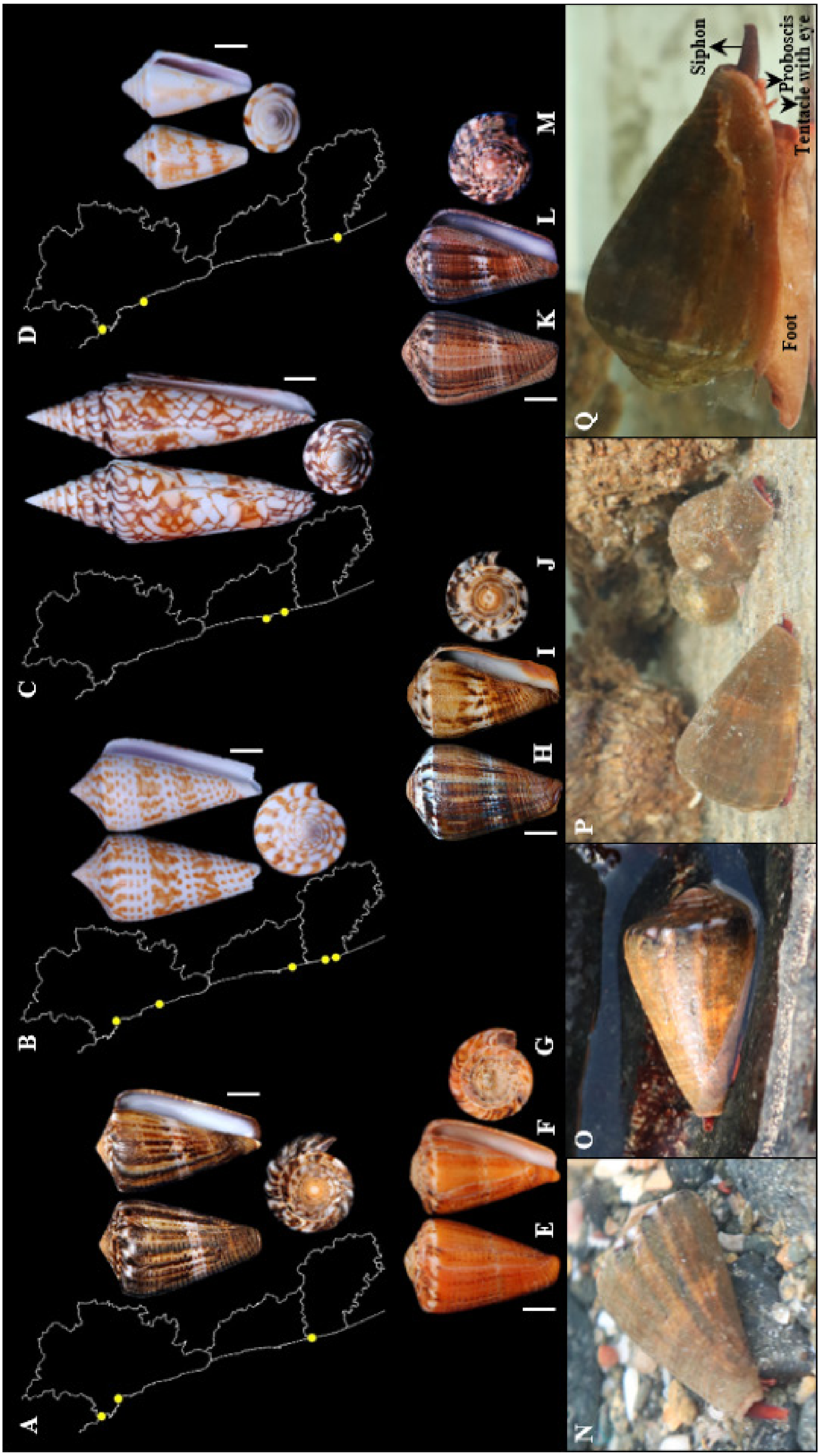


Image 2. Distribution (locality data) of Conidae in the Karnataka coast. (A)*C. biliosus*, (B)*C. inscriptus*, (C) *C. milneedwardsi*, and (D) *Conasprella dictator*. (E-M) Colour morphs in *C. biliosus*. (A-M) Dorsal, ventral, and top views of cone snail species. Scale bar is 1 cm. (N-O) *C. biliosus* in the natural habitat. (P-Q) *C. biliosus* in aquarium. © B S Chandan.

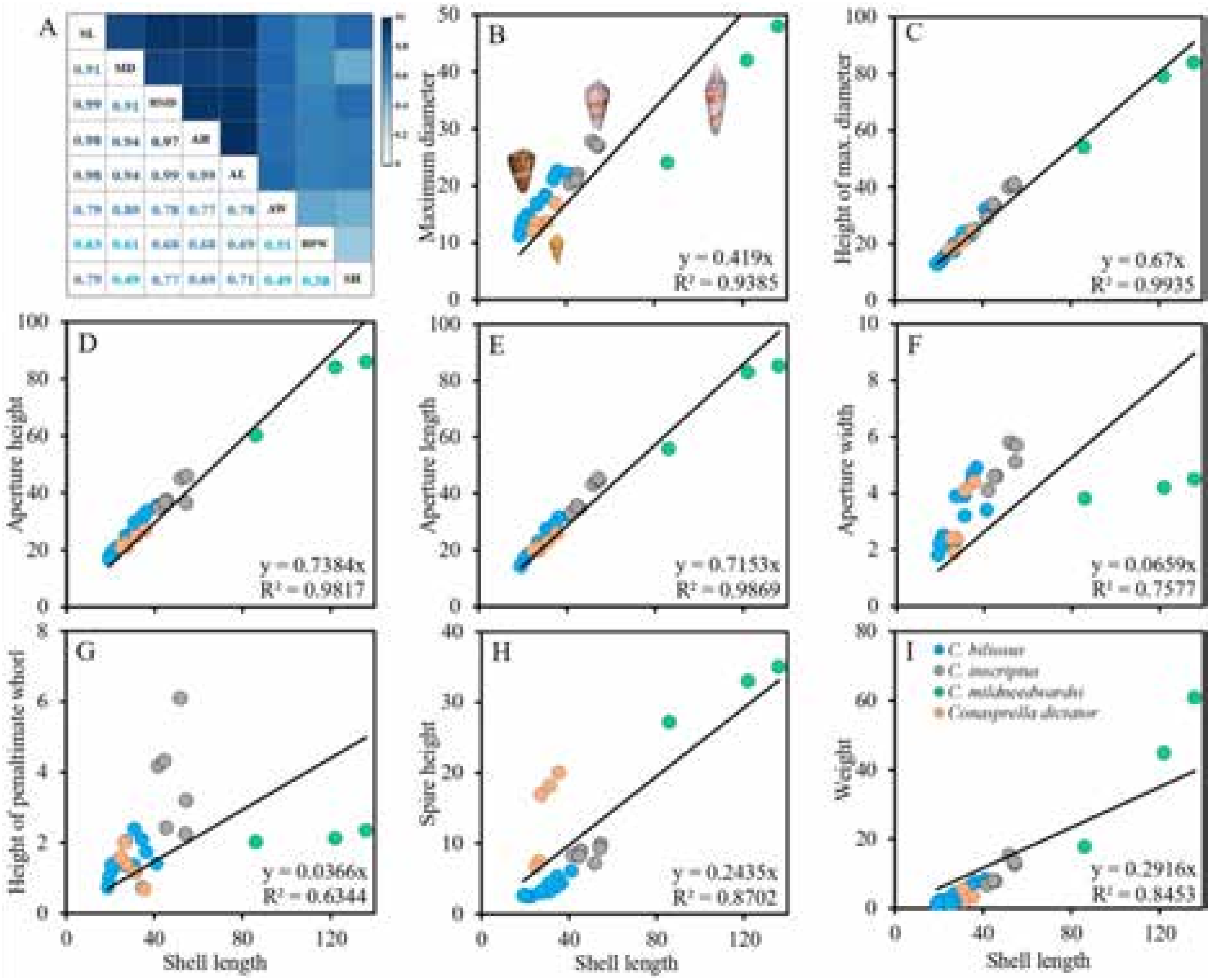


**Figure 1. (A) The Spearman's rank correlation coefficient matrix of morphometric variables. (B-I)Scatter plots of various shell measurements (all in mm, except weight which is in g) against the shell length (in mm). Abbreviations: AH - aperture height, SL - shell length, MD -maximum diameter, HMD -height of maximum diameter, AL -aperture length, AW -aperture width, APW -height of penultimate whorl, and SH -spire height.**

other factors primarily contributed to PC1, as evident by the spread of long-shelled *C. milneedwardsi* along PC1. The variables HPW, AW and SH primarily contributed to PC2.

### Phylogeny of *C. biliosus*

The mitochondrial COI sequences from two *C. biliosus* samples were obtained and submitted to NCBI (supplemental information). The phylogenetic tree based on these COI sequences revealed that *C. biliosus* samples (PQ390234 and PQ392002) from Karnataka were distinct, but clustered within the same clade of other *C. biliosus* (KJ549870.1 and KJ550138.1) from Indo-West-Pacific region (Puillandre et al. 2014) (Figure 3). The *C. shikamai* and other species formed a clear outgroup. Since COI sequences were found and used widely for cone snail databases, 12S rRNA and 16S rRNA sequences were not included in the analysis.

## DISCUSSION

This study documents four species of cone snails: *C. biliosus*, *C. inscriptus*, *C. milneedwardsi*, and *Conasprella dictator* from the Karnataka coast. While Conidae species generally share a similar shell shape (Rockel et al. 1995), morphometric measurements are widely used as distinguishing features between species. The ranges of values observed in this study are consistent with previous records of morphometric measurements for cone shells of the corresponding species from the Indo-Pacific region (Rockel et al. 1995), Lakshadweep

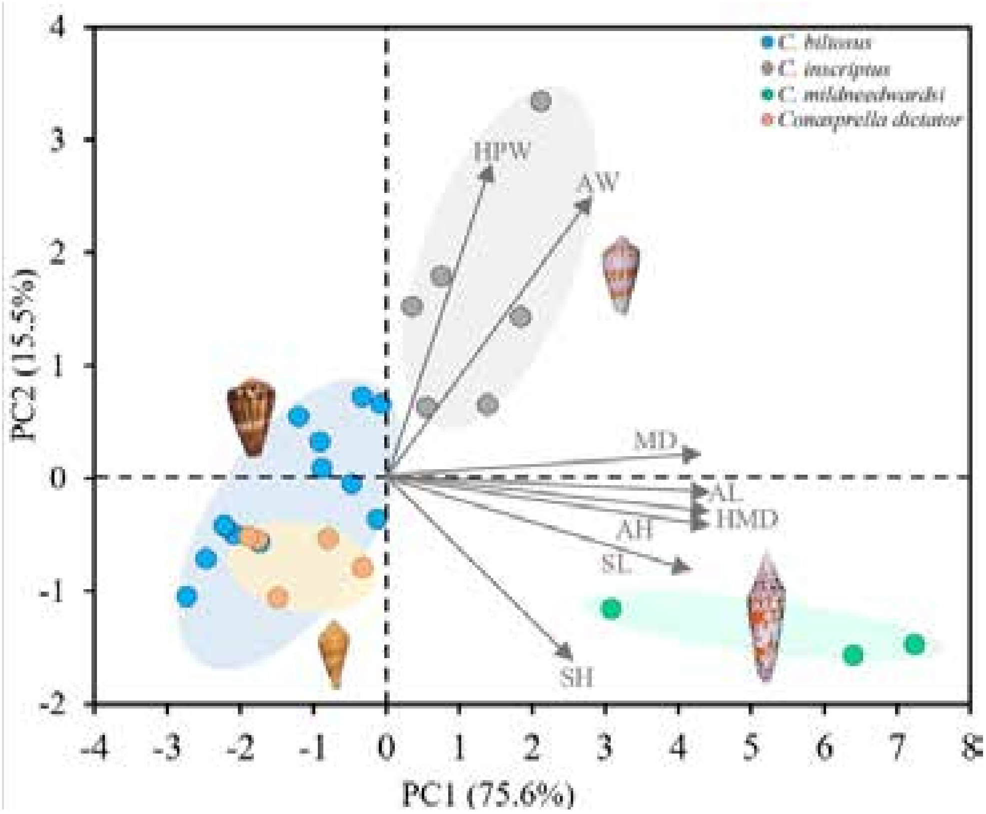


**Figure 2. PCA biplot of Conidae species based on morphometric variables. Groups based on species.The arrows indicate the relationships of the variables with species group.**

