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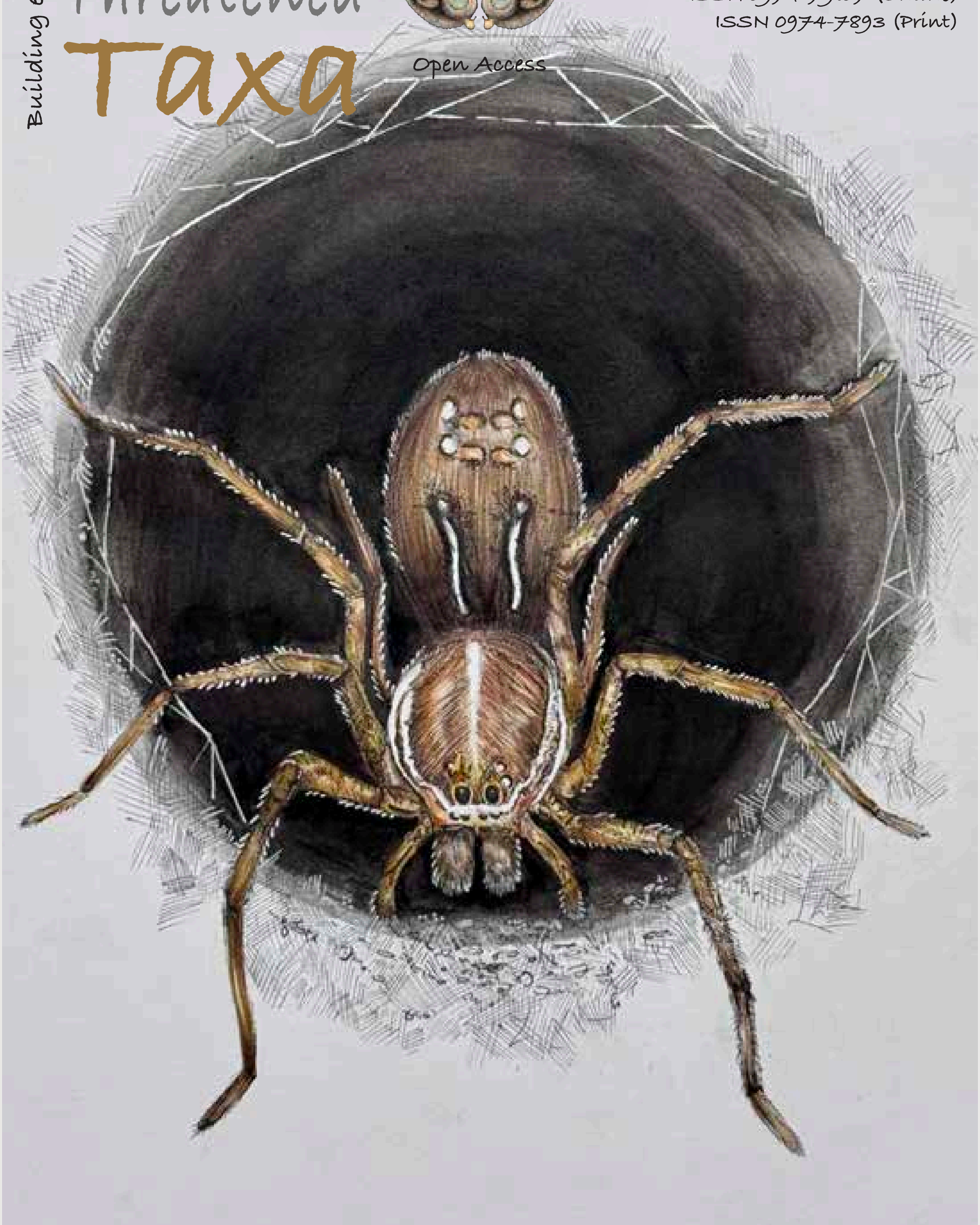
Journal of Threatened Taxa

Open Access

10.11609/jott.2024.16.11.26063-26186
www.threatenedtaxa.org

26 November 2024 (Online & Print)
16(11): 26063–26186
ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)



ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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continued on the back inside cover

Cover: Mixed media with fine liners, colour pencils, and watercolour background of an Indian funnel web spider. © Elakshi Mahika Molur.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26063–26077

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9186.16.11.26063-26077

#9186 | Received 06 June 2024 | Final received 15 September 2024 | Finally accepted 09 October 2024

ARTICLE

Endemicity and diversity of birds of the Kuvempu University Campus, Shivamogga District, Karnataka: an updated checklist

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Abstract: The updated checklist of birds at Kuvempu University Campus including present and past records, now contains 229 species, belonging to 16 orders and 62 families. A family-wise analysis showed that Accipitridae dominated the avifauna of the region (16 species), followed by Muscicapidae (14 species), Picidae (11 species), Columbidae (9 species), Strigidae (8 species), and Cuculidae, Alaudidae, Sturnidae, & Motacillidae (7 species each). The community consists of 83% (190 species) resident and 17% (39 species) winter migrant species. The study also documented four species of birds that are classified as 'Near Threatened' (Black-headed Ibis *Threskiornis melanocephalus*, Pallid Harrier *Circus macrourus*, Malabar Pied Hornbill *Anthracoceros coronatus*, and Grey-headed Bulbul *Microtarsus priocephalus*), one 'Endangered' Egyptian Vulture *Neophron percnopterus*, and one 'Vulnerable' Woolly-necked Stork *Ciconia episcopus* as per the IUCN Red List of Threatened Species. The campus harbours 69 species, of which 14 are endemic to both the Indian Subcontinent and the Western Ghats. The study highlights the impact of anthropogenic activities as the main cause for the loss of diversity of birds and their habitats and emphasizes the urgent need to conserve this biodiversity-rich area with long-term monitoring programs.

Keywords: Bird conservation, Bhadra Wildlife Sanctuary, campus birds, endangered, endemic, threatened, vulnerable species, winter migrants, Western Ghats.

Editor: H. Byju, Coimbatore, Tamil Nadu, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Harisha, M.N. & B.B. Hosetti (2024). Endemicity and diversity of birds of the Kuvempu University Campus, Shivamogga District, Karnataka: an updated checklist. *Journal of Threatened Taxa* 16(11): 26063–26077. https://doi.org/10.11609/jott.9186.16.11.26063-26077

Funding: Self-funded.

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Author contributions: MNH involved in the design of the research, survey, data collection, analysis and write up of the manuscript. BBH contributed in supervision of data collection and manuscript preparation.

Competing interests: The authors declare no competing interests.

Acknowledgements: I thank faculties of the Department of Wildlife and Management and authorities of the Kuvempu University, for granting permission to carry out this study and also thankful to our students, Ms. Amrutha Rajan (AR), Ms. V.S. Dhanyashree (VSD), Mr. Abhishek Pujari (AP), Mr. K.V. Chethan (KVC), Mr. Suraj Channabasu Revadigar (SCR), and Mr. N.J. Karthik (NJK) for their assistance while photographing birds during the field survey and also for contributing some of their valuable bird photographs for this paper.

INTRODUCTION

Birds are the best monitors of environmental changes and serve as ecological indicators to assess habitat quality (Bibby 1999; Morelli et al. 2014). The changes in their composition, population behavior patterns, and reproductive ability have most often been used to assess the long-term effects of habitat fragmentation. Hence, they are good indicators of the ecological status of any given ecosystem (Harisha & Hosetti 2009; Byju et al. 2023).

Bird species composition is highly related to the forest vegetation types and depends on stratification, canopy density, altitude, season, and disturbance (Bilgrami 1995; Das 2008; Jayson & Mathew 2003) and their assemblage structure is affected by changes in habitat either due to natural or anthropogenic disturbances (Duguay et al. 2000; Weakland et al. 2002; Rahayuningsih et al. 2007). Also, a seasonal change in the species diversity of birds occurs in forests due to their foraging behaviour (Robertson & Hackwell 1995). The diversity, abundance, and distribution of birds, particularly of native species, positively correlate with the increasing structural complexity of the vegetation and have an impact on birds in terms of their food, water, and cover (Gregory et al. 2003; Clawges et al. 2008; Rajpar & Zakaria 2011).

Bhadra Wildlife Sanctuary has more than 253 species of birds (Referred to as eBird Field Checklist Bhadra Wildlife Sanctuary - Lakkavalli, Chikkamagaluru, Karnataka, IN; ebird.org/india/hotspot/L3134967). **The** birds of Kuvempu University (KU) Campus have been documented since 1997. The first published systematic bird list from the KU campus reported 94 species (Nazneen et al. 2000). Later, it was updated with the addition of 41 species (Dinesh et al. 2007). Except for these reports, no detailed long-term studies have been done on the diversity of birds in the Campus. In this context, the present study was undertaken to record the status, composition, and endemicity of birds of the KU Campus, Shivamogga.

MATERIAL AND METHODS

Study area

Kuvempu University Campus (13.7359 °N & 75.6324 °E) in the tropical climatic zone is hilly and the elevation gradually varies 680–720 m (Image 1). The campus is located 24 km south-east of Shivamogga City and 4 km north of Bhadra Reservoir, amidst the dry deciduous forest, and is on the edge of Bhadra Tiger Reserve and Bhadra Wildlife Sanctuary. The University campus sprawls over an area of 132.012 ha in that around 56.48% (74.56 ha) of land is forest area (undisturbed area) and the remaining 43.51% (57.45 ha) of land is used for construction & vegetation cover of the university buildings blending naturally with the varied landscape types. Annual rainfall is around 1,000 mm; the average temperature varies 18–36 °C and the average humidity ranges 60–75 %.

Vegetation structure

The predominating vegetation of the campus is typically of southern tropical dry deciduous type with considerable similarities with the Bhadra Wildlife Sanctuary. The campus has a diverse range of habitats including:

1. Evergreen forests with species like bamboo, *Santalam album*, *Ficus religiosa*, *F. benghalensis*, *F.* ***racemosa***, *F. arnottiana*, *Syzygium cumini*, and *Artocarpus* spp.;
2. Deciduous forests dominated by trees like *Terminalia paniculata, Trema micrantha*, *Xylia xylocarpa*, *Anogeissus latifolia*, *Diospyros montana*, *Acacia* spp., *Lagerstroemia* spp., *Radermachera xylocarpa*, *Careya arborea*, *Lannea coromandelica*, and *Bombax ceiba*;
3. Scrublands represented by dense thickets of shrubs like *Lantana camara*, *Carissa carandas*, *Ziziphus oenoplia*, *Catunaregam spinosa* and *Erythrina stricta*;
4. Grasslands with species like *Oplismenus burmannii*, *Arthraxon lanceolatus*, *A. hispida*, *Heteropogon contortus*, *H. ritchiei*, *Apluda mutica*, *Fimbristylis lawiana*, *Ischaemum polytrias*, and *Themeda triandra* are common. While *Parthenium* spp., *Ipomoea* spp., *Amaranthus spinosus*, *Achyranthes aspera*, *Malvastrum tricuspidatum*, *Stachytarpheta indica*, *Cassia tora*, and *Senna tora* are the prominent weeds in the study area;
5. Wetlands include a man-made pond;
6. Rocky outcrops are granite rocky hills and boulders, home to specialized flora and fauna;
7. Riparian zones are areas along water body, supporting plant and animal life; and
8. Urbanized areas with buildings, roads, and other infrastructure.

Sampling methods

The avian checklist was prepared from the intensive survey and opportunistic recordings between January 2007 to February 2015. Bimonthly field surveys were carried out by walking on fixed transects (five transects were 200 m in length with a maximum of 25 m view on with side) in the morning (0600–1000 h) and in the evening

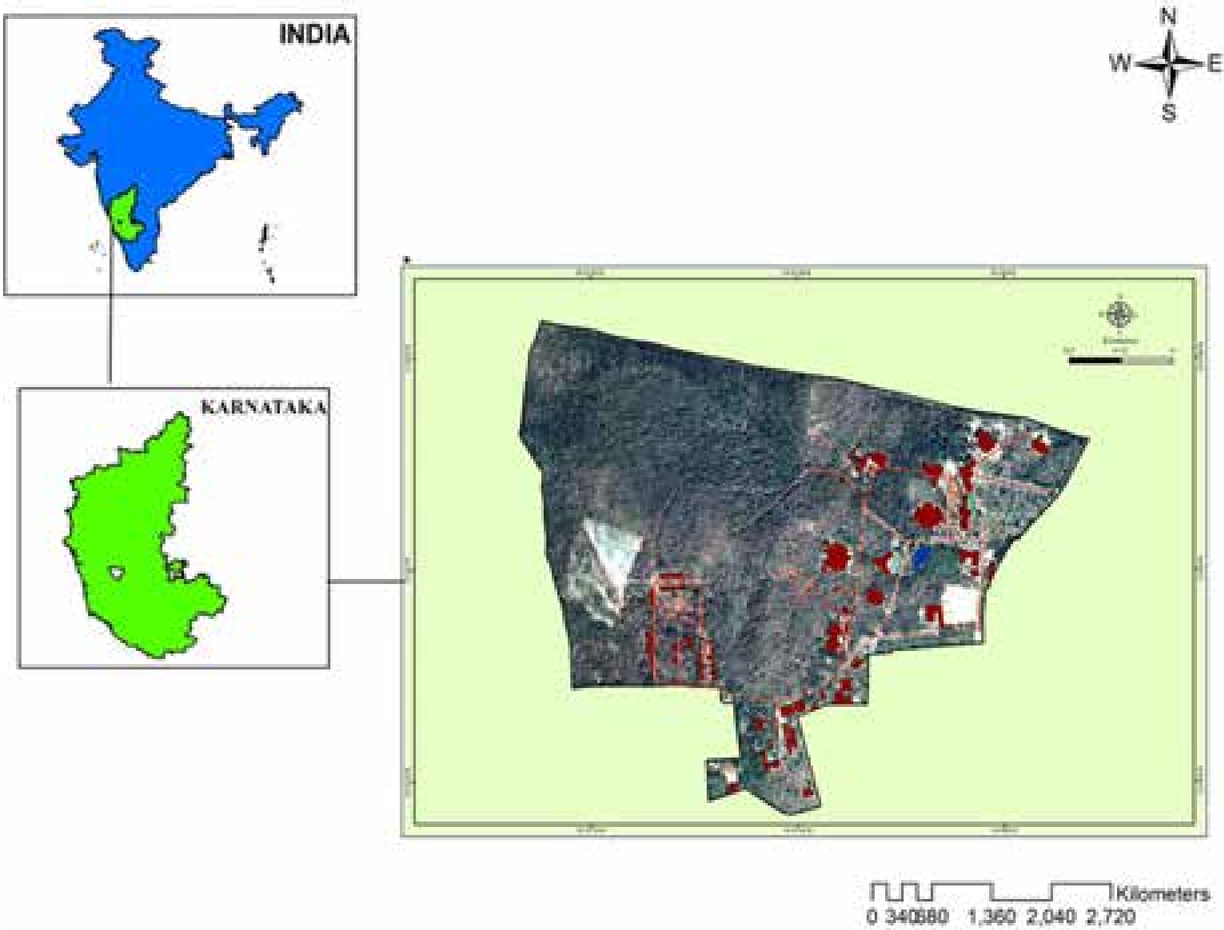

Image 1. Kuvempu University Campus.

(1600–1800 h) when birds were found to be most active (Gupta et al. 2009). A minimum of 30 minutes (speed: 8m/minute) was spent for each transect (Nazneen et al. 2001). The trails were selected and different habitats were covered (i.e., moist, dry deciduous, bamboo forest, and scrub forest). Birds were observed with a field binocular (Olympus) and photographs were taken with a Canon 400D with 75–300 mm lens for further identification. Calls of species were used to confirm the presence of species; however, species were recorded only after their sighting. Birds were identified by using field guides (Ali & Ripley 1983; Grimmett et al. 2011). Standardized common, and scientific nomenclature are following Praveen et al. (2021b & 2024). The residential status of birds was categorized as resident and winter visitors were assigned strictly regarding the study area based on the presence or absence method (Ali & Ripley 1987). The status of threatened categories was adopted from the IUCN Red List (IUCN 2019). Species richness was calculated as the total number of bird species observed in the study area.

RESULT AND DISCUSSION

Diversity and composition of avifauna

The updated checklist of birds at Kuvempu University Campus including present and past records, now contains 229 species, belonging to 16 orders and 62 families, which accounts for 42.17% of the 543 bird species of Karnataka (Praveen et al. 2021a) (Table 1). The present study alone reported 107 species new to the earlier reports. The past surveys in 1997–2000 (Nazneen et al. 2001) reported 94 species (of which 88 were new and six were not reported in the current study), and later in 2000–2001 (Dinesh et al. 2007) which was then updated by addition of 41 species, (of which 34 were new, three species such as Indian House Swift *Apus affinis*), Black-hooded Oriole *Oriolus xanthornus* and Ashy Woodswallow *Artamus fuscus* which were retaken from Nazneen et al. (2007) and four were not reported in the current survey to that of the earlier survey. Since then, more species have been added to the campus avifauna, and more information is available on species and their status. Highlights of the present survey include 107 new records of species to the area, 122 common species (88 reported by Nazneen et al. (2001), and 34 by Dinesh et al. (2007)) to that of the earlier surveys, while 11 species from the past were not reported.

Accipitridae exhibited the highest species richness (16 species), followed by Muscicapidae (14 species), Picidae (11 species), Columbidae (9 species), Strigidae (8 species), Cuculidae, Alaudidae, Sturnidae, & Motacillidae (7 species each), and Phasianidae, Estrildidae, Nectariniidae, Cisticolidae, and Dicruridae (6 species) (Table 1). Several other studies have also found a similar pattern of dominance of Accipitridae from different protected areas in India, i.e., from Lakkavalli Range Forest, Bhadra Wildlife Sanctuary, Chikkamagaluru (Harisha & Hosetti 2009), Sharavathy landscape, Shivamogga (Barve & Warrier 2013), Daroji Sloth Bear Sanctuary, Ballari, Karnataka (Harisha et al. 2021).

Residential status

The analysis of data on the residential status of avifauna revealed that 39 species were winter visitors, whereas, the remaining 190 species were residents, accounting for 17% and 83%, respectively.

Endemism

Alterations in the land use pattern of the forest patches throughout the Western Ghats have triggered the decline in the diversity of endemic bird species (Nihara et al. 2007). The campus also helps in the conservation of endemic species; in the present study 69 species endemic to the Indian Subcontinent were recorded, of which 14 species (Image 6–17) such as Grey-fronted Green Pigeon *Treron affinis*, Malabar Grey Hornbill *Ocyceros griseus*, Malabar Barbet *Psilopogon malabaricus*, White-cheeked Barbet *Psilopogon viridis*, Malabar Parakeet *Psittacula columboides*, Malabar Woodshrike *Tephrodornis sylvicola*, Malabar Lark *Galerida malabarica*, Grey-headed Bulbul *Brachypodius priocephalus*, Rufous Babbler *Argya subrufa*, Malabar Starling *Sturnia blythii*, Nilgiri Flowerpecker *Dicaeum concolor*, Crimson-backed Sunbird *Leptocoma minima*, Malabar Flameback *Chrysocolaptes socialis*, and Vigors's Sunbird *Aethopyga vigorsii* are endemic to the Western Ghats and the Indian subcontinent (Jathar & Rahmani 2006; Rasmussen & Anderton 2012; Praveen et al. 2021b, 2024) (Table 1).

IUCN Red List status

The study also revealed that the campus also supports a few threatened species such as the 'Endangered' Egyptian Vulture *Neophron percnopterus*, 'Vulnerable' Woolly-necked Stork *Ciconia episcopus*, and 'Near Threatened' species such as Black-headed Ibis *Threskiornis melanocephalus*, Pallid Harrier *Circus macrourus*, Malabar Pied Hornbill *Anthracoceros coronatus*, & Grey-headed Bulbul *Microtarsus priocephalus* were recorded from deciduous forest patch in the campus indicating their conservation significance. All the remaining species (223) are of 'Least Concern' (IUCN 2019) (Table 1).

Interesting absences

The current list of birds observed does not include 11 species of birds which were previously reported (Nazneen et al. 2001; Dinesh 2007), of which eight were wetland birds such as Little Grebe *Tachybaptus ruficollis*, Intermediate Egret *Ardea intermedia*, Great Egret *Ardea alba,* Wood Sandpiper *Tringa glareola*, Fantail Snipe *Gallinago gallinago*, Common Tern *Sterna hirundo*, Cotton Teal *Nettapus coromandelianus*, and Common Coot *Fulica atra*. The absence of these wetland birds could be due to increased anthropogenic pressure like habitat alternations, and improper, unscientific trenching and drainage systems around the water body. The area of the man-made pond is about 0.30 ha (Image 2). Earlier it was an undisturbed earthen pond and the only source of water was rainwater that came from the surrounding forest. In 2004, it was converted into a stagnant concrete pond, even though the study area is in a dry deciduous forest. Due to an unscientific trenching and drainage system, the inflow of water to the pond from the surrounding catchment area in the forest decreased, and rainwater instead of percolating into the pond flowed out of the area. Consequently, there was low retention of water in the pond and it dried at the end of winter and during summer, leading to a harsh habitat for the animals to survive (Harisha & Hosetti 2021) (Image 3).

The water birds, generally at or near the top of most wetland food chains, are highly susceptible to habitat disturbances and are therefore good indicators of the general condition of wetland habitats (Kushlan 1992; Jayson & Mathew 2002). The study also revealed that the pond has a pathway that the university staff and students use for regular walking, jogging, or exercising in the morning (0600–0800 h) and evening (1600–1900 h), posing threats to the assemblage of wetland birds.

The other three bird species not observed in the

Table 1. Updated checklist of birds of Kuvempu University Campus, Shivamogga, Karnataka.

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
	1. Order: Galliformes							
	1. Family: Phasianidae							
1	Jungle Bush Quail	*Perdicula asiatica* Latham, 1790			+	LC	R	IS
2	Rock Bush Quail	*Perdicula argoondah* Sykes, 1832			+	LC	R	IS
3	Grey Francolin	*Ortygornis pondicerianus* J.F. Gmelin, 1789			+	LC	R	
4	Red Spurfowl	*Galloperdix spadicea* J.F. Gmelin, 1789			+	LC	R	IS
5	Grey Junglefowl	*Gallus sonneratii* Temminck, 1813	+			LC	R	IS
6	Indian Peafowl	*Pavo cristatus* Linnaeus, 1758	+			LC	R	IS
	2. Order: Columbiformes							
	1. Family: Columbidae							
7	Rock Pigeon	*Columba livia* J.F. Gmelin, 1789	+			LC	R	
8	Spotted Dove	*Spilopelia chinensis* Scopoli, 1786	+			LC	R	
9	Oriental Turtle Dove	*Streptopelia orientalis*	+			LC	R	
10	Eurasian Collared Dove	*Streptopelia decaocto* Frivaldszky, 1838	+			LC	R	
11	Asian Emerald Dove	*Chalcophaps indica* Linnaeus, 1758			+	LC	R	
12	Laughing Dove	*Spilopelia senegalensis* Linnaeus, 1766			+	LC	R	
13	Grey-fronted Green Pigeon	*Treron affinis* Jerdon, 1840			+	LC	R	IS/WG
14	Yellow-footed Green Pigeon	*Treron phoenicopterus* Latham, 1790			+	LC	R	
15	Green Imperial Pigeon	*Ducula aenea* Linnaeus, 1766			+	LC	R	
	3. Order: Caprimulgiformes							
	1. Family: Caprimulgidae							
16	Jerdon's Nightjar	*Caprimulgus atripennis* Jerdon, 1845			+	LC	R	IS
17	Indian Nightjar	*Caprimulgus asiaticus* Latham, 1790			+	LC	R	
18	Jungle Nightjar	*Caprimulgus indicus* Latham, 1790			+	LC	R	IS
19	Savanna Nightjar	*Caprimulgus affinis* Horsfield, 1821			+	LC	R	
	2. Family: Apodidae							
20	Little Swift	*Apus affinis* J.E. Gray, 1830	+	+		LC	R	
21	Asian Palm Swift	*Cypsiurus balasiensis* J. E. Gray, 1829	+			LC	R	
	3. Family: Hemiprocnidae							
22	Crested Treeswift	*Hemiprocne coronata* Tickell, 1833			+	LC	R	
	4. Order: Cuculiformes							
	1. Family: Cuculidae							
23	Asian Koel	*Eudynamys scolopaceus* Linnaeus, 1758	+			LC	R	
24	Greater Coucal	*Centropus sinensis* Stephens, 1815	+			LC	R	
25	Blue-faced Malkoha	*Phaenicophaeus viridirostris* Jerdon, 1840	+			LC	R	IS
26	Common Hawk Cuckoo	*Hierococcyx varius* Vahl, 1797			+	LC	R	IS
27	Square-tailed Drongo Cuckoo	*Surniculus lugubris* Horsfield, 1821			+	LC	R	
28	Pied Cuckoo	*Clamator jacobinus* Boddaert, 1783			+	LC	R	
29	Grey-bellied Cuckoo	*Cacomantis passerines* Vahl, 1797			+	LC	R	IS
	5. Order: Gruiformes							
	1. Family: Rallidae							
30	White-breasted Waterhen	*Amaurornis phoenicurus* Pennant, 1769	+			LC	R	

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
	6. Order: Pelecaniformes							
	1. Family: Ciconiidae							
31	Woolly-necked Stork	*Ciconia episcopus* Boddaert, 1783		+		VU	R	
	2. Family: Ardeidae							
32	Little Egret	*Egretta garzetta* Linnaeus, 1766	+			LC	R	
33	Western Cattle Egret	*Bubulcus ibis* Linnaeus, 1758	+			LC	R	
34	Indian Pond Heron	*Ardeola grayii* Sykes, 1832	+			LC	R	
35	Black crowned Night Heron	*Nycticorax nycticorax* Linnaeus, 1758			+	LC	R	
	3. Family: Threskiornithidae							
36	Red-naped Ibis	*Pseudibis papillosa* Temminck, 1824			+	LC	R	IS
37	Black-headed Ibis	*Threskiornis melanocephalus* Latham, 1790		+		NT	R	
	4. Family: Phalacrocoracidae							
38	Little Cormorant	*Microcarbo niger* Vieillot, 1817	+			LC	R	
	7. Order: Charadriiformes							
	1. Family: Charadriidae							
39	Yellow-wattled Lapwing	*Vanellus malabaricus* Boddaert, 1783	+			LC	R	IS
40	Red-wattled Lapwing	*Vanellus indicus* Boddaert, 1783	+			LC	R	
41	Little Ringed Plover	*Charadrius dubius* Scopoli, 1786	+			LC	W	
	2. Family: Scolopacidae							
42	Common Sandpiper	*Actitis hypoleucos* Linnaeus, 1758	+			LC	W	
	3. Family: Turnicidae							
43	Barred Buttonquail	*Turnix suscitator* J.F. Gmelin, 1789			+	LC	R	
	8. Order: Accipitriformes							
	1. Family: Accipitridae							
44	Oriental Honey Buzzard	*Pernis ptilorhynchus* Temminck, 1821			+	LC	R	
45	White-eyed Buzzard	*Butastur teesa* Franklin, 1831			+	LC	R	
46	Black-winged Kite	*Elanus caeruleus* Desfontaines, 1789		+		LC	R	
47	Short-toed Snake Eagle	*Circaetus gallicus* J.F. Gmelin, 1788			+	LC	R	
48	Black Eagle	*Ictinaetus malaiensis* Temminck, 1822			+	LC	R	
49	Booted Eagle	*Hieraaetus pennatus* J.F. Gmelin, 1788			+	LC	W	
50	Changeable Hawk Eagle	*Nisaetus cirrhatus* J.F. Gmelin, 1788		+		LC	R	
51	Crested Serpent Eagle	*Spilornis cheela* Latham, 1790			+	LC	R	
52	Black Kite	*Milvus migrans* Boddaert, 1783	+			LC	R	
53	Brahminy Kite	*Haliastur Indus* Boddaert, 1783	+			LC	R	
54	Shikra	*Accipiter badius* J.F. Gmelin, 1788	+			LC	R	
55	Besra	*Accipiter virgatus*Temminck, 1822			+	LC	R	
56	Egyptian Vulture	*Neophron percnopterus* Linnaeus, 1758	+			EN	R	
57	Pallid Harrier	*Circus macrourus* S.G. Gmelin, 1770			+	NT	W	
58	Western Marsh Harrier	*Circus aeruginosus* Linnaeus, 1758			+	LC	W	
59	Montagu's Harrier	*Circus pygargus* Linnaeus, 1758			+	LC	W	
	9. Order: Strigiformes							
	1. Family: Tytonidae							
60	Common Barn Owl	*Tyto alba* Scopoli, 1769			+	LC	R	

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
	2. Family: Strigidae							
61	Indian Eagle Owl	*Bubo bengalensis* Franklin, 1831			+	LC	R	IS
62	Indian Scops Owl	*Otus bakkamoena* Pennant, 1769			+	LC	R	IS
63	Brown Wood Owl	*Strix leptogrammica* Temminck, 1832			+	LC	R	
64	Mottled Wood Owl	*Strix ocellata* Lesson, 1839			+	LC	R	IS
65	Brown Boobook	*Ninox scutulata* Raffles, 1822			+	LC	R	
66	Brown Fish Owl	*Ketupa zeylonensis* J.F. Gmelin, 1788			+	LC	R	
67	Jungle Owlet	*Glaucidium radiatum* Tickell, 1833			+	LC	R	IS
68	Spotted Owlet	*Athene brama* Temminck, 1821	+			LC	R	
	10. Order: Trogoniformes							
	1. Family: Trogonidae							
69	Malabar Trogon	*Harpactes fasciatus* Pennant, 1769			+	LC	R	IS
	11. Order: Bucerotiformes							
	1. Family: Bucerotidae							
70	Indian Grey Hornbill	*Ocyceros birostris* Scopoli, 1786	+			LC	R	IS
71	Malabar Grey Hornbill	*Ocyceros griseus* Latham, 1790		+		LC	R	IS/WG
72	Malabar Pied Hornbill	*Anthracoceros coronatus* Boddaert, 1783	+			NT	R	IS
	2. Family: Upupidae							
73	Common Hoopoe	*Upupa epops* Linnaeus, 1758	+			LC	R	
	12. Order: Piciformes							
	1. Family: Picidae							
74	Heart-spotted Woodpecker	*Hemicircus canente* Lesson, 1832			+	LC	R	
75	Brown-capped pygmy Woodpecker	*Yungipicus nanus* Vigors, 1832			+	LC	R	IS
76	Yellow-crowned Woodpecker	*Leiopicus mahrattensis* Latham, 1801			+	LC	R	
77	Common Flameback	*Dinopium javanense* Ljungh, 1797			+	LC	R	
78	Black-rumped Flameback	*Dinopium benghalense* Linnaeus, 1758	+			LC	R	IS
79	Greater Flameback	*Chrysocolaptes guttacristatus* Tickell, 1833			+	LC	R	
80	Malabar Flameback	*Chrysocolaptes socialis* Koelz, 1939			+	LC	R	IS/ WG
81	White-naped Woodpecker	*Chrysocolaptes festivus* Boddaert, 1783			+	LC	R	IS
82	White-bellied Woodpecker	*Dryocopus javensis* Horsfield, 1821			+	LC	R	
83	Lesser Yellownape	*Picus chlorolophus* Vieillot, 1818			+	LC	R	
84	Rufous Woodpecker	*Micropternus brachyurus* Vieillot, 1818			+	LC	R	
	2. Family: Ramphastidae							
85	White-cheeked Barbet	*Psilopogon viridis* Boddaert, 1783	+			LC	R	IS/WG
86	Brown-headed Barbet	*Psilopogon zeylanicus* J.F. Gmelin, 1788	+			LC	R	IS
87	Coppersmith Barbet	*Psilopogon haemacephalus* Statius Muller, 1776	+			LC	R	
88	Malabar Barbet	*Psilopogon malabaricus* Blyth, 1847			+	LC	R	IS/ WG
	13. Order: Coraciiformes							
	1. Family: Meropidae							
89	Blue-bearded Bee-eater	*Nyctyornis athertoni* Jardine & Selby, 1828			+	LC	R	
90	Green Bee-eater	*Merops orientalis* Latham, 1801	+			LC	R	
91	Chestnut-headed Bee-eater	*Merops leschenaultia* Vieillot, 1817	+			LC	R	

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
92	Blue-tailed Bee-eater	*Merops philippinus* Linnaeus, 1767	+			LC	W	
	2. Family: Coraciidae							
93	Indian Roller	*Coracias benghalensis* Linnaeus, 1758	+			LC	R	
	3. Family: Alcedinidae							
94	Common Kingfisher	*Alcedo atthis* Linnaeus, 1758	+			LC	R	
95	White-throated Kingfisher	*Halcyon smyrnensis* Linnaeus, 1758	+			LC	R	
96	Pied Kingfisher	*Ceryle rudis* Linnaeus, 1758	+			LC	R	
	14. Order: Falconiformes							
	1. Family: Falconidae							
97	Common Kestrel	*Falco tinnunculus* Linnaeus, 1758		+		LC	W	
	15. Order: Psittaciformes							
	1. Family: Psittaculidae							
98	Vernal Hanging Parrot	*Loriculus vernalis* Sparrman, 1787	+			LC	R	
99	Rose-ringed Parakeet	*Psittacula krameri* Scopoli, 1769	+			LC	R	
100	Plum-headed Parakeet	*Psittacula cyanocephala* Linnaeus, 1766	+			LC	R	IS
101	Malabar Parakeet	*Psittacula columboides* Vigors, 1830			+	LC	R	IS/ WG
	16. Order: Passeriformes							
	1. Family: Pittidae							
102	Indian Pitta	*Pitta brachyura* Linnaeus, 1766		+		LC	W	IS
	2. Family: Campephagidae							
103	Black-headed Cuckooshrike	*Lalage melanoptera* Ruppell, 1839		+		LC	R	
104	Large Cuckooshrike	*Coracina macei* R. Lesson, 1831	+			LC	R	
105	Orange Minivet	*Pericrocotus flammeus* J.R. Forster, 1781	+			LC	R	IS
106	Small Minivet	*Pericrocotus cinnamomeus* Linnaeus, 1766	+			LC	R	
	3. Family: Oriolidae							
107	Indian Golden Oriole	*Oriolus kundoo* Sykes, 1832	+			LC	W	
108	Black-hooded Oriole	*Oriolus xanthornus* Linnaeus, 1758	+	+		LC	R	
109	Black-naped Oriole	*Oriolus chinensis* Linnaeus, 1766		+		LC	W	
	4. Family: Artamidae							
110	Ashy Woodswallow	*Artamus fuscus* Vieillot, 1817	+	+		LC	R	
	5. Family: Vangidae							
111	Common Woodshrike	*Tephrodornis pondicerianus* J.F. Gmelin, 1789			+	LC	R	
112	Large Woodshrike	*Tephrodornis virgatus* Timminck, 1824		+		LC	R	
113	Malabar Woodshrike	*Tephrodornis sylvicola* Jerdon, 1839			+	LC	R	IS/WG
114	Bar-winged Flycatcher-shrike	*Hemipus picatus* Sykes, 1832		+		LC	R	
	6. Family: Aegithinidae							
115	Common Iora	*Aegithina tiphia* Linnaeus, 1758	+			LC	R	
	7. Family: Dicruridae							
116	Black Drongo	*Dicrurus macrocercus* Vieillot, 1817	+			LC	R	
117	Ashy Drongo	*Dicrurus leucophaeus* Vieillot, 1817	+			LC	W	
118	Bronzed Drongo	*Dicrurus aeneus* Vieillot, 1817			+	LC	R	
119	White-bellied Drongo	*Dicrurus caerulescens* Linnaeus, 1758	+			LC	R	IS
120	Greater Racket-tailed Drongo	*Dicrurus paradiseus* Linnaeus, 1766		+		LC	R	

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
121	Hair-crested Drongo	*Dicrurus hottentottus* Linnaeus, 1766			+	LC	R	
	8. Family: Rhipiduridae							
122	White-browed Fantail	*Rhipidura aureola* Lesson, 1831			+	LC	R	
123	Spot-breasted Fantail	*Rhipidura albogularis* Lesson, 1832			+	LC	R	IS
	9. Family: Laniidae							
124	Brown Shrike	*Lanius cristatus* Linnaeus, 1758	+			LC	W	
125	Long-tailed Shrike	*Lanius schach* Linnaeus, 1758	+			LC	R	
126	Bay-backed Shrike	*Lanius vittaus* Valenciennes, 1826			+	LC	R	
127	Great Grey Shrike	*Lanius excubitor* Linnaeus, 1758			+	LC	R	
	10. Family: Corvidae							
128	Rufous Treepie	*Dendrocitta vagabunda* Latham, 1790	+			LC	R	
129	House Crow	*Corvus splendens* Vieillot, 1817	+			LC	R	
130	Large-billed Crow	*Corvus macrorhynchos* Wagler, 1827	+			LC	R	
	11. Family: Monarchidae							
131	Black-naped Monarch	*Hypothymis azurea* Boddaert, 1783			+	LC	R	
132	Indian Paradise-flycatcher	*Terpsiphone paradise* Linnaeus, 1758	+			LC	R	
	12. Family: Dicaeidae							
133	Thick-billed Flowerpecker	*Dicaeum agile* Tickell, 1833			+	LC	R	
134	Pale-billed Flowerpecker	*Dicaeum erythrorhynchos* Latham, 1790	+			LC	R	
135	Nilgiri Flowerpecker	*Dicaeum concolor* Jerdon, 1840			+	LC	R	IS/WG
	13. Family: Nectariniidae							
136	Purple-rumped Sunbird	*Leptocoma zeylonica* Linnaeus, 1766	+			LC	R	IS
137	Crimson-backed Sunbird	*Leptocoma minima* Sykes, 1832		+		LC	R	IS/ WG
138	Purple Sunbird	*Cinnyris asiaticus* Latham, 1790	+			LC	R	
139	Loten's Sunbird	*Cinnyris lotenius* Linnaeus, 1766		+		LC	R	IS
140	Vigors's Sunbird	*Aethopyga vigorsii* Sykes, 1832			+	LC	R	IS/ WG
141	Little Spiderhunter	*Arachnothera longirostra* Latham, 1790			+	LC	R	
	14. Family: Irenidae							
142	Golden-fronted Leafbird	*Chloropsis aurifrons* Temminck, 1829	+			LC	R	
143	Jerdon’s Leafbird	*Chloropsis jerdoni* Blyth, 1844			+	LC	R	IS
	15. Family: Ploceidae							
144	Baya Weaver	*Ploceus philippinus* Linnaeus, 1766		+		LC	R	
145	Streaked Weaver	*Ploceus manyar* Horsfield, 1821		+		LC	R	
	16. Family: Estrildidae							
146	Red Munia	*Amandava amandava* Linnaeus, 1758			+	LC	R	
147	Tricoloured Munia	*Lonchura malacca* Linnaeus, 1766		+		LC	R	IS
148	Indian Silverbill	*Euodice malabarica* Linnaeus, 1758	+			LC	R	
149	Scaly-breasted Munia	*Lonchura punctulata* Linnaeus, 1758	+			LC	R	
150	White-rumped Munia	*Lonchura striata* Linnaeus, 1766	+			LC	R	
151	Black-throated Munia	*Lonchura kelaarti* Jerdon, 1863		+		LC	R	IS
	17. Family: Passeridae							
152	House Sparrow	*Passer domesticus* Linnaeus, 1758	+			LC	R	
153	Yellow-throated Sparrow	*Gymnoris xanthocollis* E. Burton, 1838			+	LC	R	

