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Cover: The nine vultures of India, digital art made on Krita by Dupati Poojitha.



## Floral inventory and habitat significance of riparian ecosystem along the banks of Chithari River, Kasaragod, Kerala, India

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**Abstract:** The Chithari River, spanning approximately 25 km in Kasaragod District, Kerala, originates from lateritic midlands rather than mountains, forming a riparian landscape. This study conducted a comprehensive floristic survey of its riparian zones, covering four major tributaries—Pullur Thodu, Para Thodu, Cherakkappara Thodu, and Pakkam Thodu—using line transects, and nested plots between October 2024–February 2025. A total of 340 plant species, representing 257 genera, and 75 families, were documented. Fabaceae (61 species) emerged as the dominant family, followed by Rubiaceae (20 species), Euphorbiaceae (17 species), and Moraceae (16 species). The recorded flora included 181 tree species, 45 shrubs, 69 herbs, and 45 climbers. Of these, 281 species were native, while 59 were exotic, comprising 17% of the total flora. Invasive species such as *Lantana camara*, *Chromolaena odorata*, and *Eichhornia crassipes* were found to impact various habitats significantly. The study also identified 68 endemic species, including 24 species endemic to the Western Ghats. Nine species were categorized under conservation concern, including *Crinum malabaricum* (Critically Endangered), *Pterocarpus santalinus* (Endangered), and *Strobilanthes ciliatus* (Vulnerable). The restricted distribution of *Crinum malabaricum* in lateritic streambeds of the midlands highlight the need for targeted conservation measures.

**Keywords:** Checklist, *Crinum malabaricum*, diversity, endemic species, invasive, Kanhangad, riverine, *Strobilanthes ciliatus*, survey, trees.

**Editor:** Anonymity requested.

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**Author contributions:** All the three authors contributed equally toward field data collection, manuscript writing and proof reading.

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## INTRODUCTION

Being one of the most diverse and dynamic floral habitats, riparian ecosystems form a crucial connection between land & water, playing an essential role in ecological functions, and human well-being (Davis et al. 2006; Balangen et al. 2021; Vincy & Brilliant 2024). Found along rivers, streams, lakes, and wetlands, riparian zones consist of vegetated habitats that serve as biodiversity corridors, providing habitat for a variety of plant, and animal species, and maintain the health of aquatic ecosystems (Corbacho et al. 2003; Leibowitz 2003).

The functions of riparian vegetation include filtering out chemical pollutants and acting as a natural buffer that prevents agricultural runoff from contaminating water bodies. These areas also contribute to essential processes such as organic matter decomposition, nutrient cycling, and pollution control, while supporting ecological food chains (Holmquist et al. 2011; Jonsson et al. 2017). The deep roots of large plants help reinforce banks, reducing the risk of erosion, and collapse (Perucca et al. 2007; Almadin 2020). Aquatic macrophytes, a vital component of riparian ecosystems, on the other hand, enhance habitat complexity by providing shelter, and food for invertebrates, fish, and waterbirds (Thomaz & da Cunha 2010). Additionally, these vegetation zones have influence over water temperature regulation, light availability, and affect nutrient cycles (Gregory et al. 1991; Naiman et al. 1993; Hood & Naiman 2000; Prach et al. 2001).

Studying floral association and diversity, regardless of habitat type, provides valuable knowledge for plant resource management (Cunningham et al. 2015). The southern Western Ghats, known for their unique endemic species, and rich plant life, emphasize the importance of riparian research (Vincy & Brilliant 2024). With increasing environmental changes and declining river health, a comprehensive approach to riparian zone conservation is necessary (Singh et al. 2021). Since plant species composition varies near water bodies, preserving riparian areas is crucial for maintaining landscape diversity (Sabo et al. 2005; Scalley et al. 2009).

Despite the considerable changes occurring to these habitats, which comprise wetlands and adjacent riparian systems, these areas still support the emergence of new species, as shown by the many new plant, and animal discoveries made in the past two decades (Sabu & Ambat 2007).

A high degree of disturbances such as human habitation, agricultural and industrial runoffs make the riparian zone susceptible to invasions by alien species that are generally early seral species (Richardson et al. 2007). Studies have shown that riparian plant community structure is connected to land use, and areas adjacent to agricultural, and urban stretches have been found to have high invasive species cover, and richness (Meek et al. 2010; Méndez-Toribio et al. 2014).

This study is focused on the river Chithari that flows westwards from the Western Ghats — a global biodiversity hotspot (Myers et al. 2000). The Chithari River originates in the mid-lateritic zones of Kasaragod District. The riparian vegetation of the Chithari is heterogeneous and is undergoing gradual degradation owing to the growing human disturbances, agriculture, and excessive use of chemical fertilizers, and pesticides. Local administrations have developed participatory micro-plans through active engagement with community stakeholders for the selected tributaries of the river. These plans aim to address site-specific conservation needs, promote sustainable resource use by incorporating local knowledge.

Several faunal surveys and documentation efforts have been conducted in the Chithari River and its adjacent areas. The River Research Centre (2013) carried out a preliminary assessment of fish diversity, documenting 38 species from this riverine habitat. eBird, a citizen science-based birding platform, records the presence of 169 bird species from the estuarine habitat of the Chithari River (eBird 2025). The discovery of a new tree-spider crab, *Leptarma biju*, along the riverbanks further highlights its ecological significance (Ng & Devi 2020). Additionally, GIS-based shoreline studies have provided valuable insights into the dynamic nature of the Chithari estuary (Vijayan et al. 2009). Phytological studies in and around this riverine habitat remain limited. As an initial step toward addressing this gap, we surveyed the riparian vegetation, and compiled a floral inventory, the preliminary findings of which are presented in this study.

## MATERIALS AND METHODS

The Chithari River (Figure 1), spanning approximately 25 km (River Research Centre 2013), is located in Kasaragod District, northern Kerala. Unlike most rivers in the region, it originates from the lateritic midlands rather than the mountains, resulting in a distinctive landscape. Instead of a single point of origin, the river forms from multiple smaller streams, locally known as

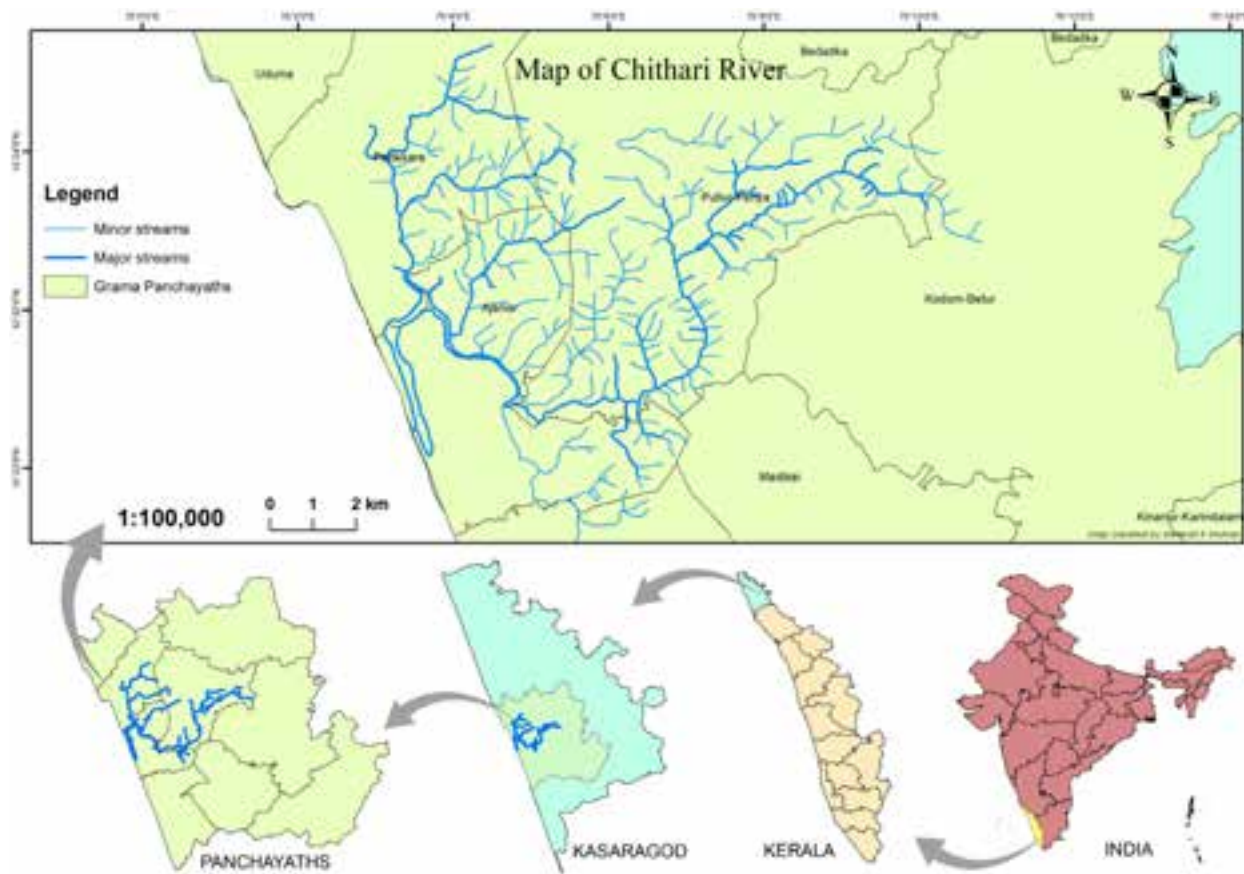


Figure 1. Map showing the Chithari River in Kasaragod, Kerala.

*Thodu*, which emerge from different locations within the midlands. The longest parent stream, Pullur Thodu, originates near Kanjiradukkam ( $12.413^{\circ}$  N,  $75.159^{\circ}$  E) at 150 m. It initially flows southwest through the sloping midlands for about 18 km before reaching the plains, where it gradually takes a U-turn near Pullur, and flows on the northwestern side. It then merges with the tributary from the northern side, attaining its full course before continuing for another 3 km and finally meeting the Arabian Sea at the Chithari estuary ( $12.342^{\circ}$  N,  $75.061^{\circ}$  E).

The land use along the river varies throughout its course. In its upper stretches, the initial streams flow through small hills, valleys, lateritic plains, and patches of forest. As it progresses, human-dominated landscapes, including plantations of coconut, rubber, arecanut, paddy fields, and sacred groves begin to influence its surroundings. Near its mouth, the river forms an estuarine habitat characterized by mangroves, marshes, estuarine creeks, and mudflats.

Four major tributaries were identified for the study. They are Pullur Thodu, Para Thodu, Cherkkappara

Thodu, and Pakkam Thodu (Figure 2). These were sampled in October 2024–February 2025. To document the riparian flora, 200 m longitudinal transects were randomly established along the course of four tributaries (Khumbongmayum et al. 2005). Each transect extended 5 m on either sides from the stream edge (Nagarajan & Bhasker 2023), and all tree species, woody climbers, and invasive plants within these belt transects were recorded. Additionally, trees observed opportunistically along the stream banks—outside the established transects—were also documented. This supplementary approach aimed to ensure a more comprehensive species inventory, especially for sparsely distributed or habitat-specific species that may not occur within the fixed transects. While the transects formed the primary framework for systematic sampling, opportunistic observations enhanced the overall floristic richness documented. Transects were established along the final stretch of the river which comprise of estuarine and coastal habitats. A total of 15 transects were considered, which were evenly spread across the tributaries and the final stretch of the river (Figure 2). At the start of each transect, a  $5 \times 5$  m

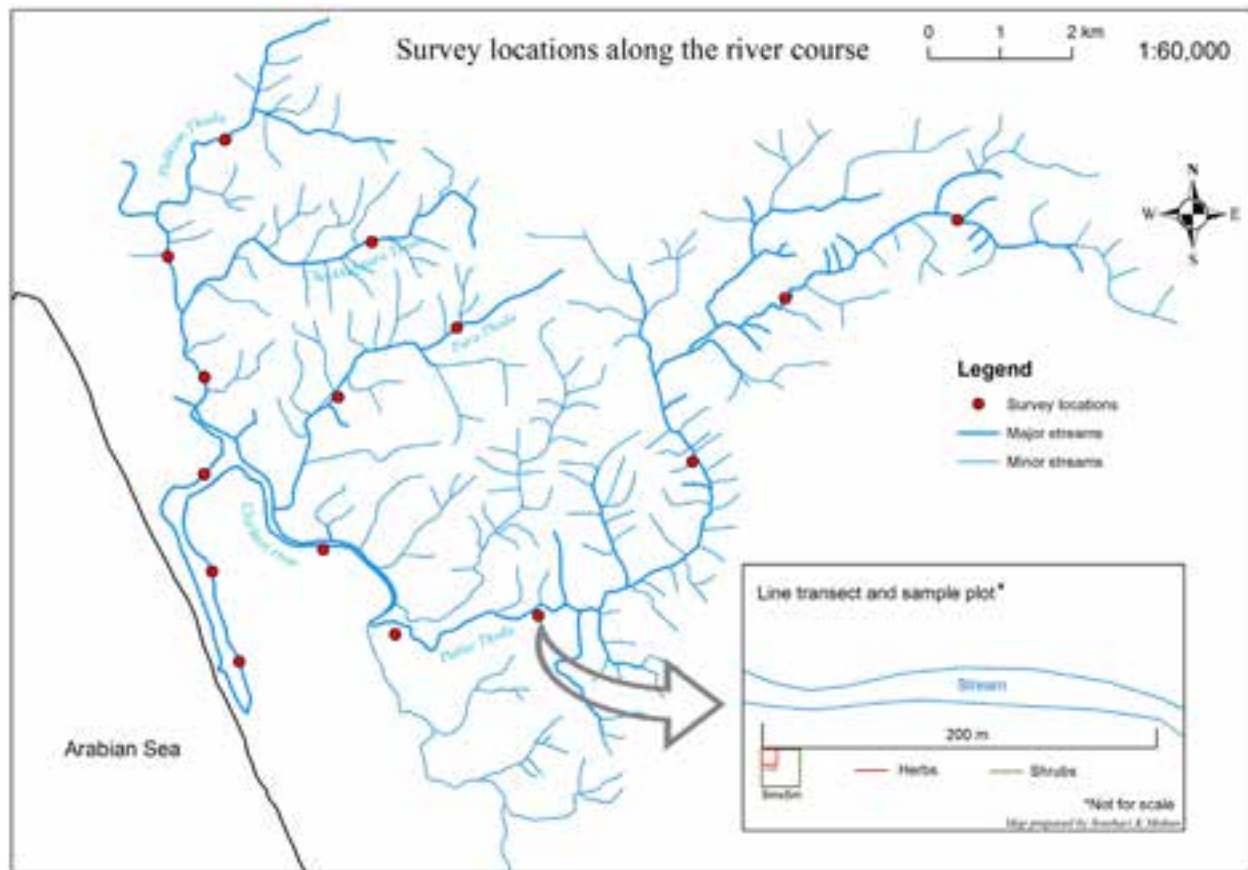


Figure 2. Survey locations along the river course.

square plot was laid out to document shrub species. A 2 × 2 m subplot, nested within this larger plot, and sharing the same starting corner (Figure 2), was used to record herbaceous species (Khumbongmayum et al. 2005; Nath et al. 2005; Jayakumar et al. 2011). The elevation across the study area ranged 0–160 m.

The observed plants were identified with the aid of regional floras (Hooker 1872–1897; Gamble 1915–1935; Sasidharan 2004, 2007, 2010; eFlora Kerala 2025). A checklist was prepared based on the APG IV system of classification and the families were arranged alphabetically with representative species within it. Information such as, habit for each species, endemism (eFlora Kerala 2025), and IUCN Red List status for the relevant taxon is also provided.

## RESULTS

During the present study, a total of 340 plant species belonging to 257 genera, and 75 families were recorded (Table 3, Images 1–32). It was observed that 43% of the recorded species belonged to seven dominant families,

namely: Fabaceae with 61 species, Rubiaceae with 20 species, Euphorbiaceae with 17 species, Moraceae with 16 species, Acanthaceae with 12 species, Apocynaceae with 11 species, and Asteraceae with 10 species (Figure 3). The study documented 181 species of trees, 45 species of shrubs, 68 species of herbs, and 46 species of climbers along the riparian stretches of the Chithari River (Figure 4). Among the 340 species recorded, 281 were recognised as native, while 59 were non-native (Table 3). Also, 308 species were found to be dicots, 29 species were monocots, and three species of pteridophytes. The study site has also shown significant diversity in terms of endemic species. A total of 68 species are endemic to this region, out of which 24 species are endemic to the Western Ghats (Table 1). This implies 7% of the total plants encountered in the study area are Western Ghats endemic species.

Nine species from the study area were found to be of critical conservation concern (Table 3). *Crinum malabaricum* is classified as ‘Critically Endangered’ (Lansdown 2016). Six species fall under the ‘Vulnerable’ category, including *Strobilanthes ciliatus* (Saha et al. 2015), *Vateria indica* (Dhyani & Barstow 2020), *Aporosa*

**Table 1. Endemic species recorded from the study area.**

	Endemic region	Number of species
1	Western Ghats	24
2	Western Ghats and Sri Lanka	2
3	Southern India	4
4	Southern India and Sri Lanka	4
5	Peninsular India	10
6	Peninsular India and Sri Lanka	7
7	India	4
8	India and Sri Lanka	13
	Total	68

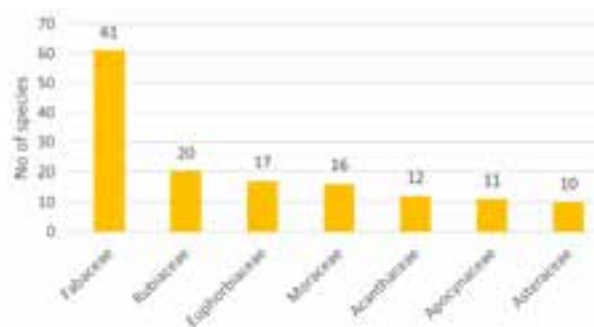
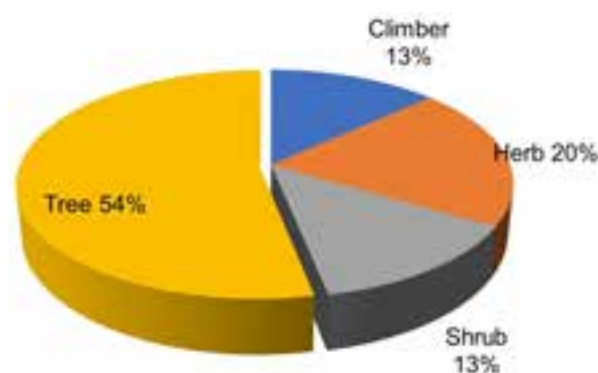
*cardiosperma* (World Conservation Monitoring Centre 2018), *Saraca asoca*, *Ochreinauclea missionis* (Barstow & Dhyani 2020), and *Santalum album* (Arunkumar 2019). *Pterocarpus marsupium* is classified as 'Near Threatened' (Barstow 2017). Critically Endangered and Western Ghats endemic *Crinum malabaricum* was observed with severely restricted distribution in the upper course of the river. The habitat was observed to be seasonal streams running through plantations and backyards located in lateritic area.

Fifteen invasive species were recorded from the study area (Table 2). Among them, *Lantana camara* and *Chromolaena odorata* were observed to severely affect open areas, while *Sphagneticola trilobata*, and *Mikania micrantha* had significant infestations in paddy fields, and along plantation edges. *Salvinia molesta* and *Eichhornia crassipes* were found to invade water bodies, though the extent of invasion by *Eichhornia crassipes* was relatively lower compared to similar habitats in urban landscapes nearby.

## DISCUSSION

The Chithari River's origin from lateritic midlands, as opposed to mountainous regions, contributes to its distinctive riparian landscape. This setting provides a mosaic of habitats, including mangroves, marshes, estuarine creeks, and mudflats near its mouth. The discovery of *Leptarma biju*, a new tree-spider crab species along the riverbanks, further emphasizes its ecological uniqueness (Ng & Devi 2020).

The floristic survey of the Chithari River's riparian zones revealed a diverse assemblage of plants. The dominance of Fabaceae aligns with studies in other riparian ecosystems, such as the Meenachil River basin, where Fabaceae was the most represented family (Vincy

**Figure 3. Number of species under dominant families recorded from the study site.****Figure 4. Percentage wise occurrence of various plants according to their habit (tree, herb, shrub, and climber).****Table 2. Invasive species recorded from the study area.**

	Species	Local name	Family
1	<i>Alternanthera brasiliana</i>	Chemcheera	Amaranthaceae
2	<i>Ageratum conyzoides</i>	Kumminnipacha, Murianpacha	Asteraceae
3	<i>Chromolaena odorata</i>	Communist pacha	Asteraceae
4	<i>Mikania micrantha</i>	Dhrutharashtra pacha	Asteraceae
5	<i>Sphagneticola trilobata</i>	Kammalpoo	Asteraceae
6	<i>Synedrella nodiflora</i>	Mudiyan Pacha	Asteraceae
7	<i>Camonea umbellata</i>	Kolavaravalli, Koravalli	Convolvulaceae
8	<i>Ipomoea triloba</i>		Convolvulaceae
9	<i>Calopogonium mucunoides</i>	Thottapayar	Fabaceae
10	<i>Centrosema pubescens</i>	Kattupayar, Poombattapayar	Fabaceae
11	<i>Eragrostis unioides</i>	Avilpullu, Karayampullu	Poaceae
12	<i>Polytrias indica</i>	Chenkodipullu	Poaceae
13	<i>Eichhornia crassipes</i>	Kulavaazha	Pontederiaceae
14	<i>Salvinia molesta</i>	African Payal	Salviniaceae
15	<i>Lantana camara</i>	Arippoo, Gulami, Konginipoo	Verbenaceae



Table 3. Checklist of floral inventory of Chithari river, Kasaragod, Kerala.

	Species	Local name	Family	Habit	Nativity	IUCN status
1	<i>Acanthus ilicifolius</i> L.	Chullikkandal	Acanthaceae	Shrub		
2	<i>Asystasia gangetica</i> (L.) T.Anderson in Thwaites	Thuppall pottas	Acanthaceae	Herb		
3	<i>Justicia gendarussa</i> Burm.f.	Vatamkolli	Acanthaceae	Shrub		
4	<i>Lepidagathis incurva</i> Buch.-Ham. ex D.Don		Acanthaceae	Herb		
5	<i>Lepidagathis</i> sp.		Acanthaceae	Herb		
6	<i>Nicotiba trinervia</i> (Vahl) Lindau		Acanthaceae	Herb	WG	
7	<i>Phaulopsis dorsiflora</i> (Retz.) Sant.		Acanthaceae	Herb		
8	<i>Rostellularia procumbens</i> (L.) Nees in A.P.de Candolle		Acanthaceae	Herb		
9	<i>Rungia pectinata</i> (L.) Nees in DC.		Acanthaceae	Herb		
10	<i>Strobilanthes ciliata</i> Nees in Wall.	Cherukurunji	Acanthaceae	Shrub	WG	VU
11	<i>Strobilanthes integrifolia</i> (Dalz.) Kuntze	Poomalakurunhi	Acanthaceae	Shrub	WG	
12	<i>Strobilanthes jomyi</i> P.Biju	Jomyi kurunhi	Acanthaceae	Shrub	WG	
13	<i>Strobilanthes</i> sp.		Acanthaceae	Shrub		
14	<i>Alternanthera brasiliana</i> (L.) Kuntze	Chemcheera	Amaranthaceae	Herb	EX	
15	<i>Alternanthera</i> sp.		Amaranthaceae	Herb		
16	<i>Cyathula prostrata</i> (L.) Blume	Cherukadaladi, Chuvannakadaladi	Amaranthaceae	Herb		
17	<i>Crinum malabaricum</i> Lekhak & S.R.Yadav	Periya polathaali	Amaryllidaceae	Herb	WG	CR
18	<i>Anacardium occidentale</i> L.	Parankimavu, Kashumavu	Anacardiaceae	Tree	EX	
19	<i>Buchanania axillaris</i> (Desr.) Ramamoorthy in Saldanha & Nicolson	Kulamavu	Anacardiaceae	Tree	ISL	
20	<i>Holigarna arnottiana</i> Hook.f.	Cheral	Anacardiaceae	Tree	WG	
21	<i>Lannea coromandelica</i> (Houtt.) Merr.	Kalashu, Uthi	Anacardiaceae	Tree		
22	<i>Mangifera indica</i> L.	Mavu	Anacardiaceae	Tree		
23	<i>Spondias pinnata</i> (L.f.) Kurz	Ambazham	Anacardiaceae	Tree		
24	<i>Annona muricata</i> L.	Mullatha	Annonaceae	Tree	EX	
25	<i>Annona reticulata</i> L.	Seethapazham	Annonaceae	Tree	EX	
26	<i>Annona squamosa</i> L.	Ramapazham	Annonaceae	Tree	EX	
27	<i>Cananga odorata</i> (Lam.) Hook.f. & Thoms. Fl. Ind. 130. 1855 & in Hook. f.	Kanakamaram	Annonaceae	Tree	EX	
28	<i>Milusa tomentosa</i> (Roxb.) Finet & Gagnep.	Kanakaittha	Annonaceae	Tree		
29	<i>Monoon longifolium</i> (Sonn.) B.Xue & R.M.K.Saunders	Aranamaram	Annonaceae	Tree	EX	
30	<i>Centella asiatica</i> (L.) Urban in Mart.	Muthil	Apiaceae	Herb		
31	<i>Alstonia scholaris</i> (L.) R.Br.	Ezhilampala	Apocynaceae	Tree		
32	<i>Carissa carandas</i> L.	Karandacherry	Apocynaceae	Shrub		
33	<i>Cosmostigma cordatum</i> (Poir.) M.R.Almeida	Kariveppilakurinja	Apocynaceae	Climber		
34	<i>Gymnema inodorum</i> (Lour.) M.R. & S.M. Almeida	Peenarivalli	Apocynaceae	Climber		
35	<i>Holarrhena pubescens</i> (Buch.-Ham.) Wall. ex G.Don	Kudakappala	Apocynaceae	Tree		
36	<i>Ichnocarpus frutescens</i> (L.) R.Br. in Ait.f.	Palvalli	Apocynaceae	Climber		
37	<i>Kametia caryophyllata</i> (Roxb.) Nicolson & Suresh	Narumarathivu	Apocynaceae	Climber	WG	
38	<i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz	Sarppaghandhi	Apocynaceae	Herb		
39	<i>Rauvolfia tetraphylla</i> (L.) Benth. ex Kurz	Pambumkolli	Apocynaceae	Herb	EX	
40	<i>Tabernaemontana alternifolia</i> L.	Kundalappala	Apocynaceae	Tree	WG	
41	<i>Wrightia tinctoria</i> (Roxb.) R.Br.	Danthappala	Apocynaceae	Tree		



	Species	Local name	Family	Habit	Nativity	IUCN status
42	<i>Cryptocoryne spiralis</i> (Retz.) C.E.C.Fisch. ex Wydler		Araceae	Herb	IN	
43	<i>Lagenandra toxicaria</i> Dalz. var. <i>toxicaria</i> ; Hook. f.		Araceae	Herb	SI	
44	<i>Pothos scandens</i> L.	Anapparuva, Paruvakodi	Araceae	Climber		
45	<i>Schefflera</i> sp.	Ettlamaram	Araliaceae	Tree		
46	<i>Acmella calva</i> (DC.) R.K. Jansen	Eripacha	Asteraceae	Herb		
47	<i>Ageratum conyzoides</i> L.	Kumminnipacha, Murianpacha	Asteraceae	Herb	EX	
48	<i>Blumea axillaris</i> (Lam.) DC.		Asteraceae	Herb		
49	<i>Blumea</i> sp.		Asteraceae	Herb		
50	<i>Chromolaena odorata</i> (L.) King & Robins.	Communist pacha	Asteraceae	Shrub	EX	
51	<i>Eclipta prostrata</i> (L.) L.	Kayyoonni	Asteraceae	Herb		
52	<i>Elephantopus scaber</i> L.	Aanayadi, Aanachuvadi	Asteraceae	Herb		
53	<i>Mikania micrantha</i> Kunth	Dhrutharashtra pacha	Asteraceae	Climber	EX	
54	<i>Sphagnetica trilobata</i> (L.) Pruski	Kammalpoo	Asteraceae	Herb	EX	
55	<i>Synedrella nodiflora</i> (L.) Gaertn.	Mudiyan Pacha	Asteraceae	Herb	EX	
56	<i>Avicennia marina</i> (Forssk.) Vierh. in Denkschr.	Chakkappoo	Avicenniaceae	Tree		
57	<i>Avicennia officinalis</i> L.	Uppatti	Avicenniaceae	Tree		
58	<i>Dolichandrone spathacea</i> (L.f.) K.Schum.	Neerpongiliyam	Bignonaceae	Tree	PI	
59	<i>Oroxylum indicum</i> (L.) Benth. ex Kurz	Palakapayyani	Bignonaceae	Tree	SISL	
60	<i>Pajanelia longifolia</i> (Willd.) K.Schum. in mplete this.Engl. & Prantl	Payyani	Bignonaceae	Tree		
61	<i>Spathodea campanulata</i> P.Beauv.	Panchasarakkaimaram	Bignonaceae	Tree	EX	
62	<i>Stereospermum colais</i> (Buch.-Ham. ex Dillw.) Mabb.	Pathiri	Bignonaceae	Tree		
63	<i>Tecoma stans</i> (L.) Kunth	Manja arali	Bignonaceae	Tree	EX	
64	<i>Bombax ceiba</i> L.	Mullilavu	Bombacaceae	Tree		
65	<i>Ceiba pentandra</i> (L.) Gaertn	Panjimaram	Bombacaceae	Tree	EX	
66	<i>Cordia dichotoma</i> G.Forst.	Naruveli	Boraginaceae	Tree		
67	<i>Lobelia alsinoides</i> Lam.	Kakkapoo (not in our part )	Campanulaceae	Herb		
68	<i>Casuarina equisetifolia</i> L.	Kaattadi	Casuarinaceae	Tree	EX	
69	<i>Lophopetalum wightianum</i> Arn.	Venkotta	Celastraceae	Tree		
70	<i>Pristimera arnottiana</i> (Wight) R.H.Archer	Mothirakurandy, Muttukorandy	Celastraceae	Climber	SI	
71	<i>Calophyllum inophyllum</i> L.	Punna	Clusiaceae	Tree		
72	<i>Garcinia gummi-gutta</i> Wight	Kudampuli	Clusiaceae	Tree	SISL	
73	<i>Garcinia indica</i> (Thouars) Choisy in DC.	Kokkum	Clusiaceae	Tree	WG	
74	<i>Combretum latifolium</i> Blume	Manjalvalli, Plakkodi, Purakkodi	Combretaceae	Climber		
75	<i>Getonia floribunda</i> Roxb.	Pullanni, Pullanji	Combretaceae	Climber		
76	<i>Terminalia arjuna</i> (Roxb. ex DC.) Wight & Arn.	Thanni	Combretaceae	Tree		
77	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Thallithenga, Badam	Combretaceae	Tree	EX	
78	<i>Terminalia catappa</i> L.	Neermaruthu	Combretaceae	Tree	ISL	
79	<i>Terminalia elliptica</i> Willd.	Karimaruthu	Combretaceae	Tree	ISL	
80	<i>Terminalia paniculata</i> Roth	Pullamaruthu	Combretaceae	Tree	PI	
81	<i>Camonea umbellata</i> (L.) A.R.Simoes & Staples	Kolavaravalli, Koravalli	Convolvulaceae	Climber	EX	
82	<i>Camonea vitifolia</i> (Burm. f.) A.R.Simoes & Staples	Manja vayaravalli	Convolvulaceae	Climber		
83	<i>Erycibe paniculata</i> Roxb.	Erumathali, Irimpiyathali	Convolvulaceae	Climber	IN	

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84	<i>Hewittia malabarica</i> (L.) Suresh in Nicolson et al.	Ohanamvalli, Vattapoomthani	Convolvulaceae	Climber		
85	<i>Ipomoea triloba</i> L.		Convolvulaceae	Climber	EX	
86	<i>Xenostegia tridentata</i> (L.) Austin & Staples	Cheruvayera, Prasarani	Convolvulaceae	Herb		
87	<i>Hellenia speciosa</i> (Koenig) S.R.Dutta	Naayi Karimbu, Aanakoova, Aanappoo	Costaceae	Herb		
88	<i>Solena amplexicaulis</i> (Lam.) Gandhi in Saldanha & Nicolson	Kuvachakka	Cucurbitaceae	Climber		
89	<i>Trichosanthes tricuspidata</i> Lour.	Kakkathondi	Cucurbitaceae	Climber		
90	<i>Cyperus haspan</i> L.		Cyperaceae	Herb	EX	
91	<i>Scleria</i> sp.		Cyperaceae	Herb		
92	<i>Dillenia pentagyna</i> Roxb.	Nayipunna	Dilleniaceae	Tree		
93	<i>Vateria indica</i> L.	Vellapayin	Dipterocarpaceae	Tree	WG	VU
94	<i>Dryopteris</i> sp.		Dryopteridaceae	Herb		
95	<i>Diospyros buxifolia</i> (Blume) Hiern	Elicheviyan	Ebenaceae	Tree		
96	<i>Diospyros candolleana</i> Wight	Karimaram	Ebenaceae	Tree	PI	
97	<i>Elaeocarpus munroi</i> (Wight) Mast. in Hook.f.	Kalrudraksham	Elaeocarpaceae	Tree	WG	
98	<i>Elaeocarpus serratus</i> L.	Kaara	Elaeocarpaceae	Tree		
99	<i>Eriocaulon dalzellii</i> Korn.	choothu	Eriocaulaceae	Herb	SI	
100	<i>Eriocaulon</i> sp.		Eriocaulaceae	Herb		
101	<i>Acalypha indica</i> L.	Naarchikkadu	Euphorbiaceae	Herb		
102	<i>Antidesma montanum</i> Blume	Asharippuli	Euphorbiaceae	Tree		
103	<i>Aporosa cardiosperma</i> (Gaertn.) Merr.	Vetti	Euphorbiaceae	Tree	PISL	VU
104	<i>Croton persimilis</i> Muell.Arg.	Pongalam, Somaraji	Euphorbiaceae	Tree		
105	<i>Euphorbia deccanensis</i> V.S.Raju	Mulachurathi	Euphorbiaceae	Herb	WG	
106	<i>Euphorbia hirta</i> L.	Nilappala	Euphorbiaceae	Herb	EX	
107	<i>Euphorbia thymifolia</i> L.	Chitrapala	Euphorbiaceae	Herb		
108	<i>Falconeria insignis</i> Royle	Kannampotti	Euphorbiaceae	Tree		
109	<i>Homonoia riparia</i> Lour.	Puzhavanchi	Euphorbiaceae	Shrub		
110	<i>Macaranga peltata</i> (Roxb.) Muell.Arg. in DC.	Vatta	Euphorbiaceae	Tree	ISL	
111	<i>Mallotus nudiflorus</i> (L.) Kulju & Welzen	Naikkumbil	Euphorbiaceae	Tree		
112	<i>Mallotus philippensis</i> (Lam.) Muell.Arg.	Sindhoori	Euphorbiaceae	Tree		
113	<i>Mallotus tetracoccus</i> (Roxb.) Kurz	Vattakkumbil	Euphorbiaceae	Tree		
114	<i>Microstachys chamaelea</i> (L.) Muell.Arg.	Kodiyavannakku, Njettavanakku	Euphorbiaceae	Herb		
115	<i>Paracroton pendulus</i> (Hassk.) Miq.	Vellamaram	Euphorbiaceae	Tree	WGSL	
116	<i>Ricinus communis</i> L.	Aavanakk	Euphorbiaceae	Shrub	EX	
117	<i>Abrus melanospermus</i> Hassk.	vella kunnikuru, Valiya kattumuthira	Fabaceae	Climber		
118	<i>Abrus precatorius</i> L.	Kunnikkuru	Fabaceae	Climber		
119	<i>Abrus pulchellus</i> Wall. ex Thwaites	Valiya Kattumuthira	Fabaceae	Climber		
120	<i>Acacia auriculiformis</i> A.Cunn. ex Benth. in Hook.'s London J. Bot. 1: 377. 1842; V.T.Antony	Acacia	Fabaceae	Tree	EX	
121	<i>Acacia mangium</i> Willd.	Mangium	Fabaceae	Tree	EX	
122	<i>Acrocarpus fraxinifolius</i> Wight & Arn.	Kurangadi	Fabaceae	Tree		
123	<i>Adenanthera pavonina</i> L.	Manjadi	Fabaceae	Tree	EX	
124	<i>Albizia amara</i> (Roxb.) Boivin	Nenmenivaka	Fabaceae	Tree		
125	<i>Albizia chinensis</i> (Osbeck) Merr.	Pottavaka	Fabaceae	Tree		
126	<i>Albizia lebbek</i> (L.) Benth. in Hook.'s London J. Bot. 3: 87. 1844; Baker in Hook. f.	Kunnivaka	Fabaceae	Tree		

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127	<i>Albizia odoratissima</i> (L. f.) Benth. in Hook.'s London J. Bot. 3: 88. 1844; Baker in Hook.f.	Pulivaka	Fabaceae	Tree		
128	<i>Albizia procera</i> (Roxb.) Benth. in Hook.'s London J. Bot. 3: 89. 1844; Baker in Hook.f.	Vellavaka	Fabaceae	Tree		
129	<i>Bauhinia malabarica</i> Roxb.		Fabaceae	Tree		
130	<i>Bauhinia purpurea</i> L.	Violet Mandaram	Fabaceae	Tree	EX	
131	<i>Bauhinia scandens</i> L. var. <i>anguina</i> (Roxb.) Ohashi	Pambuvalli	Fabaceae	Climber		
132	<i>Caesalpinia crista</i> L.	Aattuparanda	Fabaceae	Climber		
133	<i>Caesalpinia pulcherrima</i> (L.) Swartz	Rajamalli	Fabaceae	Shrub	EX	
134	<i>Calopogonium mucunoides</i> Desv.	Thottapayar	Fabaceae	Climber	EX	
135	<i>Cassia fistula</i> L.	Kanikkonna	Fabaceae	Tree		
136	<i>Centrosema molle</i> Benth.	Kattupayar	Fabaceae	Climber	EX	
137	<i>Centrosema pubescens</i> Benth.	Kattupayar, Poombattapayar	Fabaceae	Climber	EX	
138	<i>Crotalaria pallida</i> Dryand. var. <i>obovata</i> (G. Don) Polhill	Kilukkampetti	Fabaceae	Shrub		
139	<i>Dalbergia horrida</i> (Dennst.) Mabb. var. <i>glabrescens</i> (Prain) Thoth. & K.K.N. Nair		Fabaceae	Climber	WG	
140	<i>Dalbergia lanceolaria</i> L. f., Suppl. Pl. 316. 1781	Velleetti	Fabaceae	Tree		
141	<i>Dalbergia latifolia</i> Roxb., Corom. Pl. 2: 7	Eetti	Fabaceae	Tree		
142	<i>Delonix regia</i> (Boj. ex Hook.) Rafin.	Gulmohar	Fabaceae	Tree	EX	
143	<i>Derris</i> sp.		Fabaceae	Climber		
144	<i>Derris trifoliata</i> Lour., Fl. Cochinch. 433. 1790; Manilal & Sivar.	Kammattivalli	Fabaceae	Climber		
145	<i>Erythrina stricta</i> Roxb., Fl. Ind. 3: 251. 1832; Baker in Hook.f.	Mullumurikku	Fabaceae	Tree		
146	<i>Erythrina variegata</i> L. in Stickman	Murikku	Fabaceae	Tree		
147	<i>Flemingia grahamiana</i> Wight & Arn.		Fabaceae	Shrub		
148	<i>Geissaspis tenella</i> Benth. var. <i>tenella</i> ; Baker in Hook. f.		Fabaceae	Herb	WG	
149	<i>Gliricidia sepium</i> (Jacq.) Kunth ex Walp.	Sheemakonna	Fabaceae	Tree	EX	
150	<i>Grona heterocarpa</i> (L.) H.Ohashi & K.Ohashi var.	Nilathuvara	Fabaceae	Shrub		
151	<i>Hultholia mimosoides</i> Lam.	Komullu	Fabaceae	Shrub		
152	<i>Humboldtia brunonis</i> Wall.		Fabaceae	Tree	WG	
153	<i>Indigofera tinctoria</i> L.	Neelamari	Fabaceae	Shrub		
154	<i>Mimosa pudica</i> L.	Thottavadi	Fabaceae	Herb	EX	
155	<i>Mucuna bracteata</i> DC. ex Kurz	Thottapayar	Fabaceae	Climber		
156	<i>Mucuna pruriens</i> (L.) DC. var. <i>hirsuta</i> (Wight & Arn.) Wilmot-Dear	Naikkurana	Fabaceae	Climber	WG	
157	<i>Neonotonia wightii</i> (Graham ex Wight & Arn.) Lackey	Kattavarakkavalli	Fabaceae	Climber		
158	<i>Pleurolobus gangeticus</i> J.St.Hil. in Nouv. Bull. Soc. Philom. 3: 192. 1812	Orila	Fabaceae	Herb		
159	<i>Pongamia pinnata</i> (L.) Pierre	Ungu	Fabaceae	Tree		
160	<i>Pseudarthria viscida</i> (L.) Wight & Arn.	Moovila	Fabaceae	Shrub	PISL	
161	<i>Pterocarpus marsupium</i> Roxb.	Venga	Fabaceae	Tree	ISL	NT
162	<i>Pterocarpus santalinus</i> L.f.	Rakthachandanam	Fabaceae	Tree	PI	EN
163	<i>Pueraria phaseoloides</i> (Roxb.) Benth.	Thottapayar	Fabaceae	Climber	EX	
164	<i>Pycnospora lutescens</i> (Poir.) Schindl.		Fabaceae	Climber		
165	<i>Samanea saman</i> (Jacq.) Merr.	Mazhamaram	Fabaceae	Tree	EX	
166	<i>Saraca asoca</i> (Roxb.) de Wilde	Asokam	Fabaceae	Tree		VU
167	<i>Senegalia caesia</i> (L.) Maslin	Inja	Fabaceae	Climber		

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168	<i>Senegalia catechu</i> (L.f.) P. Hurter & Mabb. in Mabb.	Karingali	Fabaceae	Tree		
169	<i>Senegalia torta</i> (Roxb.) Maslin	Inja	Fabaceae	Climber		
170	<i>Senna septemtrionalis</i> (Viv.) H.S.Irwin & Barneby	Kattukonna	Fabaceae	Shrub	WG	
171	<i>Senna tora</i> (L.) Roxb.	Thakara	Fabaceae	Shrub	EX	
172	<i>Senna uniflora</i> (Mill.) H.S.Irwin & Barneby	Thakara	Fabaceae	Shrub	EX	
173	<i>Smithia conferta</i> Smith in Rees	Elakanni	Fabaceae	Herb		
174	<i>Spatholobus</i> sp.		Fabaceae	Climber		
175	<i>Tamarindus indica</i> L.	Valan puli	Fabaceae	Tree	EX	
176	<i>Teramnus mollis</i> Benth.		Fabaceae	Climber		
177	<i>Xylia xylocarpa</i> (Roxb.) Taub.	Irul	Fabaceae	Tree		
178	<i>Flacourtia jangomas</i> (Lour.) Raeusch.	Vayamkatha	Flacourtiaceae	Tree	EX	
179	<i>Flacourtia montana</i> Graham	Charalpazham	Flacourtiaceae	Tree	IN	
180	<i>Hydnocarpus pentandrus</i> (Buch.-Ham.) Oken	Marotti	Flacourtiaceae	Tree	WG	
181	<i>Canscora diffusa</i> (Vahl) R.Br. ex Roem. & Schult.	Jeerakapullu	Gentianaceae	Herb		
182	<i>Blyxa aubertii</i> L.C.Rich.		Hydrocharitaceae	Herb		
183	<i>Anisomeles indica</i> (L.) Kuntze	Chedayan, Karithumba	Lamiaceae	Herb		
184	<i>Clerodendrum infortunatum</i> L.	Peruvu, Vattapparuvalem	Lamiaceae	Shrub		
185	<i>Mesosphaerum suaveolens</i> (L.) Poit.	Naarchikkadu	Lamiaceae	Herb	EX	
186	<i>Pogostemon heyneanus</i> Benth. in Wall.		Lamiaceae	Shrub		
187	<i>Stachytarpheta jamaicensis</i> (L.) Vahl	Kadapananth, Kattupunnuthu	Lamiaceae	Shrub	EX	
188	<i>Tectona grandis</i> L.f.	Thekk	Lamiaceae	Tree		
189	<i>Vitex negundo</i> L.	Karinochi	Lamiaceae	Shrub	EX	
190	<i>Cinnamomum malabratrum</i> (Burm.f.) Blume	Vayana	Lauraceae	Tree	WG	
191	<i>Cinnamomum verum</i> Presl	Karuvappatta	Lauraceae	Tree	SISL	
192	<i>Litsea coriacea</i> (Heyne ex Meisn.) Hook.f.	Maravettithali	Lauraceae	Tree	PI	
193	<i>Persea macarantha</i> (Heyne ex Meisn.) Hook.f.	Kulamavu	Lauraceae	Tree	PISL	
194	<i>Barringtonia acutangula</i> (L.) Gaertn.	Neerpezhu	Lecythidaceae	Tree		
195	<i>Careya arborea</i> Pl. Corom. t. 218. 1811; Hook.f.	Pezhu	Lecythidaceae	Tree		
196	<i>Senna alata</i> (L.) Roxb.	Rajamalli	Leguminosae	Shrub	EX	
197	<i>Gloriosa superba</i> L.	Mendonni	Liliaceae	Climber		
198	<i>Hugonia mystax</i> L.	Mothiravalli, Kaarthotti, Modirakkanni	Linaceae	Climber	ISL	
199	<i>Fagraea ceilanica</i> Thunb.	Modakam	Loganiaceae	Tree		
200	<i>Strychnos nux-vomica</i> L.	Kanjiram	Loganiaceae	Tree		
201	<i>Strychnos potatorum</i> L. f.	Thettamparal	Loganiaceae	Tree	PISL	
202	<i>Strychnos wallichiana</i> Steud. ex A.DC. in DC.	Vallikanjiram	Loganiaceae	Climber		
203	<i>Lygodium flexuosum</i> (L.) Sw.		Lygodiaceae	Climber		
204	<i>Helicteres isora</i> L.	Edampiri-Valampiri, Eeswaramoori	Malvaceae	Shrub		
205	<i>Hibiscus hispidissimus</i> Griff.	mathi puli	Malvaceae	Shrub	EX	
206	<i>Hibiscus surattensis</i> L.	Mampazhaya, Pulichai	Malvaceae	Shrub	EX	
207	<i>Hibiscus tiliaceus</i> L.	Veliparuthi	Malvaceae	Tree		
208	<i>Thespesia populnea</i> (L.) Soland. ex Correa	Poovarashu	Malvaceae	Tree		
209	<i>Triumfetta rhomboidea</i> Jacq.	Aadaiotti, Paramutti	Malvaceae	Shrub		
210	<i>Urena lobata</i> L.	Cheeli, Kuruvachedi	Malvaceae	Shrub		
211	<i>Melastoma malabathricum</i> L.	Athirani	Melastomataceae	Shrub		



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212	<i>Memecylon umbellatum</i> Burm.f.	Kayambo	Melastomataceae	Tree	PISL	
213	<i>Aglaia elaeagnoidea</i> (A.Juss.) Benth.	Poonjava	Meliaceae	Tree		
214	<i>Azadirachta indica</i> A.Juss.	Veppu	Meliaceae	Tree	EX	
215	<i>Melia dubia</i> Cav.	Malaveppu	Meliaceae	Tree		
216	<i>Naregamia alata</i> Wight & Arn.	Nilanaragam	Meliaceae	Herb	PI	
217	<i>Swietenia macrophylla</i> King in Hook.	Mahogany	Meliaceae	Tree	EX	
218	<i>Toona ciliata</i> M. Roem.	Chandanavembu	Meliaceae	Tree		
219	<i>Cyclea peltata</i> (Burm. f.) Hook.f. & Thoms.	Padakizhangu, Padathali	Menispermaceae	Climber	ISL	
220	<i>Artocarpus heterophyllus</i> Lam.	Plavu	Moraceae	Tree		
221	<i>Artocarpus hirsutus</i> Lam.	Anjili	Moraceae	Tree	WG	
222	<i>Artocarpus zeylanicus</i> (F.M.Jarrett) E.M.Gardner & Zerega	Ondampuli	Moraceae	Tree	SISL	
223	<i>Ficus arnottiana</i> (Miq.) Miq.	Kallarayal	Moraceae	Tree	ISL	
224	<i>Ficus benghalensis</i> L.	Peral	Moraceae	Tree	EX	
225	<i>Ficus callosa</i> Willd.	Kallal	Moraceae	Tree		
226	<i>Ficus exasperata</i> Vahl	Parakam	Moraceae	Tree		
227	<i>Ficus heterophylla</i> L. f.	Vallitherakam	Moraceae	Shrub		
228	<i>Ficus hispida</i> L.f.	Erumanaa	Moraceae	Tree		
229	<i>Ficus microcarpa</i> L. f.	Ithi	Moraceae	Tree		
230	<i>Ficus racemosa</i> L.	Athi	Moraceae	Tree		
231	<i>Ficus religiosa</i> L.	Arayal	Moraceae	Tree	EX	
232	<i>Ficus tinctoria</i> G.Forst.	Ithimott	Moraceae	Tree		
233	<i>Ficus tsjahela</i> Burm.f.	Chela	Moraceae	Tree	PISL	
234	<i>Ficus virens</i> Aiton var. <i>dispersa</i> Chantaras.	Cherala	Moraceae	Tree		
235	<i>Streblus asper</i> Lour.	Paruvamaram	Moraceae	Tree		
236	<i>Moringa oleifera</i> Lam.	Muringa	Moringaceae	Tree		
237	<i>Aegiceras corniculatum</i> (L.) Blanco	Narikkandal	Myrsinaceae	Tree		
238	<i>Ardisia solanacea</i> Roxb.	Kuzhimundan	Myrsinaceae	Tree		
239	<i>Syzygium caryophyllatum</i> (L.) Alston in Trimen	Karingappazham, Kanjipazham	Myrtaceae	Tree	WGSL	
240	<i>Syzygium cumini</i> (L.) Skeels var. <i>cumini</i> ; Manilal & Sivar.	Kanhipazham, Njaval	Myrtaceae	Tree		
241	<i>Syzygium zeylanicum</i> (L.) DC.	Poochapazham	Myrtaceae	Tree		
242	<i>Chionanthus mala-elengi</i> (Dennst.) P.S. Green	Kallidala	Oleaceae	Tree	PI	
243	<i>Jasminum malabaricum</i> Wight	Kadambavalli, Kathambavalli	Oleaceae	Climber	SI	
244	<i>Tetrapilus dioicus</i> (Roxb.) L.A.S. Johnson	Edana	Oleaceae	Tree	IN	
245	<i>Ludwigia hyssopifolia</i> (G.Don) Exell	Neergrampu	Onagraceae	Herb		
246	<i>Averrhoa bilimbi</i> L.	Koyakkappuli	Oxalidaceae	Tree	EX	
247	<i>Averrhoa carambola</i> L.	Chathurapuli	Oxalidaceae	Tree	EX	
248	<i>Biophytum sensitivum</i> (L.) DC.	Mukkutti	Oxalidaceae	Herb		
249	<i>Pandanus odorifer</i> (Forsk.) Kuntze.	Kaitha	Pandanaceae	Shrub		
250	<i>Breynia vitis-idaea</i> (Burm.f.) C.E.C.Fisch.	Pavalappoola	Phyllanthaceae	Tree		
251	<i>Bridelia retusa</i> (L.) A.Juss.	Mulluvenga	Phyllanthaceae	Tree		
252	<i>Bridelia stipularis</i> (L.) Blume	Cheruka, Cherukapanachi	Phyllanthaceae	Shrub	PI	
253	<i>Cleistanthus collinus</i> (Roxb.) Benth. ex Hook.f.	Odugu	Phyllanthaceae	Tree	ISL	
254	<i>Glochidion ellipticum</i> Wight	Njanjetti	Phyllanthaceae	Tree	WG	
255	<i>Phyllanthus amarus</i> Schum. & Thonn. in C.F. Schumacher	Keezharnelli	Phyllanthaceae	Herb		

	Species	Local name	Family	Habit	Nativity	IUCN status
256	<i>Phyllanthus emblica</i> L.	Nelli	Phyllanthaceae	Tree		
257	<i>Phyllanthus reticulatus</i> Poir. in Lam.	Neernelli	Phyllanthaceae	Shrub		
258	<i>Phyllanthus virgatus</i> G.Forst. var. <i>virgatus</i> ; Manilal & Sivar.		Phyllanthaceae	Shrub		
259	<i>Arundinella</i> sp.		Poaceae	Herb		
260	<i>Axonopus compressus</i> (Sw.) P.Beauv.	Kaalappullu	Poaceae	Herb		
261	<i>Eragrostis unioides</i> (Retz.) Nees ex Steud.	Avilpullu, Karayampullu	Poaceae	Herb	EX	
262	<i>Heteropogon contortus</i> (L.) P. Beauv. ex Roem. & Schult.	Eeru pullu	Poaceae	Herb		
263	<i>Ischaemum ciliare</i> Retz.		Poaceae	Herb		
264	<i>Oplismenus</i> sp.		Poaceae	Herb		
265	<i>Polytrias indica</i> (Houtt.) Veldkamp	Chenkodipullu	Poaceae	Herb	EX	
266	<i>Eichhornia crassipes</i> Mart.	Kulavaazha	Pontederiaceae	Herb	EX	
267	<i>Ziziphus nummularia</i> (Burm.f.) Wight & Arn.		Rhamnaceae	Climber		
268	<i>Ziziphus oenopolia</i> (L.) Mill.	<u>Choori pazham</u>	Rhamnaceae	Climber		
269	<i>Bruguiera cylindrica</i> (L.) Blume	Kandal	Rhizophoraceae	Tree		
270	<i>Carallia brachiata</i> (Lour.) Merr.	Benkana	Rhizophoraceae	Tree		
271	<i>Kandelia candel</i> (L.) Druce	Cherukandal	Rhizophoraceae	Tree		
272	<i>Rhizophora apiculata</i> Blume	Kayakandal	Rhizophoraceae	Tree		
273	<i>Rhizophora mucronata</i> Poir. in Lam.	Panachikandal	Rhizophoraceae	Tree		
274	<i>Canthium coromandelicum</i> (Burm.f.) Alston in Trimen	Kandakara, Karamullu,	Rubiaceae	Shrub		
275	<i>Canthium rheedei</i> DC.	Edalimullu	Rubiaceae	Shrub	PI	
276	<i>Catunaregam spinosa</i> (Thunb.) Tirveng.	Kattunaragam	Rubiaceae	Tree		
277	<i>Chassalia curviflora</i> (Wall. ex Kurz) Thwaites	Karutha amalpori	Rubiaceae	Herb		
278	<i>Exallage auricularia</i> (L.) Bremek.	karutha tharthaval	Rubiaceae	Herb		
279	<i>Geophila repens</i> (L.) Johnst.	Karimuthil	Rubiaceae	Herb		
280	<i>Haldina cordifolia</i> (Roxb.) Hook.f. ex Brand.	Manjakkadambu	Rubiaceae	Tree		
281	<i>Hymenodictyon orixense</i> (Roxb.) Mabb.	Malamkalli	Rubiaceae	Tree		
282	<i>Ixora brachiata</i> Roxb. ex DC.	Kaatuthechi	Rubiaceae	Tree	WG	
283	<i>Ixora coccinea</i> L.	Chuvanna chekki	Rubiaceae	Shrub		
284	<i>Mitracarpus hirtus</i> (L.) DC.	Thaval	Rubiaceae	Herb	EX	
285	<i>Mitragyna parvifolia</i> (Roxb.) Korth.	Neerkadambu	Rubiaceae	Tree		
286	<i>Morinda citrifolia</i> L.	Noni	Rubiaceae	Tree		
287	<i>Morinda pubescens</i> J.E.Smith in Rees	Manjanathi	Rubiaceae	Tree		
288	<i>Mussaenda frondosa</i> L.	Vellila	Rubiaceae	Shrub	PI	
289	<i>Neolamarckia cadamba</i> (Roxb.) Bosser	Kadamb	Rubiaceae	Tree		
290	<i>Ochreinauclea missionis</i> (Wall. ex G.Don) Ridsd.	Aattuvanchi	Rubiaceae	Tree	WG	VU
291	<i>Oldenlandia corymbosa</i> L.	Parpadakappullu	Rubiaceae	Herb		
292	<i>Pavetta indica</i> L.	Pavetta, Vellachekki	Rubiaceae	Tree	ISL	
293	<i>Psychotria</i> sp.		Rubiaceae	Tree		
294	<i>Spermaceoce latifolia</i> Aubl.	Pachhapalla, Vellatharavu	Rubiaceae	Herb	EX	
295	<i>Acronychia pedunculata</i> (L.) Miq.	Orilatheppettimaram	Rutaceae	Tree		
296	<i>Aegle marmelos</i> (L.) Correa	Koovalam	Rutaceae	Tree	EX	
297	<i>Clausena anisata</i> (Willd.) Hook.f. ex Benth. in Hook.	Karambu	Rutaceae	Tree		
298	<i>Glycosmis pentaphylla</i> (Retz.) DC.	Kuttippannel, Kurumpannal	Rutaceae	Shrub		
299	<i>Melicope lunu-ankenda</i> (Gaertn.) Hartley	Kanala	Rutaceae	Tree		

	Species	Local name	Family	Habit	Nativity	IUCN status
300	<i>Murraya paniculata</i> (L.) Jack.	Naaragamulla	Rutaceae	Tree		
301	<i>Naringi crenulata</i> (Roxb.) Nicolson in Saldanha & Nicolson	Narinaragam	Rutaceae	Tree		
302	<i>Zanthoxylum asiaticum</i> (L.) Appelhans	Karamullu	Rutaceae	Climber		
303	<i>Zanthoxylum rhetsa</i> (Roxb.) DC.	Kuyili, Mullilam	Rutaceae	Tree		
304	<i>Salix tetrasperma</i> Roxb.	Vanchi	Salicaceae	Tree		
305	<i>Salvinia molesta</i> D.Mitch.	African Payal	Salviniaceae	Herb	EX	
306	<i>Santalum album</i> L.	Chandanam	Santalaceae	Tree	EX	VU
307	<i>Allophylus cobbe</i> (L.) Raeusch.	Mookkannanpezhu	Sapindaceae	Tree		
308	<i>Otonephelium stipulaceum</i> (Bedd.) Radlk.	Poripoovam	Sapindaceae	Tree	WG	
309	<i>Sapindus trifoliatus</i> L.	Soapumkayi, Ponnikkai	Sapindaceae	Tree		
310	<i>Schleichera oleosa</i> (Lour.) Oken	Poovam	Sapindaceae	Tree		
311	<i>Madhuca longifolia</i> (Koenig) Macbr.	Ilippa	Sapotaceae	Tree	ISL	
312	<i>Madhuca nerifolia</i> (Moon) H.J.Lam	Aatilippa	Sapotaceae	Tree	ISL	
313	<i>Manilkara hexandra</i> (Roxb.) Dubard	Kirni	Sapotaceae	Tree		
314	<i>Mimusops elengi</i> L.	Elengi	Sapotaceae	Tree		
315	<i>Limnophila aquatica</i> (Roxb.) Alston		Scrophulariaceae	Herb		
316	<i>Limnophila repens</i> (Benth.) Benth. in DC.	Manganaari	Scrophulariaceae	Herb		
317	<i>Ailanthus triphysa</i> (Dennst.) Alston in Trimen	Matty	Simaroubaceae	Tree		
318	<i>Smilax zeylanica</i> L.	Arikanni, Kareelanchi	Smilacaceae	Climber		
319	<i>Sonneratia alba</i> J.E.Sm. in Rees	Nakshatrakandal	Sonneratiaceae	Tree		
320	<i>Firmiana colorata</i> (Roxb.) R.Br. in Bennett & Brown	Malambarathi	Sterculiaceae	Tree		
321	<i>Sterculia guttata</i> Roxb. ex DC.	Pottakkavalam	Sterculiaceae	Tree		
322	<i>Symplocos acuminata</i> (Blume) Miq.	Paachotti	Symplocaceae	Tree		
323	<i>Grewia nervosa</i> (Lour.) G. Panigrahi	Kottappazham, Cherikotta	Tiliaceae	Tree		
324	<i>Grewia tiliifolia</i> Vahl	Chadachi	Tiliaceae	Tree		
325	<i>Triumfetta rotundifolia</i> Lam.		Tiliaceae	Herb		
326	<i>Holoptelea integrifolia</i> (Roxb.) Planch.	Aaval	Ulmaceae	Tree		
327	<i>Trema orientalis</i> (L.) Blume	Aamathali	Ulmaceae	Tree		
328	<i>Callicarpa tomentosa</i> (L.) L. in Murr.	Cheruthekk	Verbanaceae	Tree	PISL	
329	<i>Clerodendrum infortunatum</i> L.	Vattapperuvalam	Verbanaceae	Tree		
330	<i>Gmelina arborea</i> Roxb.	Kumbil	Verbanaceae	Tree		
331	<i>Premna mollissima</i> Roth	Knappa	Verbanaceae	Tree		
332	<i>Premna serratifolia</i> L.	Munja	Verbanaceae	Tree		
333	<i>Vitex altissima</i> L.f.	Myla	Verbanaceae	Tree		
334	<i>Clerodendrum paniculatum</i> L.	Hanuman Kireedam	Verbanaceae	Shrub		
335	<i>Lantana camara</i> L.	Arippoo, Gulami, Konginipoo	Verbanaceae	Shrub	EX	
336	<i>Rotheca serrata</i> (L.) Steane & Mabb.	Kurukutti	Verbanaceae	Shrub		
337	<i>Volkameria inermis</i> L.	Puzhamulla	Verbanaceae	Shrub	ISL	
338	<i>Cissus repens</i> Lam.	Chunnambu valli	Vitaceae	Climber		
339	<i>Leea indica</i> (Burm. f.) Merr.	Njalu	Vitaceae	Shrub	EX	
340	<i>Zingiber zerumbet</i> (L.) J.E.Smith	Kattinchi, Kattukolnichi	Zingiberaceae	Herb		

Index to nativity: ISL — India and Sri Lanka | SI — southern India | IN — India | WG — Western Ghats | EX — Exotic | PI — Peninsular India | SISL — southern India and Sri Lanka | PISL — Peninsular India and Sri Lanka | WGSL — Western Ghats and Sri Lanka. Index to IUCN status: CR — Critically Endangered | EN — Endangered | VU — Vulnerable | NT — Near Threatened.