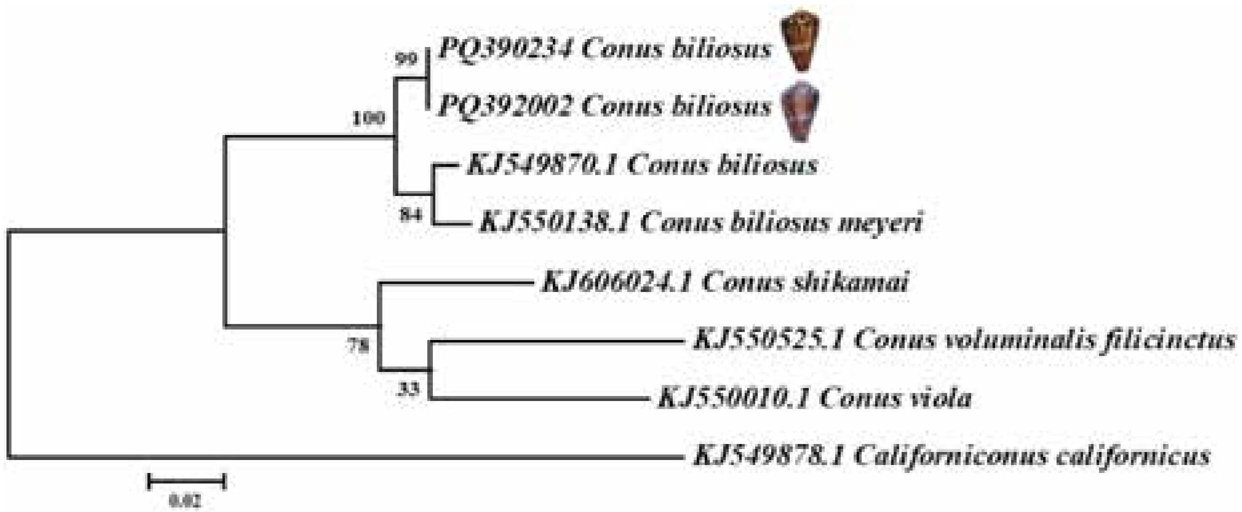


**Figure 3. Maximum likelihood (ML) based phylogenetic tree of *C. biliosus* using partial mitochondrial COI gene sequences. The *C. shikamai* and others were used as the out group. Numbers next to nodes indicate percentage bootstrap values based on 1000 iterations. Scale bar indicates the number of substitutions per site.**

(Ravinesh et al. 2018), Tamil Nadu (Venkitesan et al. 2019), and Kerala (Ravinesh et al. 2022).

The phylogenetic analysis based on mitochondrial COI sequences of live *C. biliosus* specimens collected in this study placed them in a monophyletic group with *C. biliosus meyeri*, a southern subspecies found in Indian marine habitats (Puillandre et al. 2014).

This pilot study on the previously unexplored

Karnataka coast may inspire researchers to conduct more intensive surveys and acquire accurate data on habitat and distribution of cone snails of the Karnataka coast.

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## Supplemental information

Notes:

Partial gene sequences of 12S rRNA, 16S rRNA, and COI from two specimens of *C. biliosus*. The sequences have been submitted to NCBI and the accession numbers are given.

>PQ393374|12S rRNA (partial) from *C. biliosus* 1
ATTCGACATTCCACGTTTTTCCCCGACCCTTCCTTATTCGCAGTTTCAGCCTTGTATACCGTTGTCGTCAGGTAACTATCTTAAAACATAGTAGTTAGCTACGAAAATATTATTAATTAACACGTCA
AATCAAGGTGCAGCTAATAAGAGGGAGAGGATGGGTTACAATTATATATTTATAATCACGAAAAACGTCTTAAATAAGGTGTTGGAAGGAGGACTTGAAAGTAATTTTAATTATATAAAGAGA
ATGAATAGGGCTCTAAAAACGTGCACA

>PQ394594|16S rRNA (partial) from *C. biliosus* 1
CGGACCTTGCCCAGTGAGTTTTTTCAACGGCCGCGGTACTACTCACCGTGCAAAGGTAGCATAATCATCCTGCCTTATAATTGAAGGCTGGAATGAATGGTTTGACAAGAATACAACTGTCTC
TATATGATTTCCTAGAATTTTATTTTTGGATGAAAAAGTCCAGATATTATTAAAAGACAAGAAGACCCTATCGAGCTTTAGAGAAATTAGTAGACTTAATAATAAATCAATAGAAATAAAAGAAA
AACTACTAAATACACTTTGGTTGGGGCAACCGAGGAGCAAATAAAGCCTCCTTTAAGTTTTAAATCCTGCATGTGCTGATCCAAATATTTTGATCAAAGGAATTAGTTACCGTAGGGATAACAG
CATTATCTTTTTCAAGAGCCCATATCGAAAAAAAGGTTTGTGACCTCGATGTTGGACCAGAATATCCTAAAGATGCAGAAGTCTTTAAGGGTTGGTCTGTTCGACCATTAAAATTCTACGTGAT
CTGT

>PQ390234|COI (partial) from *C. biliosus* 1
GGGTTGGTCGGTACTGCCTTAAGTTTATTGATTCGTGCAGAATTAGGTCAGCCTGGTGCATTACTCGGAGACGATCAGTTGTATAATGTAATTGTGACAGCACATGCTTTTGTTATAATTTTTTT
CTTAGTGATGCCAATAATAATTGGGGGATTTGGGAATTGATTAGTACCTCTTATATTGGGGGCTCCAGATATGGTTTTTCCTCGACTAAATAATATAAGTTTTTGGCTTCTTCCGCCTGCGTTATT
GCTTCTTCTATCATCGGCAGCGGTAGAAAGGGGTGTTGGTACAGGATGAACAGTATATCCTCCTTTAGCAGGAAATCTAGCTCATGCTGGAGGTTCTGTAGATCTGGCGATTTTCTCTCTCCAT
CTTGCTGGGGTTTCTTCTATTTTGGGTGCAGTAAATTTTATTACCACAATTATTAATATACGATGGCAGGGAATAAAATTTGAACGCCTTTCGTTGTTTGTGTGGTCCGTAAAAATTACTGCTATT
TTATTGCTTTTATCTTTACCTGTGTTAGCAGGAGCAATTACGATACTCTTAACCGATCGAAATTTTAATACTGCTTTCTTTGACCCAGCAGGAGGTGGGGATCCTATTTTATACCAGCATTTGTTC

>PQ393375|12S rRNA (partial) from *C. biliosus* 2
TACAAAAAAGAAATCATAACGTGGTAAGTCTATCCACGTCTATACCCGACCCTCTATAGCAGTTCAGCCTGTATACCGTTGTCGTCAGGTAACTTCTTAAAACATAGTAGTTAGCTCGAAAATAT
TATTAATTAACACGTCAAATCAAGGTGCAGCTAATAAGAGGGAGAGGATGGGTTACAATTATATATTTATAATCACGAAAAACGTCTTAAATAAGGTGTTGGAAGGAGGACTTGAAAGTAATT
TTAATTATATAAAGAGAATGAATAGGGCTCTGAAACGTGCACAAA

>PQ380238|16S rRNA (partial) from *C. biliosus* 2
TTTTAAACGGGCCGCGGTACTCTGACCGTGCAAAGGTAGCATAATCATTTGCCTTATAATTGAAGGCTGGAATGAATGGTTTGACAAGAATACAACTGTCTCTATATGATTTCCTAGAATTTTAT
TTTTGGATGAAAAAGTCCAGATATTATTAAAAGACAAGAAGACCCTATCGAGCTTTAGAGAAATTAGTAGACTTAATAATAAATCAATAGAAATAAAAGAAAAACTACTAAATACACTTTGGTT
GGGGCAACCGAGGAGCAAATAAAGCCTCCTTTAAGTTTTAAATCCTGCATGTGCTGATCCAAATATTATGATCAAAGGAATTAGTTACCGTATGGATAACAACGTTATC

>PQ392002|COI (partial) from *C. biliosus* 2
GGGTTGGTCGGTACTGCCTTAAGTTTATTGATTCGTGCAGAATTAGGTCAGCCTGGTGCATTACTCGGAGACGATCAGTTGTATAATGTAATTGTGACAGCACATGCTTTTGTTATAATTTTTTT
CTTAGTGATGCCAATAATAATTGGGGGATTTGGGAATTGATTAGTACCTCTTATATTGGGGGCTCCAGATATGGTTTTTCCTCGACTAAATAATATAAGTTTTTGGCTTCTTCCGCCTGCGTTATT
GCTTCTTCTATCATCGGCAGCGGTAGAAAGGGGTGTTGGTACAGGATGAACAGTATATCCTCCTTTAGCAGGAAATCTAGCTCATGCTGGAGGTTCTGTAGATCTGGCGATTTTCTCTCTCCAT
CTTGCTGGGGTTTCTTCTATTTTGGGTGCAGTAAATTTTATTACCACAATTATTAATATACGATGGCAGGGAATAAAATTTGAACGCCTTTCGTTGTTTGTGTGGTCCGTAAAAATTACTGCTATT
TTATTGCTTTTATCTTTACCTGTGTTAGCAGGAGCAATTACGATACTCTTAACCGATCGAAATTTTAATACTGCTTTCTTTGACCCAGCAGGAGGTGGGGATCCTATTTTATACCAGCATTTGTTC

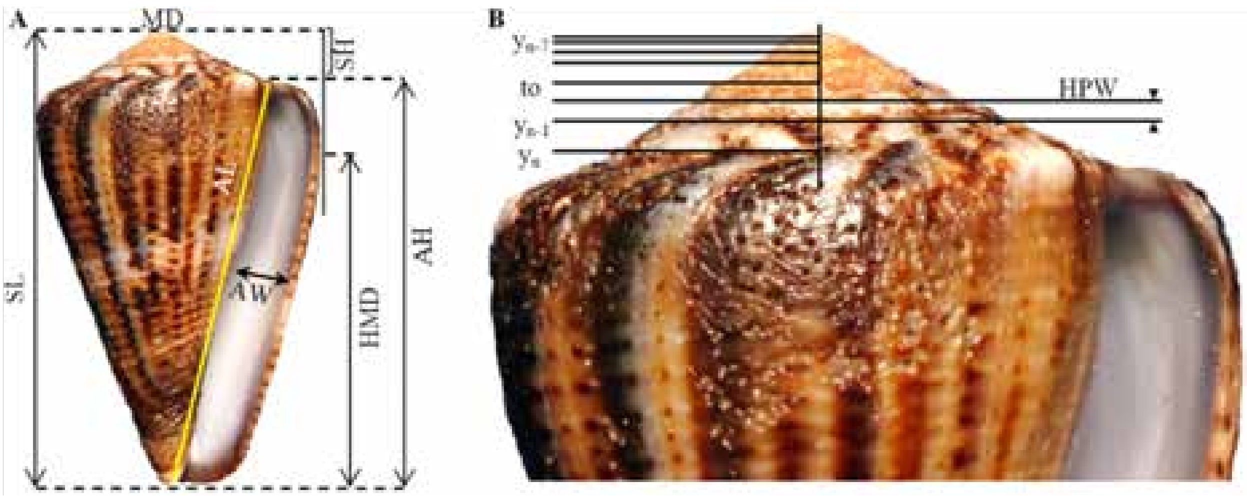


**Image S1. Morphometric measurements of the Conidae shell (*C. biliosus*): A—Ventral view | B—Posterior or spire view. Abbreviations: AH—aperture height | AL—aperture length | AW—aperture width | HPW—height of penultimate whorl | HMD—height of maximum diameter | MD—maximum diameter | SH—spire height | SL—shell length. In the spire, the successive whorls are labelled as $y_n$ to $y_{n-7}$. © B S Chandan.**

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COMMUNICATION

# New distribution record and DNA barcoding of the steno-endemic plant *Cordia diffusa* (Boraginaceae)

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**Abstract:** The research marks the first documented occurrence of *Cordia diffusa* in Kurumbapalayam, a location where it had not been reported previously. In this study, genomic markers were also utilized to document the endemic plant species *Cordia diffusa* K.C.Jacob of the Boraginaceae family. This study employed chloroplast markers such as matK, rbcL, and the psbA-tnH region to determine their efficiency in identifying the species. For the first time, *C. diffusa* has been analyzed and documented using these genetic markers. Sequences obtained from three different primer sets for each marker were submitted to GenBank. This research highlights the first genetic documentation and identification of *C. diffusa* using chloroplast markers, contributing valuable genetic data to GenBank and advancing the understanding of this endangered species distribution.