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
	18. Family: Motacillidae							
154	Paddyfield Pipit	*Anthus rufulus* Vieillot, 1818	+			LC	R	
155	Tree Pipit	*Anthus trivialis* Linnaeus, 1758			+	LC	W	
156	Olive-backed Pipit	*Anthus hodgsoni* Richmond, 1907			+	LC	W	
157	White-browed Wagtail	*Motacilla maderaspatensis* J.F. Gmelin, 1789	+			LC	R	IS
158	Western Yellow Wagtail	*Motacilla flava* Linnaeus, 1758			+	LC	W	
159	Grey Wagtail	*Motacilla cinerea* Tunstall, 1771	+			LC	W	
160	Forest Wagtail	*Dendronanthus indicus* J.F. Gmelin, 1789			+	LC	W	
	19. Family: Fringillidae							
161	Common Rosefinch	*Carpodacus erythrinus* Pallas, 1770		+		LC	W	
	20. Family: Paridae							
162	Indian Black-lored Tit	*Machlolophus aplonotus* Blyth, 1847		+		LC	R	IS
163	Cinereous Tit	*Parus cinereus* Vieillot, 1818	+			LC	R	
	21. Family: Alaudidae							
164	Ashy-crowned Sparrow Lark	*Eremopterix griseus* Scopoli, 1786	+			LC	R	IS
165	Singing Bushlark	*Mirafra javanica* Horsfield, 1821		+		LC	R	
166	Indian Bushlark	*Mirafra erythroptera* Blyth, 1845			+	LC	R	IS
167	Jerdon's Bushlark	*Mirafra affinis* Blyth, 1845			+	LC	R	IS
168	Sykes's Lark	*Galerida deva* Sykes, 1832		+		LC	R	IS
169	Malabar Lark	*Galerida malabarica* Scopoli, 1786		+		LC	R	IS/ WG
170	Rufous-tailed Lark	*Ammomanes phoenicura* Franklin, 1831			+	LC	R	IS
	22. Family: Cisticolidae							
171	Grey-breasted Prinia	*Prinia hodgsonii* Blyth, 1844		+		LC	R	
172	Zitting Cisticola	*Cisticola juncidis* Rafinesque, 1810			+	LC	R	
173	Ashy Prinia	*Prinia socialis* Sykes, 1832	+			LC	R	IS
174	Plain Prinia	*Prinia inornata* Sykes, 1832	+			LC	R	
175	Jungle Prinia	*Prinia sylvatica* Jerdon, 1840			+	LC	R	IS
176	Common Tailorbird	*Orthotomus sutorius* Pennant, 1769	+			LC	R	
	23. Family: Acrocephalidae							
177	Blyth's Reed Warbler	*Acrocephalus dumetorum* Blyth, 1849		+		LC	W	
178	Clamorous Reed Warbler	*Acrocephalus stentoreus* Hemprich & Ehrenberg, 1833			+	LC	W	
179	Booted Warbler	*Iduna caligata* Lichtenstein, 1823		+		LC	W	
	24. Family: Hirundinidae							
180	Dusky Crag Martin	*Ptyonoprogne concolor* Sykes, 1832			+	LC	R	
181	Barn Swallow	*Hirundo rustica* Linnaeus, 1758	+			LC	W	
182	Wire-tailed Swallow	*Hirundo smithii* Leach, 1818	+			LC	R	
183	Red-rumped Swallow	*Cecropis daurica* Laxmann, 1769		+		LC	W	
184	Streak-throated Swallow	*Petrochelidon fluvicola* Blyth, 1855			+	LC	R	
	25. Family: Pycnonotidae							
185	Red-whiskered Bulbul	*Pycnonotus jocosus* Linnaeus, 1758	+			LC	R	
186	Red-vented Bulbul	*Pycnonotus cafer* Linnaeus, 1766	+			LC	R	
187	White-browed Bulbul	*Pycnonotus luteolus* Lesson, 1841		+		LC	R	IS
188	Yellow-browed Bulbul	*Acritillas indica* Jerdon, 1839			+	LC	R	IS

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
189	Grey-headed Bulbul	*Microtarsus priocephalus* Jerdon, 1839			+	NT	R	IS/WG
	26. Family: Phylloscopidae							
190	Green Warbler	*Phylloscopus nitidus* Blyth, 1843			+	LC	W	
191	Greenish Warbler	*Phylloscopus trochiloides* Sundevall, 1837		+		LC	W	
192	Tickell's Leaf Warbler	*Phylloscopus affinis* Tickell, 1833			+	LC	W	
193	Large-billed Leaf Warbler	*Phylloscopus magnirostris* Blyth, 1843		+		LC	W	
	27. Family: Sylviidae							
194	Yellow-eyed Babbler	*Chrysomma sinense* J.F. Gmelin, 1789			+	LC	R	
	28. Family: Zosteropidae							
195	Indian White-eye	*Zosterops palpebrosus* Temminck, 1824		+		LC	R	
	29. Family: Timaliidae							
196	Indian Scimitar Babbler	*Pomatorhinus horsfieldii* Sykes, 1832		+		LC	R	IS
197	Tawny-bellied Babbler	*Dumetia hyperythra* Franklin, 1831			+	LC	R	IS
198	Dark-fronted Babbler	*Dumetia atriceps* Jerdon, 1839		+		LC	R	IS
	30. Family: Pellorneidae							
199	Puff-throated Babbler	*Pellorneum ruficeps* Swainson, 1832		+		LC	R	
	31. Family: Leiothrichidae							
200	Common Babbler	*Argya caudata* Dumont, 1823			+	LC	R	IS
201	Rufous Babbler	*Argya subrufa* Jerdon, 1839	+			LC	R	IS/ WG
202	Jungle Babbler	*Argya striata* Dumont, 1823	+			LC	R	IS
203	Large Grey Babbler	*Argya malcolmi* Sykes, 1832	+			LC	R	IS
204	Yellow-billed Babbler	*Argya affinis* Jerdon, 1845			+	LC	R	IS
	32. Family: Sittidae							
205	Velvet-fronted Nuthatch	*Sitta frontalis* Swainson, 1820			+	LC	R	
206	Indian Nuthatch	*Sitta castanea* Lesson, 1830			+	LC	R	IS
	33. Family: Sturnidae							
207	Chestnut-tailed Starling	*Sturnia malabarica* J.F. Gmelin, 1789	+			LC	W	
208	Malabar Starling	*Sturnia blythii* Jerdon, 1845			+	LC	R	IS/ WG
209	Brahminy Starling	*Sturnia pagodarum* J.F. Gmelin, 1789	+			LC	R	
210	Rosy Starling	*Pastor roseus* Linnaeus, 1758			+	LC	W	
211	Common Myna	*Acridotheres tristis* Linnaeus, 1766	+			LC	R	
212	Jungle Myna	*Acridotheres fuscus* Wagler, 1827	+			LC	R	
213	Southern Hill Myna	*Gracula indica* Cuvier, 1829			+	LC	R	IS
	34. Family: Muscicapidae							
214	Indian Robin	*Copsychus fulicatus* Linnaeus, 1766	+			LC	R	IS
215	Oriental Magpie Robin	*Copsychus saularis* Linnaeus, 1758	+			LC	R	
216	Bluethroat	*Luscinia svecica* Linnaeus, 1758			+	LC	W	
217	White-rumped Shama	*Copsychus malabaricus* Scopoli, 1786			+	LC	R	
218	Asian Brown Flycatcher	*Muscicapa dauurica* Pallas, 1811			+	LC	W	
219	Brown-breasted Flycatcher	*Muscicapa muttui* E.L. Layard, 1854			+	LC	W	
220	Tickell's Blue Flycatcher	*Cyornis tickelliae* Blyth, 1843			+	LC	R	
221	Verditer Flycatcher	*Eumyias thalassinus* Swainson, 1838			+	LC	W	
222	Red-breasted Flycatcher	*Ficedula parva* Bechstein, 1792			+	LC	W	
223	Blue-capped Rock Thrush	*Monticola cinclorhyncha* Vigors, 1831			+	LC	W	

	Common name/ Order/ Family	Scientific name	Nazneen et al. 2000	Dinesh et al. 2007	Harisha & Hosetti	IUCN Red List status	Residential status	Endemicity
224	Blue Rock Thrush	*Monticola solitarius* Linnaeus, 1758			+	LC	W	
225	Black Redstart	*Phoenicurus ochruros* S.G. Gmelin, 1774			+	LC	W	
226	Pied Bushchat	*Saxicola caprata* Linnaeus, 1766	+			LC	R	
227	Siberian Stonechat	*Saxicola maurus* Pallas, 1773			+	LC	W	
	35. Family: Turdidae							
228	Indian Blackbird	*Turdus simillimus* Jerdon, 1839			+	LC	R	IS
229	Orange-headed Thrush	*Geokichla citrina* Latham, 1790			+	LC	R	

LC—Least Concern | NT—Near Threatened | VU—Vulnerable | R—Resident | W—Winter Migrant | IS—Endemic to Indian Subcontinent | WG—Endemic to Western Ghats.

current survey include Crimson Sunbird *Aethopyga siparaja*, White-throated Fantail *Rhipidura albicollis*, and Eurasian Golden Oriole *Oriolus oriolus*. However, subspecies, such as Vigors's Sunbird *Aethopyga vigorsii*, and Spot-breasted Fantail *Rhipidura albogularis* have been regularly observed in the campus. The Crimson Sunbird is monotypic, its absence from the study area could be due to a true absence or due to a lack of favorable habitats for the species and because of its restricted home range (distribution range: Himalayan foothills in India, from the west in Himachal Pradesh (Kangra) to the east in Sikkim and Bhutan, south to northern West Bengal, eastern Bihar, eastern Madhya Pradesh, and Odisha (possibly northern Andhra Pradesh), and western Bangladesh (Cheke et al. 2020). The reasons for the absence of the polytypic White-throated Fantail could also be due to its restricted home range, i.e., central Himalaya (Nepal and Sikkim), and from plains of Bangladesh to eastern India (lower West Bengal) (Boles 2020). However, the Eurasian Golden Oriole *Oriolus oriolus* is a monotypic species and was formerly considered to be a subspecies of the Indian Golden Oriole *Oriolus kundoo* (Rasmussen & Anderton 2005), absence from the study area could be due to its limited distribution, which includes western, central, and southern Europe (south from southern Finland), and northern Africa (Morocco to Tunisia), east to Altai Mountains (Southern Siberia, western Mongolia, and extreme northwastern China), and south to northern Iran and locally to northern Arabian Peninsula; non-breeding in Sub-Saharan Africa (Walther & Jones 2020).

Birds with breeding activity

The campus supported breeding activities of a few species of birds such as the House Crow *Corvus splendens*, followed by the Scaly-breasted Munia *Lonchura punctulata* and White-rumped Munia *L. striata*, Blue-faced Malkoha *Phaenicophaeus viridirostris*, Greater Coucal *Centropus sinensis*, Changeable Hawk Eagle *Nisaetus cirrhatus*, Red-whiskered Bulbul *Pycnonotus jocosus*, Red-vented Bulbuls *P. cafer*, Purple Sunbird *Cinnyris asiaticus*, Purple-rumped Sunbirds *Leptocoma zeylonica*, Barn Owl *Tyto alba*, Spotted Owlet *Athene brama*, and Indian Paradise Flycatcher *Terpsiphone paradisi*. The presence of an old nest of White-rumped Munia *Lonchura striata*, besides the active nest on the same *Artocorpus* sp., indicates that the bird used the site for nesting year after year. Earlier reports (Nazneen et al. 2001; Dinesh et al. 2007) corroborating with the present findings indicate that the flat terrain with open sun-baked areas behind the employee quarters might be a traditional breeding ground for many bird species such as Indian Peafowl *Pavo cristatus*, Indian Nightjars *Caprimulgus asiaticus*, Red-wattled Lapwing *Vanellus indicus*, and Yellow-wattled Lapwing *V. malabaricus*.

The landscape with diverse habitat types provides additional opportunities for diverse avian assemblages (Karr & Roth 1971). The present study shows that the KU campus represents a sound avifaunal diversity as it lies in an important ecological zone, i.e., Bhadra Wildlife Sanctuary of the Western Ghats Mountain ranges. Therefore, a variety of habitats and environments of the campus attract and support a variety of bird species. The diversity and distribution of species within a habitat are influenced by the variation in vegetation (MacArthur et al. 1962; Karr & Roth 1971; Pearman 2002). During the flowering and fruiting seasons, the plants like *Ficus arnottiana* (December– April), *F. racemosa* (December–March), *F. benghalensis* (November–January), *F. religiosa* (November–January), *Trema orientalis* (August–January), *Lannea coromandelica* (Januray–July), *Ziziphus oenoplia* (July–January), *Z. mauritiana*

Image 2. Manmade wetland in the Kuvempu University campus estate. © M.N. Harisha.

Image 3. Drainage trench in the forest patch of Kuvempu University Campus. © M.N. Harisha.

Image 4. Forest patch with weeds cleared or uprooted. © M.N. Harisha.

Image 5. Anthropogenic activities in the forest area of Kuvempu University campus. © M.N. Harisha.

(May–June), *Muntingia calabura* (May–June), and *Securinega virosa* (December–March) were in extensive bloom with flowers and fleshy fruits. On the other hand, trees like *Bombax ceiba* (February–May), *Careya arborea* (February–July), *Butea monosperma* (February–April), *Spathodea campanulata* (December–March), and *Peltophorum pterocarpum* (September–November; March–May), though with dry non-edible fruits, were blooming with flowers of bright coloured and fine good quantity of nectar. These plant resources might also attract insects and consequently provide prey resources for insectivorous birds.

Anthropogenic disturbances on forest structure and function are well-established (Bhat & Murali 2001; Chandrashekara et al. 2006) and their impact on overall avifaunal diversity. The present study also revealed the threats to avifaunal habitats due to anthropogenic activities such as habitat alternations, improper drainage systems and land use patterns for new building constructions, road widening, frequent weed clearing, garbage dumping, and pollution (Images 4 & 5). Such disturbances adversely affect habitats and might threaten both resident and migratory bird species.

CONCLUSION

The present study revealed that varied habitats and vegetation structures on the campus attract and support a variety of resident, migrant, endemic, and threatened bird species. This reiterates the significance of academic campuses in conserving biological diversity at a regional level. The avifauna and their habitat are adversely impacted due to intensive anthropogenic activities. And more scientific studies are required to understand the season-wise population dynamics of birds in this area

Image 6. Grey-fronted Green Pigeon *Treron affinis* © M.N. Harisha.

Image 7. Malabar Barbet *Psilopogon malabaricus* © N.J. Karthik.

Image 8. White-cheeked Barbet *Psilopogon viridis* © M.N. Harisha.

Image 9. Malabar Parakeet *Psittacula columboides* © M.N. Harisha.

Image 10. Malabar Woodshrike *Tephrodornis sylvicola* © N.J. Karthik.

Image 11. Malabar Lark *Galerida malabarica* © M.N. Harisha.

Image 12. Grey-headed Bulbul *Brachypodius priocephalus* © M.N. Harisha.

Image 13. Rufous Babbler *Argya subrufa* © M.N. Harisha.

Image 14. Malabar Starling *Sturnia blythii* © V.S. Dhanyashree.

Image 15. Crimson-backed Sunbird *Leptocoma minima* © M.N. Harisha.

Image 16. Vigors's Sunbird Aethopyga vigorsii © M.N. Harisha.

Image 17. Malabar Pied Hornbill *Anthracoceros coronatus* © M.N. Harisha.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26078–26088

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9329.16.11.26078-26088

#9329 | Received 26 July 2024 | Final received 25 October 2024 | Finally accepted 18 November 2024

OPEN ACCESS

ARTICLE

Unregulated wild orchid trade in Manipur: an analysis of the Imphal Valley markets from the Indo-Burma hotspot

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Abstract: Unsustainable and illegal trade of wild orchids at local and international markets is a well-known conservation issue throughout the world. Local as well as international trade of wild orchids is under-reported and under-researched. The study assessed wild orchids traded in markets of the Imphal Valley, the northeastern Indian state of Manipur in 2022–23. Eighty-two wild orchid species from 33 genera were observed to be traded including the wild orchid species, viz., *Paphiopedilum hirsutissimum*, *Renanthera imschootiana*, and *Vanda coerulea*, protected under the Wildlife (Protection) Act, 1972 of India. The local wild orchid trade in Imphal is unregulated, unchecked and unmonitored, which is a serious concern for the conservation of wild orchid species in Manipur and within the Indo-Burma hotspot.

Keywords: CITES, Imphal valley, local wild orchid trade, Orchidaceae, orchid sanctuaries, *Renanthera imschootiana*, *Vanda coerulea,* wild orchids.

Editor: Pankaj Kumar, Florida International University & Fairchild Tropical Botanic Garden, Miami, USA. **Date of publication:** 26 November 2024 (online & print)

Citation: Kabuini, K.K. & M.D. Meitei (2024). Unregulated wild orchid trade in Manipur: an analysis of the Imphal Valley markets from the Indo-Burma hotspot. *Journal of Threatened Taxa* 16(11): 26078–26088. https://doi.org/10.11609/jott.9329.16.11.26078-26088

Funding: None.

Competing interests: The authors declare no competing interests.

Author details: Kamei Kambuikhonlu Kabuini was a master's student in the Department of Environmental Science, Manipur University. She has worked on her dissertation entitled "Unregulated commercialization of wild orchids in Manipur: An analysis of Imphal Valley markets". Dr. Maibam Dhanaraj Meitei is a faculty in the Department of Environmental Science, School of Human and Environmental Sciences, Manipur University, Canchipur, India. His research area is focussed on wetland studies, environmental pollution monitoring, phytotechnology and bioresource management.

Author contributions: MDM framed the concept of the project; KKK performed the survey; MDM and KKK were involved in analysis, manuscript writing, editing and finalizing the manuscript. All authors have read and approved the final version of the manuscript.

Acknowledgements: Kamei Kambuikhonlu Kabuini acknowledges the cooperation provided by orchid collectors and sellers during market survey. Authors also thank the local buyers for their cooperation during data collection. Authors also thank the Department of Environmental Science, Manipur University for providing the infrastructure facility.

INTRODUCTION

With an estimated 28,484 species, orchids account for 10% of angiosperms and represent the most diverse group of flowering plants, as well as the most threatened (Kumar 2024). Habitat loss coupled with climate change pose serious threats for orchids which are terrestrial, epiphytic and lithophytic (Barman & Devadas 2013; Brummitt et al. 2015). Orchids represent a significant illegally traded horticultural crop because of their beauty, rarity and popularity (Ballantyne & Pickering 2012; Phelps & Webb 2015; Hinsley et al. 2016). Consequently, all orchid species are included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in Appendix I and II, where the trade of Orchidaceae family is either legally regulated or prohibited (UNEP-WCMC 2018). Additionally, the International Union for Conservation of Nature (IUCN) has listed 2023 orchid species in the 'Threatened' category (IUCN 2024).

Around 1484 orchid species are reported from India and the northeastern states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura represent an important orchid hotspot with a total of 856 species (Kumar et al. 2022). From the Indian subcontinent, the usage of orchids for medicine is reported since ancient times with different orchids mentioned in Ayurveda (Bose et al. 2017). Today, illegal trade of wild orchid species in northern India intended for their use in local traditional medicine and international trade for the Chinese herbal medicine is pushing different rare and threatened species towards extinction (Hinsley et al. 2018). Around 1295 species belonging to 179 genera found in India are listed in the Appendix II of CITES (De 2022). Moreover, as a result of high demand in the Indian market, orchid cut flowers worth INR 2321.84 lakhs were imported in 2018–19 (De 2020). Despite the increasing demand in India for both local and international trade, most of the orchid dealers haven't explored the concepts of mass scale multiplication techniques. The native sellers largely depend on the harvest from wild to meet the supply chain (TRAFFIC 2022; WWF-India 2022). In the northeastern state of Manipur, deforestation in the hills for jhum and charcoal harvesting, forest fire and illegal overexploitation for trade are the major threats to orchids. The state is home to 407 orchid taxa belonging to 95 genera (Mao & Deori 2018). The mass scale orchid production using micropropagation is still lacking in the state. Therefore, majority of the trade is based on wild collection from tropical and subtropical forests of Manipur. Hence, in order to highlight the problem of un-monitored wild orchid trade, a project was undertaken to identify major wild orchid selling areas in the Imphal valley region of Manipur and document the wild orchid species traded locally during 2022–23.

MATERIALS AND METHODS

Study area

Manipur is a state in the northeastern India region (24.663°E & 93.906°N) of the Indo-Burma hotspot. The state with an area of 22,327 km^2 can be sub-divided into two regions; central oval shaped Imphal or Manipur Valley (constituting 10%) and surrounding hills (Image 1). The 2,238 km^2 valley is surrounded by hills with a maximum elevation of 2,994 m (Laiba 1992). The region is dominated by tropical moist deciduous vegetation and records an annual rainfall of 1,500– 1,700 mm. The minimum temperature ranges 2–21 °C and maximum of 23–36 °C, respectively.

Market survey

The market surveys were performed in Khwairamband, Pishumthong, Naoremthong, Lamlong, Sekmai, and Bishnupur markets of the Imphal Valley during September–November, 2022 and February–April, 2023 (Image 1; Table 1). The markets were visited on a weekly basis during early morning hours (Image 2). The information's were collected based on a semi structured questionnaire (Q1) and field photographs of wild orchids along with the sellers were taken with due permission. Moreover, prior permission was obtained from sellers for participation under the assurance of anonymity and confidentiality. Ten female sellers (individuals mainly from Kangpokpi and Senapati districts) from 10 vendors were questioned. During the survey, information's such as local name of wild orchids, collection methods, frequency of collection, collection season, location of orchid habitat, rarity in wild, preference by buyers, demand in market and price in market were gathered. Later, wild orchids were identified using available standard literature wealth on orchids of Manipur (Deb 1961; Mao 1999; Kumar & Kumar 2005; Nanda et al. 2013; Mao & Deori 2018; Rao & Kumar 2018). The scientific names of wild orchids were cross checked using the online website (WFO Plant List 2024) of the Royal Botanic Garden, Kew and Missouri Botanical Garden (accessed on 6 September 2024). Further, information's on endemicity and threatened status of wild orchids were gathered and compiled (IUCN 2024).

Table 1: Local bazaars surveyed for wild orchid trade.

Site	Latitude	Longitude
Sekmai Bazaar	N 24.826005	E 93.919110
Khurai Lamlong	N 24.830345	E 93.915717
Naoremthong	N 24.834325	E 93.916136
Khwairamband	N 24.817760	E 93.912400
Pishumthong	N 24.827280	E 93.911187
Bishnupur Bazaar	N 24.864179	N 93.959745

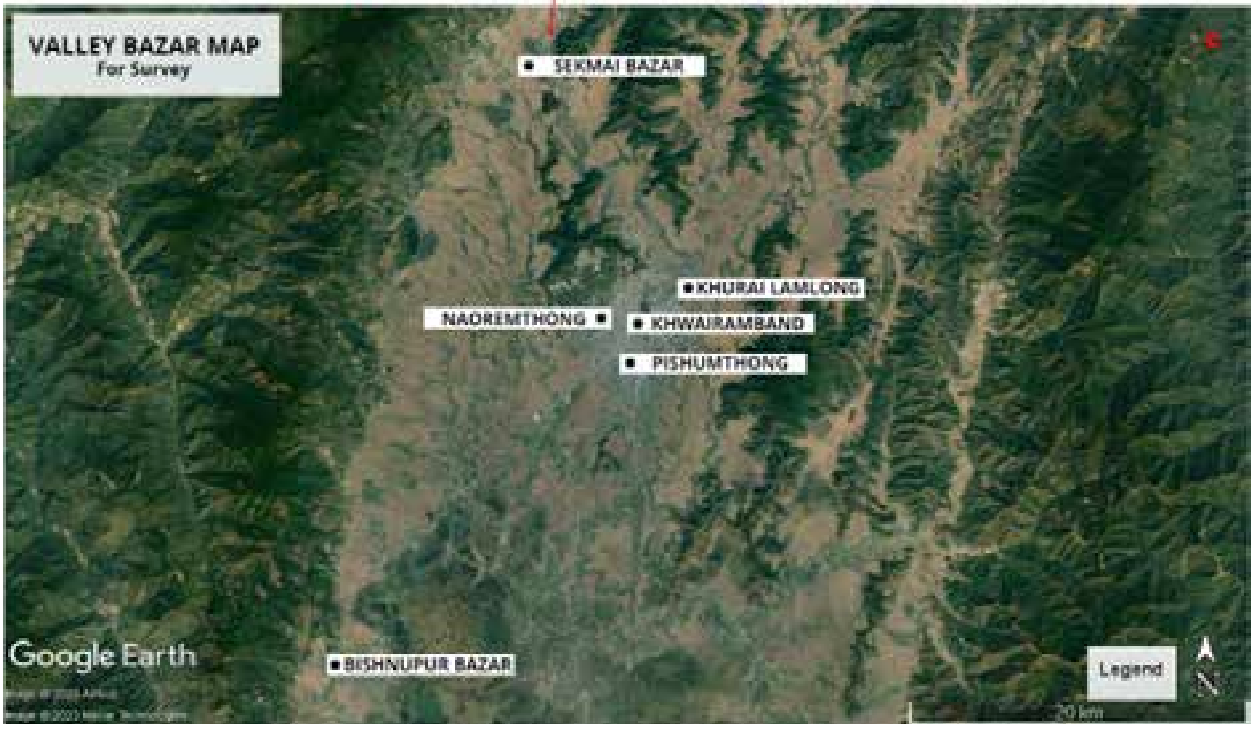

Image 1. a—Map of India | b—Manipur | c—the Imphal valley region with local bazaars surveyed for wild orchid trade documentation during 2022–2023.

Household survey

To assess the viability of wild orchids in local households, 15 local cultivators from 15 different localities of the Imphal Valley, viz., Thoubal, Kakching, Keishampat, Keishamthong, Wangoi, Namdunlong, Ragailong, Langthabal khoupum, Thongju, Khagempali, Singjamei, Chingmeirong, Kyamgei, and Sagoltongba were interviewed using semi-structured questionnaire (Q2). The buyer's questionnaire consisted of questions such as preferences of orchid, purchasing frequency, the total number of orchids purchased so far, number of orchids that died during household cultivation, the price range of orchids bought, knowledge of rare orchids and government role in orchid conservation in the state, etc.

Data analysis

The information obtained from wild orchid sellers and cultivators was analysed in the Department of Environmental Science, Manipur University. Further, the survival rate of wild orchids under cultivation in local households and knowledge of local buyers on the threatened and rare status of wild orchids were calculated from questionnaire data using Microsoft Excel 2010 for windows.

RESULTS AND DISCUSSION

Socio-demographic characteristics of orchid collectors and sellers

The wild orchid collectors and sellers belong to local tribal ethnic groups of Manipur (mainly from Kuki and Naga ethnic communities). For the individuals, wild orchid collection and their trade is a means of livelihood. The interview of local sellers revealed that the families involved in the trade during the study period were from economically weaker sections. As such, the trade of wild orchids, wild edibles, and horticultural plants collected from the jungles of Manipur serves as a significant income source for the individuals. More or less, they are agriculturalist or horticulturalists, or individuals devoted to floriculture.

Collection of wild orchids from jungles of Manipur

The orchid collection was mainly performed by villagers between 30 and 50 years old. Although the sellers were from Kangpokpi and Senapati Districts during the survey, the collectors mentioned that wild orchids came from all hill districts of Manipur, viz., Tamenglong, Churachandpur, Ukhrul, Tengnoupal, Kamjong, Pherzawl, Noney, and Chandel. Moreover, the orchid sellers interviewed were involved in trade for a minimum of 2–3 years. For local trade, wild orchid collection is performed throughout the year irrespective of flowering seasons. The epiphytic orchids were collected by experienced climbers gathering orchids by hand or using a long bamboo pole with a machete attached at the top to detach orchids from trunks and branches of tall trees. Another destructive method predominantly used by collectors is felling of host trees and gathering of all wild orchids, irrespective of demand. For terrestrial orchids, tubers were unearthed and whole plants were collected. As such, with no knowledge of sustainability among collectors, orchid habitats are often destroyed to a point with little chance for regeneration after harvest seasons. In addition, orchid collectors have little knowledge about threatened status of wild orchids. This has led to depletion of many orchid bio-resources in its natural habitats which are endemic or rare in the region.

Wild orchids traded in the Imphal Valley markets

During the survey, it was observed that Pishumthong bazaar is the main hub for local wild orchid trade in the valley (Image 1). On average, 4–5 local sellers were observed during market visits. The sellers were from Kangpokpi and Senapati Districts of Manipur. Further monitoring of other busy local bazaars at Naoremthong, Lamlong, Sekmai, and Bishnupur showed no reports of wild orchid traders opening their vendors during the study period. A total of 82 wild orchid species from 33 genera were locally traded during the study period, 2022–2023 (Table 2; Image 3). Orchids such as *Bulbophyllum reptans*, *Coelogyne alba*, *Coelogyne articulata*, *Liparis resupinata*, and *Pholidota imbricata* were marketed between price range of INR 30–50, respectively. The low-price range is associated with less fondness of local buyers. Hence, they are in low demand according to sellers. Moreover, wild orchids such as *Bulbophyllum* spp., *Liparis* spp., *Oberonia* spp., with unattractive flowers are rarely bought. The unattractive nature is concentrated on the color and size of flowers as per the buyer's opinion. On the contrary, the price of species such as *Cleisostoma simondii*, *Cymbidium bicolor*, *Cymbidium elegans*, *Dendrobium wardianum*, *Schoenorchis fragrans*, *Vanda alpina*, and *V. coerulea* ranged from INR 100–500, respectively. The higher price is associated with repeat purchases by local buyers and their rarity as per the seller's opinion. Further, most orchid species in high demand have captivating (large and colorful) unique flowers. The species such as *Coelogyne barbata*, *Cymbidium devonianum*, *C. elegans*, *C. lowianum*, *Dendrobium crepidatum*, *D. devonianum*, *D. falconeri*, *D. lituiflorum*, *D. parishii*, *D. polyanthum*, *Papilionanthe vandarum*, *Phaius flavus*, *Phalaenopsis marriottiana*, *P. taenialis*, *Pleione praecox*, *Renanthera imschootiana*, *Rhynchostylis retusa*, *Thunia alba*, *Vanda ampullacea*, and *V. coerulea* were some of the widely exploited and preferred wild orchids by local buyers as per sellers. As such, sellers fix the prices of wild orchids depending on their demand or rarity.

Further, seven threatened species were collected from wild habitats and traded locally (Table 3). Moreover, three wild orchid species, viz., *P. hirsutissimum*, *R. imschootiana*, and *V. coerulea* which are protected under the Schedule VI of the Wildlife (Protection) Act, 1972 of India was commonly and frequently traded (Image 3). Strict application of rules and regulations was not observed from the concerned authorities on this issue of legally protected wild orchid trade reported from the Imphal Valley. The statement is supported by local sellers freely trading orchids that are protected by the domestic legislation of India. Moreover, endemic species such as *Arachnis senapatianum* was also found traded. As such, the act of threatened and endemic wild orchid collection from their habitats without any regulation will pose a serious risk to population of such orchids in Manipur. Similar to the study, research on

Table 2. Wild orchid species locally traded in the Imphal valley region of Manipur during 2022–2023.

	Scientific name	Habit	Flowering season	Price (INR per piece)
1	*Acampe rigida* (Buch.-Ham. ex Sm.) P.F.Hunt	Epiphyte	May–June	100–250
2	*Acanthephippium striatum* Lindl.	Terrestrial	May–September	50–200
3	*Aerides multiflora* Roxb.	Epiphyte	May–June	250
4	*Aerides odorata* Lour.	Epiphyte	April–May	50–100
5	*Aerides rosea* Lodd. ex Lindl. & Paxton	Epiphyte	May–July	250
6	*Anthogonium gracile* Wall. ex Lindl.	Terrestrial	July	250
7	*Arachnis senapatianum* (Phukan & A.A.Mao) Kocyan & Schuit.	Epiphyte	May–June	200
8	*Arundina graminifolia* (D.Don.) Hochr.	Terrestrial	March–August	300
9	*Bulbophyllum affine* Lindl.	Epiphyte	June	100–300
10	*Bulbophyllum lobbii* Lindl.	Epiphyte	August–September	150
11	*Bulbophyllum odoratissimum* (Sm.) Lindl. ex Wall.	Epiphyte	May	150
12	*Bulbophyllum reptans* (Lindl.) Lindl. ex Wall.	Epiphyte	January–February	30–50
13	*Bulbophyllum rothschildianum* (O'Brien) J.J.Sm.	Epiphyte	August	250
14	*Calanthe masuca* (D.Don) Lindl.	Terrestrial	August–September	200
15	*Calanthe puberula* Lindl.	Terrestrial	August–October	200
16	*Cephalantheropsis longipes* Hook.f.	Terrestrial	November–December	150
17	*Chiloschista parishii* Seidenf.	Epiphyte	April–June	100–200
18	*Cleisostoma racemiferum* (Lindl.) Garay	Epiphyte	July	50–200
19	*Cleisostoma simondii* (Gagnep.) Seidenf.	Epiphyte	July–September	150–400
20	*Coelogyne alba* (Lindl.) Rchb.f.	Epiphyte	June–July	30–50
21	*Coelogyne articulata* (Lindl.) Rchb.f.	Epiphyte	April–May	30–50
22	*Coelogyne barbata* Lindl. ex Griff.	Epiphyte	October	150–300
23	*Coelogyne corymbosa* Lindl.	Epiphyte	May–June	100
24	*Coelogyne punctulata* Lindl.	Epiphyte	March	100–200
25	*Crepidium purpureum* (Lindl.) Szlach.	Terrestrial	June–July	200
26	*Cymbidium aloifolium* (L.) Sw.	Terrestrial	May–June	150
27	*Cymbidium bicolor* Lindl.	Epiphyte	April–May	100–500
28	*Cymbidium devonianum* Paxton	Epiphyte	May	100–350
29	*Cymbidium eburneum* Lindl.	Epiphyte or lithophyte	March–April	250
30	*Cymbidium elegans* Lindl.	Epiphyte or lithophyte	October–June	100–500
31	*Cymbidium iridioides* D.Don	Epiphyte or lithophyte	September–October	200
32	*Cymbidium lancifolium* Hook.	Epiphyte or lithophyte	May–June	100–300
33	*Cymbidium lowianum* (Rchb.f.) Rchb.f.	Epiphyte or lithophyte	April–May	200–300
34	*Dendrobium amoenum* Wall. ex Lindl.	Epiphyte	May–August	50–150
35	*Dendrobium aphyllum* (Roxb.) C.E.C.Fisch.	Epiphyte	April–May	50–100
36	*Dendrobium calocephalum* (Z.H.Tsi & S.C.Chen) Schuit. & Peter B.Adams	Epiphyte	August	300
37	*Dendrobium chrysanthum* Wall	Epiphyte	September–October	50–300
38	*Dendrobium chrysotoxum* Lindl.	Epiphyte	April–May	100–300
39	*Dendrobium crepidatum* Lindl. & Paxton	Epiphyte	April–May	50–100
40	*Dendrobium denneanum* Kerr	Epiphyte	May–June	50–100
41	*Dendrobium densiflorum* Lindl.	Epiphyte	April–May	50–100
42	*Dendrobium devonianum* Paxton	Epiphyte	April–May	50–200
43	*Dendrobium falconeri* Hook.	Epiphyte	April–May	50–100

	Scientific name	Habit	Flowering season	Price (INR per piece)
44	*Dendrobium formosum* Roxb. ex Lindl.	Epiphyte	May–June	50–150
45	*Dendrobium heterocarpum* Wall. ex Lindl.	Epiphyte	March	100–300
46	*Dendrobium jenkinsii* Wall. ex Lindl.	Epiphyte	April–May	50–150
47	*Dendrobium lituiflorum* Lindl.	Epiphyte	April–May	50–100
48	*Dendrobium moschatum* (Banks) Sw.	Epiphyte	May–June	50–300
49	*Dendrobium ochreatum* Lindl.	Epiphyte	April–May	50–150
50	*Dendrobium parishii* H.Low.	Epiphyte	May–June	50–100
51	*Dendrobium polyanthum* Wall. ex Lindl.	Epiphyte	May–June	100–250
52	*Dendrobium thyrsiflorum* B.S.Williams	Epiphyte	April–May	150
53	*Dendrobium wardianum* R.Warner	Epiphyte	April–May	200–500
54	*Eria coronaria* (Lindl.) Rchb.f.	Epiphyte or lithophyte	November	100–250
55	*Liparis resupinata* Ridl.	Epiphyte	November–December	30–50
56	*Oberonia acaulis* Griff.	Epiphyte	November–December	30–50
57	*Oberonia jenkinsiana* Griff. ex. Lindl.	Epiphyte	December–January	50
58	*Oberonia mucronata* (D.Don) Ormerod & Seidenf.	Epiphyte	September–October	50–100
59	*Oberonia teres* Kerr	Epiphyte	May	50–100
60	*Paphiopedilum hirsutissimum* (Lindl. ex Hook.) Stein	Epiphyte	October–November	350–500
61	*Papilionanthe vandarum* (Rchb.f.) Garay	Epiphyte	September–October	50–200
62	*Phaius flavus* (Blume) Lindl.	Terrestrial	April–June	100–300
63	*Phaius tankervilleae* (Banks) Blume	Terrestrial	March–May	150
64	*Phalaenopsis marriottiana* (Rchb.f.) Kocyan & Schuit.	Epiphyte	April–August	100–150
65	*Phalaenopsis taenialis* (Lindl.) Christenson & Pradhan	Epiphyte	April–July	150–350
66	*Pholidota imbricata* Lindl.	Epiphyte	June–July	30–50
67	*Pinalia acervata* (Lindl.) Kuntze	Epiphyte	May–June	50–200
68	*Pinalia spicata* (D.Don) S.C.Chen & J.J.Wood	Epiphyte	July–August	100
69	*Pleione praecox* (Sm.) D.Don	Epiphyte	September–October	100–300
70	*Polystachya concreta* (Jacq.) Garay & H.R.Sweet	Epiphyte	August–September	50–100
71	*Renanthera imschootiana* Rolfe	Epiphyte	April–May	100–250
72	*Rhynchostylis retusa* (L.) Blume	Epiphyte	April	150–300
73	*Schoenorchis fragrans* (C.S.P. Parish & Rchb.f.) Seidenf. & Smitinand	Epiphyte	July–August	350–500
74	*Schoenorchis gemmata* (Lindl.) J.J.Sm.	Epiphyte	May	150–350
75	*Spathoglottis pubescens* Lindl.	Terrestrial	August–September	200–300
76	*Thunia alba* (Lindl.) Rchb.f.	Epiphyte	June–July	100–300
77	*Uncifera obtusifolia* Lindl.	Epiphyte	February–March	50–200
78	*Vanda alpina* (Lindl.) Lindl.	Epiphyte	June	100–500
79	*Vanda ampullacea* (Roxb.) L.M.Gardiner	Epiphyte	April–May	100–300
80	*Vanda bicolor* Griff.	Epiphyte	August–October	100–150
81	*Vanda coerulea* Griff. ex Lindl.	Epiphyte	March–May	100–500
82	*Vanda cristata* Wall. ex Lindl.	Epiphyte	August–October	150

wild orchid collection and their commercial trade in illegal local and international markets is reported from different countries such as Vietnam (Bullough et al. 2021), Thailand, Lao PDR, & Myanmar (Phelps 2015), Nepal (Subedi et al. 2014), and China (Gale et al. 2019) etc. The research showed that illegal international trade of wild orchids is common in these countries. The illegal activities in turn posed a remarkable threat in the

Image 2. Wild orchid vendors at Pishumthong bazaar of Manipur. © Kamei Kambuikhonlu Kabuini.

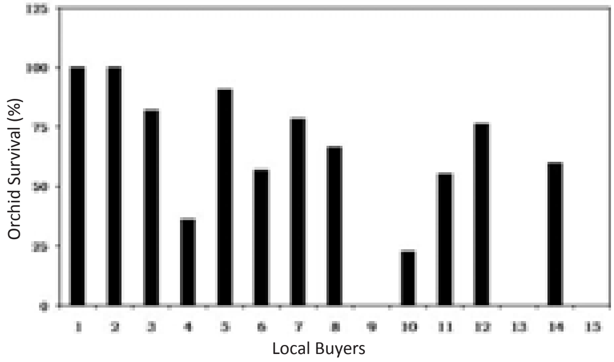

Figure 1. Wild orchid survival percentage among local orchid buyers of the Imphal Valley region.

conservation of the different wild orchids in their natural habitats.

Status of the wild orchids cultivated in local households

The interviews of local buyers showed that wild orchid customer base is diverse, encompassing people of various ages and genders, both young and old. The price of orchids they bought ranged from INR 30–500, respectively. The pricing of wild orchid is unpredictable. It was observed that survival rate varies significantly across different wild orchids when they were brought under cultivation and unexperienced buyers see varying levels of success in maintaining these plants (Figure 1). The reasons for low survival rates are change of habitat coupled with improper management due to lack of knowledge on orchid cultivation, diseases, and pest. The wild orchids grow in a particular habitat which is in the deep moist jungles of Manipur. Therefore, their removal and transplanting elsewhere forces the orchids to adapt to an entirely new set of environment where plants might not succeed. Among common host trees, buyers used Mango *Mangifera indica*, Pomelo *Citrus maxima*, Lemon *Citrus limon*, Plumeria *Plumeria rubra*, Bottle Brush *Callistemon citrinus*, and Hibiscus *Hibiscus rosa-sinensis*. However, most of the wild orchids were in plastic or terracotta pots, since majority of households didn't have good size trees (Image 4). Further, most orchids observed during the visits were not in their best health. Among buyers, only 33% had the knowledge of threatened and rare wild orchids. Most of the local buyers do not have basic awareness on legal restrictions surrounding the purchase and sale of wild orchids protected by domestic legislation. The results revealed a significant gap in awareness regarding the legality of wild orchid trade among surveyed participants.

Suggestive measures for conservation

From the market survey, it is evident that local trade of wild orchids in Manipur takes place without any inhibition in the Imphal Valley. Therefore, it becomes necessary that continuous monitoring of such situation should be a part of the concerned authorities' action plan for orchid conservation. The following measures are suggested for the conservation of wild orchids in Manipur region of the Indo-Burma hotspot:

(i) In situ conservation is the most desirable conservation strategy for wild orchids. The Government of Manipur needs to expand the Protected Areas Network (PAN) to include important orchid habitats in the state. For example, State Governments of Arunachal Pradesh, Sikkim, Karnataka, and West Bengal have designated various orchid rich areas as "Orchid Sanctuaries" under the Wildlife Protection Act, 1972 (amended in 1992). The actions will control smuggling or poaching of wild orchids. Further, there are options to establish community conservation reserves with collaboration of government agencies and local communities (Ngashangva 2021).