Image 1–8. 1—*Pandanus odorifer*, plant | 2—*Morinda citrifolia*, plant | 3—*Acanthus ilicifolius*, plant | 4—*Rhizophora mucronata*, tree | 5—*Hibiscus hispidissimus*, leaf | 6—*Limnophila repens*, plant | 7—*Ficus arnottiana*, leaf | 8—*Syzygium caryophyllatum*, flower. © 1,2,5,6—Shyamkumar Puravankara | 3,4,7,8—Sreehari K Mohan.





Image 9–16. 9—*Cyclea peltata*, plant | 10—*Acmella calva*, flower | 11—*Antidesma montanum*, fruit | 12—*Strobilanthes integrifolius*, plant | 13—*Strobilanthes jomyi*, flower | 14—*Limnophila repens*, plant | 15—*Symplocos cochinchinensis*, flower | 16—*Kandelia kandal*, flower. © 9,10,13,14—Shyamkumar Puravankara | 11,12,15,16—Sreehari K Mohan.





Image 17–24. 17—*Premna mollissima*, flower | 18—*Dolichandrone spathacea*, plant | 19—*Volkameria inermis*, flower | 20—*Ziziphus oenopolia*, flowers and leaves | 21—*Cissus repens*, plant | 22—*Helicteres isora*, flower | 23—*Alstonia scholaris*, tree | 24—*Clerodendrum infortunatum*, flowers. © 17,18,22,23,24—Sreehari K Mohan | 19,20,21—Shyamkumar Puravankara.





Image 25–32. 25—*Glycosmis pentaphylla*, plant | 26—*Cryptocoryne spiralis*, plant | 27—*Crinum malabaricum*, plant | 28—*Ochreinauclea missionis*, plant | 29—*Grewia nervosa*, plant | 30—*Calophyllum inophyllum*, plant | 31—*Chassalia curviflora*, flower | 32—*Canthium rheedei*, plant. © 25,26,28–31—Shyamkumar Puravankara | 27—Sreehari K Mohan.

et al. 2015).

The presence of exotic plants constituting approximately 17% of the total flora, poses ecological concerns. Invasive alien plant species are recognized as major drivers of biodiversity loss, affecting ecosystem services, and socio-economic conditions (Rai & Singh 2020). In the Chithari River and adjacent habitats, invasive plants have aggressively colonized various habitats, including open areas, paddy fields, plantation edges, and aquatic habitats. Such invasions suppress native vegetation through resource competition, allelopathy, and habitat modification (Rai & Singh 2020). Aquatic weeds such as *Eichhornia crassipes* were relatively lower compared to similar habitats in urban landscapes nearby, probably due to the lower levels of organic nutrients, and sewage disposal in the river. This assumption should be validated with water quality testing to arrive at a decision.

The detection of eight Red Listed species underscores the ecological significance of the Chithari River's riparian habitats. *Crinum malabaricum*, classified as 'Critically Endangered', was observed with a severely restricted distribution in the upper course of the river. The occurrences of four species of *Strobilanthes* which are endemic to Western Ghats emphasize the need for conserving the habitat. *Strobilanthes ciliatus*, one among them is categorized as 'Vulnerable' as per the latest assessment of IUCN Red List. Similarly, the presence of other vulnerable species, such as *Vateria indica*, recorded from one of the sacred groves in the habitat, highlights the need for targeted conservation measures to prevent further population decline (Nagarajan & Bhaskar 2023).

The riparian zones of the Chithari River support a unique and diverse flora, including several species of conservation concern. The proliferation of invasive species poses a significant threat to its ecological balance. Ensuring the long-term sustainability of this ecologically significant region requires tailored conservation and management strategies. A comprehensive understanding of the river's flora, coupled with ecosystem-based conservation approaches, is essential. Effective management should include regular monitoring, public awareness initiatives, and targeted control measures for invasive species to safeguard the ecological integrity of this vital riparian ecosystem.

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## Propagation through stem cutting and air layering of a Critically Endangered tree *Humboldtia unijuga* Bedd. var. *trijuga* J. Joseph & V. Chandras. (Magnoliopsida: Fabales: Fabaceae)

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**Abstract:** *Humboldtia unijuga* var. *trijuga* is an evergreen tree, endemic to the southern Western Ghats of India belonging to the family Fabaceae, and is categorized as 'Critically Endangered'. High rates of flower & fruit predation and the recalcitrant nature of seeds have detrimental effect on regeneration, and individual recruitment in the wild. Therefore, the present study aimed to produce saplings through conventional propagation methods of stem cuttings and air layering by exogenous application of auxins, with various concentrations of Indole-3-butyric acid (IBA), Indole-3 acetic acid (IAA), and  $\alpha$ -Naphthalene acetic acid (NAA). The study revealed that both IBA and IAA had developmental effects on stem cuttings and air layering, but maximum rooting was observed at 1,500 mg/l of IBA. This concentration may be used for mass multiplication and conservation of this endangered tree species.

**Keywords:** Agasthyamalai, auxin, conservation, conventional propagation method, evergreen tree, southern Western Ghats, Thiruvananthapuram.

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**Author contributions:** PSJ conceptualised and designed the work, analysed the data, SS carried out the fieldwork, data collection, and drafted the manuscript. PSJ corrected and edited the manuscript in the final form. PSJ and SS approved the final manuscript.

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## INTRODUCTION

*Humboldtia unijuga* var. *trijuga* J. Joseph & V. Chandras. is a medium-sized evergreen tree of the family Leguminosae, endemic to the southern Western Ghats. Its distribution is highly restricted to the hillocks of the Agasthyamala forests within the altitude range of 490–1,050 m (Sanjappa 1986). This species has been categorized as ‘Critically Endangered’ by the IUCN Red List (WCMC 1998). Traditional healers use the bark and leaves of this tree for skin treatment (Vijayan et al. 2007). Its crimson-coloured cauliflorous flowers, with their strikingly attractive appearance, make this plant as a potential ornamental choice for gardens, and avenues. Being a Critically Endangered species and a potential economically important plant, conservation of this species is highly essential. Studies showed that flower, fruit, and seed predation by animals along with the recalcitrant nature of seeds negatively affect the natural regeneration, and recruitment of this species (Jothish & Anilkumar 2023). Hence, it is very important to multiply the plant and reintroduce into its natural habitat. Vegetative propagation is an easy and advantageous method to obtain saplings of species with ineffective sexual reproduction (Honney & Bossuyt 2005; Jose et al. 2011).

Vegetative propagation methods are considered technically simple and cost-effective for developing exact copies and conserving the stock plants with the same genetic identity (Carmona et al. 2022). Treating with rooting hormones is an effective approach for the multiplication and vegetative propagation of any plant species (Abidin & Metali 2015). The positive effects of auxin treatment for vegetative propagation were reported by various workers (Ali et al. 2008; Kharkwal et al. 2008; Jeruto et al. 2010; Kamila & Panda 2019). Among the propagation methods, air layering has an added advantage over other techniques, as it ensures early blooming, and fruit set (Jose et al. 2010). In this context, simple vegetative propagation trials of air layering and stem cuttings were conducted with rooting hormones for the production of more planting materials for the reintroduction, and conservation programs of this species.

## MATERIALS AND METHODS

### Study species

*Humboldtia unijuga* var. *trijuga* is an evergreen under-storey species growing up to 12 m. It is located

in Bonacaud of Thiruvananthapuram District of Kerala State. The tree naturally exhibits slow growth. Leaves are compound with three to four pair of leaflets. Flowering is observed in August–January. Flowers are borne in compact racemes on branches and main trunk, and are crimson red in colour. Fruit is a legume with one or two seeds and fruits are observed in December–April. Insect larvae and arboreal animals like monkeys and squirrels predate flowers and fruits. Seeds are large and recalcitrant.

### Stem cuttings

The stem-cutting experiment was conducted during August–September at the central nursery of Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, in 2022. Each experiment had 25 cuttings for each treatment. Healthy and disease-free stem cuttings of semi-hard type having 15–20 cm of length, 8–10 mm diameter with 3–5 nodes, and leaves were collected from adult individuals from the natural habitat. Immediately after collection, the cuttings were brought to the nursery and treated with 1% Bavistin to avoid fungal infection. The basal portion of each cutting was cut at right angles and dipped in four different concentrations of auxins (500, 1,000, 1,500, and 2,000 mg/l), viz., Indole-3-butyric acid (IBA), Indole-3 acetic acid (IAA), and  $\alpha$ -Naphthalene acetic acid (NAA) for five minutes. The cuttings dipped in distilled water were treated as a control. The cuttings were planted on the same day of collection as early as possible. To avoid / reduce the transpiration rate, the leaf's surface area was subjected to a half-cut. Immediately after the treatment, the whole set was planted into the sand bed in the mist house having a temperature of  $28 \pm 2$  °C, and 70–80 % relative humidity. Intermittent mist was supplied for 40 seconds six times in a day. The experiment was observed twice a week for the first six weeks, and the cuttings that showed wilting were removed. After 12 weeks the sprouted plants were observed. Root parameters like percentage of rooting, number of roots per cutting, and root length were recorded. Cuttings with roots of  $\geq 1$  mm were considered as rooted and used for calculating rooting percentage (out of total treatments) and root length of  $\geq 1$  cm was considered for calculating mean number of roots. After measurements, they were planted in polythene bags containing a potting mixture of river sand, dried cow dung, and garden soil in a 2:1:1 ratio. Survival percentage was calculated after six months.

### Air layering

Air layering was done on 16 randomly selected

disease-free, healthy individuals of *H. unijuga* var. *trijuga* growing in its habitat. The layering experiments were conducted in August–September of 2022, which experienced an active growth phase of the plant with favorable climatic conditions. Actively growing stems were selected, and from these branches, and lateral branches of 30–50 cm length, and 1–3 cm diameter with leaves were randomly selected from each tree for layering treatments. A small strip of bark (3 cm) from the selected branches was girdled out below the nodal region using a sharp budding knife. Various concentrations (500, 1,000, 1,500, and 2,000 mg/l) of growth hormones such as IBA, IAA, and NAA were applied (absorbent cotton dipped in respective concentrations of auxins) on the girdled region, and were covered with polythene sleeves containing moist rooting compost. The rooting medium was prepared by mixing cocopeat, river sand, and dried cow dung in a ratio of 2:1:1. Both ends of the layering were tied tightly with thread to avoid drying of the medium. Small holes were made in the polythene sleeves to permit limited air exchange. The trials were carried out in such a manner that a single tree was layered with the application of a single concentration (500 or 1,000 or 1,500, or 2,000 mg/l) of different hormones including one control without hormones (4 treatments + 1 control). Accordingly, a total of 80 layers (experiment) were created under four different auxin treatments. The air layers were labelled properly and left undisturbed for eight weeks. Observations were made weekly for root emergence and the treatments were sprayed with water to maintain moisture content. After eight weeks, the air layers were cut from the parent plant and the rooting mixture was gently removed. The success of layers was assessed by recording the presence of callus, rooting percentage, number of roots, and root length as in stem cuttings. The number of roots initiated from each treatment was counted and averaged. Similarly, lengths of roots formed in a layer were measured, and mean root length was calculated. After measurements, air-layered plants were planted in polythene bags containing potting mixture of river sand, dried cow dung, and garden soil in 2:1:1 ratio and kept in the nursery. Survival percentage was calculated after six months.

### Statistical analysis

Each of the five treatments, including the control, was replicated five times. The results of root length and number of roots were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test,  $p \leq 0.05$  with SPSS software v.16. Data for mean root length and number of roots are given as mean  $\pm$

standard error of the replicates.

## RESULTS

### Propagation through stem cuttings

The stem cuttings treated with IBA and IAA only showed developmental response. The rooting percentage was varied 16–66.6 under different auxin concentrations. All IBA-treated cuttings showed rooting, while cuttings treated with 1,000 and 1,500 mg/l IAA only showed rooting. Those cuttings treated with NAA and control cuttings showed no rooting response at all (Table 1). Cuttings treated with 1,500 mg/l of IBA showed maximum rooting (66.6%), whereas 1,500 mg/l IAA resulted in 54.5% rooting. The mean root number and mean root length of IBA set ranged from  $2.4 \pm 0.24$ – $7.2 \pm 0.58$  and  $1.8 \pm 0.05$ – $6.4 \pm 0.12$  cm and were significantly higher than other treatments (one-way ANOVA). The IAA set ranged from  $2.2 \pm 0.37$ – $5.2 \pm 0.48$  and  $2.3 \pm 0.18$ – $4.7 \pm 0.12$  cm, respectively (Image 1A–C). The bud initiation was observed on the fourth week after planting in control cuttings, however after the bud break it dried off. The stem cuttings treated with 1,500 mg/l of IBA and IAA showed bud initiation after two weeks, and responded with maximum rooting, and shooting. After six weeks the buds were transformed into fully functional leaves. Finally, after 12 weeks the regenerated stem cuttings were transplanted into separate pots.

### Air layering

Air layering samples responded positively to auxins such as IBA and IAA. The root initiation was observed in hormone-treated samples after 3–4 weeks. Rooting percentage varied 20–52.6 %. The number of roots and root length were measured after eight weeks (Image 1D–E). Data indicated that layering of samples treated with 1,500 mg/l of IBA recorded higher percentage of rooting success (52.6%) and was at par with 1,500 mg/l of IAA (46.1%). The mean root number and the mean root length of IBA set ranged from  $3.2 \pm 0.20$ – $10.2 \pm 0.37$  and  $1.6 \pm 1.01$ – $7.2 \pm 0.19$  cm, and significantly higher than other treatments (one-way ANOVA). The IAA set was  $3.2 \pm 0.20$ – $6.8 \pm 0.20$  and  $2.6 \pm 0.08$ – $4.6 \pm 0.13$  cm, respectively (Table 2). After 15 weeks the layered samples were transplanted into separate pots and placed under a mist house before reintroduction.





Image 1. *Humboldtia unijuga* var. *trijuga*: A–C—Stem cuttings. A—1,500 mg/l of IBA treated stem cutting | B—1,500 mg/l of IAA treated stem cutting | C—Stem cutting established on polythene bag | D—Air layering | E—early flowering on air layered sapling. © Shintu S.

## DISCUSSION

The present study aimed to develop a vegetative propagation protocol for the multiplication and ex situ conservation of the 'Critically Endangered' species *H. unijuga* var. *trijuga*. When effective seeding is not

available, exact copies of the parent plant can be produced in large numbers using conventional propagation methods, which can be made more successful through the application of exogenous auxin, within a shorter period. The present study showed that exogenous application of specific auxins resulted in rooting in stem

**Table 1.** Effect of different concentrations of auxins on rooting percentage, number and length of roots, and survival percentage of stem cuttings of *Humboldtia unijuga* var. *trijuga* after 12 weeks.

Treatments / hormone	Concentration (mg/l)	Callus formation (%)	Number of roots (Mean $\pm$ SE)	Root length (cm) (Mean $\pm$ SE)	Rooting (%)	Survival (%)
Control	NA	16	NR	NR	NR	NA
IBA	500	32	2.4 $\pm$ 0.24 <sup>d</sup>	1.8 $\pm$ 0.05 <sup>f</sup>	25	25
	1000	56	3.8 $\pm$ 0.37 <sup>c</sup>	2.7 $\pm$ 0.12 <sup>d</sup>	54.5	36.3
	1500	68	7.2 $\pm$ 0.58 <sup>a</sup>	6.4 $\pm$ 0.12 <sup>a</sup>	66.6	40
	2000	36	4.4 $\pm$ 0.89 <sup>bc</sup>	3.5 $\pm$ 0.15 <sup>c</sup>	16	NR
IAA	500	24	NR	NR	NR	NA
	1000	40	2.2 $\pm$ 0.37 <sup>d</sup>	2.3 $\pm$ 0.18 <sup>e</sup>	44.4	25
	1500	56	5.2 $\pm$ 0.48 <sup>b</sup>	4.7 $\pm$ 0.12 <sup>b</sup>	54.5	33.3
	2000	NR	NA	NA	NA	NA
NAA	500	NR	NA	NA	NA	NA
	1000	NR	NA	NA	NA	NA
	1500	NR	NA	NA	NA	NA
	2000	NR	NA	NA	NA	NA

Note: Stem cuttings with at least one root were considered for calculating percentage of rooting: SE—standard error | ANOVA Df (n-1) = 12, F = 74.6\*\*\*, F = 573.9\*\*\*, level of significance  $P < 0.05$ ,  $n = 25$  | different letters indicate significant differences between treatments based on  $p$  value  $< 0.05$  and same letter are not significantly different from each other at the  $p < 0.05$ . NR—Not responded | NA—Not applicable.

**Table 2.** Effects of different concentrations of auxins on rooting percentage, number and length of roots, and survival percentage of air-layered branches *Humboldtia unijuga* var. *trijuga* after 12 weeks.

Treatment / hormone	Concentration (mg/l)	Callus formation (%)	Number of roots (Mean $\pm$ SE)	Root length in cm (Mean $\pm$ SE)	Rooting (%)	Survival (%)
Control		30	1.4 $\pm$ 0.24 <sup>d</sup>	1.04 $\pm$ 0.05 <sup>e</sup>	33.3	NR
IBA	500	20	3.8 $\pm$ 0.37 <sup>c</sup>	2.4 $\pm$ 0.14 <sup>c</sup>	20	NR
	1000	52	6.6 $\pm$ 0.50 <sup>b</sup>	4.3 $\pm$ 0.11 <sup>b</sup>	46.1	50
	1500	76	10.2 $\pm$ 0.37 <sup>a</sup>	7.2 $\pm$ 0.19 <sup>a</sup>	52.6	60
	2000	36	3.2 $\pm$ 0.20 <sup>c</sup>	1.6 $\pm$ 0.06 <sup>d</sup>	22.2	NR
IAA	500	NR	NA	NA	NA	NA
	1000	36	3.2 $\pm$ 0.20 <sup>c</sup>	2.6 $\pm$ 0.08 <sup>c</sup>	33.3	33.3
	1500	52	6.8 $\pm$ 0.20 <sup>b</sup>	4.6 $\pm$ 0.13 <sup>b</sup>	46.1	42.8
	2000	NR	NA	NA	NA	NA
NAA	500	NR	NA	NA	NA	NA
	1000	NR	NA	NA	NA	NA
	1500	NR	NA	NA	NA	NA
	2000	NR	NA	NA	NA	NA

Note: Air layering with at least one root was considered for calculating the percentage of rooting: SE—standard error; ANOVA Df (n-1) = 12, F = 203.7\*\*\*, F = 683.5\*\*\*, level of significance  $P < 0.05$ ,  $n = 25$  | different letters indicate significant differences between treatments based on  $p$  value  $< 0.05$  and same letter are not significantly different from each other at the  $p < 0.05$ . NR—Not responded | NA—Not applicable.

cuttings and air layering in *H. unijuga* var. *trijuga*, and the saplings survived successfully. Many studies revealed the effectiveness of vegetative propagation of endemic and endangered species used for the restoration of vegetation (Lemay et al. 2009; Ramos-Palacios et al. 2012; Duarte et al. 2018) and ex situ conservation

practices for species with inherent problems of seed germination and seedling establishment in wild (Kamila & Panda 2019). Also, this will allow large-scale production of planting materials for reintroduction programmes. The precision of auxins was found critical for the vegetative propagation success in this study. Auxins are particularly

crucial for plant cell growth and are involved in numerous biological processes including initiation of leaf primordia, and lateral root production (Bertoni 2011; Pacurar et al. 2014).

The present study confirmed that the application of auxins IBA and IAA were found ideal for *H. unijuga* var. *trijuga* which promote root formation both in air layering, and stem cuttings like *Syzygium caryophyllatum* (Hussain & Anilkumar 2016) and *Dysoxylum malabaricum* (Hussain et al. 2013), as they found that stem cuttings treated with 1,500 mg/l of IBA recorded the highest rooting success (53.3%) and 63% of success in air layering. Although rooting was observed in all the treatments, especially of IBA treatments, data revealed the maximum significant rooting response by higher concentration of IBA followed by higher concentration of IAA (Tables 1 & 2). The application of IBA may enhance the translocation of sugar to the base of cuttings and stimulate rooting in the layering process. Nanda (1975) reported that auxins promote the activity of hydrolytic enzymes, which in turn promotes stem cuttings to root by enhancing the mobilization of reserve food supplies. The present experiment revealed that stem cuttings treated with 1,500 mg/l IBA resulted in significantly higher values for sprouting of 66.6% and 54.6% survival. The application of IBA was found ideal for the allied species *Humboldtia vahliana* as reported by Jose et al. (2010). Behera et al. (2020) reported that cuttings of *Commiphora wightii* treated with concentrations of IBA shows better result at concentration of 1,000 mg/l. The present study as well as other studies showed that IBA is one of the most effective and widely used auxins in vegetative propagation over a wide range of concentrations and is effective in stimulating root growth in a large number of plant species (Hartman et al. 2011). IBA was found to be a better rooting hormone in comparison with IAA & NAA, and is nontoxic to plants (Eganathan et al. 2000). In this study, no response was found against the application of NAA in both stem cuttings and air layering. Jose et al. (2011) recorded a high percentage of rooting in *Humboldtia bourdillonii*, an allied endemic species, using 500 mg/l of NAA. This may be due to the inherent physiological differences of the species.

It was observed that the rooting response treated with 1,500 mg/l of IAA and IBA were recorded with formation of maximum number of roots and survival. The highest survival percentage of air layers (60%) was recorded in stem treated with 1,500 mg/l IBA and 42.8 % in IAA. A similar type of result was reported in *Elaeocarpus venustus* (Soorangkattan et al. 2021). According to Eganathan et al. (2000), saplings raised

via air-layering exhibit greater adaptability to field conditions. A higher rooting success was reported by Kamila & Panda (2019) when using 5,000 mg/l of IBA in *Lasiococca comberi*. Woody forest species like *Myrica esculenta* (Purohit et al. 2004) and *Quercus glauca* (Purohit et al. 2005) were easily multiplied through air layering. The rooting response varies depending on the type of cuttings used and the season may be due to the activities of hydrolytic enzymes which are reported to be highly active during monsoon and post monsoon months (Nanda 1975; Blake & Bentley 1985).

The present study showed that the application of IBA and IAA may result in rooting of stem cuttings, and air layering experiments of the endangered woody species *Humboldtia unijuga* var. *trijuga*, which could be used successfully for mass propagation, and for reintroduction programs. Also, the plants survived well in the nursery (Tables 1 & 2) and even some of the air-layered saplings flowered (Image 1E). This feature showed a good indication that these plants reintroduced in their native habitat, may survive.

## CONCLUSION

The present study revealed a mass multiplication technique through stem rooting and air layering by the application of auxins to conserve this critically endangered species. The stem cuttings and layering samples pre-treated with 1,000 & 1,500 mg/l of IBA, and IAA showed the maximum response. The present study may provide a cost-effective technique for mass production of genetically identical mature planting materials for reintroduction programmes. This technique could support the long-term survival of this species in the wild.

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## Niche characterization and distribution of Sikkim Himalayan *Begonia* (Begoniaceae), India: a niche modeling approach

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**Abstract:** Understanding species' ecological niches and distribution patterns is crucial for biodiversity conservation and management, particularly in ecologically sensitive regions. We used an NDVI-based ecological niche modeling (ENM) approach for *Begonia* species for this purpose, where we achieved high predictive accuracy (AUC: 0.82–0.97). Niche breadth analysis revealed a positive correlation ( $r = 0.747$ ,  $p = 0.003$ ) between broader niche breadth and larger predicted distribution areas, aligning with the notion that better-performing models tend to capture either highly specialized (narrow-breadth) or ecologically flexible (broad-breadth) niches. Models for *Begonia picta*, *B. panchtharensis*, *B. sikkimensis*, and *B. xanthina* were classified as fair ( $0.8 < \text{AUC} < 0.9$ ), and exhibited broader niche breadth, with ranges extending from the western Himalaya to the eastern Himalaya, encompassing Nepal, Bhutan, and China. In contrast, *B. satrapis*, *B. gemmipara*, and *B. nepalensis* showed very good model performance ( $\text{AUC} > 0.95$ ) but had the narrowest niche breadth (0.102–0.195), suggesting specialized habitat requirements and restricted distributions. Given their limited ecological flexibility and smaller suitable areas, these species warrant immediate conservation attention to mitigate extinction risks.

**Keywords:** Conservation, Darjeeling, diversity, endemic, ENM, MaxEnt, NDVI, niche overlap, niche breadth, northeastern India.

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**Author contributions:** AP conducted the field survey, collected data, performed the modeling, and drafted the manuscript. DA refined the model and contributed to manuscript revision. AC was responsible for the research design, provided overall supervision, and approved the final version of the manuscript

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## INTRODUCTION

For centuries, ecologists and biologists have been fascinated on why species vary greatly in the extent of its distribution. Some species have a narrow distribution range, whilst some closely related species have a broader distribution, ranging from the continental to the global scale (Willis 1922). It is widely believed that narrowly distributed species have specialized environmental requirements while the widely distributed species have broader environmental tolerance. Therefore, a positive correlation between environmental niche breadth and range size is widely accepted in macro ecological studies (Gaston 2000; Gaston & Spicer 2001; Slatyer et al. 2013). However, it is difficult to conclude the above hypothesis because the environmental niche of a species is usually defined by the set of occurrence records. Hence, a larger number of presence locality data are likely to have a wider distribution range, unlike species having a lesser number of occurrence records (Burgman 1989; Gaston & Blackburn 2000; Gregory & Gaston 2000; Gaston & Spicer 2001). Therefore, the species-rich genus *Begonia* in Sikkim Himalaya was chosen as the model plant to answer this question.

*Begonia* L. is the sixth largest genus of flowering plants and provides several important ecosystem services. For instance, they help stabilize soil in humid understory environments, support local invertebrates, and contribute to microhabitat maintenance in forested areas. Some *Begonias* also hold ornamental and economic value, being used in horticulture for their diverse foliage, and showy flowers. In certain regions, they have recognized medicinal uses, underscoring their cultural and economic importance. Consequently, conserving *Begonias* will not only preserve the essential ecological interactions but also safeguard potential benefits for local communities.

Being one of the largest genera of flowering plants, they provide an excellent opportunity to study the processes underlying the theory of rapid radiation. A sufficient amount of occurrence data is required to develop a robust distribution model and to test the above mentioned theory (Moonlight 2017). However, the unavailability of geo-referenced occurrence data in herbaria and other online sources such as GBIF (Global Biodiversity Information Facility) limits the use of such techniques. At present, there is a growing need to estimate the species distribution range for theoretical as well as applied reasons, e.g., understanding species geography to its conservation. Limited species occurrence data pose enormous challenge to the

researchers. Moreover, quantifying the environmental factors which contribute the most to the distribution of species becomes even more complicated and challenging (Guisan & Thuiller 2005; Colwell & Rangel 2009). The factors that govern the distribution of species are biotic factors, abiotic factors (soil and topography), species interaction, competition, predators, and parasites (Gaston 2003). In practice, the species distribution model is developed using only the occurrence data, and abiotic variables. Recently several studies have indicated the importance of biotic interaction in shaping the spatial distribution of species (Gotelli et al. 2010; Sunday et al. 2011). The factors such as biotic interaction and dispersal are usually ignored, and their effect considered negligible at broader geographical scale or spatial scales (Soberón 2007; Colwell & Rangel 2009; Gotzenberger et al. 2012; Araújo et al. 2014). Thus, abiotic factors, such as bioclimatic variables, NDVI, slope, and aspect are often used in predicting, and identifying the suitable habitat of species (Pradhan et al. 2020). The selection of predictor variables is fundamental before modelling, yet the choice of input variables is still debatable (Synes & Osborne 2011). The ecologically relevant variables are capable of generating robust models and vice versa. For example, the soil type variables might be good predictor variables for plants whilst temperature, and forest fragmentation related variables might be a good choice for animals. The use of NDVI contributes to the modelling process by providing information about the phenological status, canopy cover, and the water content variation (Amaral et al. 2007). In addition to capturing phenological status and canopy cover, NDVI also provides insights into spatial variation in plant health & productivity, reflecting factors such as vegetation stress, and soil nutrient availability. Consequently, NDVI data can be used as a proxy for detecting water deficits, drought stress, or nutrient limitations, all of which are critical for understanding *Begonia* establishment, and persistence. Thus, this study aimed to (1) predict the suitable habitat of *Begonia* species in Sikkim Himalaya, and (2) define the ecological niche of *Begonia* species and quantify the similarities between them using ENM techniques. The ENMs constructed were compared to assess the similarities of the ecological niche of the *Begonia* species, and to know if they share the same ecological niche or not.

## MATERIALS AND METHODS

### Study area

The district of Darjeeling shares a continuous geological and physiographic landscape with Sikkim, rendering the two regions inseparable in these respects (Basu 2013). The region lies adjacent to Nepal in the east, China in the north, and Bhutan in the west, making it a geopolitically, and biogeographically significant segment of the Eastern Himalaya. The physical features of Darjeeling and Sikkim are very similar, separated by rivers Teesta, and Rungit which act as a natural boundary dividing the two geographically consonant regions (Figure 1). Therefore, the state of Sikkim along with Darjeeling together constitutes the Sikkim Himalaya. The two regions from herein will be referred to as Sikkim Himalayas (Rai et al. 2000). The region lies amid the eastern Himalayan regions, roofed by a snow clad mountain in the north, and planes in the south. It is bordered by countries such as Nepal in the east, China in the north, and Bhutan in the west, and is tectonically one of the most active areas of the Himalaya.

### Collection of occurrence record

The primary occurrence data or the presence data (i.e., geographic coordinate/Latitude and Longitude) for 13 species of *Begonia* (viz., *B. satrapis*, *B. gemmipara*, *B. josephii*, *B. picta*, *B. xanthina*, *B. cathcartii*, *B. flaviflora*, *B. megaptera*, *B. nepalensis*, *B. palmata*, *B. sikkimensis*, *B. panchtharensis*, *B. roxburghii*) were collected from the hills of Darjeeling and Sikkim Himalaya using Garmin GPS (Global Positioning System). The occurrence data were collected with an accuracy of 3–10 m.

The geographic coordinate was collected in the form of Degree Minute Second (DMS) which was later converted to decimal degrees (DD) using the formula:

$$DD = D + M/60 + S/3600$$

The converted presence data was later rearranged in Microsoft Excel in the following order, i.e., species name, longitude, latitude, and then saved in CSV (comma delimited) format, and was later used for modelling.

### Predictor Variables

The model was developed using normalized difference vegetative index (NDVI) raster data for January to December obtained from GLCF (Global Land Cover Facility) (University of Maryland, USA). The NDVI is a numerical indicator that quantifies vegetation by measuring the difference between near-infrared (which vegetation strongly reflects) and red light (which vegetation absorbs), and is given by the formula:

$$NDVI = (NIR - RED) / (NIR + RED)$$

Where; NIR = near-infrared and

RED = Red light

The 12 NDVI variables were first subjected to correlated tests ( $r > 0.9$ ) using ENM Tools 1.3 software (Warren et al. 2010). Thus, out of 12 NDVI variables, 10 were used to model the distribution of *Begonia* in Sikkim Himalaya along with altitude (Table 1). Although NDVI data for August and September were initially considered, both months showed high correlation ( $r > 0.9$ ) with July NDVI, risking over fitting if included simultaneously. Following best practices to reduce multicollinearity, we retained July NDVI as representative of the monsoon peak and excluded August and September. This approach helps ensure model parsimony and avoids redundant variables.

### Ecological Niche Modelling

MaxEnt v.3.3.3k Software (Phillips & Dudík 2008) was used to model the distribution of *Begonia* species in Sikkim Himalaya. MaxEnt modelling was used because it has a high accuracy rate and performs better with small size (Elith et al. 2006). The 10 percentile training presence logistic threshold was used, with 20 replicates run, and maximum of 5,000 iterations for each species. All other settings were kept default as it has been calibrated with a wide range of species (Phillips & Dudík 2008). From 20 replicated runs for each species, the average, and maximum, minimum, median, and standard deviation was obtained. Each *Begonia* species were modelled individually using a set of NDVI variables.

Using Niche Toolbox (<http://shiny.conabio.gob.mx:3838/nichetoolb2/>) binary maps were obtained and suitable areas for each species of *Begonia* were calculated using 10 percentile training presence logistic threshold cut off values.

### Niche Overlap

ENM Tools software was used to examine the degree of niche overlap between *Begonia* species. Schoener's D and Hellinger's I metrics were used to estimate the niche overlap between the species.

Schoener's D is given by the formula:

$$D(p_X, p_Y) = 1 - \frac{1}{2} \sum_i |p_{X,i} - p_{Y,i}|$$

Where  $p_{x,i}$  and  $p_{y,i}$  are the normalized suitability scores for species X and Y in grid cell  $i$ , similarly Hellinger's I is given by the formula:

$$I(p_X, p_Y) = 1 - \frac{1}{2} \sqrt{\sum_i (\sqrt{p_{X,i}} - \sqrt{p_{Y,i}})^2}$$



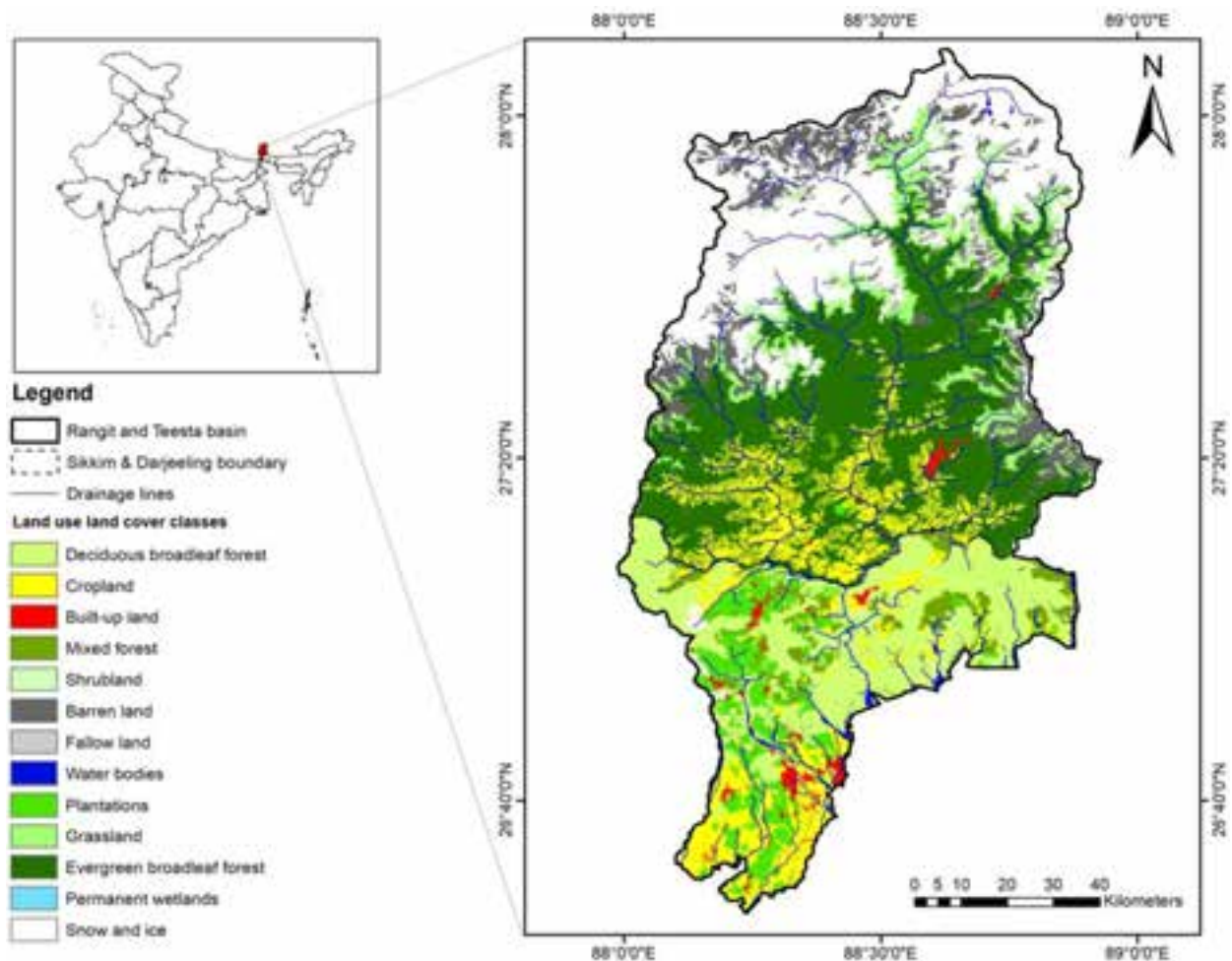


Figure 1. Land use land cover in Sikkim and Darjeeling of northeastern India. Data source for land use land cover: Roy et al. (2016), [https://daac.ornl.gov/VEGETATION/guides/Decadal\\_LULC\\_India.html](https://daac.ornl.gov/VEGETATION/guides/Decadal_LULC_India.html).

The niche similarity measures are obtained after comparing the predicted suitable habitat calculated for each grid cell from a model developed through MaxEnt. The niche overlap values range vary from 0–1. The value 0 indicates no overlap and the value of 1 indicates a complete overlap of niches. If only two ENM outputs of two species are loaded in ENM Tools, single values of *D* and *I* will be produced and if more than two populations of different species are loaded pair wise *D* and *I* values will be produced in simple Microsoft excel file (Warren et al. 2010).

#### Niche Breadth

Niche breadth was also assessed using the same set of output predicted distribution models for each species (Phillips et al. 2006; Warren et al. 2010).

#### Model evaluation and Performance

The model developed for each species was classified and evaluated based on “area under the curve” or AUC values. The model was further graded as: poor ( $AUC < 0.8$ ), fair ( $0.8 < AUC < 0.9$ ), good ( $0.9 < AUC < 0.95$ ), and very good ( $0.95 < AUC < 1.0$ ) following Thuiller et al. (2005).

#### RESULTS

##### Occurrence record

A total of 108 occurrence records or geographic coordinates (*B. gemmipara* = 8, *B. josephii* = 12, *B. satrapis* = 10, *B. picta* = 12, *B. nepalensis* = 4, *B. palmata* = 14, *B. panchtharensis* = 5, *B. sikkimensis* = 8, *B. cathcartii* = 7, *B. megaptera* = 5, *B. xanthina* = 5, *B. flaviflora* = 5, *B. roxburghii* = 13) were collected from Sikkim Himalayas.

The individual occurrence data were then correlated with the set of NDVI variables.

### Predicted habitat distribution

ENM was computed individually for each *Begonia* species. The model developed for 13 species of *Begonia* are presented in Figure 2. The 10 percentile training presence logistic threshold values for each species of *Begonia* are also provided in table 4. Using the threshold values of individuals *Begonia* species suitable habitat was calculated. Therefore based on NDVI dataset, *B. panchtharensis* had the maximum area predicted to be suitable (~4306.88 km<sup>2</sup>), followed by *B. sikkimensis* (~3,804.62 km<sup>2</sup>), *B. picta* (~3,785.4 km<sup>2</sup>), *B. cathcartii* (~2,480.01 km<sup>2</sup>), *B. xanthina* (~1,905.8 km<sup>2</sup>), *B. josephii* (~1,833.28 km<sup>2</sup>), *B. flaviflora* (~1,634.74 km<sup>2</sup>), *B. megaptera* (~1,412.58 km<sup>2</sup>), *B. satrapis* (~1,274.37 km<sup>2</sup>), *B. gemmipara* (~1,131.25 km<sup>2</sup>), *B. palmata* (~783.446 km<sup>2</sup>), *B. roxburghii* (~766.19 km<sup>2</sup>), *B. nepalensis* (~60.36 km<sup>2</sup>).

### Model evaluation and validation

Our model performance showed high accuracy and demonstrated high predictive ability based on AUC scores. The mean AUC; ranged from 0.82 in *B. panchtharensis* and *B. sikkimensis* to 0.97 in *B. satrapis* (Table 4).

### Contributing variables and Environmental constraints for *Begonia* species

The different NDVI variables used to model the distribution of *Begonia* species in Sikkim Himalaya showed a varying degree of contribution to each species

model developed. The NDVI for July contributed the most in *B. cathcartii* (68.7 %) followed by *B. sikkimensis* (62.6 %), *B. josephii* (50.5 %), *B. flaviflora* (48.5 %), and *B. gemmipara* (47.6 %). The NDVI for November contributed the most in the case of *B. picta* (32.3 %), *B. palmata* (37.3 %), *B. megaptera* (56.8 %), and *B. roxburghii* (29.6 %). The NDVI for May, January, and March each contributed the most in *B. satrapis* (63.6 %), *B. nepalensis* (24.5 %), and *B. xanthina* (53.1 %) respectively to the final predictive model (Figure 3; Table 3). Considering the permutation importance, the NDVI for July contributed the most in *B. gemmipara* (71.9 %), *B. josephii* (39.5 %), *B. cathcartii* (60.6 %) and *B. flaviflora* (67.7 %). The NDVI for November contributed the most in *B. picta* (72.7 %), *B. palmata* (40.3 %), and *B. megaptera* (53.3 %), *B. xanthina* (33.6 %). The NDVI for January, May, October, and December contributed the most in *B. nepalensis* (30.3 %), *B. satrapis* (72.1 %), *B. sikkimensis* (43.0 %), and *B. panchtharensis* (53.3 %), and altitude contributed the most in *B. roxburghii* (35.0 %) (Table 3).

### Niche overlap

The niche overlap test resulted in significantly different levels of overlaps in *Begonia* species. The Hellinger's I niche overlap values were highest between *B. picta*, *B. sikkimensis*, and *B. megaptera* (overlap value = 0.96) indicating the high level to niche overlap whereas the lowest level of niche overlap was estimated between *B. satrapis*, and *B. flaviflora* (overlap value = 0.35) (Table 2).

Similarly, the Schoener's D niche overlap values were highest between *B. picta*, *B. sikkimensis*, and *B. megaptera* (overlap value = 0.81) indicating the high

**Table 1. Correlation analysis for the 12 NDVI layers to check multicollinearity using ENM Tools 1.3 (Warren et al. 2010).**

Predictor variables	eu1 (Jan)	eu2 (Feb)	eu3 (Mar)	eu4 (Apr)	eu5 (May)	eu6 (Jun)	eu7 (Jul)	eu8 (Aug)	eu9 (Sep)	eu10 (Oct)	eu11 (Nov)	eu12 (Dec)
Alt	-0.56	-0.77	-0.24	-0.53	-0.57	-0.16	-0.76	-0.75	-0.61	-0.10	-0.53	-0.51
eu1 (Jan)		0.57	0.15	0.45	0.35	0.07	0.58	0.61	0.60	0.13	0.46	0.45
eu2 (Feb)			0.12	0.54	0.60	0.22	0.62	0.58	0.46	0.23	0.54	0.54
eu3 (Mar)				0.03	-0.01	-0.16	0.18	0.22	0.22	-0.15	0.09	0.04
eu4 (Apr)					0.67	0.56	0.69	0.67	0.60	0.59	0.69	0.75
eu5 (May)						0.47	0.58	0.54	0.43	0.45	0.63	0.65
eu6 (Jun)							0.39	0.35	0.29	0.73	0.51	0.59
eu7 (Jul)								<b>0.96</b>	0.88	0.37	0.75	0.71
eu8 (Aug)									<b>0.92</b>	0.35	0.74	0.69
eu9 (Sep)										0.33	0.71	0.63
eu10 (Oct)											0.56	0.72
eu11 (Nov)												0.75

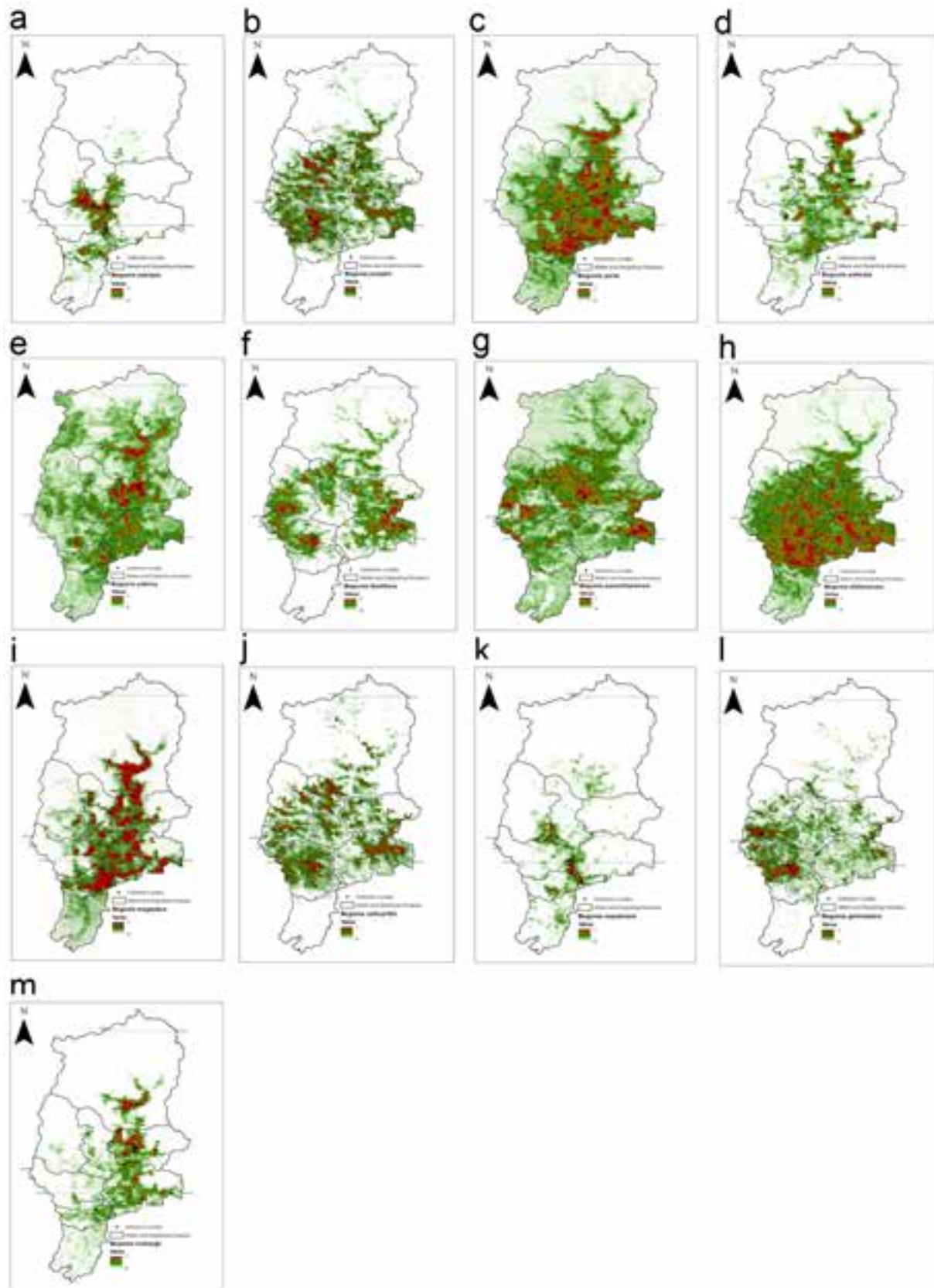


Figure 2. Predicted distribution map based on NDVI variables: a—*B. satrapis* | b—*B. josephii* | c—*B. picta* | d—*B. palmata* | e—*B. xanthina* | f—*B. flaviflora* | g—*B. panchtharensis* | h—*B. sikkimensis* | i—*B. megaptera* | j—*B. cathcartii* | k—*B. nepalensis* | l—*B. gemmipara* | m—*B. roxburghii*.



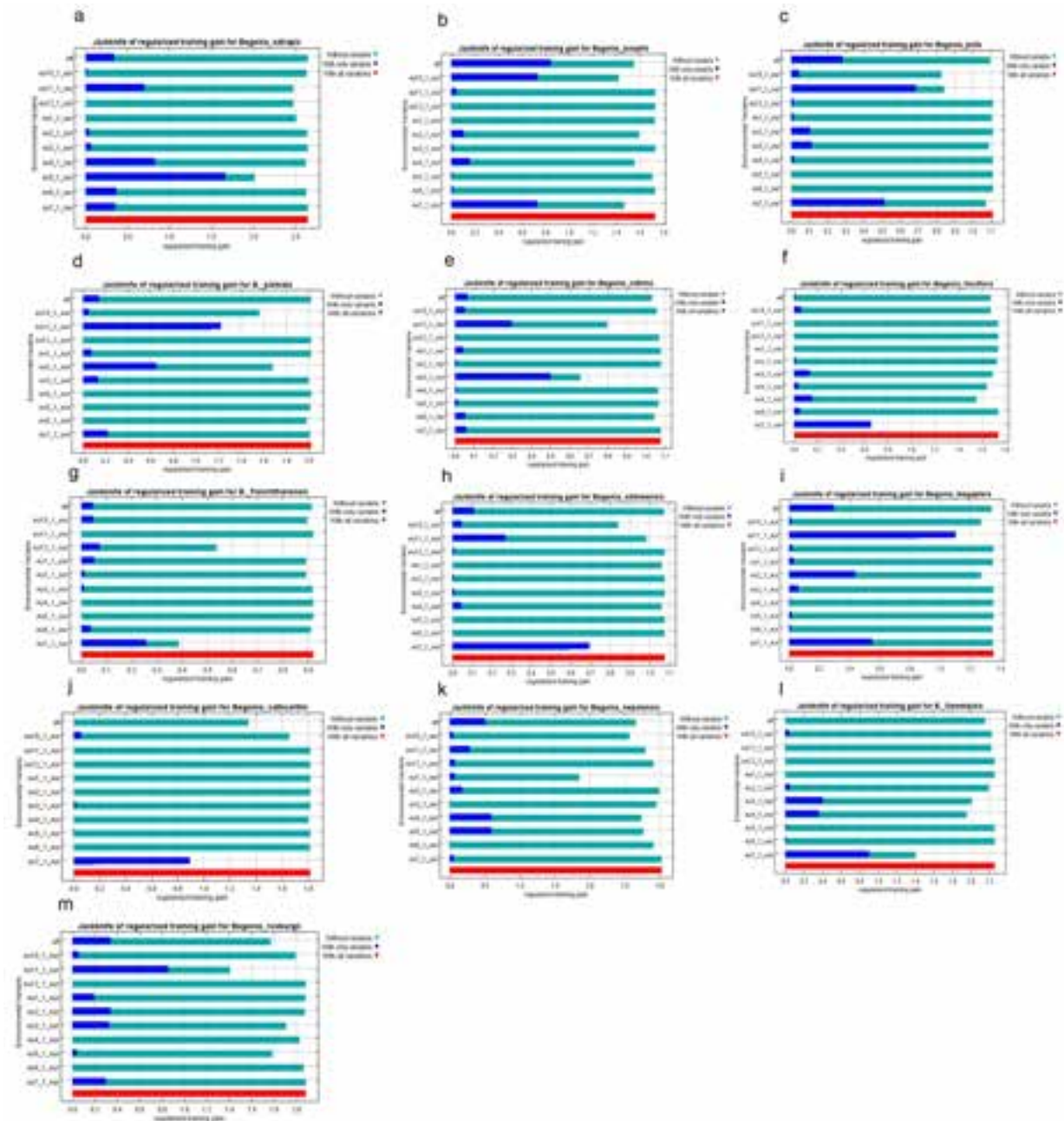


Figure 3. Results of jackknife test for variables importance in *Begonia* species using NDVI variable. a—*B. satrapis* | b—*B. josephii* | c—*B. picta* | d—*B. palmata* | e—*B. xanthina* | f—*B. flaviflora* | g—*B. panchtharensis* | h—*B. sikkimensis* | i—*B. megaptera* | j—*B. cathcartii* | k—*B. nepalensis* | l—*B. gemmipara* | m—*B. roxburghii*.

level to niche overlap whereas a low level of niche overlap was estimated between *B. satrapis*, and *B. flaviflora* (0.12) (Table 2).