**Keywords:** Boraginaceae, chloroplast markers, Endangered species, Kurumbapalayam, matk, psbA-tnH, rbcL, species identification.

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**Author contributions:** MH–data collection, data analysis, and writing-original draft. DLL–supervision, guidance in research methodology, and writing-review & editing. HA–assistance in data collection and visualization.

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## INTRODUCTION

The *Cordia* plant genus is part of the Boraginaceae family often referred to as the forget me-not family and includes herbs and trees, among its 2,700 species spread across almost 125 genera historically categorized into five subfamilies named Boraginoideae, Cordioideae, Ehretioideae, Heliotropioideae, and Wellstedioideae (De Candolle in 1845; Bentham & Hooker in 1876; Engler in 1898; Pilger & Krause in 1915). However, according to evolutionary studies, physical traits, and consistent naming conventions, these have now been expanded to 11 separate and related plant families. These include Boraginaceae sensu stricto, Codonaceae, the established Coldeniaceae family, Cordiaceae, Ehretiacaea, Heliotropceae, Hoplestigmataceae, Hydrophylacaea, Lennoaceae, Namaceae, and Wellstediaceae as detailed by Luebert et al. (2016). The genus name *Cordia* pays tribute to the botanist and pharmacist Valerus Cordus as referenced by Quattrocchi (2012).

Henry et al. (1978) reported *Cordia diffusa* K.C.Jacob, a shrub commonly known as Kovai Manjack or Sirunaruvilli, is a rare and threatened flowering plant of southern India. While it is believed to be endemic to this region (Arumugam et al. 2019), reports suggest its presence in other areas like the Gulf of Mannar (Daniel & Umamaheshwari 2001) and Chengalpattu District (Ramamurthy 1987; Nayar 1996; Singh et al. 2015). In the region of Kurumbapalayam, in Sarkarsamakulam, within Coimbatore *C. diffusa* has been recently discovered. Highlighted by its traits the identification of plant species has been a common practice. Spotting endangered plants that resemble species based on their vegetative features poses a challenge. A solution to this dilemma could lie in DNA barcoding, introduced by Hebert et al. (2003) for the purpose of species identification or discovery. The ideal DNA barcode should be a consistent short DNA sequence (400–800 bp) that can be produced and characterized efficiently for all living species (Savolainen et al. 2005). CBOL Plant Working Group (2009) evaluated the performance of psbK–psbI, atpF–atpH, and trnH–psbA spacers, and matK, rbcL, rpoB, and rpoC1 and among the seven plastid barcode loci, recommends that rbcL and matk as standard barcoding region, ITS and trnH-psbA as supplementary barcode loci (China plant BOL group et al. 2011; Pang et al. 2012) these four loci were widely accepted as plant DNA barcodes (Yu et al. 2011; Li et al. 2012; Parveen et al. 2012; Aubriot et al. 2013; Saarela et al. 2013; Yan et al. 2015). Plastome sequences have also been used widely to study the phylogenetic connections of numerous plant taxa (Lv et al. 2022; Su et al. 2022; Xu et al. 2022; Hu et al. 2023).

It should be remembered that DNA barcoding is not a substitute for taxonomy, but rather an important tool that generates information on undiscovered species (Ebach et al. 2005). Rapid economic development and its impact on the flora and fauna of numerous nations, particularly in the tropical and subtropical regions, molecular identification can speed up identification and improve the accuracy of species separation in complex species and tree plants and estimate the biodiversity of these regions to preserve the rare endemic and endangered species. In the present study, DNA barcoding of the endangered species *Cordia diffusa*, which has not been done so far, is done, and its phylogenetic relationship with other species was determined based on BLAST results.

## MATERIALS AND METHODS

### Plant collection

Plant materials were collected in 2022 in Kurumbapalayam, Coimbatore (Figure 1, Image 1). The collected specimens were authenticated, and a herbarium voucher was submitted to the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu.

### DNA isolation, PCR amplification and DNA sequencing

DNA from *C. diffusa* leaf was obtained using the Macherey Nagel Nucleospin Plant II mini spin DNA extraction kit from 100 mg of tissue. The amount of DNA extracted was measured using a Thermo Scientific Nanodrop 2000 spectrophotometer. To barcode the DNA samples, for identification purposes the specific genes matK, rbcL and trnH-psbA were used for PCR amplification using a thermal cycler (Bio-Rad, USA). PCR reactions were performed with a total volume of 30µl reaction mixture containing 2µl (100 ng) of DNA, 15 µl of EmeraldAmp GT PCR mix (TaKaRa, Bio USA, Inc.), 0.5 µl (10 pM) of each forward and reverse prime, and 12 µl of nuclease-free water. PCR cycling conditions were: one cycle of 5 min at 95°C, followed by 30 cycles at 95°C for 1 min. Annealing at different temperatures for different genes: 58°C for the matK gene, 55°C for the rbcL gene, and 57°C for the trnH psba gene for 30 seconds and extension at 72°C for a minute and the final extension step at 72°C for 10 minutes. The reaction mixture consists of 2µl (100 ng) of DNA, 15µl of EmeraldAmp GT PCR mix (TaKaRa, Bio USA, Inc.), 0.5µl (10 pM) of each forward and reverses primer, and 12µl of nuclease-free

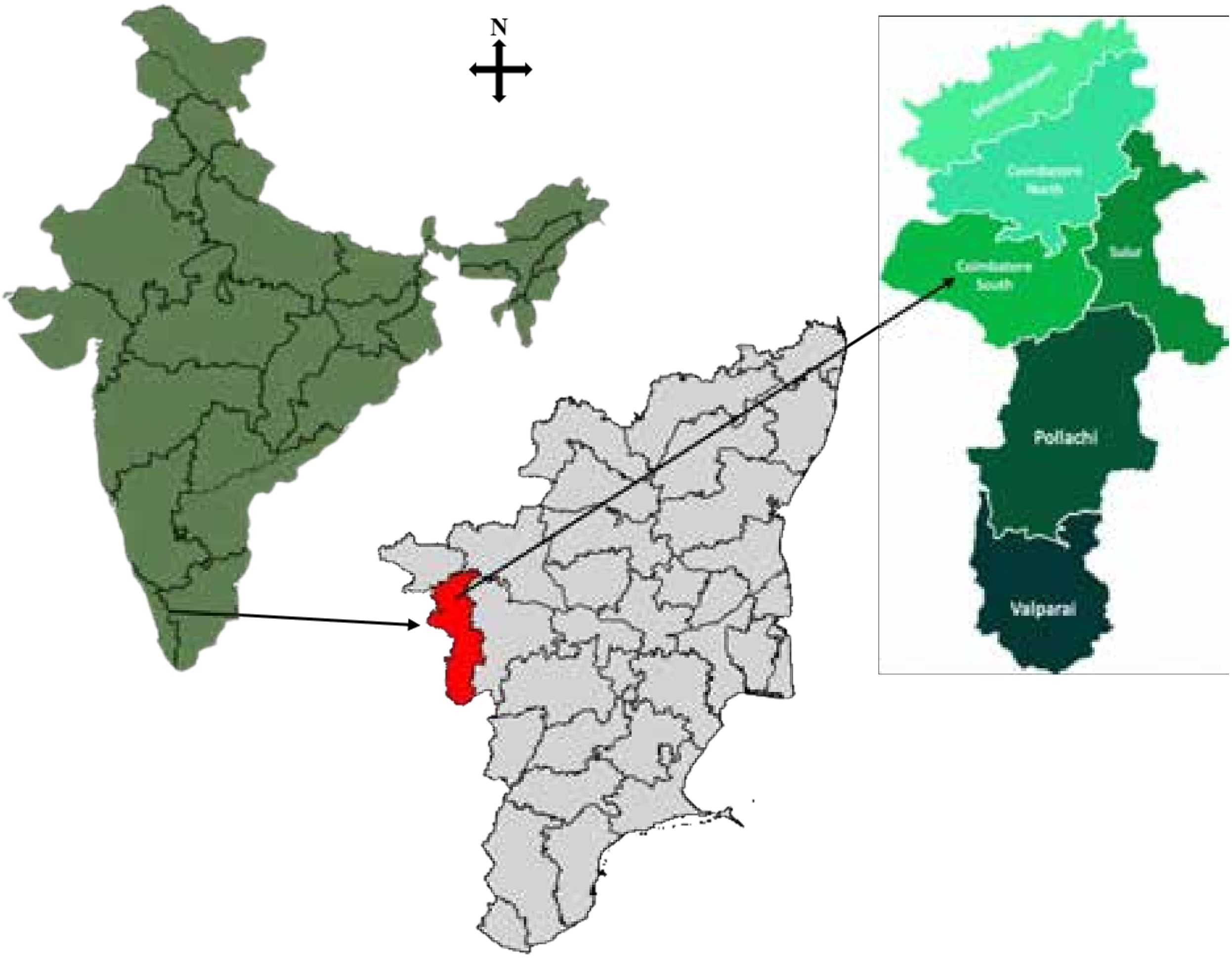


**Figure 1. Study area of the plant *Cordia diffusa*.**

water made up to 30µl. The primer set descriptions are given in Table 1. The amplified PCR products were loaded in a 1% TAE agarose gel and observed using a UV-Transilluminator. The amplified DNA was sequenced at Biokart (Karnataka, India). Sequencing was performed in both primers to obtain the full length of the targeted region.

**Sequence submission and data analysis**

The DNA sequences were assembled and edited using codon code Aligner V 2.06 (Codon Code Co, USA) to remove unclear base calls, and both sequences were assembled. The sequences were modified according to the NCBI criteria (https://www.ncbi.nlm.nih.gov/) and compared to the global sequence in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the nucleotide BLAST algorithm. Both BLASTn and BLASTx were performed sequentially. The sequences were submitted to NCBI through the Bankit submission channel (https://www.ncbi.nlm.nih.gov/WebSub/). To determine the percentage of sequence similarity, the FASTA format of *Cordia diffusa* DNA sequences was compared with the NCBI database using BLAST + 2.14.).

## RESULTS

A detailed literature survey on the occurrence of *C. diffusa* was done, and the species previously reported in Vaalankulam, Ramanathapuram (Chandrabose 1968), and IFGTB (Prabu et al. 2019) were identified in Kurumbapalayam, Coimbatore. The herbarium of the plant was submitted to the Botanical Survey of India, Coimbatore, with Voucher number MH178154 (Image 2).

**PCR Results and DNA Barcode Sequence Analysis**

Partial DNA sequencing was performed using matK, rbcL, and trnH-psbA gene regions. The accession number of the GenBank submitted sequences with their


**Image 1. The habit of *Cordia diffusa*. © Haritha, M. & D. Leena Lavanya.**

**Table 1. List of primers used for DNA amplification.**

| Primer name | Sequence |
|---|---|
| F | CTTCCGATATGAGGAATTCTTCC |
| R | CTTCCGATATGAGGAATTCTTCC |
| F | ATGTCACCACAAACAGAGACTAAAGC |
| R | TCGCATGTACCTGCAGTAGC |
| F | CGCGCATGGTGGATTCACAATCC |
| R | GTTATGCATGAACGTAATGCTC |

respective base lengths and nucleotide composition percentages are summarized in Table 2. Among the genes used for the study, the highest G+C content (42%) was observed in the rbcL gene compared to matK and trnH-psbA.

## DISCUSSION

*Cordia diffusa,* a rare, endemic shrub species geographically restricted to Tamil Nadu and Sri Lanka, was first identified by Dr. K. Cherian Jacob in 1938 and later described in 1944. Henry et al. (1978) listed *C. diffusa* as a rare and threatened plant of southern India. Later, Chandrabose & Nair (1988) expressed concerns over its potential extinction, classifying it as indeterminate on the IUCN Red List of Threatened Plants (Rao et al. 2003). Although *C. diffusa* has been reported at several locations in Coimbatore, recent observations by Prabhu et al. (2019) indicated the removal of the species from the IFGTB region where it had previously been found.

**Image 2. Herbarium of *Cordia diffusa* (MH178154).**

This study adds new findings by documenting the presence of *C. diffusa* in Kurumbapalayam, Coimbatore.

Plant species identification primarily relies on sequencing variation from reference sequences and reconstructing phylogenetic relationships (Altschul et al.