(ii) Initial ecological restoration of already degraded orchid rich habitats must be a priority of the concerned authority. The initiatives for afforestation of degraded

Image 3. Some of the wild orchid species: a—*Renanthera imschootiana* | b—*Pleione praecox* | c—*Dendrobium chrysanthum* | d—*Liparis resupinate* | e—*Cymbidium elegans* | f—*Vanda coerulea* which are traded in local bazaars of the Imphal Valley region. © Kamei Kambuikhonlu Kabuini.

Image 4. Cultivated orchids in local households of the Imphal Valley region. © Kamei Kambuikhonlu Kabuini.

areas with suitable host trees must be taken up.

(iii) Similar to Khonghampat Orchidarium, which is the only orchid *ex-situ* conservation center of Manipur, the state need more ex situ conservation centers in hill districts where wild orchid habitats are found.

(iv) It is time that a long-term population monitoring programme must be conducted by concerned authority to assess the health of wild orchid population.

(v) Endemic species such as *A. senapatianum* need immediate attention and actions. Their exploitation in an unsustainable way must be completely stopped by using various orchid conservation strategies.

(vi) Research is absent on wild orchid trade of Manipur in local and international markets. There is an urgent need of in-depth research that analyses the volume of local wild orchid market in Manipur and their illegal international trade via Myanmar.

(vii) The concerned authorities must continuously

Table 3. Wild orchid species which are threatened or protected by the Indian domestic legislation.

	Species	Rare/ Threatened	Legally protected in state and country* (Yes/No)
1	*Dendrobium chrysotoxum* Lindl.	Threatened	No
2	*Dendrobium densiflorum* Lindl.	Threatened	No
3	*Dendrobium falconeri* Hook. (Th)	Threatened	No
4	*Dendrobium parishii* H.Low.	Threatened	No
5	*Paphiopedilum hirsutissimum* (Lindl. ex Hook.) Stein	Rare	Yes
6	*Renanthera imschootiana* Rolfe	Threatened	Yes
7	*Vanda coerulea* Griff ex Lindl.	Threatened	Yes

* The Wildlife (Protection) Act, 1972.

and strictly monitor local wild orchid markets and their international trade. Further, strict actions must be taken up against illegal trade if carried out in the state. For example, trade of scheduled species such as *Paphiopedilum hirsutissimum*, *Renanthera imschootiana*, and *Vanda coerulea* is illegal.

(viii) Training programmes on mass scale multiplication of wild orchids for trade using tissue culture techniques and establishment of micropropagation units in the state will reduce stress on wild orchid population. Further, it will improve economy of the state.

(ix) The lack of awareness is an important issue in the society, which must be immediately tackled by the concerned authorities. As such, various conservation awareness programmes must be initiated to sensitize the common mass on the issue and invite the locals to be a part of conservation programmes.

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Supplementary Q1: Interview for data collection (sellers)

1. Name:
2. Age
3. Gender
4. Locality
5. Orchid collected from:
6. Did you get permission from the concerned department?
7. If so, what?
8. If not, why?
9. List of collected orchid:
10. Collection season:
11. Do you collect only orchid?
12. How much is collected?
13. How often do you collect?
14. Status of the orchid in its natural habitat? Abundant/ scarce.
15. How far do you have to walk to collect the orchid?
16. How often do you not find the orchid?
17. Harvesting technique (a). cutting whole tree. (b). climb and collect.
18. Health of the orchid at the time of harvesting?
19. What measure do you take up to improve the health of the orchid before selling?
20. Do you harvest every orchid that you find regardless of its demand?
21. Do you harvest only those orchids that are in high demand?
22. What changes can you see the population of orchid in its natural habitat?
23. How much is the demand of the orchid in the market?
24. Most sold species.
25. Least sold species.
26. What do you do with the orchids that are not sold in the market?
27. How many customers do you have?
28. How many of them are regular customer?
29. Do you have customer from outside of the state or country?
30. Do you have any knowledge on rare orchid?
31. Any measures taken up to conserve the rare orchid sp.?
32. Do you run a nursery?
33. If yes, how many sp. do you have in your nursery?

Signature of the informant

Supplementary Q2: Interview for data collection (buyers)

1. Name:
2. Age:
3. Gender:
4. Locality:
5. Profession:
6. What is the selling point?
7. What are the preferences when you buy?
8. How often do you buy?
9. Number of different orchids you have purchased.
10. Price range of the orchids bought.
11. Is the price expensive/reasonable?
12. Condition of the orchids at the time of purchasing.
13. Number of Orchids Planted.
14. Number of Orchids survived.
15. Possible reasons for the death.
16. Measures taken up to revive dying orchids.
17. Orchids with repeated purchase.
18. Reasons for repeated purchase.
19. Reasons for buying.
    For Commercialization
    For Personal use
20. Planting area a) Pots b) Trees
21. Do you have any knowledge regarding rare orchid species trade?
22. Number of rare orchids collected so far.
23. Do you know the practice adopted by the collectors for harvesting?
24. How often do you see orchids naturally growing in your locality?
25. Have you resold the orchids you have purchased?
26. How many have you resold?
27. Do you follow any propagating method to increase the number of orchid species for reselling purpose?
28. Rate at which you resold.
29. How much is the demand?
30. Do you know that the orchid trade in Manipur is via illegal way?
31. If so, what should be the mechanism to regulate the conditions? (Personal view)
32. Any comment on the conservation of orchids in the natural habitats. (Mechanisms you wish to propose)
33. Do you think that orchid conservation is possible by planting the species in households of valley?
34. Do you think orchid trade should be regulated by the government?

Signature of the informant

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26089–26103

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.8954.16.11.26089-26103

#8954 | Received 08 February 2024 | Final received 11 October 2024 | Finally accepted 28 November 2024

OPEN ACCESS

ARTICLE

Watershed survey of streams in western Bhutan with macroinvertebrates, water chemistry, bacteria and DNA barcodes

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Abstract: Bhutan in the eastern Himalaya contains some of the last pristine watersheds in the world, yet there has been limited monitoring of streams and rivers. Eighteen streams in three watersheds were surveyed for chemistry, bacteria, and macroinvertebrates in post-monsoon (2015) and monsoon (2016) seasons. Many water quality variables, including temperature, pH, specific conductivity, nitrite, nitrate, *E. coli,* and total coliform bacteria differed between seasons and between areas upstream and downstream of anthropogenic disturbance. In both seasons, total coliform bacteria and *E. coli* were significantly higher downstream of anthropogenic disturbance, with many urban sites having high coliform levels (>2000 cfu/100 ml) indicative of sewage inflow. A total of 50 insect families and six non-insect taxa were identified. During the post-monsoon, eight of 13 metrics (e.g., total richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, % EPT, % non-insects, HKHbios, BMWP1983, ASPT1983, and ASPT2021) based on kick samples (qualitative) indicated impairment, while in the monsoon season composite Surbers (quantitative) had two metrics (e.g., total richness and Shannon) that differed between sites up and downstream of disturbance. DNA barcoding for cytochrome *c* oxidase subunit I (*COI*) in 63 morphological species of mayfly, stonefly, and caddisfly indicated 18 additional species, 17 mayflies and one stonefly. Forty-two barcode species were new additions to the Barcode of Life Data database. Results suggest macroinvertebrates are a viable method for evaluating human impacts on Bhutan streams. Bhutan faces future challenges of sanitation management, climate change, and shared river systems, and monitoring will need to be expanded. The monsoon season may be an ideal time to measure water chemistry and bacteria due to increased runoff, but macroinvertebrate sampling should occur in the post-monsoon season to obtain the best sampling conditions and larger individuals. Increasing the knowledge of species in the region, potentially with the help of DNA barcodes, will document the diversity of the region and help amplify the capacity for macroinvertebrates with future biomonitoring.

Keywords: Biomonitoring, coliform, COI gene, diversity, eastern Himalaya, EPT, hotspot, seasons, water quality.

Editor: J.A. Johnson, Wildlife Institute of India, Dehradun, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Battle, J.M., B.W. Sweeney, B. Currinder, A. Aufdenkampe, B.A. Fisher & N. Islam (2024). Watershed survey of streams in western Bhutan with macroinvertebrates, water chemistry, bacteria and DNA barcodes. *Journal of Threatened Taxa* 16(11): 26089–26103. https://doi.org/10.11609/jott.8954.16.11.26089-26103

Funding: The 2015 work was supported by gifts from Peter Kjellerup, Mandy Cabot, W.B. Dixon Stroud Jr., Lisa Stroud, and the Stroud Endowment for Environmental Research. The 2016 work was funded by the Greg and Susan Walker Endowment, a Trustees' Council of Penn Women Grant, and a Graduate & Professional Student Assembly Grant from the University of Pennsylvania. Barcode work was funded with help from a donation from MilliporeSigma, as facilitated by Tim Currinder. The preparation of this publication was supported by the Stroud Endowment for Environmental Research. This is Stroud Publication 2023001.

Competing interests: The authors declare no competing interests.

Author details, Author contributions, & Declarations: See end of this article.

Acknowledgements: The Waterkeeper Alliance was instrumental in organizing this project. Nedup Tshering (Clean Bhutan) and Pem Dekhi (Waterkeeper Alliance Bhutan) provided valuable assistance in Bhutan. We are grateful for the input from Dr. Sarah Willig, Dr. Yvette Bordeaux, Heather Kostick, Sally Cardy, Amanda Johnston, and William Morrissey from the University of Pennsylvania. We thank Peter Kjellerup, Mandy Cabot, and Dixon & Lisa Stroud for their assistance with the 2015 field collection. Special thanks to Dr. John Jackson from the Stroud Center for his support and access to various resources within the Stroud Center entomological section throughout the project. Stroud Center employees Kelly McIntyre and Mike Broomall were responsible for the EPT species identification, additional help from Stroud was provided by Katie McFadden Billé, Sally Peirson, and Matt Wilson for sample processing, and Charlie Dow, Tim Smith, and Ellie Kissis for data analyses. The 2016 data was from the Capstone Projects of B. Currinder and N. Islam from the University of Pennsylvania.

INTRODUCTION

Located in the eastern Himalaya, Bhutan is mountainous, 70% forested, and considered a world biodiversity "hotspot" (Wangdi et al. 2013). Human populations historically occurred largely in rural areas throughout the low to mid-elevations of the western part of the country (Worldometers 2022). Bhutan's urban population has grown by 40% from 2005 to 2017, and it is projected to comprise half of the country's population by 2037 (NSBB 2019). As of 2020, the capital Thimphu accommodated around 28% of the total urban population and ~13% of the total population (Worldometers 2022). This increased urbanization in the western part of the country has put significant stress on Bhutan's abundant, but fragile, forests and water resources (Wangdi et al. 2013). Thus, despite the country's relatively small population (791,817 people in 38,177 km^2; Worldometers 2022) and large forested areas, significant challenges currently exist in maintaining adequate water quality, particularly for humans and aquatic wildlife living downstream of population centers (WBMP 2016).

Although urban development tends to take up less area in the watershed than agriculture, it often has a larger impact on stream conditions (WBMP 2016). A recent survey of Bhutan wastewater management reported that only eight out of 35 towns (~7% of Bhutan's population) have a public sewage system, with the majority (80%) of the remaining urban population depending on on-site sanitation systems, with many being both inadequately designed and maintained (Dorji et al. 2019). Climate change poses additional threats to water quality in Bhutan through its impact on hydrology (WBMP 2016), causing localized droughts and frequent flooding (Tariq et al. 2021). Water scarcity, construction of roads, and hydropower dams are all factors that will impact water quality in Bhutan's streams and rivers (WBMP 2016; Thapa et al. 2020; Tariq et al. 2021). Because of its shared river systems (i.e., those flowing through multiple countries; sensu Price et al. 2014), the capacity to manage water quality becomes more complicated because of political challenges.

Agricultural land use and its impacts on water quality in Bhutan, as in other watersheds worldwide, varies with factors like intensity, region, livestock, and crop type, with virtually all water quality loss being due to modified flows, degraded channel habitat, altered temperature regimes, and high inputs of nutrients, pesticides, and sediments (Allan 2004). In Bhutan, the primary mode of livelihood in rural areas has historically consisted of traditional rain-fed and irrigated row crop agriculture, but recent times have seen a transition to more intensive agriculture using inorganic fertilizers (Dorji et al. 2011). Forestry (commercial and traditional firewood collection) and industry (mining, cement industry, fishery) are also impacting water quality in Bhutan (WBMP 2016; Tariq et al. 2021). Several studies in the Bhutan region examining water quality have pointed to the discharge of untreated sewage directly into streams as the major source of pollution in urban areas, while nutrient levels have been indicative of agricultural disturbance (Korte et al. 2010; Giri & Singh 2013; Dorji et al. 2021).

The use of aquatic macroinvertebrates (i.e., insects, crustaceans, molluscs, and worms) has been shown to be a powerful tool for monitoring freshwater around the world because of their high diversity, high abundance, and spectrum of pollution tolerances (Allan 2004). In Bhutan, macroinvertebrates have been shown to be of use for monitoring agricultural and urban impacts but most studies have focused on one stream and did not use a watershed approach (Moog et al. 2008; Ofenböck et al. 2010; Wangyal et al. 2011; Giri & Singh 2012; Dorji 2014a; Dorji et al. 2014, 2021; Gurung & Dorji 2014; Wangchuk & Dorji 2018).

This study was designed to further investigate the water quality of stream and river systems in western Bhutan for many sites throughout three watersheds. The study focused on how water quality responded to the presence and activities of human development within the districts of Thimphu and Paro in the Wangchhu basin and the districts of Punakha and Wangduephodrang in the Punatsangchhu basin. Family-level identifications were used to describe the macroinvertebrate assemblage of sites while species-level data on three major aquatic insect Orders (Ephemeroptera, Plecoptera, and Trichoptera; also known as EPT) were barcoded using the mitochondrial cytochrome *c* oxidase 1 (*COI*) gene. EPT has been shown worldwide to be the most sensitive (i.e., intolerant) of pollution and therefore most indicative of stream and water health (Resh & Jackson 1993). Species-level knowledge of macroinvertebrates needs to be expanded in Bhutan and the south Asian region to better connect taxa to water quality parameters. Recent advances in the use of deoxyribonucleic acid (DNA) barcoding to identify aquatic macroinvertebrate species have enhanced their use for biomonitoring (Sweeney et al. 2011; Jackson et al. 2014; Li et al. 2022).

METHODS

In this study, 18 streams and rivers in three watersheds were selected to measure water chemistry, bacteria, and macroinvertebrates (Table 1). Water quality was measured at 16 sites from 6–13 November 2015 (post-monsoon season) and 12 of the same sites were sampled from 15–20 August 2016 (monsoon season) with an additional two sites added (Figure 1; Table 1). Stream sites represented a gradient of anthropogenic disturbance (e.g., an undisturbed, forested upstream area was contrasted with a downstream area impacted by agriculture or urbanization) and were labeled as being either upstream or downstream of major human disturbance (Table 1).

Temperature, conductivity, pH, and dissolved oxygen were measured with an Orion 5-Star portable meter and turbidity was measured with a Campbell Scientific OBS3+ turbidity sensor. Water samples were analyzed for ammonia, nitrite, nitrate, and phosphorus using API-brand freshwater test kits and quantified using an open- source colorimeter by IO-Rodeo (http://iorodeo.com/pages/colorimeter-project accessed August 2016). Total coliform bacteria and *Escherichia coli* bacteria were measured within 24 h of collection using the 3M™ Petrifilm™ *E.coli*/Coliform Count Plate kit and expressed as colony-forming units (cfu)/100ml. Wilcoxon rank-sum test (t approximation, 2-sided test) was used to examine differences in water quality variables between sites classified upstream or downstream of disturbance for each year, and both years of data were combined to examine if differences existed upstream or downstream within the Paro and Thimphu watersheds. As BT03 was a drinking well, only chemistry and bacteria were sampled (Table 1).

In 2015, macroinvertebrates were qualitatively sampled using a 500-µm D-frame net in riffle and run areas. The stream bottom was disturbed by kicking the substrate and collecting downstream, in addition, rocks, leaf packs, and woody material were examined. In the field, collected material was placed in a tray, and specimens were picked by hand before preserving in 95% ethanol, which was changed within 24 h of collection.

In 2016, macroinvertebrates were quantitatively collected with a Surber sampler (0.093 m^2; 250-µm mesh net). For each site, 16 individual Surber samples were taken in riffle areas (and some run areas if riffle habitat was scarce) and the contents (macroinvertebrates and organic debris) were split evenly between two large buckets containing stream water. The content of each of the two buckets was then transferred to a field sample splitter and the sample was split evenly into four subsamples (0.1858 m^2; Arscott et al. 2006). Two subsamples were preserved in 70% isopropyl alcohol resulting in four samples per site. In the laboratory, the entire 2015 sample was identified but, in 2016 three of the four preserved samples were further subsampled and processed under a microscope until a minimum of 200 macroinvertebrate specimens were obtained (>600 individuals per site). For three sites (BT06, BT11, BT13), only 1–2 preserved samples were processed because of limited time. Macroinvertebrate insects were identified to family level and some non-insects (e.g., oligochaetes, planarians, nematodes, bivalves, snails, and mites) were identified to order level or higher.

In order to ensure that taxon richness metrics were not biased by the number of individuals examined, samples were standardized (i.e., rarefaction) using the SAS statistical package (version 9.4, SAS Institute Inc., Cary, North Carolina). The 2015 qualitative samples were standardized to 100 individuals (except sites BT06, BT12, and BT14, which had <100 individuals), and the 2016 quantitative samples were standardized to 200 individuals/sample with both datasets being resampled to 1,000 random draws. Macroinvertebrate samples were used to calculate richness and percentage metrics, as well as the Shannon and Simpson diversity indices (Resh & Jackson 1993). Using samples in their entirety to best mimic the original index methods, the Hindu Kush-Himalaya Index (HKHbios; Ofenböck et al. 2010), the Biological Monitoring Working Party (BMWP), and the Average Score per Taxon (ASPT) were calculated. BMWP and ASPT were based on Armitage et al. (1983) method (ASPT 1983; BMWP 1983) and a Bhutan version following Dorji et al. (2021), BMWP (2021), and ASPT (2021). Within each year, a Wilcoxon rank-sum test (normal distribution, one-sided) was used to examine differences in macroinvertebrate metrics between sites classified upstream or downstream of disturbance.

Non-metric multidimensional scaling (NMS) was used to examine how macroinvertebrate taxa assemblages differed among years and in relation to various types of disturbance (i.e., upstream or downstream) using PC-ORD (version 6.22, MjM Software, Gleneden Beach, Oregon). This analysis was done using Sorenson distance, the step length was set at 0.20, and Monte Carlo was used to determine the optimal number of axes. NMS was performed using presence/absence data of 42 common taxa (i.e., taxa found in at least 2 samples) and was run with 41 iterations, an r^2 set at 0.28, a final stress of 12.0, and a final instability was <0.00001.

In an effort to better document the EPT diversity,

Table 1. Description of the Bhutan sampling locations in 2015 and 2016. Sites in similar watersheds are listed in pairs or groups indicating ones that were upstream (US) or downstream (DS) of disturbance. Stream type (tributary or mainstem), size, and land use are general descriptors. Years of water chemistry, bacteria, and macroinvertebrates were sampled are provided.

Location		US or DS	Stream type	Size (discharge m^3/s Nov 2015)	Land use	Chem & bacteria yrs	Macroinvert yrs	Elevation (m)	Latitude	Longitude
Paro River watershed										
BT03	groundwater well accessed at Tiger's Nest Tea House	US	other		Forest	2015, 2016		2976	27.4884	89.3586
BT04	Stream below Tiger's Nest	US	trib	small	Forest	2015, 2016	2016	2982	27.4859	89.3621
BT02	Holy Water stream near Chilai La pass	US	trib	small (0.03)	Forest	2015, 2016	2015, 2016	3235	27.3709	89.3620
BT07	Woo Chhu at Woo Chhu village	DS	trib	small (0.30)	Suburban/ agriculture	2015, 2016	2015, 2016	2412	27.3912	89.4244
BT05	Stream 1 by Ramzi	US	trib	small (0.13)	Forest	2015, 2016	2015, 2016	2866	27.5415	89.3295
BT06	Stream 2 by Ramzi	DS	trib	small	Forest/ agriculture	2016	2015, 2016	2692	27.5226	89.3283
BT01	Paro Chhu at Udumwara Resort	US	main	medium	Suburban/ agriculture	2015, 2016	2015	2355	27.4651	89.3558
BT08	Paro Chhu at Shaba	DS	main	large	Suburban/ agriculture	2015, 2016	2015, 2016	2432	27.3548	89.4643
Thimphu River watershed										
BT13	Thimphu Chhu at Chagri Dorjeden Monastery	US	main	medium	Forest	2015, 2016	2015, 2016	2599	27.5961	89.6304
BT09	Thimphu Chhu at Dodena	US	main	medium	Forest/ suburban	2015, 2016	2015, 2016	2523	27.5792	89.6348
BT15	Thimphu Chhu at Chanjiji Football Ground	DS	main	large	Urban	2016	2015, 2016	2293	27.4565	89.6491
BT12	Thimphu Chhu at Lungtenphug	DS	main	large	Urban	2015	2015	2296	27.4502	89.6547
BT14	Ola Rong Chhu at Semtokha	DS	trib	medium (1.94)	Urban	2015, 2016	2015	2283	27.4434	89.6603
BT11	Thimphu Chhu at Zimda	DS	main	large	Urban	2015, 2016	2015, 2016	2283	27.4302	89.6426
Paro & Thimphu watersheds										
BT10	Wangchhu at Tamchu	DS	main	large	Urban	2015, 2016	2015	2021	27.2503	89.5252
Punatsangchhu watershed										
BT16	Mochhu River upstream of Punakha Dzong	US	main	medium	Forested	2015	2015	1481	27.7117	89.7652
BT17	Punatsangchu below Khuruthang	DS	main	large	Urban/ agriculture	2015	2015	1209	27.5452	89.8699
BT18	Punatsangchu at Wangdue Phodrang	DS	main	large	Urban/ agriculture	2015	2015	1203	27.4863	89.8959

DNA was sequenced (COI gene) for a subset of EPT specimens to evaluate if species could be separated by morphology alone, or whether there were cryptic species present. The process of selecting EPT specimens for barcoding involved inspecting all individuals and choosing specimens that could be identified to genus level, and further dividing them into groups based on morphology. Common mayfly specimens were selected from forested and urban streams with the goal of barcoding four individual larvae from both stream types (undisturbed vs. disturbed) and a variety of sites and drainages where possible. Caddisflies and stoneflies were also separated based on morphology and 3–6 individuals were barcoded where possible albeit not from both stream types. The majority of the barcoded specimens were from 2015 because 2016 specimens were mostly small and immature, and therefore difficult or impossible to identify to a low level. Leg tissue from each specimen was sent to the Canadian Centre for DNA Barcoding at the University of Guelph, where genomic mitochondrial DNA was extracted and the 658-base pair (bp) barcoding region of the COI gene was amplified and sequenced (Sweeney et al. 2011). Sequences and detailed information about all specimens including photographs are stored on the GenBank and Barcode of Life Data systems (BOLD) website (https://boldsystems.

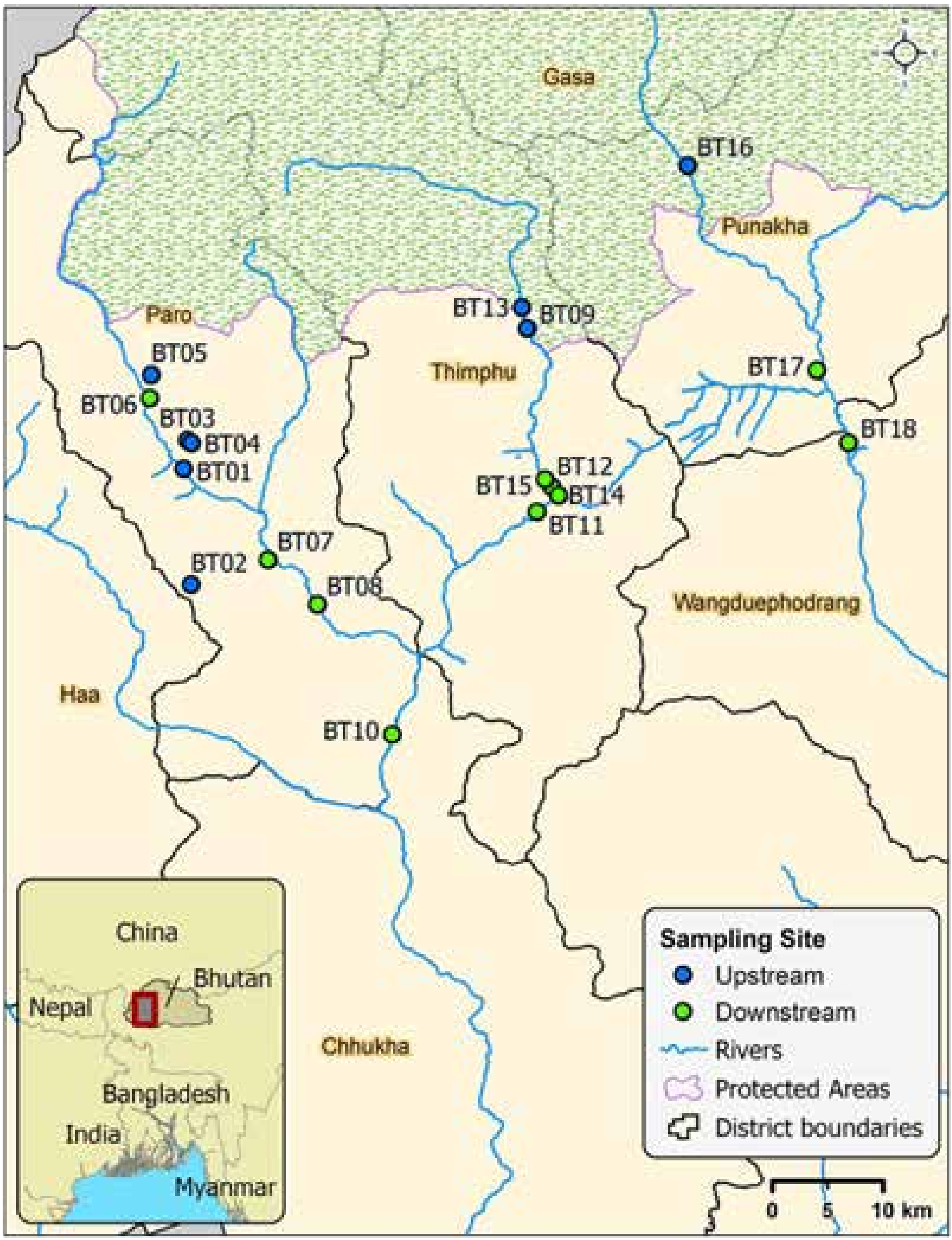

Figure 1. Map of Bhutan indicating sites sampled in 2015 and 2016. Blue circles indicate sites upstream of human disturbance and green circles are downstream.

org/). Of the 458 individuals submitted for barcoding, COI sequences ≥200 bp were determined for 281 specimens (61% of the total, 25 individuals with 200–350 bp; nine individuals with 351–450 bp; 247 individuals with 451–658 bp). The number of barcoded species and variance determined by BOLD Barcode Index Number (BIN) was based on their criteria for compliant barcode sequences (data accessed 8/2023). The study included 230 barcode-compliant individuals and 51 non-compliant (mainly because of short sequences). Sequences were aligned with a BOLD aligner and neighbor-joining trees (pairwise deletion and Kimura-2-parameter distance) were used to identify genetically distinct barcode species, which were confirmed using BINs where possible.

RESULTS

Water chemistry and bacteria variables for all study sites are summarized in Table 2. Results of the Wilcoxon rank-sum test showed that many of the 2015 water quality variables (specifically, pH, dissolved oxygen, specific conductivity, turbidity, ammonia, nitrite, nitrate, and phosphate) did not differ significantly ($p>0.05$) between sites upstream vs. downstream of disturbance. Temperature, *E. coli*, and total coliform were all significantly lower upstream of disturbance relative to downstream sites (Table 2). In 2016, pH, specific conductivity, and nitrite were all significantly higher upstream compared to sites downstream of disturbance, whereas *E. coli*, total coliform, and nitrate were significantly lower upstream than downstream. It is notable that differences in coliform bacteria, both total and *E. coli*, between upstream and downstream sites differed in both 2015 and 2016 by an average of thousands of cfu/100 ml. In contrast, for 2016 the differences in water quality variables were relatively small between upstream and downstream sites [e.g., pH (±0.2), nitrate and nitrite (±0.2 ppm)]. In the Paro and Thimphu watersheds, when the 2015 and 2016 data were combined, the coliform (total and *E. coli*) had the same patterns as the individual years with higher levels downstream than upstream. In addition, ammonia levels in the Thimphu watershed were significantly higher downstream (0.19 ppm) than upstream (0.04 ppm), while specific conductivity was higher upstream (181 µS/cm) than downstream (126 µS/cm).

A total of 50 insect families and six non-insect taxa were identified in 2015 and 2016; specifically, 36 taxa in 2015 and 49 taxa in 2016. The mayfly Baetidae was the only taxa collected from all 26 samples, while the mayflies Ephemerellidae and Heptageniidae, the caddisfly Hydropsychidae, and the true flies Chironomidae, Simuliidae, and Tipulidae were also common (>80% of the 26 samples). There were 22 rare taxa (i.e., 13 taxa were only recorded from one sample, and nine taxa were only recorded from two samples). Based on counts, Baetidae, Ephemerellidae, Heptageniidae, and Hydropsychidae were the most abundant in 2015 and Baetidae, Chironomidae, and Simuliidae were most abundant in 2016. For the 2015 data, the Wilcoxon test showed that for the 13 metrics examined, total richness, EPT richness, % EPT, % non-insects, HKHbios, BMWP 1983, ASPT 1983, and ASPT 2021 were significantly ($p \leq 0.05$) different between upstream and downstream sites (Table 3). In 2016, total richness and Shannon diversity were higher in upstream sites than downstream ones, while EPT richness and % EPT were only slightly ($p \leq 0.09$) different between the upstream and downstream sites.

The NMS revealed sites clustered by year and disturbance with years separating sites along axis 1 (32%) and disturbance separating sites along axis 2 (39%; Figure 2). Differences between years are likely related to the contrast in sampling seasons (post-monsoon vs. monsoon) and methods (qualitative dip net vs. quantitative Surber). For differences between years (axis 1), Stenopsychidae was the key taxa for 2015 whereas Acari, Empididae, Lepidostomatidae, Psychodidae, and individuals of mayflies, true flies, and caddisflies too small to identify beyond the family were the key taxa for 2016. Macroinvertebrate diversity was higher in 2016 (when samples were processed in the laboratory with a microscope) than in 2015 (when samples were processed in the field by eye). Microscope processing allows the counting of both small individuals (e.g., Acari, Ceratopogonidae, oligochaetes) as well as individuals that were too small to identify beyond order (e.g., Ephemeroptera, Trichoptera, Diptera). There were also more individuals examined in 2016 (>600 specimens per site) vs. 2015 (>100 specimens per site), increasing the likelihood of greater diversity. The NMS also showed sites upstream of disturbance were characterized by Perlodidae, Nemouridae, Rhyacophilidae, and Athericidae, whereas sites downstream of disturbance had fewer taxa and were more likely to have oligochaetes.

Although there were more morphological EPT taxa (76) than barcoded taxa (63), the actual barcode total may be underrepresented, because only 60% of the 458 individuals were successfully sequenced (Table 4). The 40% failure rate for barcoding may have resulted from the challenge of obtaining high-grade ethanol (95%, non-denatured) in Bhutan, making it difficult to properly preserve the DNA in samples. When only sequenced taxa were examined, there were 17 more taxa revealed with barcode than morphology alone, specifically 16 mayflies (in the families Baetidae, Ephemerellidae, Heptageniidae, Leptophlebiidae) and one stonefly (*Nemoura*). Barcoding indicated no additional caddisfly species. The average intraspecific variance across all groups was relatively low (average 0.36%; range = 0.0–1.18 %), in contrast to the interspecific variance (average 10.1%; range = 1.0–17.8 %). There were 19 barcoded species with <3 individuals so intraspecific variance could not be determined for those species. There were four taxa (*Acentrella* sp. C, *Drunella* sp. A, *Hydropsyche* sp. D, *Paragnetina*) that appeared to be morphologically distinct but grouped with another barcode species suggesting multiple morphotypes. Based on the BOLD database, there were 13 unique

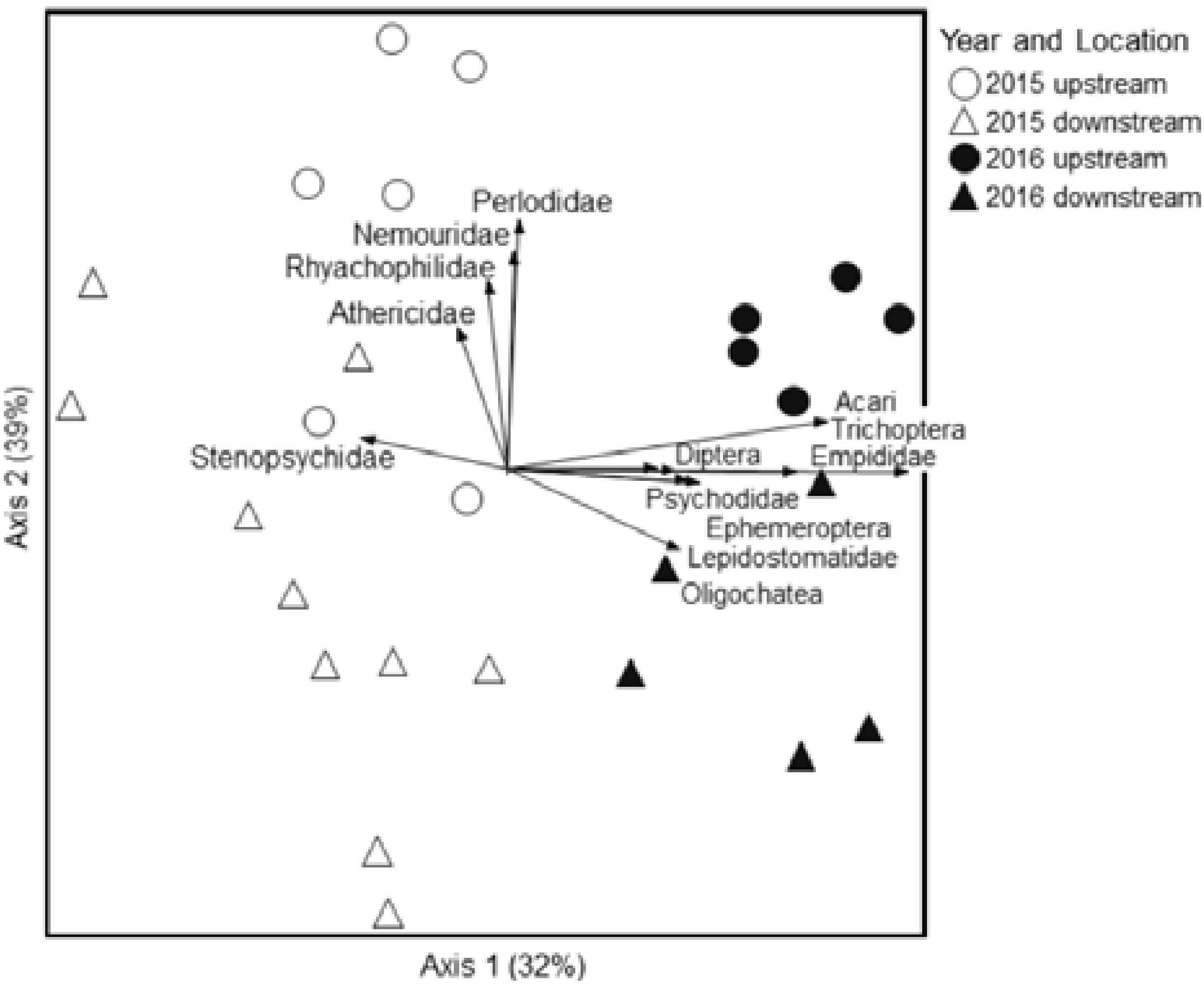

Figure 2. Non-metric multidimensional scaling of macroinvertebrates taxa groups (presence/absence) collected in Bhutan streams qualitatively in 2015 and quantitatively in 2016. Sites were classified as being upstream or downstream of disturbance.

species that were considered non-compliant, nine of them because sequences were too short (<500 bp) and they were not assigned to a BIN, and four are awaiting compliance with metadata requirements (Table 4). There were 42 barcode taxa (24 mayflies, six stoneflies, and 12 caddisflies) that were new sequences (e.g., new BINs) to the BOLD database (Table 4). One mayfly and seven caddisfly species had already been barcoded in other studies and had a species name available in BOLD (Table 4).

When mayfly sequences were compared between multiple sites (e.g., upstream vs. downstream and among drainage basins), they revealed differences that morphology failed to uncover. Overall, 201 of the 342 mayfly specimens (59%) were successfully barcoded, resulting in a total of 42 species versus 27 species based on morphology. There were several morphological taxa that looked similar but barcoding revealed that they did not occur at the same site (i.e., no spatial overlap; Figure 3). For example, barcodes indicated the presence of two species of *Epeorus* sp. C (29 individuals barcoded from eight streams) but one species was found in all the drainages (in small to medium streams) while the other species was only found at the large river sites of the Punatsangchhu drainage. This pattern of two (or more) species being morphologically similar but not overlapping geographically also occurred for *Cincticostella* sp. B (i.e., Paro and Thimphu sites vs. Punatsangchhu; 13 specimens), *Notacanthurus* sp. B (Paro vs. Punatsangchhu; 13 specimens), and *Epeorus* sp. B (i.e., upstream sites in the Paro vs. Thimphu; seven specimens). One caveat is that all of these spatial differences among species may be influenced by small sample sizes.

Barcoding revealed that one taxon, *Baetis* sp. A, was made up of five barcode species (34 specimens

Table 2. Water quality variables from November 2015 and August 2016 at Bhutan streams and rivers. Range of variables given for sites considered to be upstream (US) or downstream (DS) of a disturbance. Wilcoxon rank-sum test results (** p≤0.01; * 0.05≤p>0.01; ns not significant) within years and watersheds.

Variables	2015			2016			Paro			Thimphu		
	US (n = 8)	DS (n = 8)	t-test	US (n = 7)	DS (n = 7)	t-test	US (n = 9)	DS (n = 6)	t-test	US (n = 4)	DS (n = 8)	t-test
pH	7.47–8.39	7.55–8.56	ns	8.06–8.51	7.40–8.18	*	7.47–8.51	7.40–8.29	ns	8.06–8.39	7.41–8.56	ns
Temp (°C)	4.4–9.4	7.9–12.5	*	8.3–13.7	12.8–16.1	ns	4.4–13.7	9.2–14.9	ns	5.8–10.1	7.9–16.1	ns
Dissolved Oxygen (mg/L)	6.5–10.1	8.6–13.1	ns	7.7–8.6	7.3–8.5	ns	7.7–9.6	7.3–9.2	ns	6.5–9.0	7.3–9.8	ns
Spec. Conductivity (µS/cm)	21–198	90–156	ns	114–187	40–138	*	21–179	110–141	ns	166–198	40–156	*
Turbidity (NTU)	1–18	2–70	ns	1–13	2–20	ns	1–18	2–70	ns	1–13	2–20	ns
Total Coliform (cfu/100 ml)	0–44	21–443	**	0–267	367–16,000	**	0–267	22–2,400	*	0–100	107–16,000	*
E. coli (cfu/100ml)	0–1	<1–58	**	0	0–3933	**	0–1	1–433	*	0	14–4533	*
Ammonia (ppm)	0–0.28	0.06–0.30	ns	0.02–0.22	0.06–0.28	ns	0.07–0.28	0.07–0.28	ns	0.02–0.09	0.06–0.30	*
Nitrite (ppm)	0.03–0.41	0.03–0.10	ns	0.02–0.09	0.04–0.25	*	0.02–0.09	0.03–0.11	ns	0.04–0.41	0.05–0.25	ns
Nitrate (ppm)	0.95–2.31	1.45–1.96	ns	0.94–1.50	1.22–1.81	*	0.97–2.31	1.17–1.69	ns	0.94–1.69	1.22–1.96	ns
Phosphate (µM)	0.33–0.46	0.30–0.76	ns	0.25–0.41	0.32–0.81	ns	0.30–0.46	0.33–0.57	ns	0.25–0.49	0.30–0.81	ns

sequenced from 12 sites), with two common species being found in small to medium streams of the Paro and Thimphu watersheds, while a third common species preferred large sites in the Punatsangchhu and the confluence of the Paro and Thimphu Rivers. In addition, for some taxa only identified to a specific genus via morphology, barcoding was able to reveal multiple species, i.e., *Fallceon* (2 species), *Iron* (3 species), and *Paraleptophebia* (3 species), while in other cases morphology and barcoding were aligned (e.g.., *Acentrella* species A, B and C, *Baetis* sp. D, *Drunella* sp. A).