### Niche Breadth

The niche breadth analysis resulted in narrower niches in some *Begonia* species. *Begonia panchtharensis* had the highest niche breadth value (NBV) of 0.642, indicating broader niches compared to other related

species of *Begonia*, which also presented the broadest distribution of suitable habitat. Similarly, the niche breadth for *B. sikkimensis* (NBV = 0.412) and *B. picta* (NBV = 0.384) were also high with broader distribution of suitable habitat compared to other species of *Begonia*. The lowest niche breadth value was estimated in *B. satrapis* (NBV = 0.102) indicating a very narrow niche. Species like *B. nepalensis* (NBV = 0.110) and *B. palmata* (NBV = 0.180) also showed low niche breadth with a

Table 2. Summary of niche overlap values based on NDVI dataset [Schoener's D (above diagonal) and Hellinger's I (below diagonal)].

<div> <div>Schoener's D</div> <div>Hellinger's I</div> </div>	<i>B. gemmipara</i>	<i>B. josephii</i>	<i>B. satrapis</i>	<i>B. picta</i>	<i>B. nepalensis</i>	<i>B. palmata</i>	<i>B. panchtharensis</i>	<i>B. sikkimensis</i>	<i>B. cathcartii</i>	<i>B. megaptera</i>	<i>B. xanthina</i>	<i>B. flaviflora</i>	<i>B. roxburghii</i>
<i>B. gemmipara</i>		0.65	0.25	0.50	0.24	0.33	0.49	0.63	0.69	0.38	0.45	0.68	0.32
<i>B. josephii</i>	0.89		<b>0.22</b>	0.55	0.27	0.46	0.49	0.68	0.76	0.46	0.43	0.68	0.37
<i>B. satrapis</i>	0.53	0.49		0.43	0.55	0.38	0.32	0.37	0.20	0.43	0.34	<b>0.12</b>	0.28
<i>B. picta</i>	0.81	0.81	0.73		0.44	0.67	0.57	<b>0.81</b>	0.49	<b>0.81</b>	0.70	0.48	0.66
<i>B. nepalensis</i>	0.50	0.52	0.81	0.73		0.39	0.31	0.39	0.21	0.44	0.34	0.18	0.33
<i>B. palmata</i>	0.61	0.71	0.68	0.91	0.68		0.37	0.56	0.35	0.75	0.52	0.32	0.67
<i>B. panchtharensis</i>	0.79	0.75	0.63	0.85	0.59	0.67		0.64	0.49	0.48	0.65	0.53	0.40
<i>B. sikkimensis</i>	0.89	0.89	0.68	<b>0.96</b>	0.68	0.82	0.88		0.64	0.67	0.63	0.60	0.54
<i>B. cathcartii</i>	0.93	0.95	0.46	0.77	0.45	0.62	0.76	0.88		0.39	0.40	0.75	0.31
<i>B. megaptera</i>	0.67	0.69	0.74	<b>0.96</b>	0.73	0.95	0.79	0.89	0.65		0.65	0.37	0.70
<i>B. xanthina</i>	0.76	0.71	0.66	0.91	0.88	0.88	0.88	0.87	0.68	0.88		0.43	0.55
<i>B. flaviflora</i>	0.90	0.90	<b>0.35</b>	0.77	0.41	0.60	0.82	0.87	0.93	0.65	0.75		0.34
<i>B. roxburghii</i>	0.60	0.62	0.57	0.90	0.61	0.91	0.67	0.81	0.57	0.92	0.80	0.63	

Note: Species are grouped by sections. Highest and lowest overlap values are in bold

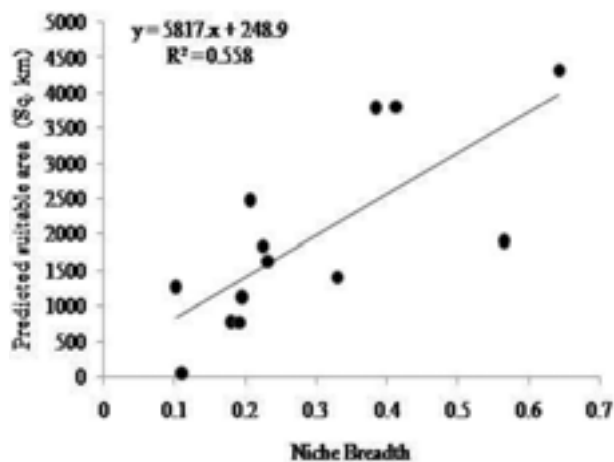


Figure 4. Correlation between predicted suitable area and niche breadth of *Begonia* species using NDVI variables.

narrow distribution of suitable habitat (Table 4).

#### Relationship between predicted suitable habitat and niche breadth

A strong positive correlation ( $r = 0.747$ ,  $p = 0.003$ ) was observed between predicted suitable habitat and niche breadth indicating that the species with higher predicted area retains broader niche breadth and vice versa (Figure 4).

## DISCUSSION

### Niche characterization in *Begonia* species

The distribution of *Begonia* species is correlated with NDVI based on niche modeling. The importance of NDVI variables contributing to the final predictive model varied across species. The model developed for *B. gemmipara*, *B. josephii*, *B. sikkimensis*, *B. cathcartii*, and *B. flaviflora* showed the highest contribution by NDVI for July. Species like *B. picta*, *B. palmata*, *B. megaptera*, and *B. roxburghii*, usually flowers late after the monsoon, and thereby NDVI for November might have been the most important predictor variables affecting the distribution of species. Interestingly NDVI for November contributed the most in predicted the distribution of *B. nepalensis*. The month of November might have contributed the most in *B. nepalensis* as the species flowers late during dry season i.e. December–January. Amongst all the 13 species of *Begonia*, *B. satrapis* is considered Critically Endangered and is endemic to Sikkim and Darjeeling District of West Bengal (Adhikari et al. 2018). Due to narrow geographic range having restricted distribution, such taxa are more sensitive to habitat disturbance leading to extinction (Peterson & Watson 1998). The distribution of *B. satrapis* is strictly affected by NDVI for May, when the species begins to regenerate from the tuber.

**Table 3.** Average contribution of input NDVI variables to model output for each species of *Begonia* distributed in Sikkim Himalaya.

Taxon	<i>B. gemmipara</i>	<i>B. josephii</i>	<i>B. satrapis</i>	<i>B. picta</i>	<i>B. nepalensis</i>	<i>B. palmata</i>	<i>B. panchtharensis</i>	<i>B. sikkimensis</i>	<i>B. cathartii</i>	<i>B. megaptera</i>	<i>B. xanthina</i>	<i>B. flaviflora</i>	<i>B. roxburghii</i>
Variables													
<b>Percentage contribution</b>													
Eu1 (Jan)	0	0.9	12.5	0.3	<b>24.5</b>	0	1.3	0.8	0	0.3	0.1	0	0.1
Eu2 (Feb)	14.1	3.8	1.2	0	0.9	30.9	6.0	0	0.3	21.2	0	4.2	5.3
Eu3 (Mar)	19.2	0	1.0	5.0	2.0	4.0	1.2	0	0	0	<b>53.1</b>	4.3	18.2
Eu4 (Apr)	11.3	4.7	3.2	0	10.8	0.2	0.1	0.8	0.5	0.3	1.3	2.0	2.6
Eu5 (May)	0	4.6	<b>63.6</b>	0	18.2	1.8	0	0	0.2	0	0.5	31.6	12.3
Eu6 (Jun)	0.1	0	1.1	0	3.5	1.2	0.7	0	0.2	1.1	4.1	0.1	0.6
Eu7 (Jul)	<b>47.6</b>	<b>50.5</b>	0	12.5	0	0.6	37.2	<b>62.6</b>	<b>68.7</b>	0.4	0.1	<b>48.5</b>	0
Eu10 (Oct)	1.6	23.2	0.7	28.2	9.6	24.1	2.4	26.9	9.3	12.1	9.6	4.4	12.0
Eu11 (Nov)	2.2	0	6.2	<b>32.3</b>	10.2	<b>37.3</b>	0	8.2	0	<b>56.8</b>	22.6	1.5	<b>29.6</b>
Eu12 (Dec)	0	0	7.0	0	2.5	0	<b>45.9</b>	0	0	0.2	1.1	0	0.1
Altitude	0	0	3.5	21.5	17.9	0.1	5.1	0.6	20.8	7.5	7.5	3.3	19.2
<b>Permutation importance</b>													
Eu1 (Jan)	0	0.9	4.5	0.2	<b>30.3</b>	0	5.7	0	0	1.3	0	0.2	0
Eu2 (Feb)	7.2	8.4	0.3	0.3	0.6	23.7	7.9	0	0	0	0	2.7	0
Eu3 (Mar)	6.2	0	0.1	2.2	0.3	1.5	0	0	0	0	17.6	0.6	7.5
Eu4 (Apr)	2.4	1.8	1.6	0	2.3	0	0.3	4.7	0.2	0.4	2.9	5.1	5.1
Eu5 (May)	0	1.4	<b>72.1</b>	0	17.0	0.1	0	0	0	0	2	7.3	19.5
Eu6 (Jun)	0.7	0	0.2	0	2.9	3.4	2.7	0.1	0.7	3.4	12.3	1	1.7
Eu7 (Jul)	<b>71.9</b>	<b>39.5</b>	0	6.2	0	3.1	25.8	41.8	<b>60.6</b>	1.9	0	<b>67.7</b>	0
Eu10 (Oct)	1.5	27.7	0.6	9.4	6.4	27.4	0.1	<b>43.0</b>	9.9	15.9	5.9	10.4	5.5
Eu11 (Nov)	0.8	0.1	14.8	<b>72.7</b>	18.5	<b>40.3</b>	0	9.7	0	<b>67.3</b>	<b>33.6</b>	0	25.6
Eu12 (Dec)	0	0	5.8	0	0.4	0	<b>53.3</b>	0	0	2.2	11.7	0	0.1
Altitude	9.2	20.2	0	8.9	21.3	0.6	4.0	0.6	28.5	7.5	14	4.9	<b>35.0</b>

### Niche overlap and Niche Breadth

It is often assumed that closely related species are morphologically and physiologically alike, and have similar environmental requirements, i.e., niche retention (Futuyma & Mitter 1996; Webb 2000; Violle et al. 2011). The niche overlap test for *Begonia* species resulted in great variability in niche overlap values between morphologically similar species. The low niche overlaps values between species of section *Diploclinium*, viz., *B. satrapis* and *B. josephii* might have resulted due to competitive interaction leading to niche partitioning (Hardin 1960). Moreover, the highest niche overlap values between species of section *Diploclinium*, viz., *B. picta* and *Platycentrum*, viz., *B. sikkimensis* and *B. megaptera* support the 'limiting similarity hypothesis' (MacArthur & Levins 1967) which posits that competitive

exclusion among closely related species leads to the frequent coexistence of more distantly related species within ecological communities.

The results of niche breadth analysis support the idea that better-performing models are associated with more specialized and narrow niche breadth and vice-versa (Fuchs et al. 2018). The model developed for *Begonia* species viz. *B. picta*, *B. panchtharensis*, *B. sikkimensis*, and *B. xanthina* were considered fair ( $0.8 < \text{AUC} < 0.9$ ), with higher niche breadth indicating more ecological flexibility compared to other species of *Begonia*. These species in addition to having broader niche breadth have larger distribution areas, ranging from Western Himalaya to entire Eastern Himalaya, covering countries like Nepal, Bhutan, and China (Rajbhandari et al. 2010; Rana 2016; Camfield & Hughes 2018; Hughes et al.



**Table 4.** Niche breadth values and predicted suitable area (10 percentile training presence logistic threshold value).

	Species	AUC	Niche breadth	Threshold value/ Area (km <sup>2</sup> )
1	<i>B. gemmipara</i>	0.93	0.195	0.304/1131.25
2	<i>B. josephii</i>	0.94	0.225	0.384/1833.27
3	<i>B. picta</i>	<b>0.89</b>	<b>0.384</b>	<b>0.396/3785.40</b>
4	<i>B. satrapis</i>	<b>0.97</b>	<b>0.102</b>	0.133/1274.37
5	<i>B. flaviflora</i>	0.91	0.231	0.416/1634.74
6	<i>B. cathcartii</i>	0.91	0.206	0.283/2480.00
7	<i>B. megaptera</i>	0.91	0.329	0.521/1412.57
8	<i>B. nepalensis</i>	<b>0.89</b>	<b>0.110</b>	<b>0.605/60.36</b>
9	<i>B. palmata</i>	<b>0.95</b>	<b>0.180</b>	<b>0.469/783.44</b>
10	<i>B. panchtharensis</i>	<b>0.82</b>	<b>0.642</b>	<b>0.333/4306.88</b>
11	<i>B. sikkimensis</i>	<b>0.82</b>	<b>0.412</b>	<b>0.445/3804.61</b>
12	<i>B. xanthina</i>	0.84	0.565	0.449/1905.79
13	<i>B. roxburghii</i>	0.94	0.191	0.448/766.19

Note: The highest and the lowest niche breadth are highlighted in bold. Values range from 0–1: 0 is equal to one grid cell being suitable (specialized niche); whereas 1 is where all grid cells are suitable (broad niche).

2018; Pradhan et al. 2019). Thus, these species have wider climatic tolerance with larger variation within and amongst the population and sometimes even recognized at a variety level (Camfield & Hughes 2018). In addition to having a wider niche breadth, these species also have a wider predicted distribution area compared to other species. A case apart in *B. xanthina* with broader niche breadth and smaller area (~1905 km<sup>2</sup>) predicted to be suitable. However, the model developed for *B. satrapis*, *B. gemmipara*, and *B. nepalensis* were considered a very good performing model with the lowest niche breadth (ranging 0.102–0.195) indicating lesser ecological flexibility. Such species with smaller niche breadth have lesser tolerance to climatic variation preferring homogenous environmental conditions (Kassen 2002; Dennis et al. 2011). The study thus displays a positive correlation between species' niche breadth and suitable predicted area, except in the case of *B. xanthina* the results were otherwise. Niche breadth of most species was consistent with their geographic distributions, as narrowly distributed species have smaller niche breadth and broader distributed species have wider niche breadth (Gaston 1993; Kunin & Gatson 1997). The narrow distribution range of *B. satrapis*, *B. gemmipara*, and *B. nepalensis* might be primarily due to narrow niche breadth. The study is in line with the study on the Mexican genus of globular cacti and numerous other similar studies (Zhu et al. 2016; Mosco 2017). Therefore,

such rare species with narrow niche breadth have a higher probability of extinction (Futuyma & Moreno 1988; McKinney 1997) and thus require immediate conservation initiatives to conserve the existing extant population.

## CONCLUSION

The predictive distribution model for *Begonia* species, like *B. picta*, *B. panchtharensis*, *B. sikkimensis*, and *B. xanthina*, showed wider niche breadths, indicating greater ecological flexibility. In addition to their broader niche breadths, these species have larger distribution areas that range from the western to eastern Himalaya. In contrast, the models for *B. satrapis*, *B. gemmipara*, and *B. nepalensis* demonstrated very strong performance with narrower niche breadths, indicating less ecological flexibility. As a result, these species require immediate attention, as their smaller suitable habitats and narrow niche breadths make them more vulnerable to extinction.

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## Diversity of snakes (Reptilia: Serpentes) in the Tezpur University Campus, Assam, India

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**Abstract:** In this study, 15 species of snakes were found in the Tezpur University campus in Assam, northeastern India. The snakes were documented by employing visual encounter survey and rescue calls. Tezpur University campus comprises of a 1.6 km<sup>2</sup> area with a green cover of approximately 75% and water bodies that serve as the home for wildlife, including reptiles. Numerous chaotic incidents of anxiety and fear due to snake sightings occurred at the campus, highlighting the need for management of snake-human negative interactions. A total of 64 snake sightings were noted during the study period, belonging to Typhlopidae (two species), Colubridae (nine species), Elapidae (three species), and Pythonidae (one species). Among them, three species, namely *Naja kaouthia*, *Bungarus fasciatus*, and *Bungarus lividus*, were venomous. These findings may make a significant contribution to the management of snake-human interactions on campus. In addition, it may serve as a reference for studies of the impact of a gradually urbanising world on snake diversity.

**Keywords:** Biodiversity, distribution, Indo-Burma hotspot, northeastern India, *Oligodon melaneus*, roadkill, snakebite, squamata, venomous snakes.

**Bodo:** বেজিৰসংনায়াব, সা-সানজা ভাৰতনি আসাম হাটৰ সায়াব থানায তেজপুৰ মুলুগসোলীসালিনি বাদায়াব, Visual Encounter Survey আৰু Rescue call আদৰ্ বাহায়ায়নি গেজেৰজোঁ গাৰ্হে মোন 15 হাৰিসানি জিৰোঁফোৰখোঁ সঁদাননানৈ মোননো হাৰ্হোমোন। তেজপুৰ মুলুগসোলীসালিনি বাদায়া 1.6 বৰ্গ কিল মিটাৰনি আনসোলখোঁ সাংলোবো, জেৰাব 75% সোমখোৰ আনসোল আৰো দৈ (ফুখি) বাহাগোফোৰ দৈ, জায় মানবায়া জিৰ (reptiles) জোঁ লোগোহে হায়ানি জিৰ-জুনাবফোৰনো থায়া খুলি হোয়ো। বৰেখানি, মুলুগসোলীসালিনি বাদায়াব জিৰোঁ নুনায়ানি জাহোনাব জিগাসিনায়া আৰো গিনায়ানি গোৰা জাথায়ফোৰ জাদোমোন। বৈ জাথায়ফোৰা জিৰোঁ আৰো মানসিনি দাডাৰাজিখোঁ সামলায়নাংগোনি গোনাথিখোঁ রেখা খালামনা দিন্দিদোমোন। বেজিৰসংনায়াব সমনি গেজেৰাব গাৰ্হে মা 64 জিৰোঁ নুনায়াখোঁ বৈগাথিখোঁ খালামনায়া জাদোমোন, জেৰাব Typhlopidae (মোন 2 হাৰিসা), Colubridae (মোন 9 হাৰিসা), Elapidae (মোন 3 হাৰিসা) আৰু Pythonidae (মোন 1 হাৰিসা) নখৰনি জিৰোঁফোৰ দোমোন। বেফোৰনি গেজেৰাব, মোনথাম হাৰিসাফোৰ - *Naja kaouthia* (জিৰোঁ ফেটিগাম), *Bungarus fasciatus* (জিৰোঁ গবাল) আৰু *Bungarus lividus* (গোসোম গাবনি জাথোহে জিৰোঁ) ফোৰা বিস গোনাংমোন। বে জিৰসংনায়াব খাৰিফোৰ কেম্পাসআব জিৰোঁ আৰো মানসি গেজেৰাব জানায়া দাডাৰাজিখোঁ সামলায়নায়াব মখ জাথাব বিহোমা হোনো হাগোঁ। বৈনি অনগায়েবো, বে খাৰিফোৰা জিৰোঁনি বায়দি রেখোম (diversity) নি সায়াব, লাসে-লাসে নোগোৰ এৰা সহৰাব গুল্‌লায়লানায়া (urbanization) মুলুগনি গোহোমখোঁ ফায়াসংনায়ানি থাখায়া মোনসে reference মৰ্হে খামানি মাননো হাগোঁ।

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## INTRODUCTION

The snake-human negative interactions is a frequently underestimated issue, presenting significant challenges to conservation, and public health. The conflict between humans and snakes has existed since time immemorial, and an innate fear of snakes is deeply rooted in humans and other primates (Öhman & Mineka 2003). As a result, snakes become one of the most misunderstood, and feared animals. Snakes feed on various invertebrates and vertebrates (Khormizi et al. 2021), and this diverse prey preference makes the ecological role of each snake species uniquely significant (Forgus 2018; Thacker 2020). Concerningly, the global population of herpetofauna is declining owing to habitat destruction (Gibbons et al. 2000), and urbanisation (Rubbo & Kiesecker 2005; McKinney 2006). Interestingly, some reptiles, including snakes, have adapted to urban settlement (Purkayastha et al. 2011; Parkin et al. 2021; Barhadiya et al. 2022). Moreover, a few studies also revealed that university campuses, can serve as a favourable habitat for snakes due to the conservation of natural habitats maintained for sustainable development (Ahsan et al. 2015; Shome et al. 2022; Janani & Ganesh 2024).

Globally, 1.8–2.7 million people are affected by snakebite each year, resulting in an estimated 80,000–1,38,000 deaths (Ralph et al. 2022). India, home to over 365 snake species (Uetz 2025), reported an average of 58,000 snakebite deaths per year between 2000 and 2019 (Suraweera et al. 2020). Though these deaths are presumably caused by four snake species, namely, *Naja naja*, *Daboia russelii*, *Bungarus caeruleus*, and *Echis carinatus* (big four), other venomous snakes prevalent in that area are also responsible. India being a geographically varied country, has different regions with medically important snakes endemic to that region. For instance, *Naja kaouthia* is distributed across Assam, Arunachal Pradesh, Uttar Pradesh, Bihar, Sikkim, West Bengal, Odisha, Tripura, Mizoram, Nagaland, Meghalaya, and Manipur. *Trimeresurus erythrurus* is distributed mainly across the northeastern states of Tripura, Meghalaya, Arunachal Pradesh, Sikkim, Mizoram, Manipur, Nagaland, and West Bengal, with isolated records from Odisha, and Andhra Pradesh (Deuti et al. 2021). Many other medically significant snakes, such as *Naja sagittifera*, *Naja oxiana*, *Bungarus fasciatus*, *Bungarus niger*, *Bungarus lividus*, *Ophiophagus hannah*, and *Gloydius himalayanus*, are distributed to some limited ranges within the country (Uetz 2025). They possess the potential of being the cause of snakebite-related medical emergencies in these geographic

ranges. This highlights the need for understanding the distribution of venomous snakes in the country for effective management of snakebite related medical emergencies. On the other hand, snakes play a crucial role in the ecosystem as predators, and mediators of biotic interactions. Despite their secretive nature, aquatic snakes can reach high densities, and consume significant amounts of prey, facilitating energy transfer between aquatic and, terrestrial habitats (Willson & Winnie 2016). Cobras, rat snakes, and snakes that typically feed on rodents contribute greatly to India's grain production and supply. By keeping the rodent population under control, they prevent crop damage, and reduce loss in crop production (Whitaker & Captain 2004). Although snake venom is a lethal mixture primarily composed of proteins and peptides, it holds outstanding therapeutic potential when structurally engineered, as evidenced by clinically used drugs like Captopril, and Tirofiban derived from venom components (Ferreira & de Silva 1965; Gan et al. 1988; Yeow & Kini 2012; Xiao et al. 2017; Munawar et al. 2018). This underscores the need to consider snake conservation for maintaining overall ecosystem integrity (Willson & Winne 2016), as well as for advancing biomedical research by harnessing snake venom as a valuable bioresource for therapeutic development. Educating people about snake identification, ecology, ethology, and distribution of venomous, and non-venomous snakes may help in avoiding snakebite incidences as well as conserving these fascinating reptiles (Whitaker & Whitaker 2012; Whitaker & Martin 2015).

In the past, studies on herpetofauna were carried out in various parts of northeastern India, including Assam, namely: Barail Wildlife Sanctuary, and the Cachar District of Assam (Das et al. 2009), the urban city of Guwahati (Purkayastha et al. 2011), Jeypore Reserved Forest of Assam (Islam & Saikia 2014), Deepor Beel (Sengupta et al. 2016), Nalbari District of Assam (Baishya & Das 2018), and Guwahati University campus (Gogoi et al. 2023). No systematic study was carried out at the Tezpur University campus, which is home to various flora, and fauna.

The present study aims to understand the diversity of snakes present in the Tezpur University campus, Assam, India. Documenting the various species of snakes found in this campus may contribute to the management of herpetofauna, and mitigation of human-snake negative interactions.



## MATERIALS AND METHODS

### Study area

Tezpur University Campus (TUC) is located in the Sonitpur District of Assam, India ( $26.696^{\circ}$  N,  $92.835^{\circ}$  E). Tezpur University Campus is on  $1.06 \text{ km}^2$  of land, bounded by concrete walls, and stands 12 km away from Tezpur City, and about 30 km away from Nameri National Park. The campus comprises various academic buildings, staff quarters, sports playground, two water bodies, a botanical garden, and green cover area of approximately 75% of the total land (Image 1). The campus houses approximately 4,000 residents. The average high temperature in Tezpur during summer is around  $31^{\circ}\text{C}$ , while the average winter low temperature is around  $13^{\circ}\text{C}$ . It receives an average annual rainfall of about 1,749 mm and has an average relative humidity of 74% (World Weather Online 2024).

### Methodology

The study was carried out from June 2021–May 2024. A visual encounter survey (Crump & Scott Jr. 1994) was employed to carry out the study. Random searches along the paths as well as active searches by flipping wood logs, tins, and leaf litter were employed to find snakes at the suitable spots. Field surveys were done in the morning at 0600–1100 h, and at 1800–2100 h in the evening to find the snakes in their natural habitat. Deceased snakes found on roads were also included in the study. Rescue calls were attended irrespective of the time, and the snakes detected were also included in the list. Coordinates of sighting points were recorded using Google Maps on a mobile phone. Specimens were photographed and identified using relevant literature, then either released into their natural habitat or handed over to forest officials for safe release into the forest.



Image 1. Study area—Tezpur University campus.

### GIS analysis

A map showing the location points of snake sightings, along with a spatial distribution density map, was created using ArcGIS 10.7.1. To create a spatial distribution density map, a 100 m<sup>2</sup> fishnet grid covering the study area was generated. This grid was overlaid with the recorded species presence points. Next, only those grid cells where the species were observed, were selected filtering out the rest. Within these selected grid cells, centroid points using the “Calculate Geometry” tool were calculated to represent the central location of species presence. These centroid points served as input for the inverse distance weighted (IDW) interpolation technique, which estimates density by weighting closer points more heavily. The IDW parameters that includes the number of snake sightings, search radius, and cell size, were carefully adjusted to optimise accuracy. The resulting raster map displayed species density distribution, highlighting areas of higher, and lower occurrence. Finally, the output was validated by comparing it with field observations to ensure the reliability of the generated spatial distribution map.

### RESULTS

During this study, a total of 64 snake sightings were obtained inside the TUC, resulting in a record of 15 species (Table 1). Among them, two species belonged to Typhlopidae, nine species were Colubridae, three were Elapidae, and one to the Pythonidae family. Among the reported species, three species, namely, *Naja kaouthia*, *Bungarus fasciatus*, and *Bungarus lividus*, were venomous. One species, *Boiga gokool* was mildly venomous and not medically important, and 11 were non-venomous. The species belonging to the Colubridae family was recorded to be the most abundant at TUC, followed by Elapidae, then Typhlopidae and Pythonidae. At the species level, *Lycodon aulicus* (n = 12) was the most abundant, followed by *Ptyas mucosa* (n = 9). Species-wise numbers of snake encounters are shown in Figure 1a. The highest number of snakes were encountered during the months of July–September in the study period (Figure 1b). Details of date, time, and microhabitat where snakes were sighted are provided in Supplementary Table 1.

One species recorded from TUC in this study, namely *Python bivittatus*, was listed as ‘Vulnerable’, while 13 species were enlisted in the ‘Least Concern’ category, and one species, *Oligodon melaneus* was enlisted under the ‘Data Deficient’ category of the IUCN Red List

(Table 1). Furthermore, from the recorded snakes, one species, *Python bivittatus* was protected under Schedule I, and three species, *Naja kaouthia*, *Ptyas mucosa*, and *Fowlea piscator* were listed under Schedule II, while the remaining others were listed under Schedule IV of the Wildlife (Protection) Amendment Act 2022 (Table 1).

During the study, different snakes were detected at various places of the campus, such as gardens, administrative building premises, staff quarters, departmental areas, unnamed roads, and hostels (Figure 2a). Highest spatial density of snakes was detected on the road near Kanchenjunga Men’s Hostel, followed by Pobitora Madam Curie Women’s Hostel, Chandrababha Saikiani Bhawan Bus Stop, and the Department of Molecular Biology and Biotechnology (Figure 2b). Photographs of snakes encountered in the TUC are shown in Image 2.

### DISCUSSION

Closed campuses like university campuses are reported to be a safe habitat for various flora and fauna, including snakes. These campuses can serve as model ecological units for studying wildlife diversity, assessing the influence of environmental, and anthropogenic factors, and extrapolating findings to broader landscapes or communities. Despite being relatively secure habitats, factors such as infrastructure development, and the resulting shrinkage of natural vegetation can negatively impact snake diversity. Such disturbances may contribute to the observed variation in species richness and composition across different university campuses. The number of species (n = 15) recorded from Tezpur University in our study represents 18.29% and 3.56% of total species of Assam and India, respectively. This level of ophidian diversity in TUC revealed by our study is relatively lower compared to other university campuses where similar studies were carried out. For instance, in a study, a total of 19 species of snakes belonging to eight families were recorded from Guwahati University campus (Gogoi et al. 2023). A total of 23 species of snakes, including the big four were recorded in an urban college campus of Madras Christian College, Chennai (Janani & Ganesh 2024). Recently, Vanlalhruaia et al. (2024) reported 42 snake species under 31 genera belonging to seven families from Mizoram University campus, Mizoram. A total of 36 species of snakes belonging to 22 genera and five families were reported from Chittagong University Campus, Bangladesh (Ahsan et al. 2015).

Table 1. List of snakes documented from Tezpur University Campus.

Family	Scientific name	Common name	Venom type	IUCN Red List status	Wildlife (Protection) Amendment Act 2022 status	Distribution in India	No. of sightings obtained
Typhlopidae	<i>Argyrophis diardii</i>	Diard's Blind Snake	Non-venomous	Least Concern	Schedule IV	Tripura, Sikkim, Manipur, Meghalaya, Assam, Mizoram, Arunachal Pradesh, Nagaland	Roadkill: 02
	<i>Indotyphlops braminus</i>	Brahminy Blind Snake	Non-venomous	Least Concern	Schedule IV	Throughout India	Live: 04
Colubridae	<i>Lycodon aulicus</i>	Indian Wolf Snake	Non-venomous	Least Concern	Schedule IV	Throughout India, including Lakshadweep but not the Andaman & Nicobar Islands	Live: 08 Roadkill: 03
	<i>Ptyas mucosa</i>	Indian Rat Snake	Non-venomous	Least Concern	Schedule II	Throughout India, from sea level to 4,000 m	Live: 08 Roadkill: 01
	<i>Fowlea piscator</i>	Checkered Keelback	Non-venomous	Least Concern	Schedule II	Throughout India	Live: 03 Roadkill: 02
	<i>Coelognathus radiatus</i>	Copper-headed Trinket Snake	Non-venomous	Least Concern	Schedule IV	Tripura, Manipur, Meghalaya, Assam, Arunachal Pradesh, Uttarakhand, Madhya Pradesh, Chhattisgarh, Odisha, West Bengal, Sikkim, Bihar, Himachal Pradesh, Mizoram, Nagaland	Live: 03 Roadkill: 03
	<i>Coelognathus helena</i>	Common Trinket Snake	Non-venomous	Least Concern	Schedule IV	Throughout India, up to Jammu & Kashmir (Poonch) in the north, to Manipur and the Naga Hills in the Northeast.	Roadkill: 01
	<i>Dendrelaphis biloreatus</i>	Painted Bronzeback Snake	Non-venomous	Least Concern	Schedule IV	West Bengal, Assam, Arunachal Pradesh, Mizoram	Live: 02 Roadkill: 01
	<i>Oligodon melaneus</i>	Blue-bellied Kukri Snake	Non-venomous	Data Deficient	Schedule IV	West Bengal (Tindharia, Darjeeling), Assam (Barengabari, Manas National Park).	Roadkill: 01
	<i>Amphiesma stolatum</i>	Buff Striped Keelback	Non-venomous	Least Concern	Schedule IV	Tripura, Sikkim, Manipur, Meghalaya, Kerala, Tamil Nadu, Andhra Pradesh, Karnataka, Gujarat, Madhya Pradesh, Chhattisgarh, Odisha, Uttar Pradesh, Assam, Bihar, Maharashtra, Arunachal, Punjab, Himachal Pradesh, Jammu and Kashmir, Mizoram, Telangana, Nagaland	Live: 04 Roadkill: 01
	<i>Boiga gokool</i>	Arrowback Tree Snake	Venomous, not medically important	Least Concern	Schedule IV	West Bengal (Darjeeling), Assam, Manipur, Meghalaya, Nagaland Arunachal Pradesh, Odisha, Uttar Pradesh, Tripura	Roadkill: 01
Elapidae	<i>Bungarus lividus</i>	Lesser Black Krait	Venomous	Least Concern	Schedule IV	Meghalaya, Assam, Arunachal Pradesh, Nagaland	Live: 03
	<i>Bungarus fasciatus</i>	Banded Krait	Venomous	Least Concern	Schedule IV	Meghalaya, Assam, West Bengal, Bihar, Odisha, Uttar Pradesh, Maharashtra, Haryana, Madhya Pradesh, Arunachal Pradesh, Andhra Pradesh, Tripura, Mizoram, Telangana	Live: 03
	<i>Naja kaouthia</i>	Monocled Cobra	Venomous	Least Concern	Schedule II	Manipur, Meghalaya, Assam, Arunachal Pradesh, Uttar Pradesh, Bihar, Sikkim, West Bengal, Odisha, Tripura, Mizoram, Nagaland	Live: 03 Roadkill: 01
Pythonidae	<i>Python bivittatus</i>	Burmese Python	Non-venomous	Vulnerable	Schedule I	Assam, Tripura, Sikkim, Meghalaya, Mizoram, Arunachal Pradesh, Nagaland, Uttar Pradesh	Live: 06

Note: Distribution data was adopted from Whitaker & Captain 2004; Ahmed et al. 2009; Basfore et al. 2024; and Uetz 2025.

*Oligodon melaneus*, a species documented in the study site was an interesting finding. This species was originally described from Darjeeling, West Bengal in 1909 (Wall 1909). Then it was rediscovered from Barengabari, a village situated on the southern border of Manas National Park, Assam, in 2022 after 112 years of its original discovery (Das et al. 2022). The report stated that the discovery was based on a fresh roadkill specimen and that was the third known specimen of that species. So far, no other report of the sighting of *Oligodon melaneus*

has been reported. The roadkill specimen documented in this study might be the fourth documented specimen of the *Oligodon melaneus*. Finding such a rarely sighted snake in the TUC highlights the biodiversity significance of the campus. Records of the numbers of snakes killed on the road revealed the risk for herpetofauna and conservation issues in the campus. Findings of this study will serve as a reference for future studies dealing with the assessment of biodiversity at the Tezpur University campus as well as other gradually urbanising localities.

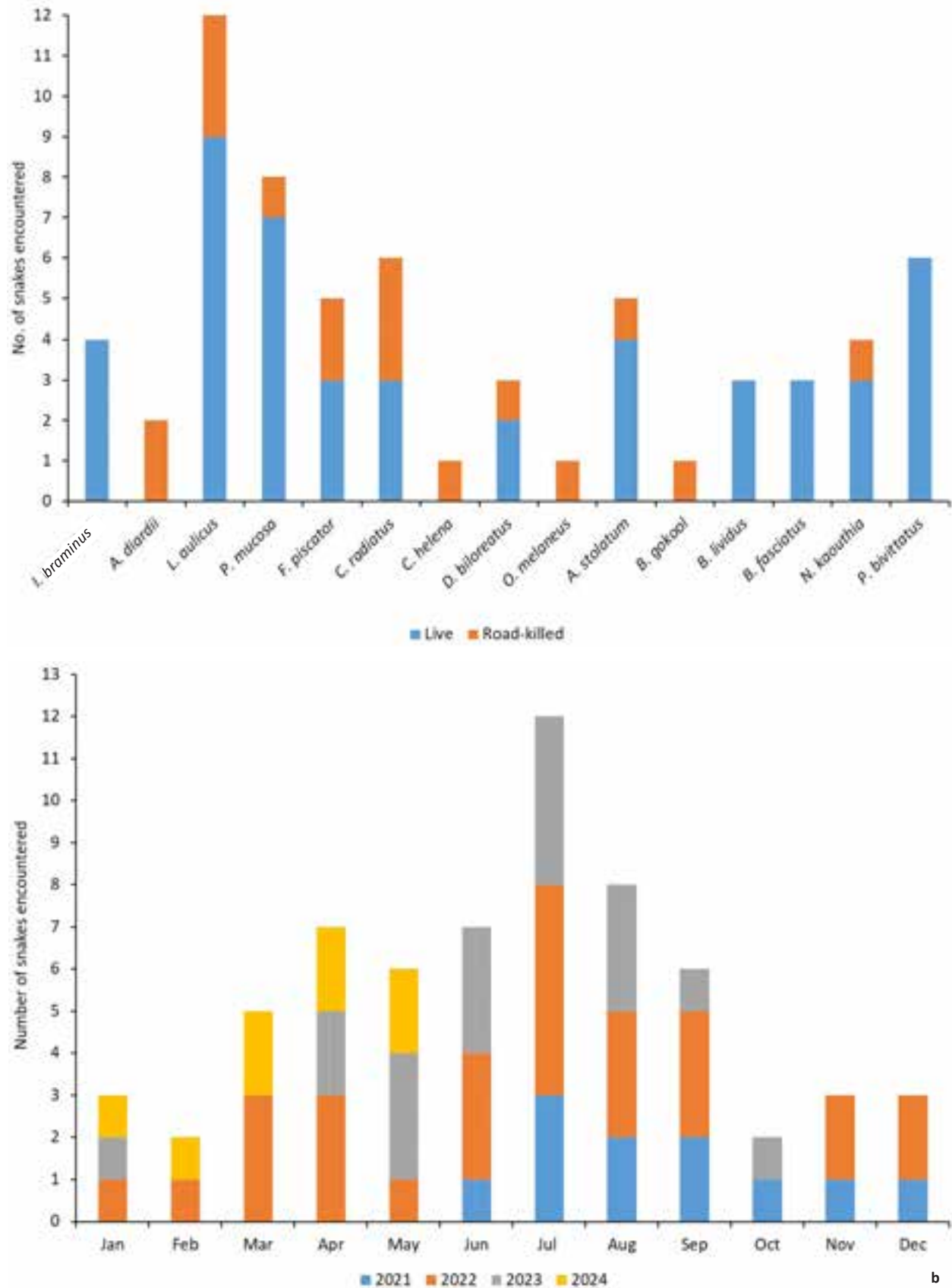


Figure 1. a—bar diagram showing the number of species-wise snakes encountered at Tezpur University campus during the study period | b—bar diagram showing number of month-wise snakes encountered at Tezpur University campus during the study period.





Figure 2. a—map showing location of snakes encountered at Tezpur University campus during the study | b—map showing density of spatial documented snakes.



Image 2. Photographs of snakes documented at Tezpur University campus: A—*Indotyphlops braminus* (Daudin, 1803) | B—*Argyrophis diardii* (Schlegel, 1839) | C—*Lycodon aulicus* (Linnaeus, 1758) | D—*Fowlea piscator* (Müller, 1887) | E—*Ptyas mucosa* (Linnaeus, 1758) | F—*Coelognathus radiatus* (Boie, 1827) | G—*Coelognathus helena* (Daudin, 1803) | H—*Oligodon melaneus* (Wall, 1909) | I—*Amphiesma stolatum* (Linnaeus, 1758) | J—*Dendrelaphis biloreatus* (Wall, 1908) | K—*Python bivittatus* (Kuhl, 1820) | L—*Boiga gokool* (Gray, 1834) | M—*Bungarus fasciatus* (Schneider, 1801) | N—*Bungarus lividus* (Cantor, 1839) | O—*Naja kaouthia* (Lesson, 1831). Green label indicates non-venomous species, orange label indicates venomous but not medically important, and red label indicates venomous species. © Mahari J. Basumatary.

Three venomous species of snakes documented at the TUC in the present study are *Naja kaouthia*, *Bungarus fasciatus*, and *Bungarus lividus*. These venomous snakes are prevalent in many parts of northeastern India and possess the potential to cause snakebite-related medical emergencies. A recent study reported that elapid snakes, including *Naja kaouthia*, *Bungarus fasciatus*, and *Bungarus niger* were responsible for 21.5% of snakebite cases presented to the Demow Model Hospital, Sivasagar, Assam (Kakati et al. 2023).

### Suggested conservation strategies

Snakes play an important role in the ecosystem as a predator as well as prey for some animals. They are biocontrol agents of pests like mice and rats. The present study revealed that 73% of encountered species of snakes in the present study were non-venomous. They were harmless, if not beneficial. Still, the presence of three venomous species of snakes (*Naja kaouthia*, *Bungarus fasciatus*, and *Bungarus lividus*) found in campus has the potential to cause medical emergencies. Therefore, to avoid unfortunate medical emergencies related to snakebite, campus dwellers are suggested to be aware of the identity, and diversity of snakes at the campus.

Translocation of animals to their own natural habitat is the best practice to conserve wildlife and to avoid negative interactions. However, in our case, almost all of the snakes rescued in the campus were released back into the forested area of the campus. In one case, a rescued *Naja kaouthia* was handed over to forest officials with the purpose of releasing it to the wild. In two cases, large individuals of *Python bivittatus* were also handed over to the forest officials for translocation.

Notably, the green coverage within the campus is shrinking gradually as a greater number of buildings are being built. This may impact the diversity of snakes and other wildlife from the campus. Additionally, many roadkill snakes detected during the study revealed that there is a challenge for the herpetofauna for coexistence in the campus. Therefore, vehicle owners, and drivers are urged to exercise greater caution while navigating the area.

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**Author contributions:** MJB—designed and conducted the study, carried out photographic documentation, collected data, and prepared the manuscript. AB—performed the GIS analysis and contributed to the manuscript review. RD—conceptualized and supervised the study, provided guidance throughout all stages of the research, and reviewed the manuscript.



Supplementary Table 1. Snake sighting details: coordinates, date, time, and microhabitat.

Snake	Latitude (° N)	Longitude (° E)	Condition	Date	Time (h)	Place and microhabitat where snake was observed
<i>Indotyphlops brahminus</i>	26.697	92.832	Live	05.vii.2021	1903	On a tarmac road near department of Environmental science
<i>Indotyphlops brahminus</i>	26.696	92.829	Live	03.vi.2022	1803	On a tarmac road near bus stand
<i>Indotyphlops brahminus</i>	26.701	92.833	Live	21.xii.2022	1925	On a roadside patch of grass
<i>Indotyphlops brahminus</i>	26.698	92.835	Live	11.viii.2023	1905	Under a pile leaf litter near Kendriya Vidyalaya
<i>Argyrophis diardii</i>	26.697	92.832	Road-kill	03.vi.2021	1815	On tarmac road behind department of physics
<i>Argyrophis diardii</i>	26.699	92.832	Road-kill	08.vii.2022	2115	On tarmac road near department of Mass Communication and Journalism
<i>Lycodon aulicus</i>	26.699	92.832	Road-kill	24.viii.2021	1915	On tarmac road near cafeteria
<i>Lycodon aulicus</i>	26.697	92.829	Road-kill	03.i.2022	2000	Parking area in front of Scholars home
<i>Lycodon aulicus</i>	26.703	92.829	Road-kill	11.i.2022	2035	On a tarmac road near department of Business Administration
<i>Lycodon aulicus</i>	26.697	92.831	Live	03.ii.2022	Not recorded	In the garden in front of department of Molecular Biology and Biotechnology
<i>Lycodon aulicus</i>	26.699	92.831	Live	10.vi.2022	2215	Inside a room on the second floor of PMCWH
<i>Lycodon aulicus</i>	26.700	92.831	Live	19.xi.2022	2330	Inside a bathroom on the second floor of PMCWH
<i>Lycodon aulicus</i>	26.700	92.832	Live	14.xii.2022	1910	In a hallway, Kapili Women's Hostel
<i>Lycodon aulicus</i>	26.702	92.827	Live	03.i.2023	1716	In the grass covered play ground near school of engineering
<i>Lycodon aulicus</i>	26.702	92.833	Live	17.iv.2023	2115	Inside a bathroom of staff quarter near essential
<i>Lycodon aulicus</i>	26.705	92.829	Live	07.vii.2022	2025	Inside a bathroom, staff quarter
<i>Lycodon aulicus</i>	26.700	92.837	Live	16.ii.2023	2205	In a hallway on the third floor of Saraighat CV Raman Men's Hostel
<i>Ptyas mucosa</i>	26.699	92.833	Road-kill	14.viii.2021	1957	On a tarmac road near gymnasium
<i>Ptyas mucosa</i>	26.697	92.831	Live	07.iii.2022	1305	In the garden in front of department of Molecular Biology and Biotechnology
<i>Ptyas mucosa</i>	26.703	92.828	Live	24.iv.2022	1135	In a secondary forest near department of Electronics and Communication Engineering
<i>Ptyas mucosa</i>	26.699	92.834	Live	10.viii.2022	1456	In a garden near Chemical Sciences
<i>Ptyas mucosa</i>	26.698	92.828	Live	12.viii.2022	1530	Inside a room, staff quarter
<i>Ptyas mucosa</i>	26.697	92.827	Live	21.iv.2023	1930	In the garden of driver's colony
<i>Ptyas mucosa</i>	26.702	92.832	Live	23.v.2023	0930	Courtyard, Bordoichila Women's Hostel
<i>Ptyas mucosa</i>	26.701	92.833	Live	17.viii.2023	0845	Grass covered playground
<i>Ptyas mucosa</i>	26.703	92.831	Live	26.v.2024	0730	Secondary forest , near water tank, B type quarter
<i>Fowlea piscator</i>	26.699	92.833	Live	30.x.2021	1750	In a garden near the department of Chemical Science
<i>Fowlea piscator</i>	26.699	92.836	Live	07.iii.2022	1000	Secondary forest, near the Saraighat CV Raman Men's Hostel
<i>Fowlea piscator</i>	26.699	92.832	Live	24.iv.2022	1315	In a the garden along the road in front of Pobitora Madam Curie Women's Hostel
<i>Fowlea piscator</i>	26.699	92.833	Road-kill	09.xi.2022	Not recorded	On a tarmac road near Gymnasium
<i>Fowlea piscator</i>	26.701	92.831	Road-kill	17.i.2024	Not recorded	On a tarmac road near Niribili pond
<i>Coelognathus radiatus</i>	26.698	92.836	Live	11.vii.2021	1103	Secondary forest near animals welfare Club
<i>Coelognathus radiatus</i>	26.700	92.836	Live	03.iii.2022	1610	In a hallway
<i>Coelognathus radiatus</i>	26.704	92.830	Live	17.viii.2022	Not recorded	In a staircase of building
<i>Coelognathus radiatus</i>	26.700	92.830	Road-kill	27.ix.2022	Not recorded	On a tarmac road, near Vice Chancellor's residence
<i>Coelognathus radiatus</i>	26.700	92.830	Road-kill	09.v.2023	1945	On a tarmac road, near Vice Chancellor's residence

Snake	Latitude (°N)	Longitude (°E)	Condition	Date	Time (h)	Place and microhabitat where snake was observed
<i>Coelognathus radiatus</i>	26.700	92.835	Road-kill	29.iii.2024	Not recorded	On a tarmac road, bus stop, near Patkai Men's Hostel
<i>Coelognathus helena</i>	26.699	92.833	Road-kill	23.v.2022	2003	On a tarmac road, near the office of Dean, Students' Welfare
<i>Dendrelaphis biloreatus</i>	26.699	92.830	Live	16.vi.2023	1130	In a room on the ground floor, department of Assamese
<i>Dendrelaphis biloreatus</i>	26.700	92.829	Live	08.iii.2024	1530	On a branch of a Hibiscus plant, in a garden, Quarter B16
<i>Dendrelaphis biloreatus</i>	26.700	92.830	Road-kill	11.ix.2021	2006	On a tarmac road, bus stop near Chandraprabha Saikiani Bhawan
<i>Oligodon melaneus</i>	26.701	92.833	Road-kill	24.vi.2022	2104	On a tarmac road, near the originating point of the path leading to Jiri Women's Hostel
<i>Amphiesma stolatum</i>	26.697	92.833	Live	24.iv.2022	0815	Garden near shopping complex
<i>Amphiesma stolatum</i>	26.697	92.833	Live	17.viii.2022	1045	Garden near amenity centre
<i>Amphiesma stolatum</i>	26.700	92.832	Live	07.iii.2024	0844	Garden in front of Subansiri Womens Hostel
<i>Amphiesma stolatum</i>	26.697	92.836	Live	23.iv.2024	0730	Garden, Kendriya Vidyalaya
<i>Amphiesma stolatum</i>	26.697	92.832	Road-kill	18.v.2024	1345	On a tarmac road, between the amenity centre and the electric substation
<i>Boiga gokool</i>	26.696	92.832	Road-kill	16.vi.2023	1537	On a tarmac floor, near Department of Environmental Science
<i>Bungarus lividus</i>	26.701	92.833	Injured	19.viii.2022	2055	On a tarmac road, near Kanchenjunga Men's Hostel
<i>Bungarus lividus</i>	26.701	92.833	Live	18.viii.2023	1905	On a roadside grass patch near Essentials
<i>Bungarus lividus</i>	26.698	92.832	Live	21.x.2023	Not recorded	On a courtyard in front of Academic Building II
<i>Bungarus fasciatus</i>	26.701	92.831	Live	04.xii.2021	Not recorded	On a tarmac road, near Niribili pond
<i>Bungarus fasciatus</i>	26.701	92.830	Live	08.vii.2022	Not recorded	On a grass patch near Niribili pond
<i>Bungarus fasciatus</i>	26.699	92.831	Live	19.viii.2023	Not recorded	On a grass patch near Academic Building 1
<i>Naja kaouthia</i>	26.698	92.834	Live	17.ix.2021	1635	In the courtyard of the administrative building
<i>Naja kaouthia</i>	26.700	92.837	Live	23.i.2022	1826	In the courtyard of Saraighat CV Raman Men's Hostel
<i>Naja kaouthia</i>	26.701	92.836	Live	16.vi.2023	1940	In a hallway of Choraideu Men's Hostel
<i>Naja kaouthia</i>	26.699	92.835	Road-kill	11.viii.2023	Not recorded	On a tarmac road, Near Community Hall
<i>Python bivittatus</i>	26.703	92.832	Live	13.vii.2021	1630	Secondary forest near Jiri Women's Hostel
<i>Python bivittatus</i>	26.701	92.832	Live	10.vii.2022	1955	Garden near swimming pool
<i>Python bivittatus</i>	26.704	92.830	Live	15.ix.2022	1825	In a drain along the road leading to B-type quarters
<i>Python bivittatus</i>	26.699	92.829	Live	03.v.2023	1905	On a tarmac road, C-Type quarter
<i>Python bivittatus</i>	26.699	92.829	Live	11.vii.2023	1445	On a branch of a Litchee plant near the C-type quarters
<i>Python bivittatus</i>	26.698	92.828	Live	12.ix.2023	1230	In a secondary forest near the children park



## INTRODUCTION

Shorebirds predominantly inhabit wetland areas, coastal zones, mudflats, marshes, and other shallow aquatic ecosystems (Byju et al. 2024). Wetlands include marshes, peatlands, and aquatic habitats with depths not exceeding six meters that can be natural or artificial, and have permanent or temporary water ranging from fresh to saline. They are among the most productive ecosystems (Ramsar Convention Secretariat 2013) and provide habitats that contribute significantly to biodiversity, and play vital role in sustaining natural systems, and ensuring ecological stability globally (Ten et al. 2012; Draïdi et al. 2019).

Estuaries and coastal zones, known for their high productivity due to nutrient-rich shallow waters (Nixon et al. 1986; Reshmi et al. 2024), are essential for shorebirds, and seabirds (Byju & Raveendran 2022), which often occupy apex predator roles in ecosystems. When these areas become recreational and socio-economic hubs, there can be significant negative impacts on plant, and animal communities (Draïdi et al. 2023; Kutir et al. 2024). Human activities around estuaries, such as urbanization, pollution, and habitat alteration, have profoundly affected shorebirds (Jackson et al. 2024), which are particularly sensitive to changes in ecosystem health since their distribution, and abundance are closely tied to the availability of food resources.

Globally, coastal birds face growing threats from anthropogenic pressures, including habitat degradation, pollution, overharvesting of prey, and the loss of essential foraging, roosting, and breeding sites (Otieno 2011; Byju et al. 2025). In Algeria, wetlands and estuaries serve as critical staging posts, and wintering grounds for migratory birds (Gill et al. 2001). Despite this, urbanization's direct impact on avian diversity in the estuaries of Algiers remains understudied.

While studies have documented shorebird diversity in various parts of Algeria, limited information exists on the distribution and diversity of waders in urban estuaries, particularly in the Algiers region. To address this knowledge gap, the present study aimed to investigate the temporal distribution and diversity of wader bird species in the estuaries of Réghaïa, Sablette, and Zéralda, and to establish a checklist of migratory, and non-migratory shorebird species, and identify factors influencing their distribution.

## MATERIALS AND METHODS

### Study site

Algiers, the capital of Algeria, is located in the north-central part of the country along the Mediterranean coast, between 36.0000° N & 3.0000° E and 36.7667° N & 5.0000° E (Image 1). The city is bordered by a coastal strip approximately 80 km long, which is part of Algeria's 1,622-km coastline. Algiers features three main estuaries:

**Réghaïa Estuary:** Located within the Mitidja plain at coordinates 36.800° N & 3.316° E–36.800° N & 3.350° E (Image 1). This estuary is recognized internationally as a Ramsar site of wetlands of global importance. It is bordered to the east by the Makin region, to the west by agricultural lands, and to the north by a strip of sand dunes.

**Sablette Estuary:** Located at coordinates 35.895° N and 0.047° E (Image 1). This area stretches for 4.5 km in the middle of the Gulf of Algiers, between the estuaries of the Oued El Harrach, and the desalination plant. Sablette is an urban public space facing the Mediterranean Sea.

**Zéralda Estuary:** Located at coordinates 36.711° N and 2.842° E (Image 1), this estuary is an important part of the coastal system of Algiers, and is situated near the town of Zéralda.

### Bird survey

This study was conducted from February 2022–December 2023, with observations made twice a month during early morning (0730–1000 h) and late afternoon (1530–1800 h), as bird activity is highest during these periods. Observations were made using binoculars (Zenith 10 × 50) and a Nikon P900 camera. Digital photographs were taken to aid in species identification when direct observation in the field was insufficient or inconclusive. Birds flying over the survey points without a clear association with the site were excluded to avoid overestimation, following the methodology of (Buckland & Elston 1993). At each estuary, five fixed survey points were established, spaced at least 200 apart to avoid double counting. Observations were conducted for 15 minutes at each point during each visit.