**Table 2. Average AT and GC nucleotide composition of *Cordia diffusa* species in three primers.**

| Primers | Accession number | Total base pairs obtained after sequencing | A (%) | T (%) | G (%) | C (%) | AT (%) | GC (%) |
|---|---|---|---|---|---|---|---|---|
| matK | OQ852800.1 | 727 bp | 36 | 32 | 17 | 15 | 68 | 32 |
| rbcL | OR096230.1 | 548 bp | 28 | 30 | 21 | 21 | 58 | 42 |
| trnH-psbA | OR096231.1 | 315 bp | 28 | 45 | 17 | 10 | 73 | 27 |

1997). But as Smith et al. (2008) pointed out, in order to comprehend the detected species, it is crucial to combine plant DNA data with other traits, including morphology, ecology, and development. Because the chloroplast genomes of angiosperms are so well conserved, the chloroplast regions matK, rbcL, and trnH-psbA were used in this study to barcode *C. diffusa* (Chevenet et al. 2006; Amiryousefi et al. 2018; Mehmood et al. 2020). Reliable molecular markers for species identification are provided by these conserved areas.

Across the plant taxa, rbcL is highly conserved, enabling the development of universal primers for PCR amplification. However, the variation within the rbcL region allows for species identification and phylogenetic studies (Kress & Erickson 2007; Hollingsworth et al. 2009). *C. diffusa* (OR096230.1) has a base pair length of 548. The main purpose of DNA barcoding is to tell species apart effectively due to its significant variability, which helps in identifying plant species, especially when dealing with diverse groups where other markers might struggle with distinguishing closely related taxa as mentioned by Kress et al. (2005) and Shaw et al. (2007).

## CONCLUSION

The study serves a significant part in documenting the molecular identification of *Cordia diffusa*, an endangered and steno-endemic plant species. In addition to enriching molecular databases, DNA sequences submitted to GenBank are a useful resource for upcoming studies in conservation biology and plant taxonomy. The new occurrence of the plant in Kurumbapalayam, Coimbatore, extends its known distribution and shows the need for continuous research and observation of regional plants. Further studies will concentrate on creating plans for the plant's conservation and sustainable management.

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SHORT COMMUNICATION

# First record of the sea slug *Lobiger serradifalci* (Calcara, 1840) (Gastropoda: Sacoglossa: Oxynoidae) from the Indian coast

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**Abstract:** The present study confirms the presence of a Sacoglossan (Mollusca) species, *Lobiger serradifalci* (Calcara, 1840), from India. The distribution of this species has not yet been documented in India. It has been reported from Turkey, Italy, France, Greece, Israel, and now, for the first time, from the western coast of India. *L. serradifalci* was discovered under green seaweeds in the lower intertidal zone. The species was found to remain hidden within various types of green seaweeds in the region. The study provides comprehensive details on its morphology, habitat, and taxonomy.

**Keywords:** Intertidal Sacoglossans, marine gastropods, sap-sucking sea slugs, western coast of India.

Gastropod molluscs, which comprise snails and slugs, are incredibly diversified in terms of species diversity, morphology, habitat, and numerous other characteristics. They have significant body plan differences and extend to freshwater, marine, and terrestrial habitats (Bieler 1992). Indian sea slugs were classified into 361 distinct species (Apte & Desai 2017), with an additional 33 species reported in the Gulf of Kachchh (Apte et al. 2010) and 24 species reported on a checklist from several islands and reefs in the Gulf of Kachchh of the Gujarat coast (Rao & Sastry 2005). There are 95 species found throughout the Gujarat coast; seven of these are found in Saurashtra (Poriya et al. 2015; Vadher et al. 2020). The food habits of gastropods include herbivores, omnivores, carnivores, and parasites, some of which drill through the shells of other animals to feed. One of the few examples of specialized herbivores in the marine environment is the fascinating clade of opisthobranch molluscs known as the Sacoglossans. Each sacoglossan species is generally associated with a single genus or species of macroalgae and shows a close evolutionary relationship with its algal host(s) (Krug 2011). Sacoglossa, also known as the “solar-powered sea slugs,” are divided into two clades: the shelled families, Oxynooidea, and the shell-free families, Plakobranchacea (Handeler et al. 2009). The superfamily Oxynooidea comprises four families, including the Oxynoidae, which includes sea snails and bubble snails (Bouchet et al. 2005). Out of the three genera of the Oxynoidae family, *Lobiger* (Krohn 1847) comprises only five species: *L. nevilli* Pilsbry, 1896; *L. sagamiensis* Baba, 1952; *L. serradifalci* Calcara, 1840; *L. souverbii* P. Fischer, 1857; *and L. viridis* Pease, 1863 (MolluscaBase 2023). The present study marks the first record of *L. serradifalci* from the Indian coast, specifically along the Adri coast of Gujarat. Given its highly cryptic nature, the observation of *L. serradifalci* proves to be particularly challenging, especially when concealed beneath green algae. The elusive nature of *L. serradifalci,* combined with its habitats with green algae, imparts an added layer of difficulty to its discovery. The species’ predominantly green coloration further camouflages

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it within its surroundings, making it one of the most challenging Sacoglossan species to discern in this coastal ecosystem. This research has provided information on the habitat, morphology, and systematics of this species.

## Material and Method

The specimens of sacoglossans, referred to as the genus *Lobiger*, were occasionally observed in the intertidal zone of the coast during field visits. The specimen was collected from a tide pool on the Adri coast (21.60194 ˚ N, 70.47611 ˚E) on January 22, 2024 (Image 1A). The Adri coast is located on the west coast of India and has a mixed type of intertidal habitat with rocky outcrops studded with sand (Image 1B). The supratidal zone is covered with sand; the upper, middle, and lower intertidal zones are rocky-sandy. A single sample was collected, cleaned and preserved in a 10% buffered formalin solution. The morphological characteristics of the species were observed using a stereo zoom microscope (Model S.N.-391). The voucher specimen was deposited at the Museum of the Department of Zoology, Bahauddin Government Science College, Junagadh. Standard references were used for identification, including Calcara (1840), Gonor (1961), Thompson (1988), Jensen (1996), and Furfaro et al. (2020). We used the WoRMS database (2023) to determine this species' current taxonomic status on MolluscaBase.

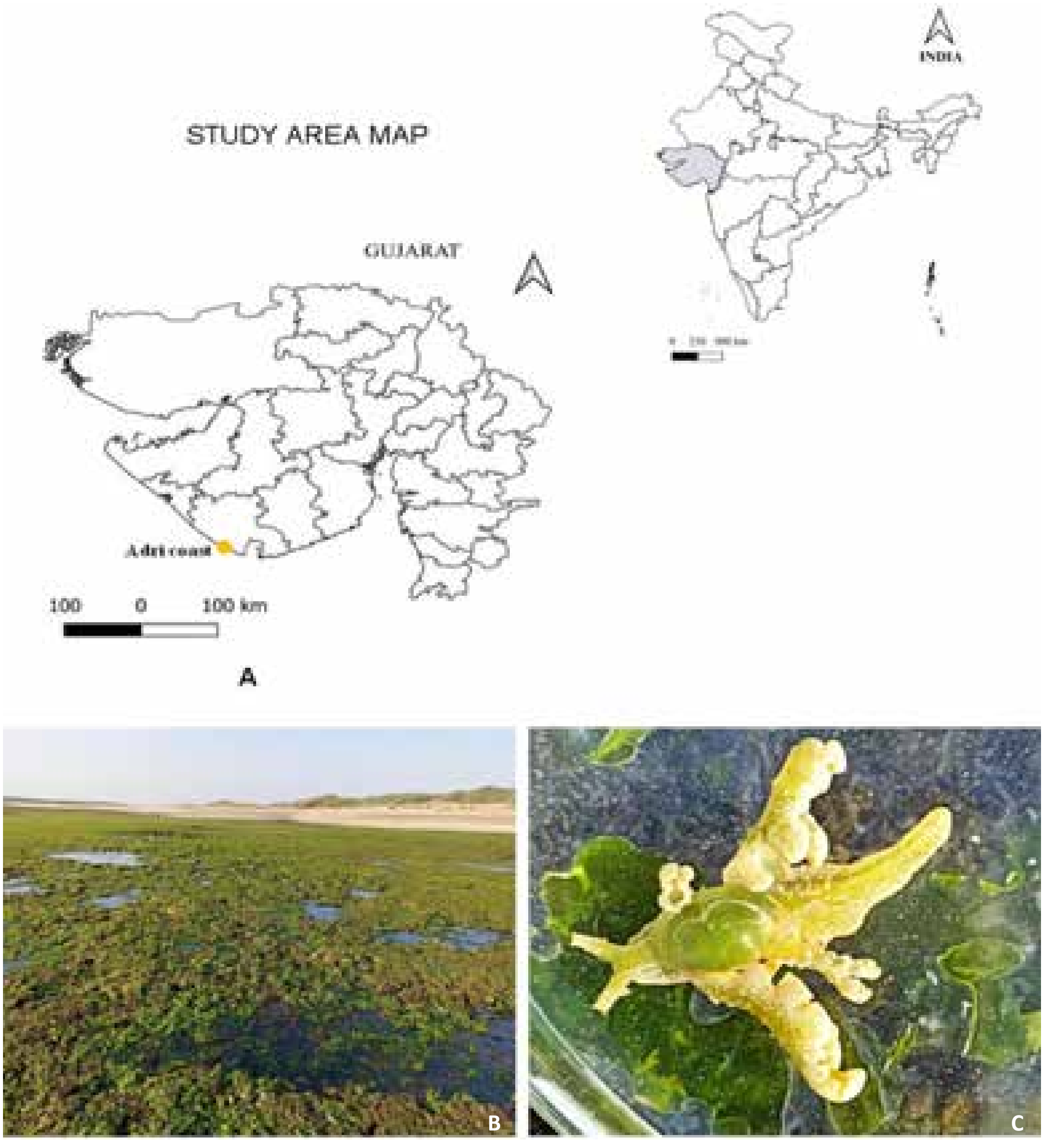


**Image 1. A—Map of the study site, Adri Coast (Gujarat), western coast of India | B—Intertidal habitat | C—*Lobiger serradifalci* associated with green seaweed. © Dimpal Dodiya.**

## Results and Discussion

### Systematics

Phylum: Mollusca Linnaeus, 1758
Class: Gastropoda Cuvier, 1797
Order: Sacoglossa Ihering, 1876
Family: Oxynoidae Stoliczka, 1868
Genus: *Lobiger* Krohn, 1847
Species: *Lobiger serradifalci* (Calcara, 1840)

### Synonyms (taken from WoRMS)

*Lobiger serradifalci* (Calcara 1840), *Bulla serradifalci* (Calcara 1840), *Lobiger philippi* (Krohn, 1847)

### Morphological Description

The specimen is 20 mm in length, with a light yellow coloration across its entire body and a greenish hue beneath the shell (Image 2B). The external shell is translucent, showing the clear grass-green colour of the underlying mantle and the rhinophores are one pair (Image 2A). There are a few scattered white papillae on the head and tentacles (Image 2A). The foot and perfectly smooth body were completely covered in green seaweed, indicating that the species prefers seaweed-covered rocky substrates. The two pairs of parapodia are large and small, smooth, stumpy, and flattened, with white on surface and green & yellow inside, almost invariably fused together, and have pinkish dorsal edges (Image 2A,B). The parapodia lobe is long compared to the shell, which is highly elevated and slightly tipped backward and the outer side is white with brown speckling (Image 2D). The foot's region features tubercles, and in addition, the tail has a median ridge covered by two comparative, less lateral ridges (Image 2A,C). The body sides are smoother than those above the level of the parapodia lobes and have less developed tubercles.

### Habitat

The specimen was found beneath green seaweeds *Caulerpa racemosa* and *Ulva* Lactuca (Image 1C) in the lower intertidal zone, which had large, deep-tide pools and areas covered with various kinds of seaweed.

### Geographical Distribution

The present study confirms the presence of *Lobiger serradifalci* on the Indian coast. It was first reported from the Bay of Palermo, Mediterranean Sea (Calcara 1840); the Turkish coast (Pruvot-Fols 1954); Palermo, Messina, Naples, Marseilles, the Balearic Islands, and Banyuls (Wirz-Mangold & Wyss, 1958); the Bay of Mersin (formerly Icel) in the Eastern Mediterranean and on the southern coast of Turkey (Swennen 1961); Villefranche-sur-Mer (Alpes-Maritimes), France (Gonor 1961); Israel (Barash & Danin 1982); Taranto (Perrone 1983); Aghios Andreas (Ionian Sea), Greece (Thompson 1988); the Salento Peninsula in Southeast Italy (Furfaro et al. 2020).