DISCUSSION

Based on health concerns and other management reasons, the World Health Organization (WHO 2017) has provided guidelines for drinking water that can be used as a baseline for stream conditions. They set levels not to be exceeded for nitrite (3 mg/L), nitrate (50 mg/L), and *E. coli* (zero cfu/100 ml) based on health concerns, a pH range (6.50–8.50) for sewage treatment operation, and an ammonia level (1.5 mg/L) for odor (WHO 2017). Using WHO criteria, many water quality parameters in this study were largely within the acceptable range (Table 2): specifically, nitrite, nitrate, and ammonia levels were below these limits and only one site had a pH slightly above the limit (8.56). *E. coli* was the only variable that was above the recommended limit (WHO 2017) and it was exceeded for the majority of the downstream sites (94%), while all the upstream sites registered no *E. coli* or extremely low levels (1 cfu/100 ml). This is good news for those living upstream, given that much of Bhutan's rural population draws untreated water for consumption directly from stream or river systems (Giri et al. 2010; Rahut et al. 2015) and *E coli* is a measure of fecal coliform and thus an indicator of fecal contamination. This is not good news for those living downstream of disturbance, because high fecal coliform indicates an increased risk of pathogen-borne illnesses (USEPA 2012; WHO 2017). In the United States, the Environmental Protection Agency (USEPA 2012) guideline for streams is that *E. coli* should not exceed a geometric mean of 126 cfu/100 ml to be considered safe for swimming. Unfortunately, 20% of the Bhutan samples exceeded this level, and for four of the 2016 sites (BT11, BT10, BT14, BT15) in the urban area of Thimphu watershed, levels were anywhere from 23 to 35 times higher (2900–4533 cfu/100 ml). This indicates that untreated sewage was entering the river at or near those sites. The results suggest fecal coliform or *E. coli* could be a powerful, yet easy and inexpensive tool

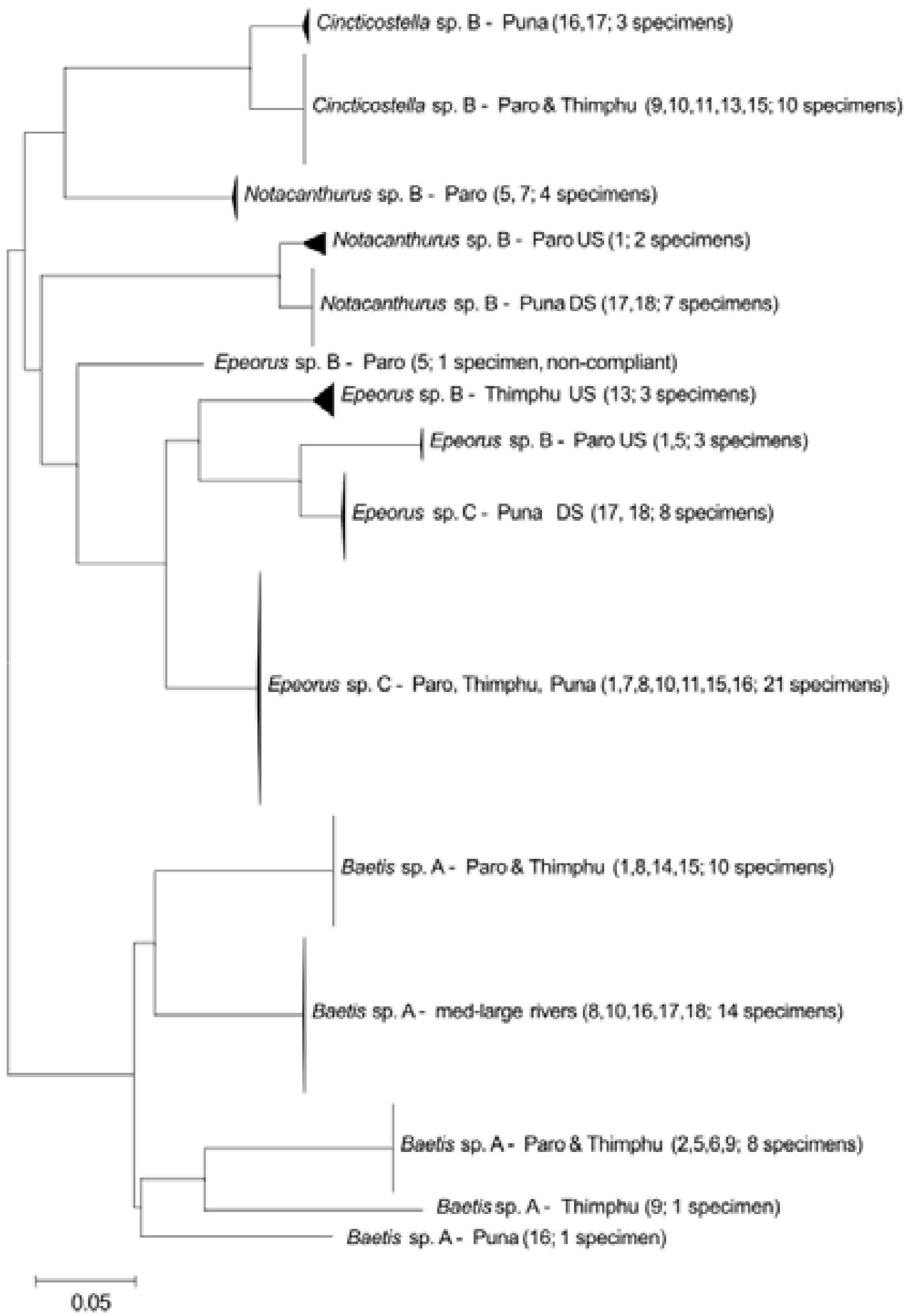

Figure 3. Neighbor-joining tree of five morphological mayfly species and their designations based on barcode. Taxa name is followed by the watershed it was found in [Paro, Thimphu, and Puna (-tsangchhu)], the specific sites (see Table 1), and the number of specimens barcoded. For each taxon, the vertical distance of the line indicates the number of individuals, and the horizontal distance is the maximum genetic diversity within the branch.

Table 3. Macroinvertebrate metrics for 2015 qualitative (dip nets) and 2016 quantitative (Surbers) sampling in Bhutan. Range of metrics provided for sites considered to be upstream (US) or downstream (DS) of disturbance. Wilcoxon rank-sum test results (* p≤0.001; ** 0.01≤p>0.001; * 0.05≤p>0.01; ● 0.09≤p>0.05; ns not significant) indicate if metrics differed based on disturbance.**

Metrics	2015			2016		
	US (n = 6)	DS (n = 10)	t-test	US (n = 5)	DS (n = 5)	t-test
Richness	12–14	9–16	*	14–18	12–15	*
EPT Richness	8–11	5–8	***	6–8	4–7	●
Diptera richness	2–4	2–3	ns	3–6	3–5	ns
% EPT	79–95	35–95	*	38–46	21–80	●
% Chironomidae	0–10	0–20	ns	13–47	5–19	ns
% Non-insects	0–4	0–25	*	5–10	4–28	ns
Shannon Diversity	1.88–2.14	1.42–2.38	ns	1.81–2.40	1.58–2.06	*
Simpson Diversity	0.79–0.86	0.64–0.88	ns	0.70–0.88	0.70–0.81	ns
HKHbios	7.6–8.7	6.2–7.6	***	5.9–7.8	6.7–7.8	ns
BMWP 1983	68–111	48–76	**	47–71	42–71	ns
BMWP 2021	46–86	41–77	ns	46–69	47–79	ns
ASPT 1983	6.5–7.6	6.0–7.3	**	5.4–7.4	5.2–6.8	ns
ASPT 2021	6.1–7.2	5.5–7.4	**	4.5–6.7	5.0–6.2	ns

to regularly monitor the safety of Bhutan streams for various public activities.

A previous study of four headwater streams in Bhutan reported that most environmental variables (i.e., temperature, conductivity, stream width, depth, velocity) did not differ between monsoon and pre-monsoon seasons (Dorji 2014b). Similarly, a 2008–2009 study of the river Wang Chhu near Thimphu city sampled in pre-monsoon, monsoon, and post-monsoon indicated similar patterns in response to urban pollution in all three seasons and that nitrate, total coliform, and biochemical oxygen demand [BOD] were the best parameters for monitoring urban impacts (Giri & Singh 2013). The results suggest that water chemistry in the monsoon season was better able to discern impacts than in the post-monsoon season. A study examining agricultural practices in a Bhutan stream in the Samtse district also indicated that the monsoon season was the optimal time to measure the highest levels of nitrate, BOD, and total dissolved solids (Giri et al. 2010).

It is important to note for this study that not all the same sites were measured in both years (e.g., Punatsangchhu sites were only sampled in 2015). Nevertheless, there were more water quality variables that differed between upstream and downstream sites in the monsoon season than the post-monsoon (3 vs. 6). This might be related to the fact that higher discharge in the monsoon season may result in more pollution entering the stream than for the pre- or post-monsoon seasons (Giri et al. 2010). Typically, 70% of the annual precipitation is concentrated during the monsoon season that occurs from June to September and a major portion of the water volume in the basins is attributed to rain-fed recharge (WBMP 2016).

In Bhutan, septic tanks are commonly reported to overflow into the environment due to poor design and maintenance and this problem is exacerbated by heavy monsoon rains because soak-pits and waste stabilization ponds can become full and overflow (Taylor-Dormond et al. 2018; Dorji et al. 2019). Also, because agriculture across this country occurs in steep topography, erosion is extensive in Bhutan and is exacerbated by heavy rain showers during the pre-monsoon season falling on bare soils prior to crop emergence (Dorji et al. 2011; WBMP 2016). Although the dominant soil type, gneissic, is resistant to erosion, the loss of fertile soils during storms results in increased nutrients and sediments washing into streams and rivers (Baillie et al. 2004; Dorji et al. 2011). Rapid runoff into Bhutan waters during flood events is further exacerbated by forest fires and overgrazing (Tariq et al. 2021). The above factors suggest that most of the water quality differences measured in this study (Table 2) between upstream and downstream sites were indicative of pollution (i.e., higher temperatures, coliform, and *E. coli* downstream than upstream in 2015 and higher coliform, *E. coli,* nitrite, and nitrate downstream than upstream in 2016). In contrast, the higher pH and conductivity levels in the upstream sites

Table 4. Morphological name followed by a letter is the designation of unique species. Variance is show as maximum within a barcode species (% intra) and distance to nearest neighbor (% inter). Number of barcode species and variance determined by BOLD BINs based on their criteria for compliant barcode (data accessed 8/2023). Intraspecific variance was listed as not available (na) if there was only one individual in the BIN. If there were multiple barcode species for a single morphological name then the range for % variance is shown. Instances where barcode species was based on noncompliant specimens are denoted with "b" followed by a number of basepairs (bp) in sequence; all of these were 1 individual with the exception of Skwala and Mystacides (2 individuals). Asterisks indicate a new sequence to the BOLD library.

Morphological name	No. individual barcoded	No. sequences (>200 bp)	No. barcode species	% Variance Intra	Inter	[a] Not unique sequence [b] Not barcode compliant [c] Name on BOLD BIN
EPHEMEROPTERA - 27 of 31 morphological taxa sequenced						
Total	**342**	**201**	**42**			
Baetidae						
Acentrella sp. A	17	14	1	1.96	15.2	
Acentrella sp. B	29	19	1	0.51	17.0	
Acentrella sp. C*	4	1	1	na	13.5	
Acentrella sp. D	1	1	0			[a] *Acentrella* sp. C
Baetis sp. A*	47	34	5	0–2.51	5.8–15.6	
Baetis sp. B*	2	1	1	na	15.5	
Baetis sp. C*	7	1	1	na	14.6	
Baetis sp. D	13	6	1	2.16	15.4	
*Fallceon***	14	9	2	Na–0	16.5–16.9	
Caenidae						
Caenis sp. A	4	0	—			
Caenis sp. B*	2	2	1	na	12.7	
Ephemerellidae	27	12	2	0–0.73	16.3–16.5	[c] *Spinorea gilliesi*
Cincticostella sp. A	3	0	—			
Cincticostella sp. B	27	13	2	0.36–0.92	4.8	
Cincticostella sp. C	6	0	—			
Drunella sp. A*	10	9	1	0.18	11.1	
Drunella sp. B	1	1	0			[a] *Drunella sp. A*
Drunella sp. C	2	1	1			[b] 268 bp
Teloganopsis	4	0	—			
Ephemeridae						
*Ephemera**	1	1	1	na	4.7	
Heptageniidae						
Afronurus	1	1	1	0.17	13.4	
*Cinygmula**	6	4	1	0.7	9.2	
Epeorus sp. A	11	1	1			[b] 329 bp
Epeorus sp. B*	10	7	3	1.61–1.77	3.7–11.8	[b] 217 bp, [c] *E. aculeatus*
Epeorus sp. C*	38	29	2	0–1.46	7.8–11.5	
*Iron****	4	4	3	na–0.96	12.2–14.3	
Notacanthurus sp. A*	6	5	1	1.0	17.0	
Notacanthurus sp. B***	19	13	3	na–0	2.5–14.0	
*Rhithrogena***	7	4	2	na–0.16	3.9	
Leptophlebiidae						
*Paraleptophlebia***	9	5	3	0.17–0.89	9.6	[b] 484 bp
Neoephemeridae*	10	3	1	0.96	9.6	
PLECOPTERA - 10 of 11 morphological taxa sequenced						
Total	**29**	**19**	**10**			
Capniidae	1	1	1			[b] 260 bp
Leuctridae						
Paraleuctra	1	1	1			[b] 441 bp
Nemouridae						
*Amphinemura**	4	2	1	0.2	10.7	
*Nemoura***	3	2	2	na	6.6	

Morphological name	No. individual barcoded	No. sequences (>200 bp)	No. barcode species	% Variance Intra	Inter	[a] Not unique sequence [b] Not barcode compliant [c] Name on BOLD BIN
Peltoperlidae						
*Cryptoperla**	3	3	1	0.37	12.7	
Perlidae						
Calineuria	3	0	—			
Kiotina sp. A*	2	2	1	0	15.7	
Kiotina sp. B *	2	2	1	na	15.6	
Paragnetina	5	3	1	2.5	14.6	
Tetropina	1	1	0			[a] *Paragnetina*
Perlodidae						
Skwala	4	2	1			[b] 202 & 459 bp
TRICHOPTERA - 26 of 34 morphological taxa sequenced						
Total	**87**	**61**	**25**			
Brachycentridae						
*Brachycentrus**	3	3	1	0	6.0	
*Micrasema**	1	1	1	na	10.5	
Glossosomatidae						
*Agapetus**	6	6	1	1.37	13.0	
Glossosoma	3	3	1	1.12	9.1	[c] *Glossosoma dentatum*
Hydropsychidae						
Arctopsyche	3	3	1	0.48	5.8	[c] *Arctopsyche lobata*
Hydropsyche sp. A	5	0	—			
Hydropsyche sp. B*	2	2	1	0.17	8.5	
Hydropsyche sp. C	4	1	0			[a] *Hydropsyche* sp. D
Hydropsyche sp. D*	3	3	1	0.33	2.7	
Hydropsyche sp. E	3	2	1	0.17	5.8	
Hydropsyche sp. F*	3	2	1	0.17	11.7	
Hydropsyche sp. G*	3	3	1	0.64	2.7	
*Lepidostoma**	3	3	1	1.36	10.1	
*Mystacides**	2	2	1	na	3.1	[b] 586 & 594
Limnephilidae	2	2	1	0.18	10.8	[c] *Phylostenax himalus*
*Chimarra**	3	3	1	0.34	2.7	
*Neurocyta**	1	1	1	na	3.6	[b] 637 bp
Psychomyiidae	1	0	—			
Rhyacophilidae						
Himalopsyche sp. A	3	3	1	1.19	2.5	[c] *Himalopsyche digitata*
Himalopsyche sp. B	2	1	1	1.81	8.5	
Himalopsyche sp. C	3	1	1	0.17	11.0	[c] *Himalopsyche horai*
Himalopsyche sp. D	1	0	—			
Rhyacophila sp. A*	3	1	1	na	2.3	
Rhyacophila sp. B	3	3	1	0.38	7.5	
Rhyacophila sp. C	1	0	—			
Rhyacophila sp. D	1	0	—			
Rhyacophila sp. E	1	1	1			[b] 317 bp
Rhyacophila sp. F	2	0	—			
Rhyacophila sp. G	2	2	1	0.17	1.0	[c] *Himalopsyche tibetana*
Rhyacophila sp. H	4	0	—			
Rhyacophila sp. I	1	0	—			
Rhyacophila sp. J	2	2	1			[b] 202 & 257 bp
Stenopsychidae						
Stenopsyche sp. A	4	4	1	0.64	6.2	
Stenopsyche sp. B	3	3	1	0.32	10.6	

versus the downstream sites in 2016 are likely due to a geological influence since these variables typically increase with pollution but were found to decrease downstream of disturbance areas.

For the post-monsoon season, the 2015 kick samples sorted in the field resulted in larger, more mature macroinvertebrate specimens, and many metrics indicated significant differences between upstream and downstream sites. The best metrics were related to sensitive groups known to become less abundant in response to disturbance (i.e., EPT richness and % EPT; Table 3). Other important metrics capable of measuring disturbance in 2015 were taxon richness (on average having two more taxa upstream than downstream, often families belonging to EPT) and % non-insects (averaging 1% upstream vs. 6% downstream). In addition, the metric BMWP1983 indicated the upstream sites had better environmental conditions than the downstream sites in 2015. It is noteworthy that although the BMWP1983 was initially designed for European streams, it worked better than the version (BMWP 2021) modified specifically for Bhutan (Dorji et al. 2021). To this end, BMWP1983 characterized some insect families (i.e., Ephemerellidae and Heptageniidae) as sensitive to disturbance even though they were found in nearly all the sites (including degraded sites) suggesting those families contain taxa somewhat pollution-tolerant, while other families (e.g., Perlidae and Perlodidae) seemed to be better indicators of "good" water quality or sites that lack major human disturbance. Also, both the 1983 and 2021 versions of the metric ASPT, which is the BMWP modified to account for richness, were sensitive to disturbance in 2015. The HKHbios was designed to monitor streams in the region (Bangladesh, Bhutan, Nepal, India, and Pakistan) and worked well in indicating impact in 2015, although it rated all the sites as "good", even the disturbed ones, but the sampling method in this study was modified, which may have inflated the scores (Ofenböck et al. 2010).

In 2016, the fact that taxon richness and Shannon were the only metrics associated with the Surber sampling to indicate a disturbance is likely related to multiple factors (Table 3). The monsoon season is a difficult time to sample, presenting a safety issue, and high-water levels may have scoured some streams more than others. Also challenging is achieving equal sampling effort at sites across a gradient of small streams to large rivers, especially since high flow limited sampling in some cases to only the stream edges. Regional studies of monsoon effects on macroinvertebrates are not all in agreement (Brewin et al. 2000; Ofenböck et al. 2010; Dorji 2014b; Wangchuk & Dorji 2018; Thapa et al. 2020). Most studies in tropical Asian streams suggest a tendency for an overall decline in macroinvertebrates abundance and richness during the monsoon versus drier seasons (see Dudgeon 1999; Brewin et al. 2000). In Bhutan, one study reported macroinvertebrate abundance in headwater streams also decreased after flash floods but found no difference in macroinvertebrate diversity between pre- and post-monsoon seasons (Dorji 2014b). In contrast, a study of springs in nearby Nepal found EPT richness was higher in the post-monsoon versus the pre-monsoon season (Thapa et al. 2020). In a relatively large survey, Ofenböck et al. (2010) studied 198 streams in the Hindu Kush-Himalayan region and found that both pre- and post-monsoon macroinvertebrate data were able to differentiate non-impacted and impacted sites. To evaluate disturbance, they recommended sampling in the pre-monsoon season to avoid the many complications (noted above) associated with flooding effects in the post-monsoon period (Ofenböck et al. 2010).

The NMS indicated distinct differences in the 2015 and 2016 macroinvertebrate assemblages, which may be attributed to both time of year and sampling methods (Figure 2). More importantly, both sampling years, independent of the method, resulted in the separation of upstream and downstream sites. Given that for 2016, only two of the 12 metrics showed a significant difference between upstream and downstream sites (Table 3), perhaps metrics more specific to the Bhutan macroinvertebrate assemblages like % Baetidae or % Plecoptera (or possibly % Nemouridae and % Perlodidae), might be more sensitive measures of disturbance but this would require a larger dataset to put it to the test.

The level of disturbance was not well-defined in this study. Not all sites designated downstream of disturbance had the same level of degradation. Hopefully, going forward, land use types may be quantified to better understand the relationship between disturbance in the watershed and its impact on macroinvertebrate assemblages (Giri & Singh 2013). Many macroinvertebrate studies in Bhutan are still using the higher family level identification, and although this level of identification is useful in instances of high degradation (Giri & Singh 2012; Dorji 2014a, Dorji et al. 2014; Gurung & Dorji 2014; Wangchuk & Dorji 2018), it has been shown in other studies not to be as sensitive as genus or species level identification in discerning small levels of disturbance (Arscott et al. 2006). Although progress has begun in creating species-level checklists for Bhutan (Wangdi et al. 2018; Dorji et al. 2021; Gyeltshen & Prasad 2022), research on the taxonomy of most of the aquatic macroinvertebrate groups is very limited and lacks baseline data. Bhutan seems to have a high diversity of macroinvertebrates belonging to 18 orders and 89 families (Dorji & Gurung 2017), with current species counts of 38 stoneflies, 172 caddisflies, 33 dipterans, 41 beetles, five mites, 12 hemipterans, 114 dragonflies and damselflies, and one megalopteran (Wangdi et al. 2018). As of 2017, at least 566 new species of flora and fauna have been recorded for Bhutan, including 77 aquatic species (Takaoka & Somboon 2008; Gyeltshen et al. 2018).

The biggest challenge in species-level identification for aquatic macroinvertebrates is that taxonomic keys still need to be expanded or developed for many groups. This study shows that DNA barcoding may help in this regard. DNA barcoding expanded the EPT list by 17 species and highlighted the presence of cryptic taxa (e.g., four species for *Baetis sp. A*; Table 4, Figure 3). Moreover, it suggested that morphologically similar species of mayflies often segregate according to either drainage or disturbance. Other studies have shown DNA barcoding improves macroinvertebrate monitoring (Jackson et al. 2014; Li et al. 2022) and have shown that morphologically similar mayfly species were spatially separated within the same river based on pollution (Sweeney et al. 2011). The barcoding results (42 "new" DNA sequences) represent only a start for EPT and highlight the need for further additions to the DNA reference library for the region.

The largest water quality challenges Bhutan faces going forward are sanitation management, climate change, and shared river systems (WBMP 2016). Urban areas of Bhutan will have to provide adequate sanitation infrastructure and sufficient regulatory pollution control measures to be enforced to protect water quality (Karn & Harada 2001; Dorji et al. 2019). For example, macroinvertebrate monitoring, in conjunction with chemical and bacteria parameters, could help evaluate the effectiveness of the new 2021 biological processing plant in Thimphu city that replaced their outdated sewage facility (Lhaden 2021). Bioassessment with macroinvertebrates could also help in managing changes in hydrology due to climate change and guide policy in managing river systems shared with neighboring countries. Given its inexpensive and straightforward nature, biomonitoring of streams with macroinvertebrates seems to be an accessible tool for both public officials and community/citizen science. The study shows water chemistry and bacteria were best sampled in the monsoon season to have the greatest measure of human disturbance, while macroinvertebrates were most effective in detecting impacts when sampled in the post-monsoon season. The DNA findings (e.g., 18 more EPT species using barcode versus morphology and 42 new sequences added to the BOLD database) suggest the diversity of stream macroinvertebrates in this region is presently underestimated and the continued expansion of species identifications (either morphologically or through DNA barcoding) will greatly aid in the future assessments of Bhutan waterways.

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Declarations: Data availability any data presented in this paper will be available from the corresponding author upon request. Barcode data is available on GenBank and Barcode of Life Data systems.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26104–26109

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9255.16.11.26104-26109

#9255 | Received 01 July 2024 | Final received 09 November 2024 | Finally accepted 19 November 2024

COMMUNICATION

Indian Leopard predation on the sub-adult Himalayan Griffon Vulture (Accipitridae: Accipitriformes)

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Abstract: This study documents instances of predation on Himalayan Griffon Vulture *Gyps himalayensis* by Indian Leopards *Panthera pardus fusca* Meyer, 1794 near the pre-release aviary of the Buxa Vulture Conservation Breeding Centre within the Buxa Tiger Reserve. Camera trap monitoring revealed leopard predation on sub-adult vultures, highlighting a previously unreported threat to vulture populations. The adaptability of leopards to diverse ecosystems, coupled with their varied diet, underscores the need for further research to understand the implications of leopard predation on vulture conservation efforts. This documentation provides insights into the prey-predator interactions and emphasizes the importance of continued monitoring and conservation measures to safeguard vulnerable vulture populations.

Keywords: Buxa Tiger Reserve, camera trap, *Gyps himalayensis,* leopard food, *Panthera pardus fusca,* post-release monitoring, vultures, vulture predation, wildlife conservation, wintering grounds.

Editor: L.A.K. Singh, Bhubaneswar, Odisha, India.

Date of publication: 26 November 2024 (online & print)

Citation: Chakraborty, S.S., D. Ray, A. Sen, P.J. Harikrishnan, N.K. Jha & R. Ghosh (2024). Indian Leopard predation on the sub-adult Himalayan Griffon Vulture (Accipitridae: Accipitriformes). *Journal of Threatened Taxa* 16(11): 26104–26109. https://doi.org/10.11609/jott.9255.16.11.26104-26109

Funding: Ministry of Environment, Forest and Climate Change (MoEFCC), Government of India. West Bengal Forest Department, Government of West Bengal.

Competing interests: The authors declare no competing interests.

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Author contributions: SSC—conceptualization, methodology, investigation, writing- original draft. DR—project administration, writing – review and editing. AS—project administration, writing - review and editing. PJH—project administration, writing – review and editing. NKJ—writing, literature review and editing. RG—investigation and data collection.

Acknowledgements: This work was carried out under the Vulture Conservation Breeding Programme of West Bengal, funded by the Ministry of Environment, Forest and Climate Change (MoEFCC), Government of India, and the West Bengal Forest Department, Government of West Bengal. We extend our sincere gratitude to the Bombay Natural History Society, Mumbai for managing the project until March 2023 and to the West Bengal Zoo Authority for their active support in running the programme from April 2023 onwards. We are deeply grateful for their invaluable contributions to the conservation of vultures in the region.

INTRODUCTION

Vultures are nature's most efficient scavengers. They occupy a crucial ecological niche that helps in maintaining a healthy and natural environment. They play useful role in the ecosystem by efficiently disposing of carcasses. Once very common, vultures are on the verge of extinction in Indian subcontinent. Populations of three species of vultures, viz., the Oriental White-rumped, the Long-billed, and the Slender-billed, have declined drastically between the mid 1990s and the mid-2000s (Prakash et al. 2003, 2007; Pain et al. 2008).

The Vulture Conservation Breeding Centre at Rajabhatkhawa is an initiative of the West Bengal Forest Department, with the goal of saving three *Gyps* species of vultures, namely the Oriental White-rumped Vulture *Gyps bengalensis*, Long-billed Vulture *Gyps indicus*, and the Slender-billed Vulture *Gyps tenuirostris*, from looming extinction. This centre also works as a rescue and rehabilitation centre for vultures for the entire West Bengal state. Since the establishment of the centre, a total of 95 Himalayan Griffon Vultures have been rescued, 80 individuals successfully rehabilitated and subsequently released back into their natural habitat. The present documentation of leopard predation on Himalayan Griffon is the byproduct of intense monitoring of the habitat and behaviour of wild vultures in the study area by the centre. The Himalayan Griffon Vulture *Gyps himalayensis* is considered a resident of the mountains of central Asia, the Himalaya, southern and eastern Tibet, and China. Post breeding, the adults remain, for most of the year, in the breeding grounds while juveniles migrate to the plains of south and southeast Asia in winter (Naoroji 2006; Rasmussen & Anderton 2012). These migrating Himalayan Griffons frequently visit the pre-release aviary site of the Buxa Vulture Conservation Breeding Centre where captive bred vultures from the conservation breeding centre are kept for acclimatization before release into the wild. Visit of Himalayan Griffon at the site and close interaction with the captive-bred vultures indicate the social behaviour of the vulture and success of the conservation breeding and reintroduction programme.

Study area

The observed predation on vulture by leopard has been documented near pre-release aviary, on the bank of the Bala River near 22nd Mile anti-poaching camp, (26.6178N & 89.5612E) of the Buxa Vulture Conservation Breeding Centre located within the Buxa Tiger Reserve. The Tiger Reserve is located in the northeastern corner of West Bengal and covers an area of 760.87 km^2. The northern boundary of the reserve borders Bhutan while the eastern side borders Assam. The western and southern boundaries are bordered by tea gardens and agricultural fields (Figure 1). Biogeographically, the tiger reserve lies in two major zones: the central Himalaya and Gangetic Plains. The elevation of the reserve ranges 60–1,750 m. The forest type is primarily tropical moist deciduous dominated by Sal *Shorea robusta*. The temperature ranges 10–32 °C; and the average annual rainfall is about 4,100 mm.

MATERIALS AND METHODS

The first captive-bred vulture was released into wild as part of reintroduction in the year 2020. Since then, 31 captive-bred White-rumped Vultures along with 45 rescued Himalayan Griffons have been released using the soft release methodology. In this method, the birds earmarked for release are kept in pre-release aviary for acclimatization and socialization followed by opening the gate of pre-release aviary in presence of wild vultures. This method was initially developed and applied in France in 1980s (Terrasse & Choisy 2007). It was found in earlier studies that soft release protocols tend to have a positive outcome and are 40% more successful than hard release protocol in conservation translocation (Resende et al. 2021). In addition, animals remain at or near the release site during initial period in wild which increase the chance of conservation translocation success by 77%.

Following the methodology described by Terrasse et al. (2004), food was frequently provided at feeding sites located just outside the pre-release aviary. Besides, carcass of wild animals that died naturally inside the tiger reserve were also provided after necessary medical checks for disease. This attracted wild vultures near the pre-release aviary and also supported released vultures during initial days. The site is closely monitored through trap-camera. Trap-camera photographs are downloaded twice a week and analysed to document various activities and behaviour of the released and wild vultures. Professional color model 1347 of Cuddeback Digital motion sensor cameras were used to collect photographs. A research team remain stationed day and night at a nearby camp to observe and understand behaviour of vultures including feeding time and pattern, preference of body parts and interaction with wild birds. The intense monitoring also included other scavengers feeding on the carcass.

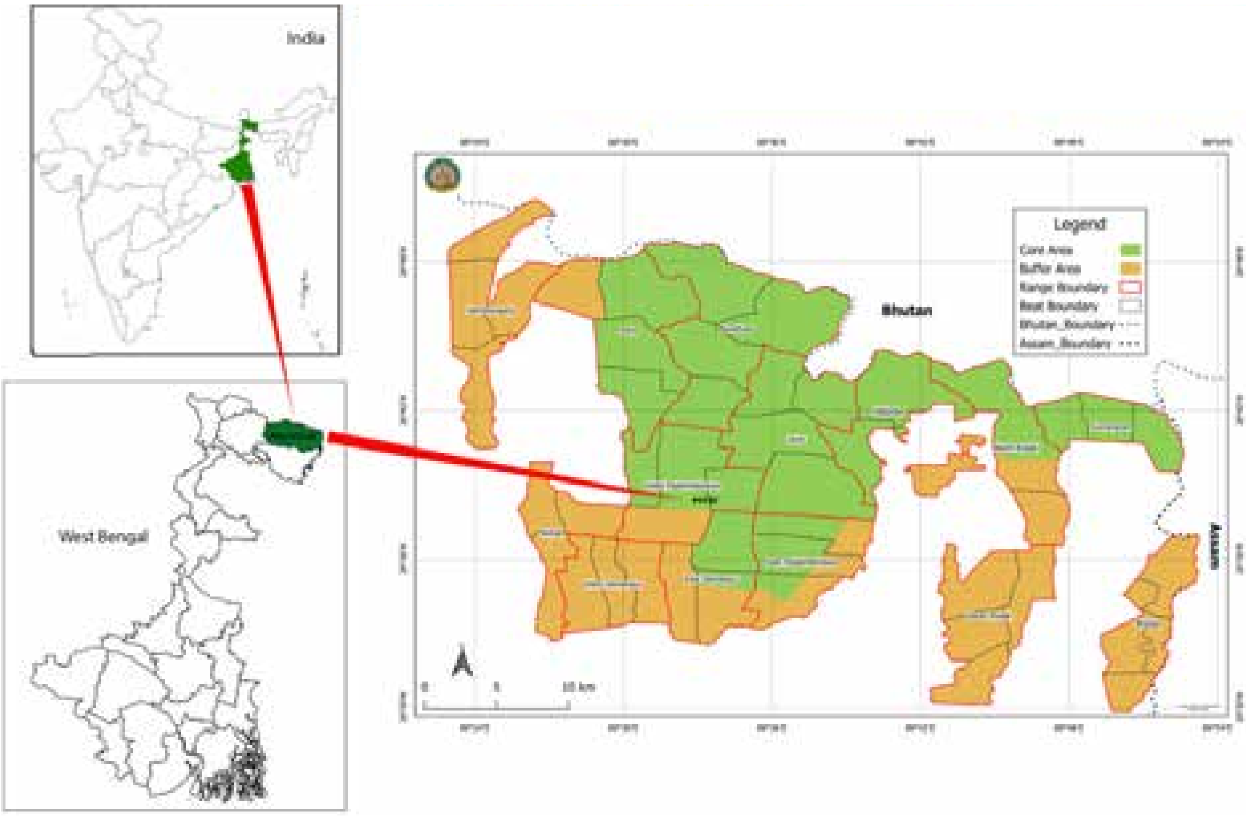

Figure 1. Location of Buxa Vulture Conservation Breeding Centre, Rajabhatkhawa.

RESULTS AND DISCUSSION

In February 2020, when screening and analysing trap camera images from the supplementary feeding site near 22nd Mile pre-release aviary, an unusual event of an Indian Leopard *Panthera pardus fusca* (Meyer, 1794) preying a sub-adult Himalayan Griffon Vulture was recorded on 10 February 2020. The leopard approached the griffon feeding on a Sambar Deer *Rusa unicolor* carcass and despite aggression shown by the vulture the leopard killed the vulture and dragged it out of the frame of the trap camera. Later, the almost consumed carcass of the griffon was observed in a nearby bush.

Later on, in March 2024, two more carcasses of Himalayan Griffon vultures were found in the Bala riverbed near the supplementary feeding site. Both carcasses were almost fully consumed, and only some bones and feathers were left. Presumably, these vultures were also predated by leopards.

Vultures which are thought to have very few predators at their adulthood are evidently not that safe in its natural habitat.

The Indian Leopard has a wide range of distribution in India, except above the treeline in the Himalaya and desert areas (Daniel 1996). In addition to their natural habitats, the elusiveness and behavioural flexibility of the leopard allow them to survive near villages and human settlements (Daniel 1996; Nowell & Jackson 1996). Studies on the food habits of leopard suggest that they have a more diverse diet, ranging from small rodents and birds to medium-sized wild ungulates weighing less than 50 kg (Eisenberg & Lockhart 1972; Bothma & Le Riche 1986; Santiapillai et al. 1982; Johnsingh 1983; Rabinowitz 1989; Seidensticker et al. 1990; Karanth & Sunquist 1995, 2000; Sankar & Johnsingh 2002; Henschel et al. 2005; Andheria et al. 2007).

Leopards in and around Buxa Tiger Reserve (BTR) are highly adaptable as they inhabit a diverse range of ecosystems that includes dense forests of central BTR, hilly terrain along Indo-Bhutan border, and tea garden areas adjoining the forest land. As per the latest report published by National Tiger Conservation Authority of India, there are 61 leopards inhabiting the territory of BTR while 74 are utilizing the Reserve (Qureshi et al. 2024). Carnivore sign survey data of Buxa Tiger Reserve from the year 2022 reveals 16 individuals/100 km^2 (Annual Report 2022). Diversity of terrain has provided diverse prey base for leopard in the region from small

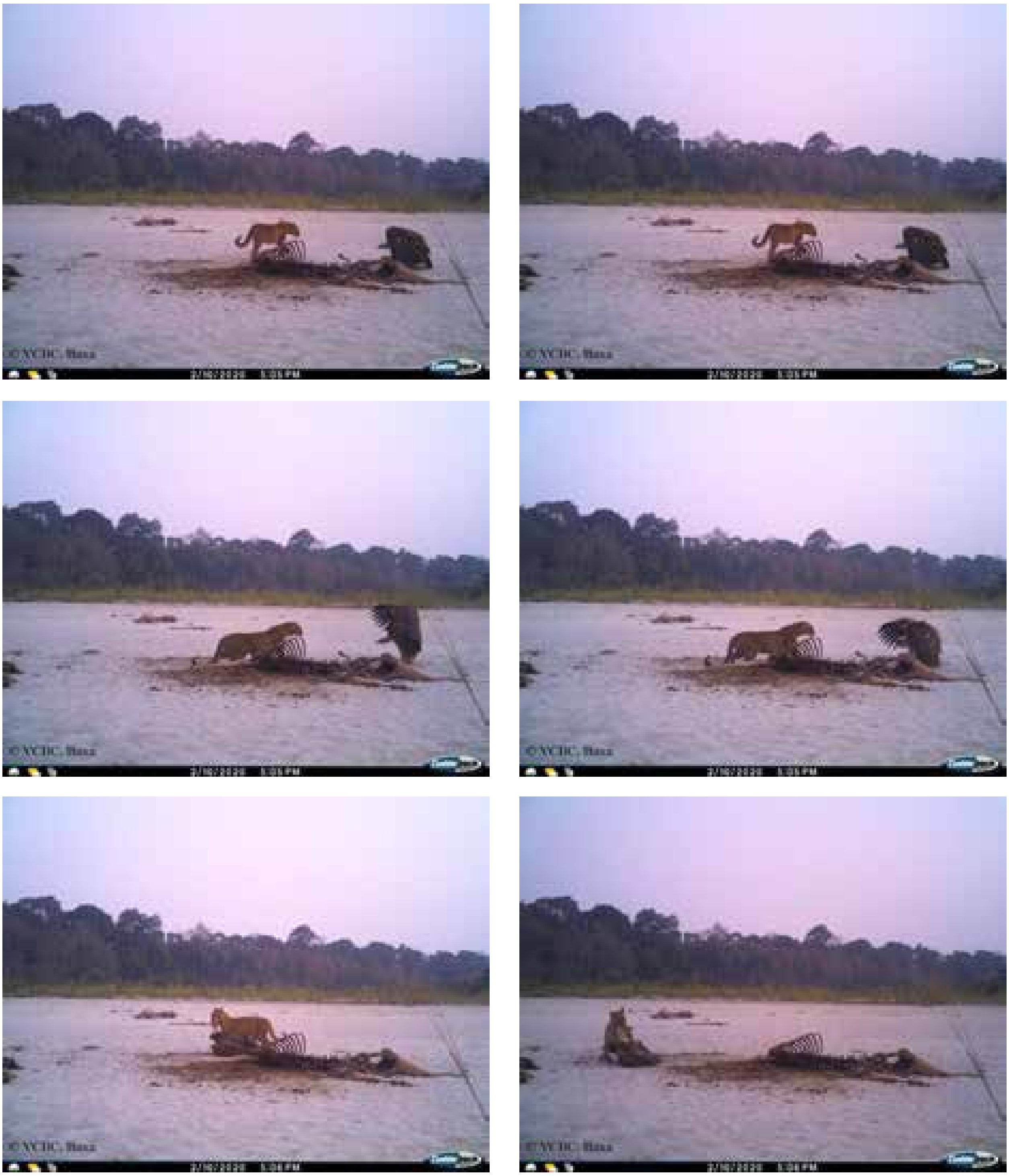

Image 1–6. The sequence of a sub-adult Himalayan Griffon Vulture being preyed by an Indian Leopard on 10 February 2020.

livestock in tea garden labour settlement to wild prey in the core area, making the reserve suitable for leopard habitation.

Indian leopards are known to hunt and feed on wild birds (Ahmed et al. 2008; Selvan et al. 2013) including peafowls (Mondal et al. 2011) on a regular basis in different protected areas of India. However, predating on Himalayan Griffon Vultures has seldom been reported. Thompson et al. (2020) reported killing of free-ranging vultures (Cape Vulture *Gyps coprotheres*) by two captive leopards when the birds landed inside the leopard-enclosure in South Africa's Limpopo Province. It appears

Image 7. Remains of Himalayan Griffon Vulture at Bala Riverbed captured on 21 March 2024.

Image 8. Remains of another Himalayan Griffon Vulture at Bala Riverbed captured on 21 March 2024.

that leopard predation on vultures is either very rare or has gone unreported.