Surveys were not conducted during heavy rainfall, or when wind speeds exceeded normal levels, to ensure data reliability. The methodology followed the standard protocols for bird monitoring (Howes & Bakewell 1989; Bibby et al. 1998).

The scientific names and classification of the species in this study adhere to the most recent version of the IOC



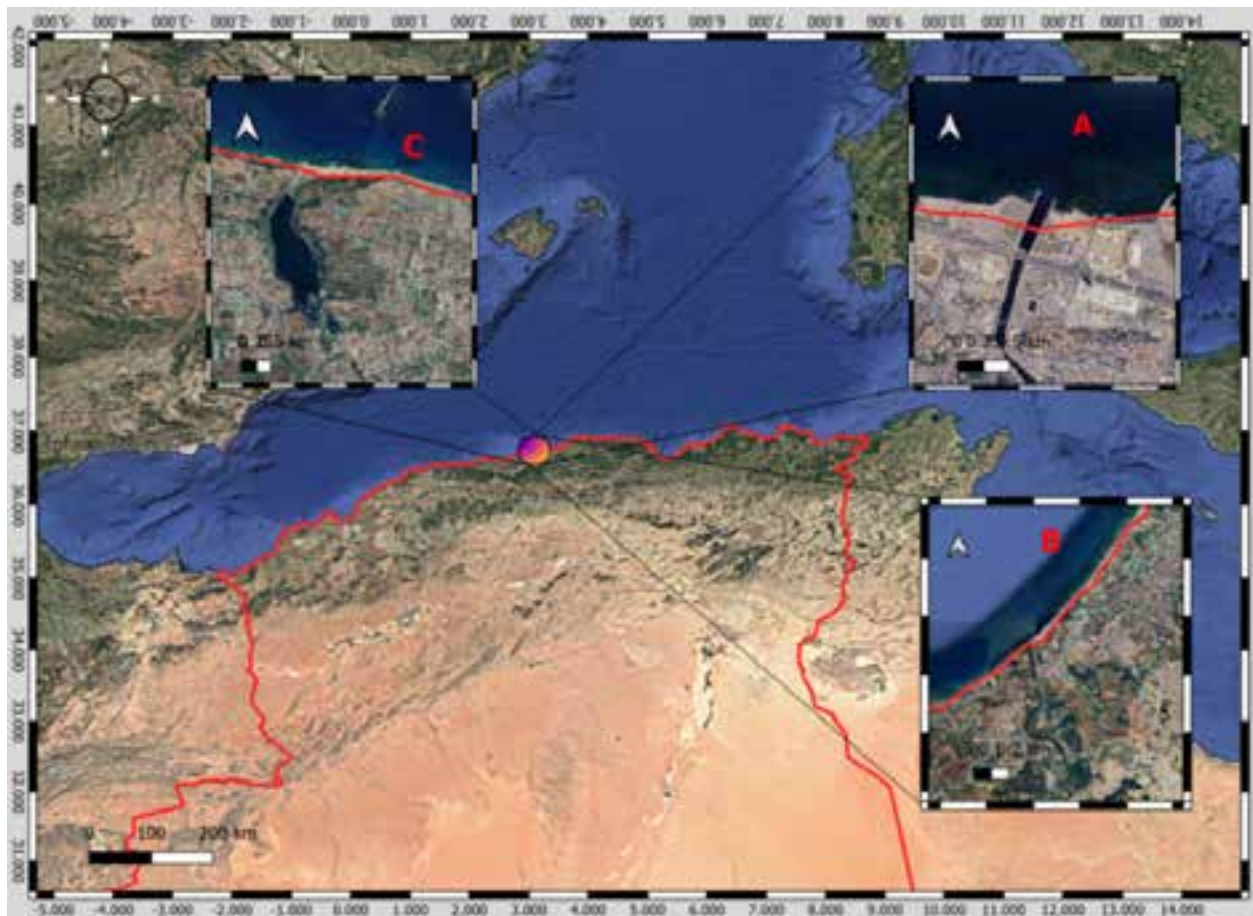


Image 1. Location of study areas in Algiers Province: A—Réghaïa Estuary | B—Sablette Estuary | C—Zéralda Estuary.

World Bird List (Gill et al. 2023). Regarding conservation status, Algeria follows Executive Fiat 12–235 of 24 May 2012, which lists protected nondomestic animal species, and Ordinance n° 06–05 of 15 July 2006, which governs the protection of endangered species. For international conservation status, we referred to the IUCN Red List (IUCN 2024).

### Data analysis

Whittaker's Beta Diversity Index was used to measure species turnover between sites, highlighting variations in species composition across habitats. Jaccard's index of similarity was calculated to evaluate the degree of similarity in species assemblages between sites, providing a comparative perspective on community composition. the following formula:

$$J = c / (A+B-c) * 100$$

where: "A" is the Total number of species in the first site., "B" is the Total number of species in the second site, "c" Number of species common to both sites (Castilheiro et al. 2017).

Additionally, correspondence analysis (CA) was performed to explore relationships between species and sites, identifying ecological groupings, and habitat-specific preferences. R software version 4.0.2 was used to create the data visualizations, with the ggplot2, viridis, and Facto Miner packages.

## RESULTS

### Species Diversity

A total of 27 shorebird species were recorded across the three estuaries during the study period, with each site showing distinct patterns of distribution, phenology, and conservation status. Réghaïa supported the highest bird diversity, with 21 species recorded, reflecting its ecological richness, and suitability as a stopover, and wintering site for birds (Bakhouché et al. 2019). In contrast, Sablette exhibited the lowest diversity, with eight species, likely due to significant human activity and a lack of suitable habitats. Zéralda recorded 13 species,

indicating a moderate diversity, and potential urban impact on the habitat.

### Conservation Status

The conservation status of these species highlights critical insights. According to the IUCN Red List of Threatened Species (IUCN 2024), eight species, including Eurasian Oystercatcher *Haematopus ostralegus*, Northern Lapwing *Vanellus vanellus*, Eurasian Curlew *Numenius arquata*, Bar-tailed Godwit *Limosa lapponica*, Black-tailed Godwit *Limosa limosa*, Ruddy Turnstone *Arenaria interpres*, Red Knot *Calidris canutus*, and Dunlin *Calidris alpina* are classified as 'Near Threatened' (NT), while two species, Grey Plover *Pluvialis squatarola*, and Curlew Sandpiper *Calidris ferruginea* are classified as 'Vulnerable' (VU). At the national level, under Algerian legal frameworks, six species, such as Black-winged Stilt *Himantopus himantopus*, Eurasian Curlew *Numenius arquata*, Pied Avocet *Recurvirostra avosetta*, Common Ringed Plover *Charadrius hiaticula*, Green Sandpiper *Tringa ochropus*, and Collared Pratincole *Glareola pratincole* are legally protected. These findings underscore the need for both national and international conservation efforts for these species (Figure 1).

### Phenological patterns

Phenological analyses indicate the importance of these estuaries for migratory birds. Most species were identified as winter visitors (WV), demonstrating the role of these sites as stopover points along migratory routes (Table 1; Figure 3). Only a few species, such as *Anarhynchus alexandrinus*, *Charadrius dubius*, and *Recurvirostra avosetta* were observed as residents and breeding birds (RB), highlighting their limited presence in the region (Table 1).

### Site-Specific differences

Distinct patterns were observed at each estuary. Zéralda exclusively recorded species such as *Haematopus ostralegus* and *Pluvialis squatarola*, highlighting its role in hosting specific wintering birds. In contrast, Réghaïa sustained species restricted to this site, including *Limosa lapponica*, and *Gallinago gallinago*, emphasizing its ecological importance and diversity.

Our results show a diversity of species across the three study sites. This suggests that there is partial overlap in species across sites, as we observed differences in species composition between the Réghaïa and Sabalat regions, as well as Zéralda. The Whittaker's Beta Diversity Index ( $\beta = 1.97$ ) (Table 2) reveals heterogeneity between sites with approximately 50.7% of shared species. Moreover,

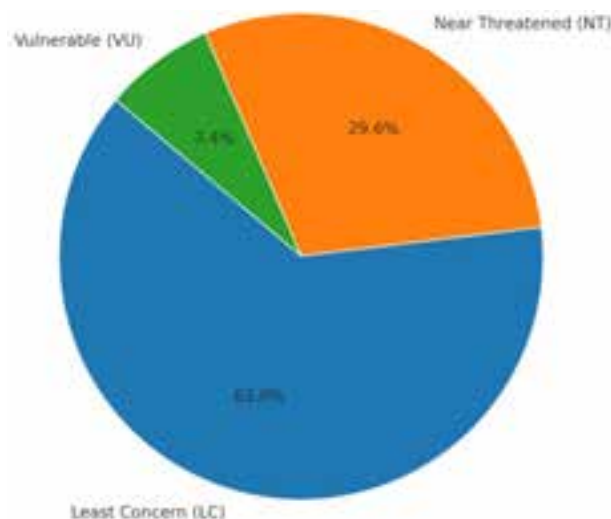


Figure 1. IUCN Red List status of shorebirds observed.

each site has its distinct species, and species turnover across sites accounts for 49.5% of diversity.

The Jaccard's Index of Similarity showed that Réghaïa and Zéralda had more similar bird species than the other sites ( $J = 0.45$ ), while Réghaïa and Sablette ( $J = 0.21$ ) as well as Zéralda and Sablette ( $J = 0.33$ ) show less similarity in terms of distribution and species overlap. The correspondence analysis (CA) (Figure 2) effectively reflects variations in shorebird species distribution across the estuaries studied, with the Ruff *Calidris pugnax*, Sanderling *Calidris alba*, and Grey Plover showing their presence exclusively at Zéralda. Eurasian Curlew was present only in Sablette. The following species, including the Black-winged Stilt, Pied Avocet, Bar-tailed Godwit, Black-tailed Godwit, Common Snipe, Green Sandpiper, Wood Sandpiper, Common Redshank, Common Greenshank, Red Knot, and Collared Pratincole, were exclusively seen in Réghaïa Estuary.

Additionally, Zéralda, and Sablette share one common species: the Eurasian Oystercatcher. Réghaïa and Sablette have Common Sandpiper as the common species. Réghaïa and Zéralda also share four species in common: European Golden Plover, Curlew Sandpiper, Dunlin, and Little Stint. All three sites of Réghaïa, Sablette, and Zéralda are characterized by the presence of four common species. namely, Common Ringed Plover, Little Ringed Plover, Kentish Plover, and Ruddy Turnstone, reflecting the similarity of ecological conditions that shape their bird assemblages.

Table 1. Presentation of recorded species.

Scientific name	Common name	Phenological status (Isenmann & Moali 2000)	Réghaïa	Sablette	Zéralda	Red List status (IUCN 2024)	Algerian law
<i>Haematopus ostralegus</i>	Eurasian Oystercatcher	WV	-	+	+	NT	-
<i>Himantopus himantopus</i>	Black-winged Stilt	MB	+	-	-	LC	+
<i>Recurvirostra avosetta</i>	Pied Avocet	RB	+	-	-	LC	+
<i>Pluvialis squatarola</i>	Grey Plover	WV	-	-	+	VU	-
<i>Pluvialis apricaria</i>	European Golden Plover	WV	+	-	+	LC	-
<i>Charadrius hiaticula</i>	Common Ringed Plover	WV	+	+	+	LC	+
<i>Charadrius dubius</i>	Little Ringed Plover	RB	+	+	+	LC	-
<i>Vanellus vanellus</i>	Northern Lapwing	WV	+	-	-	NT	-
<i>Anarhynchus alexandrinus</i>	Kentish Plover	RB	+	+	+	LC	-
<i>Numenius phaeopus</i>	Eurasian Whimbrel	WV	-	+	-	LC	-
<i>Numenius Arquata</i>	Eurasian Curlew	WV	-	+	-	NT	+
<i>Limosa lapponica</i>	Bar-tailed Godwit	WV	+	-	-	NT	-
<i>Limosa limosa</i>	Black-tailed Godwit	WV	+	-	-	NT	-
<i>Gallinago gallinago</i>	Common Snipe	WV	+	-	-	LC	-
<i>Actitis hypoleucos</i>	Common Sandpiper	WV	+	+	-	LC	-
<i>Tringa ochropus</i>	Green Sandpiper	WV	+	-	-	LC	+
<i>Tringa glareola</i>	Wood Sandpiper	WV	+	-	-	LC	-
<i>Tringa tetanus</i>	Common Redshank	WV	+	-	-	LC	-
<i>Tringa nebularia</i>	Common Greenshank	WV	+	-	-	LC	-
<i>Arenaria interpres</i>	Ruddy Turnstone	WV	+	+	+	NT	-
<i>Calidris canutus</i>	Red Knot	PV	+	-	-	NT	-
<i>Calidris pugnax</i>	Ruff	WV	-	-	+	LC	-
<i>Calidris ferruginea</i>	Curlew Sandpiper	WV	+	-	+	VU	-
<i>Calidris alba</i>	Sanderling	WV	-	-	+	LC	-
<i>Calidris alpina</i>	Dunlin	WV	+	-	+	NT	-
<i>Calidris minuta</i>	Little Stint	WV	+	-	+	LC	-
<i>Glareola pratincole</i>	Collared Pratincole	MB	+	-	-	LC	+

NT—No definite status | VB—Vanished breeder | AV—Accidental visitor | CB—Casual breeder | MB—Migrant breeder | RB—Resident breeder | PV—Passage visitor | WV—Winter visitor | VU—Vulnerable | NT—Near Threatened | LC—Least Concern (Isenmann & Moali 2000).

## DISCUSSION

Birds serve as excellent bioindicators, since their populations are influenced by factors such as climate, vegetation, food availability, rainfall, prey presence, and other ecological conditions (Aarif et al. 2025). Our research underscores the significance of understanding habitat diversity and species distribution for effective conservation strategies in Algeria's estuarine ecosystems. We identified 27 shorebird species across various threatened categories, including winter visitors and breeding populations, which highlights the seasonal abundance of shorebirds, and the ecological importance

of Algeria's estuarine habitats (Hong et al. 2024). Local habitat characteristics, such as distribution, vegetative structure, moisture levels, biomass availability, and cover patterns, strongly influence shorebird distribution, and abundance during wintering, and stopover periods (Cerdeña-Peña & Rau 2023).

A recent study, Palacios et al. (2022), illustrated the impact of human disturbance on the distributions of shorebirds during migratory, and non-migratory periods. This study showed negative correlations between human disturbance and the abundance of this group of birds, suggesting that Sablette has high human activity due to the low abundance in this area compared to

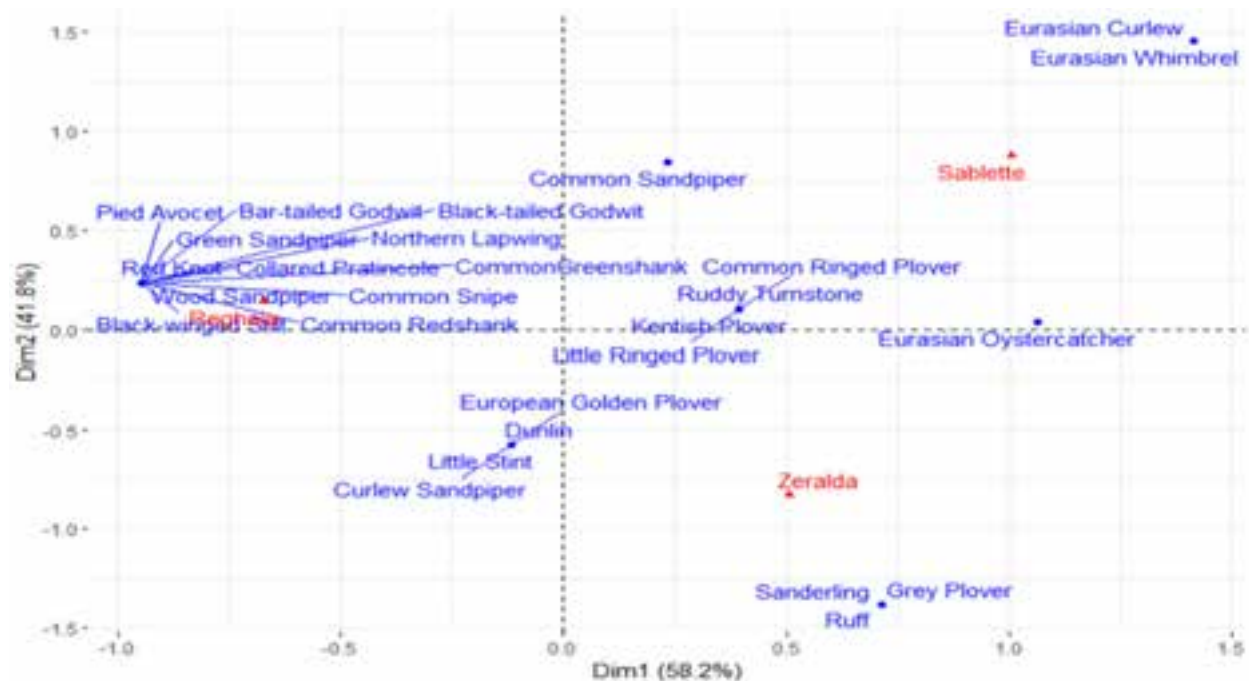


Figure 2. Correspondence analysis map (CA) of shorebird distribution in sites from February 2022 to December 2023.

Raghiya. The effects of foraging, vigilance, and aggressive behaviour of shorebirds, and seagulls in the presence of human disturbance were also compared (Burger et al. 2007). It was observed in this study that within a few minutes, seagulls returned to pre-disturbance levels; however, shorebirds did not return to pre-disturbance levels within the same time frame. These observations suggest that shorebirds respond more strongly to human disturbance despite the presence of food competition. This explains the difference in bird distribution in this study, as the Sablette is a tourist area, and the rate of human activity is very high compared to other areas. Human disturbances, such as habitat alteration, foraging disruption, and reproductive interference, significantly affect shorebird populations (Gibson et al. 2018; Setsaas et al. 2018; Rao et al. 2022). The decline in species abundance in Sablette and Zéralda could be further accentuated if disturbance levels increase, as they decreased by 50% between 1972 and 1989 at the Atlantic coast of the United States between Florida and Cape Cod Bay (Pfister et al. 1992). In general, the diversity of shorebirds in the Réghaïa estuary was much higher than in the Sablette area, potentially due to the fact the latter is at risk due to human intervention through the construction of canyons & platforms, and the installation of sea walls on the inlet beaches (Reed et al. 2012; VanDusen et al. 2012), unlike in the Réghaïa area, which is still a natural area.

Table 2. Summary statistics of Whittaker beta diversity index, gamma diversity, and turnover (%).

Whittaker beta diversity index	1.97561
Alpha diversity	13.6667
Gamma diversity	27
Turnover (%)	49.4%

Additionally, the presence of invertebrate prey plays a crucial role in shorebird abundance (Cerdeña-Peña & Rau 2023). Factors influencing the distribution of shorebirds in search of food among the mudflats were studied (VanDusen et al. 2012), and it was found that the spatial structure of the benthic invertebrate prey community is correlated with the density and composition of the community of shorebirds. In our study, it was in a similar line that none of the three studied locations is ideal for the entire shorebird community, which explains the low species similarity between Réghaïa, and Sablette in terms of distribution. Comparative analyses of species richness across study areas reveal that Réghaïa and Zéralda share higher species similarity, whereas Sablette shows lower similarity in species composition compared to both Réghaïa and Zéralda. This highlights the necessity for localized conservation efforts, habitat protection, and community engagement (Gibson et al. 2018; Setsaas et al. 2018).

Despite the scarcity of comprehensive regional or



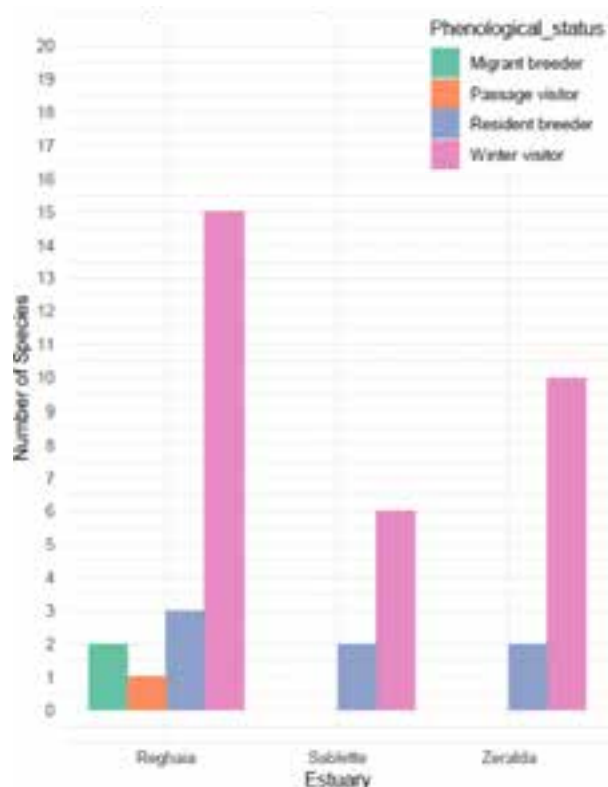


Figure 3. Phenological status of bird species across estuaries.

national assessments for shorebird species under the IUCN Red List criteria in Algeria, species like the Curlew Sandpiper and Grey Plover are now listed as Vulnerable (IUCN 2024). Furthermore, eight shorebird species, including the Eurasian Oystercatcher, Northern Lapwing, Bar-tailed Godwit, and Eurasian Curlew, Bar-tailed Godwit, Black-tailed Godwit, Ruddy Turnstone, Red Knot and Dunlin, are categorized as Near Threatened (NT) (IUCN 2024). Conservation strategies should be adapted to the specific ecological conditions of each site. At Sablette, where human disturbance is high, efforts should focus on regulating tourist activities, restricting access during sensitive periods, and restoring degraded habitats to improve their suitability for shorebirds. In contrast, at Réghaïa, which still maintains a relatively natural state, the priority should be to preserve its ecological integrity by preventing urban expansion, infrastructure development, or any modifications that could alter the estuarine ecosystem.

## CONCLUSION

The variation in species presence among study sites can reasonably be linked to habitat differences such

as the availability of suitable foraging areas, nesting areas, and less disturbed areas. To ensure effective and sustainable conservation of these estuarine habitats, it is essential to implement long-term population monitoring, habitat restoration programmes, community education initiatives, and robust local protection policies. These measures are particularly important to safeguard key zones used by migratory shorebirds for foraging, breeding, and wintering. In addition, it is necessary to conduct specific research on natural and anthropogenic disturbances to gain a greater understanding of their effects on estuarine habitats. By implementing these efforts, we can help reduce negative impacts and maintain the environmental integrity and long-term conservation of these vital coastal areas.

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## INTRODUCTION

Flying foxes are crucial for maintaining the ecological balance of many tropical regions (Fujita & Tuttle 1991; Aziz et al. 2017a; Parolin et al. 2021; Kingston et al. 2023). With their ability to fly long distances, flying foxes play pivotal roles in seed dispersal and pollination (Aziz et al. 2017b,c, 2021; Chen et al. 2017; Oleksy et al. 2017; Todd et al. 2022; Selan et al. 2023). Such an ecological role also has a positive economic impact since fruits like durian and kapok trees rely on the species for their reproduction (Fujita & Tuttle 1991; Nathan et al. 2005; Aziz et al. 2017b,c, 2021). Despite their ecological importance, over half of all flying foxes are classified as vulnerable, endangered, or critically endangered (Pulscher et al. 2021; Kingston et al. 2023). The species population decline is driven mostly by overhunting, habitat loss, and habitat degradation caused by extensive land-use changes (Tsang 2022; Kingston et al. 2023). Conservation efforts are further complicated by conflicting views towards these animals (Mo et al. 2022; Tsang 2022; Charentantanakul et al. 2023). Issues such as crop raiding, fear of zoonotic diseases, and negative attitudes have led to conflicts between humans, and flying foxes (Aziz et al. 2016; Shapiro et al. 2020; Low et al. 2021; Yabsley et al. 2021; Mohd-Azlan et al. 2022a,b; Charentantanakul et al. 2023; Kingston et al. 2023). For example, the Mauritian Flying Fox *Pteropus niger* has faced significant population declines over 50% since 2015 due to large-scale culling driven by perceived damage to commercial fruit crops (Kingston et al. 2018; Seegobin et al. 2022). As a result, flying foxes are often viewed as pests and face legal persecution (Florens 2016; Florens & Baider 2019; Seegobin et al. 2022). This undermines support for conservation and highlights the urgent need to address public perceptions and knowledge gaps regarding flying foxes.

The theory of reasoned action highlights how attitudes towards behaviours can shape, intentions, and actions (Albarracín et al. 2018; Hagger et al. 2018). Understanding threats to species, their conservation status, and public knowledge, attitudes, and perceptions can enhance outreach efforts aimed at promoting conservation and mitigating negative human-wildlife interactions (Bennett et al. 2019; Boso et al. 2021; Basak et al. 2022; Li et al. 2023). Assessing conservation attitudes is crucial as the views of local communities toward wildlife can significantly influence conservation outcomes (Li et al. 2023; Fotsing et al. 2024). Positive attitudes from local communities lead to greater support for conservation efforts, regulatory compliance, and

active participation (Loyau & Schmeller 2016; Merz et al. 2023; Tang et al. 2023). Although negative attitudes towards flying foxes pose challenges, they also offer a chance to correct misconceptions, raise awareness, and promote coexistence (Aziz et al. 2016; Tsang et al. 2022; Kingston et al. 2023). To foster effective conservation, it is essential first to understand local communities' attitudes, perceptions, knowledge, and experiences with the species, in order to design conservation policies through the engagement, and participation of the communities (Bennett et al. 2019; Mubalama et al. 2020; Fotsing et al. 2024).

Borneo's diverse ecosystems provide vital habitats for flying foxes, ranging from coastal mangroves to dense rainforests. Sabah, Malaysia, is home to two species, including *Pteropus hypomelanus* and the regionally at-risk *Pteropus vampyrus* (Phillipps & Phillipps 2018; Mildenstein et al. 2022). Habitat loss caused by deforestation, agriculture, and urban expansion (Gaveau et al. 2014) increases their vulnerability, and leads to heightened human-wildlife conflicts, particularly fruit raiding. Despite flying foxes' protected status under the Sabah Wildlife Enactment 1997, hunting licences are still issued creating conflicting legal signals. In addition, Sabah is geographically positioned within a wider flying fox heavy trade region that includes North Sulawesi and Kalimantan (Harrison et al. 2011; Latinne et al. 2020). The absence of empirical data on public attitudes and behaviours in Sabah presents a barrier to effective, locally informed conservation planning, particularly as the state moves forward with the Sabah Biodiversity Strategy 2024–2034.

Given these gaps in understanding and the conservation importance of Sabah's flying fox populations, this study was designed to explore local community-level dynamics. This study addresses three core objectives: (1) to assess public attitudes toward flying fox conservation in Sabah, (2) to evaluate knowledge levels, and identify common misconceptions about flying fox ecology, and legal protection, and (3) to identify key predictors of conservation support, including demographic variables, human-flying fox experiences, and cognitive or ethical perceptions. To explore these dynamics across diverse segments of the population, data were collected by combining in-person interviews in high-contact areas with broader-reaching online surveys. This methodological approach was designed to optimise data quality, maximise response rates, and expand demographic reach across diverse geographic regions in Sabah, aiming to capture both direct, and general public perspectives to inform a more inclusive



conservation response. The findings are intended to guide the development of targeted strategies to improve conservation awareness and promote the protection of flying fox populations in Sabah.

## METHODS

### Data collection

A self-administered survey was conducted using both in-person and online methods between September 2021 and September 2023, covering various districts in Sabah (Image 1). The questionnaire was designed in Bahasa Melayu using simple, non-technical language, and was pilot-tested on 10 individuals prior to full deployment. Feedback from the pilot informed minor revisions for clarity. Informed consent was obtained from all participants, who were briefed on the study's objectives, confidentiality, and voluntary participation. The study was approved under the Sabah Biodiversity Access Licence (Ref. JKM/MBS.1000-2/2 JLD.10 (25)) and supported by local district offices, and village heads. Two approaches were employed to maximise reach

and improve representativeness: (1) In-person surveys, administered in five districts where *P. vampyrus* is known to occur, and (2) Online surveys, disseminated via Google Forms through social media, and local networks.

Given the context-specific nature of flying fox conservation, prioritising participants with firsthand interactions was considered essential. To achieve this, an in-person survey was conducted in September 2021 in Bahasa Melayu across five districts (Tambunan, Ranau, Telupid, Tongod, and Kinabatangan), where the IUCN Red List 'Endangered' *P. vampyrus* is distributed. Three trained surveyors administered the survey in key community areas, including villages and local markets. Participants were selected through snowball sampling, a method well-suited for accessing individuals with specific knowledge or experience, despite its lack of randomisation (Atkinson & Flint 2001; Palinkas et al. 2015). Village heads were first informed of the study and helped coordinate recruitment through local committee members. In market settings, participants were selected randomly from among sellers & buyers, and were briefed on the study's objectives, structure, and confidentiality measures. Particular emphasis was placed on the

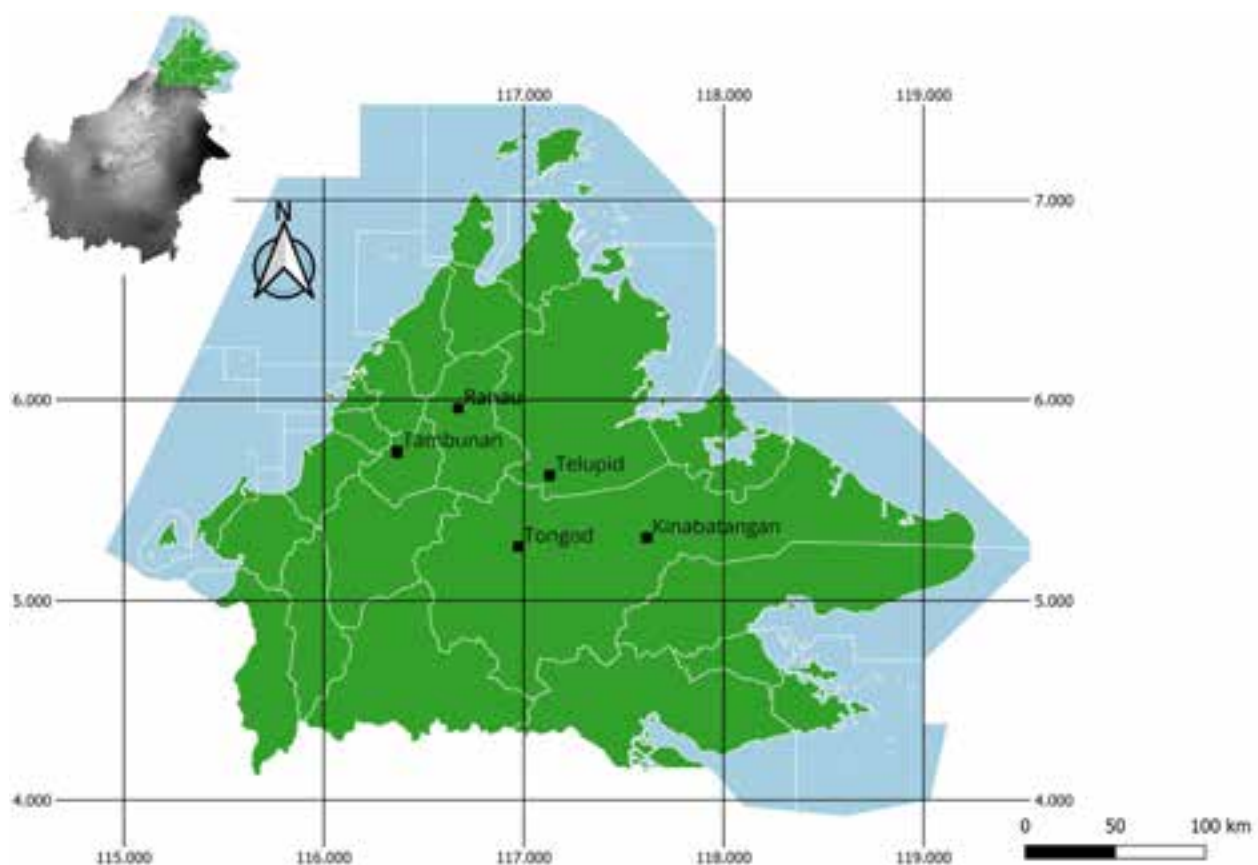


Image 1. Geographic distribution of in-person survey locations across five districts in Sabah, Malaysia.

sensitive nature of topics such as hunting, consumption, and fruit raiding, with surveyors reassuring participants of strict confidentiality to encourage honest responses. Participants completed the survey independently within 15 to 20 minutes, and surveyors were available to provide clarification or assistance to those with limited literacy to ensure full comprehension of the questions, and response options.

Due to a resurgence of COVID-19 cases and the reintroduction of movement restrictions, surveyors were unable to continue distributing questionnaires in person. As a result, the survey was shifted online via Google Forms, available in Bahasa Melayu and restricted to one response per email to ensure data integrity (Teitcher et al. 2015). While online surveys tend to attract more educated and environmentally aware individuals, combining both approaches helped improve representativeness (Kaplowitz et al. 2004). From October 2021 to September 2023, the survey was distributed via Facebook, and WhatsApp by local volunteers, who monitored participation to ensure demographic diversity. Targeted outreach through business associations, educational institutions, local social networks was employed to reach underrepresented groups, and minimise bias. Snowball sampling was used alongside strategic recruitment to ensure balanced participation across districts, age groups, and occupations.

### Questionnaire design

A semi-structured questionnaire was used to collect data on four areas: (1) socio-demographics, (2) experience, (3) knowledge, and (4) attitudes and perceptions. Questions were adapted from Aziz et al. (2017a) and neutrally phrased (see Supplementary Material 1). The demographics section covered residential district, gender, age, education, monthly household income and ethnicity. Age and education were grouped into categorical ranges, and monthly income ranged from RM500– above RM3000.

The experience section assessed participants' direct interactions with flying foxes, specifically in relation to fruit-raiding, hunting, and consumption. Follow-up questions validated claims by investigating the types of fruit affected, and places the bats experienced it (for fruit-raiding); motivations, hunting locations, and methods used; and consumption frequency, and reasons. These responses enhanced contextual understanding of human–flying fox experience. Only data relevant to the present study objectives were analysed. Experience variables were coded as binary ("Yes" for confirmed experiences; "No" for inconsistent

or negative responses).

The respondents' knowledge of key aspects of flying fox biology was assessed through five questions covering ecological roles (seed dispersal and pollination), roosting sites, legal considerations (hunting permits), and dietary habits. Some uncertain participants might have guessed instead of admitting a lack of knowledge, and social desirability bias could lead others to overestimate their expertise (Boso et al. 2021). To mitigate forced guessing, the survey included a "Don't know" option. Response options for each question were "Yes", "Don't know", and "No," with only correct answers scoring one point; incorrect and don't know responses received zero. Total knowledge scores ranged 0–5 points and were categorised based on previous conservation education research. Participants scoring below 50% (0–2 points) were classified as having limited knowledge of flying fox conservation, while those scoring above 50% (3–5 points) were deemed to have a sufficient understanding. This classification followed the frameworks established by Wendeye (2009) and Lubos (2019), with scores below 50% labelled as "Below Mastery Level", and scores above 50% as "Above Mastery Level".

The study included five fixed-response questions designed to assess perceptions and attitudes, using a mix of positive and negative statements to enhance response consistency. Attitudes reflect individuals' feelings and predispositions toward bats, which can influence their behaviours, while perceptions relate to people's beliefs, and awareness of bats in their environment (Castilla et al. 2020). This research specifically examined perceptions and attitudes towards flying foxes, addressing conservation-related aspects such as their importance, views on them as pests, attitudes toward culling, perceptions of population decline, and beliefs regarding extinction. Additionally, it explored the value of flying foxes in relation to tourism, feelings of fear, and awareness of diseases, including public perceptions of disease and attitudes towards COVID-19.

### Questionnaire response validity

A systematic data-cleaning process was conducted before statistical analysis to ensure the validity and reliability of survey responses. This involved identifying and removing unreliable data, including duplicate entries, inconsistent answers, patterned responses, and excessive missing data, following best practices in survey research (Meade & Craig 2012; DeSimone et al. 2014; Curran 2016). To minimise misinterpretation and ensure response relevance, a visual screening step was incorporated at the start of the questionnaire. This

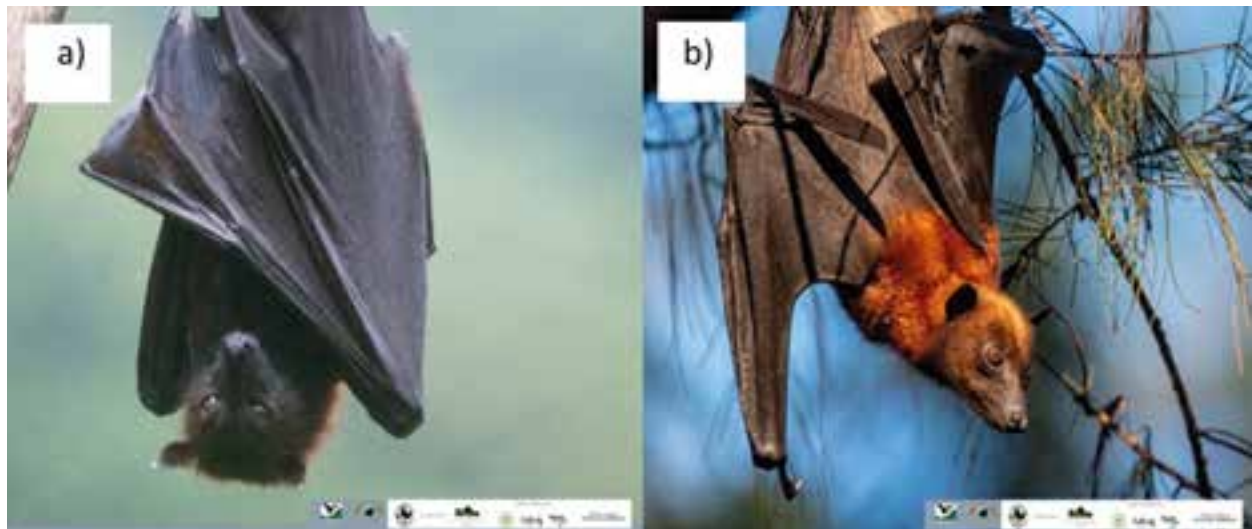


Image 2. Images of two flying foxes used in the survey to assess whether participants could recognise flying foxes as target species: a—*Pteropus vampyrus* | b—*Pteropus hypomelanus*. Source: Rimba (2021).

involved presenting two unlabelled images of flying foxes (Image 2) to assess whether participants could recognise the target species. Given the potential for confusion with other bat species or animals, this step served to clarify the survey context and confirm that responses pertained to the intended taxon.

Building upon this, further steps were taken to detect inattentive or careless responses that could compromise data quality. One common issue, known as patterned responses or straight-lining, occurs when respondents select the same answer for all questions without properly reading them (DeSimone et al. 2014). To identify such behaviour, some questions were reworded and reverse-coded across different sections (Meade & Craig 2012). For instance, the statement “Flying foxes should not be killed” was rephrased as “Should flying foxes be killed?”. Contradictory responses to these items were flagged as potentially careless (Huang et al. 2015). Inconsistent answers, showed contradictory responses to logically related items such as supporting and opposing the killing of flying foxes in different questions, were also flagged. Participants were removed from the dataset if they displayed contradictions in more than 50% of the reworded items or failed attention-check questions (Huang et al. 2015; Curran 2016). In the knowledge section, participants were also evaluated on their ability to correctly identify flying foxes and demonstrate a basic understanding of the species.

To further ensure data integrity, a post hoc comparative analysis was carried out between online and in-person participants. Although not part of the original study objectives, this analysis was deemed

necessary to assess potential biases introduced by the dual-mode sampling approach. Survey mode can influence response patterns due to factors such as perceived anonymity, literacy levels, and self-selection biases (Kaplowitz et al. 2004; Rand et al. 2019). Thus, differences in demographic characteristics and survey responses were examined between the two groups. These comparisons help validate the decision to pool responses and provide a clearer understanding of the sample’s representativeness.

### Data analysis

To address the study objectives, attitudes (Objective 1) were measured using structured questions and statistical tests; knowledge and misconceptions (Objective 2) through a scored knowledge section; and predictors of conservation support (Objective 3) via GLMMs using demographic, experiential, and cognitive-ethical variables.

All analyses were conducted using R 4.3.3 (R Core Team 2024). Participants were categorised into two groups: in-person and online. To identify significant differences between these groups, chi-square was used to assess variations in attitudes, perceptions, fruit raiding, hunting, and flying fox consumption, while t-tests were used to compare knowledge scores. Effect sizes were calculated using the *rstatix* package in R (Kassambara 2021). Conservation attitudes were evaluated based on whether participants believed flying foxes should be conserved. Factors influencing these attitudes were examined using a Generalised Linear Mixed Model (GLMM; see Supplementary material 2)

and the “lme4” package in R (Bates et al. 2015). Before running the GLMMs, socio-demographic covariates were assessed for significant correlations ( $|\text{coefficient values}| > 0.5$ ) to ensure stable and interpretable parameter estimates (Aziz et al. 2017a). The correlation between conservation attitudes and socio-demographic covariates was assessed using the “vcd” package in R, leading to the selection of age, gender, and education as covariates (summary in Supplementary material 3).

To identify the best predictors of conservation attitudes toward flying foxes, three GLMMs were generated based on socio-demographic factors, experience (fruit raiding, hunting, consumption), and conservation-related parameters (knowledge and perception). These models were compared using Akaike’s Information Criterion (AIC) and log-likelihood values. The corrected Akaike information criterion (AICc) was calculated using the “MuMin” package in R, and the Akaike weights (wAICc) were used to quantify the likelihood of each model being the best. The variance explained by fixed effects in each GLMM was assessed with R<sup>2</sup>m (Nakagawa & Schielzeth 2012). Model 1 included demographic factors (age, gender, education); Model 2 added experience-based predictors; and Model 3 further included knowledge levels, importance perception, and opposition to killing. “Method” was included in the model to address potential non-independence of responses from different sampling methods. Multicollinearity among predictors was assessed using Generalised Variance Inflation Factors (GVIFs) with the “car” package in R.

Binomial (logit-link) GLMMs were employed to model the binary response variables. Gender was coded as 1 for men and 2 for women; age as 1 for young (<35 years) and 2 for adult (>35 years); and education as 1 for secondary education or below and 2 for tertiary education. Experience with flying foxes was coded as 1 for those with experience and 0 for those without. Attitudes and perceptions were coded in binary form: “Yes” responses as 1 and “No” or “Unsure” responses were coded as 0, with negative statements recoded to ensure positivity. Important perceptions (coded as Important) and opposition to killing (coded as “Nokill”) were included as covariates, along with knowledge scores, coded as 1 for scores below three and 2 for scores above three. Knowledge, importance perception, and anti-killing attitudes were the primary predictors in the models.

## RESULTS

### Socio-demographic information

Of the 330 participants, 320 were selected for analysis after screening, comprising 220 online, and 100 in-person participants. The demographic data collected from both in-person and online methods reveal some notable trends. Regarding gender distribution, both methods show an almost equal split, with males constituting 53% of in-person participants and 51% of online participants. The age distribution highlights a significant proportion of older individuals, with the 45–54 years age group being the largest in both methods (31% in-person, 24% online). The highest education level for both in-person (62%) and (45%) online participants were secondary education. Occupation reveals a substantial presence of self-employed individuals (43% in-person, 28% online). Ethnicity data indicate Kadazandusun as the predominant group, especially online (74%). The residential data point to a diverse geographic spread, with notable concentrations in Ranau (23% in-person), and Tambunan (28% online). Detailed social-demographic results are listed in Supplementary material 4.

### Attitude on flying foxes among local participants

The study’s results indicated no statistically significant differences in the distribution of responses regarding flying fox conservation, extinction, killing, fear, and perceived links to COVID-19 between in-person and online participants. Specifically, chi-squared tests revealed no significant differences for conservation ( $\chi^2(320) = 4.64$ ,  $p = 0.10$ ), Cramér’s  $V = 0.12$ ). This was similar with other attitudes, extinction ( $\chi^2(320) = 0.60$ ,  $p = 0.74$ ), killing flying foxes ( $\chi^2(320) = 3.07$ ,  $p = 0.22$ ), fear of flying foxes ( $\chi^2(320) = 2.44$ ,  $p = 0.30$ ), and COVID-19 ( $\chi^2(320) = 0.65$ ,  $p = 0.72$ ).

Most participants expressed positive attitudes toward flying fox conservation (Figure 1). Among in-person participants, 68% ( $n = 68$ ) supported conservation, 25% ( $n = 25$ ) were unsure, and only 7% ( $n = 7$ ) opposed it (see Figure 1a). Similarly, 77% ( $n = 170$ ) of online participants favoured conservation, 15% ( $n = 33$ ) were unsure, and 8% ( $n = 17$ ) opposed it. Concerning flying fox extinction, 69% ( $n = 69$ ) of in-person participants, and 72% ( $n = 159$ ) of online participants opposed it. Additionally, the fear of flying foxes was not a significant concern for most participants, with both 64% ( $n = 64$ ) of in-person and 64% ( $n = 141$ ) of online participants reporting no fear of these animals.

The majority of participants also opposed killing flying



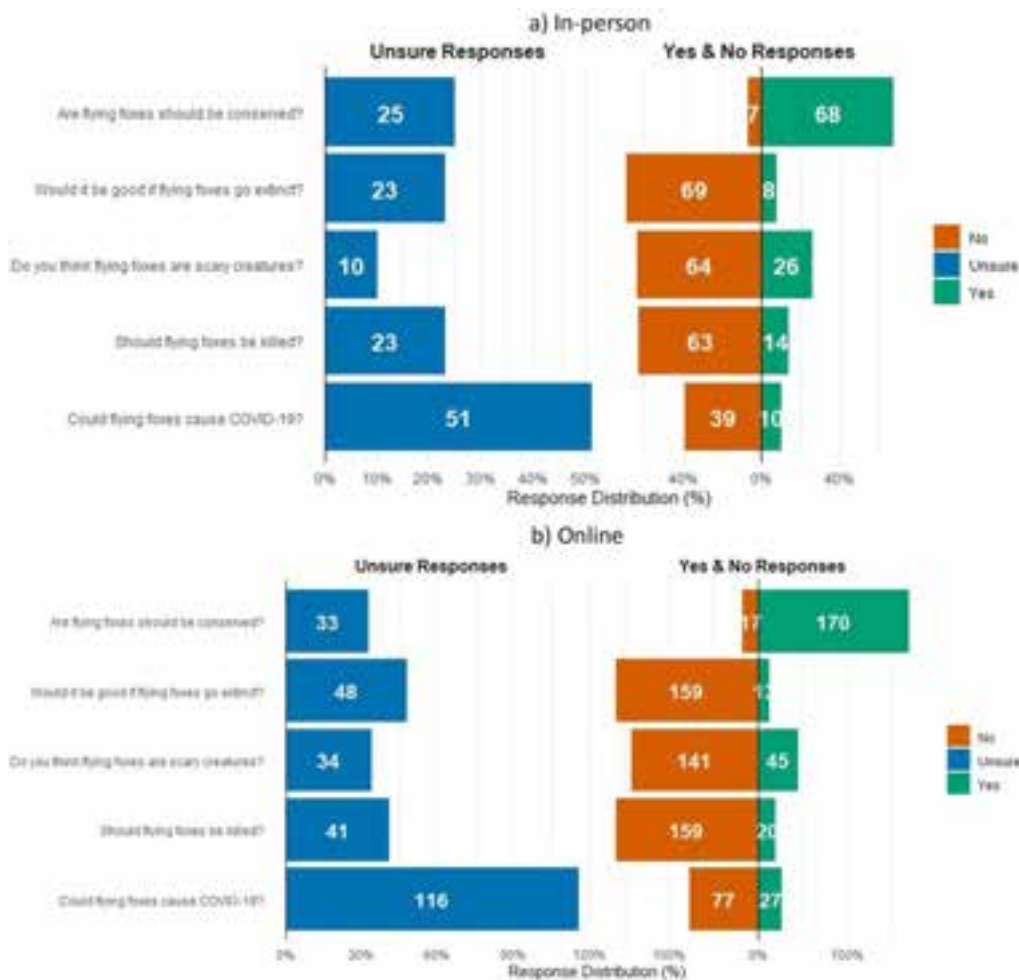


Figure 1. Conservation attitudes of in-person (Figure 1a,  $n = 100$ ) and online (Figure 1b,  $n = 220$ ) participants toward flying foxes in Sabah, categorised by responses to five key questions ("Yes," "Unsure," "No").

foxes, with 63% ( $n = 63$ ) of in-person and 72% ( $n = 159$ ) of online participants against this practice. Regarding the link between flying foxes and COVID-19, a slight majority were unsure: 51% ( $n = 51$ ) of in-person participants and 53% ( $n = 116$ ) of online participants. Disagreement with the idea that flying foxes are responsible for the virus was more prominent among in-person participants (39%,  $n = 39$ ) compared to 35% ( $n = 77$ ) of online participants, patterns which are illustrated in Figure 1.

#### Perception of flying fox among local participants

Chi-squared tests revealed no significant differences between participant types in their views on the importance of flying foxes ( $\chi^2(320) = 3.41$ ,  $p = 0.18$ ) and disease transmission ( $\chi^2(320) = 5.63$ ,  $p = 0.06$ ). Significant differences were observed regarding perceptions of flying foxes as pests ( $\chi^2(320) = 6.88$ ,  $p = 0.03$ ), population decline ( $\chi^2(320) = 39.16$ ,  $p < 0.05$ ), and tourist attractions ( $\chi^2(320) = 9.33$ ,  $p = 0.03$ ).

A majority, 66% ( $n = 66$ ) of in-person, and 76% ( $n = 167$ ) of online participants recognised the environmental importance of flying foxes (see Figure 2). On the issue of pest perception, in-person participants were divided, with 39% ( $n = 39$ ) rejecting the notion that flying foxes are pests, while 38% ( $n = 38$ ) agreed, and 23% ( $n = 23$ ) were unsure. In comparison, 42% ( $n = 93$ ) of online participants did not view flying foxes as pests, while 25% ( $n = 54$ ) did, and 33% ( $n = 73$ ) were unsure. Regarding disease transmission from flying foxes to humans, 51% ( $n = 51$ ) of in-person participants and 41% ( $n = 91$ ) of online participants were uncertain. Notably, more online participants (45%,  $n = 99$ ) believed in disease transmission than in-person participants (31%,  $n = 31$ ). Nearly half of the participants believed that flying fox populations were declining, with 52% ( $n = 84$ ) of online, and 42% ( $n = 42$ ) of in-person participants expressing this concern. A higher percentage of participants, 61% ( $n = 61$ ) of in-person, and 68% ( $n = 139$ ) of online participants,

viewed flying foxes as potential tourist attractions, while 27% (n = 27) of in-person, and 21% (n = 43) of online participants did not. A smaller proportion (12%, n = 12 in-person; 11%, n = 22 online) were unsure about this, as summarised in Figure 2. Among online participants, 204 responded to item on flying foxes as potential tourism and 163 to the item on population decline, with fewer responses due to skipped questions.

### General knowledge of flying foxes among local participants

The mean knowledge score was 2.8 (Standard deviation, SD = 1.1) for in-person respondents and 3.0 (SD = 1.0) for online respondents. Statistical analysis using a t-test revealed no significant differences in knowledge between in-person and online participants ( $t(178.46) = -0.79$ ,  $p = 0.43$ ). Less than 10% of participants (7% in-person, 5% online) correctly answered all five knowledge questions, and over half of all participants scored below three, indicating low overall knowledge of flying foxes (see Figure 3).

Most participants recognised the importance of flying foxes in seed dispersal, with 58% (n = 58) of in-person and 68% (n = 150) of online participants acknowledging this. However, fewer participants were aware of their role in pollination, only 37% (n = 37) in-person compared

to 52% (n = 114) online. Misconceptions were common, particularly concerning flying foxes' habitats, and feeding habits. Many participants incorrectly believed that flying foxes live in caves (48%, n = 48 in-person; 55%, n = 122 online), and some were unsure of their feeding habits. A minority believed that flying foxes feed on blood (10%, n = 10 in-person; 16%, n = 35 online). There was also a gap in knowledge about hunting regulations: 46% (n = 46) of in-person and 47% (n = 103) of online participants were unsure whether a licence is required to hunt flying foxes. In comparison, 43% (n = 43) of in-person and 36% (n = 79) of online participants knew about the licensing requirement, as illustrated in Figure 4.

### Fruit raiding, hunting, and consuming experience

A chi-squared test revealed a significant difference between in-person and online participants in reported fruit raiding experiences ( $\chi^2(1) = 10.16$ ,  $p = 0.01$ ), whereas no significant differences were observed for hunting ( $\chi^2(1) = 0.03$ ,  $p = 0.85$ ) or consumption ( $\chi^2(1) = 0.70$ ,  $p = 0.40$ ). Fruit raiding was the most commonly reported experience, with significantly more in-person participants (44%) encountering crop losses than online (24%). Hunting experience was slightly higher in in-person surveys (11%) compared to online (8%). Consumption experience was around 23% for in-person

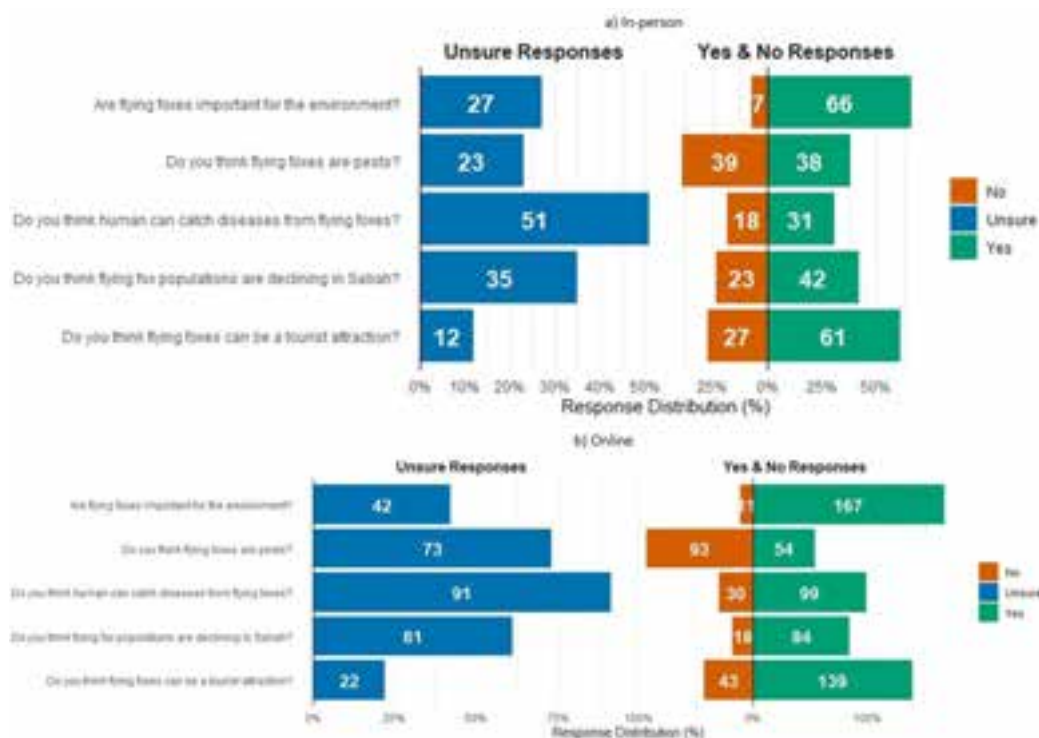


Figure 2. Participants' perceptions of flying foxes in Sabah are presented in Figure 2a (in-person) and Figure 2b (online), with responses categorised as "Yes", "Unsure", or "No".