### Remarks

The morphological characteristics of *L. serradifalci* align with previous observations made by Thompson (1988). Externally, *L. serradifalci* bears a resemblance to the *Lobiger souverbii* species, as both inhabit *Caulerpa* fronds and exhibit an elongated green body with a shell (Marcus 1977; Jensen & Clark 1983). *L. serradifalci* has a light grass-green body, flattened & in-rolled parapodia, and a pink-white exterior with brown speckling. In contrast, *L. souverbii* presents distinctive morphological features, including parapodial margins that are red with dots. The mantle is yellowish-green in color beneath its transparent shell, with several shorter blue lines scattered throughout the dorsal surface. *L. souverbii* also possesses two pairs of long, upright parapodia (Ichikawa 1993). *L. serradifalci* resembles another saccoglossan species, *Lobiger viridis*. Its whole body is bright yellow in colour and one pair of short rhinophores are present above the animal's mouth. Species have been commonly found on the Indian coast, like Poshitra Reef, Ashaba Island, the Gulf of Kachchh, and Andaman Island (Parasharya 2012; Apte & Desai 2017). It is possible that *L. serradifalci* is present in intertidal habitats but due to its close resemblance, it may have been overlooked and misidentified as *L. viridis* in a previous study.

The earliest study indicated *Caulerpa racemosa* as food of *L. serradifalci* (Marcus 1977; Jensen & Clark 1983). Few studies have also reported the phylogenetic relationship between Sacoglossans and their food plants (Kay 1968; Clark & Busacca 1978). All shelled Sacoglossans use *Caulerpa sp.* as their main food source, which demonstrates the phylogenetic relationships between Sacoglossa and their food plants (Jensen 1997; Parsons 1994). *L. serradifalci* also uses *Caulerpa* sp. (Swennen 1961), including *Caulerpa prolifera* (Gonor 1961; Gavagnin et al. 1994), and *C. racemosa* (Marcus 1977; Jensen & Clark 1983) as a main food source. During the present study, species were also found beneath the green seaweed *C. racemosa* habitat in the lower intertidal zone of the Adri coast.

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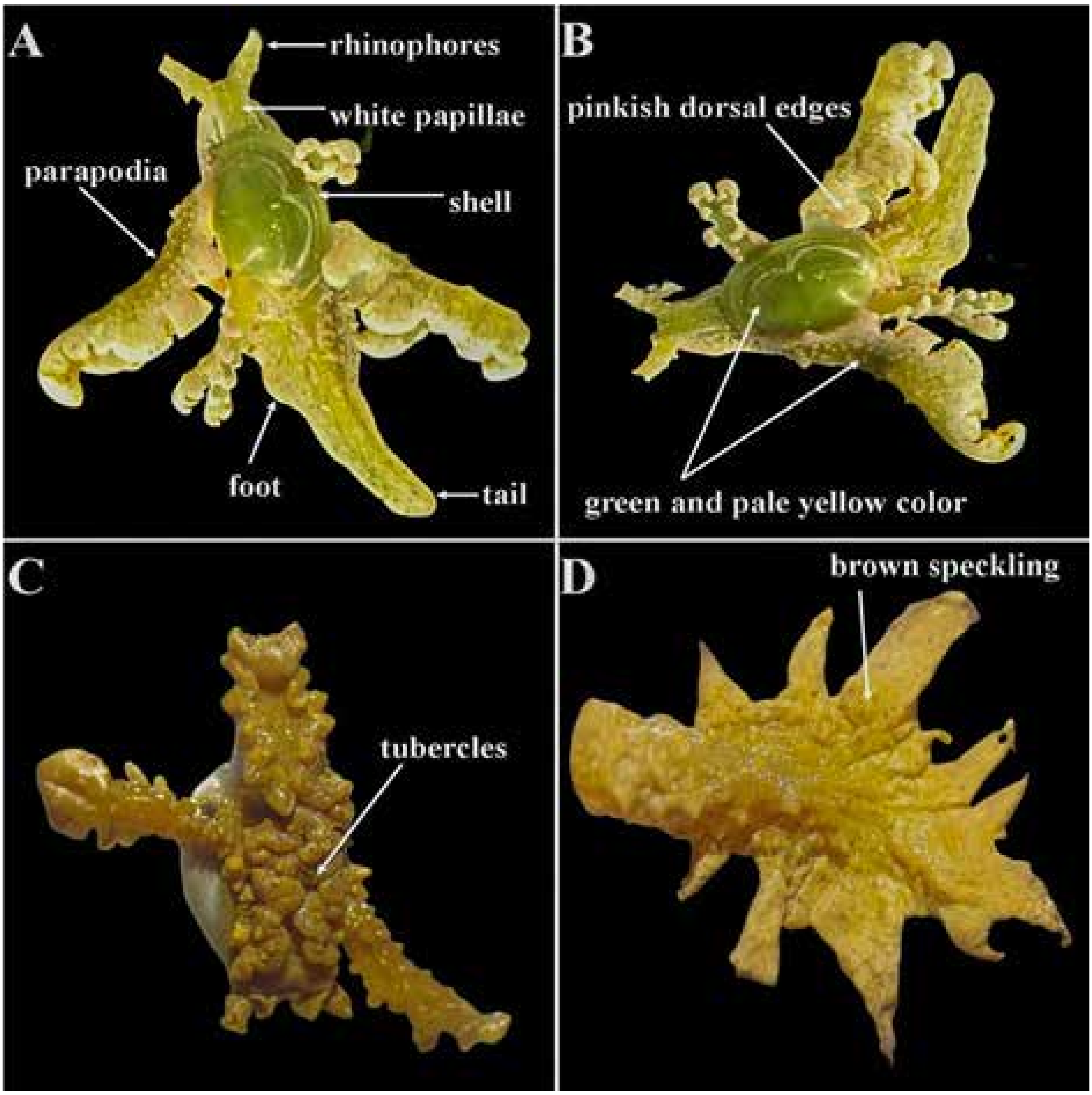


**Image 2. *Lobiger serradifalci* specimen: A—dorsal view, morphological characters (rhinophores, white papillae, parapodia, shell, foot, tail) | B—pinkish dorsal edges, green and pale yellow color | C—tubercles | D—brown speckling. © Dimpal Dodiya.**

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SHORT COMMUNICATION



# *Impatiens damrongii* (Balsaminaceae), a new record for the flora of Vietnam

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**Abstract:** *Impatiens damrongii* Shimizu, previously known as a native species of southern China, northern Laos, and northern Thailand, is found first in Vietnam, in the Nam Dong Conservation Area (Thanh Hoa Province). The voucher herbarium specimens of this species collected in Vietnam are housed in the herbarium of the Vietnam National University of Forestry. A morphological description of this species, accompanied by color photographs, data on ecology and phenology in Vietnam are additionally provided.

**Keywords:** Limestone flora, new distribution, Nam Dong Conservation Area, plant taxonomy, Thanh Hoa Province.

The genus *Impatiens* L. (Linnaeus 1753), a member of the Balsaminaceae family, is one of the most diverse groups of angiosperms, with 1000–1121 species distributed mainly in the tropical and subtropical Africa and Asia, with the center of diversity and probable origin in southern China (Grey-Wilson 1980; Chen et al. 2007; Mabberley 2017; POWO 2024). In Vietnam, 36 species of this genus were recorded before 2003 (Tardieu 1944; Nguyen & Kiew 2000; Pham 2003). Over the last two decades, seven species were additionally reported, namely: *I. purpureifolia* S.H.Huang & Y.M.Shui (Shui et al. 2011), *I. kamtilongensis* Toppin (Vu et al. 2015), *I. parvisepala* S.X.Yu & Y.T.Hou (Hoang et al. 2015), *I. napoensis* Y.L.Chen (Nguyen et al. 2018), *I. siculifera* Hook.f. (Pham et al. 2019), *I. monticola* Hook.f. (Nguyen et al. 2021), and *I. lobulifera* (Quan et al. 2024). During the botanical fieldwork in limestone areas of the Nam Dong Conservation Area in northern Vietnam in December 2023, the authors collected an unusual species of *Impatiens*. After careful morphological examination of the species and analysis of available literature as well as collections of different herbaria, namely HN, LE, VNF, and VNM (all acronyms following Thiers 2024), this plant was identified as *Impatiens damrongii* Shimizu, which was previously reported from Thailand (Shimizu 1969; Suksathan & Ruchisansakun 2022), Laos (Souvannakhoummane et al. 2021), and southern China (Chen et al. 2023). This is the first record of this species in the flora of Vietnam, the data for which are presented in this communication.

## Material and Methods

All collected and studied herbarium specimens of the newly discovered species are submitted and presently stored in the herbarium of the Vietnam National University of Forestry (VNF). Color photographs of plants were taken in natural habitats. Morphological

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observations and measurements were made on living plants, dried specimens, and alcohol-preserved materials. Morphological characters were described using the terminology proposed by Shui et al. (2011) and Ruchisansakun et al. (2018). Provide an insert map if possible.

## Results

### Taxonomic Treatment

*Impatiens damrongii* Shimizu, Acta Phytotax. Geobot. 24: 38. 1969); S.E. Asian Stud. 8, 2: 215 (1970); Souvannakhoummane et al., Edinburgh J. Bot. 78: 6 (2021); Suksathan & Ruchisansakun, Impatiens Thailand 128 (2022). – Type: Thailand, Phitsanulok Province, Phu Miang Mountain, Shimizu, T.11634 (holotype KYO!, isotype BKF!).

**Description:** Herbs perennial, up to 70 cm tall. Stem green to dark green with purple tint, erect or procumbent in basal part, laxly branched at lower nodes, smooth and glabrous. Leaves petiolate, alternate, aggregated near the stem apex; petiole (1.5) 2–4 cm long; lamina chartaceous, oblong-elliptic, 6.5–11 x 2–3.5 cm, lateral veins 3–4(5) pairs. Inflorescences arising from upper axils, 1–2 flowered; peduncle 1–3.5 cm long; rachis short; bracts persistent, ovate, 7–8 × 4–5 mm, apex mucronate, margin entire to serrate, with 2–4 pairs of glands at base. Flowers yellow, 1.7–2.1 cm wide; pedicel 10–13 mm long, glabrous. Lateral sepals 4; outer pair white with a yellow or greenish tint, obliquely orbicular or broadly ovate, 10–12 × 8–10 mm, apex mucronate, margin almost entire to sparsely serrate, with 2–4 pairs of grands at the base, glabrous to sparsely pubescent; inner pair linear to narrowly lanceolate, 18–20 × 2–3 mm. Lower sepal ca. 2.3 cm long, yellow, bucciniform, 1.2–1.4 cm long, apex apiculate, distal part abruptly constricted into a yellow, helicoid spur, spur apex bifid, gray- brown. Dorsal petal light yellow, 1.8–2.0 × 1.2–2.5, deltoid, apex emarginate, midvein adaxially with a thick crest along all its length. Lateral united petal up to 2 cm wide, yellow with red longitudinal lines in basal half, upper petals suborbicular to obovate, 20 × 5–12 mm, apex rounded; lower petals obovate, 24–28 × 10–15 mm, apex emarginate, at the base with oblong auricle. Stamens 5; filaments 7–8 mm long, flat; anthers reddish pink. Ovary fusiform, glabrous. Capsule narrowly oblong, about 2 cm long, abruptly pointed at apex. (Images 1&2).

**Flowering and fruiting:** October–December.

**Distribution:** Southern China (Yunnan), northern Laos (Louangphabang), Thailand (Phitsanulok Province), Vietnam (Thanh Hoa Province, Nam Dong Conservation Area).

**Habitat:** This species has been found in Thailand, China, and Laos, at elevations of 1200–1900 m, in humid evergreen forests often near streams. In Vietnam it was observed in primary and secondary evergreen broad-leaved lowland dry forests, along watershed in association with *Miliusa sinensis* Finet & Gagnep., *Garcinia* sp., *Begonia* sp., *Costus tonkinensis* Gagnep., *Dracaena cochinchinensis* (Lour.) S.C.Chen, *Ficus* sp., *Ocimum* sp., and *Diospyros mollis* (Kurz) Gürke at elevations of elevations of 900–950 m.

**Specimens examined:** Vietnam: Thanh Hoa Province, Nam Dong Conservation Area, on crystalline highly eroded rocky limestone along stream, around point 20.307 N & 104.888 E, at elevation 950 m, 09 December 2023, Nguyen Huu Cuong (VNF: NHC20231209001). Vietnam: Thanh Hoa Province, Nam Dong Conservation Area, dry evergreen broad-leaved secondary forest on cliffs composed by white crystalline marble-like highly eroded limestone along lake shore around point 20.306 N & 104.888 E, at elevation of about 900 m, not common, 10 December 2023, Nguyen Huu Cuong, Nguyen Van Ly (VNF: CL20231210012).