To the best of the knowledge of the authors, this is the first documentation of the predation and subsequent consumption of Himalayan Griffon Vulture by an Indian Leopard in a natural habitat. More research on the food habits of Indian Leopards particularly in the Himalayan Griffon's wintering grounds, may show that vultures are a more common prey than previously thought, or this account may represent an extremely unusual occurrence.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26110–26115

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7791.16.11.26110-26115

#7791 | Received 24 January 2022 | Final received 03 September 2024 | Finally accepted 09 November 2024

OPEN ACCESS

COMMUNICATION

Diet composition and diet choice of Lesser Mouse-tailed Bat *Rhinopoma hardwickii* (Gray, 1831) (Rhinopomatidae: Chiroptera)

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Abstract: The food composition and food choice of *R. hardwickii* were assessed through guano analysis at different seasons and geographical locations. Guano samples of *R. hardwickii* were collected from the roost sites of the Gangetic plains and arid region of Uttar Pradesh. Each intact pellet was dissolved and recognizable insect body parts like legs, antennae, wings, and mouth parts were separated and photographed. Each insect remnant was identified to its lowest rank as much as possible. A total of 10 roost sites of *R. hardwickii* were observed in the arid region and Gangetic plains of Uttar Pradesh and all of them were found in historical monuments. A total of 61 pellets of 10 sites yielded 1,035 remnants of insects. The highest percentage of remnants belongs to legs, followed by wings, antennae, abdominal segments, and mouthparts. The remnants belong to eight insect orders such as Coleoptera, Hemiptera, Orthoptera, Hymenoptera, Dermaptera, Diptera, Lepidoptera, and Plecoptera. The remnants of order Hemiptera showed the highest frequency of occurrence followed by orders Coleoptera, Orthoptera, and Hymenoptera, and these four orders of insects constitute the major portion of the diet of *R. hardwickii*. The orders Dermaptera, Diptera, Lepidoptera, and Plecoptera contributed a small proportion to the diet of *R. hardwickii*. The remnants of orders Lepidoptera and Plecoptera were occasional. The result of the current study shows that the food choice of *R. hardwickii* did not differ significantly across roost sites, while differed seasonally. Further, it reveals that the Lesser Mouse-tailed Bat acts as a potential and natural insect balancing agent.

Keywords: Agricultural pest, arid zone, biological pest controller, guano analysis, insect remnant, insectivorous bat, seasonal food habit.

Editor: H. Raghuram, Sri. S. Ramasamy Naidu Memorial College (Autonomous), Virudhunagar, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Misra, P.K., S. Farheen, S. Singh & V. Elangovan (2024). Diet composition and diet choice of Lesser Mouse-tailed Bat *Rhinopoma hardwickii* (Gray, 1831) (Rhinopomatidae: Chiroptera). *Journal of Threatened Taxa* 16(11): 26110–26115. https://doi.org/10.11609/jott.7791.16.11.26110-26115

Funding: The financial assistance of Science and Engineering Research Board, Department of Science and Technology, New Delhi through a major research project (No. EEQ/2018/000104) to VE is acknowledged.

Competing interests: The authors declare no competing interests.

Ethical statement: Relevant ethical permits were secured for data collection vide Letter No. 214/11/DAAS/BBAU/2011 of Babasaheb Bhimrao Ambedkar University and Archaeological Survey of India, Lucknow circle for bat survey (F. No. 10-16/23/2013-M 11535).

Author details: Pawan Kumar Misra did his PhD on "Isolation and characterization of guanophilic fungi of the bats of Uttar Pradesh" and currently working on educational sector. Sayma Farheen has completed her postgraduation from Babasaheb Bhimrao Ambedkar University and currently pursuing her PhD in Lucknow University, Lucknow, UP. Shaktivardhan Singh has completed his PhD on "Behavioural ecology of the Mouse-tailed Bat, *Rhinopoma hardwickii*" and currently working on educational sector. Vadamalai Elangovan is professor in the Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh and working on behavioural ecology of bats since 1996. VE is currently working on "conventional and alternative reproductive strategies of the Indian Flying Fox".

Author contributions: PKM and SF performed the experimental work, analysed the data and drafted the manuscript. SS supported the field work and data collection. VE designed the experiment and edited the manuscript.

Acknowledgements: The financial assistance of Science and Engineering Research Board, Department of Science and Technology, New Delhi through a major research project (No. EEQ/2018/000104) to VE is acknowledged. We thank the Archaeological Survey of India for permitting us to conduct the field survey in old monuments of Uttar Pradesh.

INTRODUCTION

Bats are voracious in nature and feed on large scale of insects in a night. Insectivorous bats are the primary consumers of many nocturnal insects (Kunz & Pierson 1994). They prey on a variety of agricultural insect pests such as tobacco budworms, corn borers, plant hoppers, and oriental armyworms (Whitaker 1993). Noctuid moths are major agricultural pests which are popular for long-distance and seasonal migrations (Wolf et al. 1990; Westbrook et al. 1995), they are abundantly eaten by bats (Thompson 1982; Robinson 1990). Insectivorous bats can suppress the pest population to its lowest level than other known natural enemies (Van Driesche & Bellows 1996). A large colony of insectivorous bats can deplete the insect pest at large scale; therefore, they act as potential biological pest control agents (Lee & McCracken 2005). Several genera of bats including *Taphozous*, *Rhinopoma*, *Tadarida* and *Miniopterus* form large colonies, from few hundreds up to several million individuals (Constantine 1967; McCracken et al. 1994; Elangovan et al. 2018).

The genus *Rhinopoma* is monophyletic with only four known species such as *R. hardwickii* (Gray, 1831), *R. microphyllum* (Brünnich, 1792), *R. muscatellum* (Thomas, 1903), and *R. macinnesi* (Hayman, 1937). They preferred to live in groups, forming colonies of hundreds to thousands of individuals (Elangovan et al. 2018). Very few studies have been carried out on the diet selection of *R. hardwickii*. Feldman et al. (2000) reported that they foraged exclusively in open areas but did not discuss about diet choice. Advani (1981) reported that *R. microphyllum kinneari* (Wrougthon, 1912) mainly fed on Coleoptera, Lepidoptera, and Orthoptera throughout the year, while Isoptera was the preferred diet during summer and monsoon seasons. No detailed report is available on diet composition and diet selection of *R. hardwickii* at various seasons and habitats in India. Thus, to fulfil the lacuna, a study on diet composition and diet selection of *R. hardwickii* was carried out in arid zones of Bundelkhand and adjoining area of Gangetic plains in Uttar Pradesh.

MATERIALS AND METHODS

Faecal pellets collection and analysis

Field surveys were carried from April 2019 to February 2020 at arid zones of Bundelkhand (i.e., Hamirpur, Lalitpur, Jalaun, and Jhansi) and its adjacent districts of Gangetic plains (i.e., Lucknow and Barabanki) in Uttar Pradesh. Guano samples were collected from the roost sites by spreading 2 x 2 m polythene sheet beneath the roost. In addition, the bats were captured using mist net, each individual was kept in a cotton bag until defecation, and thereafter they were released at the site of capture. Fresh faecal pellets were collected seasonally, i.e., summer (March–June), monsoon (July–August), and winter (November–February). Guano samples were kept in sample vials and stored at -20°C until analysis. Each intact pellet was soaked and dissolved in distilled water, teased gently using a fine brush and the insect remnants were separated using forceps. The recognizable insect body parts like legs, antennae, wings and mouth parts were separated and photographs were taken under a stereo microscope (RSMr3, Radical Scientific) using Digital Camera. Each insect remnant was identified to its lowest rank as much as possible by following Brues et al. (1954) and online resources. The identified remnants of different sites were grouped into legs, antennae, wings, and mouth parts and the frequency of occurrence was obtained.

Statistical analysis

Normality tests were performed to determine the distribution of the data set ($p < 0.05$), therefore non-parametric test (Kruskal Wallis H test) was applied to determine the seasonal and regional variations in the food choice of *R. hardwickii*. Guidelines of the American Society of Mammologists for the care and use of mammals were followed (Sikes et al. 2011).

RESULTS

A total of 10 roost sites of *R. hardwickii* were observed in the arid region (Hamirpur, Lalitpur, Jalaun, and Jhansi) and Gangetic plains (Lucknow and Barabanki) of Uttar Pradesh (Figure 1). All the roosts of *R. hardwickii* were found in historical monuments of the Uttar Pradesh. A total of 61 pellets of 10 roost sites yielded 1035 remnants of insects. The highest proportion of remnants was legs (47.29%) followed by wings (26.44%), antennae (7.62%), abdominal segments (5.31%), and mouth parts (0.19%), while the proportion of unidentified body parts of insects was 13.12%.

The insect remnants belong to eight insect orders such as Coleoptera, Hemiptera, Orthoptera, Hymenoptera, Dermaptera, Diptera, Lepidoptera, and Plecoptera. Further, the valuable diagnostic features of the remnants allowed us to identify up to family level, e.g., Scarabaeidae, Carabidae and Staphylinidae

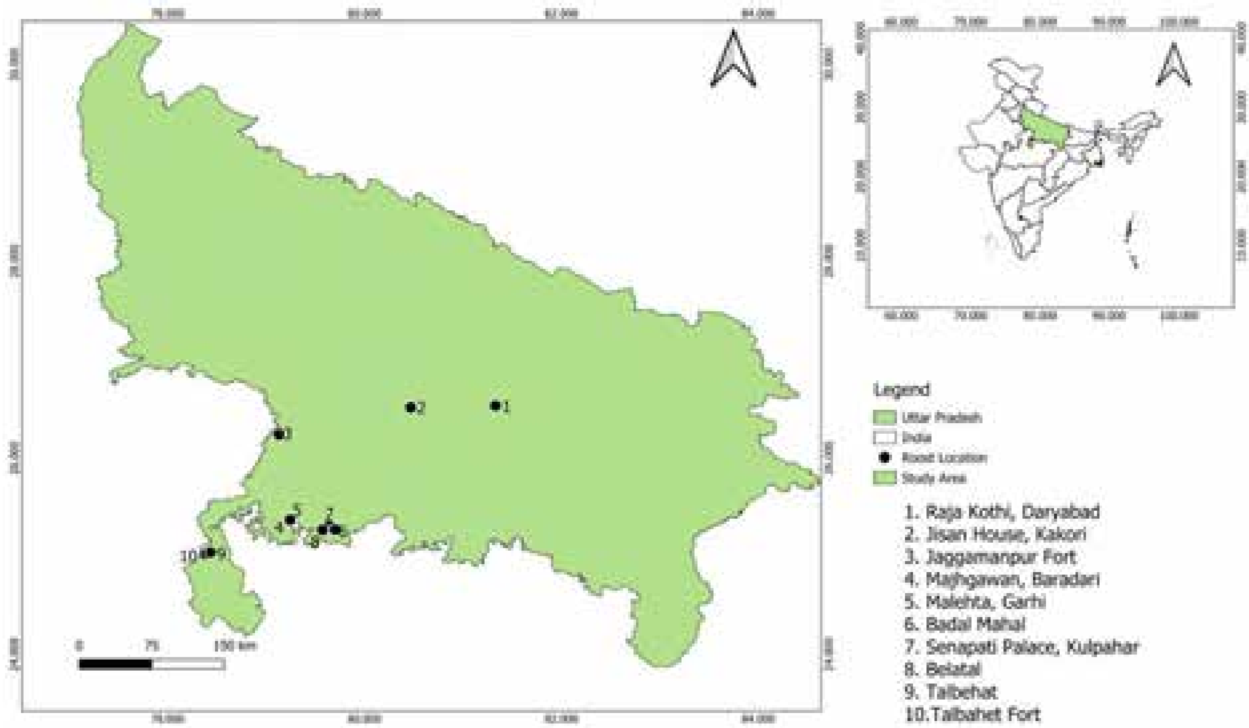

Figure 1. Roost sites of *Rhinopoma hardwickii* in Uttar Pradesh.

Table 1. The insect remnants retrieved from faecal pellets of *Rhinopoma hardwickii* at different roost locations. The values are given in percentage.

Roost locations/ insects order	Lucknow	Barabanki	Lalitpur	Jalaun	Jhansi	Mahoba	Hamirpur
Coleoptera	29.55	9.09	7.58	8.33	9.85	15.91	19.70
Hemiptera	5.34	0.00	13.74	1.53	6.11	14.50	58.78
Orthoptera	7.89	2.63	13.16	10.53	39.47	7.89	18.42
Hymenoptera	3.45	0.00	93.10	0.00	3.45	0.00	0.00
Dermaptera	33.33	0.00	33.33	0.00	33.33	0.00	0.00
Diptera	0.00	100.00	0.00	0.00	0.00	0.00	0.00
Lepidoptera	100.00	0.00	0.00	0.00	0.00	0.00	0.00
Plecoptera	0.00	100.00	0.00	0.00	0.00	0.00	0.00

(Coleoptera), Gryllidae and Gryllicrididae (Orthoptera), Cynidae (Hemiptera), Formicidae (Hymenoptera). The remnants of order Hemiptera consist of legs (tarsi with claw; Images 1AH–AI) and wings (hemi-elytra; Images 2L–O). The remnants of order Coleoptera consist of legs (femur, coxae and tibia, tarsi with claw; Images 1A–V), and wings (elytra; Images 2A–K), while the order Orthoptera consists of coxae and tibia with claw (Images 1W–AG) and leathery non–membranous wings (Images 2P–S). Tarsi of Coleoptera were usually heteroamorous and apparently with three to five segments and one pair of claws (Images 1A–V), while of Hemiptera with three segments and claw (Images 1AH–AI). The wing remnants of Orthoptera were membranous, venation rather complete but not complex with pentagonal or quadrant shape cells (Images 2P–S). They had large legs with spines, tibia with stout spines and movable spur, tympanum located in front of tibia/rarely spinose and tarsi with 4–5 segmented claws (Images 1W–AG).

The remnants of Hemiptera showed the highest frequency in faeces (14.69%) followed by Coleoptera (13.27%), Orthoptera (5.21%), and Hymenoptera (2.65%). The remnants of orders Dermaptera (0.26%), Diptera (0.17%), Lepidoptera (0.088%), and Plecoptera

Table 2. Seasonal variation in food preference of *Rhinopoma hardwickii*. Values are given as Mean ± SD. The dash (-) indicates the absence of particular insect order during the season.

Season	Summer	Monsoon	Winter	χ^2	p -value
Coleoptera	3.70 ± 0.75	1.88 ± 0.49	2.80 ± 1.94	3.42	0.18
Hemiptera	1.75 ± 0.25	2.27 ± 0.798	3.58 ± 3.18	0.38	0.82
Orthoptera	0.95 ± 0.08	1.22 ± 0.86	1.08 ± .12	1.293	0.52
Hymenoptera	-	7.00 ± 6.00	-	0.50	0.48
Dermaptera	-	-	-	-	-
Diptera	1	1	1	-	1
Lepidoptera	1	-	-	-	-
Plecoptera	-	-	-	-	-
Unidentified	8.42 ± 5.45	16.1 ± 8.92	11.45 ± 4.42	1.5	0.47
χ^2	15.285	9.414	9.106	-	-
p-value	0.018	0.152	0.059	-	-

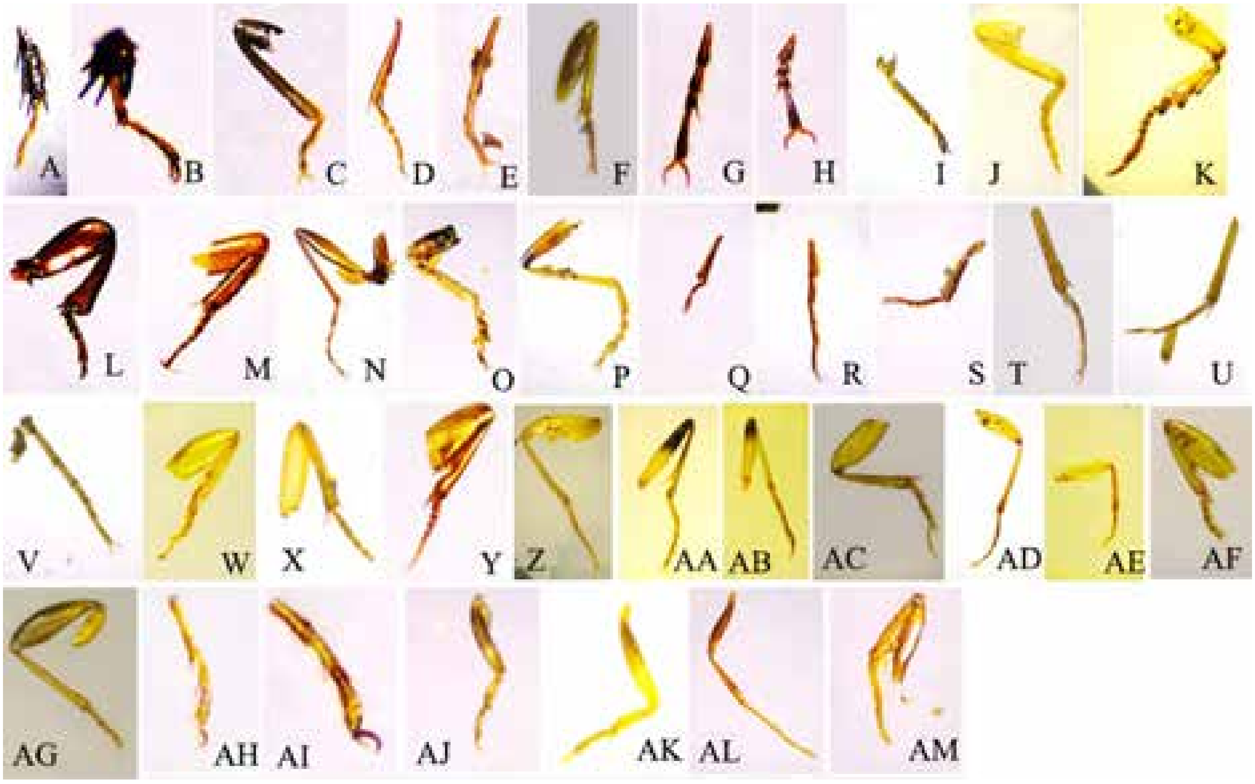

Image 1. Legs of insects isolated from the guano of *Rhinopoma hardwickii*: A–V—Coleoptera | W–AG—Orthoptera | AH–AI—Heteroptera | AJ–AK—Dermaptera | AL–AM—Hymenoptera. © Pawan Kumar Misra & Sayma Farheen.

(0.088%) were found in a small proportion. Further, a major proportion of insect remnants was unidentifiable (63.53%) because they were either broken or incomplete. The remnants of orders Lepidoptera and Plecoptera were occasional (Table 1). The remnants retrieved from faecal pellets showed variation in diet choice of *R. hardwickii* at different localities, i.e., coleopterans were highest in Kakori, hemipterans in Hamirpur (Maleta), orthopterans in Jhansi and hymenopterans in Lalitpur (Table 1). There was no significant difference observed in the occurrence of remanence of various insect orders in 10 different roost sites, Coleoptera (χ^2 = 0.800, p = 0.999), Hemiptera (χ^2 = 0.788, p = 0.990), Orthoptera (χ^2 = 4.50, p = 0.342), Hymenoptera (χ^2 = 0.330, p =

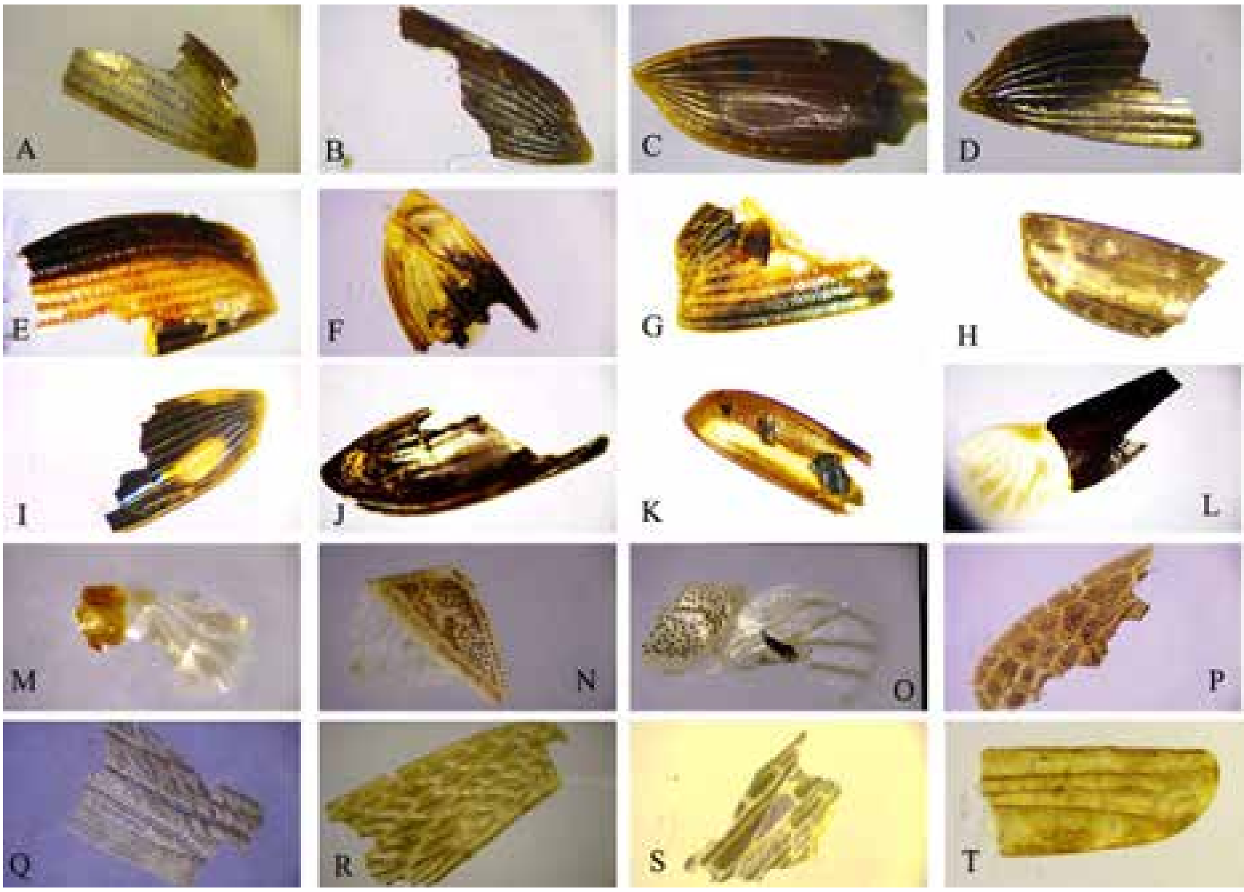

Image 2. Remnants of insect wings isolated from the guano of *Rhinopoma hardwickii*: A–K—Coleoptera | L–O—Hemiptera | P–S—Orthoptera | T—Plecoptera. © Pawan Kumar Misra & Sayma Farheen.

0.563), Diptera (χ^2 = 1.00, p = 0.317), Lepidoptera and Plecoptera (χ^2 = 0.00, p = 1.00).

The food choice of *R. hardwickii* varied with seasons, the remnants of Coleoptera were higher during summer, Hemiptera during winter, and Orthoptera during monsoon seasons (Table 2). The food choice of *R. hardwickii* showed a significant difference during summer (χ^2 = 15.285, p = 0.018), while the food choice did not differ during monsoon and winter seasons (p >0.05, Table 2).

DISCUSSION

The results of present study showed that the Lesser Mouse-tailed Bats consumed insects belonging to eight insect orders across the geographical locations and seasons. The most preferred food items belong to orders Hemiptera, Coleoptera, Orthoptera and least preferred items belong to orders Dermaptera, Diptera, Lepidoptera and Plecoptera. Feldman et al. (2000) and Whitaker & Yom-Tov (2002) investigated the habitat utilization and dietary composition of *R. hardwickii* and found that they used open habitat and fed coleopteran insects which contributed about 51% of the diet. Heteropteran (order Hemiptera) insects were the second most commonly found food items and contributed 30.4% of the diet of *R. hardwickii* (Whitaker & Yom-Tov 2002). The diet selection of many insectivorous bats depends upon dental and cranial morphology, wing shape, and echolocation call (Neuweiler 2000; Altringham 2011; Weterings & Umponstira 2014). The results of faecal pellet analysis revealed that the legs and wings constituted more than 74% of the remnants isolated, while antennae, abdomen, and mouth parts contributed less than 15%. The highest percentage of legs and wings in the isolated remnants probably be due to the composition of chitin in legs and wings.

The Bundelkhand region of Uttar Pradesh comes under dry-arid zone wherein scarcity of water occurs except rainy season, while Lucknow and Barabanki are fertile Gangetic plains. The flora and fauna also vary naturally among the regions; no difference was observed in the obtained remnants and food choices of *R. hardwickii*. Although, the food choices vary across roost sites (geographical regions) but did not differ significantly. The food choice of *R. hardwickii* was influenced by seasons. The earlier studies deduced that the prey availability and prey selection of most insectivorous bats were probably influenced by temporal, seasonal, and geographical factors (Whitaker 1995; Whitaker et al. 1996).

Arthropods destroy over 18% of the annual production of crops worldwide (Culliney 2014). The use of agricultural insecticides causes harmful impact on consumer and environment. Therefore, use of biocontrol agents for the suppression of insect pest is very important and the insectivorous bats are good source of insect pest suppressors as they consume a large number of insects of various orders. According to Boyles et al. (2013), insectivorous bats decreased the cost of pesticide about USD 22.9 billion a year and also reduced the development of pesticide resistance. Similarly, the current study revealed that the mouse-tailed bats consume a wide range of insects belong to eight orders across seasons, and geographical areas of Uttar Pradesh. Further, the food choice of *R. hardwickii* varies with seasons, and Coleoptera was the most preferred food item in summer, while Hemiptera and Orthoptera were preferred food items during winter and in monsoon seasons, respectively. Since, the mouse-tailed bats consume a lot of insects and play active role as insect suppressor, their roost sites and populations need adequate conservation for their sustenance and human welfare.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26116–26123
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.9368.16.11.26116-26123
#9368 | Received 05 September 2024 | Final received 05 October 2024 | Finally accepted 31 October 2024
OPEN ACCESS
COMMUNICATION

DNA barcoding and distribution of *Osteobrama peninsularis* (Teleostei: Cyprinidae) in India

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Abstract: *Osteobrama peninsularis* has been reported in southern Indian drainages, particularly in the Krishna River drainage. This study provides new findings of *O. peninsularis* in two distant locations: the Kangsabati River (Suvarnarekha River drainage) in West Bengal and Wyra Lake (Godavari River drainage) in Telangana. This marks the first record of *O. peninsularis* in eastern India, specifically in West Bengal. The species can be distinguished from other *Osteobrama* members by its 28–31 branched anal-fin rays and 55–60 lateral line scales, along with other unique morphological features. Mitochondrial cytochrome oxidase C subunit I gene sequences from specimens collected in Suvarnarekha and Godavari River drainages cluster together in the phylogenetic analysis, indicating that the clade of *O. peninsularis* is distinct and maintains significant genetic distance from its congeners.

Keywords: Carp, genetic analysis, minnow, new record, peninsular India, range extension, ray-finned fish, taxonomy, West Bengal.

Editor: Mandar Paingankar, Government Science College Gadchiroli, Maharashtra, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Laskar, B.A., A.K. Tudu, S. Rath & L. Kosygin (2024). DNA barcoding and distribution of *Osteobrama peninsularis* (Teleostei: Cyprinidae) in India. *Journal of Threatened Taxa* 16(11): 26116–26123. https://doi.org/10.11609/jott.9368.16.11.26116-26123

Funding: The research is funded by the Core Funding of Zoological Survey of India (ZSI), Kolkata, Ministry of Environment, Forest and Climate Change (MoEF&CC), New Delhi.

Competing interests: The authors declare no competing interests.

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Author contributions: BAL & AKT did field surveys and collected the specimens. BAL, AKT & SR studied the morphology and meristics of the specimens for taxonomic identification. BAL generated the DNA data, and did the molecular analysis. BAL, AKT, SR & LK wrote the article. BAL & LK reviewed the article.

Acknowledgements: We would like to express our heartfelt gratitude to Dr. Dhriti Banerjee, Director of the Zoological Survey of India, for graciously granting us permission to undertake this important work.

INTRODUCTION

The genus *Osteobrama* includes several key food fish species in India, thriving in both lentic and lotic systems. The species in the genus *Osteobrama* (type species *Cyprinus cotio* Hamilton, 1822) are characterized by their laterally compressed bodies, elevated dorsum, absence of procumbent predorsal spines, rounded abdomens in front of the pelvic fins, keeled abdominal edges from the pelvic-fin origin to the vent, and long anal fins with more than 10 branched rays (Talwar & Jhingran 1991). Hamilton (1822) described the type species as having a row of "5–6 black spots below the fore part of the lateral line, around which is a bluish shining depression" (plate 207).

Sykes described two species, *Rohtee vigorsii* and *R. ogilbii*, from the Deccan region of India (Sykes 1838), categorizing them under the genus *Rohtee*, which he characterized by long dorsal and anal fins, a posteriorly serrated last undivided dorsal-fin ray, and minute scales. Bleeker (1863) designated *R. ogilbii* as the type species of the genus, a classification upheld in later taxonomic work (Tilak & Husain 1989). The placement of species within the genera *Osteobrama* and *Rohtee* has been addressed by Jordan (1919), Hora (1921), and Mukerji (1934). Hora (1937) distinguished *R. ogilbii* from species now classified as *Osteobrama* by its unique procumbent predorsal spine and the long anal fin with 13 to 14 branched rays, Consequently, *Rohtee* is now recognized as valid with *R. ogilbii* as its sole species, while *Osteobrama* currently includes 10 valid species (Laskar et al. 2024).

Among the 10 valid species, the type species *O. cotio* is widespread in the Ganga basin of India and Bangladesh (Rahman et al. 2018). Three congeners, *O. feae*, *O. cunma*, and *O. belangeri* are distributed in Myanmar and the Irrawaddy drainage in India and China (Rahman et al. 2018). Doi (1997) reported *O. alfredianus*, *O. belangeri*, and *O. feae* from the Salween basin, but Laskar et al. (2024) questioned the validity of *O. alfredianus*. Silas (1952) described *O. peninsularis* from peninsular India. Two more species, *O. neilli* and *O. bakeri* are found in extreme southern peninsular India (Talwar & Jhingran 1991; Jadhav et al. 2011; Rahman et al. 2018). Shangningam et al. (2020) described *O. tikarpadaensis* from the Mahanadi River in Odisha and recognized *O. dayi* as a valid species. Laskar et al. (2024) states that *O. vigorsii* is limited to the Krishna River system in southern India, while the distribution of *O. tikarpadaensis* extends to the Godavari River drainages of South India. Morphologically, *O. peninsularis* closely resembles *O. cotio*, leading to frequent misidentifications due to their narrow range of morphological variations. The species is currently listed as Data Deficient on the IUCN Red List, raising questions about its record from Kerala (Dahanukar 2011).

This study suggests the extension of the distribution of *O. peninsularis*, which was previously documented only in southern India, by identifying the species from a new location in eastern India: Kangsabati River in the state West Bengal. DNA barcoding of specimens from southern India and eastern India further confirms the identification of *O. peninsularis*. These findings align with the original description of the species, with minor variations, and is a first report of *O. peninsularis* in eastern Indian drainage.

MATERIALS AND METHODS

Morphometric and meristic data were recorded following Jayaram (1999). Measurements were taken on the left side of the specimens using digital calipers to the nearest 0.1 mm. Fin rays and scale counts were performed under transmitted light with a stereomicroscope. All pored scales were counted for reporting the lateral line scale. The count of transverse scale rows, between the lateral line and the origin of the dorsal-fin, include the lateral line scale. Body subunits are expressed as a percentage of standard length (SL), while head subunits are presented as a percentage of head length (HL). The specimens have been deposited at the Zoological Survey of India (ZSI), Kolkata, and at the Freshwater Biology Regional Centre of the Zoological Survey of India, Hyderabad.

Genetic analysis. Tissue samples were obtained from freshly collected specimens of *O. peninsularis* and preserved in 90% ethanol. The genomic DNA was extracted through QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The published primer pair (Ward et al. 2005): FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1-5'TAGACTTCTGGGTGGCCAAAGAATCA3' was used to amplify the partial segment of mitochondrial cytochrome oxidase C subunit I gene (COI). The 30 µl PCR mixture contains 10 pmol of each primer, 100 ng of DNA template, 1 × PCR buffer, 1.0–1.5 mM of MgCl2, 0.25 mM of each dNTPs, and 1U of Taq polymerase (Takara BIO Inc., Japan). The thermal profile was set to initial 2 min at 95 °C followed by 35 cycles of 0.5 min at 94 °C, 0.5 min at 54 °C, and 1 min at 72 °C, followed in turn by 10 min at 72 °C and subsequent hold at 4°C. The PCR products were further purified using QIAquickR Gel extraction Kit

(Qiagen, Valencia, CA).

The cycle sequencing and Sanger sequencing was executed commercially. Both forward and reverse chromatograms were checked through SeqScanner V1.0 (Applied Biosystems Inc., CA, USA), nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/), and ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/) to trim the low-quality reads and gaps. The DNA sequences generated as part of the current study have been deposited in GenBank with accession No. MT896379 & PQ333057 for *O. peninsularis*; MZ854239 & MZ854240 for *O. cotio*. We retrieved all the available COI sequences of *Osteobrama* species from GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/, assessed on 28 August 2023) and ran a test of neighbor-joining phylogeny. Based on the cohesive clustering, a maximum of five representative database sequences from each conspecific clade were used in the refined analysis. Following Rahman et al. (2018), uncertain sequences of *O. cotio* from Narmada River basin as well as from Karnafuli and Sangu Rivers were not included in the dataset. Further, a maximum of five representative sequences of three congeners used in Rahman et al. (2018), *O. belangeri*, *O. cunma*, and *O. feae* were used in the dataset. The dataset was aligned using ClustalX (Thompson et al. 1997) and the Kimura 2 parameter (K2P) genetic distances were estimated by using MEGAX (Kumar et al. 2018).

RESULTS

Material examined

Osteobrama peninsularis Silas 1952 (Image 1, and Table 1 and 2): ZSI FF 9901, 1, 69.4 mm SL, Kangshabati (or Kansai) River, Paschim Medinipur District, West Bengal, India (22.406^{0}N & 87.307^{0}E), collected by S. Rath, 14 October 2022. Genbank accession for mtCOI sequence: PQ333057; FBRC/ZSI/F3549, 1, 68.0 mm SL, Wyra lake, Godavari River drainage, Khammam District, Telangana, India, collected by Sudipta Mandal, 20 July 2020. Genbank accession for mtCOI sequence: MT896379.

Description

Body deep, laterally compressed. Dorsal profile sloping upward linearly to nape, then in a broad curve to dorsal fin origin, forming a distinct hump, then sloping gradually downward towards caudal peduncle. Ventral profile strongly curved from tip of snout to origin of anal fin. Head compressed longer than deep. Eye large situated anteriorly on head, visible from dorsal and ventral side. Mouth terminal, obliquely directed upwards. Barbels absent.

Table 1. Morphometric Measurements of *Osteobrama peninsularis* from West Bengal (ZSI FF 9901). The table presents various body dimensions, expressed in millimeters and as percentages of standard length (SL) and head length (HL), providing a detailed overview of the species' morphological characteristics.

	Parameters	value
1	Standard Length	69.4 mm
	% SL	
2	Body Depth	42.22
3	Head Length (Lateral)	23.92
4	Head depth (Occiput)	19.45
5	Snout Length	6.20
6	Eye Diameter	8.36
7	Inter orbital Width	8.79
8	Max. Head width	12.39
9	Gape Width	5.48
10	Internerial space	4.76
11	Body width at anal fin origin	9.51
12	Body width at dorsal fin origin	11.67
13	Caudal Peduncle Length	9.37
14	Caudal Peduncle Depth	12.54
15	Dorsal-fin base Length	13.40
16	Dorsal-fin Length	26.37
17	Pectoral-fin Length	17.00
18	Pelvic-fin Length	14.99
19	Anal-fin base Length	37.03
20	Anal-fin Length	40.35
21	Caudal fin length	24.93
22	Median caudal fin Length	11.53
23	Predorsal Length	51.30
24	Prepectoral Length	22.05
25	Prepelvic Length	39.48
26	Preanal Length	53.31
27	Pelvic anal distance	15.85
	% HL	
28	Snout Length	25.90
29	Eye Diameter	34.94
30	Inter Orbital Width	36.75
31	Max. Head Width	51.81
32	Gape Width	22.89
33	Internerial space	19.88

Dorsal fin with iii unbranched and eight branched rays, last unbranched ray stiff and serrated. Pectoral fin with i unbranched and 14 branched rays. Pelvic fin i unbranched and eight branched rays. Anal fin long with iii unbranched rays and 29 branched rays. Caudal fin deeply forked with 9+8 branched rays. Scales small in size. Pre-dorsal scale 24. Lateral line complete with 58 scales.

Coloration

In preserved specimens, dorsal and dorsolateral surfaces of head and body faint brown, lateral surface of body greyish, become lighter ventrally. Dorsal, pectoral, pelvic, anal and caudal fin is pale white. An oblique black streak immediately posterior to opercle, parallel to upper opercular margin present.

Genetic analysis

The mtCOI sequences (*denovo*) of both the specimens of *Osteobrama peninsularis* in the study, cluster together. In the phylogram (Figure 1), the *denovo* sequences of *O*. *peninsularis* along with a few sequences borrowed from GenBank (with taxa name *O*. *cotio*) form a distinct clade. The sequences in the *O*. *peninsularis* clade show 0.0 to 0.62 % pairwise genetic distance (intraspecies divergence) and maintain 5.28 to 5.68% genetic distance (interspecies divergence) with the sequences in the clade of *O*. *cotio*. The clade of *O*. *peninsularis* corresponds with one of the subclades of Clade A referred in Rahman et al. (2018). Notwithstanding to having a considerable range of genetic divergence among the three subclades in Clade A of *O*. *cotio*, Rahman et al. (2018) stated that "The haplotype group represented by the sequences from the Narmada, Karnafuli, Sangu, and Godavari drainages may represent a distinct species but not necessarily undescribed. Based on the very brief description (Silas 1952) and data on topotypes in Jadhav et al. (2011), the oldest alternative available name may be *O*. *peninsularis*, with type locality Pune (Maharashtra, India) in the upper Krishna River drainage". Based on the COI sequences, we consider that the three sequences (KF550101 to KF550103) with no locality information but identified as *O*. *cotio* in NCBI are in fact *O*. *peninsularis*.

Image 1. *Osteobrama peninsularis*: i—ZSI FF 9901, 69.4 mm SL, Kangsabati River, West Bengal, India (© Shibananda Rath) | ii—FBRC/ZSI/F/3549, 68.0 mm SL; Wyra lake, Godavari River drainage, Khammam District, Telangana, India (© Boni Amin Laskar).

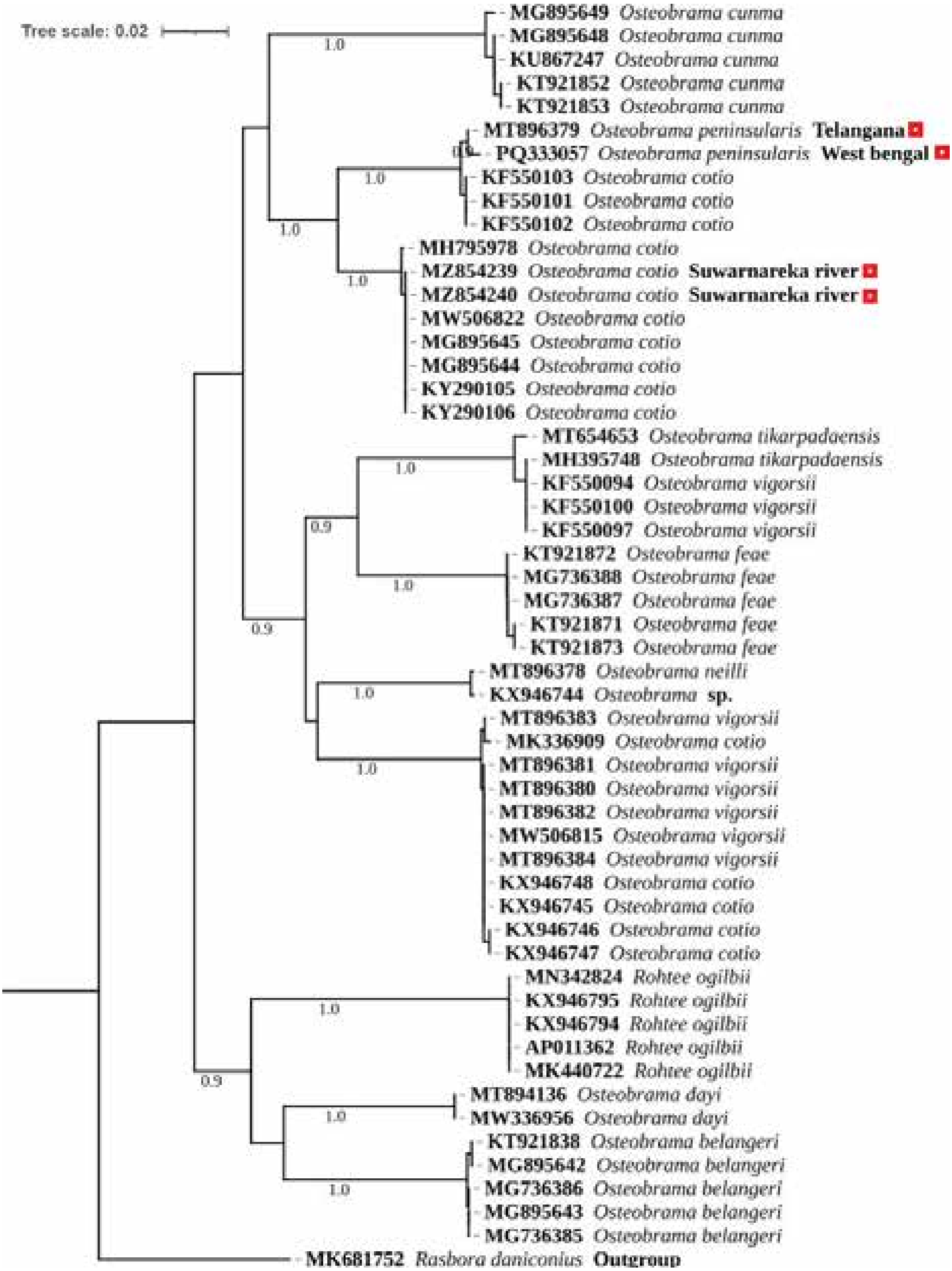

Figure 1. Neighbour-joining phylogram of the *Osteobrama congeners* based on mtCOI partial shows a distinct clade of the studied species, *O. peninsularis*. Numbers at branches show bootstrap. The NCBI accession numbers are given with the organism's name, the de novo sequences are marked with red square.