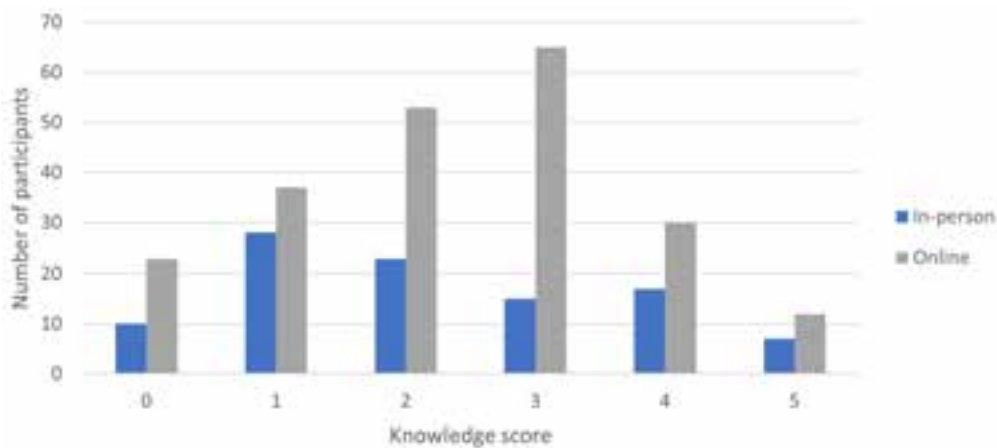


Figure 3. Summary of knowledge scores (from lowest score 0 to highest score 5) based on participant types, covering 100 in-person and 220 online participants in Sabah.

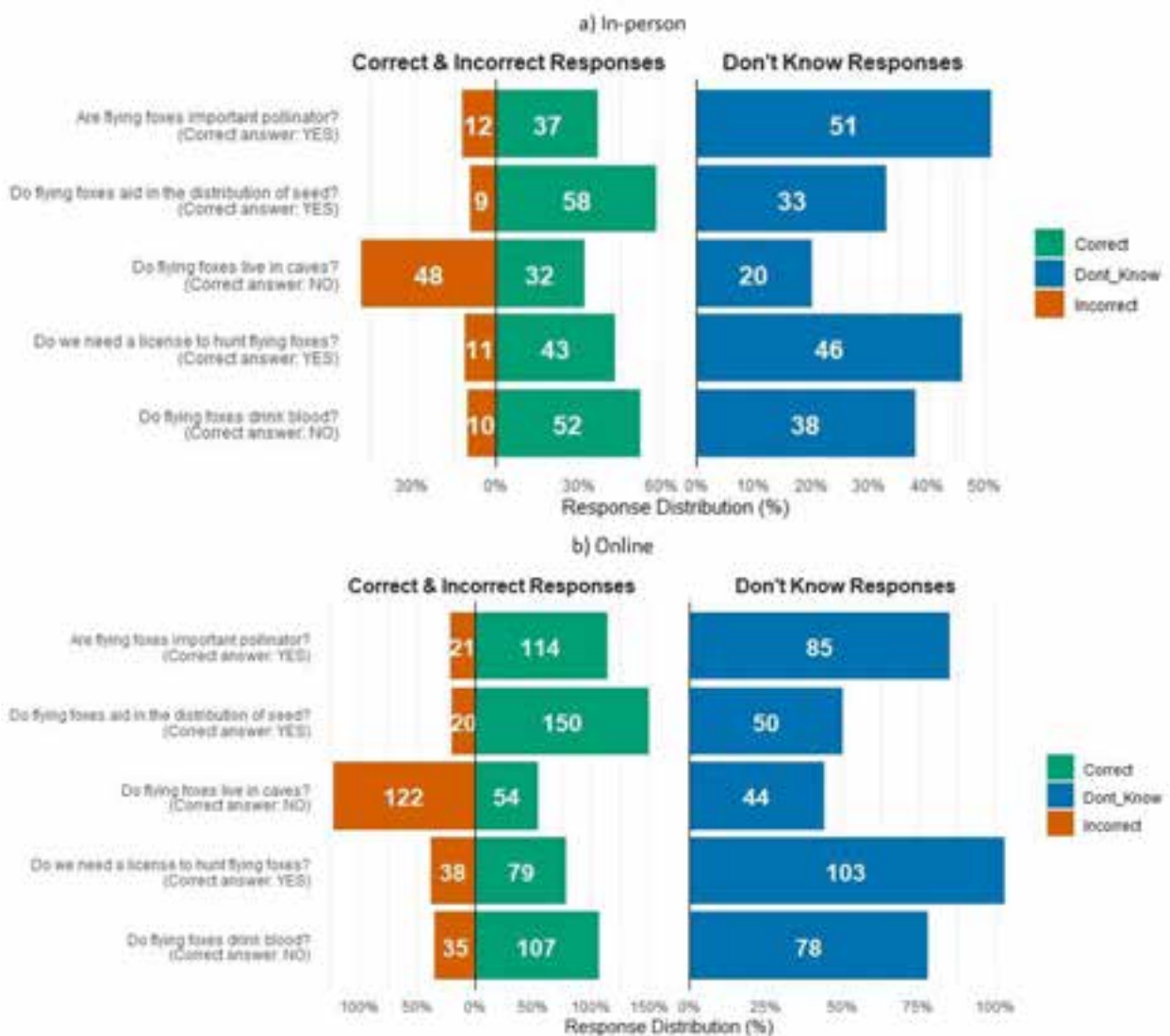


Figure 4. The knowledge of in-person (Figure 4a, n = 100) and online (Figure 4b, n = 220) participants toward flying foxes in Sabah is based on five questions grouped by "Yes", "Don't know", and "No".

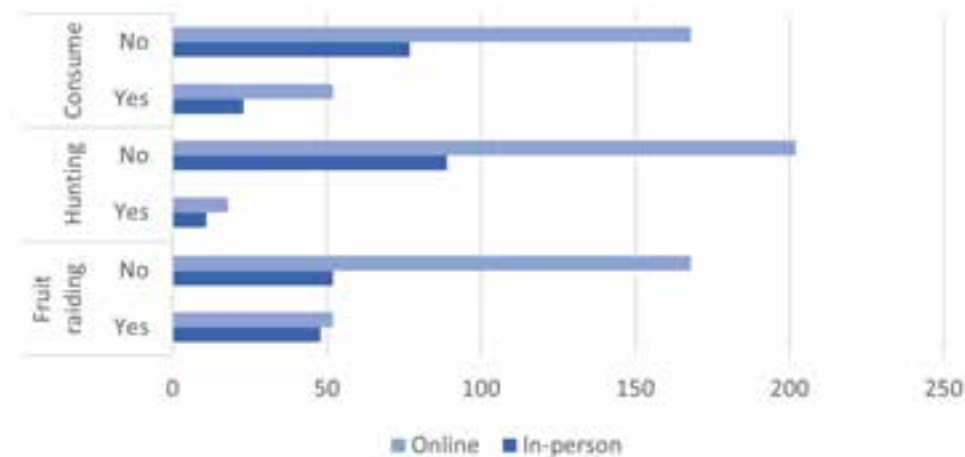


Figure 5. Proportion of in-person (n = 100) and online (n = 220) participants who reported direct experiences with flying foxes in Sabah, including fruit raiding (in-person, n = 48; online, n = 52), hunting (in-person, n = 11; online, n = 18), and consumption (in-person, n = 23; online, n = 52).

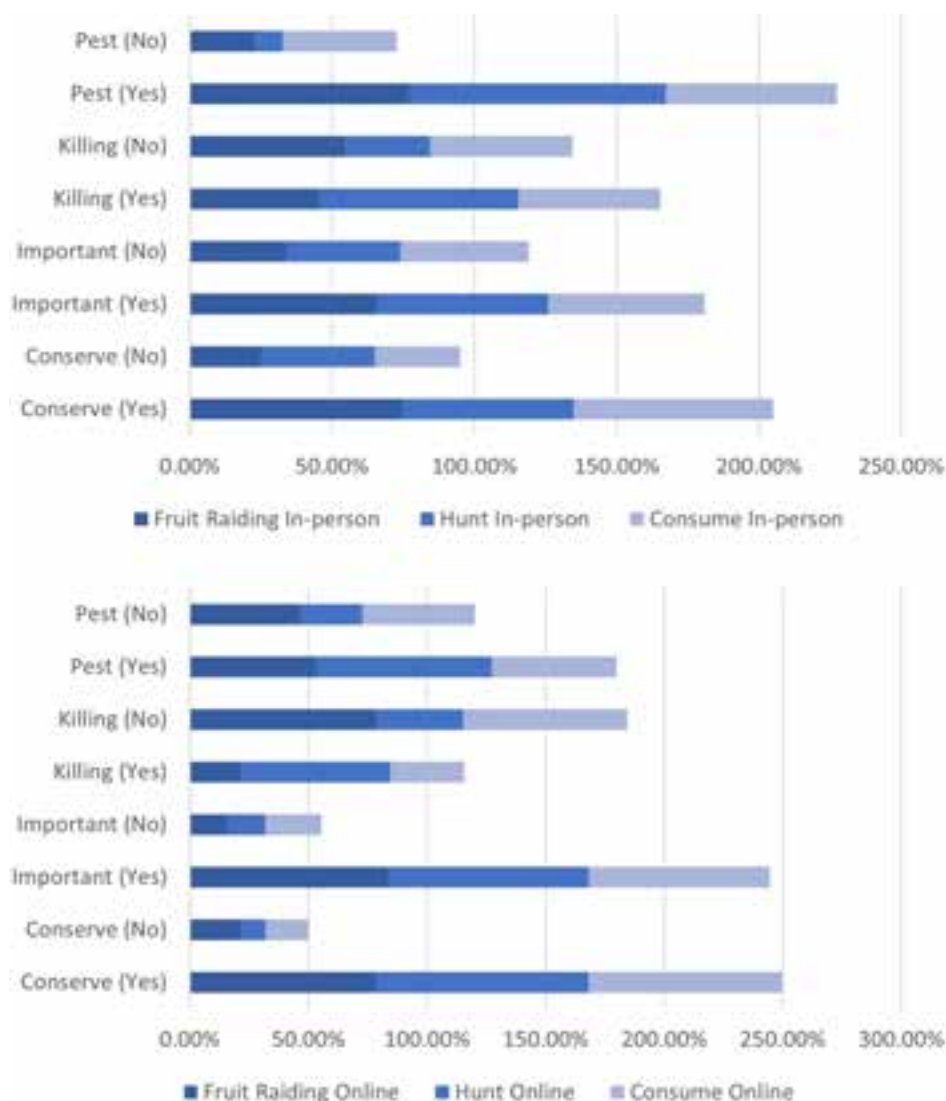


Figure 6. Proportion of participants experienced fruit raiding (in-person, n = 48; online, n = 52), hunting (in-person, n = 11; online, n = 18), consumption (in-person, n = 23; online, n = 52) with their attitudes (conservation and killing), and perceptions (important and pest).



**Table 1.** The comparison of the GLMM model is based on demographic, negative experience, and conservation-related variables.

Model		K	LL	AIC	BIC	dAICc	wAICc	R <sup>2</sup> m
Model 1	Conserve ~ Age + Gender + Education + (1 Method)	3	396.50	362.60	411.30	55.04	~0	0.09
Model 2	M1 + Fruit raiding + Hunt + Consume + (1 Method)	6	-171	366.10	396.50	58.99	~0	0.11
Model 3	M2 + Knowledge score + Important + No Kill + (1 Method)	9	-133.80	305.60	377.20	0	1.00	0.39

Term abbreviations: k—number of parameters | LL—maximum log-likelihood | AIC—The Akaike Information Criterion | BIC—Bayesian Information Criterion | dAICc—difference in AICc for each model from the most parsimonious model | wAICc—AICc weight | R<sup>2</sup>m—marginal R<sup>2</sup>.

**Table 2.** Key predictors of conservation attitudes in the final generalised linear mixed model (Model 3).

Predictor	Odds ratio (OR)	95% CI (OR)	p-value
Knowledge score = 3	7.43	(2.33, 23.65)	<0.05
Knowledge score = 5	8.40	(0.85, 83.33)	<0.05
Importance perception	4.30	(2.11, 8.77)	<0.05
Opposition to killing	3.62	(1.90, 6.90)	<0.05

and online participants (see Figure 5).

In-person respondents (77%) were more likely to perceive fruit raiding as a pest problem than online respondents (54%). In-person respondents (45%) may have more direct conflicts with fruit raiding, leading to a higher acceptance of killing as a solution. Online respondents were less likely to associate fruit raiding with pest issues. There was high support for killing among hunters (In-Person: 70%, Online: 63%). For consumer participants, both in-person and online respondents lean more towards conservation rather than supporting killing (summarised in Figure 6).

#### Factors influencing the conservation attitude

The random effect for survey method (online vs. in-person) had near-zero variance across all models (Variance =  $2.0766 \times 10^{-17}$ , SD =  $4.55 \times 10^{-9}$ ), indicating that survey mode did not meaningfully influence conservation attitudes. This suggests that responses were consistent across both survey formats. Given this result, including the survey method as a random effect does not improve model performance.

Model 3 had the lowest AIC (305.60) and highest log-likelihood (-133.80), indicating the best model fit (Table 1). Consequently, Model 3 was selected as the final model for predicting conservation attitudes. The final model revealed that age, gender, education level, and experiences with flying foxes (hunting, fruit raiding, consumption) were not significant predictors of conservation attitudes ( $p > 0.05$  for all).

The final model (Model 3) identified knowledge level, importance perception, and opposition to killing as the strongest predictors of conservation attitudes (Table 2). All variables had Generalized Variance Inflation Factor,  $GVIF^{1/(2 \times Df)}$  values below 2 (Min: 1.05, Max: 1.20), indicating that multicollinearity was not a concern in the model (Fox & Monette 1992). Therefore, all predictors were retained in the final analysis. Higher knowledge levels significantly increase the likelihood of conservation support (OR = 7.43,  $p < 0.05$ ). Perceiving flying foxes as ecologically important is a strong predictor of conservation attitudes (OR = 4.30,  $p < 0.05$ ). Opposition to killing flying foxes significantly increases conservation support (OR = 3.62,  $p < 0.05$ ). Knowledge Score 5 also exhibited a strong positive effect (OR = 8.40), though it was marginally significant ( $p < 0.05$ ), suggesting that higher knowledge levels may play an increasing role in conservation attitudes.

## DISCUSSIONS

#### Flying fox conservation attitudes and knowledge gaps among locals in Sabah

The findings reveal broad public support for flying fox conservation in Sabah, with 68–77% of respondents expressing favourable attitudes. This strong sentiment provides a valuable foundation for community-led initiatives, particularly when paired with education, and engagement strategies aligned with public values. Attitudes toward flying foxes vary across other regions in Malaysia; for instance, negative perceptions were more prevalent among orchard farmers on Tioman Island (Aziz et al. 2017a), while more favourable attitudes were documented in western Sarawak, where respondents recognised the species' ecological value, and eco-tourism potential (Mohd-Azlan et al. 2022a). Research indicates that positive attitudes, while not always directly translating into behaviour, are critical precursors to conservation action when supported by enabling factors such as incentives, emotions, and social norms



Figure 7. Summary of recommended intervention areas for flying fox conservation in Sabah.

(Bennett et al. 2019; Nguyen-Van et al. 2021; Vaske et al. 2021). Although the present study focused on attitudes rather than behaviours, the level of public goodwill suggests a promising readiness for outreach efforts aimed at fostering long-term conservation engagement.

Overall attitudes toward flying fox conservation were generally supportive, the study revealed notable gaps in ecological knowledge, with mean knowledge scores ranging from 2.8– 3.0 out of 5. The results revealed that many respondents were unaware of the species' roosting sites, diet, and protected status, with common misconceptions including beliefs that flying foxes inhabit caves or feed on blood. Nearly half of the respondents (46%) were also unaware of existing hunting regulations. Comparable trends have been documented in other regions of Malaysia and internationally. For example, Aziz et al. (2016) reported widespread misconceptions about pteropodid bats, commonly perceived as pests, while orchard farmers on Tioman Island demonstrated limited awareness of the species' ecological roles despite frequent encounters (Aziz et al. 2017a). In western Sarawak, Mohd-Azlan et al. (2022a) found that although 76% of respondents acknowledged the ecological importance of flying foxes, 47% still considered them pests and 52% regarded them as a food source. Similarly, on Japan's Ishigaki Island, respondents were familiar with flying foxes but remained unaware of their ecological significance (Vincenot et al. 2015). These findings underscore the variability of knowledge across different regions, influenced by species visibility, and local interactions, and highlight the critical need for context-specific, narrative-based education to improve conservation awareness and outcomes.

#### Factors affecting conservation attitudes among locals in Sabah

Model 3 showed that demographic factors (age, gender, education) and experiences (hunting, fruit raiding, consumption) were not significant predictors of conservation attitudes ( $\Delta AIC_c > 10$ ,  $R^2 < 11\%$ ). This non-significance is plausible when between-group variation, such as survey mode, is minimal or captured by fixed effects. In such cases, random effects with near-zero variance add little value and are often excluded to enhance model parsimony (Bates et al. 2015). This decision is supported by prior chi-square and t-tests, which found no significant differences in attitudes or knowledge between online and in-person participants.

In contrast to findings from a similar study where demographic factors such as age and occupation predicted conservation attitudes in Tioman Island (Aziz

et al. 2017), current findings highlight that cognitive and moral variables, namely knowledge, perceived importance, and ethical opposition to killing, are more consistent, and stronger predictors of conservation-positive attitudes. This should be interpreted cautiously, as non-significant results may reflect limitations such as small sample size, variable design, or contextual factors specific to Sabah. Demographic effects may only become apparent through interactions with psychological or cultural variables, which are often stronger predictors of conservation attitudes than demographics alone (Schultz 2011; Kansky & Knight 2014; Wilbur et al. 2018; Bhatia et al. 2019; Clayton et al. 2021). Likewise, the weak predictive power of conflict-based experiences such as fruit raiding, indicates that personal encounters alone may not drive attitudes without mediation by prior knowledge, social norms, or media exposure (Dickman 2010; Slagle et al. 2013). This may also reflect the use of binary coding, which can mask variation, and reduce statistical power (MacCallum et al. 2002). Beyond these demographic and experiential factors, broader contextual factors including education, income, civic engagement, and participation in environmental activities have been shown to influence conservation attitudes (Oliveira et al. 2024). Future research should prioritise mixed-methods approaches to better capture the nuanced sociocultural, psychological, and contextual drivers of conservation attitudes beyond demographic, and experiential factors.

Knowledge emerged as the strongest predictor of conservation attitudes, with higher scores significantly associated with conservation-positive views. This association is consistent with findings in conservation psychology, where greater understanding of conservation issues is often correlated to stronger support for wildlife protection (Bennett et al. 2019). This insight can support flying fox conservation by highlighting that increased knowledge may lead people to tolerate negative experiences when they recognise the wildlife species' overall ecological benefits (Deshpande & Kelkar 2015; Hallwass et al. 2024). While knowledge is important, it may be insufficient on its own, especially in contexts shaped by utilitarian views or disease-related fears. Reid (2016) found that even knowledgeable individuals in Costa Rica expressed intentions to kill bats, influenced by fear, and cultural norms. This reinforces the view that knowledge must be complemented by value-based or emotionally resonant messaging to effectively shape conservation attitudes (Otto & Pensini 2017). This underscores the need for targeted, culturally resonant education efforts that combine factual information with

ethical framing to correct misconceptions, and enhance conservation outcomes.

In addition to knowledge, moral variables such as perceived ecological importance and ethical opposition to killing also significantly predicted conservation attitudes in Sabah. Similar patterns were observed in western Sarawak, where communities recognised the ecological roles of flying foxes and their potential for eco-tourism, despite the absence of formally measured cognitive or moral predictors (Mohd-Azlan et al. 2022). Internationally, studies in Greece, and Vietnam similarly found that moral values (such as opposition to killing and recognition of species importance) had a greater influence on conservation attitudes than demographic factors (Liordos et al. 2017; Huong et al. 2024). These findings are consistent with the Value-Belief-Norm theory (Stern et al. 1999), which emphasises moral obligation as a central driver of pro-environmental behaviour, and reinforce critiques of demographic-based outreach. Consequently, conservation strategies are more effective when grounded in ethical responsibility and aligned with locally shared values (Kollmuss & Agyeman 2002), an approach that may be particularly relevant in the Sabah context.

### Enhancing Flying Fox Conservation through Conservation Initiatives in Sabah

The current results indicate that local communities demonstrate substantial conservation support, influenced by knowledge and moral values, highlighting the need to engage them through targeted awareness campaigns, participatory initiatives, and policy interventions to sustain conservation outcomes. Effective strategies should prioritise public education, the dissemination of ecological knowledge, protection policy, and the promotion of non-lethal methods to mitigate human flying fox conflict. These approaches are fundamental to fostering sustainable coexistence between humans and flying fox populations. This aligns with the Sabah Biodiversity Conservation Strategy 2024–2034, which emphasises the importance of enhancing the capacities of all stakeholders, including local communities to manage, and conserve biodiversity effectively (Sabah Biodiversity Centre 2024). With this, the current study suggests some key areas for flying fox conservation initiatives in Sabah:

#### 1. Educational tools: Conservation Awareness and Knowledge Gaps

Findings from the study indicate that baseline knowledge regarding flying foxes among participants was generally low. To address these challenges, it is

essential to develop educational tools that are accessible and culturally appropriate for local communities. Such materials should aim to improve understanding of flying fox ecology, particularly their roles in seed dispersal, and pollination, while also clarifying existing hunting regulations, and licensing procedures. Enhancing public knowledge in these areas may contribute to stronger community support for conservation policies and practices.

## 2. Flying Fox Conservation Programme: Capitalise on Existing Positive Attitudes

Although several respondents reported fruit raiding by flying foxes, most expressed opposition to lethal control. This indicates that, despite human–wildlife conflict, there is a foundation of positive attitudes that can be harnessed to support conservation. Effective efforts should therefore address community concerns such as crop damage and hunting, through inclusive dialogue and participatory strategies. Public biodiversity awareness campaigns can further strengthen support, while the adoption of non-lethal crop protection methods, such as those demonstrated by Berthinussen et al. (2021), offers a practical solution that balances conservation objectives with local needs.

## 3. Misinformation on Disease Transmission and Public Perception

The findings also revealed substantial misinformation about disease transmission, with many participants believing flying foxes spread illnesses like COVID-19 or expressing uncertainty about zoonotic risks. Some respondents reported hunting or consuming flying foxes, practices linked to increased risk of zoonotic diseases such as the Nipah virus. Addressing these misconceptions is critical. Public communication strategies should aim to provide evidence-based information that distinguishes between actual and perceived risks associated with flying foxes. Emphasis should be placed on correcting inaccurate beliefs about direct transmission of diseases to humans, while simultaneously promoting a more informed and nuanced understanding of zoonotic pathways. Accurate risk communication may help to mitigate fear-based attitudes and reduce retaliatory behaviours that undermine conservation efforts.

## 4. Conservation Strategies and Community Involvement

The results indicate strong community interest in conservation, with many respondents supporting flying fox monitoring. Flying foxes were also widely viewed as potential tourist attractions, suggesting opportunities to align conservation with ecotourism, provided public health concerns are addressed. Conservation strategies

should prioritise community involvement through school-based programmes, citizen science, and participatory initiatives to raise awareness, and encourage stewardship (Ballard et al. 2017). Events like Bat Appreciation Days can help shift public perceptions, while ecotourism offers a sustainable, incentive-driven model that supports both local livelihoods, and species protection.

Together, these pillars provide a framework for action. linking the study key findings to broader contributions in conservation science and policy. As shown in Figure 7, the colour coded framework illustrates coordinated multi-sectoral strategies: purple—educational tools and awareness building | blue—community conservation programmes | yellow—misinformation mitigation and risk communication| green—local community participatory initiatives and ecotourism opportunities.

## Challenges and limitations

The study faced post-COVID-19 constraints, particularly movement restrictions and health concerns in 2021, which impacted in-person participation. Although surveys were conducted during Phase Four of the National Recovery Plan, privacy, and health worries remained a barrier. To maximise reach and sample diversity, a dual-mode strategy combining in-person interviews and online surveys was adopted. This approach is supported by methodological research showing that mixed formats improve demographic and behavioural representativeness (De Leeuw 2017; Rand et al. 2019; González & Revilla 2020). While online surveys tend to overrepresent younger or more conservation-aware individuals, in-person formats may suffer from interviewer effects, and social desirability bias (Bethlehem 2010). By leveraging the strengths of both, the study aimed to create a more balanced dataset.

Both in-person and online survey methods introduced distinct but complementary biases. Face-to-face surveys reached individuals with direct experience of flying foxes, as seen in higher reports of fruit-raiding; however, this did not translate to greater knowledge of flying foxes, suggesting that personal exposure does not necessarily improve conservation literacy. Online surveys offered broader demographic reach but were more prone to voluntary response bias, often attracting conservation-leaning participants. To minimise this, distribution included non-environmental channels. Importantly, conservation attitude scores were consistent across both methods, indicating that survey mode did not significantly affect responses. Nonetheless, some bias may remain, online anonymity may reduce social desirability bias, whereas in-person responses could



be influenced by social expectations. Future research could apply indirect questioning techniques such as the Unmatched Count Technique (UCT) or Randomised Response Technique (RRT) to further minimise bias, though these require larger samples (Coutts & Jann 2011; Hinsley et al. 2019). Overall, the mixed-method approach enhanced representativeness and provided a more balanced perspective on human–flying fox interactions (Nissen et al. 2018).

Although this study focuses on Sabah, its findings may be applicable to other regions where flying foxes face similar threats. Key predictors (knowledge, perceived ecological importance, and ethical opposition to killing) can inform conservation strategies elsewhere. Adaptation to local contexts, including cultural beliefs and legal frameworks, is essential. Future studies can test these predictors in other regions to support broader, community-based conservation efforts.

## CONCLUSION

This study examined conservation attitudes, flying fox knowledge, and the key factors influencing public support for flying fox conservation in Sabah. The findings demonstrate a strong foundation of public support, but also reveal significant knowledge gaps and persistent misconceptions. Importantly, positive conservation attitudes were closely linked to flying fox knowledge, and ethical norms, while demographic, and experiential variables played a comparatively minor role.

Key contributions of the study include:

1. A novel framework—four actionable pillars tailored to Sabah: targeted education, enhanced conservation programmes, disease misinformation mitigation, and locally driven participatory strategies to address critical gaps in current efforts.
2. Theoretical insight—confirmation that conservation attitudes are primarily shaped by ethical and ecological considerations, consistent with global literature but newly contextualised for flying foxes in Southeast Asia.
3. Policy relevance—direct alignment with the Sabah Biodiversity Strategy 2024–2034, offering practical guidance for strengthening stakeholder capacities, and fostering inclusive biodiversity management.

By integrating scientific evidence with community perspectives, this study offers a replicable model for advancing conservation outcomes, and promoting coexistence with ecologically important yet vulnerable species.

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**Abstract vernacular:** Keluang (*Pteropus* spp.) merupakan agen pendebungaan dan penyebar biji benih yang penting dalam ekosistem tropika, namun lebih separuh daripada spesies ini diancam kepupusan, menjadikan pemuliharaan mereka sebagai keutamaan global. Di Sabah, Malaysia, pemahaman terhadap sikap komuniti tempatan terhadap kelawar buah amat penting bagi merangka strategi pemuliharaan yang berkesan. Kajian ini menggunakan tinjauan soal selidik sendiri yang disasarkan kepada komuniti Sabah (n = 320; 100 bersemuka, 220 atas talian merentasi pelbagai daerah di Sabah) bagi menilai sikap pemuliharaan, jurang pengetahuan, dan faktor yang mempengaruhi sikap tersebut. Ujian statistik menunjukkan anggaran 70% responden Sabah (68% bersemuka; 77% atas talian) mempunyai sikap positif terhadap pemuliharaan keluang, sekali gus menyediakan asas kukuh untuk memperluaskan usaha pemuliharaan berasaskan komuniti, walaupun lebih separuh masih mempunyai kefahaman ekologi yang terhad atau salah tanggapan tentang keluang. Generalised linear mixed models (GLMM) mengenal pasti tahap pengetahuan (OR = 7.43,  $p < 0.05$ ), pengiktirafan kepentingan ekologi (OR = 4.30,  $p < 0.05$ ), dan penentangan beretika terhadap pembunuhan keluang (OR = 3.62,  $p < 0.05$ ) sebagai peramal paling kuat kepada sokongan pemuliharaan keluang. Faktor sosio-demografi mahupun pengalaman konflik tidak menunjukkan pengaruh yang signifikan terhadap sikap pemuliharaan. Dapatan ini menekankan keperluan bagi pendidikan berfokus dan penglibatan komuniti untuk meningkatkan pengetahuan dan memperbaiki salah tanggapan, meningkatkan kesedaran tentang perlindungan undang-undang terhadap keluang, seperti keperluan permit memburu, serta usaha proaktif untuk menangani maklumat tidak sahih mengenai risiko penularan zoonosis daripada keluang. Pembangunan instrumen pendidikan, program jangkauan komuniti, dan strategi mitigasi konflik tanpa pembunuhan perlu diberikan keutamaan sebagai titik intervensi utama untuk mempromosikan pemuliharaan keluang. Langkah-langkah ini, walaupun berasaskan konteks Sabah, boleh dijadikan panduan untuk memperkukuh usaha pemuliharaan keluang dalam konteks komuniti lain di tempat lain.

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**Author contributions:** LAB, NHH, and MP conceptualised the study, with LAB leading data collection, data curation, and contributing resources together with NHH. LAB, LF, and SGS performed the formal analysis, while MP, LF, SGS, and NHH validated the findings. The original draft was written by LAB, MP, LF, SGS, and NHH, with all authors contributing to review and editing.



## Supplementary Material 1. Questionnaire Sample

### SECTION A: SOCIO-DEMOGRAPHY

Residential: \_\_\_\_\_

Age:

18 – 29 years old  
30 – 39 years old  
40 – 49 years old  
50 years old and above

Sex:

Male  
female

Ethnic:

Kadazandusun  
Rungus  
Bajau  
Cina  
Melayu  
Sungai  
Others (State):  
\_\_\_\_\_

Highest education level:

SRP/PMR/PT3  
SPM  
STPM/Diploma/Certificate  
Bachelor Degree  
Master/ PhD

Occupation:

Own (State):  
\_\_\_\_\_  
Government servant (State):  
\_\_\_\_\_  
Private (State):  
\_\_\_\_\_  
Others (State):  
\_\_\_\_\_

Gross Income:

RM500- RM1000  
RM1001-RM2000  
RM2001-RM3000  
>RM3000

Please refer to the following figure:



1. These are flying foxes. Can you differentiate them from other animals, especially small bats and birds?

Yes / No

## SECTION B: EXPERIENCE

2a. Do you experience flying fox raiding your fruiting trees?

Yes / No

2b. List the name of your fruiting trees affected by the flying fox fruit raiding.

---

2c. Where is/are district/s that you experience this fruit raiding by flying fox?

---

3a. Based on your experience, have you hunt flying foxes?

Yes / No

3b. What is/are the reason/s of hunting flying foxes?

---

3c. Where do you usually hunt flying foxes? (For example: forest, mangrove)

---

3d. What do you use for flying fox hunting?

---

4a. Do you consume flying foxes? (if the answer is No, skip the remaining questions and go to part 3)

Yes / No

4b. What is/are the reason/s of consuming flying foxes?

---

4c. What is the frequency of you consuming the flying foxes?

Often: A few times in a year

Frequent: Subject to availability/once a year

Sometimes: Once in several years

Rare: Last ten years ago

### SECTION C: KNOWLEDGE, ATTITUDE & PERCEPTION

**\*\*Additional Note:** Local names for flying foxes are *gawir* or *mengkawot*. Respondents need to identify the flying fox pictures before answering the questioners.

#### C1: KNOWLEDGE

No	Item	No (0)	Don't know (0)	Yes (1)
1	Are flying foxes important pollinators?			
2	Do flying foxes aid in the distribution of seed?			
3	Do flying foxes live in caves?			
4	Do license is required to hunt flying foxes?			
5	Do flying foxes drink blood?			

#### C2: ATTITUDE

No	Item	No (0)	Unsure	Yes (1)
6	Are flying foxes should be conserved?			
7	Would it be good if flying foxes go extinct?			
8	Do you think flying foxes are scary creature?			
9	Should flying fox be killed?			
10	Could flying fox cause COVID-19?			

#### C3: PERCEPTION

No	Item	No (0)	Unsure	Yes (1)
11	Are flying foxes important for the environment?			
12	Do you think flying foxes are pests?			
13	Do you think human can catch diseases from flying foxes?			
14	Do you think flying fox populations are declining?			
15	Do you think flying foxes can be tourist attractions?			

## Supplementary Material 2. R scripts for GLMM.

```

library(openxlsx)
library(dplyr)
library(tableone)
library(lme4)
library(MuMIn)
library(performance)
setwd("C:/Users/USER/data objective 3")
datreg <- read.xlsx("regall.xlsx")
nrow(datreg)
datreg$Age <- as.factor(datreg$Age)
datreg$Gender <- as.factor(datreg$Gender)
datreg$Education <- as.factor(datreg$Education)
datreg$Knowledge.Score <- as.factor(datreg$Knowledge.Score)
datreg$Highest.monthly.income <- as.factor(datreg$Highest.monthly.income)
datreg$Fruit.raiding <- as.factor(datreg$Fruit.raiding)
datreg$Hunt <- as.factor(datreg$Hunt)
datreg$Consume <- as.factor(datreg$Consume)
datreg$Important <- as.factor(datreg$Important)
datreg$Kill <- as.factor(datreg$NoKill)
datreg$Conserve <- as.factor(datreg$Conserve)

model1a <- glmer(Conserve ~ Age + Gender + Education + (1 | Method), family = binomial, data = datreg)
summary(model1)
model1b <- glmer(Conserve ~ Age + Gender + Education + Hunt + Fruit.raiding + Consume + (1 | Method), family = binomial,
data = datreg)
summary(model2)
model1c <- glmer(Conserve ~ Age + Gender + Education + Hunt + Fruit.raiding + Consume + Knowledge.Score + Important +
NoKill + (1 | Method), family = binomial, data = datreg)
summary(model3)

AICc(model1)
AICc(model2)
AICc(model3)
# Compute AICc
aicc_values <- c(AICc(model1), AICc(model2), AICc(model3))
min_aicc <- min(aicc_values)
dAICc <- aicc_values - min_aicc
dAICc
# Compute wAICc
wAICc <- exp(-0.5 * dAICc) / sum(exp(-0.5 * dAICc))
wAICc
#compute R²m
r.squaredGLMM(model1)[1]
r.squaredGLMM(model2)[1]
r.squaredGLMM(model3)[1]
#multicollinearity check among covariates
library(car)
vif_values <- vif(model1c)

```



### Supplementary Material 3. Summary of association between demographic variables and conservation attitude.

Demographic Variable	Group	Pearson $\chi^2$ (df)	p-value	Cramér's V
Education	Online	15.75 (2)	0.0004	0.268
Income	Online	3.84 (4)	0.427	0.132
Ethnicity	Online	13.64 (13)	0.400	0.249
Residential Area	Online	18.69 (18)	0.411	0.291
Occupation	Online	5.25 (6)	0.512	0.154
Gender	Online	1.08 (1)	0.299	0.070
Age	Online	7.99 (4)	0.052	0.191
Gender	In-person	2.89 (1)	0.039	0.170
Age	In-person	3.55 (4)	0.471	0.188
Education	In-person	0.58 (2)	0.750	0.076
Income	In-person	4.79 (3)	0.188	0.219
Ethnicity	In-person	16.23 (8)	0.089	0.403
Residential Area	In-person	7.57 (8)	0.477	0.275
Occupation	In-person	11.85 (6)	0.065	0.344

## Supplementary Material 4. Demographic characteristics of in-person and online participants.

	Category	In-person (n)	Online (n)	n (%)
Gender	Male	53	111	164 (51)
	Female	47	109	156 (49)
Age	<24 years old	18	29	47 (14.7)
	25-34 years old	11	36	47 (14.7)
	35-44 years old	16	54	70 (21.9)
	45-54 years old	31	53	80 (26.2)
	>55 years old	24	48	72 (22.5)
Highest education level	No formal/ Primary education	25	21	46 (14.4)
	Secondary education	62	100	162 (50.6)
	Tertiary education	13	99	112 (35)
Highest Monthly Income	<RM500	0	23	23 (17)
	RM500-RM1000	15	69	84 (26.2)
	RM1001-RM2000	20	30	50 (15.6)
	RM2001-RM3000	1	32	33 (10.3)
	>RM3000	64	66	130 (40.6)
Occupation	Students	9	23	32 (10)
	Self-employed	43	61	104 (32.5)
	Private sectors	13	35	48 (15)
	Government servants	26	76	102 (31.9)
	Pensioners	1	10	11 (3.4)
	Non-employed	8	15	23 (6.8)
Ethnicity	Kadazandusun	52	162	214 (66.7)
	Sungai	35	3	38 (11.9)
	Bajau	3	24	27 (8.4)
	Others	10	131	141 (43.9)
Current residential area	West Coast Division	25	91	116 (36.3)
	Interior Division	22	91	113 (35.3)
	Kudat Division	1	23	24 (7.5)
	Sandakan Division	52	10	62 (19.4)
	Tawau Division	0	5	5 (1.6)



## INTRODUCTION

Agarwood-producing species, specifically the *Aquilaria* spp. in the Thymelaeaceae family, are primarily distributed in the Asian region (Li et al. 2023; Xie et al. 2024; Bora et al. 2025). The *Aquilaria* genus has 21 species, of which 13 species are reported to be agarwood producers (Lee & Mohamed 2016; Xie et al. 2024). They produce agarwood in their trunks and primary branches due to wounding by worms, lightning or wind-broken branches, natural microbial or fungal infections, or infections that are artificially induced by drilling holes, cutting the bark, and injecting chemicals (Jim 2015; Azren et al. 2019; Wang et al. 2020).

The infection court of the fungal infection of *Aquilaria* spp. is in the heartwood, where *Aquilaria* spp. would generate a high commercial value (Zhang et al. 2024). The increase in levels of trade over the past decade has resulted in overexploitation throughout the range of this species (Chowdhury et al. 2024; Xie et al. 2024). Despite the challenges, such as illegal harvesting in the wild, it is difficult to cultivate *A. malaccensis* due to its sensitivity index in terms of survival rate and environmental conditions where this species is compatible (Kharnaier & Thomas 2021; Latifah et al. 2024).

*Aquilaria cumingiana* and *A. malaccensis* are two closely related species of the family Thymelaeaceae. Globally, *A. malaccensis* was categorized as 'Critically Endangered' while *A. cumingiana* was categorized as 'Vulnerable' in the IUCN Red List (Harvey-Brown 2018). These *Aquilaria* sp. are considered as a problematic species in terms of species identification due to lack of scientific studies on species identification. Using leaf architecture is one way of baseline identification of the species (Mercado et al. 2024). Leaf architecture refers to the form and position of elements in leaf structure, including venation pattern, marginal configuration, and leaf shape. Maulia & Susandarini (2019) reported that venation patterns show significant differences in leaf architecture that distinguish the closely related species of *Aquilaria*.

In the present study, the leaf architecture in *Aquilaria cumingiana* and *A. malaccensis* was examined. This study aimed to evaluate the role of leaf architecture in species identification of *A. cumingiana* and *A. malaccensis* growing in Mindanao areas. To date, there is no published report on the characterization of leaf architecture of *A. cumingiana* and *A. malaccensis* as useful taxonomic evidence, especially for species identification.

## MATERIALS AND METHODS

**Study area:** Samples of plant materials were obtained from two provinces in Mindanao, Philippines. *A. cumingiana* leaf samples were collected from Davao Oriental, while *A. malaccensis* leaf samples were collected from Agusan del Sur (Image 1). These two species were later propagated in a backyard nursery situated in Makar, Baliok, Toril, Davao City, Davao del Sur, Philippines (Figure 1). Laboratory analysis of collected leaf material was performed at the Forestry Laboratory of the University of Mindanao, Matina Campus, Davao City, Davao del Sur, Philippines (Image 2). Data were analyzed on 01 August 2022.

### Material collection

Materials used in this study were leaves from seedlings of *A. cumingiana* and *A. malaccensis* collected from the two provinces, Davao Oriental, and Agusan del Sur. There were 30 juvenile leaves of each species of *A. cumingiana* and *A. malaccensis* collected for the statistical data analysis. Some of the leaves was added to the herbaria collection for the taxonomical evidence. The leaves of each species were collected from different provenances. Foresters and a local parataxonomist confirmed the identification of tree species. The herbaria were deposited in the Department of Forestry of the University of Southeastern Philippines – Mabini Campus.

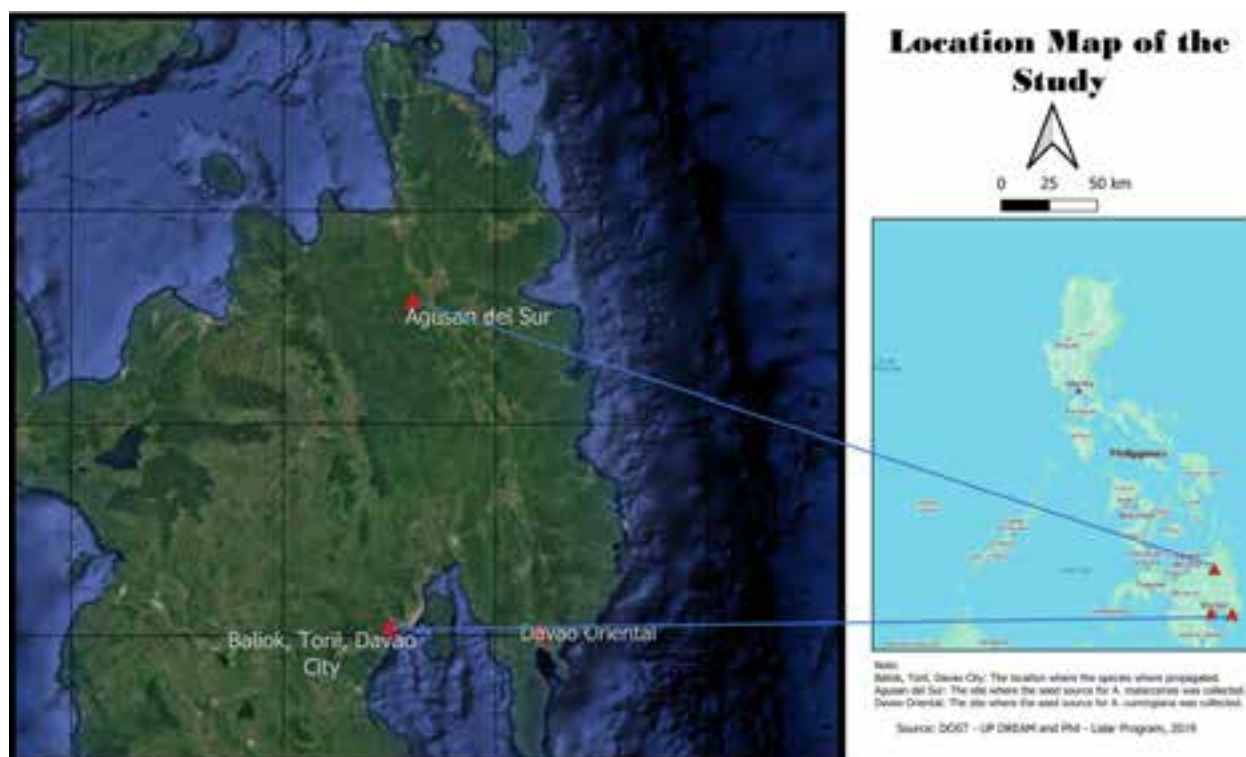
### Leaf architecture traits

There were 21 leaf architectural traits employed in this study, covering both general morphological traits and detailed venation features. Traits such as base shape, apex shape, laminar shape, and angles (base and apex) describe the overall form of the leaf, while traits like tooth apex, lobation, marginal development, and leaf margin account for edge modifications. Venation-related traits, including primary to quaternary vein categories, vein spacing, and venation pattern, provide critical information on vascular architecture, which is highly diagnostic in distinguishing species. Additionally, the areole and laminar blade contribute to identifying structural variations at finer scales. These traits follow the standardized classification of leaf architecture proposed by Hickey (1973) and further refined in the Manual of Leaf Architecture by Ellis et al. (2009).

### Measurement

The leaf architecture data were recorded based on manual leaf architecture (Table 1) with modifications and





**Image 1.** The location map shows where the *Aquilaria* species propagated and the areas where the species were collected.

several additional traits developed by the Smithsonian Institution (1999). The general morphological traits (laminar shape, base, apex, margin, lobation, leaf size, and area) of *A. cumingiana* and *A. malaccensis* were measured using ruler, calipers, and image analysis (Hickey 1979). Venation traits were examined under compound OptiLab microscope camera for digital image capturing.

**Analysis:** Evaluating the leaf architecture in *A. cumingiana* and *A. malaccensis* was analyzed to cluster analysis using the PAST (Paleontological Statistics) software version 3.23 to determine the hierarchical relationships among the different species variations.

## RESULTS AND DISCUSSION

### Leaf architecture of *Aquilaria cumingiana*

Leaves of *A. cumingiana* were alternate and simple in terms of leaf attachments (Image 3a). Laminar shape was lanceolate, with laminar size varying 754–5,600 mm (Image 3b). The leaves are symmetrical, glabrous, cuneate, entire, acute both in leaf shape, base angle, apex shape, and apex angle (Image 3a–e). The leaf texture was smooth and shiny, light green in colour, while the leaf margin was untoothed, and no distinguished

lobation (Image 3). The leaf venation was pinnate, weak in primary vein size, regular polygonal reticulate, vein spacing increasing towards the base (Image 3). The primary venation is straight to slightly curved (Image 3f–g,i), the secondary venation is festooned semi-craspedodromous, secondary vein angle uniform (Image 3g), and the tertiary venation is opposite percurrent (Image 3h–m). The areolation and the quaternary venation were not observed. The marginal development was arranged in a looped formation (Image 3i). There were variations in midrib width, marginal vein width, and the blade class. Trichomes in the laminar area were observed, but strong evidence is required (Image 3i–j).

### Leaf architecture of *Aquilaria malaccensis*

*Aquilaria malaccensis* displays its variation in terms of leaf architecture as compared to *A. cumingiana*. The leaves of *A. malaccensis* were alternate, simple, lanceolate, symmetrical, acute, obtuse, acuminate, entire, glabrous, untoothed, and no lobation (Image 4a–e). The venation characteristics of *A. malaccensis* are pinnate, weak, reticulodromous, straight to slightly curved for the primary vein course, with irregular venation spacing (Image 4f). The secondary vein category is semi-craspedodromous, the tertiary vein is categorized as random, while the quaternary vein is dichotomizing

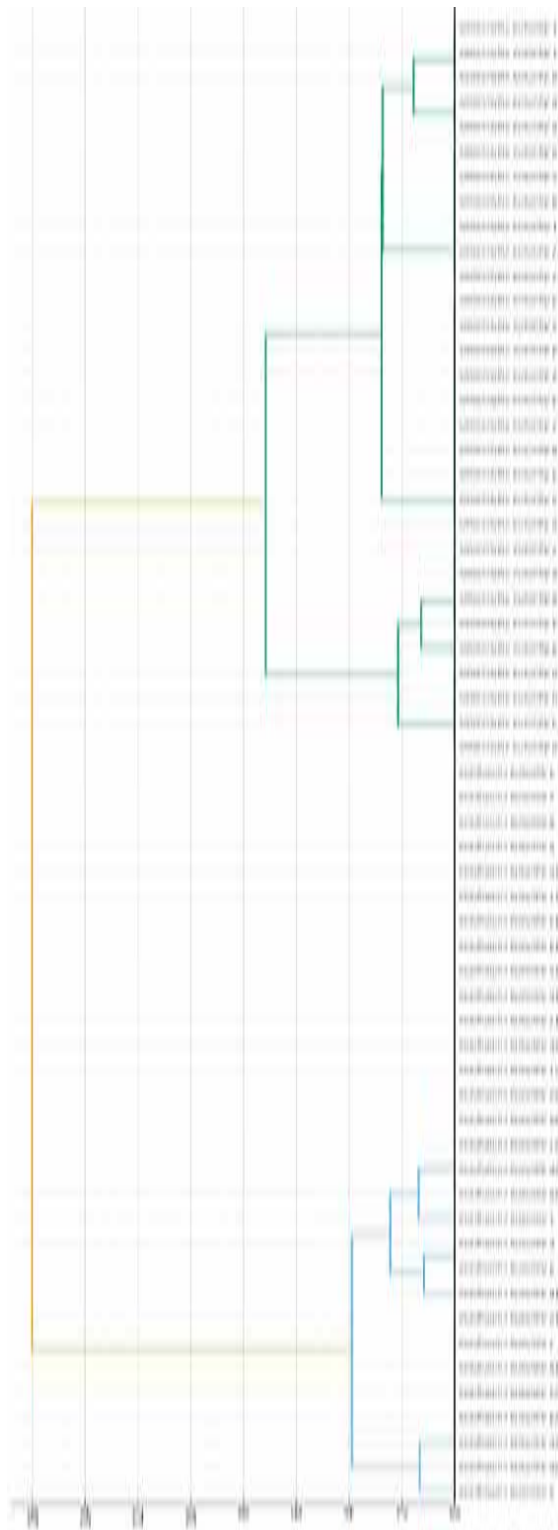


Figure 1. Dendrogram of *Aquilaria cumingiana* (left) and *A. malaccensis* (right) samples based on 21 leaf architectural traits used in this study, using PAST (Paleontological Statistics) software version 3.23.

(Image 4). The areolation was not observed, while the marginal development was looped (Image 4h–k). There was a notable occurrence of trichomes in the below leaf surface (Image 4i). This result has a similarity assessment to the study of Maulia & Susandarini (2019) on the leaf architecture of *A. malaccensis*.

#### Variations between *Aquilaria cumingiana* and *Aquilaria cumingiana*

The dendrogram (Figure 1) clearly distinguishes *A. cumingiana* from *A. malaccensis* based on 21 leaf architectural characteristics, with *A. cumingiana* forming a compact cluster that reflects its morphological uniformity, while *A. malaccensis* displays broader sub-clustering, indicative of greater intraspecific variation. The correlation (Figure 2) further shows that only 10 traits strongly influenced this clustering, particularly base shape, apex shape, and venation-related traits such as secondary, tertiary, and quaternary vein categories, while other traits like leaf margin, lobation, and tooth apex contributed little to species identification. These results highlight that venation and lamina form are the most reliable diagnostic features for separating the two *Aquilaria* sp.

#### Summary of key findings

The comparative study of leaf architecture in *A. cumingiana* and *A. malaccensis* is important for their morphological and taxonomic identification. These species have smooth texture and pinnate venation that includes festooned semi-craspedodromous secondary veins, and a symmetrical, and lanceolate lamina. The stable morphological profile suggested by the invariant features in the sample over different times could be the result of the adaptation to an ecological niche.

*Aquilaria malaccensis* has higher leaf variability. The secondary venation, mostly dichotomous, but there is also random tertiary venation with possible irregular spacing, arc venation, and other morphological plasticity, is a testament to its greater morphological plasticity. The presence of trichomes under the *A. malaccensis* leaves (as opposed to the smooth surface of *A. cumingiana*) could also be an adaptation to different environmental pressures.

Cluster analysis of 21 traits of leaf form revealed clear taxonomic separation between *A. cumingiana* and *A. malaccensis*. From this, it could be concluded that the variation in *A. malaccensis* is driven to a greater extent, suggesting that genetics or environment has a greater effect on the morphology of these specimens. These findings underscore the importance of leaf architecture

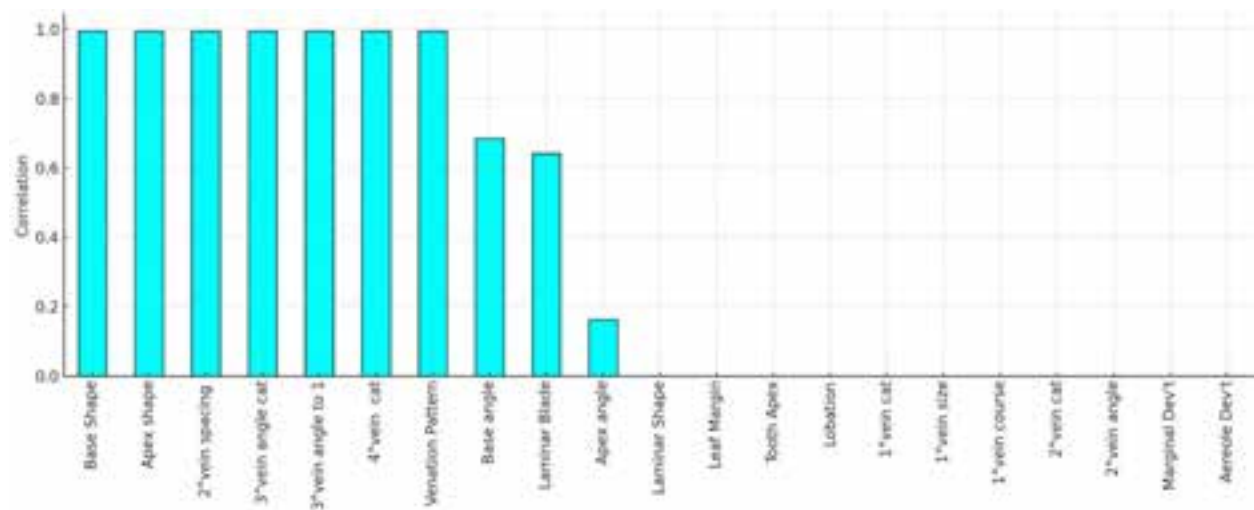


Figure 2. Correlation variation in *Aquilaria cumingiana* and *A. malaccensis*, using PAST (Paleontological Statistics) software version 3.23.



Image 2. Examination of venation pattern using photo microscope (Olympus CH40). © RL Germo.

in distinguishing closely related species, particularly where morphological similarities blur taxonomic boundaries.

The fixed differences were observed in 10 characteristics, including laminar blade, base angle, and apex angle between both species. These dissimilarities suggest that these characteristics could serve as diagnostic markers for taxa identification. While the fixed nature of other traits reinforces the genealogical relationship among these species, morphological divergence may result from ecological divergence but may reflect genetic divergence.

## CONCLUSION

This study underscores the relevance of a comprehensive leaf architectural study toward the identification of closely related species in the genus *Aquilaria*. The study suggests that *A. malaccensis* is more morphologically variable compared to *A. cumingiana* and is likely to have a broader ecological amplitude or population genetic diversity. In contrast, the stable morphology observed in *A. cumingiana* suggests a stable taxonomic relationship that may be dictated by particular environmental demands. These findings serve as original data for taxonomic identification and for the conservation and sustainable management of these economically valuable agarwood-producing species.



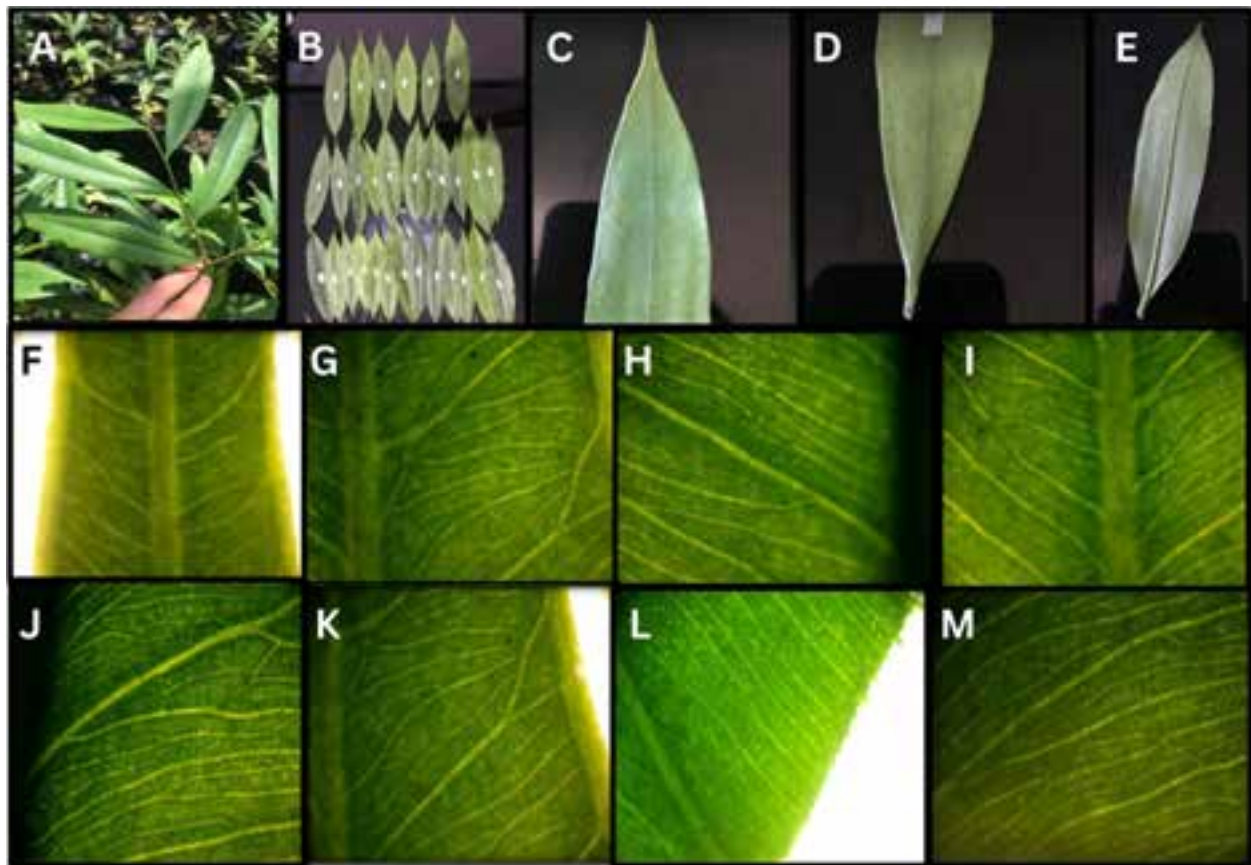


Image 3. *Aquilaria cumingiana*: a—leaf composition | b—leaf shape | c—leaf apex | d—leaf base | e—leaf margin and leaf surface coverings | f—primary vein | g—secondary vein | h—tertiary vein | i—vein spacing | j—leaf venation | k—marginal leaf venation | l—trichomes | m—basal venation arrangement. © RL Geramo.