**Note:** Since *I. damrongii* is somewhat similar to *I.*

**Image 1.** ***Impatiens damrongii*** **Shimizu. A&B—flowering plants in natural habitat. © Cuong Huu Nguyen**

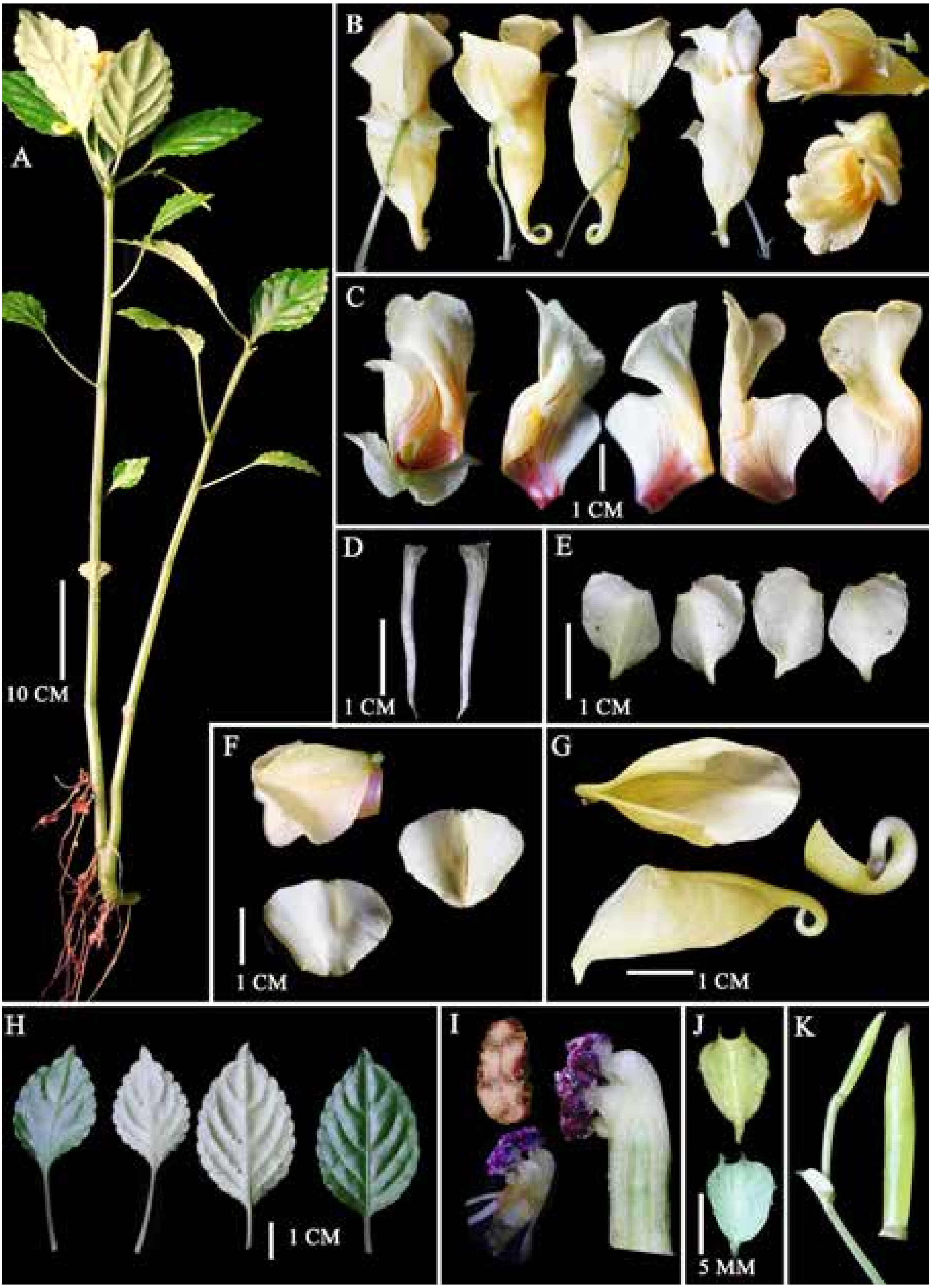


**Image 2.** *Impatiens damrongii* Shimizu: A—habit | B—flowers, views from different sides | C—lateral united petals, ventral and dorsal side | D—inner lateral sepal | E—outer lateral sepal | F—dorsal petal | G—lower sepal | H—abaxial and adaxial surface of leaf | I—stamens | J—bract | K—capsule. © Cuong Huu Nguyen

*linearisepala* S.Akiyama, H.Ohba & S.K.Wu (1996) in morphology and distribution range, further molecular study is needed to clarify their relationship. Plants of *I. damrongii* in Vietnam have minor differences from the Thailand, China, and Laos specimens, including gray-brown apex spur (vs. yellow), and habitat in the mountainous area at an altitude of 850–950 m alt. (vs. 1200–1900 m altitude).

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SHORT COMMUNICATION

# Invasive record of Brazilian Petunia *Ruellia elegans* Poir. (Acanthaceae) from northeastern India

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**Abstract:** The present study reports the invasive occurrence of *Ruellia elegans* Poir. from northeastern India. The beautiful red flowered species flourishing in Digboi, Assam is marked as its new distribution extension. The occurrence has been documented as a new distribution record for the flora of Assam as well as northeastern India. A detailed morphological depiction, photographic images, characterization and taxonomic notes have been provided to aid in its identification and recognition.

**Keywords:** Assam, biodiversity, conservation, distribution record, flora, floral attributes, identification, morphology, native, taxonomy, threatening.

The Brazilian Petunia *Ruellia elegans* Poir. belongs to a morphologically diversified tribe *Ruellieae* Dumortier. According to Angiosperm Phylogeny Group (APG), *Ruellieae* appertain in the sub-family *Acanthoideae* Eaton of family *Acanthaceae* Jussieu nom. cons. (Stevens 2001 onwards). As stated in Plants of World Online (POWO 2024) database, *Acanthaceae* contributes 208 genera and is one of the diverse plant families with estimation of approximately 4,900 species (Manzitto-Tripp et. al. 2021). Members of the genus *Ruellia* Plum. ex. L. are mostly herbs, shrubs, rarely trees or lianas (Tripp 2007). The genus is horticulturally important having 350 species (Tripp et. al. 2013). However, according to POWO (2024), the genus is portrayed with 365 species distributed in tropics to subtropics to North America. As mentioned by Tripp (2007), the species of *Ruellia* exhibit remarkable diversity in floral color, size and shape of corolla lobes, tubes, throat. According to Zhuang & Manzitto-Tripp (2022), the genus *Ruellia* manifests diversification in floral color due to anthocyanin biosynthetic pathway (ABP) and *R. elegans* was characterized with both pelargonidin and cyanidin pigments. The species *R. elegans* Poir. is native to northern, northeastern, and southeastern Brazil, predominantly thriving in wet tropical biome. Further, it has been introduced in regions of Andaman Islands, Puerto Rico, and Jamaica (POWO 2024).

In accord with POWO (2024), India is represented with six native species of *Ruellia* viz., *R. beddomei* C.B.Clarke., *R. ciliata* Hornem., *R. malabarica* Kostel., *R. patula* Jacq., *R. pseudopatula* Ensermu., *R. sibua* (Nees) I.M. Turner., and *R. sivarajanii* Sreedevi, Remadevi & Binojk. Whereas, *R. ciliatiflora* Hook., *R. simplex* C.Wright., *R. tuberosa* L. are non-native and introduced species of India. *R. elegans* is an indigenous species of Brazil (north-east, south, south-east) and introduced in the regions of Andaman Islands, Jamaica, and Puerto Rico. However, the species existence has been marked from Odisha, as a new recorded distribution for eastern India (Kalidass et. al. 2016). According to Ezcurra (1993), the species distribution has extended due to human activities.

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**Competing interests:** The author declares no competing interests.

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**Materials and Methods**

On 19 September 2023, the specimen has been allocated from the vicinity of Dehing Patkai rainforest of Digboi, Assam. Field photographs and GPS location were recorded with help of Canon IXUS 190. The vegetative and reproductive features of the collected specimen were examined through a stereo-zoom binocular microscope (Labomed CZM4). The identity of the species was confirmed through its protologue, and herbarium records. The photoplates were prepared using Adobe Photoshop software (version 7.0). The voucher specimen has been prepared following the protocol of Jain & Rao (1977) and deposited at Gauhati University Botanical Herbarium (GUBH). The map depicting the location of *Ruellia elegans* collected from Tinsukia District of Assam has been shown **(Figure 1)**.

**Taxonomic Treatment**

***Ruellia elegans* Poir.** in J.B.A.M.de Lamarck, Encycl., Suppl. 4: 727.1816. – *Ruellia formosa* Andrews in Bot. Repos. 10:t.610.1810. nom. illeg. – *Ruellia superba* D. Dietr. in Nachtr. Vollst. Lex. Gärtn. 17:307.1821. – *Arrhostoxylum formosum* Nees in A.P. de Candolle, Prodr. 11:215.1847. – *Arrhostoxylum roseum* Nees in C.F.P. von Martius & auct. suc. (eds.), Fl. Bras. 9:61.1847. – *Arrhostoxylum silvaccola* var. *montanum* Nees in C.F.P. von Martius & auct. suc. (eds.), Fl. Bras. 9:60.1847. – *Ruellia speciosa* Mart. ex Nees in A.P.de Candolle, Prodr. 11: 215.1847. – *Asystasia formosa* T. Anderson in J. Agric. Soc. India, n.s., 1: 270.1868. – *Stemonacanthus formosus* T. Anderson in J. Agric. Soc. India, n.s., 1: 270.1868. – *Ruellia rosea* (Nees) W. Bull in Nursery Cat. (William Bull) 143:8.1878. – *Arrhostoxylum elegans* (Poir.) Bremek. & Nann. Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 45(1):12.1948.

**Description**

Annual erect herbs, 30–60 cm tall, ascending, sparsely branched, pubescent, quadrangular; Leaves 4–10 × 2–4 cm in size, acuminate, simple, entire, elliptic-oblong, coriaceous, cuneate base, sparsely pubescent adaxially, glabrous abaxially, exstipulate, petiole 1–2 cm long, flat, slender, pubescent; Inflorescence axillary, arising from upper leaves, raceme, 12–15 cm long, few

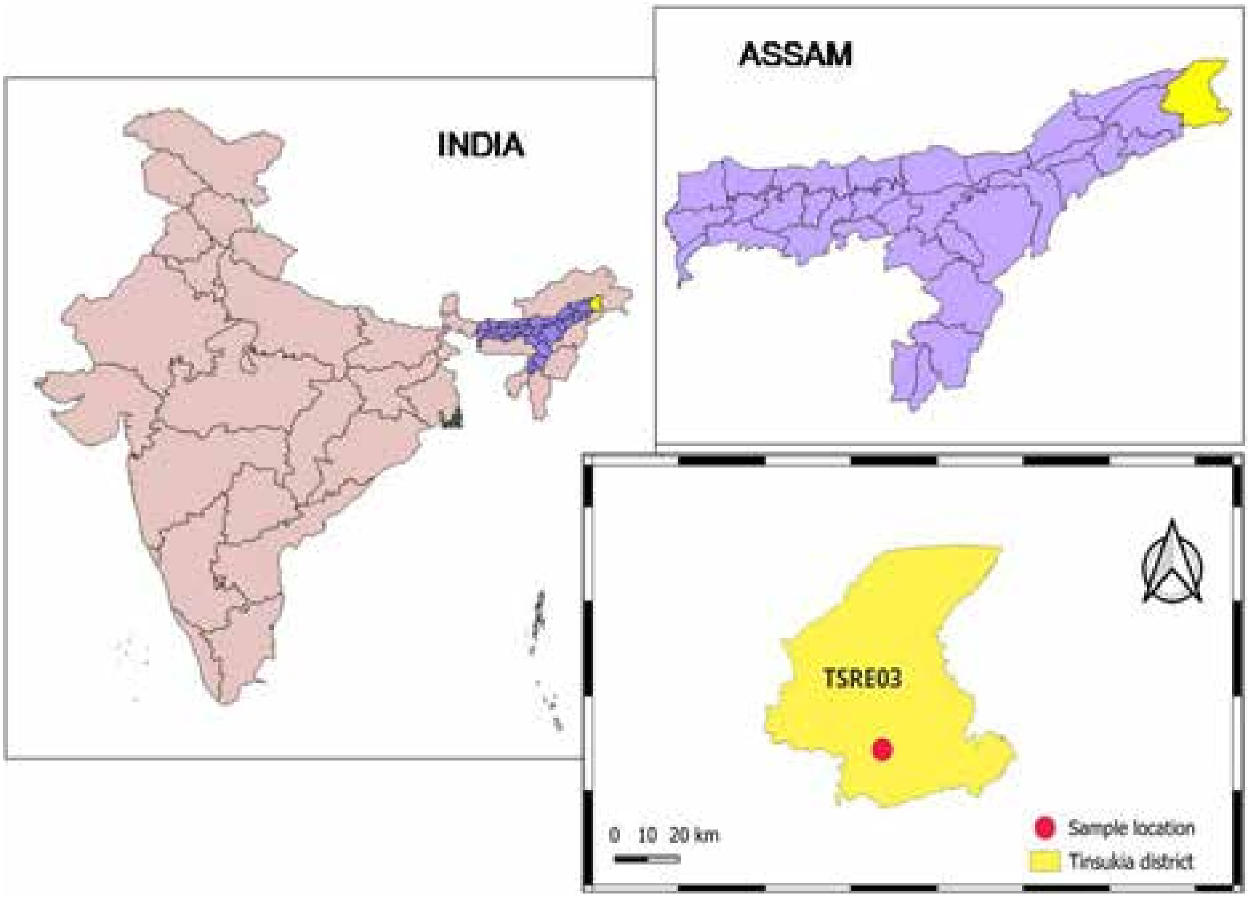


**Figure 1. Geographic location of *Ruellia elegans* Poir. in Tinsukia District of Assam (Map prepared through QGIS software 3.40.0).**