Table 2. Meristic counts of *Osteobrama peninsularis* from different locations. This table summarizes the meristic characteristics of *O. peninsularis* specimens from West Bengal (ZSI FF 9901) and Wyra Lake in Telangana (FBRC/ZSI/F3549), alongside counts from previous literature (Silas 1952).

Parameters	*O. peninsularis* from W.B.: ZSI FF 9901	*O. peninsularis* from, Wyra Lake, Godavari Drainage, Telangana: FBRC/ZSI/F3549	*O. peninsularis* original descriptions by Silas (1952)
Dorsal fin	iii 8	iii 8	iii 9
Pectoral fin	i 14	i 14	16
Pelvic fin	i 8	i 9	i 9
Anal fin	ii 29	ii 30	iii 28-31
Lateral line scales	58	58	55-60
Predorsal scales	24	24	21-24
Pre-anal scales	21	-	-

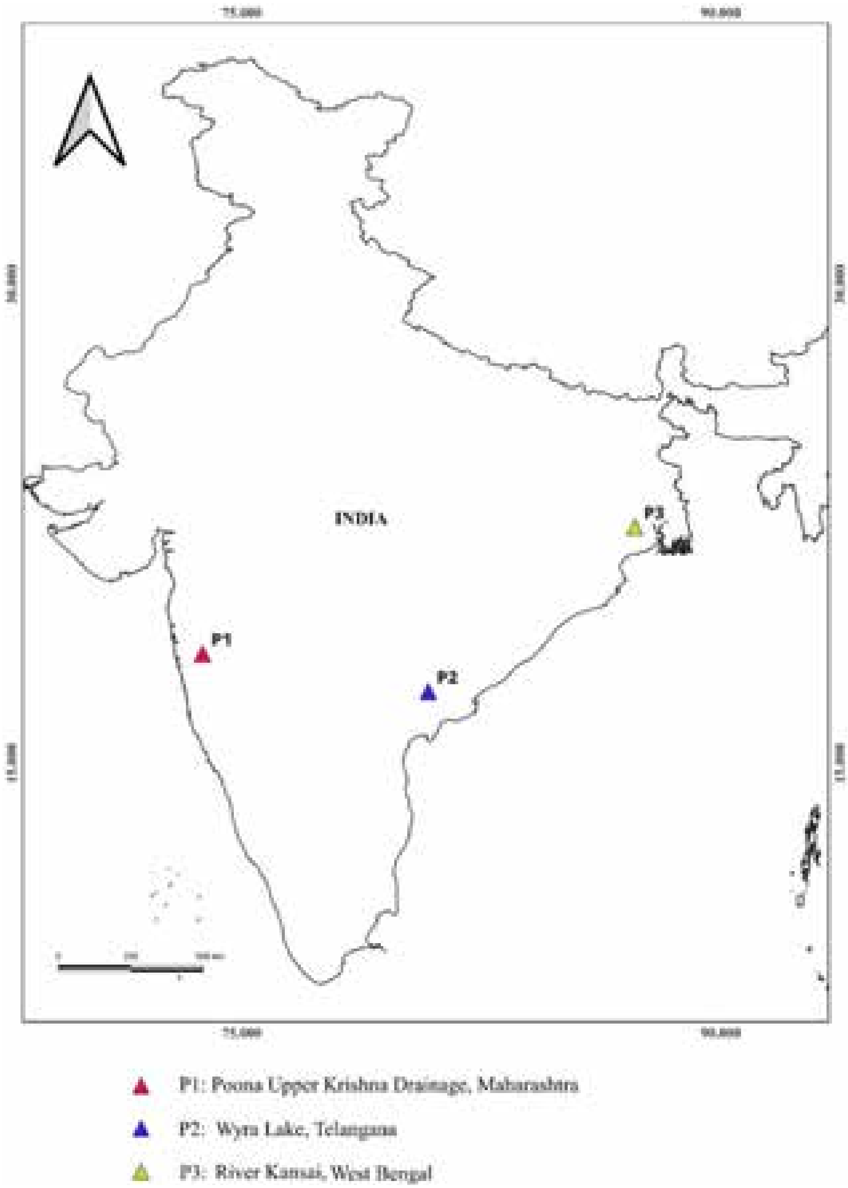

Figure 2. *Osteobrama peninsularis* known distribution across southern Indian drainages, highlighting its presence in the Krishna River drainage, as well as newly recorded locations in the Kangsabati River (Suvarnarekha River drainage) in West Bengal and Wyra Lake (Godavari River drainage) in Telangana.

DISCUSSION

In this study, specimens were identified as *Osteobrama peninsularis* based on morphological characteristics, including 58 lateral line scales, the absence of barbels, and an anal fin with 29 branched rays. This species, originally described from Pune (Poona) in the upper Krishna River drainage of Maharashtra, has been frequently reported in Maharashtra (Silas 1952; Tonapi & Mulherkar 1963; Kharat et al. 2000, 2003; Arunachalam et al. 2002; Wagh & Ghate 2003; Chandanshive et al. 2007; Heda 2009; Jadhav & Yadav 2009).

Biju et al. (1999) reported *O. peninsularis* from the Periyar River in Central Kerala, indicating a range extension into Kerala. They, along with Talwar & Jhingran (1991) and Jayaram (1999), recognized the species as distributed solely in peninsular India, including Maharashtra, Odisha, Andhra Pradesh (erstwhile), and Kerala. The species has also been documented in the Tungabhadra River, Karnataka (Shahnawaz & Venkateshwarlu 2009; Shahnawaz et al. 2010). Although previous studies (Jayaram & Mazumdar 1976; Mohanty et al. 2015) noted its occurrences in Odisha, Dutta et al. (1993) did not include it in the state fauna series of Odisha.

Morphologically, *O. peninsularis* is superficially similar to *O. belangeri*, *O. cotio*, *O. cunma* because of lack of barbels. However, it is distinguished from all the three congeners in having pre-dorsal 21 to 24 scales and Lateral line scales 55 to 60. Furthermore, it is distinguished from *O. belangeri* in having more branched anal-fin rays (28–31 vs. 17–18), less pre-dorsal scales (21–24 vs. 31–34), less lateral line scales (55–60 vs. 70–78); from *O. cotio* in having less branched anal-fin rays (28–31 vs. 33–38), less lateral line scales (55–60 vs. 65); from *O. cunma* in having more branched anal-fin rays (28–31 vs. 25–29), less pre-dorsal scales (21–24 vs. 28–30) and more lateral line scales (55–60 vs. 42–53). This study largely aligns with the original description of *O. peninsularis*, noting only minor variations in body morphometry (see Table 1). Parameters of meristic counts, provide insights into the species' morphological consistency across different populations. The specimens examined were smaller than the type specimens, and minor variations may relate to their distribution. The findings indicate that the distribution of *O. peninsularis* extends through the river basins of the Godavari and Krishna in Maharashtra, Telangana, Andhra Pradesh, and into the Mahanadi basin in Odisha and the Subarnarekha river basin in West Bengal. In a recent study, amendment of description of *O. vigorsii* and the expansion of distribution of *O. tikarpadaensis* have also been reported (Laskar et al. 2024). With the addition of *O. peninsularis*, the state fauna of West Bengal now includes two species of *Osteobrama*. A distribution map of *O. peninsularis* is given in Figure 2.

The presence or absence of barbels is a crucial taxonomic feature in *Osteobrama* (Hora & Misra 1940; Shangningam et al. 2020). When present, the barbels may be either one pair of maxillary barbels or both maxillary and rostral, sometimes being minute or rudimentary. The rostral barbels can be hidden or barely visible, while in some species, they extend to the base of the maxillary barbels. *Osteobrama* species are categorized into three groups based on their barbels: (i) with four well-defined barbels, (ii) with two rudimentary maxillary barbels, and (iii) without barbels (Hora & Misra 1940). Recently, *O. vigorsii* was revised and placed in Group (i), alongside *O. bakeri*, *O. feae*, *O. neilli*, and *O. tikarpadaensis* (Laskar et al. 2024).

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26124–26130

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.8963.16.11.26124-26130

#8963 | Received 13 February 2024| Final received 18 September 2024 | Finally accepted 14 November 2024

OPEN ACCESS

COMMUNICATION

Diving into diversity: aquatic beetles of Sukhna Wildlife Sanctuary, Chandigarh, India

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Abstract: The Sukhna Wildlife Sanctuary, located in the Shivalik Hills of Chandigarh, India, encompasses several freshwater ecosystems, including ponds, streams, and marshes. The objective of this study is to fill the current information void regarding the populations of aquatic beetles in the sanctuary. During the period from June to September 2023, a comprehensive study was conducted in the Sukhna Wildlife Sanctuary, resulting in the identification and documentation of 164 specimens of aquatic Coleoptera. These specimens belonged to six species, five genera, and two families in which the Dytiscidae family was found to be the most prevalent. The study findings offer significant insights into the population magnitude and geographical dispersion of aquatic beetles within the investigated settings and show the need for comprehensive surveys to understand the intricate interplay of factors governing beetle distribution.

Keywords: Biodiversity, Coleoptera, Dytiscidae, ecological indicators, freshwater ecosystems, Hydrophilidae, *Hydroglyphus*, *Laccophilus*, Shivalik hills, species richness.

Editor: Hasko F. Nesemann, Hofheim am Taunus, State of Hesse, Germany.

Date of publication: 26 November 2024 (online & print)

Citation: Chaudhary, K.O. (2024). Diving into diversity: aquatic beetles of Sukhna Wildlife Sanctuary, Chandigarh, India. *Journal of Threatened Taxa* 16(11): 26124–26130. https://doi.org/10.11609/jott.8963.16.11.26124-26130

Funding: None.

Competing interests: The author declares no competing interests.

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Acknowledgements: The author is grateful for his encouragement and assistance, director of Zoological Survey India, and the Chandigarh Forest Department for providing access to Sukhna Wildlife Sanctuary. The author is also grateful to Mr. Narbir Khalon, Mr. Pragye Om Chaudhary, Mr. Navjit Singh, Mrs. Rima Dhillon, R.F.O Devender Chauhan, and Forester Sanjay Yadav for their assistance in the field work and collection of specimens.

INTRODUCTION

Aquatic beetles belonging to the Coleoptera order and its Adephaga and Polyphaga suborders are a remarkably diverse and ecologically significant group of insects that exert a substantial impact on freshwater ecosystems worldwide. With over 13,000 species worldwide, these insects inhabit a variety of aquatic habitats, including rivers, lakes, ponds, and marshes (Short 2017). In India, a nation renowned for its abundance of species, aquatic beetles are no exception to the abundance of species. India is a hotspot of aquatic beetle diversity, with approximately 776 species distributed across 137 genera and 17 families (Chandra et al. 2017). Aquatic beetles play crucial roles in freshwater ecosystems by engaging in nutrient cycling and serving as integral components of aquatic food webs. Furthermore, their sensitivity to environmental changes provides valuable insights into ecosystem health (Ribera et al. 2003).

The Sukhna wildlife sanctuary in the Shivalik Hills of Chandigarh, India (Figure 1) contains a variety of freshwater habitats, such as ponds, streams, and wetlands, and is of great ecological significance. Its strategic location, which acts as a link between the Himalaya and the northern plains, increases its ecological significance. Despite the environmental significance of the sanctuary and the crucial role aquatic beetles play in shaping freshwater ecosystems, there has been a dearth of research on the composition and distribution of aquatic beetle communities within the sanctuary. Understanding the intricate structure of these communities is crucial for conserving the sanctuary's aquatic ecosystems. Several environmental factors, including microhabitat characteristics, habitat size, vegetation structure, and ecological habitat types, are known to influence these communities (Lundkvist et al. 2003; Akunal & Aslan 2017). Prior research on Indian aquatic beetles has focused primarily on taxonomic aspects, providing limited insight into their habitats and ecology (Sheth et al. 2018). Given the unique biodiversity of the Shivalik region, the significance of this knowledge gap increases.

This study represents the first investigation into the aquatic beetle population within the Sukhna Wildlife Sanctuary, aiming to bridge the existing knowledge gap in this particular field. The survey findings indicate that a total of 164 specimens were observed, encompassing seven distinct species belonging to two separate families and five genera. This investigation aims to contribute to the expanding body of knowledge on aquatic beetles in India and compile a comprehensive baseline dataset on aquatic Coleoptera for the union territory of Chandigarh. This research on the aquatic beetle fauna of Sukhna Wildlife Sanctuary hopes to shed light on their ecological significance, contribute to the sanctuary's conservation efforts, and increase our knowledge of the region's freshwater ecosystems.

METHODS & MATERIALS

Study Area

The Sukhna Wildlife Sanctuary is located within the geographical coordinates of $30^0$171'–$30^0$110' N and $76^0$162'–$76^0$291' E. It is situated in the Shivalik Hills of Chandigarh, India, and is renowned for its untouched ecological environment. On 16 March 1998, the region was officially established as a wildlife refuge, covering a vast land area of around 25.98 km^2 (equal to 6,420.99 ac), with the primary purpose of protecting a wide range of plant and animal species. Located in close proximity to the renowned Sukhna Lake, this sanctuary serves as a crucial contributor to the region's endeavours in conserving biodiversity. The Sukhna Wildlife Sanctuary encompasses forests, shrub fields, and sections of the Nepli Forest, resulting in a distinctive and vital ecological environment for various wildlife species. The ecological value of the area is enhanced by its position inside the outermost Shivalik Range, which is distinguished by geological formations and an altitude range spanning from 346 m to 620 m .

Sampling

The survey was conducted in the Nepli Range of Sukhna Wildlife Sanctuary from June to September 2023. The data collection efforts were primarily directed towards four prominent water bodies which act as siltation dams in the sanctuary, as shown in Figure 1. A 20-cm-diameter, pond net with mesh size of 500 µm and an extendible telescopic handle was used for the aquatic beetle collection (Dudgeon 1999; Merrit & Cummins 1978). At each body of water, samples were collected by meticulously sweeping the net six times in opposite directions across a 1-m distance (Subramanian & Sivaramakrishnan 2007). This strategy guaranteed the capture of aquatic beetles from a variety of microhabitats along the water bodies coastlines. After collecting the contents of the sweep net, they were gently poured into a spill tray. The aquatic beetles were then counted visually, and one representative specimen of each species was collected and preserved in an ethanol solution containing 90% ethanol. To minimise

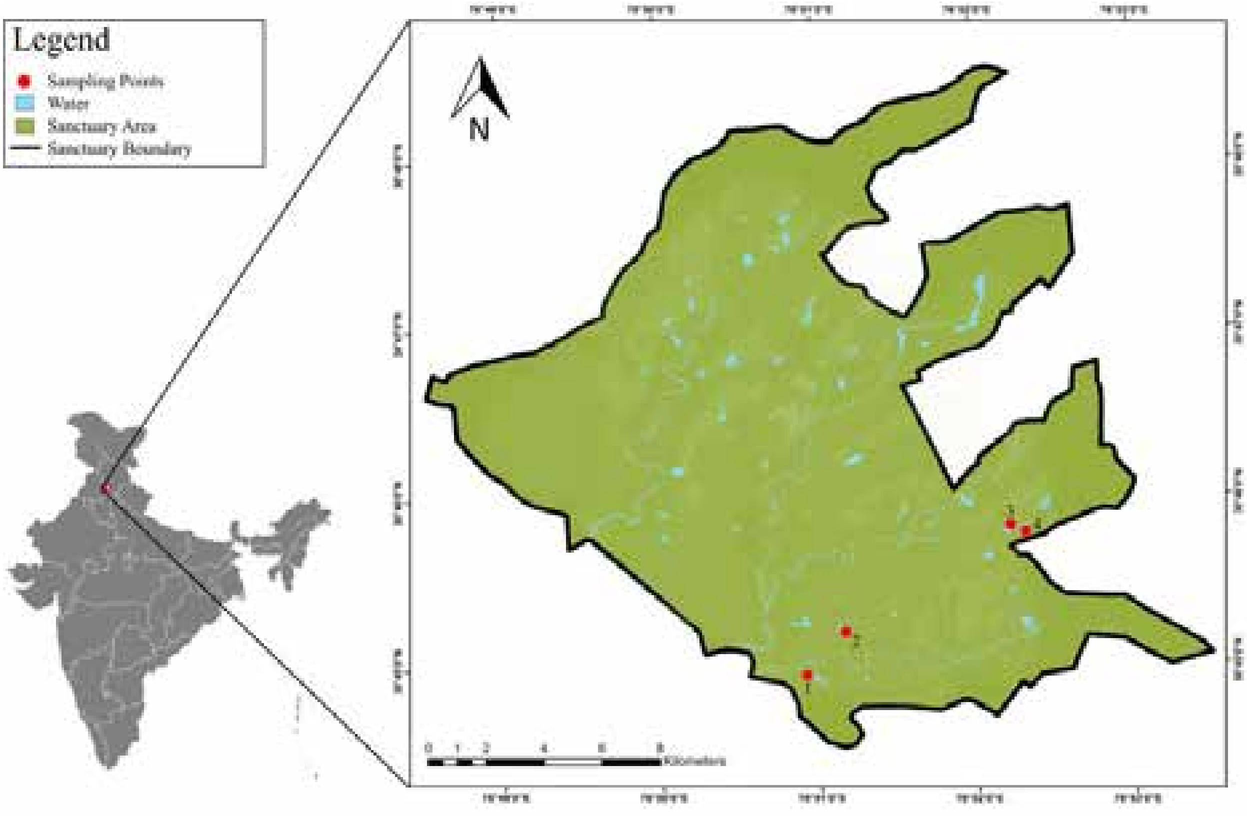

Figure 1. Sampling locations within Sukhna Wildlife Sanctuary.

Table 1. Sampling locations of aquatic beetles within Sukhna Wildlife Sanctuary.

Sampling point	Name of water body	Description of water body	Latitude (N)	Longitude (E)	Elevation (in m)	Area (in m2)
Point 1	Majla Wala Dam No.3	Check the dam with little vegetation on the shoreline and high depth.	30.752	76.853	366	7648
Point 2	Julahe Wala Dam No.4	Check the dam with decent vegetation and rocky substrate.	30.755	76.857	390	9174
Point 3	Kandalewla Dam No.2	Check dam with low vegetation, high turbidity and deep water.	30.765	76.875	426	5848
Point 4	Kandalewla Dam No.1	Check dam with high vegetation, low turbidity and shallow water.	30.764	76.876	450	4217

disturbance, the remaining contents of the spill tray were returned to their natural habitat.

Taxonomic Identification

Each specimen was photographed using a Wadeo Digital Microscope. Subsequently, the samples were forwarded to the Zoological Survey of India (ZSI) in order to undergo taxonomic identification. The process of identification was accomplished by means of dissecting the collected specimens and conducting a comparison of male genitalia along with the use of reliable identification keys and original descriptions (Vazirani 1968, 1984, Ghosh & Nilsson 2012).

RESULTS

In the present investigation, a total of 164 aquatic Coleoptera specimens belonging to six species, five genera, and two families were identified and documented at Sukhna Wildlife Sanctuary (Image 1, Table 2). All species were recorded for the first time from the union territory of Chandigarh.

The most numerous family was discovered to be Dytiscidae, followed by Hydrophilidae. As seen in the figure below, the species *Laccophilus parvulus* demonstrated the greatest overall abundance, with a significant presence specifically at point 4, where

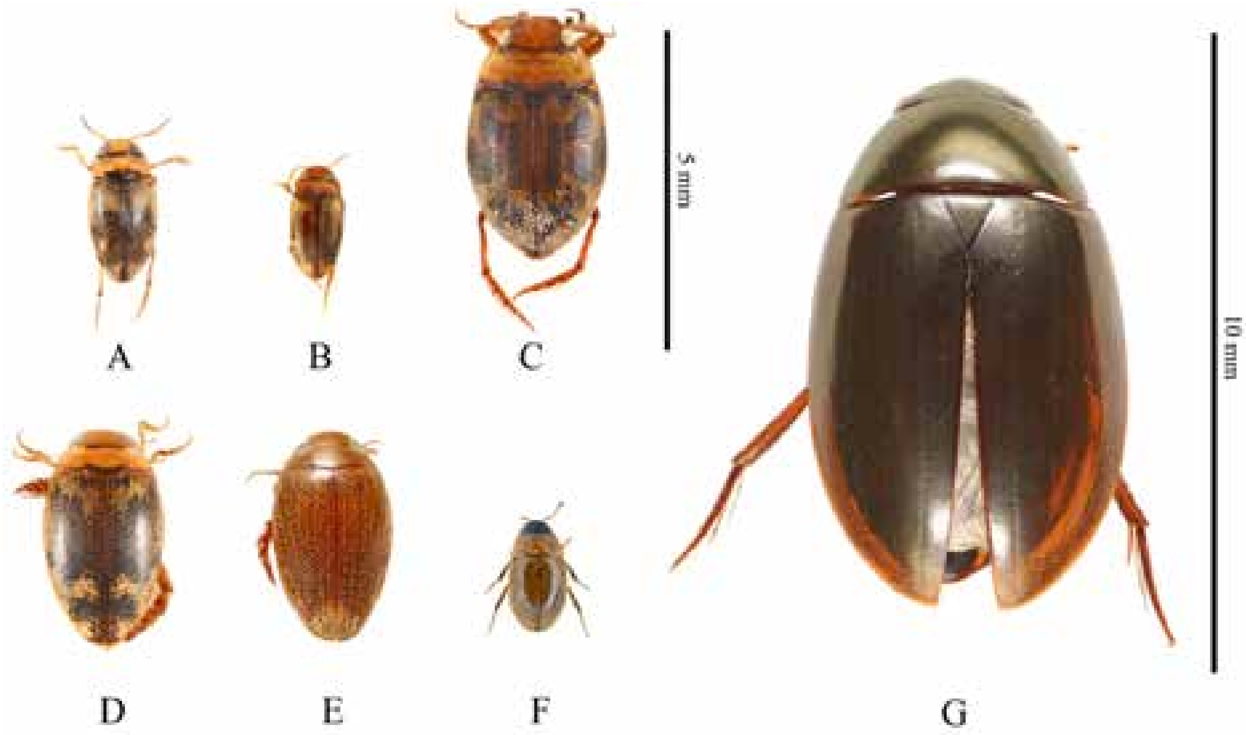

Image 1. A—*Hydroglyphus flammulatus* (Sharp, 1882) | B—*Hydroglyphus pendjabensis* (Guignot, 1954) | C—*Hyphoporus* sp. | D—*Laccophilus sharpi* (Régimbart, 1889) | E—*Laccophilus parvulus* (Aubé, 1838) | F—*Enochrus* (*Methydrus*) *esuriens* (Walker, 1858) | G—*Sternolophus inconspicuus* (Nietner, 1856). © Karmannye Om Chaudhary.

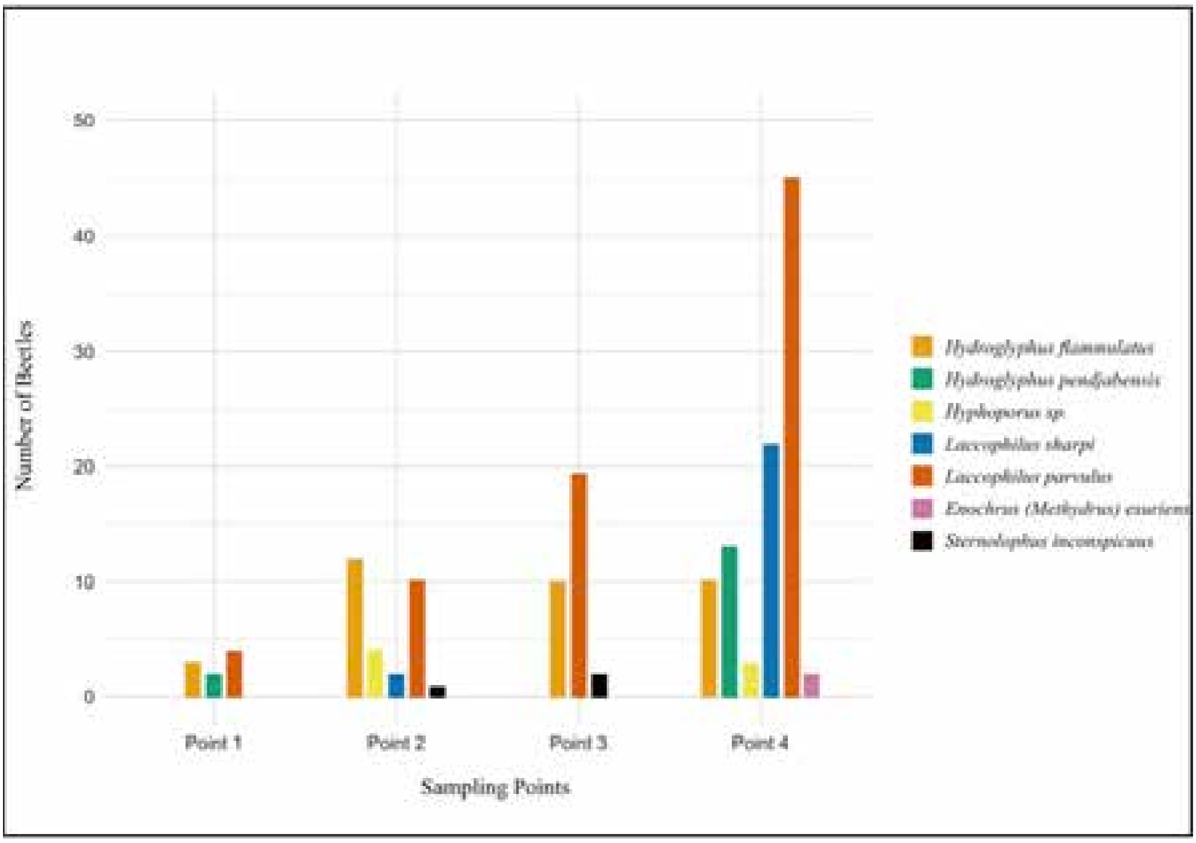

Figure 2. Distribution of aquatic beetles through the various sampling points.

Table 2. A systematic inventory of water beetles found in Sukhna Wildlife Sanctuary, Chandigarh, India and their distribution through India and the world (Ghosh & Nilsson 2012; Chandra et al. 2017; Gupta et al. 2022; Sonali et al. 2022).

Family	Scientific name	Distribution through India	Distribution through the world
Dytiscidae	*Hydroglyphus flammulatus (Sharp, 1882)*	Andhra Pradesh, Assam, Bihar, Delhi, Gujarat, Himachal Pradesh, Jharkhand, Kerala, Maharashtra, Manipur, Meghalaya, Madhya Pradesh, Odisha, Punjab, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal.	Bangladesh, China, Indonesia, Iran, Japan, Sri Lanka, Cambodia, Malaysia, Nepal, Pakistan, Thailand, Taiwan, Vietnam.
	Hydroglyphus pendjabensis (Guignot, 1954)	Andhra Pradesh, Bihar, Delhi, Goa, Gujarat, Jharkhand, Maharashtra, Madhya Pradesh, Odisha, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal.	Bangladesh, Myanmar, Nepal, Pakistan
	Hyphoporus sp.	-	Palearctic and Oriental, from Iran to India and southeastern Asia;
	Laccophilus sharpi (Régimbart, 1889)	Andaman & Nicobar Islands, Assam, Bihar, Delhi, Gujarat, Himachal Pradesh, Haryana, Jharkhand, Kerala, Maharashtra, Meghalaya, Manipur, Madhya Pradesh, Odisha, Puducherry, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal.	Sri Lanka, Myanmar, Nepal, Pakistan.
	Laccophilus parvulus (Aubé, 1838)	Andaman & Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Goa, Gujarat, Himachal Pradesh, Jharkhand, Karnataka, Kerala, Maharashtra, Manipur, Odisha, Puducherry, Rajasthan, Sikkim, Tamil Nadu, Tripura, Uttarakhand, Uttar Pradesh, West Bengal.	Bangladesh, Bhutan, China, Indonesia, Sri Lanka, Myanmar, Malaysia, Nepal, Philippines, Pakistan, Singapore, Thailand, Vietnam.
Hydrophilidae	*Enochrus (Methydrus) esuriens (Walker, 1858)*	Andaman & Nicobar Islands, Andhra Pradesh, Dadra and Nagar Haveli, Jammu & Kashmir, Jharkhand, Maharashtra, Manipur, Madhya Pradesh, Odisha, Punjab, Sikkim, Telangana, Uttarakhand, Uttar Pradesh, West Bengal.	Australia, Bangladesh, China, Fiji, Indonesia, Japan, South Korea, Sri Lanka, Malaysia, Philippines, Papua New Guinea, Saudi Arabia, Vietnam
	Sternolophus inconspicuus (Nietner, 1856)	Madhya Pradesh, Maharashtra, Meghalaya, Tamil Nadu, Uttar Pradesh.	Cambodia, China, Hong Kong, Indonesia, Japan, Laos, Myanmar, Nepal, Philippines, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam.

a total of 45 individuals were recorded. The species *Hydroglyphus pendjabensis* exhibited a higher concentration at point 3, where a total of 13 individuals were seen. In contrast, the species *Hyphoporus* sp. demonstrated a more uniform distribution, with four individuals observed at point 1 and six individuals observed at point 3. The species *Laccophilus sharpi* had a notable presence at point 2, with a total of 22 individuals being seen. *Enochrus* (*Methydrus*) *esuriens* had a predominant distribution at point 1, where a total of four individuals were seen. The species *Sternolophus inconspicuus*, which was quite uncommon, was observed as a single specimen at point 1 and as two specimens at point 2. *Hydroglyphus flammulatus* exhibited a very homogeneous spatial distribution over the entirety of the four designated sample locations, with a range of abundances spanning 2–12 individuals.

As seen in Figure 2, sampling point 1 had the lowest number of aquatic beetle individuals and similar species richness to point 3. Sampling point 2 had a higher species richness than point 2 and point 3 had a lower number of individuals as compared to point 3. Point 4 had the highest number as well highest species richness of aquatic beetles.

DISCUSSION

The results obtained from this research provide valuable information regarding the population size and spatial distribution of aquatic Coleoptera species in the Sukhna Wildlife Sanctuary. The majority of species that were recorded are widely distributed and often found throughout various regions of India. According to the data presented in Table 2, it has been observed that four out of the seven aquatic beetle species found in Chandigarh have not yet been reported in the neighbouring states of Punjab and Haryana (Ghosh & Nilsson 2012).

The results show that the Dytiscidae family is dominant in aquatic habitats, which is consistent with global trends in aquatic ecosystems. Predatory diving beetles, scientifically known as Dytiscidae, are amazing adapters to a wide range of aquatic settings and frequently hold the top predatory positions within them (Miller & Bergsten 2016). The abundance of these species in the Sukhna Wildlife Sanctuary emphasises their biological relevance in the aquatic food chain, as they act as important predators and nutrient recyclers. The comparatively uniform distribution of *Laccophilus parvulus* and *Hydroglyphus flammulatus* throughout the sampling sites is a noteworthy observation, which implies that these species have less specialized habitat preferences and are capable of adjusting to a diverse array of environmental conditions because specific environmental conditions, including vegetation type, water profundity, and temperature, are preferred by particular species (Lundkvist et al. 2003). Different species of aquatic beetles were found in different numbers and areas at each of the four sampling sites, which shows that the aquatic habitats in the sanctuary are not all the same. The observed discrepancies may be ascribed to distinct microhabitat attributes, habitat dimensions, vegetation configuration, and additional ecological variables that are recognized to impact communities of aquatic beetles (Ribera et al. 2003; Akunal & Aslan 2017; Sharma et al. 2019).

Figure 2 shows a steady increase in the population size of aquatic beetles from point 1 to point 4 of the Sukhna Wildlife Sanctuary, which corresponded to a significant elevation gradient, where point 1 was positioned at the lowest altitude and point 4 was at the highest. This corresponds to the fact that altitude may have a significant impact on the composition of aquatic insects by influencing the distribution of species (Taher & Heydarnejad 2020). The results also exhibit that points 2 & 4 had greater species richness than points 1 & 3. The differences seen may be due to the different biological features of the places where samples were taken. With higher vegetation, detritus, and shallower water, points 2 & 4 were suitable water bodies to harbour a variety of beetle species (Molnar et al. 2009), while points 1 & 3 exhibited low vegetation cover, high turbidity, and stagnant water, which may have led to a lack of species diversity (Gomezlutz et al. 2017). A detailed and extensive examination of the temporal variations and habitat preferences of aquatic beetles might aid in statistically validating the findings. This is required since the current study has a small sample size and was completed over a short period of time. Completing such an investigation would present substantial challenges since it would require removing a large population of aquatic beetles from their environment, which might alter the balance of the aquatic ecosystems. Furthermore, precisely identifying beetle species requires microscopic inspection of their genitalia, which would require their euthanasia. The fact that only seven species from two families were observed during this time period calls for further surveying and building upon the data that this paper offers. Aquatic beetles from the families Gyrinidae, Noteridae, and Elmidae remain absent from this habitat.

CONCLUSION

In conclusion, this study lays down the baseline data for the aquatic Coleoptera found in Chandigarh and provides insights into the functioning of aquatic beetle communities in Sukhna Wildlife Sanctuary. A considerable abundance of the family Dytiscidae was revealed by the systematic identification and categorization of 164 specimens representing seven species, revealing insight into its critical role as the principal predator and contributor to nitrogen cycling within aquatic environments. A pattern in the diversity and number of beetles at different altitudes influenced by different factors, such as habitat quality, was also observed; it is critical to undertake more extensive surveys and long-term surveillance of aquatic Coleoptera populations in order to acquire an understanding of their responses to environmental changes and the complex interrelationships between biotic and abiotic factors. The findings not only add to our understanding of the richness present in the Sukhna Wildlife Sanctuary but also highlight the need for coordinated conservation efforts to maintain these essential freshwater habitats because, as indicators of habitat features and ecological variety, water beetles are vital members of the biotic community in all wetland environments (Eyre & Foster 1989; Fairchild et al. 2000).

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26131–26149

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.8741.16.11.26131-26149

#8741 | Received 14 September 2023 | Final received 24 October 2024 | Finally accepted 03 November 2024

OPEN ACCESS

REVIEW

An updated checklist of snakes (Reptilia: Squamata) in northeastern India derived from a review of recent literature

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Abstract: This paper reviews studies of the snakes of northeastern India published between 2001 and 2024 identified from searchable databases, covering diversity, range extension, distribution records, new genus, new species, redescription, rediscovery, and taxonomic revision. This analysis of the literature and publicly available information presents an updated checklist of 126 snake species representing 12 families and 46 genera, along with their distribution across states in northeastern India and their IUCN Red List status. The study also reveals a research gap in some northeastern states that provides opportunities for further regional studies.

Keywords: Assam, Arunachal Pradesh, herpetofauna, Manipur, Meghalaya, Mizoram, Nagaland, ophidian, Sikkim, Tripura.

Editor: S.R. Ganesh, Kalinga Foundation, Agumbe, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Basfore, B., M.J. Kalita, N. Sharma & A.R. Boro (2024). An updated checklist of snakes (Reptilia: Squamata) in northeastern India derived from a review of recent literature. *Journal of Threatened Taxa* 16(11): 26131–26149. https://doi.org/10.11609/jott.8741.16.11.26131-26149

Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: BB drafted the manuscript, compiled data from various sources, and prepared the checklist of snakes along with their regional distribution. MJK conceptualized the manuscript and contributed to the study's design. NS enhanced the manuscript's fluency and supervised its preparation. ARB assisted in designing the study.

Acknowledgements: The authors are indebted to the Department of Zoology, Pandu College for providing the resources to carry out this project. They sincerely thank friends, families, and colleagues for their support and guidance.

INTRODUCTION

The documentation and evaluation of snakes in India date back to the 19th century. Some of the pioneering works on Indian snakes include Günther (1864), Jerdon (1870), Boulenger (1890), Wall (1909a, 1910a, 1911, 1918, 1922a), Cazaly (1914), Smith (1943), Sharma (1976), Das (1991), and Murthy et al. (1993). The reptilian fauna of the country was reported by Aengals et al. (2018), who listed 572 species of reptiles, comprising 304 species of snakes representing 16 families. Later, Deuti et al. (2022) reported the occurrence of 471 amphibians and 681 reptile species from India.

Northeastern India consists of the states of Assam, Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Tripura, and Sikkim, which is bordered by Myanmar in the east, Nepal in the west, Bangladesh in the south-west, Bhutan in the north-west, and China in the north. This region covers an area of about 0.26 million km^2 (Jain et al. 2012). The altitude varies from sea level to 8,600 m at the peak of Kanchenjunga in Sikkim (Chib & Lodrick 2023). Northeastern India has an impressive biodiversity and harbors high endemicity in plant and vertebrate species, resulting in its recognition as a biodiversity hotspot (Das & Khosla 2018). Most of the genera and species diversity of snakes in the country are confined to northeastern India and the Western Ghats (Hmar et al. 2020), making conducting proper surveys in these areas important. Some of the pioneering works on the snakes of northeastern India include Cantor (1839), Blyth (1851, 1853, 1854), Gray (1853), Günther (1860), Anderson (1871), Wall (1908, 1909b, 1910b,c, 1922b), Acharji & Kripalani (1951), Talukdar & Sanyal (1978), Mathew (1983, 1992, 1995, 1998), Sanyal & Gayen (1987), Shamungou (1995), Sengupta et al. (2000), and Captain & Bhatt (2000).

Since the start of the 21st century, many scientific articles and guidebooks have been written about the snakes in northeastern India. Some states in the region have been thoroughly studied in the last 23 years, but others still need more research. For instance, some of the northeastern states have seen a lot of research on snake diversity, distribution, and species description, whereas in other states, there hasn't been as much research, so our understanding of snake biodiversity there is limited. The present study aims to address such disparities by providing a comprehensive review of ophidian studies in northeastern India, encompassing well-studied areas and those requiring more attention. Through this review, we endeavor to shed light on the current state of snake research in the region and identify areas for future study and conservation action.

MATERIALS AND METHODS

We examined 111 research papers published between January 2001 and September 2024. These articles were obtained from online searchable databases such as 'Google Scholar', the 'Reptile Database' and various journals using a combination of keywords such as "snakes", "northeastern India", "reptiles", "herpetofauna", "new records", "new species", "diversity", "rediscovery", and "taxonomic revision of snakes". In our segregation and allocation process, we categorized studies based on the states they focused on or where the snakes were studied. Each article was carefully reviewed to determine its geographic relevance and allocated accordingly. If an article covered multiple states, we reviewed it separately and included it in the appropriate section, ensuring a clear state-wise review. The analysis of previously published papers, especially the recent ones, and the data gathered from other publicly available sources were used to prepare an updated checklist of snakes in northeastern India. We also searched the recent version of the IUCN Red Data List to check their conservation status.

RESULTS

Diversity

In the last two decades or so, several studies have been conducted to understand the diversity of snakes at the district or state level and in the protected and non-protected areas of northeastern India. Pawar & Birand (2001) surveyed to document the herpetofaunal diversity in northeastern India and reported 57 snake species. Later, Ahmed et al. (2009) published a photographic field guide illustrating 101 species out of the 274 species of herpetofauna known from the region. The study also presented a photo gallery of 48 reptile species and a checklist of herpetofauna for the northeast region, enlisting 102 species of snakes. However, our analysis reveals that certain species listed in their checklists have undergone taxonomic revisions, and the distribution of some species has been confined to specific localities. This highlights the dynamic nature of taxonomic status and distribution patterns, emphasizing the importance of periodic reassessment in biodiversity studies.