These morphological differences should be further explored in terms of their ecological and genetic basis using more molecular approaches and by sampling more habitat types in the future. Indeed, exploring the environment where trichome and venation patterns develop could also help in deciphering the adaptive strategies of these species.

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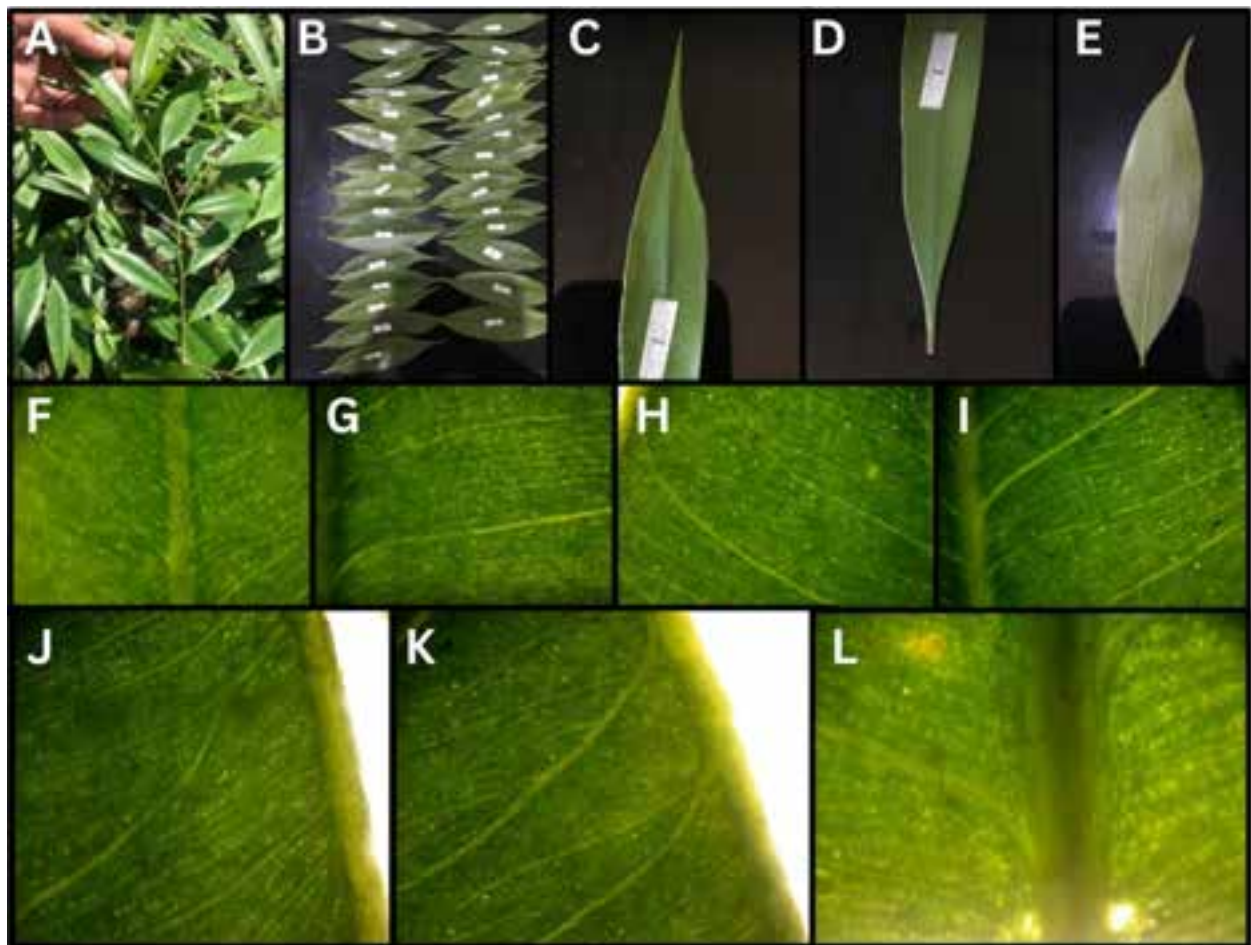


Image 4. *Aquilaria malaccensis*: a—leaf composition | b—leaf Shape | c—leaf Apex | d—leaf Base | e—leaf margin and leaf surface coverings | f—primary vein | g—secondary vein | h—tertiary & quaternary vein | i—vein spacing | j—pattern of leaf venation | k—marginal leaf characteristics | l—trichomes. © RL Germo.



Image 5. Seeds of: a—*Aquilaria cumingiana* | b— *Aquilaria malaccensis*. © First Pirico Farmers Association Inc.

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## First record of *Euclimacia nodosa* (Westwood, 1847) and two species of the genus *Mantispilla* Enderlein, 1910 (Neuroptera: Mantispidae) from the sub-Himalayan foothills of West Bengal, India

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**Abstract:** A year-long light trap study records three neuropteran mantidfly species, namely *Euclimacia nodosa*, *Mantispilla indica*, and an unidentified *Mantispilla* sp. from the sub-Himalayan Terai region of West Bengal. Among the three species, a colour variant of rare wasp-mimicking mantidfly *E. nodosa*, and an unknown species of *Mantispilla*, are significant. A redescription of all three species, with illustrations, is provided, which will enrich information about neuropteran fauna from this biodiverse landscape.

**Keywords:** Darjeeling, Mantidfly, Mantispids, Neuropteran fauna, NBU, new distribution records, taxonomic update, terai region.

**Editor:** Anonymity requested.

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**Author details:** SAHA, A, DAS, S and DAS, P are researchers under supervision of Saha, D, professor and head, Department of Zoology, University of North Bengal. Their work primarily focuses on studying mosquito-vectors, while also exploring the rich insect diversity of the area. SARKAR, R worked as a researcher at ATREE and NBU, cherishes his close interest in insects.

**Author contributions:** AS, RS—conceptualization, data curation, formal analysis, methodology, writing—original draft; SD, PD—investigation, writing-review and editing; DS—supervision, writing-review and editing.

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## INTRODUCTION

Mantispids (Neuroptera: Mantispidae) or mantidflies are interesting creatures of the insect world who feature mantid-like traits, particularly the raptorial forelegs used for catching prey. The intra-specific colour pattern, size variability, polymorphism, and sexual dimorphism are very common in the members of Mantispidae (Handschin 1961; Opler 1981; Redborg & MacLeod 1983; Snyman et al. 2018; Kaur et al. 2021). Some groups of mantidflies, for example of the genus *Euclimacia* Enderlein, 1910, mimic the colour pattern and morphology of certain vespid wasps (Hymenoptera: Vespidae) (Ohl 2004; Bhattacharjee et al. 2010). These insects display a variety of behaviours too and carry out important ecological functions (Ohl 2004). Mantispids are cosmopolitan in distribution and explore a wide range of habitats, e.g., arboreal to aquatic and forests to grasslands (Snyman et al. 2020). Their larvae exhibit remarkable predatory behaviour at different life history stages. This developmental process is known as ‘hyper-metamorphosis’, each stage of which has a distinct shape, and behaviour (Redborg 1998; Snyman et al. 2018). Their food habits include spider eggs, larvae & pupae of different insect orders (Mansell 2010). They clearly have a parasitic connection since they go through several growth stages while inside egg sacs of spiders and eat the eggs or spiderlings (Redborg 1998). Adults are typically observed preying on other insects or consuming pollen grains, and nectar on flowers (Redborg 1998; Mansell 2010). As mantidflies are both parasites and predators, their ecological effects on other arthropod populations are substantial (Redborg 1998; Ohl 2004). This dual role helps to maintain ecological balance by managing the number of various insect species, particularly the agricultural pests (Ohl 2004).

Across the world, 395 neuropteran species, described under 44 genera, are known, of which 22 species under eight genera were recorded from India (Oswald & Machado 2018; Kaur et al. 2021; Pandher 2024) and all extant mantidflies are represented by a single subfamily Mantispinae. The remarkable works on mantidflies from our country were by Westwood (1848, 1852), Walker (1853), Needham (1909), Enderlein (1910), Banks (1933), Ghosh & Sen (1977), Ghosh (1977, 1998, 2000a,b), mostly from northeastern India and a few southern Indian states. In the post-independence period, Ghosh (1977, 1998, 2000a,b) pioneered in studying neuropteran diversity from the nine northeastern Indian states, including Darjeeling (West Bengal), and Sikkim. As a result of these exploratory surveys, four mantidfly

species, namely—*Austroclimaciella quadrituberculata* Westwood, 1852, *Mantispilla indica* Westwood, 1852, *Mantispilla coorgensis* Ohl, 2004, and *Mantispa alicante* Banks, 1913 (Ghosh 1998; Bhattacharjee et al. 2010) were reported from West Bengal (Table 1).

Darjeeling-Sikkim Himalayan region (part of the central Himalaya), situated at the northernmost part of West Bengal, holds six mantispid species (Halder et al. 2018). Despite this historical assemblage of mantispid fauna in Darjeeling-Sikkim Himalayan region and northeastern Indian states, studies on this group from West Bengal remain extremely limited. Apart from checklists, there are no studies that document detailed photos, biology, and young stages of the species. Since 2000, only a single record by Bhattacharjee et al. (2010), included a rarely known species, *Euclimacia nodosa* Westwood, 1847, for the first time from Buxa Tiger Reserve (BTR) (Table 1). The species was first recorded from the Garo hills, Meghalaya, erstwhile in the Assam State, as ‘*Mantispa nodosa*’ (Ghosh 2000a; Bhattacharjee et al. 2010; Kaur et al. 2021). In later studies, all the concerned species of genus *Mantispa* were transferred under genus *Mantispilla* Enderlein, 1910. Our present paper records *Euclimacia nodosa* from the sub-Himalayan foothills (Terai region) for the first time and with revised nomenclature for the other two *Mantispilla* species (Table 1).

## MATERIALS AND METHODS

The University of North Bengal (26.709° N, 88.354° E) is well known for its vast biodiversity in the terms of flora as well as fauna, spanning around 315.99 acres, located in the sub-Himalayan region of West Bengal. The university campus is surrounded by lush vegetation, including natural & plantation forests of deciduous, evergreen plants, grasslands, and wetlands. Within the campus, two semi-perennial streams gave rise to ecotone

**Table 1. An updated list of Mantispidae from West Bengal, India.**

	Species name	Literature source
1.	<i>Austroclimaciella quadrituberculata</i> Westwood, 1852	Ghosh 1998, 2000a,b
2.	<i>Mantispilla indica</i> Westwood, 1852	Ghosh 1998, 2000a,b
3.	<i>Mantispilla coorgensis</i> Ohl, 2004	Ghosh 1998; Kaur et al. 2021
4.	<i>Mantispa alicante</i> Banks, 1913	Ghosh 1998; Kaur et al. 2021
5.	<i>Euclimacia nodosa</i> Westwood, 1847*	Bhattacharjee et al. 2010

\*First time record from Terai region in this study.



zones between grasslands, and riversides. Notably, a considerable portion of the campus experiences seasonal accumulation of water during the monsoon. Biological richness of the area has been highlighted by earlier studies of Mukhopadhyay et al. (2015), Pal (2017), and Saha et al. (2023). However, there were no previous records of any neuropterans from this area or the terai region.

For the collection of adult mantidflies, a year-long light trap study using mercury vapour bulb (160 Watt) was carried out once a month from September 2023–August 2024 in North Bengal University (NBU) Campus. The study within the campus was conducted with permission from the Department of Zoology and the Watch and Ward Department, NBU. In each month, three consecutive nights (one new moon night and the next two nights) were chosen for light trap study. We used small-sized insect nets for capturing the species when they were found sitting on the light sheet or near the trap during the survey. After capturing, the specimens were killed using ethyl acetate vapour in a killing jar. The specimens were subsequently pinned and stretched to study in dry condition under a stereoscopic binocular microscope Magnus MS-24 and measurements of different parts were taken in mm using an ocular micrometre. All the specimens were dissected for genitalia identification and preserved in 80% ethyl alcohol. Permanent preservation was carried out as per Ghosh (1998, 2000b). Terminalia of all collected specimens were externally examined for the identification of sexes.

Specimen identification was done using identification keys (Ghosh & Sen 1977; Ghosh 1998, 2000a,b; Bhattacharjee et al. 2010; Snyman et al. 2018; Kaur et al. 2021; Choudhury 2023), relevant websites (<https://www.inaturalist.org>; <https://bugguide.net>) and in consultation with neuropteran specialists (Dr. Manpreet Singh Pandher, ZSI) for a conclusive identification. The genus-level identification was done as described by Snyman et al. (2018) and species-level based on keys by Ghosh & Sen (1977), Bhattacharjee et al. (2010), and Choudhury (2023). Photographs of dried specimens were taken using a cell-phone camera (Google Pixel 6a). The wasp species was identified using key provided by Kumar & Sharma (2015). The photo plates were prepared in Adobe® Photoshop® 10.

## RESULTS

Three mantidfly species, belonging to different genera, were found in NBU campus during the study period. These were: a single individual of *Euclimacia nodosa* (Image 1) and *Mantispilla* sp. (Image 4) each, and three individuals of *Mantispilla indica* (Image 3) collected (Table 2).

### Taxonomic account

#### Order Neuroptera

Suborder Planipennia

Family Mantispidae Westwood, 1840

Subfamily Mantispinae Enderlein, 1910

#### I. Genus *Euclimacia* Enderlein, 1910

*Euclimacia* Enderlein, 1910: 362.

Type species: *Euclimacia partita* Enderlein, 1910: 366 (original designation).

Diagnosis: Members of genus *Euclimacia* are distinguished from other genera in having the following characteristics: Antennae perfoliate; symmetrical, perfoliate flagellomeres; pro-thorax distinctly very short, wrinkled and 'humped'; wing venation and colouration are prominent with extended pterostigma; large sized species; species are often found in bright and dark colours; resemble several vespid wasps (*Vespa* spp.) species.

Distribution of the genus: Australasian, Oriental, and Palearctic regions.

#### 1. *Euclimacia nodosa* (Westwood, 1847)

*Mantispa nodosa* Westwood, 1847: 70.

(Image 1A–H)

Material examined: Male, 28.vi.2024, ground of animal house, Dept. of Zoology, NBU, Darjeeling, West Bengal, India. Collected by Abhirup Saha and Ratnadeep Sarkar.

Diagnostic characters: Antenna reddish-brown; prothorax very short and 'nodose'; costal part of forewing brownish or orange yellow; large sized species, body colour rust red to brownish-black, abdomen wasp-like.

Redescription: Wings. Costal portion of fore and hindwings with dark colouration; wing venation prominent, veins brownish (Image 1C,D). Head. Head triangular with broad vertex; eyes large (Image 1H); antenna reddish brown in colour, proximally dark, and distally reddish-yellow, consisting 48 articles (Image 1G). Body. Head, antenna, prothorax, abdomen, and legs rust red (Image 1A,B); neck constricted, prothorax short, distally broader, wrinkled or 'nodose' and 'humped'

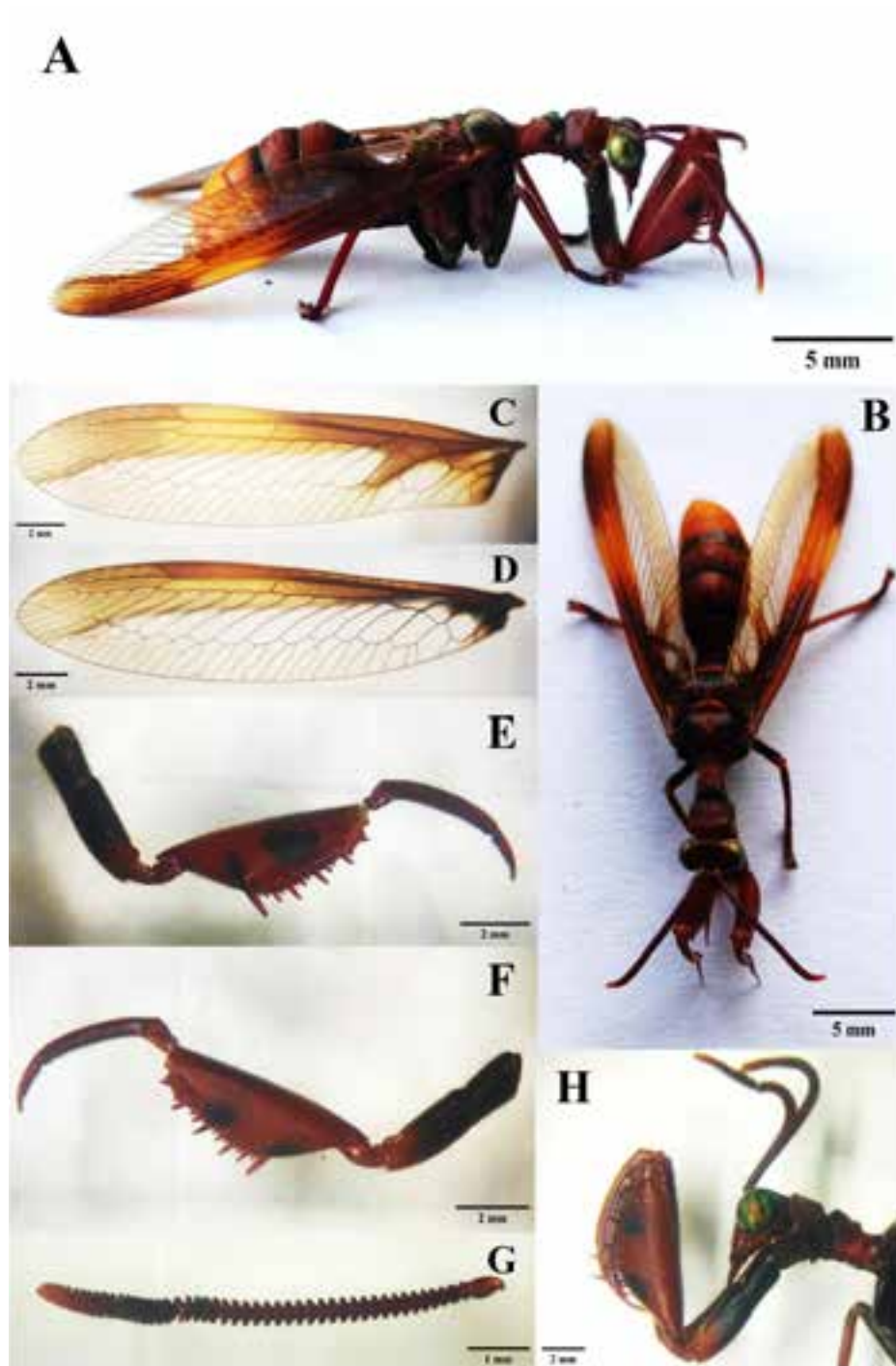


Image 1. *Euclimacia nodosa*, male (1A–H): A—General habitus in lateral view | B—General habitus dorsum | C—Forewing | D—Hindwing | E—Raptorial foreleg in inner view | F—Raptorial foreleg in outer view | G—Antenna | H—Head and thorax in lateral view. © Abhirup Saha & Ratnadeep Sarkar.

(Image 1H). Terminal four segments of abdomen yellowish-orange (Image 1A,B). Legs. Coxa of fore legs brownish-black, fore femur rust red with two black spots at the base of anteroventral spines (Image 1E,F). All measurements of different body parts are presented in

Table 2.

Distribution: India (Figure 1): Assam (Ghosh 2000a,b), Chhattisgarh (Kaur et al. 2021), Kerala, Madhya Pradesh (Kaur et al. 2021), Meghalaya (Ghosh 2000a,b), West Bengal (Bhattacharjee et al. 2010).

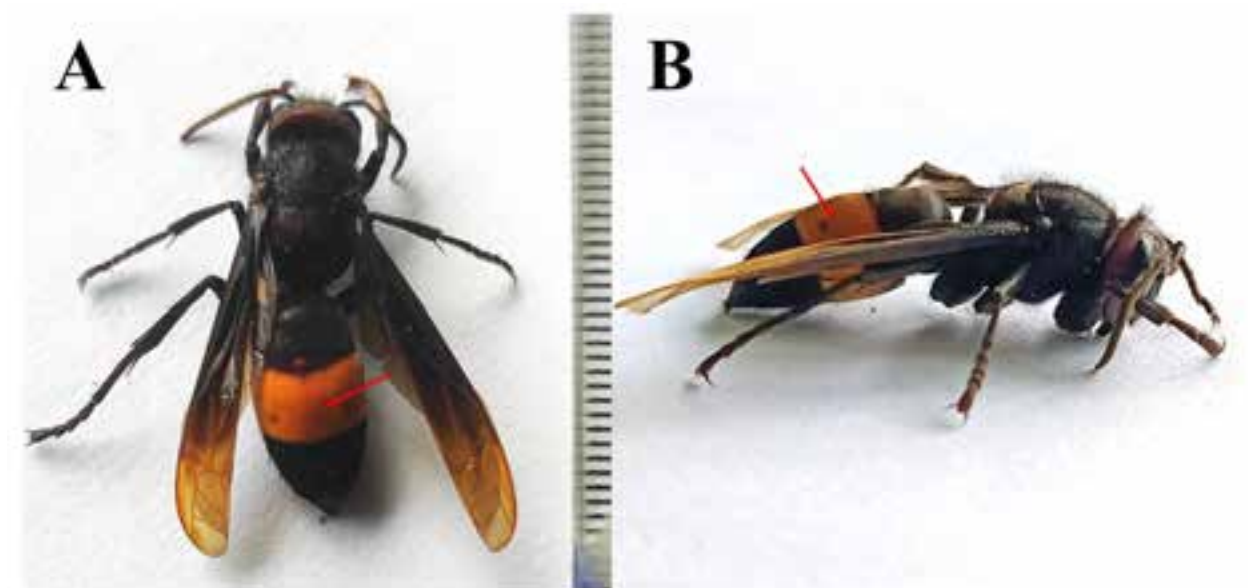


Image 2. *Vespa tropica* L., a putative model for *Euclimacia nodosa*: A—General habitus dorsum | B—General habitus in lateral view. Arrow showing typical orange colour band in abdomen. © Abhirup Saha, NBU.

Remarks: After reviewing available description of the *Euclimacia* species given by Kaur et al. (2021), this species is found to be *Euclimacia nodosa*, recorded from Meghalaya (Ghosh 2000a,b), and BTR, West Bengal (Bhattacharjee et al. 2010) as it matches with the descriptions and illustrations given by Kaur et al. (2021) and the images on iNaturalist websites ([https://www.inaturalist.org/observations?taxon\\_id=1100298](https://www.inaturalist.org/observations?taxon_id=1100298)). Intraspecific colour pattern variability is very common in the different genera under family Mantispidae but not profoundly known for the genus *Euclimacia* (Ehlers et al. 2024). Here, the individual recorded from NBU campus possesses a yellowish-orange colour in the terminal four segments of abdomen, while yellowish banding was found on the abdominal segments II and III in the record of BTR or its first record from Meghalaya. *Euclimacia nodosa* mimics the vespoid wasp *Vespa tropica* L. and similarity lies in the structure, and colouration of the abdominal segments (Image 1A–H & Image 2A–B). The male specimen from NBU is larger in all body-size metrics with a comparatively broader abdomen than the male specimen from BTR (Table 2).

## II. Genus *Mantispilla* Enderlein, 1910

*Mantispilla* Enderlein, 1910: 346.

Type species: *Mantispa indica* Westwood, 1852: 268 (original designation).

Diagnosis: *Mantispilla* lacks pronotal setae, mesothorax bald or pubescent. Longitudinal line (pigmentation) on dorsum or inner lateral side of fore

coxae present. *Mantispilla* species are generally yellow coloured accompanied by black or brown.

Distribution of the genus: Afrotropical, Oriental, and Palearctic region.

## 2. *Mantispilla indica* (Westwood, 1852)

*Mantispa indica* Westwood, 1852: 268.

(Image 3A–H)

Material examined: 3 females, 15.ix.2023 and 28.vi.2024, ground of animal house, Dept. of Zoology, NBU, Darjeeling, West Bengal, India. Collected by Abhirup Saha and Ratnadeep Sarkar.

Diagnostic characters: Antenna black except two basal segments, prothorax yellow with two brown lines anterior, and postero-laterally brown, pterostigma elongate and red, head yellow with brown patterns, abdomen with alternate black & yellow bands in lateral view.

Redescription: Wings. Pterostigma elongate and reddish in colour, hyaline, and veins black (Image 3B,C). Head. Head yellow with brown patterns; antenna dark and consists of 28 articles; eyes large with metallic appearance (Image 3F,G). Body. Prothorax dorsally yellow with two brown lines at the anterior part and ventro-laterally dark brownish (Image 3F,G), meso & metathorax yellow with black lines (Image 3A). Eight segmented abdomens, with black lines at the junction of each tergite (Image 3A). Legs. Fore femur with a small black spot anteriorly and inner side brownish (Image 3D,E); mid and hind legs yellow with brown claws (Image

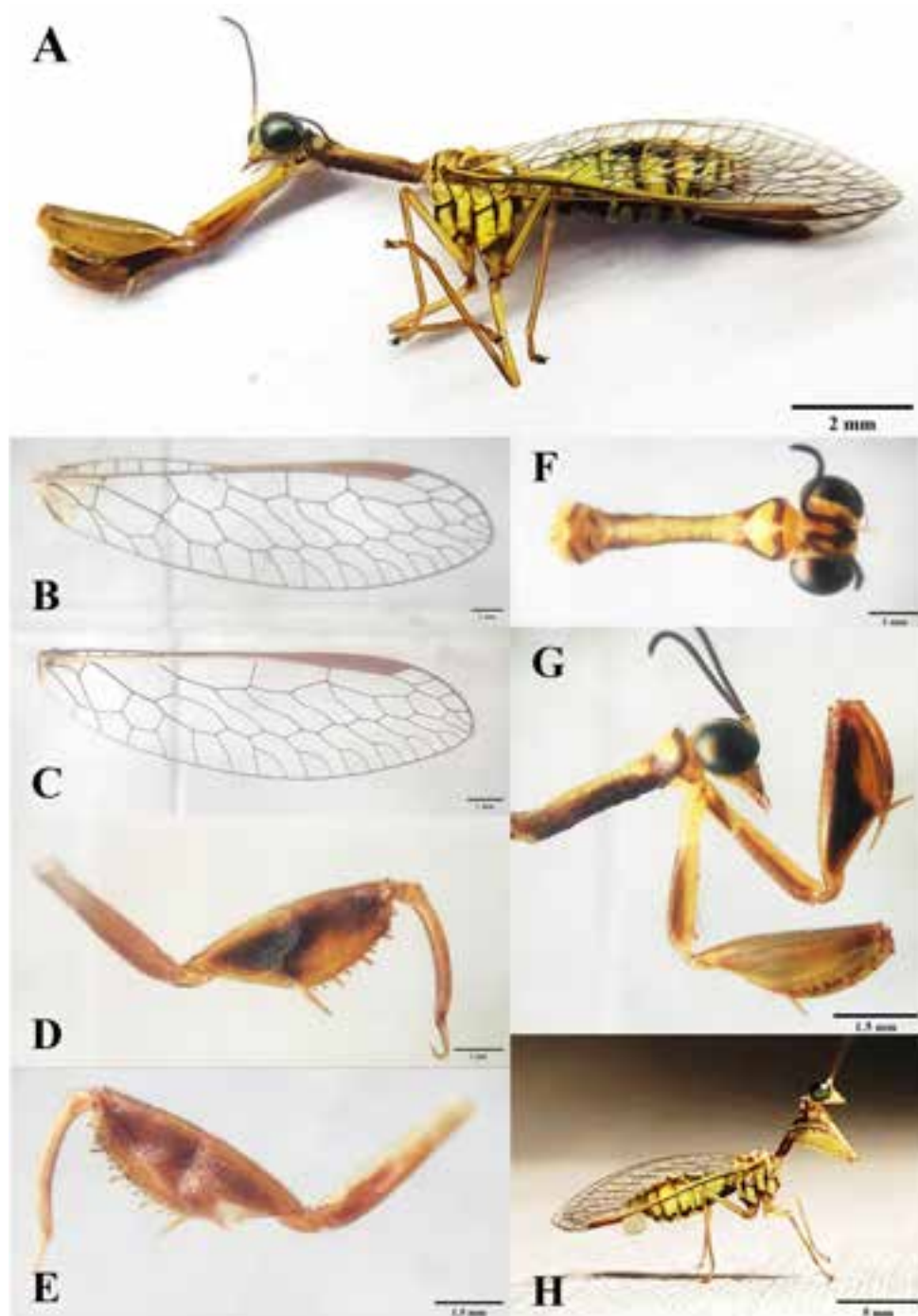


Image 3. *Mantispilla indica*, female (3A–H): A—General habitus in lateral view | B—Forewing | C—Hindwing | D—Raptorial foreleg in inner view | E—Raptorial foreleg in outer view | F—Head and antennae dorsum | G—Head and antennae in lateral view | H—Alive individual. © Abhirup Saha & Ratnadeep Sarkar.

3A). All measurements of different body parts are presented in Table 2 and are compatible with those of Suryanarayanan & Bijoy (2021), and Choudhury (2023).

Distribution: India (Figure 1): Assam (Ghosh 1998; Choudhury 2023); West Bengal (Kolkata and Darjeeling) (Ghosh & Sen 1977); Meghalaya (Ghosh, 1998); Sikkim

(Ghosh 2000a,b); Karnataka; Himachal Pradesh (Western Himalaya); Rajasthan (Ghosh 1977); Western Ghats (Suryanarayanan & Bijoy 2021).

Remarks: *Mantispilla indica* is one of the most widely distributed species of the genus *Mantispilla* but after consulting relevant literature, no good quality



illustrations of this species were found. Colour pattern variability has also been observed in this species (Kaur et al. 2021).

### 3. *Mantispilla* sp. (close to *indica*)

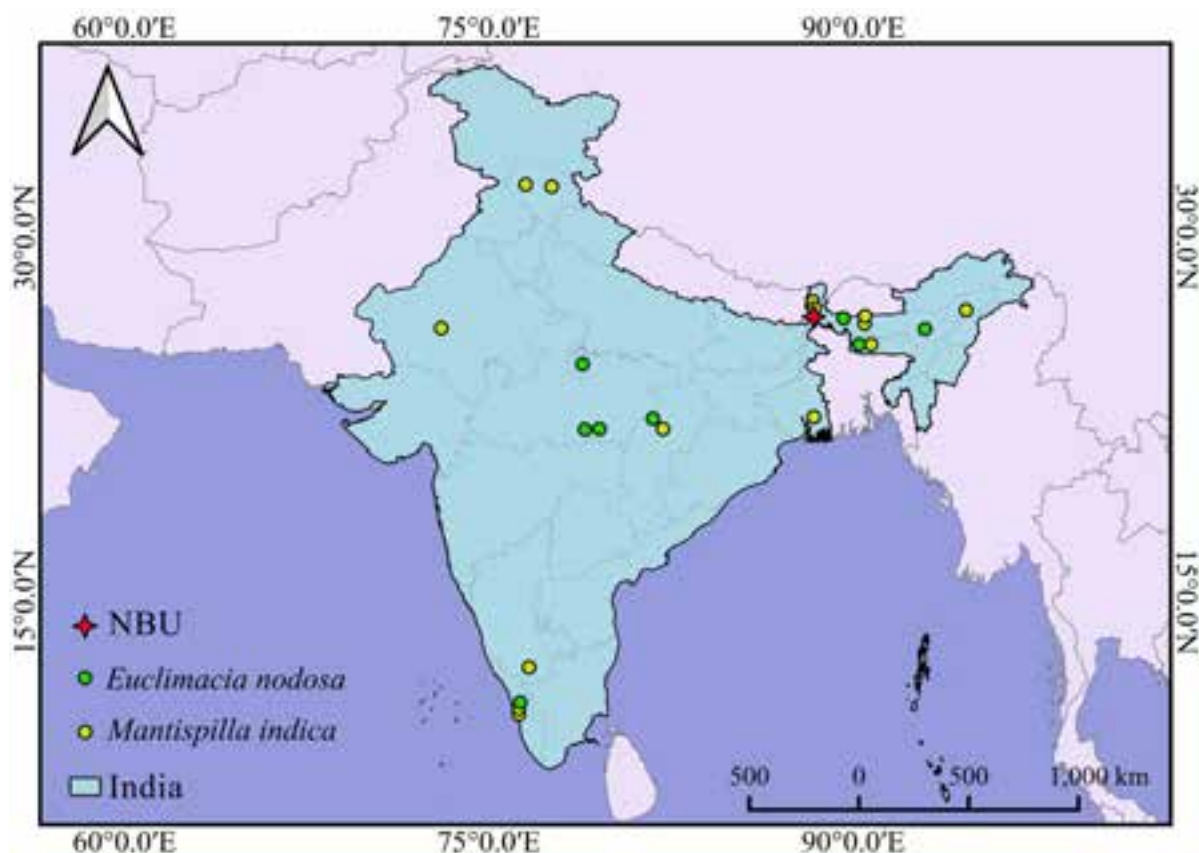
(Image 4A–G)

Material examined: Female, 28.vi.2024, ground of animal house, Dept. of Zoology, NBU, Darjeeling, West Bengal, India. Collected by Abhirup Saha and Ratnadeep Sarkar.

Redescription: Wings. Pterostigma elongate and reddish, hyaline with prominent black veins, venation almost similar to *Mantispilla indica* (Image 4B,C). Head. Head brownish-yellow with brown marks, antenna brownish, consists of 28 articles. Body. Specimen with darker complexion in comparison *Mantispilla indica* (Image 4A,G), prothorax dorsum is not yellow but light brownish (Image 4E), the anterior surface has two brown lines but are not as prominent as *M. indica* (Image 4F); meso and metathorax also brownish. Abdomen. Dark yellow with black lines at the junction of tergites, additionally, a pair of black lines extended

**Table 2.** Measurements of different body parts of the collected specimens (all measurements in mm; for *Euclimacia nodosa*, one individual was measured; for *Mantispilla indica*, the average of three individuals and for *Mantispilla* sp., one individual, were measured).

Different body attributes	<i>Euclimacia nodosa</i>	<i>Mantispilla indica</i>	<i>Mantispilla</i> sp.
(Length from head to tip of abdomen)	24	12.5	12
Total antennal length	9	3	2.5
Eye	2	1.25	1
Prothorax length	4.5	3.5	3
Mesothorax length	2.3	1	1
Metathorax length	2.3	1	1
Abdomen	13	6	5.5
Fore coxa	4.8	3	3
Fore femur	6.3	3.4	3.4
Fore tibia	3.8	2	2
Fore tarsus	1.5	0.9	0.6
Total midleg length	11	7	6
Total hindleg length	14	9	8
Total forewing length	20	10.5	11
Total hindwing length	18.5	9	9.5



**Figure 1.** Distribution map of *Euclimacia nodosa* and *Mantispilla indica* in India along new records from NBU, West Bengal. The map was created using QGIS software version 3.30.0.

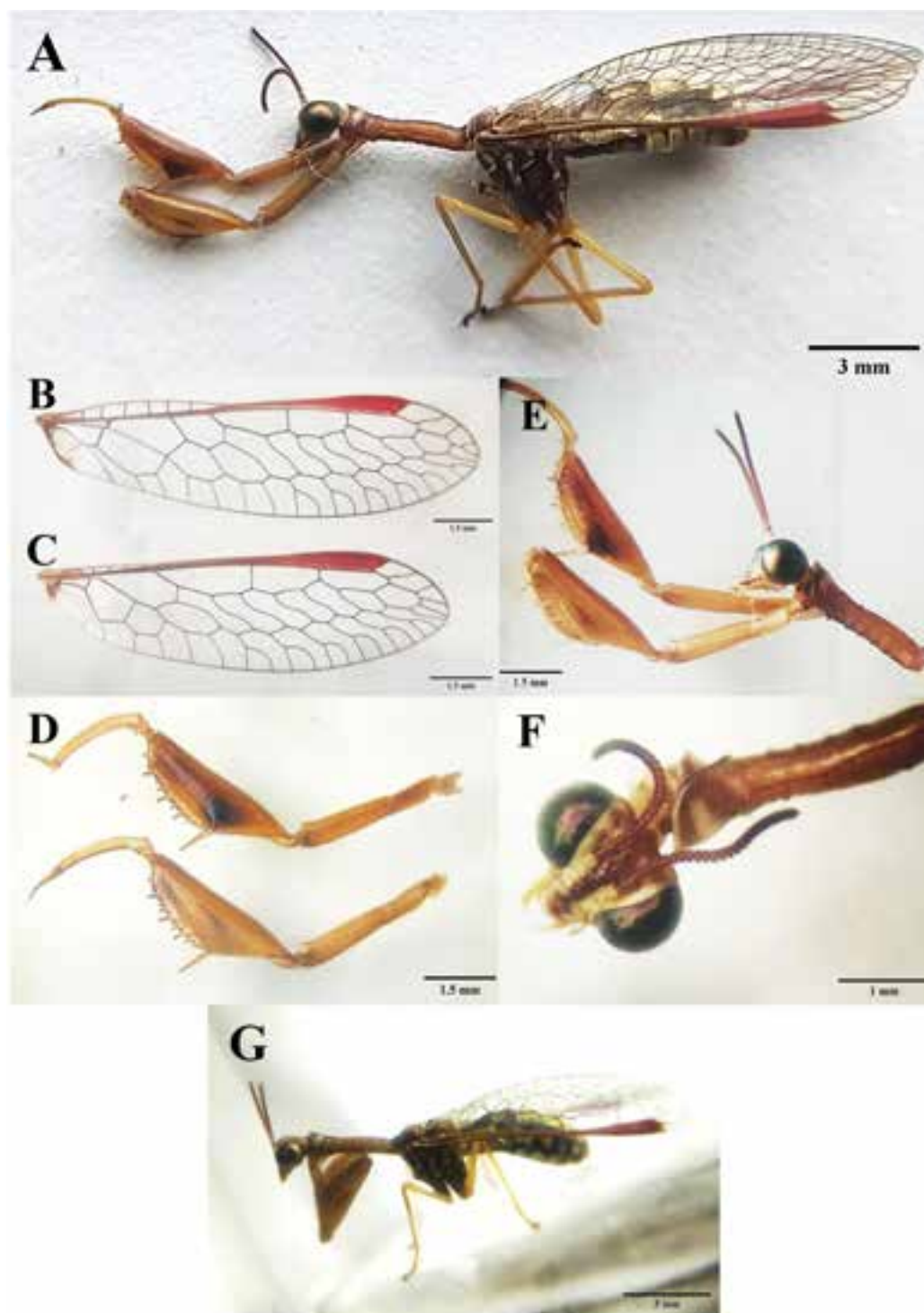


Image 4. *Mantispilla* sp. female (4A–G): A—General habitus in lateral view | B—Forewing | C—Hindwing | D—Raptorial foreleg in inner view (upper) and in outer view (lower) | E—Head and thorax dorsum | F—Head and antennae in lateral view | G—Alive individual. © Abhirup Saha & Ratnadeep Sarkar.

on both lateral surfaces when seen from the sides. Fore coxae and femora dull yellowish but inner side brownish (Image 4D); mid and hind legs yellowish with brown claws (Image 4A). All measurements of different body

parts are presented in Table 2.

Remarks: All measurements of the specimen are found very close to *Mantispilla indica*, but the body colouration and colour pattern are different. Colour of

the pro, meso, and metathorax, and colour patterns of abdomen are noticeably different. Since 'colour pattern variability' is commonly observed in *Mantispilla indica* (Kaur et al. 2021), may be this specimen is a colour-variant.

## DISCUSSION

During this year-long study, only five individuals were found, probably due to relatively small population, and complex life-history traits (Ohl 2007; Suryanarayanan & Bijoy 2021; Choudhury 2023). Though *Euclimacia nodosa* is rare, *Mantispilla indica* is relatively more common. All the species were recorded between June–September, which is late summer and monsoon season, and corroborated with the other findings across India (Bhattacharjee et al. 2010; Kaur et al. 2021).

In *Euclimacia nodosa*, the colour pattern variation was also found in several other records of the species across India (Snyman et al. 2018; Kaur et al. 2021; <https://www.inaturalist.org/>). This phenomenon might proclaim, the variety of *Euclimacia nodosa* found in this region mimics greater banded hornet, *Vespa tropica* L. which is widespread in India, and southeast Asia (Image 2). Mantids and mantidflies are broadly similar due to convergent evolution. Although the identity of first two species, *Euclimacia nodosa*, and *Mantispilla indica* can be established, the identity of third one is still uncertain. Is it a different species under the genus *Mantispilla* or a colour variant of *Mantispilla indica* still needs more investigation.

Considering these species recorded for the first time in Terai, an updated distribution map of the two mantidfly species in India, namely—*Euclimacia nodosa* and *Mantispilla indica* in India—are included (Figure 1) at the end. All available previous records from the various Indian states (Ghosh 1977, 1998, 2000a,b; Ghosh & Sen 1977; Bhattacharjee et al. 2010; Sharma & Chandra 2013; Kaur et al. 2021; Suryanarayanan & Bijoy 2021; Choudhury 2023), along with the new records from the sub-Himalayan region of West Bengal are included in this map.

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## Butterfly diversity in Jitpur Simara Sub-metropolitan City, Bara District, Nepal: a preliminary checklist

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**Abstract:** Butterflies are among the most charismatic creatures, a well-studied group of insects, and significant bioindicators of the terrestrial ecosystem. Despite several studies conducted in different regions of Nepal, research in the tropical areas remains sparse. Hence, this study aimed to prepare a checklist of butterflies in the tropical region of the Bara District, Nepal. We conducted opportunistic butterfly surveys using the checklist method over a complete annual cycle (May 2023–April 2024) across three habitat types: forests, agricultural lands, and human settlements. A total of 85 butterfly species from 66 genera belonging to six families were recorded. Among them, the Nymphalidae family was the richest in terms of species ( $S = 41$ ), followed by Lycaenidae ( $S = 19$ ), Pieridae ( $S = 12$ ), Hesperidae and Papilionidae ( $S = 6$ ), and Riodinidae ( $S = 1$ ). Among the recorded species, 12 are classified as Least Concern, and 73 are Not Evaluated, according to the IUCN Red List. The record of such a vast number of species from a small area underscores the importance of tropical regions for butterfly habitats. This study highlights the biodiversity value of the central Terai and provides a baseline for future research and conservation planning.

**Keywords:** Bio-indicator, butterfly survey, habitats, insect, Lepidoptera, Nymphalidae, tropical region.

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**Author contributions:** ALISHA MULMI: conceptualization, investigation, methodology, data curation, resources, validation, writing– original draft, review, and editing. PRAKRITI CHATAUT: investigation, writing– original draft, review, and editing. MAHAMAD SAYAB MIYA: supervision, methodology, formal analysis, validation, visualization, writing– original draft, review, and editing.

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## INTRODUCTION

Butterflies are charismatic insects belonging to Lepidoptera, the second-largest insect order, which also comprises moths (Khan et al. 2023). Approximately 19,000 species of butterflies have been described worldwide (van Nieukerken et al. 2011). They play a crucial role in ecosystem functioning, especially in plant pollination and the food chain, serving as a food source for birds, amphibians, and reptiles (Tiple & Bhagwat 2023). Similarly, they function as important bio-indicators for terrestrial ecosystems because of their sensitivity to environmental changes such as habitat loss, fragmentation, disturbance, and climate change (Wilson & Maclean 2011; Syaripuddin et al. 2015; Ellis et al. 2019; Forsberg et al. 2020; Hill et al. 2021).

Butterflies are one of the most studied groups of insects worldwide (Pinkert et al. 2022). Tropical regions harbor the highest biodiversity, so the insect (Lepidoptera) diversity around the globe (Slade & Ong 2023), which is due to factors such as long evolutionary history, higher productivity, spatial heterogeneity, and increased ecological or niche specialization (Dyer et al. 2007; Ricklefs & Marquis 2012; Brown 2014). Although a large proportion of butterflies reside in the tropics, there is a notable deficiency in the study of tropical butterfly ecology and diversity compared to temperate butterflies, which hinders effective conservation efforts (Bonebrake et al. 2010; Pinkert et al. 2022). Local or regional studies can bridge the information gap and offer support for their conservation.

Nepal is home to 695 species of butterflies (Sajan et al. 2025) likely thriving due to its varied geography and climate, which ranges from tropical in the southern plains to tundra, and nival in the snow-covered north (Paudel et al. 2021). Studies on this group in Nepal are primarily focused on subtropical to temperate regions, such as (Miya et al. 2021; Subedi et al. 2021), with very little research in the tropical region (Khanal 2006, 2009; Suwal 2015; Tamang et al. 2019; Miya et al. 2025). Bara District, located in the tropical Terai region of Nepal, encompasses diverse habitats that potentially harbour a rich diversity of insects, including butterflies. To our knowledge, there is no published information on butterflies in the Bara District, which necessitates studies regarding this group in the region.

To document butterfly diversity, researchers employ various sampling techniques depending on study objectives and habitat characteristics. Those include the Pollard walk survey (Pollard 1977), point count (Henry et al. 2015), tapping & netting, mark-recapture (Kral et al.

2018), and, less frequently, the checklist method (Royer et al. 1998). Pollard walk and mark-recapture are popularly used for long-term monitoring of butterflies, while point count is suitable when other traditional methods are difficult to employ (Henry et al. 2015). In comparison, tapping & netting, as well as the checklist method, are more practical for the initial determination of the species (Royer et al. 1998). In the checklist method, a preliminary list of butterflies is prepared to mark the presence or absence, created based on the hypothetically complete list of regularly breeding resident species in the region (Royer et al. 1998).

Given the ecological importance of butterflies and the knowledge gap in Nepal's tropical lowlands, this study aimed to document butterfly diversity in Jitpur Simara Sub-metropolitan City of Bara District using a year-round checklist method. The resulting checklist serves as a foundational reference for future ecological studies and biodiversity monitoring in the region.

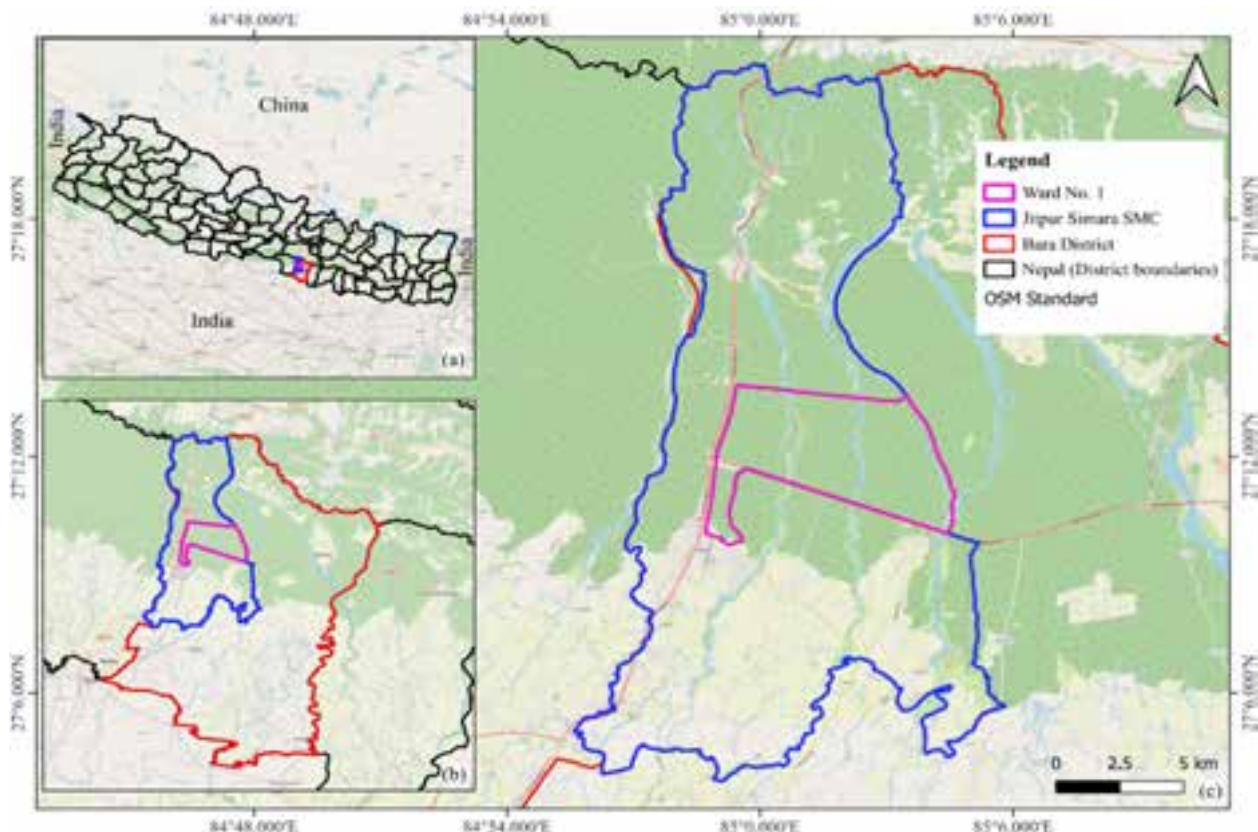
## MATERIALS AND METHODS

### Study area

The study was conducted in Jitpur Simara Sub-metropolitan City (JSSMC), Ward No. 1 Bara District, Nepal (Figure 1). The JSSMC covers an area of 311.67 km<sup>2</sup>, and Ward No. 1, covers an area of 42.75 km<sup>2</sup>. The sub-metropolitan city is situated between 26.850°–27.850° N, 84.850°–85.267° E, with an altitude ranging from 152–915 m. The study area encompasses three major land use types: forests, agricultural lands, and human settlements (JSSMC 2020). The forest of the Bara District is dominated by *Sal Shorea robusta*, along with the linear patches of riverine forests (Baral et al. 2022). The study area's fertile soil supports seasonal crops, primarily paddy in summer, and wheat & mustard in winter. The human settlement area includes home gardens with some seasonal flowers. The study area exhibits four distinct seasons: pre-monsoon (March–May), monsoon (June–September), post-monsoon (October–November), and winter (December–February), the four seasonal categories of Nepal. The region's average annual temperature is 24.5°C, with July being the hottest month at 36°C, and January the coldest at 8.6°C. Likewise, the average annual precipitation is 1,653 mm, with most of the rainfall occurring between mid-June and mid-July (>83%) (Baral et al. 2022).

### Data collection

The study was conducted from May 2023–April 2024, covering all seasons of the year, with sampling occurring



**Figure 1.** Study area map showing: a—District boundaries of Nepal | b—Bara District | c—Jitpur Simara Sub-metropolitan City with ward number one boundary.

twice a month. Using a checklist method, we documented butterflies to determine their presence in the region, serving as a baseline for future studies. Butterflies were opportunistically surveyed across three habitat types: forests, agricultural lands, and human settlements. Two observers explored the study area from 1000–1500 h on sunny days to maximize butterfly detection (Sajan & Sapkota 2024; Miya et al. 2025). This period is deemed appropriate for sampling butterflies because their activity is linked to the day's temperature (Wittman et al. 2017). We recorded the presence or absence of species, based on species expected to occur in central Terai, compiled from Smith (2011) and other regional records. The first author photographed all butterflies in natural settings using a Nikon Coolpix L340 camera to aid in identification. Initial field identifications of species were later verified through comparison with reference guides (Smith 2011; Smith et al. 2016), published literature (Tamang et al. 2019; Neupane & Miya 2021; Van der Poel & Smetacek 2022), internet sources, and consultation with expert lepidopterists when identification was uncertain.

### Data analysis

We followed Van der Poel & Smetacek (2022) for the scientific name of the butterfly species. The IUCN status of butterflies is based on the IUCN Red List of Threatened Species (IUCN 2025).

### RESULTS

A total of 85 butterfly species from 66 genera belonging to six families (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, and Riodinidae) were recorded. Nymphalidae was the richest family with 41 species, followed by Lycaenidae ( $S = 19$ ), Pieridae ( $S = 12$ ), Papilionidae ( $S = 6$ ), Hesperiidae ( $S = 6$ ), and Riodinidae ( $S = 1$ ) (Figure 2). The complete list of butterflies, including their families, scientific names, common names, and IUCN Red List status, is provided in Table 1. The butterfly images are shown in photo plates (Images 1–85). Among the total species, 12 are classified under the 'Least Concern' (LC) category of the Red List, while 73 were 'Not Evaluated' (NE) (Table 1).

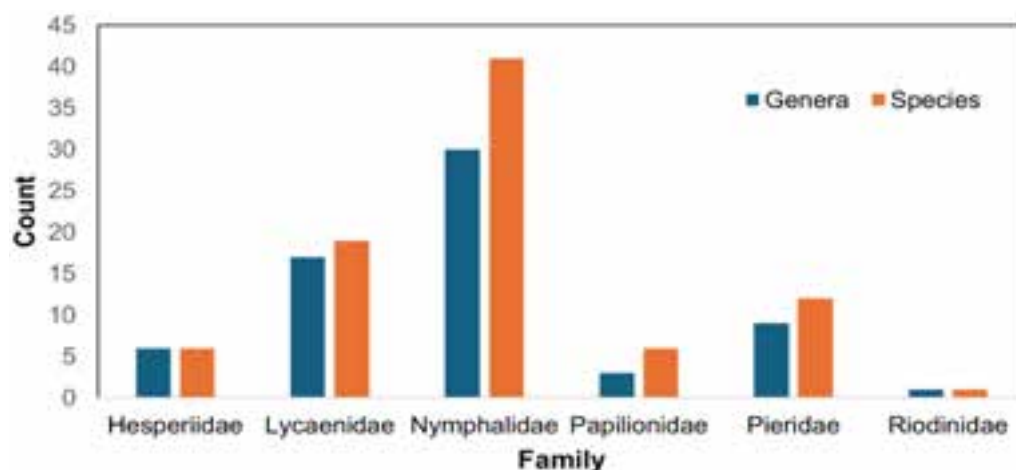


Figure 2. Family-wise distribution of butterfly genera and species in the study area.

## DISCUSSION

In this study, we documented the butterfly fauna from a small region of the tropical climate of Nepal. The recorded species account for 12.23% of all butterfly species found in Nepal, reflecting their rich diversity in the study area. The species number in our study area is quite comparable to studies from similar climatic regions of Nepal. While our species richness was lower than the 133 species reported from the eastern lowland (Tamang et al. 2019) and central lowland Nepal (Hetuuda;  $S = 115$ ) (Miya et al. 2025), it exceeded findings from other tropical sites such as Parsa National Park ( $S = 31$ ) (PNP 2018) and Surkhet District ( $S = 33$ ) (Oli et al. 2023).