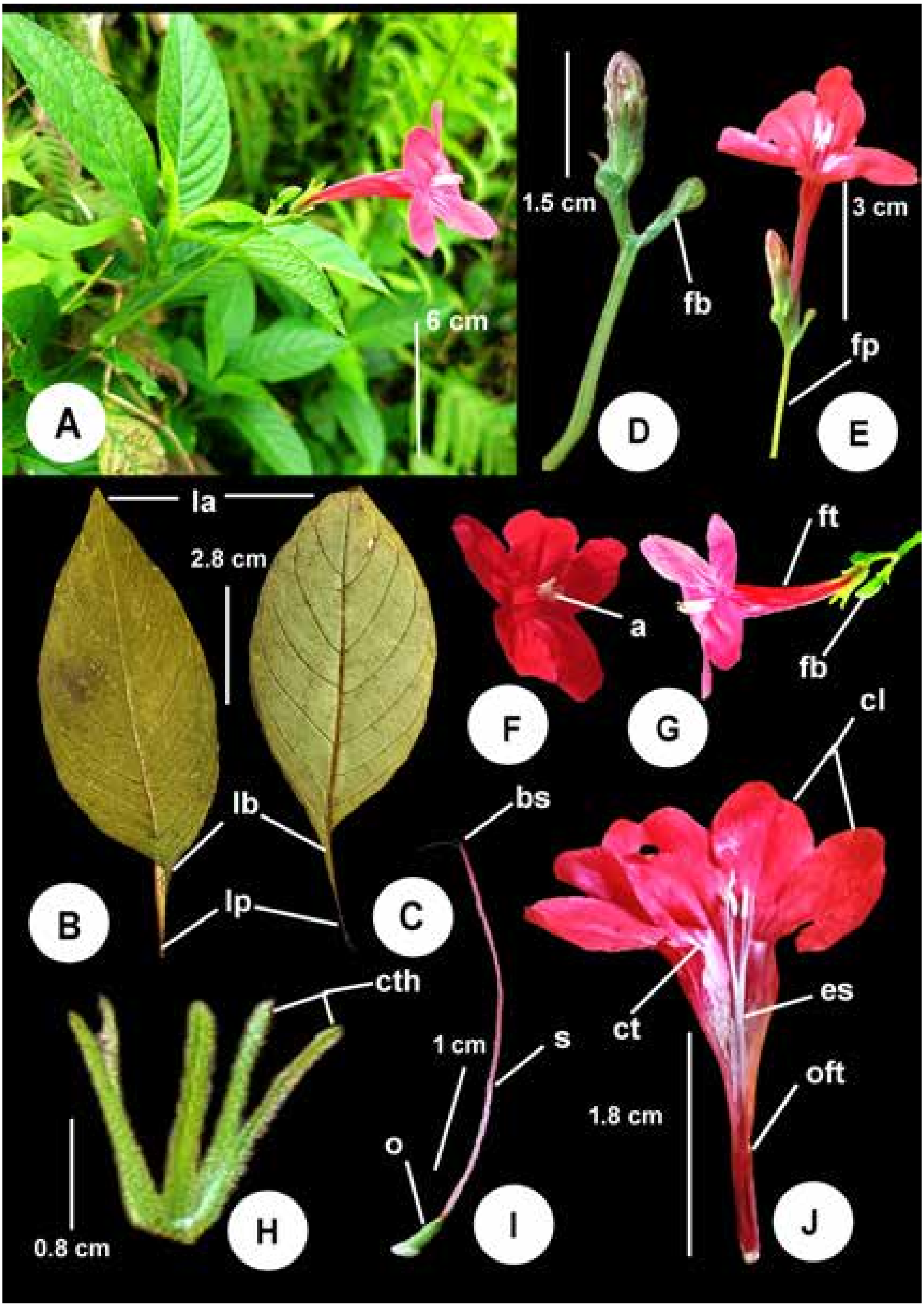


**Image 1.** ***Ruellia elegans*** **Poir.: A—habit of the plant | B—adaxial side of the leaf surface | C—abaxial leaf surface showing leaf apex (la), leaf base (lb) and petiole (lp) | D—flower bud with floral bract (fb) | E—complete flower displayed on its floral pedicel (fp) | F—frontal view of the flower exhibiting anthers (a) | G—side view of the flower portraying floral tube (ft) and floral bract (fb) | H—calyx displaying five tooth (cth) | I—gynoecium with ovary (o), style (s) and bifid stigma (bs) | J—dissected corolla manifesting open floral tube (oft), epipetalous stamen (es), corolla lobes (cl) and corolla throat (ct). © Mamita Kalita.**

flowered; Bract spathulate, pubescent, 0.8–1.2 × 0.4–0.6 cm; Flower pedicellate, bright red trumpet shaped, 4–5.5 cm long, bisexual, regular; Calyx 1.5–1.8 cm, five sepals, valvate, green, woolly, unequal lobes, united at base; Corolla with five petals, 1–1.5 cm segregated limbs above, lower part united to form 3–4 cm long floral tube, throat curved, pink-white patch inside, minutely hirsute outside, peduncle 0.5–1 cm long; Stamens 4, didynamous, 3.2–3.8 cm long, anthers white, 2.5–3 mm long, sub-extrorse, versatile, dithecous; Gynoecium 3.8–4 cm long, stigma bifid, pink, style pinkish white, ovary 2.8–3.7 mm, elliptic, glabrous, ovary disc 0.8–1 mm, cream-white. (Image 1)

**Common names:** Brazilian Petunia, Christmas pride, Elegant Ruellia, Red Ruellia, Wild Petunia.

**Habitat:** Terrestrial species found in high rainfall regions in wild or semi-wild patches/ margins of forest. Also, it grows well in understory of forests where partial sunlight falls.

**Phenology:** The flowering and fruiting has been observed during September.

### Specimen examined

**Brazil:** South Brazil, Prov. St. Paul and Rio, Leg. Weir, J. 1861–62. #s.n. (K001048032! K001048033!); Environs de Rio de Janeiro et D´Ouro Preto, Leg. Moss A. Glaziou, v.1885. Coll.No.15297 (K001048034!); São Paulo, Leg. M. Kuhlman, 13.xi.1947. Coll.No.1518 (K001048038!); Minas Gerais, 20.3294°S, 46.3052°W, altitude 780 m, Leg. Souza, V.C. 14.i.1994. Coll.No.5059 (K001048037!); Near Rio, Leg. Graham. #s.n. (K001048035!) Determined by C. Kameyama, 1995; São Paulo, Leg. M. Groppo Jr. 26.ii.2001. Coll.No.595 (K001048036!). **India:** Andaman & Nicobar Islands, Port Blair, Nayagaon, South Andamans, Dr. S. Kumar, 15.iii.2008. Coll.No.26343 (PBL0000015227! PBL0000015228!). **Assam:** Tinsukia, Digboi, Near Dehing Patkai WS, Mamita Kalita, 19.ix.2023. Coll.No. TSRE03 (GUBH20634).

### Taxonomic note

The specific epithet `*elegans*´ propound the pleasing appearance or bounteous nature of the species. The herbarium specimen (K001048032, K001048033, K001048038) were initially identified as *Ruellia formosa* and (K001048032, K001048033, K001048034, K001048035) as *Arrhostoxylum formosum.* Later, the correct identification was provided as *Ruellia elegans* and the former two names are now treated as synonyms. A write up over the herbarium sheet K001048035 describe the species as plants with bright red flowers having four anthers (two long and two short ones). As mentioned by Ezcurra (1993), the calyx and corolla morphology of *R. elegans* is somewhat similar to *R. sceptrum-marianum* (Vell.) Stearn., and *R. acutangula* Nees, *R. silvaccola* (Nees) Lindau. Further, he described the species as red flowers having horizontal corolla forming a narrow tube with weak peduncles and pedicels. The presently examined species have similar floral attributes as described.

### Conclusion

At the location site, altogether 8–10 individuals of the plant species were seen, each differing in their reproductive growth. Few other species growing along with *Ruellia elegans* are: *Diplazium* sp., *Dryopteris* sp., *Isodon* sp., *Oxalis debilis*, *Plantago major*, and *Rostellularia* sp. According to Rao & Kumar (2024), the species conservation status is not assessed in Brazil. The impacts of this invasive species should be monitored to take appropriate measures to control its spread threatening the native biodiversity.

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NOTE

# *Cuphea carthagenensis* (Jacq.) J.F.Macbr. (Lythraceae) — a new non-native plant record for the Eastern Ghats of India

Prabhat Kumar Das [1], Bishal Kumar Majhi [2], Shashi Sourav Hansda [3], Samarendra Narayan Mallick [4], Purnendu Panda [5] & Pratap Chandra Panda [6]

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During the ongoing plant diversity inventory of the Eastern Ghat region of Odisha, the authors collected specimens from two populations of an interesting species of *Cuphea* with glandular-pubescent aerial parts and pale-purple to deep pink flowers from Sunabeda Plateau and Pendajam waterfall of Koraput District of Odisha. After examination of plant specimens, consultation of relevant literature, and matching with authentic herbarium specimens, it is identified as *Cuphea carthagenensis* (Jacq.) J.F.Macbr. (Lythraceae), a species native to South America.

In India, half-a-dozen species of *Cuphea* P.Browne have so far been reported, such as *C. hyssopifolia* Kunth, *C. carthagenensis* (Jacq.) J.F.Macbr.*, C. ignea* A.DC.*, C. lanceolata* W.T.Aiton*, C. micropetala* Kunth, and *C. procumbens* Ortega, which are commonly grown as garden ornamental plants or found as alien weeds in waste places, roadsides and degraded forest areas (Das & Chanda 1987; Singh et al. 2000; Paul & Kumar 2023).

Among these, the species *C. carthagenensis* is so far been reported from northeastern states such as Assam, Arunachal Pradesh, Nagaland (Naithani 1990; Naithani & Bennet 1990), Mizoram (Singh et al. 2002), Darjeeling Hills of West Bengal (Paul & Kumar 2012), and Manipur (Panmei et al. 2019) as a naturalized weed in cultivated lands, disturbed habitats and also sometimes in open forests. There is no report of its occurrence in the Eastern Ghats of India, which covers the states of Odisha, Andhra Pradesh, Tamil Nadu, and Karnataka nor from peninsular India. The present report of its occurrence from Odisha is very interesting in view of its naturalization extending to the eastern parts of the country (Image 1).