Multiple research investigations have also

Table 1. Distribution list of the snake species in northeastern India.

	Scientific name	Common name	Distribution in northeastern Indian states	References	IUCN Red List status
	Family: Colubridae				
1	*Ahaetulla flavescens* (Wall, 1910)	Yellow Vine Snake	[1]AS, [1]AR, [1]ML, [1]MZ	[1]Srikanthan et al. (2022)	NA
2	*Ahaetulla longirostris* Mirza, Pattekar, Verma, Stuart, Purkayastha, Mohapatra & Patel, 2024	Long-snouted Vine Snake	[1]AS, [1]ML	[1]Mirza et al. (2024b)	NA
3	*Archelaphe bella* (Stanley, 1917)	Bella Rat Snake	[1]AS, [2]AR, [1]NL,	[1]Uetz et al. (2023) [2]Jayaramaiah (iNaturalist observation, 2023)	LC
4	*Boiga cyanea* (Duméril, Bibron & Duméril, 1854)	Green Cat Snake	[1]AS, [2]AR, [3]ML, [4]MN, [5]MZ, [6]NL, [7]SK	[1]Smith (1943), [2]Borang et al. (2005) [3]Mathew (1995), [4]Premjit et al. (2024) [5]Lalremsanga et al. (2011), [6]Ao et al. (2004), [7]Jha &Thapa (2002)	LC
5	*Boiga gokool* (Gray, 1834)	Eastern Cat Snake	[1,3,4]AS, [2,3,4]AR, [3,4]ML, [3,4]MN, [5]MZ, [3]NL, [6]TR, [4]SK	[1]Smith (1943), [2]Sanyal & Gayen (2006), [3]Das et al. (2010) [4]Ahmed et al. (2009), [5]Lalremsanga & Lalronunga (2017), [6]Nath et al. (2021c)	LC
6	*Boiga multifasciata* (Blyth, 1861)	Many-banded Cat Snake	[1]AR, [1,2]SK,	[1]Das et al. (2010), [2]Jha & Thapa (2002),	LC
7	*Boiga multomaculata ochracea* (Theobald, 1868)	Tawny Asian Cat Snake	[1]MZ	[1]Köhler et al. (2023)	NA
	Boiga multomaculata septentrionalis Kohler, Charunrochana, Mogk, Than, Kurniawan, Kadafi, Das Tillack & O'Shea, 2023	Northern Polymorphic Asian Cat Snake	[1]AS, [1]NL	[1]Köhler et al. (2023)	
8	*Boiga quincunciata* (Wall, 1908)	Assamese Cat Snake	[1]AS, [2]AR, [3]MN, [4]MZ	[1]Wall (1908), [2]Sanyal & Gayen (2006), [3]Sinate et al. (2022), [4]Lalremsanga et al. (2011)	LC
9	*Boiga siamensis* Nutaphand, 1971	Thai Cat Snake	[1,4]AS, [2]AR, [4]ML, [3]MZ, [4,6]NL, [5]SK	[1]Sarkar et al. (2022), [2]Borang et al. (2005), [3]Lalremsanga et al. (2011), [4]Smith (1943), [5]Jha & Thapa (2002), [6]Ao et al. (2004)	LC
10	*Boiga stoliczkae* (Wall, 1909)	Stoliczka's Asian Cat Snake	[1]AS, [1]AR, [1]SK	[1]Köhler et al. (2023)	NA
11	*Boiga trigonata* (Schneider, 1802)	Common Cat Snake	[1]ML, [2]SK	[1]Mathew (1995), [2]Smith (1943)	LC
12	*Calamaria pavimentata* Duméril, Bibron & Duméril, 1854	Collared Reed Snake	[1]AS, [2,3]ML, [1]MN, [4]MZ	[1]Smith (1943), [2]Mathew (1995), [3]Ranade (2022), [4]Lalremsanga et al. (2011),	LC
13	*Chrysopelea ornata* (Shaw, 1802)	Ornate Flying Snake	[1]AS, [2]AR, [3]ML, [4]MZ, [5]NL, [6]SK, [7]TR	[1]Sengupta et al. (2016), [2]Sanyal & Gayen (2006), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Bhupathy et al. (2013), [6]Jha & Thapa (2002), [7]Majumder et al. (2010)	LC
14	*Coelognathus helena* (Daudin, 1803)	Indian Trinket Snake	[1]AS, [2]MN, [2,3]NL	[1]Das et al. (2007), [2]Whitaker & Captain (2004), [3]Bhupathy et al. (2013)	LC
15	*Coelognathus radiatus* (Boie, 1827)	Copper-headed Trinket Snake	[1,6]AS, [2,6]AR, [3,6]ML, [4,6]MN, [5,6]MZ, [6]NL, [2,6,7]SK, [8]TR	[1]Das et al. (2007), [2]Whitaker & Captain (2004), [3]Mathew (1995), [4]Mathew (2005), [5]Lalremsanga et al. (2011), [6]Ao et al. (2004), [7]Smith (1943), [8]Majumder (2012)	LC
16	*Cyclophiops doriae* (Boulenger, 1888)	Green Grass Snake	[1]AS, [1]MN	[1]Wall (1924)	LC

	Scientific name	Common name	Distribution in northeastern Indian states	References	IUCN Red List status
17	*Dendrelaphis biloreatus* Wall, 1908	Gore's Bronzeback	[1]AS, [2]AR, [3]MZ, [4]SK	[1]Wall (1908), [2]Sanyal & Gayen (2006), [3]Biakzuala et al. (2022), [4]Jha & Thapa (2002)	LC
18	*Dendrelaphis cyanochloris* (Wall, 1921)	Blue Bronzeback	[1]AS, [1]AR, [2]MZ, [3]SK	[1]Ahmed et al. (2009), [2]Lalremsanga et al. (2011), [3]Jha & Thapa (2002)	LC
19	*Dendrelaphis proarchos* (Wall, 1909)	Painted Bronzeback	[1]AS, [2]AR, [3]ML, [4]MZ, [5]NL, [6]TR, [7]SK	[1]Mahananda et al. (2023), [2]Borang et al. (2005), [3]Chandramouli et al. (2021), [4]Malsawmdawngliana et al. (2022a), [5]Bhupathy et al. (2013), [6]Purkayastha et al. (2020b), [7]Jha & Thapa (2002)	NA
20	*Elaphe cantoris* (Boulenger, 1894)	Eastern Trinket Snake	[1,3]AR, [2,3]ML, [3]NL, [3,4]SK	[1]Borang et al. (2005), [2]Mathew (1995), [3]Ao et al. (2004), [4]Chettri et al. (2011)	LC
21	*Elaphe hodgsoni* (Günther, 1860)	Himalayan Trinket Snake	[1]ML, [1]SK	[1]Whitaker & Captain (2004)	LC
22	*Elaphe taeniura* Cope, 1861	Striped Trinket Snake	[1]AR, [2]MZ, [3]SK	[1]Borang et al. (2005), [2]Harit (2010), [3]Jha & Thapa (2002)	VU
23	*Euprepiophis mandarinus* (Cantor, 1842)	Mandarin Rat Snake	[1]AR, [2]MZ, [3]NL	[1]Borang et al. (2005), [2]Ashaharraza et al. (2019), [3]Bhupathy et al. (2013)	LC
24	*Gongylosoma scriptum* (Theobald, 1868)	Common Ringneck	[1]MZ	[1]Lalremsanga et al. (2018)	LC
25	*Gonyosoma frenatum* (Gray, 1853)	Khasi Hills Trinket Snake	[1]AR, [2]ML	[1]Borang et al. (2005), [2]Smith (1943)	LC
26	*Gonyosoma prasinum* (Blyth, 1854)	Green Trinket Snake	[1,4]AS, [2,4]AR, [4]ML, [4]MN, [4]MZ, [3,4]NL, [5]SK	[1]Smith (1943), [2]Borang et al. (2005), [3]Bhupathy et al. (2013), [4]David et al. (2022), [5]Jha & Thapa (2002)	LC
27	*Liopeltis frenata* (Günther, 1858)	Günther's Reed Snake	[1]AR, [2]ML, [3]MZ, [4]NL	[1]Borang et al. (2005), [2]Mathew (1995), [3]Lalremsanga et al. (2011), [4]Ao et al. (2004)	LC
28	*Liopeltis rappi* (Günther, 1860)	Himalayan Reed Snake	[1]SK	[1]Jha & Thapa (2002)	LC
29	*Liopeltis stoliczkae* (Sclater, 1891)	Stoliczka's Reed Snake	[1]AR, [2]NL, [3]SK	[1]Borang et al. (2005), [2]Ao et al. (2004), [3]Jha & Thapa (2002)	LC
30	*Lycodon aulicus* (Linnaeus, 1758)	Common Wolf Snake	[1,6]AS, [1,6]AR, [2,6]ML, [1,6]MN, [6]MZ, [3,6]NL, [4,6]SK, [5,6]TR	[1]Smith (1943), [2]Mathew (1995), [3]Bhupathy et al. (2013) [4]Chettri et al. (2011), [5]Majumder et al. (2012), [6]Whitaker & Captain (2004)	LC
31	*Lycodon fasciatus* (Anderson,1879)	Banded Wolf Snake	[1]AS, [2]AR, [3]MZ, [2]NL	[1]Smith (1943), [2]Ao et al. (2004), [3]Lalbiakzuala & Lalremsanga (2017)	LC
32	*Lycodon gammiei* (Blanford, 1878)	Sikkim False Wolf Snake	[1]AR, [2]SK	[1]Mistry et al. (2007), [2]Jha & Thapa (2002)	NT
33	*Lycodon jara* (Shaw, 1802)	Twin-spotted Wolf Snake	[1]AS, [2]AR, [3]ML, [4]MN, [5]MZ, [6]TR, [7]SK	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Elangbam et al. (2022), [5]Lalremsanga et al. (2011), [6]Purkaysatha et al. (2020b), [7]Chettri et al. (2011)	LC
34	*Lycodon septentrionalis* (Günther, 1875)	White-banded Wolf Snake	[1,2]AS, [2]AR, [3]ML, [2]MZ, [2,4]NL, [5]SK	[1]Smith (1943), [2]Biakzuala et al. (2020b), [3]Mathew (1995), [4]Ao et al. (2004), [5]Jha & Thapa (2002)	LC
35	*Lycodon zawi* Slowinski, Pawar, Win, Thin, Gyi, Oo & Tun, 2001	Zaw's Wolf Snake	[1]AS, [1]ML, [1]MZ	[1]Slowinski et al. (2001)	LC

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36	*Oligodon albocinctus* (Cantor, 1839)	Light-barred Kukri Snake	[1,5]AS, [2,5,]AR, [3,5]ML, [5]MN, [4]MZ, [5]NL, [6]TR, [5,7]SK	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Ao et al. (2004), [6]Purkayastha et al. (2020b), [7]Jha & Thapa (2002)	LC
37	*Oligodon catenatus* (Blyth, 1854)	Assam Kukri Snake	[1]AS, [2]MZ	[1]Blyth (1854), [2]Lalbiakzuala & Lalremsanga (2020)	LC
38	*Oligodon cinereus* (Günther, 1864)	Günther's Kukri Snake	[1]AS, [2]AR, [3]ML, [4]MZ	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011)	LC
39	*Oligodon cyclurus* (Cantor, 1839)	Cantor's Kukri Snake	[1]AS, [2]ML, [3]MZ, [4]NL	[1]Smith (1943), [2]Mathew (1995), [3]Lalremsanga et al. (2011), [4]Bhupathy et al. (2013)	LC
40	*Oligodon dorsalis* (Gray, 1834)	Bengalese Kukri Snake	[1]AS, [2]AR, [3]ML, [4]MN, [5]MZ, [3]NL	[1]Dey et al. (2022), [2]Wall (1910), [3]Smith (1943), [4]Mathew (1995), [5]Lalremsanga et al. (2011)	LC
41	*Oligodon kheriensis* Acharji & Ray, 1936	Coral Red Kukri Snake	[1,2]AS	[1]Sutradhar & Nath (2013), [2]Nath et al. (2021a)	LC
42	*Oligodon erythrogaster* Boulenger, 1907	Nagarkot Kukri Snake	[1]SK	[1]Jha & Thapa (2002)	NT
43	*Oligodon erythrorhachis* Wall, 1910	Red-striped Kukri Snake	[1,2]AS, [1,2]AR	[1]Wall (1910c), [2]Borang et al. (2005)	VU
44	*Oligodon juglandifer* (Wall, 1909)	Darjeeling Kukri Snake	[1]ML, [2]SK	[1]Chandramouli et al. (2021), [2]Jha & Thapa (2002)	VU
45	*Oligodon melaneus* Wall, 1909	Blue-bellied Kukri Snake	[1]AS, [2]SK	[1]Das et al. (2022), [2]Jha & Thapa (2002)	DD
46	*Oligodon melanozonatus* Wall, 1922	Abor Hills Kukri Snake	[1]AR	[1]Wall (1922b)	DD
47	*Oligodon theobaldi* (Günther, 1868)	Theobald's Kukri Snake	[1,2]ML, [3]NL	[1]Smith (1943), [2]Mathew (1995), [3]Ao et al. (2004)	LC
48	*Oreocryptophis porphyraceus* (Cantor, 1839)	Black-banded Trinket Snake	[1]AS, [2,4]AR, [3,4]ML, [4]MN, [5]MZ, [4]NL, [4,6]SK	[1]Mahananda et al. (2023), [2]Borang et al. (2005), [3]Mathew (1995), [4]Whitaker & Captain (2004) [5]Harit (2016), [6]Jha & Thapa (2002)	LC
49	*Ptyas korros* (Schlegel, 1837)	Indo-chinese Rat Snake	[1]AS, [1]AR, [2]ML, [3]MN, [4]MZ, [5]NL, [6]SK, [7]TR	[1]Smith (1943), [2]Mathew (1995), [3]Acharji & Kripalani (1951), [4]Lalremsanga et al. (2011), [5]Ao et al. (2004), [6]Jha & Thapa (2002), [7]Giri et al. (2017)	NT
50	*Ptyas mucosa* (Linnaeus, 1758)	Oriental Rat Snake	[1]AS, [2]MZ, [3]SK, [4]TR	[1]Das et al. (2007), [2]Lalremsanga et al. (2011), [3]Jha & Thapa (2002), [4]Purkaysatha et al. (2020b)	LC
51	*Ptyas nigromarginata* (Blyth, 1854)	Green Rat Snake	[1]AS, [2]AR, [1]ML, [1]NL, [1]SK	[1]Smith (1943), [2]Borang et al. (2005)	LC
	Family: Elapidae				
52	*Bungarus bungaroides* (Cantor, 1839)	Northeastern Hill Krait	[1]AS, [4]AR, [1,2]ML, [1]SK, [3]NL	[1]Smith (1943), [2]Mathew (1995), [3]Ao et al. (2004), [4]Agarwal et al. (2010)	LC
53	*Bungarus fasciatus* (Schneider, 1801)	Banded Krait	[1]AS, [1]AR, [1]ML, [1]MZ, [2]TR	[1]Ahmed et al. (2009), [2]Majumder et al. (2012)	LC
54	*Bungarus lividus* Cantor, 1839	Lesser Black Krait	[1]AS, [2,5]AR, [3]ML, [4]NL	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Bhupathy et al. (2013), [5]Agarwal et al. (2010)	LC

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55	*Bungarus niger* Wall, 1908	Greater Black Krait	[1,2]AS, [1,2,3]AR, [1,2]ML, [4]MN, [2]MZ, [1]NL, [1]SK, [2]TR	[1]Ahmed et al. (2009), [2]Biakzuala et al. (2021), [3]Agarwal et al. (2010), [4]Sinate et al. (2021)	LC
56	*Bungarus suzhenae* Chen, Shi, Vogel, Ding & Shi, 2021	Suzhen's Krait	[1]MN, [1]NL	[1]Gerard et al. (2024)	NA
57	*Naja kaouthia* Lesson, 1831	Monocled Cobra	[1,2]AS, [1,2]AR, [2]ML, [3]MN, [4]MZ, [2]NL,[1,2]SK, [5]TR	[1]Whitaker & Captain (2004), [2]Ao et al. (2004) [3]Acharji & Kripalani (1951), [4]Lalremsanga et al. (2011) [5]Purkayastha et al. (2020b)	LC
58	*Naja naja* (Linnaeus, 1758)	Spectacled Cobra	[1]AS, [2]ML	[1]Uetz et al. (2023), [2]Mathew (1995)	LC
59	*Ophiophagus hannah* (Cantor, 1836)	King Cobra	[1]AS, [2]AR, [3]ML, [4]MZ, [5]SK, [6]TR	[1]Das et al. (2007), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011) [5]Jha & Thapa (2002), [6]Majumder et al. (2012)	VU
60	*Sinomicrurus gorei* (Wall, 1909)	Gore's Coral Snake	[1]AS, [1]MN, [1]MZ, [1]NL, [2]TR	[1]Biakzuala et al. (2023b), [2]Deb et al. (2024a)	NA
61	*Sinomicrurus macclellandi* (Reinhardt, 1844)	Macclelland's Coral Snake	[1,4]AS, [2,4]AR, [3,4]ML, [4]MN, [4,5]MZ, [4]NL, [6]SK	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Ao et al. (2004), [5]Lalremsanga et al. (2011), [6]Jha & Thapa (2002)	LC
	Family: Homalopsidae				
62	*Enhydris enhydris* (Schneider, 1799)	Rainbow Water Snake	[1]AS, [1]ML, [1]NL	[1]Ahmed et al. (2009)	LC
63	*Ferania sieboldii* (Schlegel, 1837)	Siebold's Water Snake	[1]AS, [2]AR, [2,3]MZ, [2,4]NL, [1]TR	[1]Deb et al. (2023), [2]Agarwal et al. (2010), [3]Lalremsanga et al. (2011), [4]Smith (1943)	LC
	Family: Natricidae				
64	*Amphiesma stolatum* (Linnaeus, 1758)	Buff-striped Keelback	[1,6]AS, [2,6]AR, [3,6]ML, [4]MN, [5]MZ, [6]NL, [6]SK, [6,7]TR	[1]Das et al. (2007), [2]Borang et al. (2005), [3]Mathew (1995), [4]Acharji & Kripalani (1951), [5]Lalremsanga et al. (2011), [6]Ao et al. (2004), [7]Majumder et al. (2012)	LC
65	*Fowlea piscator* (Schneider, 1799)	Checkered Keelback	[1,6]AS, [2,6]AR, [3,6]ML, [4]MN, [5,6]MZ, [6]NL, [7]SK, [6,8]TR	[1]Das et al. (2007), [2]Borang et al. (2005), [3]Mathew (1995), [4]Mathew (2005), [5]Lalremsanga et al. (2011), [6]Ao et al. (2004) [7]Jha & Thapa (2002), [8]Majumder et al. (2012)	LC
66	*Fowlea sanctijohannis* (Boulenger, 1890)	St. John's Keelback	[1]AR, [2]SK	[1]Borang et al. (2005), [2]Chettri et al. (2011)	LC
67	*Fowlea schnurrenbergeri* (Kramer, 1977)	Bar-necked Keelback	[1]AS	[1]Sengupta et al. (2016)	LC
68	*Hebius clerki* (Wall, 1925)	Yunnan Keelback	[1,3]AR, [2,3]ML, [3]MZ, [3]NL, [4]SK	[1]Borang et al. (2005), [2]Mathew (1995), [3]Ao et al. (2004), [4]Chettri et al. (2011)	LC
69	*Hebius khasiensis* (Boulenger, 1890)	Khasi Hills Keelback	[1,3]AR, [2,3]ML, [3]MZ, [3]NL	[1]Borang et al. (2005), [2]Boulenger (1890), [3]Ao et al. (2004)	LC
70	*Hebius lacrima* Purkayastha & David, 2019	Crying Keelback	[1]AR	[1]Purkayastha & David (2019)	DD
71	*Hebius modestus* (Günther, 1875)	Modest Keelback	[1]AR, [2]ML, [3]MZ	[1]Borang et al. (2005), [2]Boulenger (1890), [3]Lalremsanga et al. (2011),	LC

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72	*Hebius parallelus* (Boulenger, 1890)	Striped Keelback	[1]AS, [2,3]ML, [1,2]SK	[1]Smith (1943), [2]Boulenger (1890), [3]Mathew (1995)	DD
73	*Hebius taroensis* (Smith, 1940)	Kachin Keelback	[1]AR	[1]David et al. (2021)	NT
74	*Hebius venningi* (Wall, 1910)	Chin Hills Keelback	[1]AR, [2]MN, [1]MZ, [1]NL	[1]David et al. (2021), [2]Hakim (2023)	LC
75	*Herpetoreas murlen* Lalremsanga, Bal, Vogel & Biakzuala, 2022	Murlen Keelback	[1]MZ	[1]Lalremsanga et al. (2022)	NA
76	*Herpetoreas pealii* (Sclater, 1891)	Assam Keelback	[1]AS, [1]AR	[1]Das et al. (2020b)	DD
77	*Herpetoreas platyceps* (Blyth, 1854)	Himalayan Keelback	[1]AR, [1]ML, [2]SK	[1]Smith (1943), [2]Jha & Thapa (2002),	LC
78	*Herpetoreas sieboldii* Günther, 1860	Sikkim Keelback	[1]SK	[1]Günther (1860)	DD
79	*Herpetoreas xenura* (Wall, 1907)	Wall's Keelback	[1]ML, [1]MZ, [2]TR	[1]Lalronunga (2020), [2]Giri et al. (2017)	NT
80	*Rhabdophis bindi* Das, Smith, Sidik, Sarker, Boruah, Patel, Murthy & Deepak, 2021	Bindee Keelback	[1]AS, [1]MZ, [1]TR	[1]Das et al. (2021)	NA
81	*Rhabdophis helleri* (Schmidt, 1925)	Heller's Red-necked Keelback	[1]AS, [2]AR, [1]ML, [3]MN, [1]MZ, [1]NL, [1]SK, [4]TR	[1]David & Vogel (2021), [2]Borang et al. (2005), [3]Mathew (1995), [4]Purkayastha et al. (2020b)	NA
82	*Rhabdophis himalayanus* (Günther, 1864)	Himalayan Keelback	[1]AS, [2]AR, [3]ML, [4]MZ, [1]SK, [5]NL, [6]TR	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Bhupathy et al. (2013), [6]Giri et al. (2017)	LC
83	*Rhabdophis nuchalis* (Boulenger, 1891)	Hubei Keelback	[1]NL	[1]Ahmed & Das (2006)	LC
84	*Smithophis arunachalensis* Das, Deepak, Captain, Wade & Gower, 2020	Arunachal Rain Snake	[1]AR	[1]Das et al. (2020a)	NA
85	*Smithophis atemporalis* Giri, Gower, Das, Lalremsanga, Lalronunga, Captain & Deepak, 2019	Mizo Rain Snake	[1]MZ	[1]Giri et al. (2019)	DD
86	*Smithophis bicolor* (Blyth, 1854)	Two-coloured Rain Snake	[1]AR, [1,2]ML, [2]MZ	[1]Smith (1943), [2]Giri et al. (2019)	LC
87	*Smithophis mizoramensis* Mirza, Bhardwaj, Lalmuanawma, Choure, Lalremsanga, Vabeiryureilai, Captain, Zagade & Patel, 2024	Mizo Brook Snake	[1]MZ	[1]Mirza et al. (2024a)	NA
88	*Trachischium apteii* Bhosale, Gowande& Mirza, 2019	Apte's Slender Snake	[1]AR	[1]Bhosale et al. (2019)	NA
89	*Trachischium fuscum* (Blyth, 1854)	Darjeeling Slender Snake	[1]AS, [2]AR, [3]SK	[1]Smith (1943), [2]Agarwal et al. (2010) [3]Jha & Thapa (2002),	LC
90	*Trachischium guentheri* Boulenger, 1890	Rosebelly Slender Snake	[1]SK	[1]Jha & Thapa (2002)	VU
91	*Trachischium monticola* (Cantor, 1839)	Assam Slender Snake	[1]AR, [2]ML	[1]Borang et al. (2005), [2]Mathew (1995)	LC
92	*Trachischium tenuiceps* (Blyth, 1854)	Orange-bellied Slender Snake	[1]AR, [2]SK	[1]Borang et al. (2005), [2]Jha & Thapa (2002)	DD
93	*Trimerodytes yunnanensis* Rao et Yang, 1998	Yunnan Annulate Keelback	[1]AR	[1]Nguyen & Vogel (2024)	LC
94	*Xenochrophis cerasogaster* (Cantor, 1839)	Painted Keelback	[1]AS, [2]ML	[1]Smith (1943), [2]Mathew (1995)	VU
	Family: Pareidae				
95	*Pareas andersonii* Boulenger, 1888	Anderson's Slug Snake	[1]MZ, [1]NL	[1]Vogel et al. (2020)	NA
96	*Pareas kaduri* Bhosale, Phansalkar, Sawant, Gowande, Patel & Mirza, 2020	Kadur's Slug Snake	[1]AR	[1]Bhosale et al. (2020)	NA
97	*Pareas modestus* Theobald, 1868	Mountain Slug Snake	[1]MZ	[1]Vogel et al. (2020)	NA

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98	*Pareas monticola* (Cantor, 1839)	Assam Slug Snake	[1]AS, [2]AR [1,3]ML, [4]MZ, [1]NL, [1]SK, [5]TR	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Deb et al. (2024b)	LC
	Family: Pseudaspididae				
99	*Psammodynastes pulverulentus* (Boie, 1827)	Mock Viper	[1]AS, [1]AR, [1]ML, [1]MZ, [2]SK, [3]TR	[1]Ahmed et al. (2009), [2]Chettri et al. (2011), [3]Purkayastha et al. (2020b)	LC
	Family: Pseudoxenodontidae				
100	*Pseudoxenodon macrops* (Blyth, 1854)	Mock Cobra	[1,5]AS, [2,5]AR, [3,5]ML, [4,5]MZ, [5]NL, [5,6]SK	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Ao et al. (2004), [6]Jha & Thapa (2002)	LC
	Family: Pythonidae				
101	*Malayopython reticulatus* (Schneider, 1801)	Reticulated Python	[1]MZ	[1]Lalremsanga et al. (2024)	LC
102	*Python bivittatus* Kuhl, 1820	Burmese Python	[1]AS, [2]AR, [3]ML, [4]MZ, [5]NL, [6]SK, [7]TR	[1]Purkayastha et al. (2011), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Yanthungbeni et al. (2018), [6]Jha & Thapa (2002), [7]Purkayastha et al. (2020b)	VU
	Family: Sibynophiidae				
103	*Sibynophis collaris* (Gray, 1853)	Collared Black-headed Snake	[1,2]AR, [2]ML, [1,2]MZ, [2]NL, [2]SK	[1]Pawar & Birand (2001), [2]Ao et al. (2004)	LC
	Family: Typhlopidae				
104	*Argyrophis bothriorhynchus* (Günther, 1864)	Günther's Blind Snake	[1]AS	[1]Smith (1943),	DD
105	*Argyrophis diardii* (Schlegel, 1839)	Diard's Blind Snake	[1,5]AS, [1,5]AR, [2,5]ML, [3,5]MN, [4]MZ, [5]NL, [5,6]TR, [7]SK	[1]Whitaker & Captain (2004), [2]Mathew (1995), [3]Acharji & Kripalani (1951), [4]Lalremsanga et al. (2011), [5]Ao et al. (2004), [6]Majumder et al. (2012), [7]Sinha et al. (2020)	LC
106	*Indotyphlops braminus* (Daudin, 1803)	Brahminy Blind Snake	[1]AS, [1]AR, [2]ML, [1]MZ, [3]SK, [4]TR	[1]Pawar & Birand (2001), [2]Mathew (1995), [3]Jha & Thapa (2002), [4]Purkayastha et al. (2020b)	LC
107	*Indotyphlops jerdoni* (Boulenger, 1890)	Jerdon's Worm Snake	[1,4]AR, [2,4]ML, [3]MZ, [4]NL, [5]SK	[1]Sanyal & Gayen (2006), [2]Mathew (1995), [3]Lalremsanga et al. (2011), [4]Ao et al. (2005) [5]Jha & Thapa (2002)	LC
108	*Indotyphlops tenuicollis* (Peters, 1864)	Samagutin Worm Snake	[1]ML, [2]NL	[1]Mathew (1995), [2]Smith (1943)	DD
	Family: Viperidae				
109	*Daboia russelii* (Shaw & Nodder, 1797)	Russell's Viper	[1]AS, [2]MN, [3]SK	[1]Nath et al. (2019), [2]Acharji & Kripalani (1951), [3]Jha & Thapa (2002)	LC
110	*Ovophis monticola* (Günther, 1864)	Mountain Pit Viper	[1]AS, [1]AR, [1]ML, [1]MN, [2]MZ, [1]NL, [1]SK	[1]Whitaker & Captain (2004) [2]Lalremsanga et al. (2011),	LC
111	*Ovophis zayuensis* (Jiang, 1977)	Zayuan Mountain Pit Viper	[1]AR	[1]Gerard et al. (2024)	LC
112	*Protobothrops himalayanus* Pan, Chettri, Yang, Jiang, Wang, Zhang & Vogel, 2013	Himalayan Pit Viper	[1]SK	[1]Pan et al. (2013)	LC
113	*Protobothrops jerdonii* (Günther, 1875)	Jerdon's Pit Viper	[1]AR, [2]ML, [3]MN, [4]NL, [5]SK	[1]Borang et al. (2005), [2]Mathew (1995) [3]Elangbam et al. (2023), [4]Ao et al. (2004) [5]Jha & Thapa (2002)	LC

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114	*Protobothrops kaulbacki* (Smith, 1940)	Kaulback's Lance-headed Pit Viper	[1]AR	[1]Uetz et al. (2023)	DD
115	*Protobothrops mucrosquamatus* (Cantor, 1839)	Brown Spotted Pit Viper	[1]AS, [2,3]AR, [3]MZ, [3]NL	[1]Dutta et al. (2024), [2]Borang et al. (2005), [3]Ao et al. (2004)	LC
116	*Trimeresurus arunachalensis* Captain, Deepak, Pandit, Bhatt & Athreya, 2019	Arunachal Pit Viper	[1]AR	[1]Captain et al. (2019)	DD
117	*Trimeresurus erythrurus* (Cantor, 1839)	Red-tailed Pit Viper	[1]AR, [2]ML, [3]MN, [4]MZ, [5]NL, [5,6]SK, [7]TR	[1]Borang et al. (2005), [2]Mathew (1995) [3]Elangbam et al. (2023), [4]Lalremsanga et al. (2011), [5]Whitaker & Captain (2004) [6]Jha & Thapa (2002), [7]Purkayastha et al. (2020b)	LC
118	*Trimeresurus mayaae* Rathee, Purkayastha, Lalremsanga, Dalal, Biakzuala, Muansanga & Mirza, 2022	Maya's Pit Viper	[1]MN, [2]MZ	[1]Elangbam et al. (2023), [2]Rathee et al. (2022)	NA
119	*Trimeresurus medoensis* Zhao, 1977	Green Bamboo Leaf Pit Viper	[1]AR	[1]David et al. (2001)	DD
120	*Trimeresurus popeiorum* Smith, 1937	Pope's Green Pit Viper	[1]AR, [2]ML, [3]MZ, [4]SK	[1]Borang et al. (2005), [2]Mathew (1995), [3]Lalremsanga et al. (2011), [4]Jha & Thapa (2002)	LC
121	*Trimeresurus salazar* Mirza, Bhosale, Phansalkar, Sawant, Gowande & Patel, 2020	Salazar's Pit Viper	[1, 4]AS, [1, 4]AR, [2, 4]ML, [3]MN, [4]MZ, [4]NL, [4]SK, [5]TR	[1]Mirza et al. (2020), [2]Rathee et al. (2021), [3]Elangbam et al. (2023) [4]Vogel et al. (2022), [5]Chowdhury et al. (2024)	NA
122	*Trimeresurus uetzi* Vogel, Nguyen & David, 2023	Uetz's Pit Viper	[1]MZ	[1]Biakzuala et al. (2024)	NA
	Family: Xenodermidae				
123	*Blythia hmuifang* Vogel, Lalremsanga & Vanlalhrima, 2017	Mizoram Ground Snake	[1]MZ	[1]Vogel et al. (2017)	DD
124	*Blythia reticulata* (Blyth, 1854)	Blyth's Reticulated Snake	[1]AS, [2]AR, [3]ML, [1]MN, [4]MZ, [5]NL	[1]Smith (1943), [2]Borang et al. (2005), [3]Mathew (1995), [4]Lalremsanga et al. (2011), [5]Ao et al. (2004)	DD
125	*Stoliczkia khasiensis* Jerdon, 1870	Khasi Earth Snake	[1,2]ML	[1]Smith (1943), [2]Mathew (1995)	DD
126	*Stoliczkia vanhnuailianai* Lalronunga, Lalhmangaiha, Zosangliana, Lalhmingliani, Gower, Das & Deepak, 2021	Lushai Hills Dragon Snake	[1]MZ	[1]Lalronunga et al. (2021b)	NA

The numbers 1, 2, 3... in the 'Reference' column correspond with the numbers assigned to the states listed in the 'Distribution in northeastern states' column, confirming the presence of the species in those states.
AR—Arunachal Pradesh | AS—Assam | DD—Data Deficient | LC—Least Concern | ML—Meghalaya | MN—Manipur | MZ—Mizoram | NA—Not Assessed | NL—Nagaland | NT—Near Threatened | SK—Sikkim | TR—Tripura | VU—Vulnerable.

undertaken the task of systematically documenting the diversity of snakes within northeastern states or specific localities, contributing to the broader understanding of regional herpetofauna. These are summarised state-wise as follows:

In Assam, Das et al. (2007) documented the incidence of road mortality among reptiles and recorded the fatalities of 26 snake species along a highway in close proximity to Kaziranga National Park. Das et al. (2009) reported 45 species of reptiles from the Barail Wildlife Sanctuary and the surrounding Cachar District of Assam, which included 23 snake species representing five families and 19 genera and the study also included the documentation of four individuals of the genus *Rhabdophis* whose species identity remained uncertain. Purkayastha et al. (2011) reported 59 species of reptiles and amphibians from the urban city of Guwahati, which included 23 species of snakes representing five families. Islam & Saikia (2014) recorded 35 snake species in the Jeypore Reserved Forest of Assam and documented the road mortality of 17 of those species. Sengupta et al. (2016) reported 36 species of reptiles including 19 species of snakes from the Deepor Beel wetland of Assam. Baishya & Das (2018) reported the presence of

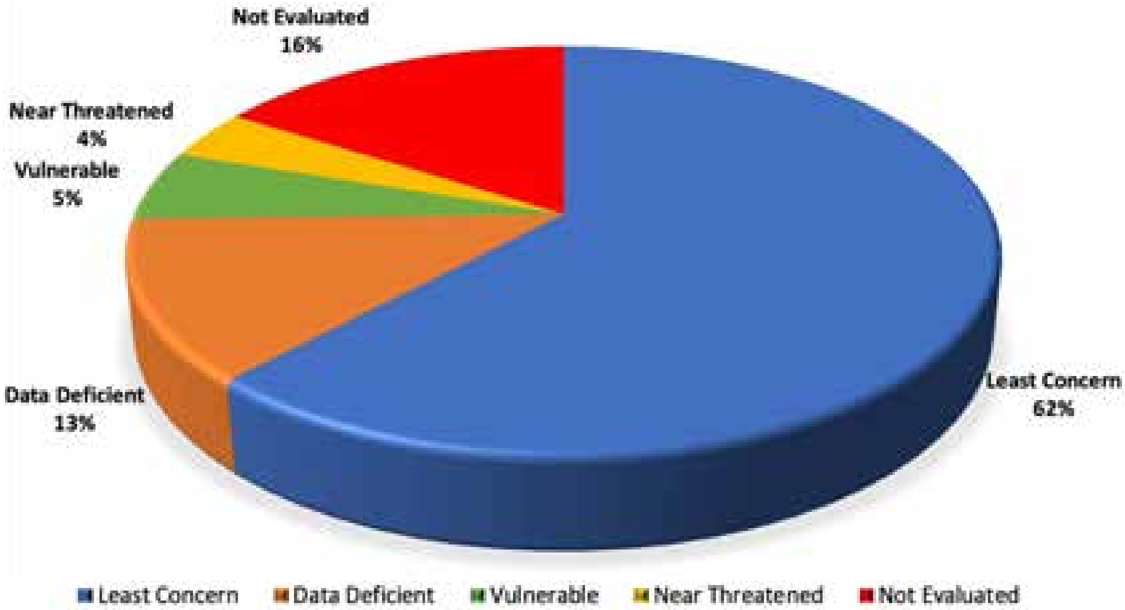

Figure 1. Distribution of snake species of northeastern India across various IUCN Red List categories.

15 snake species belonging to five families in the Nalbari district. Purkayastha (2018) presented the terrestrial vertebrate diversity of Guwahati wherein he reported 29 species of snakes from the city. Sengupta et al. (2019) reported 17 species of snakes from the Dibrugarh and Tinsukia districts of Assam. Purkayastha et al. (2020a) reported 41 species of reptiles comprising 23 species of snakes belonging to five families from Amchang Wildlife Sanctuary, Assam and Mahananda et al. (2023) presented a checklist of the terrestrial vertebrate diversity of the Garbhanga landscape wherein they reported 39 species of snakes.

In Mizoram, Laltanpuia et al. (2008) reported 24 species of snakes from the Mizoram University Campus of Tanhril, Aizawl. Harit (2010) reported 16 species of non-venomous snakes from the Champhai district of the state. Lalremsanga et al. (2011) reported 49 species of snakes representing five families, along with a note on the effects of environmental factors in the distribution of snakes throughout the state. Lalremsanga et al. (2014) reported 19 snake species from the Tamdil National Wetland of Mizoram, including the presence of *Herpetoreas xenura* which is endemic to the Indo-Burma biodiversity hotspot. Lalremsanga & Lalronunga (2017) documented 52 species of snakes from the state. Hmar et al. (2020) reported 28 species of snakes belonging to 20 genera representing seven families from the Reiek Community Reserved Forest in the Mamit district of Mizoram. Also, Malsawmdawngliana et al. (2022a) reported 23 snake species, while Gouda et al. (2023) reported 20 snake species from the Dampa Tiger Reserve of Mizoram.

In Meghalaya, Chandramouli et al. (2021) documented a comprehensive inventory of herpetofauna, identifying 75 species. The study claimed to report 29 amphibian species, 17 lizard species, and 29 snake species. However, upon our meticulous analysis of their paper, we observed a slight discrepancy, noting 18 species of lizards and 28 species of snakes.

In Arunachal Pradesh, Biswas et al. (2005) conducted a comprehensive examination of the vertebrate fauna within the D'Ering Wildlife Sanctuary, wherein they identified and documented nine distinct species of reptiles, including both venomous and non-venomous snakes. Borang et al. (2005) provided a checklist for the ophidian fauna of the state, which included 67 species representing five families and 31 genera. Sanyal & Gayen (2006) reported 78 species of reptiles from the state, which included 55 species of snakes. Agarwal et al. (2010) reported 23 species of snakes representing 16 genera and belonging to four families from Eaglenest Wildlife Sanctuary, and Sinha et al. (2021) provided a checklist of the herpetofauna diversity of the Zoological Survey of India campus of Itanagar wherein they reported the occurrence of 20 species of snakes representing five families.

In Tripura, Majumder et al. (2012) reported 55 species of reptiles and amphibians from the state, which included 18 species of snakes. Giri et al. (2017) reported 28 species of reptiles, including 12 species of snakes, from nine biodiversity hotspots, and Purkayastha et al. (2020b) reported 33 species of reptiles, including 19 species of snakes from Rowa Wildlife Sanctuary, Tripura.

In Nagaland, Ao et al. (2004) reported 41 species of snakes with 19 new records from the state. Later, Bhupathy et al. (2013) reported 31 species of snakes with eight new records from the state, and Yanthungbeni et al. (2018) reported five species of snakes from Dimapur.

In Sikkim, Jha & Thapa (2002) published the book "Reptiles & Amphibians of Sikkim" wherein they reported 59 species of snakes belonging to five families. Later, Chettri et al. (2011) conducted surveys in Teesta Valley and Maenam Wildlife Sanctuary and reported 42 species of reptiles including 28 species of snakes. Additionally, they also presented a checklist of 50 amphibian and 88 reptilian species occurring in the region.

In Manipur, Mathew (2005) mentioned the deposition of nine species of reptiles from the state at the Eastern regional station, Zoological Survey of India, Shillong. This collection represented only two species of snakes, *Coelognathus radiatus* and *Fowlea piscator*.

Range Extension and Distribution Records

In the 21st century, several studies reported the discovery of different species of snakes from parts of northeastern India where they were previously not known to exist and this extended the distributional range for those species.