Among the six families recorded, Nymphalidae was the richest family in terms of species, followed by Lycaenidae. Nymphalidae is one of the most diverse families of butterflies, with more than 6,152 species distributed worldwide (van Nieuwerkerken et al. 2011). Approximately, 34% of the butterflies found in Nepal belong to this group (Van der Poel & Smetacek 2022). Moreover, they are generalists with a large range of host plants and geographic ranges (Slove & Janz 2011; Nylin et al. 2014), which is also presumably favored by their large wingspan, active flight, and higher dispersal ability (Marini-Filho & Martins 2010; Freire et al. 2021). In our study, Nymphalidae represented 48.23% of the total species recorded, which aligns with global patterns, and reflects the ecological adaptability of this family to tropical environmental conditions.

Our findings are similar to studies from different locations of tropical regions of Nepal, such as Jhapa and Illam Districts (Tamang et al. 2019), Makwanpur District (Miya et al. 2025), Morang District (Subba & Tumbahangfe 2015), eastern Siwalik (Bhusal & Khanal 2009), and western

lowlands (Khanal 2009). Similar findings were also noted in the subtropical region of Nepal in the Tanahun, Syangja, Kaski, and Kathmandu Districts (Miya et al. 2021; Neupane & Miya 2021; Subedi et al. 2021). Our findings also align with the butterfly studies conducted in Bhutan (Singh & Chib 2015) and India (Raut & Pendharkar 2010).

Species such as *Danaus chrysippus*, *Graphium nomius*, and *Papilio polytes* were the most common, while species like *Abisara bifasciata*, and *Junonia orithya* were rarely observed. Likewise, the checklist includes several butterfly species that are considered relatively uncommon in Nepal, such as *Arhopala eumolphus*, *Caleta decidia*, *Euripus consimilis*, and *Odontoptilum angulata*. This underlines the need for more detailed ecological studies and better protection of these habitats, especially in the central Terai, a region often underrepresented in butterfly research. Moreover, the conservation status assessment revealed that 14.12% are categorized as 'Least Concern' by Red List, while the majority (85.88%) remain 'Not Evaluated' (NE), highlighting significant knowledge gaps in butterfly conservation assessments for the region.

## CONCLUSIONS

This study provided a preliminary checklist of butterflies from a small region of Jitpur Simara Sub-metropolitan City, central Terai, Nepal, comprising a total of 85 species belonging to six families. This finding highlights the significance of tropical regions of Nepal for butterfly diversity, emphasizing the need to conserve butterfly habitats in these areas. Nymphalidae was the richest family regarding the species, consistent with findings from similar or dissimilar climatic regions, denoting their adaptation to



Table 1. List of butterflies recorded in the study area with their family, scientific name, common name, and IUCN Red List status.

	Scientific name	Common name	IUCN Red List
	<b>Family Hesperidae</b>		
1	<i>Badamia exclamationis</i> (Fabricius, 1775)	Brown Awl	NE
2	<i>Matapa aria</i> (Moore, 1866)	Common Red Eye	NE
3	<i>Odontoptilum angulata</i> (C. Felder, 1862)	Chestnut Angle	NE
4	<i>Parnara guttatus</i> (Bremer & Grey, 1852)	Straight Swift	NE
5	<i>Sarangesa dasahara</i> (Moore, 1866)	Common Small Flat	NE
6	<i>Spialia galba</i> (Fabricius, 1793)	Indian Skipper	NE
	<b>Family Lycaenidae</b>		
7	<i>Acytolepis puspa</i> (Horsfield, 1828)	Common Hedge Blue	NE
8	<i>Arhopala eumolpus</i> (Cramer, 1780)	Green Oakblue	NE
9	<i>Caleta decidia</i> (Hewitson, 1876)	Angled Pierrot	LC
10	<i>Castalius rosomon</i> (Fabricius, 1775)	Common Pierrot	NE
11	<i>Catochrysops strabo</i> (Fabricius, 1793)	Forget-me-not	NE
12	<i>Chilades lajus</i> (Stoll, 1780)	Lime Blue	NE
13	<i>Chliaria othona</i> (Hewitson, 1865)	Orchid Tit	NE
14	<i>Curetis acuta</i> (Moore, 1877)	Angled Sunbeam	NE
15	<i>Jamides alecto</i> (C. Felder, 1860)	Metallic Cerulean	NE
16	<i>Jamides celeno</i> (Cramer, 1775)	Common Cerulean	NE
17	<i>Lampides boeticus</i> (Linnaeus, 1767)	Pea Blue	LC
18	<i>Leptotes plinius</i> (Fabricius, 1793)	Zebra Blue	NE
19	<i>Luthrodes pandava</i> (Horsfield, 1829)	Plains Cupid	NE
20	<i>Poritia hewitsoni</i> (Moore, 1866)	Common Gem	NE
21	<i>Prosotas nora</i> (C. Felder, 1860)	Common Line Blue	NE
22	<i>Pseudozizeeria maha</i> (Kollar, 1844)	Pale Grass Blue	NE
23	<i>Rapala manea</i> (Hewitson, 1863)	Slate Flash	NE
24	<i>Rapala varuna</i> (Horsfield, 1829)	Indigo Flash	NE
25	<i>Surendra quercetorum</i> (Moore, 1858)	Common Acacia Blue	NE
	<b>Family Nymphalidae</b>		
26	<i>Acraea violae</i> (Fabricius, 1793)	Tawny Coster	NE
27	<i>Aglais caschmirensis</i> (Kollar, 1844)	Indian Tortoiseshell	NE
28	<i>Ariadne merione</i> (Cramer, 1777)	Common Castor	NE
29	<i>Athyma nefte</i> (Cramer, 1780)	Color Sergeant	NE
30	<i>Athyma perius</i> (Linnaeus, 1758)	Common Sergeant	NE
31	<i>Charaxes bernardus</i> (Fabricius, 1793)	Tawny Rajah	NE
32	<i>Cupha erymanthis</i> (Drury, 1773)	Rustic	NE
33	<i>Cynitia lepidea</i> (Butler, 1868)	Grey Count	NE
34	<i>Cyrestis thyodamas</i> (Boisduval, 1846)	Common Map	NE
35	<i>Danaus chrysippus</i> (Linnaeus, 1758)	Plain Tiger	LC
36	<i>Danaus genutia</i> (Cramer, 1779)	Common Tiger	NE
37	<i>Discophora sondaica</i> (Boisduval, 1836)	Common Duffer	NE
38	<i>Elymnias hypermnestra</i> (Linnaeus, 1763)	Common Palmfly	NE
39	<i>Euploea core</i> (Cramer, 1780)	Common Indian Crow	LC
40	<i>Euripus consimilis</i> (Westwood, 1850)	Painted Courtesan	NE
41	<i>Euthalia aconthea</i> (Cramer, 1777)	Common Baron	NE
42	<i>Hypolimnas bolina</i> (Linnaeus, 1758)	Great Eggfly	NE
43	<i>Junonia almana</i> (Linnaeus, 1758)	Peacock Pansy	LC
44	<i>Junonia atlites</i> (Linnaeus, 1763)	Grey Pansy	NE
45	<i>Junonia iphita</i> (Cramer, 1779)	Chocolate Pansy	NE
46	<i>Junonia lemonias</i> (Linnaeus, 1758)	Lemon Pansy	NE
47	<i>Junonia orithya</i> (Linnaeus, 1758)	Blue Pansy	LC
48	<i>Kallima inachus</i> (Boisduval, 1846)	Orange Oakleaf	NE
49	<i>Kaniska canace</i> (Linnaeus, 1763)	Blue Admiral	NE
50	<i>Lethe europa</i> (Fabricius, 1775)	Bamboo Treebrown	NE
51	<i>Melanitis leda</i> (Linnaeus, 1758)	Common Evening Brown	LC
52	<i>Melanitis phedima</i> (Cramer, 1780)	Dark Evening Brown	NE
53	<i>Moduza procris</i> (Cramer, 1777)	Commander	NE
54	<i>Mycalesis mineus</i> (Linnaeus, 1758)	Dark-brand Bushbrown	NE
55	<i>Mycalesis perseus</i> (Fabricius, 1775)	Common Bushbrown	NE
56	<i>Mycalesis visala</i> (Moore, 1858)	Long-brand Bushbrown	NE
57	<i>Neptis hylas</i> (Linnaeus, 1758)	Common Sailer	NE
58	<i>Neptis soma</i> (Moore, 1858)	Creamy Sailer	NE
59	<i>Orsotriaena medus</i> (Fabricius, 1775)	Jungle Brown	NE
60	<i>Pantoporia hordonia</i> (Stoll, 1790)	Common Lascar	NE
61	<i>Parantica aglea</i> (Stoll, 1782)	Glassy Tiger	NE
62	<i>Phalanta phalantha</i> (Drury, 1773)	Common Leopard	LC
63	<i>Vagrans egista</i> (Cramer, 1780)	Vagrant	NE
64	<i>Vanessa indica</i> (Herbst, 1794)	Indian Red Admiral	NE
65	<i>Ypthima baldus</i> (Fabricius, 1775)	Common Five-ring	NE
66	<i>Ypthima huebneri</i> (Kirby, 1871)	Common Four-ring	NE
	<b>Family Papilionidae</b>		
67	<i>Graphium doson</i> (C. & R. Felder, 1864)	Common Jay	NE
68	<i>Graphium nomius</i> (Esper, 1799)	Spot Swordtail	NE
69	<i>Pachliopta aristolochiae</i> (Fabricius, 1775)	Common Rose	LC
70	<i>Papilio clytia</i> (Linnaeus, 1758)	Common Mime	NE
71	<i>Papilio demoleus</i> (Linnaeus, 1758)	Lime Swallowtail	NE
72	<i>Papilio polytes</i> (Linnaeus, 1758)	Common Mormon	NE

	Scientific name	Common name	IUCN Red List
	<b>Family Pieridae</b>		
73	<i>Appias lalage</i> (Doubleday, 1842)	Spot Puffin	NE
74	<i>Appias libythea</i> (Fabricius, 1775)	Striped Albatross	NE
75	<i>Belenois aurota</i> (Fabricius, 1793)	Pioneer	LC
76	<i>Catopsilia pomona pomona</i> (Fabricius, 1775)	Lemon Emigrant	NE
77	<i>Delias hyparete</i> (Linnaeus, 1758)	Painted Jezebel	NE
78	<i>Delias pasithoe</i> (Linnaeus, 1767)	Red-base Jezebel	NE
79	<i>Eurema blanda</i> (Boisduval, 1836)	Three-spot Grass Yellow	NE
80	<i>Eurema hecabe</i> (Linnaeus, 1758)	Common Grass Yellow	LC
81	<i>Leptosia nina</i> (Fabricius, 1793)	Psyche	NE
82	<i>Pareronia hippia</i> (Fabricius, 1787)	Indian Wanderer	NE
83	<i>Pieris canidia</i> (Linnaeus, 1768)	Indian Cabbage White	NE
84	<i>Pontia daplidice</i> (Linnaeus, 1758)	Bath White	LC
	<b>Family Riodinidae</b>		
85	<i>Abisara bifasciata</i> (Moore, 1877)	Double-banded Judy	NE

a wide range of habitats, and climate. This checklist will serve as an essential reference for future research in the region. We recommend: (1) expanding sampling efforts to nearby areas to capture landscape-level patterns in butterfly diversity, (2) incorporating standardized measures of abundance in future research, and (3) examining host plant relationships to better understand butterfly habitat requirements and inform conservation planning.

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Image 1–20. 1—Brown Awl | 2—Common Red-eye | 3—Chestnut Angle | 4—Straight Swift | 5—Common Small Flat | 6—Indian Skipper | 7—Common Hedge Blue | 8—Green Oakblue | 9—Angled Pierrot | 10—Common Pierrot | 11—Forget-me-not | 12—Lime Blue | 13—Orchid Tit | 14—Angled Sunbeam | 15—Metallic Cerulean | 16—Common Cerulean | 17—Peablu | 18—Zebra Blue | 19—Plains Cupid | 20—Common Gem.  
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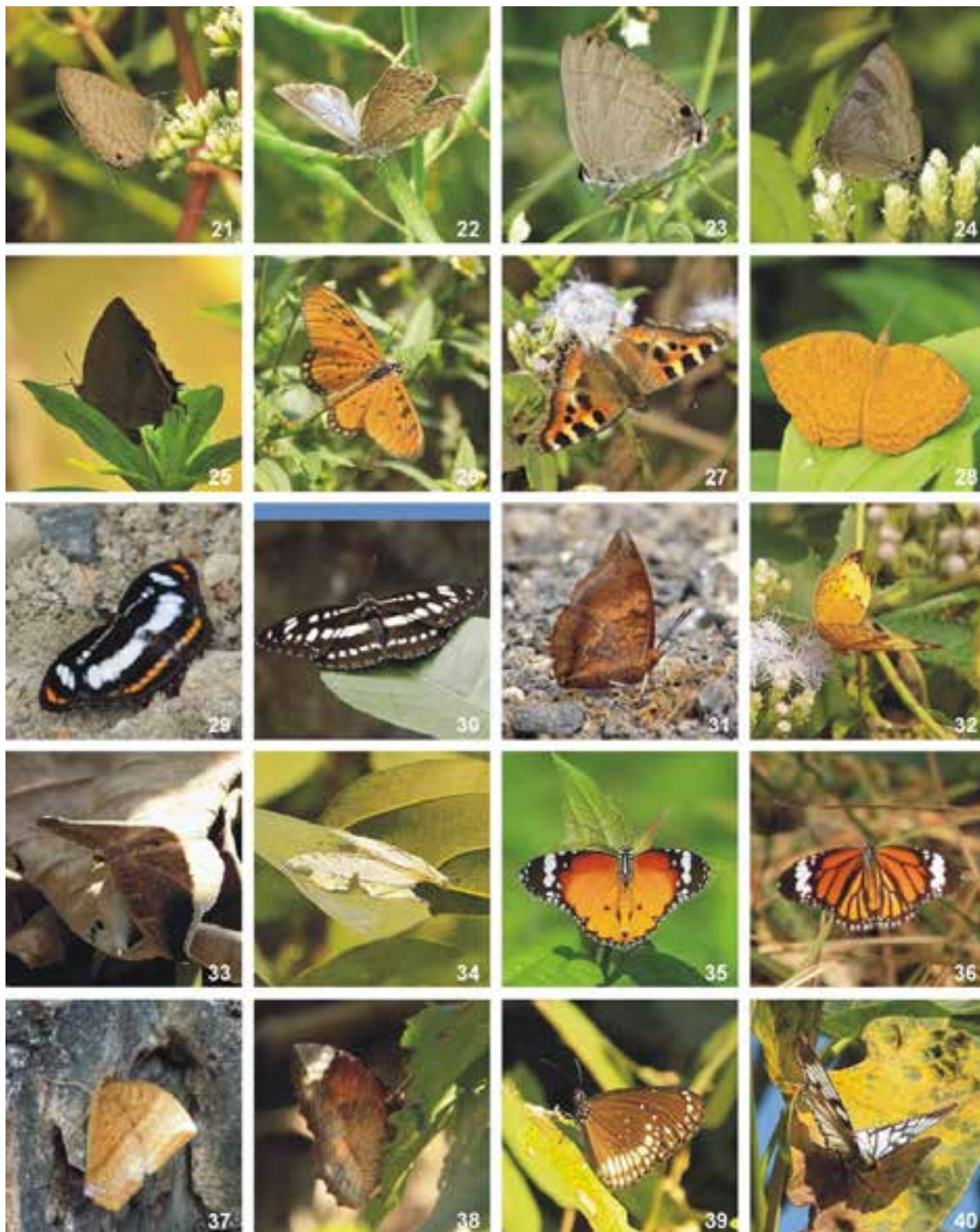


Image 21–40. 21—Common Lineblue | 22—Pale Grass Blue | 23—Slate Flash | 24—Indigo Flash | 25—Common Acacia Blue | 26—Tawny Coster | 27—Indian Tortoiseshell | 28—Common Castor | 29—Color Sergeant | 30—Common Sergeant | 31—Tawny Rajah | 32—Rustic | 33—Grey Count | 34—Common Map | 35—Plain Tiger | 36—Common Tiger | 37—Common Duffer | 38—Common Palmfly | 39—Common Indian Crow | 40—Painted Courtesan. © Alisha Mulmi.





Image 41–60. 41—Common Baron | 42—Great Eggfly | 43—Peacock Pansy | 44—Grey Pansy | 45—Chocolate Pansy | 46—Lemon Pansy | 47—Blue Pansy | 48—Orange Oakleaf | 49—Blue Admiral | 50—Bamboo Treebrown | 51—Common Evening Brown | 52—Dark Evening Brown | 53—Commander | 54—Dark-brand Bushbrown | 55—Common Bushbrown | 56—Long-brand Bushbrown | 57—Common Sailer | 58—Creamy Sailer | 59—Jungle Brown | 60—Common Lascar. © Alisha Mulmi.



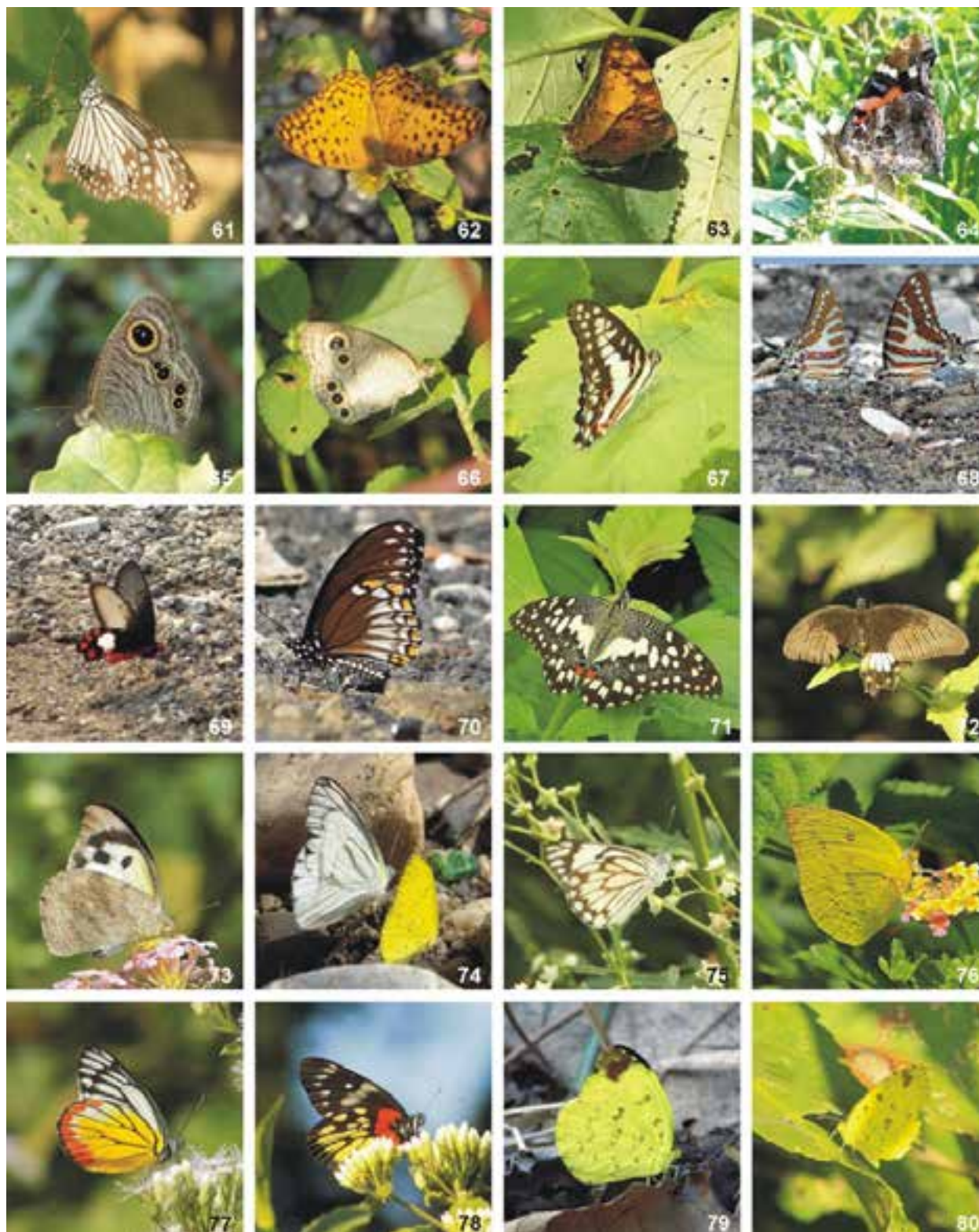


Image 61–80. 61—Glassy Tiger | 62—Common Leopard | 63—Vagrant | 64—Indian Red Admiral | 65—Common Five-ring | 66—Common Four-ring | 67—Common Jay | 68—Spot Swordtail | 69—Common Rose | 70—Common Mime | 71—Lime Swallowtail | 72—Common Mormon | 73—Spot Puffin | 74—Striped Albatross | 75—Pioneer | 76—Lemon Emigrant | 77—Painted Jezebel | 78—Red-base Jezebel | 79—Three-spot Grass Yellow | 80—Common Grass Yellow. © Alisha Mulmi.



Image 81–85. 81—Psyche | 82—Indian Wanderer | 83—Indian Cabbage White | 84—Bath White | 85—Double-banded Judy. © Alisha Mulmi.

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## First documented case of flunixin residue in a Himalayan Vulture *Gyps himalayensis* Hume, 1869 (Aves: Accipitriformes: Accipitridae) in India: conservation and veterinary implications

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**Abstract:** Non-steroidal anti-inflammatory drugs (NSAID), particularly diclofenac, have been widely identified as a major cause of vulture deaths across Asia, leading to significant population declines. The impact of other veterinary NSAIDs, including flunixin, remains poorly documented. This study reports the first confirmed case of flunixin residue in a wild Himalayan Vulture *Gyps himalayensis* (Hume, 1869) in India. A juvenile vulture was rescued from Jaldapara National Park, West Bengal, and transferred to the Buxa Vulture Conservation Breeding Centre & Aviary at Rajabhatkhawa (West Bengal) for treatment and rehabilitation. Despite medical intervention, the bird died. Necropsy revealed extensive visceral gout, indicative of renal failure. Toxicological analysis confirmed the presence of flunixin residues in the tissues (stomach contents showed the highest level of flunixin with 903.9 ng/g, followed by the kidney with 214.3 ng/g, and the liver with 67.6 ng/g). This report highlights the requirement for careful monitoring of veterinary NSAID usage in India by trained professionals for the conservation of endangered vulture populations.

**Keywords:** Buxa Vulture Conservation Breeding Centre and Aviary, non-steroidal anti-inflammatory drugs (NSAIDs), renal failure, veterinary pharmaceuticals, visceral gout, vulture conservation, West Bengal, wildlife toxicology.

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## INTRODUCTION

Vultures play a crucial ecological role as obligate scavengers, preventing the spread of diseases by efficiently disposing of animal carcasses. The Himalayan Vulture *Gyps himalayensis* (Hume, 1869) is a resident of the mountains of central Asia, the Himalaya, southern & eastern Tibet, and China (Ali & Ripley 1978). While breeding adults remain in their nesting territories for most of the year, juveniles, and sub-adults migrate to the plains of southern and southeastern Asia during the winter (Naoroji 2006; Rasmussen & Anderton 2012). This seasonal movement is primarily driven by reduced food availability at the high-altitude regions due to whiteout, leading to lesser chance of success in securing food in competition with dominant adults (BirdLife International 2024), and the need to conserve energy in harsh winter conditions. Additionally, young vultures, not yet engaged in breeding, exhibit dispersal behaviour as part of their survival strategy and future range expansion (Yong & Kasorndorkbua 2008). With the return of favourable conditions, they migrate back to their breeding grounds in summer.

As juveniles and sub-adults migrate to the plains of southern and southeastern Asia, they become exposed to anthropogenic threats, including veterinary drug residue in livestock carcasses, the primary food source. In contrast, breeding adults that remain in the high-altitude regions of the Himalaya are relatively shielded from this threat, as NSAID-laced carcasses are less common in these remote and sparsely populated regions. The indiscriminate use of NSAIDs in veterinary medicine has had catastrophic consequences for vulture populations worldwide. Diclofenac, in particular, has been linked to the catastrophic decline of several *Gyps* species in southern Asia (Oaks et al. 2004; Pain et al. 2008). Although diclofenac, along with three other NSAIDs (aceclofenac, ketoprofen, and nimesulide), are banned for veterinary use in India (Ministry of Health and Family Welfare, Government of India 2008, 2023, 2024), there are reports (Cuthbert et al. 2011; Down To Earth 2022) of continued illegal use of these NSAIDs meant for human use for veterinary purposes.

Flunixin, a potent NSAID, is commonly administered to livestock for pain management, and inflammation control. Flunixin, similar to diclofenac, aceclofenac, ketoprofen, and nimesulide, is suspected to induce renal failure in *Gyps* vultures, leading to fatal visceral gout (Zorrilla et al. 2014). Although flunixin is legally approved for veterinary use in India, its toxicity to vultures is suspected, highlighting the need for experimental

testing of the drug's toxicity in vultures (Galligan et al. 2020). Until now, there has been no documented case of flunixin poisoning in Himalayan Vultures in India. The present case reports the necropsy and toxicological findings of the first documented instance of flunixin-associated mortality in a wild Himalayan Vulture in India, highlighting a significant conservation concern for this species, and an urgent need for comprehensive monitoring of the use of this NSAID in veterinary practice.

## MATERIALS AND METHODS

### Case details and clinical presentation

The Buxa Vulture Conservation Breeding Centre & Aviary, situated at Rajabhatkhawa of Alipurduar District of West Bengal in India, serves as a conservation breeding centre for three Critically Endangered *Gyps* species of vultures, including White-rumped Vulture *Gyps bengalensis*, Long-billed Vulture *Gyps indicus*, and Slender-billed Vulture *Gyps tenuirostris*. Additionally, the centre functions as a rescue, and rehabilitation facility for vultures in the region. Since its establishment in 2006, the centre has received 95 rescued Himalayan vultures, and successfully released 80 individuals back in their natural habitat after treatment (Chakraborty et al. 2024). On 19 December 2024, a juvenile Himalayan Vulture *Gyps himalayensis* was rescued in a weakened state in Jaldapara National Park, West Bengal. The bird was promptly transported to the centre for treatment and rehabilitation. The vulture exhibited symptoms of lethargy, dehydration, and anorexia, and was unable to fly. It was identified as a juvenile Himalayan Vulture based on its overall dark plumage (except for a whitish head), distinctly darker than juvenile Eurasian Griffons *Gyps fulvus*, and lacking their rufous tinge. The bird had a long, pointed buffy-brown ruff with pale shaft streaks, dark brown upperparts, and conspicuously streaked buff-white scapulars, and upper wing coverts. Its flight feathers and tail were blackish-brown, with a dark brown crop patch, and the underparts were heavily streaked buffy-white especially on the body. These plumage features are consistent with juvenile *Gyps himalayensis* as described by Naoroji (2006).

Symptomatic treatment was initiated to stabilise the bird's condition. The treatment regime included:

- 40 ml of Dextrose Normal Saline (DNS) intravenously (IV) for rehydration and electrolyte replenishment.
- 0.5 ml of Atropine Sulphate intravenously (IV) to alleviate respiratory distress and stabilise cardiac function.

- 100 mg of Intacef Tazo intravenously (IV) a combination antibiotic (Ceftriaxone and Tazobactam) to treat suspected bacterial infections.

- 1 ml of Tribivet intravenously (IV) is used as a supportive multivitamin injection to treat vitamin B-complex deficiencies and boost recovery of the vulture.

Condition of the bird progressively deteriorated despite administration of supportive care. It succumbed to its illness on 22 December 2024. A necropsy was subsequently conducted to determine the underlying cause of death.

### Necropsy examination

A comprehensive necropsy examination was performed. The major findings of the necropsy examination included:

- Extensive deposition of uric acid crystals on visceral organs (visceral gout), indicating renal failure (Image 1).

- No evidence of external trauma or underlying diseases.

- An empty gastrointestinal tract, suggesting prolonged anorexia.

Tissue samples were collected for further toxicological analysis to determine the underlying cause of death.

### Toxicological analysis

The tissue samples (liver, kidney, and stomach contents) from the carcass were collected, labelled, and frozen immediately for further analysis. The samples were then transported to the Salim Ali Centre for Ornithology and Natural History (SACON) at Coimbatore, India, for ecotoxicological screening. Liquid chromatography-mass spectrometry (LC-MS/MS) was used to detect any residue of NSAIDs. The samples were screened for residues of 14 NSAIDs, including - diclofenac, aceclofenac, ketoprofen, ibuprofen, naproxen, paracetamol, mefenamic acid, meloxicam, nimesulide, piroxicam, tolfenamic acid, indomethacin, flunixin, and carprofen. This comprehensive screening aimed to detect any potential NSAID contamination that may have contributed to the vulture's death.

### RESULTS

Toxicological analysis revealed presence of notable levels of flunixin in the samples. Residues of other targeted NSAIDs were below detection limit in all the samples. The findings are summarised in Table 1.

Among the tissues analyzed, stomach contents showed the highest level of flunixin (903.9 ng/g), followed



Image 1. Presence of visceral gout characterised by extensive deposition of uric acid crystals on the liver of the rescued Himalayan Vulture's carcass.

by the kidney (214.3 ng/g), and liver (67.6 ng/g), which had the lowest concentration. The presence of uric acid crystal deposition in the viscera (Image 1), indicative of visceral gout, was also noted during the necropsy.

Although pharmacokinetics of flunixin in *Gyps* vultures are poorly documented, Ramzan et al. (2012) demonstrated flunixin meglumine toxicity in broiler chickens, with dose-dependent mortality (20–60%) and associated increases in serum uric acid, and creatinine. The study indicated that flunixin meglumine caused similar toxicity in birds as diclofenac. Previous studies have linked diclofenac residues ( $0.051\text{--}0.643^{-1}$  µg/g in kidneys) in *Gyps* vultures to renal failure and visceral gout (Oaks et al. 2004). During post-mortem examination, clear visceral gout, as extensive deposition of uric acid, was observed in the vulture. Further, toxicological analysis of tissue samples for 14 NSAIDs (table 1), only flunixin was detected at significant concentrations in the liver, and kidney. Therefore, it can be inferred that flunixin was one of the reasons for the death of the vulture in the present case.

Diclofenac, a non-steroidal anti-inflammatory drug inhibits cyclooxygenase (COX) enzymes. In vultures, COX inhibition impairs renal prostaglandin synthesis, reducing glomerular filtration, and uric acid excretion.

**Table 1. Concentration of flunixin found in the tissues of the Himalayan Vulture.**

NSAIDs concentration in tissue samples				
Unit = ng/g				
	NSAIDs screened	Tissue samples		
		Liver	Kidney	Stomach content
1	Diclofenac	BDL	BDL	BDL
2	Aceclofenac	BDL	BDL	BDL
3	Ketoprofen	BDL	BDL	BDL
4	Ibuprofen	BDL	BDL	BDL
5	Naproxen	BDL	BDL	BDL
6	Paracetamol	BDL	BDL	BDL
7	Mefenamic acid	BDL	BDL	BDL
8	Meloxicam	BDL	BDL	BDL
9	Nimesulide	BDL	BDL	BDL
10	Piroxicam	BDL	BDL	BDL
11	Tolfenamic acid	BDL	BDL	BDL
12	Indomethocin	BDL	BDL	BDL
13	<b>Flunixin</b>	<b>67.6</b>	<b>214.3</b>	<b>903.9</b>
14	Carprofen	BDL	BDL	BDL

BDL—Below detection limit | Detection limit—20 ng/g



**Image 2. Presence of uric acid crystals on the interior wall of the trachea of the Himalayan Vulture's carcass.**



This leads to hyperuricemia, urate crystal deposition, and visceral gout (Oaks et al. 2004; Naidoo & Swan 2009). Oaks et al. (2004) reported the range of diclofenac residues in the kidneys of vultures that died of visceral gout from 0.051–0.643 µg/g. Flunixin is also a non-steroidal anti-inflammatory drug, and in the present case the concentration of flunixin has been detected 214.3 ng/g (equivalent to 0.2143 µg/g) in the kidney of the affected Himalayan Vulture. However, there is a lack of information on whether flunixin, like diclofenac, inhibits COX enzymes in vultures.

The detection of flunixin residues in the tissue samples, confirmation by the testing agency about the probable cause of death, and the observed symptoms of gout, lead us to conclude that flunixin poisoning was the most probable cause of death in this Himalayan Vulture.

## DISCUSSION

Visceral gout is characterised by the extensive deposition of uric acid crystals on visceral organs, leading to inflammation, tissue damage, and organ dysfunction. In this present case, visceral gout was found on the liver surface (Image 1). Notably, uric acid crystals were also present on the inner wall of the trachea (Image 2), indicating a severe case that compromised the respiratory system. Uric acid crystals in the trachea can cause inflammation, blockage of the airways, and respiratory distress. The presence of visceral gout, coupled with uric acid crystals in the trachea, suggests that the vulture's death was likely caused by complications arising from kidney disease, and visceral gout developed on the liver as a consequence of flunixin poisoning. Cuthbert et al. (2007), also reported that flunixin has the potential to cause renal damage in birds. Therefore, in this case also, flunixin could be a precipitating factor. However, high flunixin residue in the stomach indicates a recent exposure. The Himalayan Vulture is currently listed as Near Threatened on the International Union for Conservation of Nature (IUCN) Red List. Their global population is estimated to be between 66,000–334,000 mature individuals (BirdLife International 2021), and is protected in India under Schedule-I of the Wild Life (Protection) Amendment Act, 2022 (Government of India 2022). This case underscores the pressing need to limit the veterinary use of flunixin along with other NSAIDs.

This study provides the first confirmed evidence of flunixin residue in a Himalayan Vulture *Gyps himalayensis* in India. The study could not identify the

source of exposure to flunixin, which could have been anywhere within its former range.

## CONCLUSION

The Himalayan Vulture's ecological importance cannot be overstated, and the drastic decline in its population is alarming. As a scavenger, it plays a crucial role in maintaining the health and balance of ecosystems by disposing of dead animals, and preventing the spread of diseases. This first reported case of flunixin residue in a Himalayan Vulture in India highlights the urgent need for monitoring of flunixin usage in veterinary use. Further research on flunixin toxicity in scavenging raptors is required to establish safe veterinary drug policies and to ensure a steady supply of safe food sources, such as carcasses, to ensure their survival.

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## INTRODUCTION

Ecological niche modelling (ENM), also known as species distribution modelling (SDM) or habitat suitability modelling, is a computational approach used in ecology, conservation biology, and biogeography to predict the potential geographic distribution or habitat suitability of a species or ecological niche under various environmental conditions. There is a rising opinion that both ENM and SDM vary in certain aspects (Melo-Merino et al. 2020); yet these concepts have been used extensively in the disciplines of ecology, biogeography, and conservation to forecast how a changing climate may affect species. ENM/SDM has also been used to manage invasive species, plan protected area management, and estimate the effects of climate change in evolutionary biology and ecology. The greater accessibility of digital data, user-friendly software, and instructional resources, as well as the growing interest & focus on these techniques, have supported the development of this field. Recent developments in data analysis and information technology have provided an edge to ecologists and conservationists to use this computational approach to a greater extent.

The origins of this ecological approach can be found in earlier works that connected biological patterns with environmental changes like geographic gradients. Also, the studies that showed how individuals, rather than groups, responded differently to environmental factors, inspired the creation of methods to represent individuals as species. In order to provide a picture of possible distributions of species at the landscape level, ENM/SDM infers correlations between species distributions (as records of occurrence or abundance), and environmental characteristics at selected study sites. These models have also been referred to in the literature as habitat models, climate envelopes, range maps, ecological niche models (ENMs), resource selection functions (RSFs), correlative models, and spatial models.

The occurrence data on species, environmental covariates, and a modelling technique are three important components that can influence the SDM outputs. Typically, the modelling is done at two levels— a) single model algorithm technique and b) ensemble technique. The foundation of ensemble modelling is the idea that each model algorithm exhibits some meaningful “signal” regarding relationships in the real world, as well as some noise brought on by the data and the limitations of the algorithm. As a result, ensemble modelling uses many models to separate the signal from the noise more effectively. Therefore, the choice

of algorithm matters and the algorithms are categorised (Rathore & Sharma 2023) as

- i) Regression Models - Generalized Linear Models (GLMs), Generalized Additive Models (GAMs), Multivariate Adaptive Regression Splines (MARS)
- ii) Classification Models - Flexible Discriminant Analysis (FDA) and Classification and Regression Tree (CART)
- iii) Complex Models - Random Forest (RF), The Genetic Algorithm for Rule-set Production (GARP), The Maximum Entropy (MaxENT) method, and Artificial Neural Network (ANN).

Among these algorithms, one stands as a popular choice for SDM modelling, i.e., MaxENT. It is an algorithm for general-purpose machine learning that calculates target probabilities by identifying the distribution that is most entropic (i.e., uniform) while adhering to the requirement that each environmental variable's expected value match its empirical average (i.e., the average value of the variable at a sample of points from species distribution). After the first publication on MaxENT by Phillips et al. (2006), who introduced the MaxENT application as a tool/software based on the maximum entropy method for SDM with presence-only data; there are several publications that have used MaxENT. In this paper, we have made efforts to explore and comprehend the preference and usage trend of SDM in the Indian context with the following questions: i) What is the extent, i.e., number of publications based on MaxENT in India? ii) What are the different aspects where MaxENT has been used and the lessons learnt from it? The extent of publications based on MaxENT in India will indicate the subject area where it was used, while also providing an overall perspective, and insights for using MaxENT in upcoming works.

## MATERIALS AND METHODS

The literature corpus was collected from the Web of Science (WoS) database. It was selected owing to its authentic and comprehensive coverage. A keyword search TC= “MaxEnt” or “MaxENT” was used to collect the data from the Web of Science, for the period between 2000–2023 (accessed on 01.x.2023). Considering the broader nature of research publications from different disciplines, it was decided to use a string keyword search. Further, studies involving topic-specific searches have recounted the increased specificity and recovery of information (Aleixandre et al. 2015; Sweileh et al. 2016). The search in WoS yielded 5232 publications from which

articles were screened based on the countries, i.e., INDIA – 214 manuscripts were sorted, and 210 manuscript metadata were used in the analysis (Nakagawa et al. 2019). The metadata was downloaded in the BibTeX format and analyzed in R version 4.0.1 along with Rstudio Version 1.3.959 using the bibliometrix R-package (<http://www.bibliometrix.org>) (Aria & Cuccurullo 2017). It provides a range of tools for importing, cleaning, and organizing bibliographic data, and for conducting various types of bibliometric analysis. The biblioshiny tool based on the bibliometrix R-package was used in the analysis.

## RESULTS AND DISCUSSION

Across the timespan, there were 210 scientific publications published in 103 journals with an annual growth rate of 27.81% (Figure 1) and the publications peaked in 2013. About 778 authors contributed with an average of 4.79 authors per document and 32.34 % international collaboration for publishing. There were only three authors who published single-authored scientific documents, which indirectly indicated the level of collaboration among authors. Almost all states were covered with at least 5–10 publications, with hotspots of the studies being Karnataka, Kerala, Tamil Nadu (Western & Eastern Ghats), Uttarakhand, and Jammu & Kashmir (Himalayan region). The least studied will be the western part of India (arid & semi-arid regions). With regard to the spatial scale, the study area in many of the studies has not been confined to selected regions within the state but even pan-India level studies have also been reported. For instance, the invasion potential of the mango fruit borer (Choudhary et al. 2019), prediction of *Boswellia serrata* in the year 2050 for two climate change scenarios - IPSL-CM5A-LR and NIMR-HADGEM2-AO (Rajpoot et al. 2020), and potential area for cultivation of *Melia dubia* (Sundaram et al. 2023) were studied at country level; whereas predicting the potential distribution of *Justicia adhatoda* was carried out at district level (Yang et al. 2013).

It is pertinent to point out that apart from the java based MaxENT software, some of the studies have used MaxEnt tool in other formats like a plugin in the QGIS, an interface based on GRASS GIS, and numerous R packages like dismo, ENMeval, SDMPlay, rmaxent, MIAMaxent, kuenm, ENiRG, and maxlike, which clearly indicate the dominance of MaxENT algorithm. There are a good number of scholarly publications that might not be captured in WoS. The usage of a single database (the WoS) and exclusion of articles in other languages may

have hampered the accessibility of all research papers. Yet, the wider coverage in Web of Science reduces the “indexer effect”, thus making the findings significant (Orimoloye & Ololade 2021). Figure 2 shows the number of publications on MaxENT from India indexed in different databases. As evident, the total number of publications using MaxENT from India was only 4% of the global output as recorded in the WoS. However, we can presume that there will be more publications related to Niche Modelling or Species Distribution Modelling using the MaxENT tool in the future. Lotka's law is used to assess productivity levels by examining the relationship between several authors and the number of articles published. The constant and beta coefficients of Lotka's law were 0.61 and 2.77, respectively. The goodness of fit test (Komogorov-Smirnoff) value was 0.91 and the p-value was 0.541 (Figure 3). This implies that Lotka's law is valid and thus there is a good possibility for an increase in the number of publications in the future (Rathinam et al. 2022).

To understand the changes in the MaxENT-based studies based on institutes, keywords, and journals over the last two decades, the three-field plot from bibliometrix tools was used (Figure 4). The left side indicates the top 20 institutes in India; the right side indicates the name of journals and the middle field indicates the keywords. Figure 4 provides a bird's eye view of the interlinkage in the published studies between 2000 and 2023. It reveals that ecological informatics, ecological engineering, and current science are some of the journals where dedicated research on ENM/SDM based on MaxENT in India is being published. The central segment indicates sides the keywords from the published papers; it is clear that the Western Ghats and Himalayas are two significant regions where SDM-based studies are being carried out.

To understand the different aspects where MaxENT has been used, the publications were sorted out on the thematic study subjects (Figure 5). SDM modelling was widely used to study trees, herbs, and mammals in the Indian context. More particularly, the MaxENT tool has been also used for some landscape-level studies. For instance, Pandey et al. (2020) assessed the landslide susceptibility along river models along the Tipari to Ghuttu highway corridors in the Garhwal Himalaya by coupling MaxENT output with DEM, NDVI, Slope, Aspect, and drainage density datasets. Unlike SDM models for flora or fauna where the presence locations of the species of interest are deployed along with environmental parameters such as temperature and rainfall, the studies on landscape (Pandey et al. 2020),



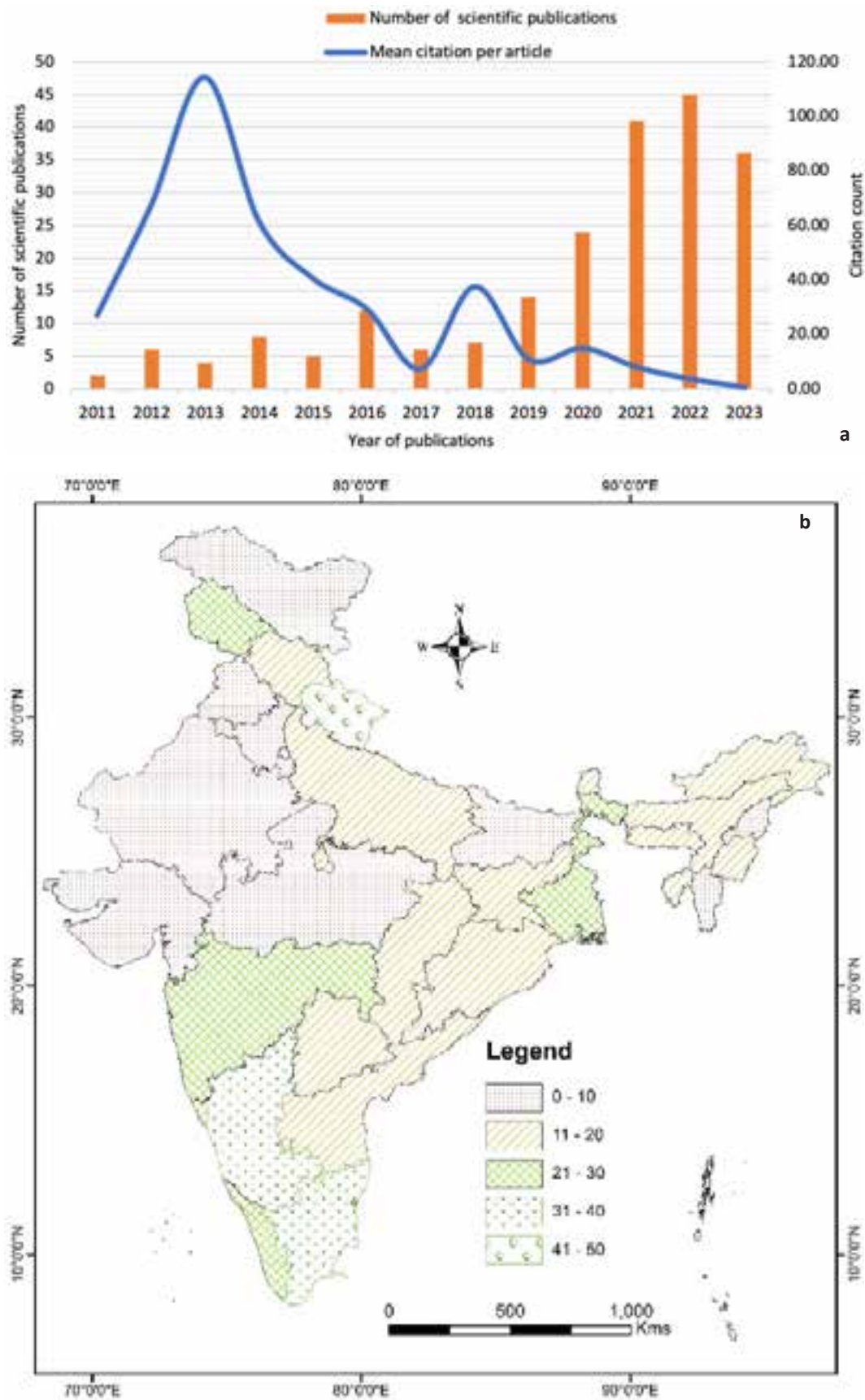


Figure 1. Scientific publications based on MaxENT over the years: a—across timescale | b—across the country.

forest fire prediction (Banerjee 2021), and transition in lagoon ecosystem (Santhanam et al. 2022) are some of the new methodologies by tuning the MaxENT tool with additional remote sensing & GIS datasets to meet the desired objectives. It is pertinent to point out that all of the studies were carried out after 2020 which indicates that new horizons using MaxENT are being explored and there will be more publications, as indicated by Lotka's law. All studies focus on the fundamental principle, i.e., the MaxENT model/tool is based on theory of statistical mechanics, and information concept which gives an approximation of a likelihood phenomenon based on known events.

Recently, Rathore & Sharma (2023) reported that SDM can be utilised for forecasting, restoration planning, climate change effect assessment, critical habitat identification, fishing zone identification, pollinator range prediction, disease spread prediction, fire regime, corridor identification, conservation status prediction, conservation planning, habitat range shift prediction, protected area management, hotspot identification, and Invasive species range identification. The recent studies have attempted to diversify the MaxENT analysis coupled with other applications and softwares (He et al. 2024; Asadollahzadeh & Torkaman 2025; Mao et al. 2025; Wang et al. 2025). More specifically, the category 'Others' mentioned in Figure 5 which are based on the application of the MaxENT tool for gully erosion and land subsistence susceptibility mapping, predicting the expansion of dengue vectors, predicting the monkey fever risk, assessing the impact of overuse of groundwater for agriculture, and many other works..

Our results indicate that MaxENT can be used in many other areas and it is up to the researchers to apply the tool with combination of other models or methods. For instance, the fluctuation of ecosystems services owing to conservation of a keystone species has been studied by combining MaxENT with Co\$ting Nature and DINAMICA EGO modelling approaches (Hemati et al. 2020). It is also coupled with InVEST models to estimate benefit of conservation effort in Chongqing Municipality (Wang et al. 2024). There are specific R packages like Dismo, Maxlike, and Biomod2. that can perform niche modelling and species distribution (Sillero et al. 2023). Some R package like MIAMaxent is created to improve the predictive performance and ecological interpretability (Vollering et al. 2019) and these packages aim to address the limitations of MaxENT (Yackulic et al. 2013; Renner et al. 2015; Sillero & Barbosa 2021). Even Python based tools are also combined with MaxENT for additional information such as the SDMtoolbox for landscape level

genetic and biogeographic model (Brown 2014).

All these studies show that this Java-based software has aided in the application of information theory and related statistical concepts for predicting factors. The use of presence/occurrence-only data (both for continuous and categorical data) has been regarded as one of the MaxENT tool limitations. Jha et al. (2022) have proved that MaxENT performs better than occupancy models which use both presence and absence data.

All the research works have invariably used bioclimatic data from the worldclim (<https://www.worldclim.org/data/worldclim21.html>) apart from additional datasets like altitude, Digital Elevation Model, NDVI, Enhanced Vegetation Index, Landsurface Temperature, Landuse & landcover, Compounded Topographic Index, Forest Type map & Forest Cover map, Direct Normal Irradiance, evapotranspiration, fraction of absorbed photosynthetically active radiation, water vapour, Leaf Area Index, Ozone, NOx, albedo, aerosol absorbing index, biodiversity indices, hill shade, habitat heterogeneity index, distance from road, soil properties, flow accumulation, Ivlev's index of selection and even human footprint have also been used. All these indicate the flexibility and wider application of MaxENT tools for identifying the niche and distribution of the species in present as well as future climatic conditions. However, the datasets are mostly open-accessible or generated for the particular study site and the inference generated directly depends on the number of occurrences datapoints used. Studies from the Indian context, are primarily accessed from databases like GBIF, Ebird Atlas or data points generated from the field survey. One particular aspect is the range of occurrence data points which can range from ~30 to 3,500 as indicated in Figure 6. It is pertinent to point out that there are a few studies with more than 3,500 occurrence points that are not included here in the figure. For instance, a study assessing the impact of climate change on the 10 hornbill species had about 93,184 points total from GBIF, however only 5,055 points were included for modelling to avoid bias, and cluttering (Sarkar & Talukdar 2023). There are certain taxa such as the Mollusca where the published studies supplement the field survey datasets and therefore mentioning the GPS coordinates in the study reports/publications will be useful in a larger context (Bharti & Shanker 2021).

Studies with small number of occurrence points in MaxEnt have made modifications in settings to prevent overfitting and ensure reliable predictions. For instance, increasing the regularization multiplier from 1 to 1.5 (maximum 3–4) to produce more generalized models

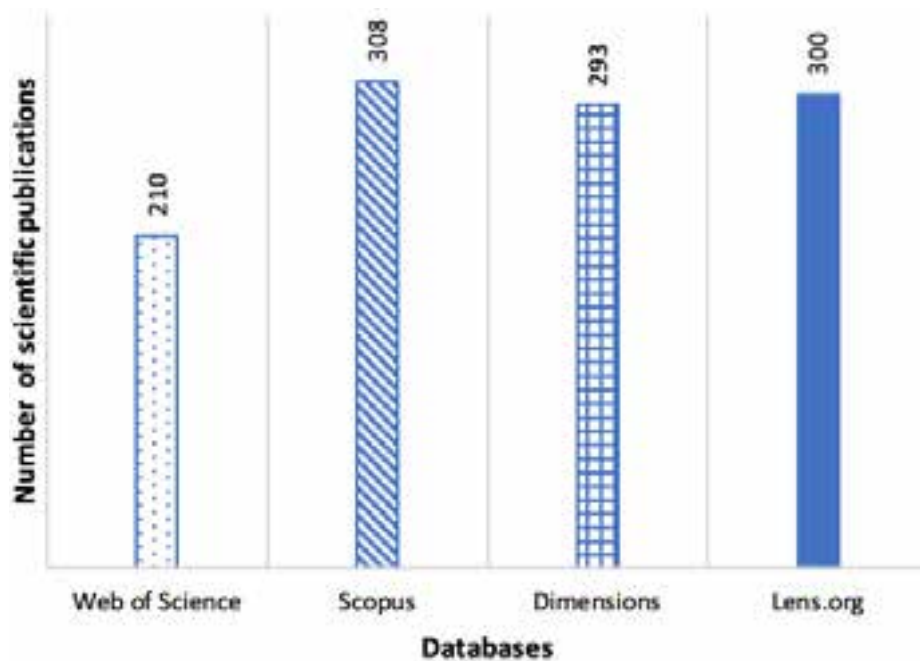


Figure 2. Number of publications in different scholarly databases.

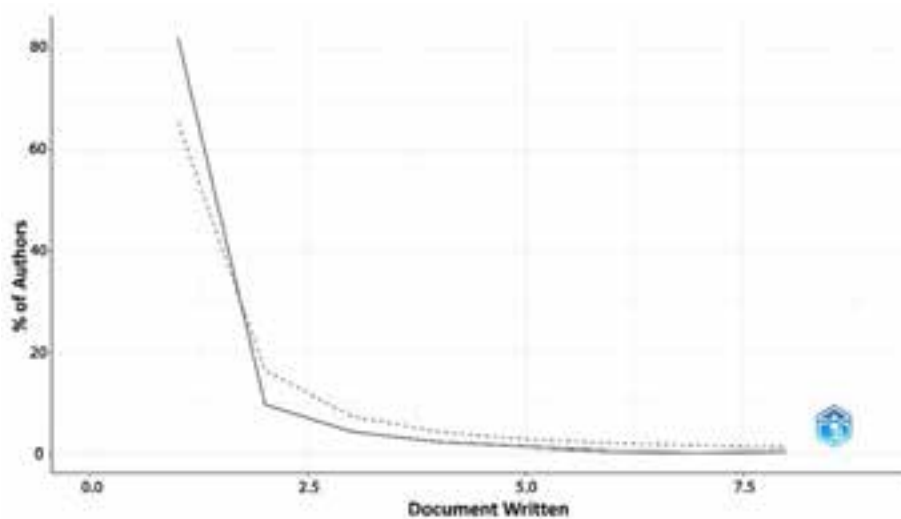


Figure 3. Theoretical and observed scientific productivity (Lotka's law) of research articles published on MaxENT over the years.

(Radosavljevic & Anderson 2014). Feature selection is also refined by restricting complex polynomial and threshold functions, often limiting the model to hinge and linear features for better interpretability.

Cross-validation methods, such as leave-one-out cross-validation (LOOCV), are commonly used in such cases to assess model robustness (West et al. 2016). Additionally, background (pseudo-absence) sampling is fine-tuned by adjusting the number of background points (default ~10,000) and incorporating bias files to correct for sampling effort and presence-only data

bias. To improve model reliability with small datasets, cross-validation techniques are essential. LOOCV is particularly useful for small sample sizes (less than 10 occurrences), as it systematically tests each occurrence point while training the model on the remaining data. For slightly larger datasets, k-fold cross-validation (with  $k = 5$  or  $10$ ) helps estimate model variance and robustness. These approaches ensure that the model is evaluated effectively despite data limitations. When choosing between logistic and cloglog output functions, logistic output (default) provides probability estimates ranging

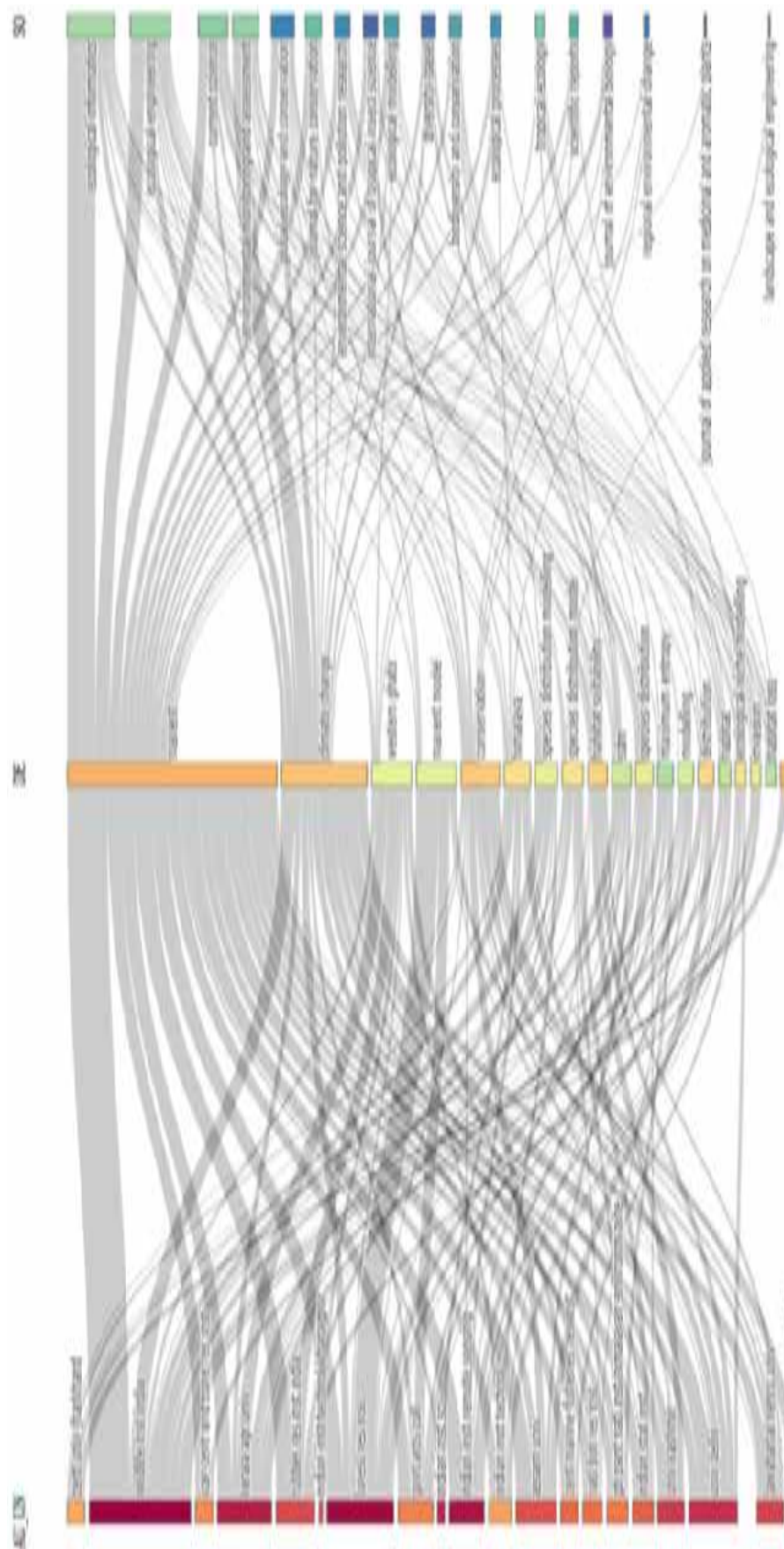


Figure 4. Three-field plot depicting linkage of the top 20 institutes, keywords, and journals for MaxENT-based studies 2000–2023.