***Cuphea carthagenensis*** (Jacq.) J.F.Macbr.,Publ. Field Columb. Mus., Bot. Ser. 8: 124. 1930; Naithani, Fl. Pl. India, Nepal & Bhutan: 172. 1990; Naithani & Bennet, Ind. For. 116(5): 423. 1990; Chowdhery & al. in P.K. Hajra

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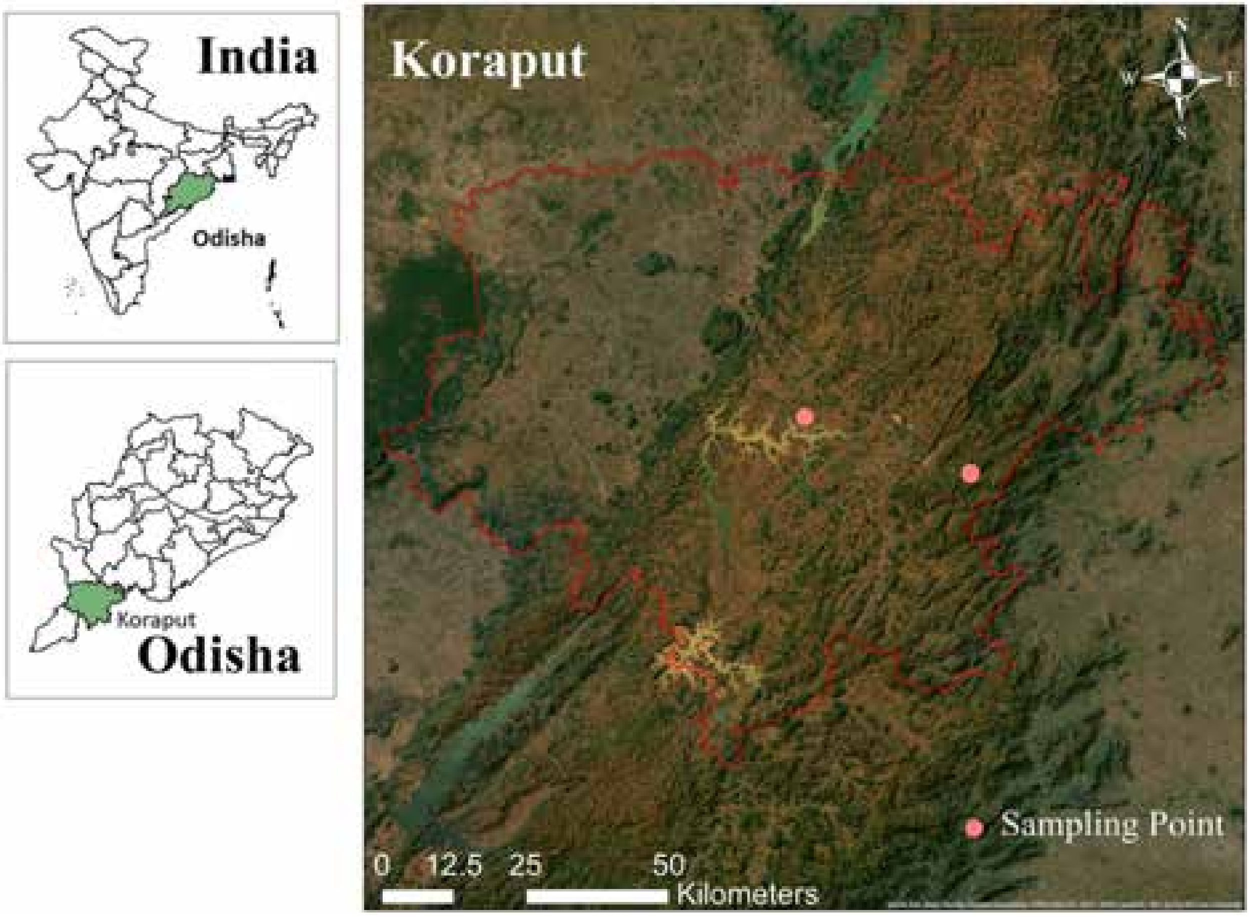


**Image 1. Map showing the localities of collection of *Cuphea carthagenensis* in Koraput District, Odisha.**

& al., Mater. Fl. Arunachal Pradesh 1: 496. 1996; Paul & Kumar, Pleione 6(1): 244. 2012; Graham, Syst. Bot. 42(4): 876. 2017; T. Mathew & al. in A.A. Mao & S.S. Dash, Fl. Pl. India Annot. Checkl. Dicot 1: 558. 2020; Paul & Kumar, Fl. Mahananda Wildlife Sanctuary: 69. 2023. *Lythrum carthagenense* Jacq., Enum. Syst. Pl. 22. 1760. *Cuphea pinto* (Vand.) Kuntze, Revis. Gen. Pl. 3(3): 96. 1898. *Cuphea balsamona* Cham. & Schlecht. in Linnaea 2: 363. 1827. *Cuphea prunellifolia*, FI. Bras. Mer. 3: 108. 1833. (Images 2 & 3)

Common name: Colombian Cuphea, Colombian wax-weed.

Perennial herb, erect or spreading, much branched from near the base, 10–40 cm tall, young parts hairy. Stem viscid-pubescent, with intermixed glandular and non-glandular hairs; trichomes sparse to abundant, erect, reddish-purple. Leaves opposite, spreading, subsessile to shortly petiolate, gradually reduced in size in inflorescence; broadly to narrowly elliptic or elliptic-oblong, rarely ovate, 0.9–3.2 X 0.6–2.0 cm, base acute to attenuate, apex acute or acuminate, margin entire; upper surface green, lower surface pale or both surfaces with scattered trichomes, midvein very prominent, secondary veins 5–6, prominent on dorsal side. Flowers extra axillary, solitary or in an indistinct leafy raceme, 4–6 mm long; pedicels 1–2 mm long; bracteoles lanceolate-linear. Floral tube, including a descending spur, sparsely pubescent with glandular hairs, distally rose-purple, otherwise green, ampullaceous with neck and mouth contracted in fruit. Calyx tubular, 3–5 mm long, longitudinally ribbed with a short spur at the base of posterior side; lobes short, equal, deltate, apex acute or short bristle-tipped. Petals 6, light pink to pink-purple, subequal, oblong, elliptic, or sub-spatulate, up to 3 mm long. Stamens 11, slightly longer than the floral tube; 2 short dorsal most inserted deepest, other 9 alternately unequal in length, inserted at the same level; filaments cream coloured, anthers light yellow. Ovary oblong, 0.5 mm long, glabrous; ovules 4–6; stigma capitate. Capsules enclosed by hypanthium, dehiscing on one side; seeds brownish, 2–6, 1–1.5 mm across, obovate to elliptic or subcircular, apex obtuse to shallowly retuse,

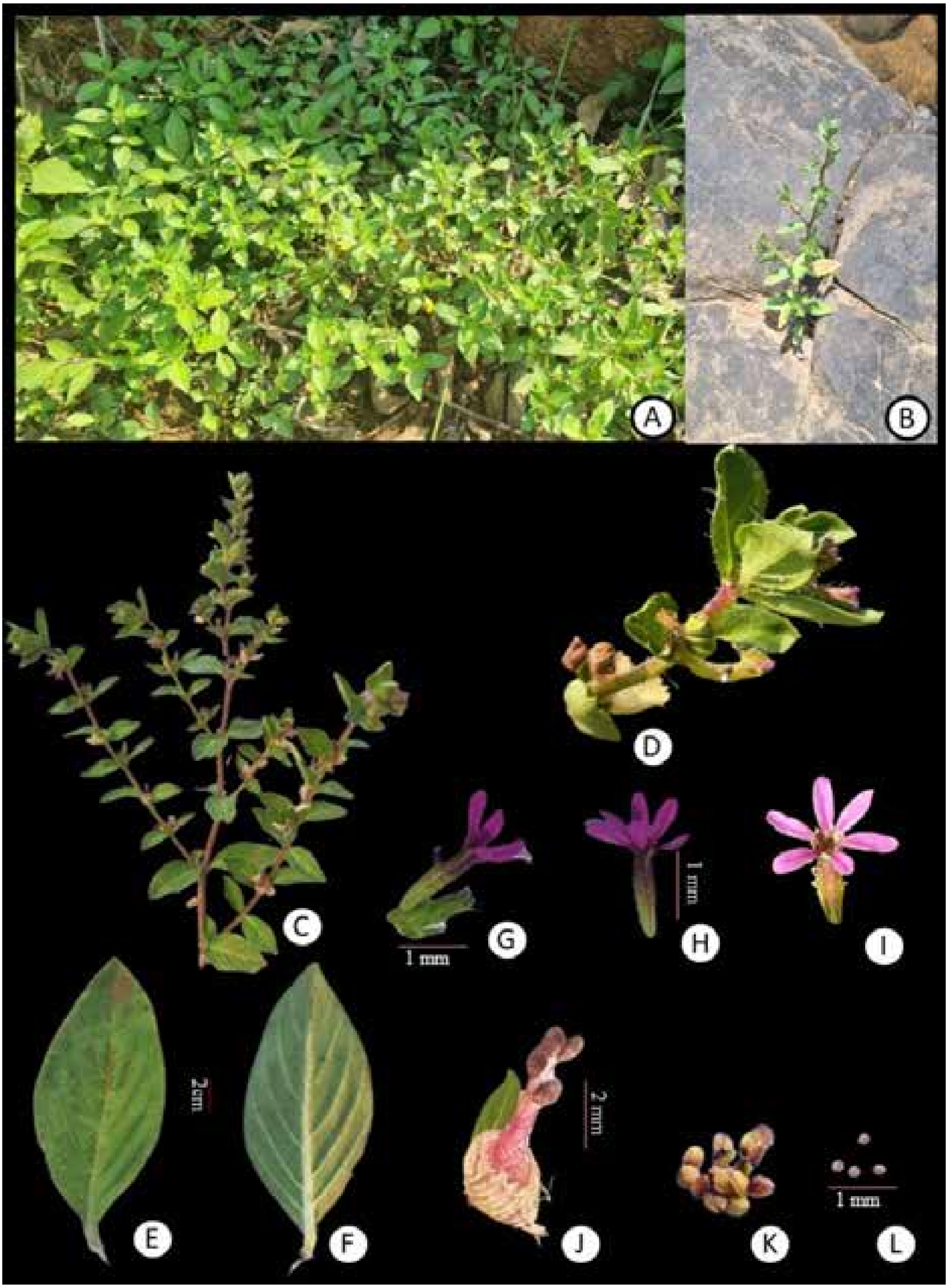


**Image 2. *Cuphea carthagenensis*: A&B—habitat | C&D—flowering and fruiting branch | E&F—abaxial and adaxial view of leaf | G—flower | H&I—flower cut open to show sepals, petals and anthers | J & K—seeds exposed on the placenta | L—seeds.**


**Image 3. Herbarium specimen of *Cuphea carthagenensis* (Jacq.) J.F.Macbr. (Image credit: P.K. Das).**

margin thin, flattened, especially at micropylar end, surface verrucose.

Flowering and fruiting: February–May.

Distribution: Though the native range of *Cuphea carthagenensis* is South America covering Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guyana, Paraguay, Peru, Suriname, and Venezuela (Graham 2017), it has widely naturalized outside its native range, in the subtropical and tropical parts Central America, North America, the Caribbean, Oceania, and Asia. In Asia, the species is reported to occur in China, East Timor, India, Indonesia, Japan, Malaysia, Myanmar, Philippines, Singapore, and Taiwan. In Oceania, *C. carthagenensis* is known from American Samoa, Australia, Micronesia, Fiji, French Polynesia, New Caledonia, Papua New Guinea, Western Samoa, Tonga, and Vanuatu. While it is a dominant weed of rice fields in Assam, India; it invades corn (*Zea mays*) plantings in Indonesia, Coconut *Cocos nucifera* groves in Vanuatu and Taro *Colocasia esculenta* fields in Fiji (Bradley 2022).

In India, the species has been previously reported from northeastern states (Assam, Arunachal Pradesh, Mizoram, Meghalaya, Nagaland, Manipur), higher hills of Darjeeling in West Bengal, and now from Koraput, Odisha located in the eastern region of the country indicating its spread as an invasive species.

Specimens examined: 2544/CBT, 20.iv.2024, India, Odisha, Koraput, Sunabeda, Central University of Odisha campus, 18.701 N & 83.012 E, 2,112 m, coll. P.K. Das & P.C. Panda; 2545/CBT, 14.v.2024, India, Odisha, Koraput, Pendajam waterfall, on the way to Putsil, 18.739 N & 82.808 E, 1,976 m, coll. P.K. Das and S.N. Mallick.

Specimen images viewed online: 1650 (K), 600 m, coll. M. Etuge, Mt. Kupe, Cameroon (K000026893); 9500 (K), 2,000 m, coll. M. Cheek, Muanenguba, Cameroon (K000026892); 9559 (K), 1,200 m, coll. M. Cheek, Muambong to Mwabag, Cameroon (K000026894).

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