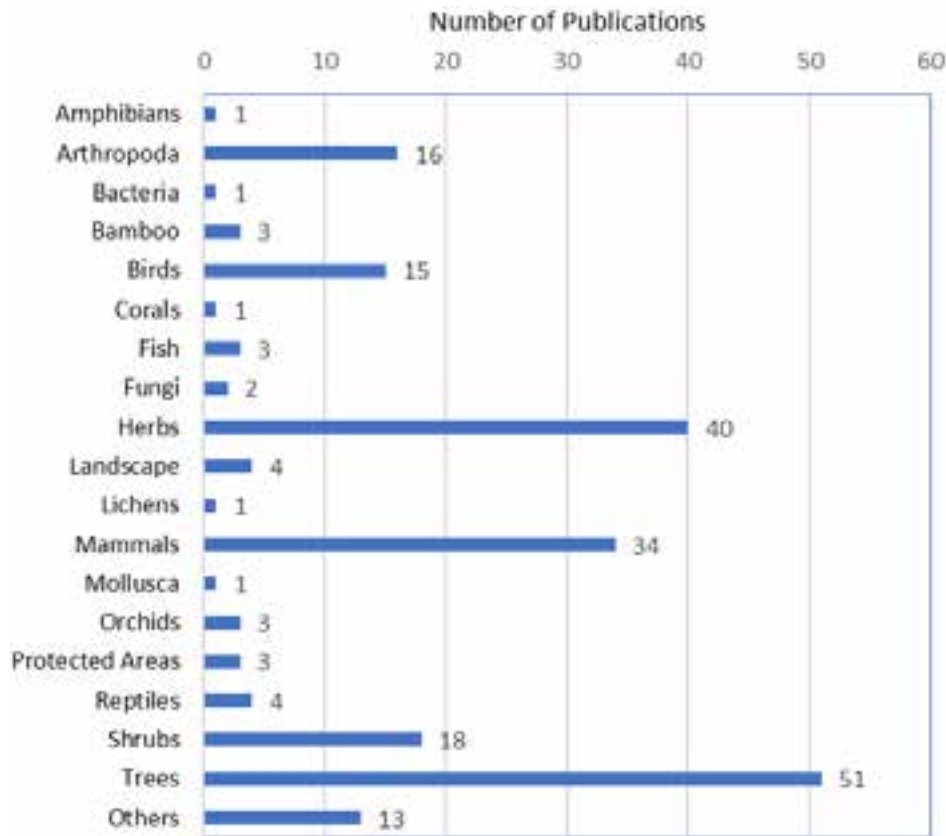


Figure 5. Different thematic groups and their corresponding number of publications from 2000 to 2023.

from 0–1 and is widely used for species distribution studies. The cloglog function is preferred when adjusting for background prevalence, especially when dealing with spatial bias in small datasets. The accuracy of predictions tends to decrease when using limited presence data, as smaller sample sizes increase model uncertainty, reduce generalizability, and may lead to overfitting. This can also create challenges in transferring predictions to new environments (Merow et al. 2013; Renner et al. 2015; Pasanisi et al. 2024).

With regard to the MaxENT modelling techniques, the feature class and regularization multiplier are the two parameters that can be modified to reduce complexity and overfitting of the model prediction (Warren & Seifert 2011). Typically, the MaxENT prediction output is a distribution of a function of the occurrence datapoint and environmental variables for each grid cells of the study area. The auto features enable selection of the output distribution having the maximum entropy from the series of output generated. Studies have indicated the need for defining the feature class and regularization parameters according to the objectives of the study (Morales et al. 2017). In this regard, only 25.25% of

studies from India have customised the regularization multiplier value for better interpretation of the results. The regularization values are tuned to give good predictive performance on a large collection of species from diverse regions. There is quite a variation and some discrepancies in the occurrence data, and a fair amount of diversity in the environmental data, so the default regularization values should be reasonable for the data to be analyzed.

A critical aspect will be usage of error-free occurrence data for MaxEnt modelling, as it depends on the rigorous validation, and preprocessing of occurrence data. Poor-quality inputs, such as duplicate records, spatially biased samples, or misaligned raster layers, can lead to misleading predictions, and overfitting. Ensuring spatial thinning of presence points, harmonizing environmental variables, and using an appropriate background extent or bias file are crucial for model reliability. Another important aspect of MaxEnt modelling is the number and type of predictor variables (i.e., environmental variables) used. Most studies typically employ 19 bioclimatic variables, often supplemented with other environmental, and anthropogenic factors such as

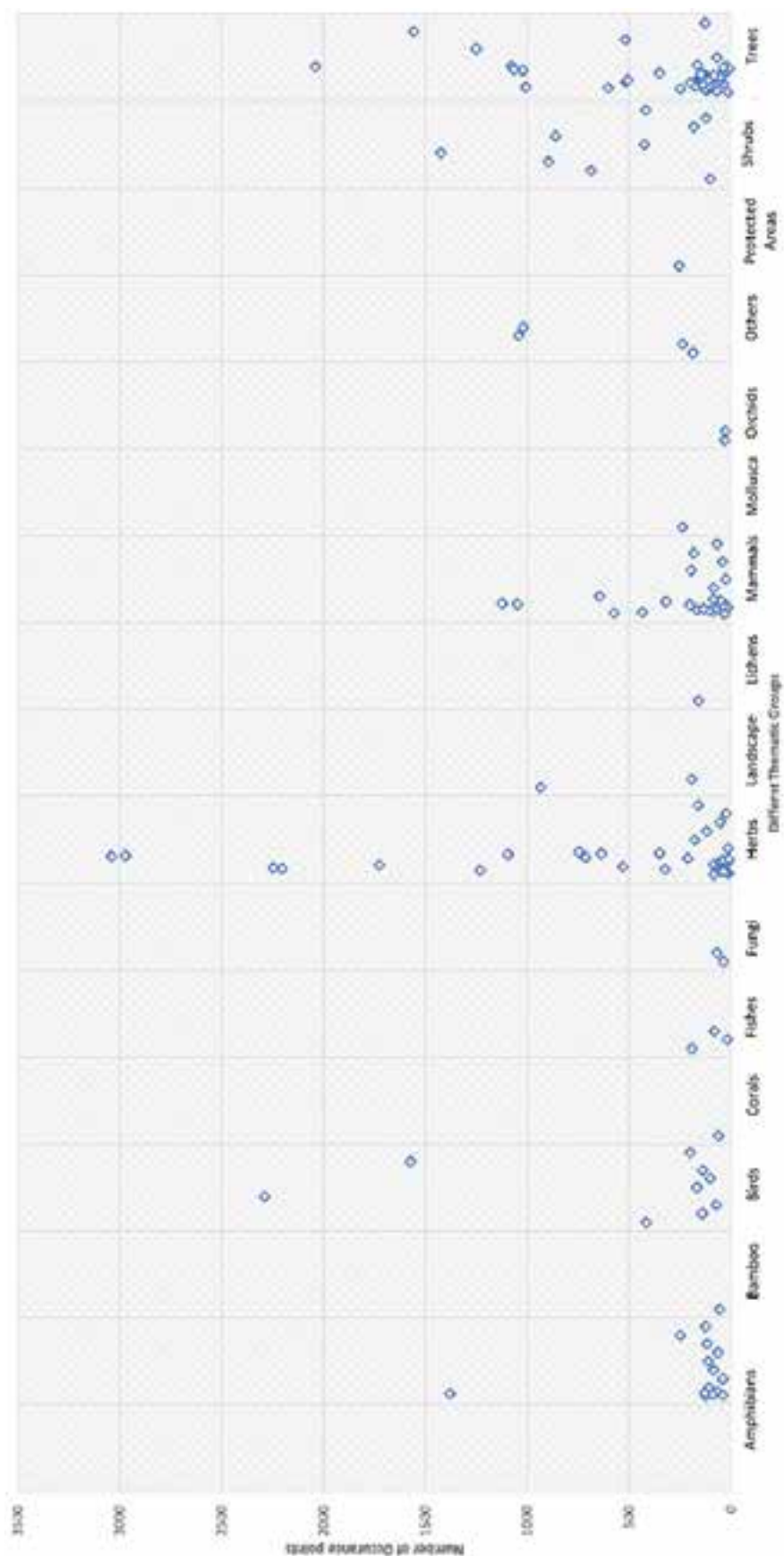


Figure 6. Overview of occurrence data points used for Indian studies.

slope, aspect, elevation, soil type, proximity to water bodies, human settlements, roads, and fire frequency. It is important to note that including a larger number of predictor variables does not necessarily lead to a better-fitting model. A key concern arises when these variables are correlated—an issue known as multicollinearity. Among the 210 publications reviewed, the number of predictor variables used varied depending on the target species. For instance, Banerjee et al. (2017) used only six bioclimatic variables for modelling *Mikania micrantha*, selecting them based on the specific climatic requirements of the species. In contrast, Thakur et al. (2021) initially considered 41 variables—a combination of bioclimatic, topographical, and land cover parameters. After testing for multicollinearity using cluster analysis based on Spearman's rank correlation ( $\rho$ ) and the average agglomeration method, the list was refined to just seven variables. While many researchers are selective in their variable choice, several studies still fail to adequately address multicollinearity, raising concerns about biased estimates, overfitting, and reduced model interpretability. It is worth noting that the issue of multicollinearity in ecological niche modelling predates the widespread adoption of niche modelling (Benito et al. 2009). Feng et al. (2019) offer a nuanced perspective, challenging the assumption that multicollinearity significantly hampers MaxEnt model performance. Disputing the commonly held belief that correlated predictor variables significantly undermine model performance. They argue that MaxEnt has an inherent mechanism for handling redundancy among predictors during the training process, which enables it to maintain robustness even in the presence of high multicollinearity. This robustness has its limits—particularly when models are projected across different spatial or temporal contexts. In such cases, shifts in environmental conditions and changes in the relationships between variables (i.e., collinearity shifts) can introduce uncertainty. To address this, the authors recommend that researchers explicitly quantify and assess these shifts to better interpret model outcomes. Interestingly, they also note that the frequent strategy of removing highly correlated variables may have minimal impact on model accuracy or predictive power, given MaxEnt's capacity to down-weight redundant information, and the lack of a direct link between predictor multicollinearity, and transferability-related issues. These insights suggest that while variable selection remains important, MaxEnt's design inherently mitigates some of the challenges posed by multicollinearity during model calibration. Nevertheless, many studies continue to assess multicollinearity among

predictor variables, and incorporating such analysis into the niche modelling process requires relatively little additional effort. Thus, it can be inferred that this capability may be one of the reasons behind the widespread preference for the MaxEnt.

It is also recommended that while projecting a species for different regions or climate conditions, there is a need to make some adjustments to the default regularization, and feature types (Sutton & Martin 2022). The other aspect of MaxEnt modelling will be the choice of global climate system (GCM) and the scenario selection. Typically, the 2 GCM models under different climatic scenarios are taken up in MaxEnt based studies and similar trend was also seen MaxEnt based studies in the Indian context. Predominantly, studies have used the Representative Concentration Pathways (RCPs) for their studies, and few studies have used the Shared Socioeconomic Pathways (SSPs). Given that SSP was adopted for the Sixth IPCC assessment report (2023), it is not being applied widely. Accounting for the influence of parameters like population, economic growth, education, urbanization, and the rate of technological development in the future greenhouse gas emission is the advantage of SSPs compared to the RCPs, in the number of scenarios used for modelling matters for better understanding, and planning for conservation, and management.

The museums, herbariums, and institutional collections have been reported as sources for occurrence data points, and there is a need to bring these occurrence datasets into a common platform. Given that many of the environmental predictors and other predictors are available in open-access platforms, ensuring the easy accessibility of the dataset will pave robust application of ENM/SDM in real-time decision-making. Relying on a single dataset might be regarded as limitation of this study. This work provides an overview as well as insight for beginners on ENM/SDM.

### Supplementary files

The metadata of the publications used in the analysis is listed in supplementary file S1 <[https://www.threatenedtaxa.org/index.php/JoTT/\\$\\$call\\$\\$/api/file/file-api/download-file?submissionFileId=69590&submissionId=8916&stageId=5](https://www.threatenedtaxa.org/index.php/JoTT/$$call$$/api/file/file-api/download-file?submissionFileId=69590&submissionId=8916&stageId=5)>.

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## MATERIALS AND METHODS

We used a SongMeter Micro, which was hung in the enclosure and set for continuous recording in uncompressed waveform audio file format. Recordings of the single male cat took place from 24 to 26 January 2022 at a sampling rate of 24,000 Hz. Recordings of the mother with kittens were taken at a sampling rate of 32,000 Hz, first at irregular intervals between April and August 2024, and then in December 2024 after the separation of mother and offspring. The higher sampling rate was chosen to ensure successful recording of higher frequency calls, which we expected from the kittens. The recording dates are presented in Table 1. Call spectrograms were created with the software Raven Pro (Bioacoustics Research Program 2017).

## RESULTS

We recorded two bouts of “meow” calls of good quality from the single male (Wav 1; Image 2A). These calls were similar to the calls from the mother after separation from her offspring (Image 2B). From the

**Table 1. Dates of acoustic recordings of Rusty-spotted Cats in Frankfurt Zoo.**

Individual	Start	End	Duration in days
Male	24.i.2022 1600 h	26.i.2022 0800 h	1.7
Female with kittens	30.iv.2024 1400 h	06.v.2024 0500 h	5.6
	14.v.2024 1000 h	17.v.2024 0600 h	2.8
	13.vi.2024 1900 h	16.vi.2024 1240 h	2.7
	19.vi.2024 1640 h	22.vi.2024 1300 h	2.8
	25.vi.2024 1115 h	26.vii.2024 0715 h	0.8
	22.vii.2024 1500 h	25.vii.2024 1115 h	2.8
	21.viii.2024 1435 h	24.viii.2024 1105 h	2.9
Female in isolation	12.xii.2024 1147 h	15.xii.2024 0547 h	2.8

mother and her kittens, we recorded more than 400 calls, and several different call types (Image 2C–G). There was a “murmuring” sound (Wav 2), probably the mother calling her kittens or the kittens answering the calls of



**Image 1. Female Rusty-spotted Cat and her kittens in Frankfurt Zoo. © Silja Fiedler**

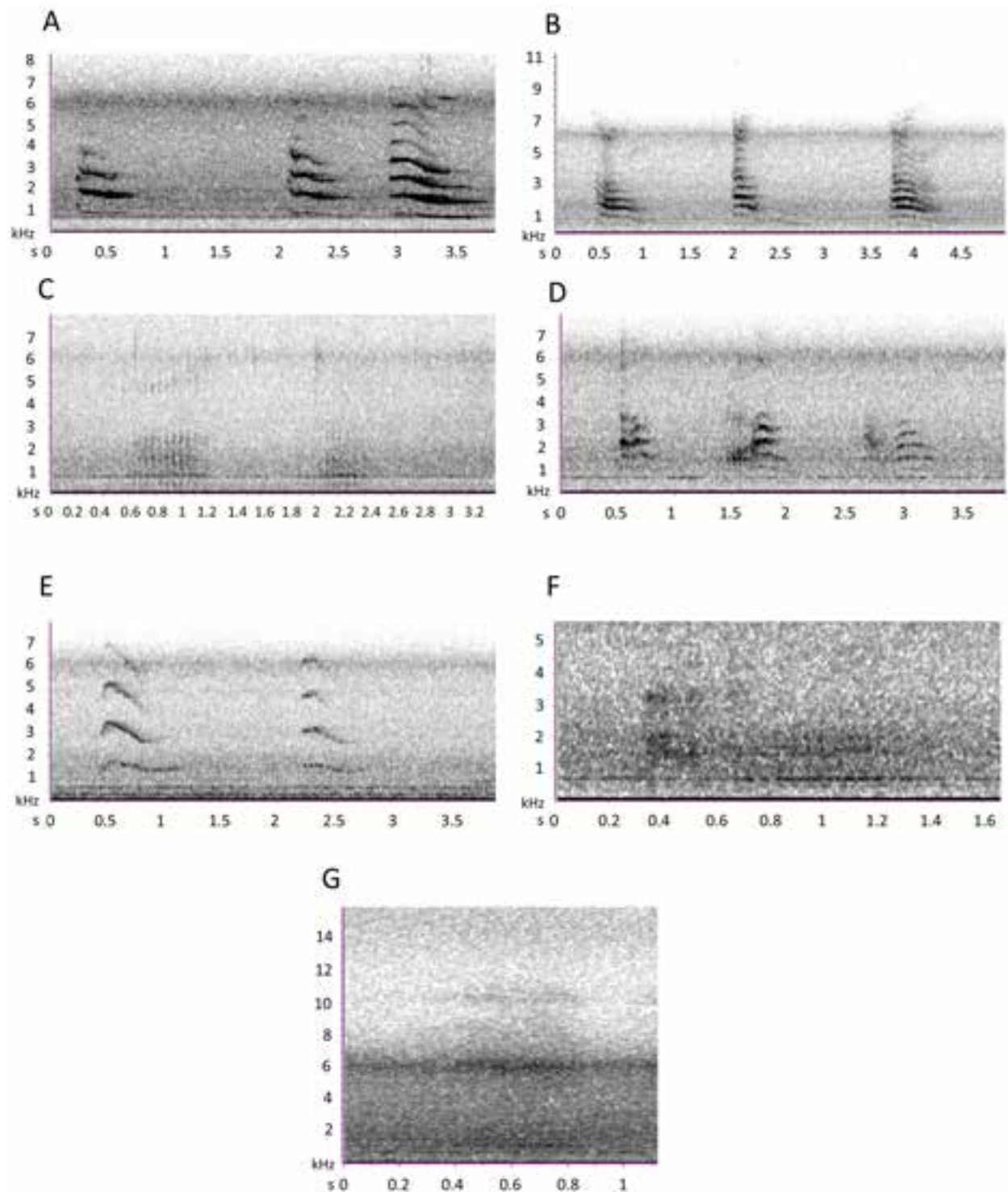


Image 2. A—"Meow" call of an adult male Rusty-spotted Cat in isolation from other cats | B—"Meow" call of an adult female Rusty-spotted Cat after separation from her young | C—"Murmur" call probably from an adult female Rusty-spotted Cat communicating with her young | D—"Meow" call probably from an adult female Rusty-spotted Cat communicating with her young | E—"Meow" call probably from a juvenile Rusty-spotted Cat | F—"Snarl" call from a Rusty-spotted Cat | G—"Hiss" call probably from an adult female Rusty-spotted Cat. All recordings were made at Frankfurt Zoo.

their mother. This was the call type with the highest number of recordings, more than 400. A second call type

with more than 30 recordings was a "meowing" sound, sometimes clearly from the kittens ([Wav\\_3](#)) because



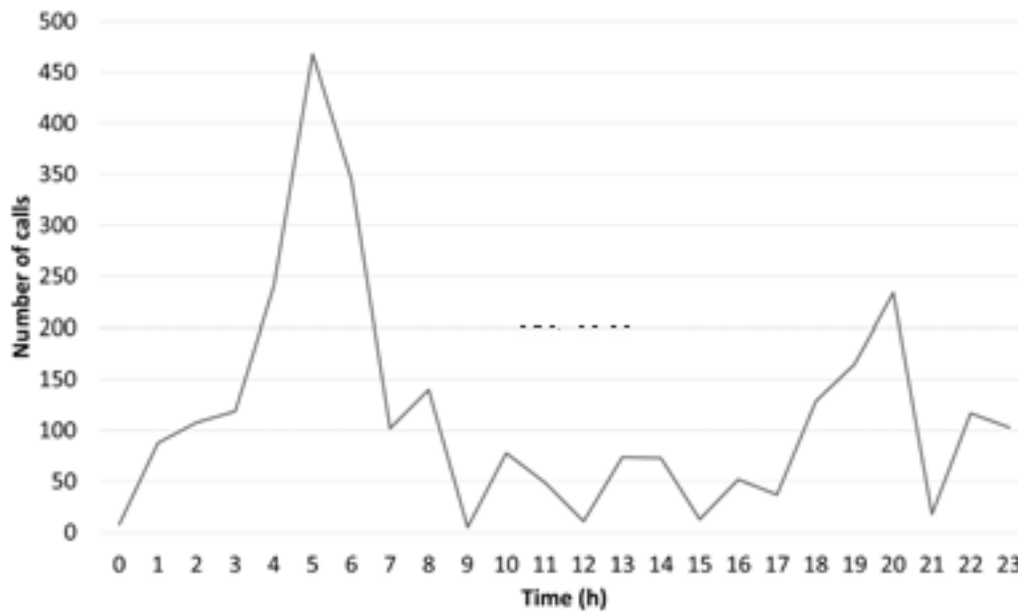


Figure 1. Number of recorded calls of a mother Rusty-spotted Cat with her two kittens in Frankfurt Zoo. Sound recordings were made over three consecutive days and the number of calls per hour were summed up.

it was very high-pitched, sometimes in a lower pitch probably from the mother (Wav 4). A third call type with less than 10 recordings was an aggressive “snarling” sound (Wav 5). Furthermore, we recorded a “hissing” sound in less than five recordings, which occurred only when keepers entered the enclosure. Most of the calls were recorded during the night, early morning or in the evening (Figure 1). We roughly counted the number of calls per hour by hand and found peaks in the number of vocalisations at 0300–0700 h and 1800–2000 h, with the highest number of calls recorded at 0500 h.

## DISCUSSION

Our dataset is the first to include close-range vocalisations of Rusty-spotted Cat kittens with their mother. This dataset shows that the vocal repertoire of the Rusty-spotted Cat encompasses at least four different sounds. Peters & Tonkin-Leyhausen (1999) recorded short “gurgling” sounds of 10 adult female Rusty-spotted Cats, which they described as being common to all the 30 Felinae species in their dataset of close-range felid vocalisations.

The captive female and her kittens communicated by using both a “murmuring”, and a “meowing” sound. In contrast, Deshmukh et al. (2020) reported that wild kitten’s “meow” during reunions with their mother after having been separated from her for more than one hour. The “murmuring” call is also one of the most frequent vocalisations of the Domestic Cat in friendly contexts

(Tavernier et al. 2020). The mother utters this call when approaching the nest, or to guide the kittens when they leave the nest (Tavernier et al. 2020). Therefore, we assume that this call is a close-distance call of the mother reassuring the kittens of her presence.

The “meow” calls of the single male Rusty-spotted Cat and of the female after separation from her offspring are likely long-distance calls in search for contact with other individuals (Peters et al. 2009). The “meow” calls from the kittens could be vocalisations that have been named “chirp”, “tweedle” or “tweet” that Domestic Cat kittens utter when they ask for something (Tavernier et al. 2020). They might have been hungry or needed body contact.

In an experiment with Domestic Cats, females reacted faster to kitten calls when the kittens were in a higher arousal state (Konerding et al. 2016). The vocalisations of the Domestic Cat encode arousal and individuality but without cues to its sex (Scheumann et al. 2012; Rutovskaya et al. 2024). Its “hiss” and “snarl” sounds are associated with defensive and aggressive reactions, respectively (Tavernier et al. 2020). We assume that these associations also hold true for similar vocalisations of the Rusty-spotted Cat.

Studying call acoustics and comparing them to known cat vocalisations could help identify the cats’ behavioural states during vocalisations. Using camera traps alongside acoustic recordings might also clarify these behavioural states. Our recordings can serve as reference material

to identify Rusty-spotted Cat vocalisations from acoustic monitoring data in their natural habitat. We encourage further analysis of the acoustic parameters of the recorded calls, which was not possible within the timeframe of this project. Our data are available on the following data repository: <https://doi.org/10.5281/zenodo.14592115>.

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## Supplementary material

Wav 1. “Meow” call from the adult male Rusty-spotted Cat



Wav 2. “Murmur” call probably from the adult female Rusty-spotted Cat communicating with her young



Wav 3. “Meow” call probably from a Rusty-spotted Cat kitten



Wav 4. “Meow” call probably from the female Rusty-spotted Cat



Wav 5. “Snarl” call from a Rusty-spotted Cat





## Effect of schistosomiasis on captive elephants in Madhya Pradesh, India

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**Abstract:** Schistosomes are parasitic flukes that reside in blood vessels and various host organs. Health monitoring of 51 captive elephants in tiger reserves of Madhya Pradesh revealed a 35% overall incidence of blood flukes. The highest levels of *Bivitellobilharzia nairi* eggs were recorded in elephants in Satpura (67%), Kanha (47%), Bandhavgarh (33%), Pench (25%), and the lowest in Panna Tiger Reserve (14%). Infected animals showed decreased haemoglobin (7.5–11.8 g/dl), and elevated aspartate aminotransferase (65–102 U/L), alanine aminotransferase (85–105 U/L), and blood urea nitrogen (46–65 mg/dl). They also showed symptoms that included dullness/depression and emaciated body condition, which were especially evident in elephants with high *B. nairi* egg counts > 1200–2300 eggs/g.

**Keywords:** Asian Elephants, *Bivitellobilharzia nairi*, blood flukes, haematobiochemistry, granuloma, Schistosomiasis.

Schistosomiasis is a devastating tropical disease, affecting humans and many animal species including African and Asian Elephants (Brant et al. 2013). Schistosomes are unisexual and dimorphic flukes that develop in the blood vessels, and their spiny eggs are responsible for the erosion of parenchymatous tissue which forms granuloma and necrosis in the liver followed by small intestine of the host. The transmission of infection in the definitive host occurs through active skin penetration of furcocercal cercariae, which develops further as an adult parasite in the portal veins. The worm load may lead to morbidity and mortality depending

upon intake of furcocercal cercariae of blood flukes, and their sustenance in the host (Agrawal & Shah 1998). In Asian Elephants, *Bivitellobilharzia nairi* has been reported consistently from Indian subcontinent, albeit without information about the disease manifestations (Bhoyar et al. 2014). The study of pathogenic effect of schistosomiasis in elephants is still scanty as only Kalapesi & Purohit (1957) have described the disease manifestations characterized by granuloma in the hepatic parenchyma, followed by necrosis or sometimes gastrointestinal bleeding with obstructive uropathy, and severe anaemia. Subsequently, infected elephants become clinically unfit for forest-oriented work owing to a reduction in their agility and potential (Singh & Agrawal 2000). Seeking biodiversity conservation mission and their need-based programmes, the trained elephants of different tiger reserves and national parks have been used for patrolling of wild animals, monitoring distant places, and immobilization of big cats (Shrivastav & Singh 2017). Thus, agility and alertness of trained elephants is a must for such important tasks of wildlife management in protected and non-protected forest areas of India. The present research article deals with disease manifestation, effects on haematology, and serum biochemistry in captive elephants of different tiger reserves.

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## MATERIALS AND METHODS

Before the collection of biological samples of each elephant from different tiger reserves of Madhya Pradesh, the information on their age, sex, and area from where they were captured or purchased was gathered. For coprodiagnostics, 20 g fecal samples from freshly defecated dung bolus were collected from each elephant in a sterilized container and divided into two parts; one part was collected without preservatives and another was kept in 10% buffered formalin and brought to the laboratory of School of Wildlife Forensics and Health for qualitative, and quantitative analysis of fecal samples. Sedimentation method of Soulsby (1982) was used to screen the *Bivitellobilharzia nairi* eggs in the processed samples of elephants. Furthermore, 4–5 ml of blood of each elephant was also collected by ear vein puncture using 18-gauge needles aseptically in a vacutainer both in EDTA (Ethylene Diamine Tetra Acetate) coated, and non EDTA serum tubes following the guidelines of Jain (1986). The blood smears were prepared soon after the blood collection and stained with Romanowsky stain for differential leukocytes counts (DLC) as well as screening of haemoprotozoans. Haematological parameters were conducted using semi-auto haematology analyzer (PG–6800 VET) within 12 h of collection of blood, while harvested serum samples were stored at 4–8 °C until further analysis. The serum biochemicals were mainly attributed to liver and kidney function tests and estimated using ERBA diagnostic kits with semi auto analyzer (ARK diagnostics, Mumbai), and

the findings were interpreted based on Benjamin (1978).

## RESULTS AND DISCUSSION

Impact of schistosomiasis on health status with reference to haematological and biochemical parameters due to blood fluke was envisaged. Overall, 35.2% prevalence of elephant schistosomiasis was recorded in different tiger reserves of Madhya Pradesh. The occurrence of *B. nairi* infection in elephants is indicative of a natural nidus of intermediate hosts for animal schistosomiasis that might be owing to presence of the water snails, i.e., *Indoplanorbis exustus* and *Lymnaea luteola* in these national parks, and adjoining endemic areas. Agrawal & Shah (1998) also observed water snails (*I. exustus* and *L. luteola*) in central India, which are responsible for mammalian schistosomiasis in livestock. Nonetheless, stagnant water resources are

**Table 1. Occurrence of *Bivitellobilharzia nairi* eggs in captive elephants.**

Tiger reserves	No. examined	Found positive	EPG range	Mean EPG±SD
Kanha	15	7 (46.6 %)	1800–2300	2000 ± 64.41
Bandhavgarh	12	4 (33.3%)	800–1200	1000 ± 34.62
Panna	14	2 (14.2 %)	600–1400	1000 ± 50.85
Satpura	6	4 (67.0%)	1200–1800	1500 ± 74.8
Pench Tiger	4	1 (25.0%)	800–1200	1000 ± 0.51
Total	51	18 (35.2 %)	600–2300	1500 ± 42.8

**Table 2. Haematological profile of *Bivitellobilharzia nairi* infected and non-infected captive elephants.**

	Parameters	Unit	Non-infected elephants		Infected elephants	
			Range	Mean ± SD	Range	Mean ± SD
1.	Total Erythrocyte Count	10 <sup>6</sup> /μl	2.61–5.34	3.57 ± 0.15	2.66–4.77	3.43 ± 0.13
2.	Haemoglobin	g/dl	12–18.4	14.25 ± 0.69	7.5–11.8	10.06 ± 1.47
3.	Pack Cell Volume	%	33.1–64.3	44.59 ± 1.67	33.5–50.2	40.70 ± 1.22
4.	Mean Corpuscular Volume	fl	107.6–153.3	124.54 ± 1.99	107.6–128.2	120.88 ± 1.47
5.	Mean Corpuscular Haemoglobin	pg	33.3–45.8	40.40 ± 0.63	33.3–40.6	38.122 ± 0.45
6.	Mean Corpuscular Haemoglobin Concentration	g/dl	26.6–37.4	32.54 ± 0.55	26.6–34.6	31.57 ± 0.40
7.	Blood Platelets	10 <sup>3</sup> /μl	226–744	458.65 ± 29.04	263–665	433 ± 28.90
8.	Total Leukocyte Count	10 <sup>3</sup> /μl	7.5–41.1	23.01 ± 1.63	11.1–31.6	20.57 ± 1.34
9.	Differential Leukocyte Count					
i.	Polymorph	%	43–71	55.21 ± 1.66	69–85	75.27 ± 1.38
ii.	Lymphocytes	%	15–38	26.52 ± 1.22	15–34	24.5 ± 1.21
iii.	Monocytes	%	4.0–24	11.65 ± 1.09	6–17	10.88 ± 0.83
iv.	Eosinophils	%	2.0–12	6.60 ± 0.63	8.0–16	10.88 ± 0.72
v.	Basophiles	%	00	00	00	00





**Image 1.** a—Showing grooming of captive elephants beside stagnant water resource | b—Body condition of schistosomes infected elephant | c—Spiny egg of *Bivitellobilharzia nairi* in faeces (400 x). © Dr. K. P. Singh.

used for grooming and bathing of captive elephants in different tiger reserves of central India where snail populations breed (Image 1a). The highest occurrence of infection was recorded in Satpura (67.0%) followed by Kanha (46.6%), Bandhavgarh (33.3%), Pench (25%), and lowest (14.2%) in Panna Tiger Reserve of Madhya

Pradesh (Table 1). Singh & Agrawal (2000) have also recorded higher prevalence (32.8%) of *B. nairi* infection in captive elephants of Kanha Tiger Reserve while Islam (1994) recorded only 16% infection of schistosomiasis in captive elephants of Kaziranga National Park. This might be owing to difference in geographical distribution and timing of wallowing, and bathing of elephants in the stagnant water resources as release of furcocercal cercariae takes place during morning hours (Agrawal & Shah 1998). Hence, the prevalence rate of Schistosomes differs with seasonal variations and might be owing to the availability of stagnant waterholes.

The complete blood count (CBC) analysis showed decreases in hemoglobin percentage ( $7.5\text{--}11.8$ ,  $10.06 \pm 1.47$  g/dl) in schistosome-infected elephants. Changes in differential leukocyte count, including eosinophilia, were recorded, particularly in elephants in which higher EPG of *B. nairi* eggs was reported. Biochemical changes included elevation of aspartate aminotransferase (AST =  $65\text{--}102$   $85 \pm 21.6$  U/L), alanine aminotransferase (ALT =  $85\text{--}105$   $91 \pm 17.4$  U/L) and blood urea nitrogen (BUN =  $46.2\text{--}65.5$   $58.2 \pm 13.7$  mg/dl), indicated in the obstruction in the liver and kidney function showed chronic phase of the disease (Table 2). Singh & Agrawal (2000) have also encountered an increased level of SGPT and SGOT in infected elephants of Kanha National Park. The considerable alteration in the blood profiles are indicative of effects of parasitemia on liver, small intestine, mesenteric veins probably causing granuloma followed by necrosis. Similar findings have been reported by Bhoyar et al. (2014) during haematobiochemical studies of elephants infected by *B. nairi*. Therefore, schistosomiasis may be controlled specifically in captive elephants through change in routine bathing in the stagnant river pockets as the water snails mostly release mammalian cercariae after sunrise early in the morning hours (Dorsey et al. 2002). The present study may be useful in determination of haematobiochemical parameters of infected and non-infected elephants with diagnosis of schistosomiasis in elephants to control the disease burden in different tiger reserves in addition to health monitoring, and diseases diagnosis of captive elephants in different tiger reserves for smooth functioning, and conducting the wildlife health management aspects.

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Table 3. Serum biochemistry of *Bivitellobilharzia nairi* infected and non-infected captive elephants.

	Parameters	Unit	Non-infected elephants		<i>B. nairi</i> infected elephants	
			Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
1.	Aspartate Transaminase	IU/L	10.56–81	40.30 $\pm$ 5.08	65–102	85 $\pm$ 21.60
2.	Alanine Amino Transaminase	IU/L	12.17–45.4	6.31 $\pm$ 0.54	85–105	91 $\pm$ 17.40
3.	Alkaline Phosphatase	IU/L	34–118.8	65.30 $\pm$ 4.63	34.2–101.4	66.06 $\pm$ 4.74
4.	Total Bilirubin	mg/dl	0.1–1.4	0.68 $\pm$ 0.08	0.1–2.9	0.96 $\pm$ 0.17
5.	Total Protein	g/dl	4.68–10.2	7.72 $\pm$ 0.28	4.52–9.8	7.89 $\pm$ 0.27
6.	Creatinine	mg/dl	1.2–2.44	1.89 $\pm$ 0.08	1.2–3.2	1.89 $\pm$ 0.12
7.	Blood Urea Nitrogen	mg/dl	9.5–40.1	23.13 $\pm$ 1.96	46.2–65.5	58.2 $\pm$ 13.70
8.	Uric Acid	mg/dl	10.4–12.8	11.08 $\pm$ 0.13	12.4–16.4	13.41 $\pm$ 0.26

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## Recent additions and taxonomic changes in the liverwort and hornwort flora of India

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Singh et al. (2016) in their Checklist of Liverworts & Hornworts in India included 930 species, and infraspecific taxa. Later, Majumdar & Dey (2021) provided lists of taxa added to Indian bryoflora during 2016–2020 along with the list of taxa synonymized during this period. The updated list of taxa thus comprised 953 species and infraspecific taxa, belonging to 141 genera, 59 families, 17 orders, and four classes from two phyla. Of these, 854 species, 17 subspecies, 41 varieties, and two forma belonged to 135 genera in 56 families of liverworts and 39 species representing six genera in three families of hornworts.

However, just within a span of four years, 18 new species, one new subspecies have been described from India and 22 species have been newly reported from India. At the same time, three species and four varieties have been synonymized. Ali et al. (2024) while doing taxonomic studies on Indian Balantiopsidaceae, excluded the occurrence of *Isotachis armata* (Nees) Gottsche from India. According to Ali et al. (2024), *I. armata* was erroneously reported by Das & Singh (2007) from Arunachal Pradesh, India, as the specimens studied by them belongs to *Isotachis japonica* Steph. During this time, six genera, viz., *Barbilophozia* Loeske,

*Dinckleria* Trevis., *Diplasiolejeunea* (Spruce) Schiffn., *Gymnocolea* (Dumort.) Dumort., *Mesoptychia* (Lindb.) A.Evans, and *Saccogyna* Dumort. of liverworts and the genus *Phymatoceros* Stotler, W.T.Doyle & Crand.-Stotl. of hornworts have been added to the liverwort & hornwort flora of the country. Two families, one each from hornwort (*Phymatocerotaceae*), and liverwort (*Saccogynaceae*) were also recorded for the first time in India.

Presently, after all these changes, the number of taxa has soared to 986 species and infraspecific taxa belonging to 148 genera, 61 families, 17 orders, and four classes in two phyla. Of these, 890 species, 18 subspecies, 37 varieties, and two forma belongs to 141 genera in 57 families of liverworts, and 39 species representing six genera in four families of hornworts (See Tables 1, 2, & 3).

**\*Note:** Li et al. (2011) synonymised the genus *Hattorioceros* (J.Haseg.) J.Haseg. under *Phaeoceros* Prosk., but Majumdar & Dey (2021) missed out. So, presently in India there are six genera under Anthocerotophyta. Majumdar & Dey (2021) also missed out the occurrence of *Barbilophozia hatcheri* from India (Gupta et al. 2019). The same has been presently incorporated.

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**Table 1. Alphabetic lists of taxa added to Indian bryoflora after publication of Majumdar & Dey (2021).**

	Taxa	Family	Reference
1	<i>Acrolejeunea aulacophora</i> (Mont.) Steph.	Lejeuneaceae	Daniels 2021
2	<i>Barbilophozia barbata</i> (Schmidel ex Schreb.) Loeske	Anastrophyllaceae	Kour & Singh 2023
3	<i>*Barbilophozia hatcheri</i> (A.Evans) Loeske	Anastrophyllaceae	Gupta et al. 2019
4	<i>Barbilophozia lycopodioides</i> (Wallr.) Loeske	Anastrophyllaceae	Kour & Singh 2023
5	<i>Cheilelejeunea occlusa</i> (Herzog) T.Kodama & N.Kitag.	Lejeuneaceae	Kumar & Singh 2025a
6	<i>Cololejeunea devendrae</i> Shashi Kumar & D.Singh	Lejeuneaceae	Kumar & Singh 2025b
7	<i>Cylindrocolea devendrae</i> Manju & P.P.Nishida	Cephaloziellaceae	Manju et al. 2023
8	<i>Cylindrocolea mizoramensis</i> Sushil K.Singh	Cephaloziellaceae	Singh 2022
9	<i>Cyathodium udarii</i> D.Singh & D.K.Singh	Cyathodiaceae	Singh & Singh 2023a
10	<i>Dinckleria singularis</i> (Schiffn.) M.A.M. Renner, Schäfer-Verw. & Heinrichs	Plagiochilaceae	Singh Deo & Singh 2020
11	<i>Diplasiolejeunea cavifolia</i> Steph.	Lejeuneaceae	Chandini et al. 2021
12	<i>Diplasiolejeunea cobrensis</i> Gottsche ex Steph.	Lejeuneaceae	Chandini et al. 2021
13	<i>Frullania bolanderi</i> Austin	Frullaniaceae	Rawat et al. 2021
14	<i>Frullania sikkimensis</i> D.Singh & D.K.Singh	Frullaniaceae	Singh & Singh 2023a
15	<i>Gymnocolea inflata</i> (Huds.) Dumort.	Anastrophyllaceae	Arya et al. 2021
16	<i>Gymnomitrium udarii</i> D.Singh & D.K.Singh	Gymnomitriaceae	Singh & Singh 2023b
17	<i>Heteroscyphus planus</i> (Mitt.) Schiffn.	Lophocoleaceae	Zheng 2024
18	<i>Megaceros gracilis</i> (Reichardt) Steph.	Dendrocerotaceae	Majumdar & Singh 2025
19	<i>Mesoptychia morrisoncola</i> (Horik.) L.Söderstr. & Váňa	Jungermanniaceae	Singh & Singh 2021
20	<i>Phymatoceros binsarensis</i> A.K.Asthana, D.Shukla & Reesa Gupta	Phymatocerotaceae	Asthana et al. 2023
21	<i>Plagiochila dampensis</i> Sushil K.Singh & K.K.Rawat	Plagiochilaceae	Singh & Rawat 2020
22	<i>Plagiochila meagheriana</i> S.Majumdar & M.Dey	Plagiochilaceae	Majumdar & Dey 2025
23	<i>Plagiochila mizoramensis</i> Sushil K.Singh & K.K.Rawat	Plagiochilaceae	Singh & Rawat 2020
24	<i>Plagiochila ponmudiana</i> A.E.D.Daniels & S.R.Aishwarya	Plagiochilaceae	Daniels et al. 2024
25	<i>Plagiochila sirumalaiana</i> A.E.D.Daniels & Z.H.Williams	Plagiochilaceae	Daniels et al. 2024
26	<i>Plagiochila squamulosa</i> Mitt. subsp. <i>tamas-pocsii</i> Sajitha, Manju & Rawat	Plagiochilaceae	Sajitha et al. 2024
27	<i>Riccia atromarginata</i> Levier	Ricciaceae	Karadakatti & Betageri 2023
28	<i>Riccia boliviensis</i> Jovet-Ast	Ricciaceae	Asthana & Srivastava 2021
29	<i>Riccia junghuhniana</i> Nees & Lindenb.	Ricciaceae	Suresh & Cargill 2024
30	<i>Riccia keralensis</i> Manju, Chandini, Sushil K.Singh & K.P.Rajesh	Ricciaceae	Manju et al. 2021
31	<i>Riccia lamellosa</i> Raddi	Ricciaceae	
32	<i>Riccia okahandjana</i> S.W.Arnell	Ricciaceae	Suresh & Cargill 2022
33	<i>Riccia sarieae</i> A.E.D.Daniels & D.T.T.Daniels	Ricciaceae	Daniels & Daniel 2022
34	<i>Riccia stenophylla</i> Spruce	Ricciaceae	Athira & Daniels 2022
35	<i>Saccogyna darjeelingensis</i> M.Dey & S.Majumdar	Saccogynaceae	Dey & Majumdar 2022
36	<i>Scapania bhutanensis</i> Amak.	Scapaniaceae	Rawat et al. 2024; Zheng 2025
37	<i>Scapania karl-muelleri</i> Grolle	Scapaniaceae	Rawat et al. 2024
38	<i>Scapania lachungensis</i> D.Singh & D.K.Singh	Scapaniaceae	Singh & Singh 2023b
39	<i>Scapania spiniloba</i> Potemkin	Scapaniaceae	Singh & Singh 2023b
40	<i>Solenostoma vidyasagarianum</i> "vidyasagariensis" Sk. R.Islam, A.K.Mondal & D.Singh	Solenostomataceae	Islam et al. 2025
41	<i>Telaranea mizoramensis</i> Sushil K.Singh	Lepidoziaceae	Singh 2022



Table 2. Alphabetic lists of taxon synonymized.

	Taxa	Family	Reference
1	<i>Folioceros paliformis</i> D.K.Singh = <i>Folioceros kashyapii</i> S.C.Srivast. & A.K.Asthana	Anthocerotaceae	Singh Deo & Majumdar 2021
2	<i>Jubula hattorii</i> Udar & V.Nath = <i>Jubula hutchinsiae</i> (Hook.) Dumort. subsp. <i>javanica</i> (Steph.) Verd.	Jubulaceae	Singh & Singh 2023a
3	<i>Jubula hattorii</i> Udar & V.Nath var. <i>mutukuzhiana</i> A.E.D.Daniels & P.Daniel = <i>Jubula hutchinsiae</i> (Hook.) Dumort. subsp. <i>javanica</i> (Steph.) Verd.	Jubulaceae	Singh & Singh 2023a
4	<i>Lopholejeunea sikkimensis</i> var. <i>kumaunii</i> Kushwaha = <i>Lopholejeunea sikkimensis</i> Steph. var. <i>sikkimensis</i>	Lejeuneaceae	Priyanka et al. 2023
5	<i>Lopholejeunea sikkimensis</i> var. <i>tenuicostata</i> Sushil K.Singh & D.K.Singh = <i>Lopholejeunea sikkimensis</i> Steph. var. <i>sikkimensis</i>	Lejeuneaceae	Priyanka et al. 2023
6	<i>Metzgeria furcata</i> (L.) Corda var. <i>ulvula</i> Nees = <i>Metzgeria furcata</i> (L.) Corda var. <i>furcata</i>	Metzgeriaceae	Singh & Singh 2023a
7	<i>Metzgeria assamica</i> S.C.Srivast. = <i>Metzgeria macrocellulosa</i> Kuwah.	Metzgeriaceae	Singh & Singh 2023a

Table 3. Taxon excluded from India.

	Taxa	Family	Reference
1	<i>Isotachis armata</i> (Nees) Gottsche sensu Sudipa Das & D.K. Singh (2007)	Balantiopsidaceae	Ali et al. 2024

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## First photographic record of the Smooth-coated Otter *Lutra perspicillata* in Polavaram Forest Range, Andhra Pradesh, India

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The Godavari is the largest river in Peninsular India, flows eastwards from Maharashtra to Andhra Pradesh before finally entering the Bay of Bengal (Babar & Kaplay 2018). The river basin covers nearly 9.5% of India's total land area, falling between 73.43°–83.12° E and 16.27°–23.72° N, extending over an area of approximately 312,812 km<sup>2</sup> (Hussain et al. 2017; Babar & Kaplay 2018). Along its stretch, the Godavari River supports diverse riparian and aquatic habitats that could potentially be suitable for *Lutra perspicillata* (Nagulu et al. 1998; Sivakumar et al. 2014; Hussain et al. 2017). The study documents photographic evidence of the Smooth-coated Otter in Polavaram Range of Eluru Division of Papikonda National Park, Andhra Pradesh.

The Smooth-coated Otter *Lutra perspicillata* inhabits a wide range of aquatic ecosystems across the Oriental region, including paddy fields, lakes, rivers, perennial water bodies, and mangroves (Khoo et al. 2021; de Ferran et al. 2022). India is home to three otter species: The Asian small-clawed Otter *Aonyx cinereus*, the Eurasian Otter *Lutra lutra*, and the Smooth-coated Otter

*Lutra perspicillata* (Rath et al. 2023). The Smooth-coated Otter is distributed across the Indian states of Assam, Arunachal Pradesh, Andhra Pradesh, Bihar, Gujarat, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Uttar Pradesh, Tamil Nadu, and West Bengal (Nagulu et al. 1998; Hussain 1999; Anoop & Hussain 2005; Baskaran et al. 2022; Trivadi & Patel 2022; Narasimmarajan et al. 2024). In Andhra Pradesh, its occurrence is recorded in areas such as East Godavari, West Godavari, Visakhapatnam, and the Krishna Wildlife Sanctuary (Nagulu et al. 1998; Kantimahanti & Allaparthi 2017; Tamarapalli et al. 2022).

The Smooth-coated Otter is facing significant threats due to habitat loss, poaching, pollution, and eutrophication, which may lead to population declines exceeding 30% (Rath et al. 2023). This species holds significant conservation importance as a key ecological indicator of freshwater ecosystem health (Ali et al. 2010; Baskaran et al. 2022). It is currently listed as 'Vulnerable' on the IUCN Red List and receives legal protection under Schedule I of the Indian Wildlife (Protection) Act, 1972

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(amended 2022) and Appendix II of CITES (Nagulu et al. 1998; Gautam & Kaur 2023; Narasimmarajan et al. 2024).

On 12 June 2024, a team from Wildlife Institute of India (WII) recorded a sighting of one individual of Smooth-coated Otter in the Polavaram Forest Range, within the boundaries of Papikonda National Park, Andhra Pradesh. This opportunistic yet significant sighting occurred during a field survey at Papikonda National Park. The otter was photographed using a Sony Alpha 6100 camera in the Kothuru Beat of Polavaram Forest Range, Eluru Forest Division in close proximity to the Godavari River; the river separates the Eluru Forest Division from the Alluri Sitharama Raju District Forest Division (Daveedu Raju pers. comm. 2024). The coordinates of the sighting were 17.330° N, 81.620° E (Figure 1). The sighting was noted at 1754 h IST and lasted for less than a minute. The otter was seen resting on an exposed sandbank (Image 1), most likely because of the low water in the summer season (Image 1). This observation took place in the southern portion of Papikonda National Park, along the Godavari River, about five kilometres from the Indira Sagar Multipurpose Project (Polavaram Project).

While anecdotal accounts and local reports have suggested the presence of otters in this area, no visual or photographic documentation had previously confirmed their existence. During field assessments, consultations were conducted with forest officials, and local fishing communities, both of whom were familiar with the species but reported infrequent sightings, indicating its rarity in the area (Nagulu et al. 1998). Although prior research has found Smooth-coated Otters in the Godavari River (Nagulu et al. 1998; Tamarapalli et al. 2022), this is the first photographic record in the Polavaram Range of Eluru Division.

Otters, which mostly feed on fish, have a big impact on aquatic ecosystems, particularly in places where the availability of prey is impacted by human expansion (Khoo et al. 2021; Narasimmarajan et al. 2024). With 89 fish species from 26 families, the Godavari River is an essential feeding environment that is encircled by the Eluru and Alluri Sitharama Raju Forest Divisions (Sivakumar et al. 2014). The ichthyofauna that otter populations consume includes species like *Wallago attu*, *Labeo rohita*, and *Catla catla*, highlighting the ecological significance of otter

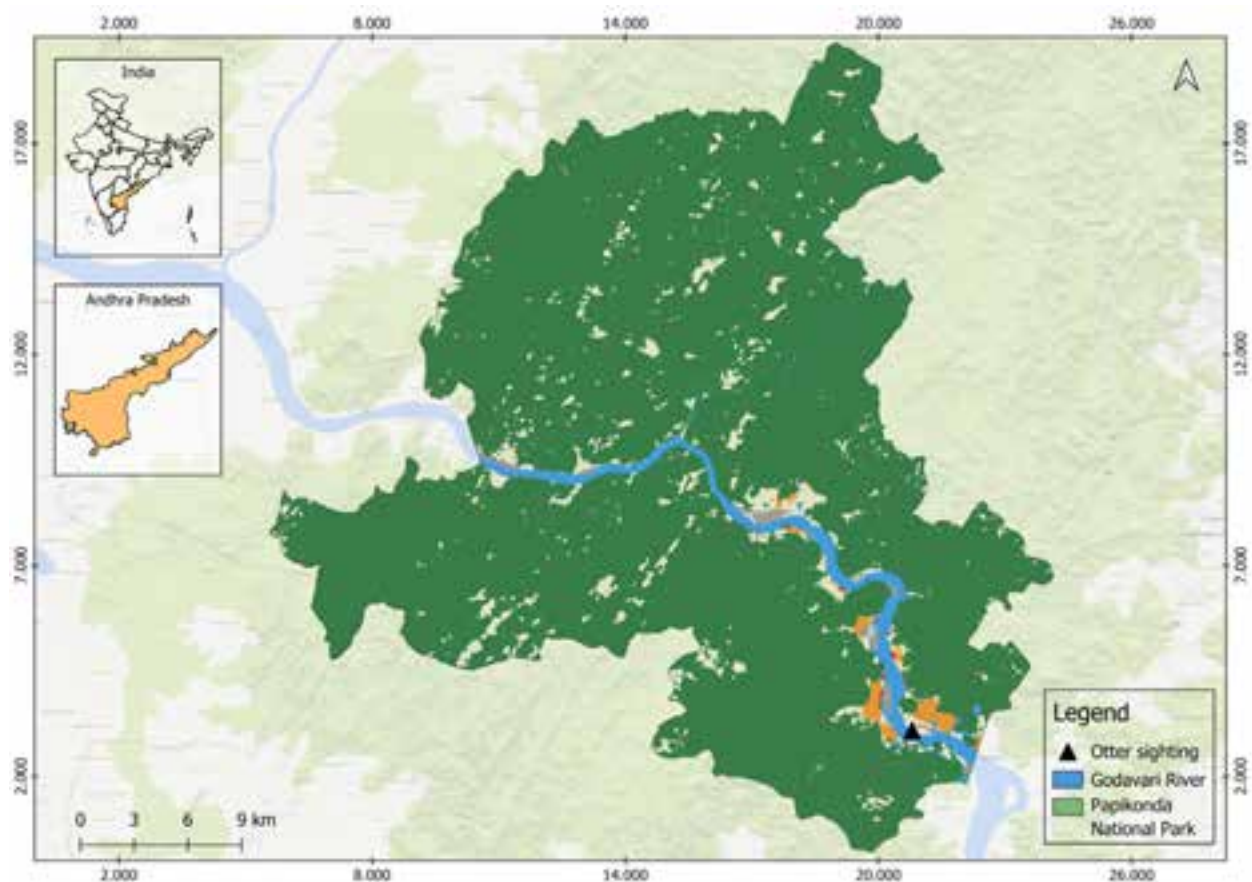


Figure 1. Sighting location of the Smooth-coated Otter *Lutra perspicillata*.





Image 1. Smooth-coated Otter *Lutra perspicillata* in Polavaram Forest Range of Eluru Division. © Arun Kumar Gorati.

populations in maintaining fish community structures (Anoop & Hussain 2005; Narasimmarajan et al. 2024).

This observation not only confirms the presence of this elusive and ecologically sensitive species in the Polavaram Forest Range of Papikonda National Park but may also extend the known geographic distribution range of this species in this stretch of Godavari River (Nagulu et al. 1998). The sighting underscores the significant gap in our understanding of freshwater biodiversity in this region. Also, the record serves as a baseline for initiating systematic ecological studies on otters' habitat suitability, occupancy trends, and potential anthropogenic threats. The detection of this apex aquatic predator highlights the need for increased freshwater biodiversity research and long-term monitoring to comprehend the ecology and population viability in this dynamic landscape (Nagulu et al. 1998; Sivakumar et al. 2014; Narasimmarajan et al. 2024).

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