In Assam, Sutradhar & Nath (2013) reported the first record of *Oligodon kheriensis* near Kachugaon Reserved Forest, Kokrajhar district. Nath et al. (2019) reported the first confirmed record of *Daboia russelii* from the state. Nath et al. (2021a) reported new distribution records for *Oligodon kheriensis* from the Bongaigaon, Chirang, Baksa, and Kokrajhar districts of Assam. Dey et al. (2022) reported the first confirmed record of *Oligodon dorsalis* from Assam and Sarkar et al. (2022) presented the distribution records of three cat snake species of the genus *Boiga*, from Assam. Also recently, Dutta et al. (2024) reported the first record of *Protobothrops mucrosquamatus* from Assam based on a live specimen observed in the Karbi-Anlong district of the state.

In Meghalaya, Gayen (2001) reported the first record of *Hebius venningi* from the state based on a specimen collected from the Jaintia Hills district. However, on further analysis, Mathew & Meetei (2004) identified the specimen from Jaintia Hills as *Herpetoreas xenura*. Also, Rathee et al. (2021) reported the occurrence of *Trimeresurus salazar* in the state along with an additional note on its hemipenis.

In Mizoram, Lalremsanga et al. (2011) provided 14 new records of snake species. Lalremsanga et al. (2015) reported four new records for the state. Harit (2016) extended the distribution of *Oreocryptophis porphyraceus* to Mizoram. Lalbiakzuala & Lalremsanga (2017) reported the first record of *Lycodon fasciatus* for Mizoram state. Lalremsanga et al. (2018) reported the first-ever record of *Gongylosoma scriptum* in India from Mizoram. Khan et al. (2019) provided the first-ever record of *Euprepiophis mandarinus* from Mizoram. Lalbiakzuala & Lalremsanga (2019) reported for the first time, the occurrence of *Hebius venningi* in Mizoram. Additionally, Biakzuala et al. (2020a) reported new distributional records for *Lycodon zawi* from all eight districts of Mizoram. Biakzuala et al. (2020b) extended the distribution of *Lycodon septentrionalis* to 10 new localities in Mizoram. Biakzuala et al. (2020c) updated new distributional records for *Blythia reticulata* in Mizoram and Biakzuala et al. (2021) reported new distributional records for *Bungarus lividus* and *Bungarus niger*. Also, Lalbiakzuala et al. (2021) contributed new distributional records of *Amphiesma stolatum* in the state. Later, Lalronunga et al. (2021a) reported new locality records of *Smithophis atemporalis* in Mizoram which extended the distributional range of the species. Lalremsanga et al. (2022) provided three new distributional records for *Gongylosoma scriptum* from Mizoram. Ruatpuii et al. (2022) reported new distributional records for *Smithophis atemporalis* and *Smithophis bicolor* from Mizoram. Malsawmdawngliana et al. (2022a) reported new distributional records for 16 species of snakes from the Dampa Tiger Reserve of Mizoram. Biakzuala et al. (2022) evaluated the genus *Dendrelaphis* in Mizoram and provided new distributional records for *D. proarchos* and the first record of *D. biloreatus* from the state. Lalrinsanga et al. (2022) contributed to the morphology, molecular phylogenetics, and new localities of *Gonyosoma prasina* from the state. Malsawmdawngliana et al. (2022b) reported new distributional records of *Stoliczkia vanhnuailianai*, which extended the distributional range of the species, and Bal et al. (2023) reported a new locality record of *Hebius venningi* in Murlen National Park of Mizoram. Recently, Biakzuala et al. (2024) reported the first-ever record of *Trimeresurus uetzi* for India, from the state of Mizoram, and Lalremsanga et al. (2024) confirmed the presence of *Malayopython reticulatus* in the state through molecular identification.

In Arunachal Pradesh, David et al. (2001) reported *Trimeresurus medoensis* from the Changlang District of the state. Zambre et al. (2009) reported the occurrence of one of the three subspecies of *Protobothrops jerdonii* from the Eaglenest Wildlife Sanctuary which was the first record for India. Sheth & Zambre (2012) reported records of *Boiga gokool* for the first time from the western Kameng district of Arunachal Pradesh. Nath et al. (2021b) reported the first record of *Elaphe taeniura* from Itanagar Wildlife Sanctuary based on a road-killed specimen, and Nath et al. (2023) reported the first record of *Trachischium monticola* in the Lower Subansiri district of Arunachal Pradesh.

In Tripura, Nath et al. (2021c) reported the occurrence of *Boiga gokool* in the state for the first time based on a live specimen observed in the Khowai district. Deb et al. (2023) reported the first record of *Ferania sieboldii* from Udaipur town of Tripura. Also, recently, Chowdhury et al. (2024) and Deb et al. (2024a,b) provided new state and district records for *Trimeresurus salazar*, *Sinomicrurus gorei*, and *Pareas monticola* from Tripura.

In Manipur, Sinate et al. (2021) reported the first record of *Bungarus niger* from Moulbem village, Churachandpur district. Subsequently, Sinate et al. (2022) reported the first record of *Boiga quincunciata* from Rovakot village, Churachandpur district. Elangbam et al. (2022) reported the first record of *Lycodon jara* from Khoijuman Khullen village, Bishnupur district. Elangbam et al. (2023) reported the first record of *Trimeresurus mayaae*, *T. salazar*, *T. erythrurus*, and *Protobothrops jerdonii* from Manipur, which extended the geographical range of these species and Hakim (2023) reported the first record of Chin Hills Keelback *Hebius venningi*, from Manipur based on a sighting of the species in the Senapati district. Additionally, Premjit et al. (2024) confirmed the occurrence of *Boiga cyanea* in Manipur, leaving Tripura as the only state where this species is yet to be recorded.

Additionally, Ahmed & Das (2006) for the first time reported the occurrence of *Rhabdophis nuchalis* in India from the Nagaland state. Dutta et al. (2013) presented new locality records for *Lycodon zawi* from Mizoram, Meghalaya, and Assam, extending the species' distributional range. Kundu et al. (2021) conducted molecular identity assessments and provided an updated distribution of *Psammodynastes pulverulentus* in northeastern India, and Vogel et al. (2022) extended the distribution of *Trimeresurus salazar* in northeastern India to Mizoram, Nagaland, and Sikkim. Also recently, Gerard et al. (2024) documented the first record of two venomous snake species from India: *Bungarus suzhenae* from the Nagaland-Manipur border and *Ovophis zayuensis* from Arunachal Pradesh.

New Genus and New Species

Over time, northeastern India has consistently played a significant role in enriching the global biodiversity by introducing numerous new species of snakes. This is evident from the fact that in the last decade, one new genus and 16 new snake species have been described from the region.

In Assam, Das et al. (2021) described a new natricine species, *Rhabdophis bindi,* which, until its discovery, was included within the *R. himalayanus* complex. The description of the species was based on specimens collected from the Cachar district of the state, and the distinctiveness of the species from its congeners was validated by morphological and molecular evidence.

In Mizoram, Vogel et al. (2017) described *Blythia hmuifang* with specimens recorded in Aizawl and Serchhip districts using morphological differences with its congeners. Giri et al. (2019) proposed a new genus, *Smithophis*, and added a new species, *S. atemporalis*, to the genus using morphological and molecular data. Lalronunga et al. (2021b) described the new species *Stoliczkia vanhnuailianai* belonging to the Xenodermidae family from the Aizawl district, which was distinguished from its congener, *Stoliczkia khasiensis* and from *Paraxenodermus borneensis* using distinct morphological features. Rathee et al. (2022) described a new green pit viper species, *Trimeresurus mayaae* and Lalremsanga et al. (2022) described the new species *Herpetoreas murlen*, both from the Champhai district of Mizoram. Also recently, Mirza et al. (2024a) described *Smithophis mizoramensis*, the fifth species within the genus with specimens collected from Mizoram.

In Arunachal Pradesh, Bhosale et al. (2019) described the new species *Trachischium apteii* based on specimens collected from Pange camp, Talle Valley Wildlife Sanctuary. Captain et al. (2019) described the new species *Trimeresurus arunachalensis* based on a single specimen collected from Ramda, West Kameng district. Purkayastha et al. (2019) described the new species *Hebius lacrima* with a single specimen from Arunachal Pradesh. Bhosale et al. (2020) described the new species *Pareas kaduri* belonging to the Pareidae family. Das et al. (2020a) described the new natricine species *Smithophis arunachalensis*, restricting its presence to the Lower Dibang Valley and Changlang district. Later, Mirza et al. (2020) described the new pit viper species *Trimeresurus salazar* from Arunachal Pradesh.

Also, Pan et al. (2013) described a new pit viper species *Protobothrops himalayanus* based on specimens collected from southern Tibet, China, and Sikkim, and Slowinski et al. (2001) described a new wolf snake species *Lycodon zawi* from Myanmar and northeastern India (Mizoram, Assam, and Meghalaya). Additionally, Mirza et al. (2024b) described a new species of vine snake, *Ahaetulla longirostris* from India with specimens collected from Bihar, Meghalaya, and Assam. This discovery invalidates the occurrence of *A. laudankia* in Assam reported by Purkayastha et al. (2021).

Rediscovery and Redescription

Field surveys by different workers have led to the rediscovery of some species believed to be extinct or not seen for a long time. Also, with the advancement in genetic science and with detailed observations, researchers could reexamine and redescribe some previously described species.

In Assam, Das et al. (2022) reported the rediscovery of *Oligodon melaneus* based on a male specimen collected from the Baksa district 112 years after its original description.

In Mizoram, Giri et al. (2019) transferred the species *Rhapdophis bicolor* to the genus *Smithophis*. Biakzuala et al. (2020b) claimed the rediscovery of *Lycodon septentrionalis* after 60 years of its first collection from Mizoram. They confirmed the occurrence of the species in five different districts of the state. Lalbiakzuala & Lalremsanga (2020) rediscovered *Oligodon catenatus* after 165 years and briefly described a specimen collected from Tamdil wetland, Mizoram. Also, Lalronunga et al. (2020) redescribed the species *Hebius xenura* and transferred it to the genus *Herpetoreas*.

In Arunachal Pradesh, David et al. (2001) reported the occurrence of *Trimeresurus medoensis* in the Changlang district of the state. They also redescribed the type specimens along with an elaborated note that included information on distribution, diagnosis, variation, hemipenis, similar species, sexual dimorphism, etc., of the species. Mistry et al. (2007) redescribed the species *Lycodon gammiei* and provided evidence of it being a valid species. Das et al. (2020b) reported the rediscovery of the species *Hebius pealii* and used phylogenetic results as evidence to transfer it to the genus *Herpetoreas*.

Taxonomic Revision

Many snake species of northeastern India lie in complex groups that are hard to resolve due to their indistinguishable morphology. Various studies have tried to resolve these complexes with the help of advanced molecular techniques and morphological data. Vogel et al. (2011) evaluated the *Dendrelaphis pictus* population of India and resurrected the taxon *Dendrelaphis proarchos* for the northwestern (Indochina) form. Vogel et al. (2020) assessed the *Pareas margaritophorus-macularius* complex and based on the morphological and genetic data, resurrected the name *Pareas andersonii* for the Indian (Mizoram & Nagaland), Myanmar and China populations as well as also resurrected the taxon *Pareas modestus* for the Indian (Mizoram) and southern Myanmar populations and elevated *P. macularius* to species level. Furthermore, Srikanthan et al. (2022) evaluated the *Ahaetulla prasina* population of northeastern India with which they resurrected the taxon *A. flavescens* and stated that the population of *A. prasina* complex found in northeastern India, in fact, represents *A. flavescens*. Biakzuala et al. (2022) evaluated the genus *Dendrelaphis* in Mizoram using morphological and molecular data. Based on this analysis, they validated the resurrection of *Dendrelaphis proarchos* and proposed that the specimen of *D. pictus* from Hainan, China be transferred to *D. proarchos*. Biakzuala et al. (2023a) evaluated the population of *Bungarus fasciatus* in Asia and suggested that there must be at least three different clades of *B. fasciatus* in Asia and that the distribution of true *B. fasciatus* is limited to Indo-Myanmar zone. Mirza et al. (2023) evaluated the Asian pit vipers (genus *Trimeresurus*) along with the revision of *Trimeresurus popeiorum* and restricted the distribution of the species to northeastern India, southern China, Bangladesh, and northern Myanmar. Additionally, the authors synonymized *T. yingjiangensis with T. popeiorum* based on shared morphological, genetic, and distributional data between the two species. Recently, Köhler et al. (2023) conducted molecular analysis and based on the low genetic divergence, placed *Boiga ochracea* in the synonymy of *Boiga multomaculata,* thereby identified two subspecies of *B. multomaculata* namely *B. multomaculata multomaculata* and *B. multomaculata ochracea* and described another new subspecies, *B. multomaculata septentrionalis*. Further, based on significant differences in morphology and genetic data, the authors resurrected the taxon *B. stoliczkae* elevating it to the species level and confined its distribution in India to Sikkim, Arunachal Pradesh, and Assam. Also recently, Biakzuala et al. (2023b) resurrected and elevated *Sinomicrurus gorei* to the species level restricting its distribution to eastern Assam, Manipur, Mizoram, and Nagaland in India. Additionally, Nguyen & Vogel (2024) revised the distribution of *Trimerodytes yunnanensis* and clarified that the specimen previously identified as *T. percarinatus* from northeastern India is, in fact, *T. yunnanensis*. Thus, this revision removes *T. percarinatus* from India's snake fauna list.

DISCUSSION

In the last two decades, comprehensive research efforts have been directed toward understanding the snake fauna of northeastern India. Several studies from different states and regions within the state have successfully reported species that were not recorded earlier, thereby extending the species' geographical range. Other studies have utilized morphological and molecular data and described 16 new species and one new genus from northeastern India within a span of 10 years (2013–2024) highlighting the potential of the region.

Moreover, studies conducted in some of the northeastern states have led to the rediscovery of four species believed to be extinct or undocumented for several decades. These species include *Oligodon melaneus* from Assam, *Herpetoreas pealii* from Arunachal Pradesh, and *Lycodon septentrionalis* and *Oligodon catenatus* from Mizoram. Additionally, studies using molecular data have also reexamined and redescribed four species from northeastern India, namely *Herpetoreas xenura*, *Smithophis bicolor*, *Trimeresurus medoensis*, and *Lycodon gammiei*.

Various workers have also contributed by carrying out taxonomic revisions of different complex species groups found in northeastern India, resulting in the discovery of cryptic species and the resurrection of several taxa, thus enhancing our knowledge of the snake fauna of the region.

From our overall analysis of existing literature, we estimate that northeastern India is home to 126 species of snakes, representing 46 genera and 12 families. According to the IUCN Red List, 78 of these species are of 'Least Concern', 16 species are 'Data Deficient', seven species are 'Vulnerable', five species are 'Near Threatened', and 20 species are yet to be evaluated. Additionally, certain genera, such as *Cyclophiops*, *Gongylosoma*, *Herpetoreas*, *Hebius*, *Liopeltis*, *Traschischium*, *Trimerodytes,* and *Stoliczkia*, have received minimal attention and require further investigation. Also, there exists a discernible gap in studies conducted in states such as Manipur, Meghalaya, and Tripura. Despite being home to diverse ecosystems and potential habitats for various snake species, these states have received limited attention in terms of systematic studies. The dearth of comprehensive research in these states poses a considerable challenge in accurately assessing their snake diversity and understanding the ecological dynamics within their respective habitats.

Northeastern India, renowned for its distinctive and captivating geographical features is predominantly characterized by enchanting hilly terrain. It is well-known for being home to 160 scheduled tribes and over 400 other tribal and sub-tribal communities and groups (D'Souza 2018). Every part of the region, including Cherrapunji, with the highest annual rainfall at 11,465 mm, receives over 1,000 mm of rainfall (Dikshit & Dikshit 2014). The combination of these factors collectively impacts the accessibility to a significant part of the region, thereby imposing limitations on extensive fieldwork and comprehensive studies.

Furthermore, the stringent wildlife laws to safeguard the region's diverse flora and fauna pose challenges in designing and conducting studies, as specimen collection for bona fide reasons is a cornerstone of taxonomic studies. Nevertheless, establishing rules and initiatives to better streamline the permit acquisition process and fund allocations for conservation-oriented academic research and conservation projects would act in the interest of inventorying, monitoring, and conserving biodiversity, and hence, prove immensely advantageous to the region and science as a whole. This would facilitate ophidian studies in the region and enhance our understanding of the subject, to identify and resolve the current priorities of snake conservation in northeastern India.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26150–26162
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.9110.16.11.26150-26162
#9110 | Received 19 April 2024 | Final received 27 August 2024 | Finally accepted 30 October 2024

OPEN ACCESS

VIEWPOINT

Decades of IUCN recommendations for biocontrol of invasive pest on the Guam cycad: you can lead policy-makers to conservation proposals but you cannot make them follow

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Abstract: Guam's cycad known as *Cycas micronesica* has been threatened by a coalition of invasive herbivore species, and the armored scale *Aulacaspis yasumatsui* has emerged as the primary threat. This lethal cycad pest invaded Guam in 2003, and the Species Survival Council of the International Union for Conservation of Nature (IUCN) began publishing recommendations addressing protection of the cycad population in 2005. Sustained epidemic mortality caused the addition of *C. micronesica* to the United States Endangered Species Act in 2015. The need to establish a sustainable coalition of biological control organisms has been the constant advice throughout almost two decades of recommendations, yet the decision-makers who controlled the direction of policy and funding have not responded to the advice with success. Therefore, we describe the history of publications in which the IUCN has asserted that this singular conservation action is urgently required to save the cycad species. We then summarize contemporary recommendations to address the ongoing threats to this and other insular cycad species.

Keywords: *Aulacaspis yasumatsui*, biological control, conservation science, *Cycas micronesica*, *Rhyzobius lophanthae.*

Abbreviations: CAS—Aulacaspis yasumatsui, Cycad Aulacaspis Scale | CSG—Cycad Specialist Group, Species Survival Council, IUCN | ESA—United States Endangered Species Act | GBF—The Kunming-Montreal Global Biodiversity Framework | IUCN—International Union for Conservation of Nature | USFWS—United State Fish & Wildlife Service.

Editor: Mike Maunder, Biodiversity, Restoration and Conservation, Crediton, United Kingdom. **Date of publication:** 26 November 2024 (online & print)

Citation: Marler, T.E., A.J. Lindström, L.I. Terry & B.E. Deloso (2024). Decades of IUCN recommendations for biocontrol of invasive pest on the Guam cycad: you can lead policy-makers to conservation proposals but you cannot make them follow. *Journal of Threatened Taxa* 16(11): 26150-26162. https://doi.org/10.11609/jott.9110.16.11.26150-26162

Funding: None.

Competing interests: The authors declare no competing interests.

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Acknowledgements: We thank John Donaldson and Patrick Griffith for able administration of the Cycad Specialist Group, and all contributing members who have devoted their time and knowledge toward the advancement of cycad conservation.

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INTRODUCTION

The cycad *Cycas micronesica* K.D.Hill grows among numerous disjunct ecological niches in the Mariana Islands, the Federated States of Micronesia, and the Republic of Palau (Hill 1994, Figure 1). The arborescent cycad species exhibits morphological traits that are typical of cycads, with large pinnately compound leaves radiating from the stem apex (Image 1). The species was the most abundant tree on Guam in 2002 when an estimated 1.57 million healthy mature trees existed (Donnegan et al. 2004). At that time, there were no identifiable threats throughout its indigenous range.

The absence of threats changed in 2003–2005 when Guam was invaded by the armored scale *Aulacaspis yasumatsui* Takagi (Cycad Aulacaspis Scale, CAS), the leaf miner *Erechthias* sp., and the *Cycas*-specific butterfly *Luthrodes pandava* Horsfield (Deloso et al. 2020). These specialist herbivorous insects arrived in Guam without their natural predators, finding an abundant population of hosts that evolved in the absence of native leaf herbivores. The rapid decreases in health of the attacked cycad trees generated unprecedented infestations by the native longhorn beetle *Acalolepta marianarum* Aurivillius, which employs the common stem borer behavior of preferentially attacking unhealthy trees (Marler 2013).

Plant mortality in the urban landscape was immediate, and plant mortality among in situ habitats began in 2005 (Marler & Lawrence 2012). A 2013 forest survey revealed only 624,000 *C. micronesica* trees remained alive, and most of these were heavily infested with CAS at the time (Lazaro et al. 2020). These findings indicated 60% of the mature tree population was killed within an eight year period. *Cycas micronesica* was listed as 'Endangered' under the IUCN Red List in 2006 (Bösenberg 2022a), only three years after the invasion. Members of the Cycad Specialist Group (CSG) within the Species Survival Commission of the IUCN have provided informal and formal published recommendations concerning the threats to and recovery needs of *C. micronesica* since 2005 when the CAS population began spreading into forest habitats on Guam. The decades of publications since the beginning of the invasion carry a common theme: exploit the successes of classical introduction biological control (Hoddle et al. 2021) of CAS has always been and continues to be the most important conservation action required for this species recovery.

Most nations do not possess the financial resources to lead the way in invasion biology adaptive management research. When a wealthy nation such as the United States experiences a consequential invasion that foreshadows similar invasions in other nations, the global community looks to that wealthy nation for knowledge that evolved from their early adaptive management iterative learning process. Three recent documents highlight how misdirection of conservation activities since the 2003 Guam invasion has led to a failure to fully establish biological control of CAS. First, the United States Fish & Wildlife Service (USFWS) is required to publish a national recovery plan and five-year status reports informing taxpayers about how the Endangered Species Act (ESA) is being honored for each ESA-listed species. *Cycas micronesica* was added to the ESA in 2015 (USFWS 2015). The national recovery plan for *C. micronesica* has not been formulated to date despite published documentation of widespread ongoing mortality, and the first status report for *C. micronesica* was published in 2020 (USFWS 2020). This status report described the death of an estimated 947,556 Guam trees between 2002 and 2012 and highlighted the need for more research to reduce the impact of cycad pests. Unfortunately, the report did not list any ongoing or planned conservation actions addressing the emergency need to establish adequate biological control of CAS. Second, the United States military owns more land on the island of Guam than any other party, and the United States Sikes Act requires the publication of a multi-year Integrated Natural Resource Management Plan to steer conservation efforts. This plan guides federal resource managers with top-down directives that are used for developing funded projects. The current plan does not include any information concerning the emergency need to establish effective biological control of CAS in Guam (DON 2022). Third, a Habitat Conservation Plan for Guam is being developed by biologists in the island's territorial government agencies, and updates of the draft document are available for review (http://www.guamhcp.com). The current draft describes numerous expensive *C. micronesica* conservation actions including plant translocations and nursery operations, the need for more research on how to manage CAS, but again no plans for exploiting the heavily communicated best available science to establish an effective biological control program. Moreover, this plan includes the proposed creation of *C. micronesica* plants that are genetically resistant to CAS herbivory. While genetic resistance is a possible explanation for why some of Guam's *C. micronesica* trees are still alive today, this has not been verified to date. The current status of knowledge indicates that intraspecific or interspecific genetic

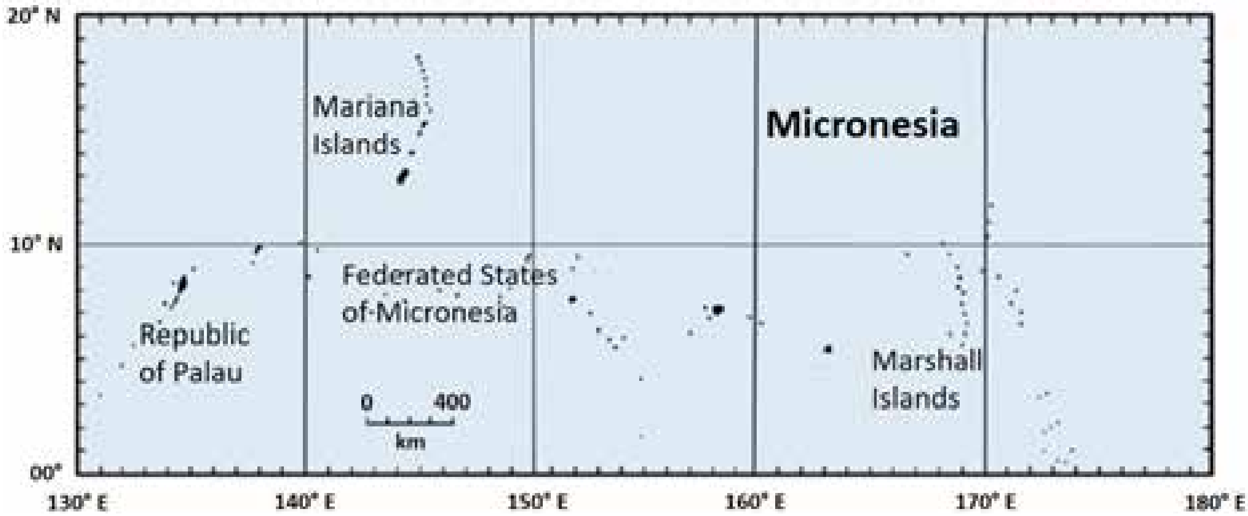

Figure 1. ***Cycas micronesica*** **is the only cycad species native to Micronesia, and exhibits an indigenous range that includes the Mariana Islands, the western limits of the Federated States of Micronesia, and the Republic of Palau.**

resistance to CAS herbivory within the *Cycas* genus has never been identified. In all countries where CAS is native, no genetic resistance among the host population has been detected. The CAS is always controlled by native predators and parasitoids in its native states. Clearly, the federal and territorial decision-makers who have been empowered to define the direction of Guam's recent and impending conservation actions have steered planning toward activities that have not honored the recommendations from the CSG since 2005.

These Guam developments have created a case study where the best available science has been ignored and evidence available to inform urgent conservation actions has been disregarded (Lindström et al. 2023). The recent invasion of Japan by CAS (Takagi 2023) has caused a repeat of the initial years following the Guam invasion, with entire crowns of leaves of the host *Cycas revoluta* Thunb. population being killed by the CAS herbivory as the first step in the process that ends in plant death (Image 2). We predict there will be sustained plant mortality that will endanger *C. revoluta* if the Japan decision-makers follow in the footsteps of the Guam decision-makers by failing to heed the IUCN's recommendation to establish immediate biological control of CAS.

Our objective herein is to plainly outline what was communicated within each of the publications that included germane recommendations from CSG members since the 2003 invasion in order to reemphasize that sustainably managing a classical biological control program of CAS remains the most important conservation endeavor needed to enable persistence of *C. micronesica*. Every citation within the chronological review contained at least one member of the CSG on the authoring team, ensuring the collective knowledge from the international experts representing the IUCN directly informed the recommendations. Thereafter, we provide contemporary recommendations for funding informative adaptive management conservation actions that acknowledge the current best management practices based on evidence from the best available science.

Chronological review of recommendations prior to ESA-listing

2005

The invasions of Taiwan and Guam by CAS generated the first two case studies in which a native *Cycas* species was threatened by non-native CAS herbivory (Tang & Cave 2016). The threats to Taiwan's *Cycas taitungensis* C.F.Shen, K.D.Hill, C.H.Tsou & C.J.Chen and Guam's *C. micronesica* led the CSG to form a new subgroup in 2005 to address the growing threat to wild cycad populations posed by the artificial spread of insect pests and pathogens affecting cycads. This new subgroup immediately published a recommendation paper in hopes of informing decision-makers in Guam and other locations (Tang et al. 2005). In addition to respecting the need for employing methods that reduce the risk of spreading CAS, the need for immediate identification of biological control organisms was discussed in detail as the most important permanent response for establishing classical biocontrol in the location of every new CAS invasion.

A commentary style article was authored by several resident biologists from Guam (Moore et al. 2005). This article detailed the initial attempts at establishing introduction biological control on Guam with the successful establishment of the predator *Rhyzobius lophanthae* Blaisdell and the unsuccessful introduction of the parasitoid *Coccobius fulvus* Compere & Annecke. The authors included the mandate that ultimate construction of effective multi-species biological control of CAS was the only conservation action that could ensure the survival of *C. micronesica*. A second commentary style article communicated the predicted demise of Guam's forests if CAS persisted without biological control into the future, the unfortunate lack of initial response by the conservation community which allowed the CAS population to become so well established by 2005, and that a multi-pronged approach rooted in biological control of CAS would be required to save the insular cycad species (Terry & Marler 2005).

2012

The Guam community operated during the first few years of conservation actions without local evidence or relevant data from other countries. Although numerous countries outside of the native range of CAS had been invaded prior to 2003 (Marler et al. 2021), no in situ *Cycas* habitats had been invaded prior to the Guam invasion. Several adaptive management projects were initiated which began to inform the conservation decisions by 2012. The first look at plant mortality from benchmarked permanent plots in northern Guam was published (Marler & Lawrence 2012), revealing 92% plant mortality within the first six years of CAS herbivory. This article pointed to the fact that in situ *Cycas* species that thrive within the native range of CAS do not experience lethal threats because of native biological control, and that ex situ *C. micronesica* plants growing in Thailand where CAS is controlled by natural enemies do not exhibit a decline in health despite experiencing CAS herbivory. The first of numerous recommendations to establish parasitoid biocontrol of CAS on Guam to augment the predator biocontrol was outlined.

Guam's urban landscape contained many *Cycas revoluta* Thunb. plants at the time of the 2003 invasion. The stem apex of this popular cycad species is covered with dense tomentum. This plant trait allowed CAS individuals to become established on cataphyll surfaces because the tomentum excluded the *R. lophanthae* predators (Marler 2012). The results verified that most *Cycas* plants contain microsites on various organ surfaces within which CAS can become established where *R. lophanthae* cannot physically navigate (Marler et al. 2021). The recommendation to introduce a smaller biological control organism such as a parasitoid species was the primary actionable recommendation from this research, as these smaller CAS enemies may be able to navigate to all CAS infestation sites.

The failures to adequately pursue biological control of CAS led to the publication of a commentary article in which the ongoing negative cycad population developments were discussed (Marler & Terry 2012). Some of the limitations of the *R. lophanthae* predator were outlined along with the emergency recommendation of establishing at least one parasitoid species to augment the established *R. lophanthae* predation.

2013

The levels of infestation of CAS, *L. pandava*, *Erechthias*, and *A. marianarum* were followed from 2005-2013 and the interplay among the four arthropod herbivores became more fully understood (Marler 2013a). Increases in CAS damage led to subsequent increases in *A. marianarum* damage and subsequent decreases in *Erechthias* damage. Alternatively, increases in CAS damage led to concurrent decreases in *L. pandava* damage. The need for a parasitoid biological control organism was reiterated, along with the prediction that future improvements in CAS control may lead to increases in *L. pandava* damage.

Experimental elevation of container-grown *C. micronesica* seedlings within in situ forest settings revealed that the predator *R. lophanthae* was more effective at controlling CAS at higher strata and less effective at lower strata (Marler et al. 2013). The findings were discussed along with the recommendation of establishing parasitoid biological control organisms which may not be constrained by the same stratification issues.

A commentary article analyzed various issues regarding stratification of *R. lophanthae* predation success (Marler 2013b). The reasons for the persistence of greater prevalence of CAS on *C. micronesica* leaves close to the soil surface were discussed in length. Accurate sampling methods are required to fully assess biocontrol efficacy, and the vertical heterogeneity in CAS incidence one decade after the Guam invasion indicated *R. lophanthae* biocontrol efficacy was clearly impaired when cycad leaves persisted close to the soil surface.

A comprehensive listing of known biological control agents was published to provide the Guam decision-

Image 1. *Cycas micronesica* is an attractive, arborescent cycad species with large pinnately compound leaves that radiate from the stem apex. © Thomas Marler.

makers the names of the organisms that could be pursued for immediate introduction to Guam (Cave et al. 2013). The need to introduce at least one parasitoid to augment the *R. lophanthae* biological control was repeated.

2014

The sustained lack of concern toward the need to biologically control CAS was addressed in another commentary style article (Marler & Lindström 2014). This opinion article proposed approaches to address stakeholder apathy or outright objection to the need for urgent conservation interventions when a native tree species is threatened with extinction. The limitations of *R. lophanthae* biological control were discussed in the context of global invasion science, whereby the Guam case study unfolded as an example that may inform conservation efforts in other invaded islands within which initial biological control efforts were unsuccessful.

Summation of recommendations prior to ESA-listing

Preemptive conservation endeavors may be highly effective for ensuring a proposed species is not ultimately added to a national endangered list such as the ESA (Treakle et al. 2023; Stanley et al. 2024). The CAS invasion that caused the ultimate ESA-listing of *C. micronesica* was predicted in 2000 (Marler 2000) and occurred in 2003 (Deloso et al. 2020; Marler et al. 2021). As outlined

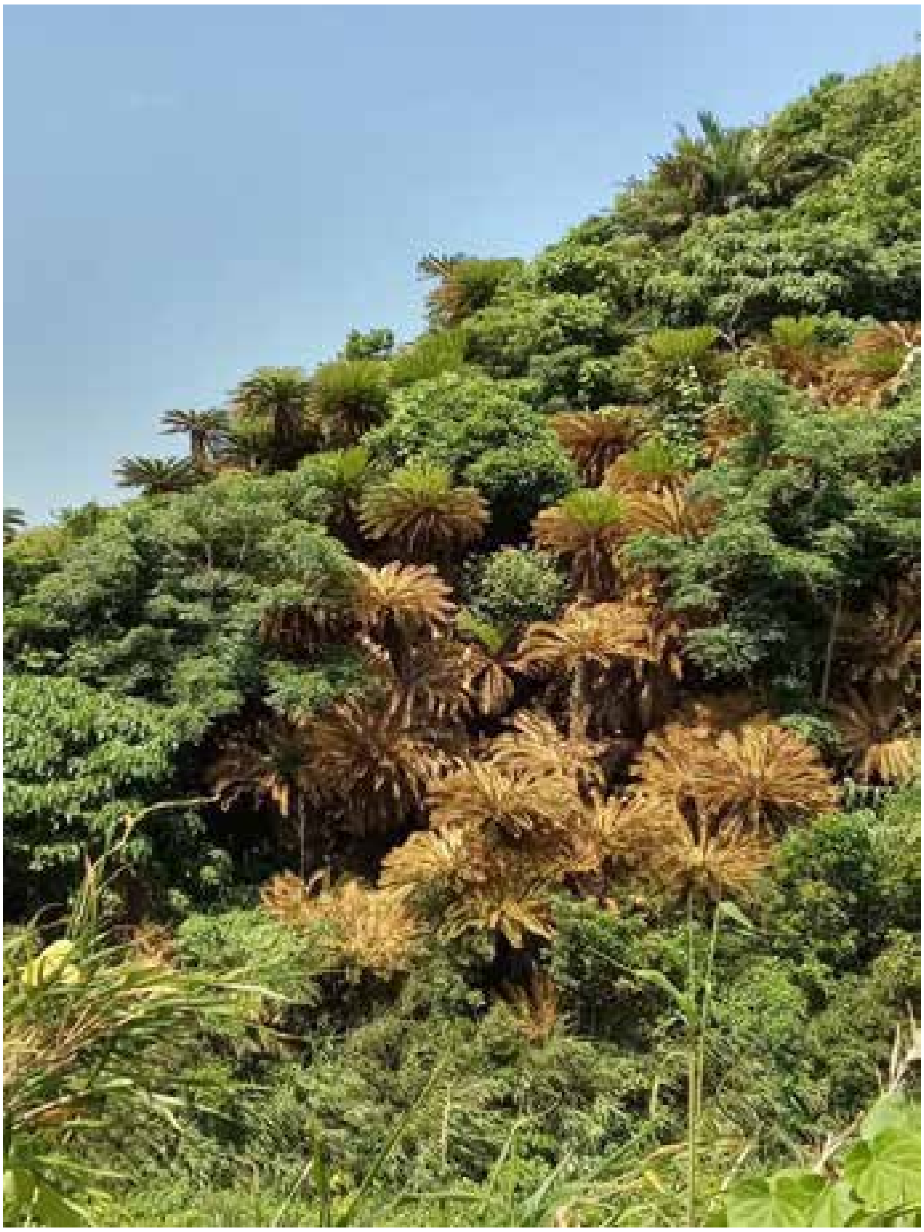

Image 2. The armored scale *Aulacaspis yasumatsui* has recently invaded natural habitats of *Cycas revoluta*. This 16 May 2024 photograph from Japan's Amami-Ôsima island reveals the rapid death of every pre-existing leaf as the first step that begins sustained damage that leads to ultimate plant death. © Thomas Marler.

above, the formal recommendations explicating the emergency conservation actions required to save *C. micronesica* from extinction risk began in 2005 and continued throughout the years prior to the ESA-listing. Moreover, the United States military was the landowner with the greatest number of *C. micronesica* plants within their custody at the time of the invasion. The deciders responsible for management decisions concerning federal lands are required by law to use evidence-based management decisions that respect the best available science. These deciders who controlled the policy and budget directions were provided a full decade of IUCN recommendations based on best available science prior to the ESA-listing. The decisions instead directed planning and considerable funding into conservation actions that did not address the recommended biological control of CAS, ensuring the addition of *C. micronesica* to the ESA.

Chronological review of recommendations after the ESA-listing

Cycas micronesica was added to the United States ESA in 2015 (USFWS 2015). Based on United States Forest

Service surveys of mature tree populations, almost 100,000 of Guam's *C. micronesica* trees died each year within one decade after the CAS invasion (Donnegan et al. 2004; Lazaro et al. 2020) and during a timeframe in which the CSG had been recommending emergency establishment of multi-species biological control of CAS (Marler & Terry 2005; Moore et al. 2005; Tang et al. 2005). These explicit biocontrol recommendations from the scientific community continued into the years following the ESA-listing.

2016

The need to provide another detailed listing of potential biological control organisms led to another publication that enumerated the available CAS predators and parasitoids along with their attributes and limitations (Tang & Cave 2016). This publication provided the Guam deciders with the latest adaptive management recommendations derived from global biocontrol research concerning which organisms carried the greatest potential for introduction to save *C. micronesica* from continued CAS-induced mortality.

2017

The first project designed to evaluate methods of salvaging mature trees from military construction sites resulted in a description of the moderate success in producing adventitious roots on large *C. micronesica* stem cuttings obtained from CAS-damaged trees (Marler & Cruz 2017). In discussing the conservation implications, the authors noted the emergency need of establishing effective biological control of CAS on Guam, and due to limited conservation funds all available public funds should not be spent on expensive salvage projects unless efficacious classical biological control is first established.

The sustained lack of concern for the need to establish biological control of CAS was addressed in another opinion style article in which the ill-informed focus on salvage of *C. micronesica* trees from construction sites was discussed (Marler & Lindström 2017). Again, recommendations to refrain from spending more conservation funding on plant translocation projects were communicated along with the assertions that redirecting those funds to expanded biological control efforts such that "…the plant mortality will cease and the species can be removed from the ESA-listing." The need to collect parasitoids within the native range of CAS was discussed along with how to maneuver through the problem that many of these parasitoids would be new to science which would require that they be described and named prior to introduction to Guam.

2018

The ongoing inability of *R. lophanthae* to adequately control Guam's CAS population led to an olfactometer study which demonstrated the preferential navigation of the predator toward mature leaves infested with CAS (Marler & Marler 2018). Guam's *C. micronesica* seedling population was rapidly killed by CAS herbivory (Marler & Lawrence 2012; Marler & Krishnapillai 2020), and the results of the olfactometer study illuminated another potential explanation for why the established predator had been ineffective in stopping the seedling mortality. The findings were discussed in the context that parasitoid biocontrol was urgently needed on Guam because parasitoids may not be constrained by the same issues that caused the predator biocontrol to be inadequate.

The results of a second study that refined methods to improve adventitious root formation on large stem cuttings were published (Marler 2018). The findings verified that reduced stem carbohydrates resulting from long-term CAS infestations were correlated with reduced asexual propagation success. Again, the recommendations included the need to refrain from expending human and budgetary resources on expensive salvage projects, as these resources should instead be spent on sustainably controlling the ubiquitous CAS infestations using classical biological control protocols.

2020

The influence of inadequate biological control of CAS on Guam was shown to reduce *C. micronesica* height increment among surviving trees (Marler et al. 2020). These data were combined with population-level mortality data to estimate that at least 70 years of demographic depth had been removed from Guam's *C. micronesica* population by 2020. Recommendations that developed from the study included the cessation of funding expensive salvage projects and that use of all available funds to "…launch biological control of the primary threats would establish the road to species recovery."

A comprehensive look at island-wide *C. micronesica* survival was published from benchmarked permanent plots (Marler & Krishnapillai 2020). The results confirmed the complete mortality of seedlings, saplings, and juveniles shortly after CAS herbivory, and 96% population mortality by 2020. The primary recommendation was to "…establish a complex integrated biological control program under the direction of scientists with appropriate international expertise" as the only conservation action that may enable recovery of *C. micronesica*.

The adaptive management literature from Guam had continued to accumulate throughout the years since 2003, and the first of several formal review articles was published as a comprehensive outline of herbivore and omnivore threats to *C. micronesica* survival (Deloso et al. 2020). Although the list of cycad consumers had grown by this time, CAS was identified as the single greatest threat to Guam's cycad population. The need for the conservation community to stop funding salvage projects and instead invest unreservedly into classical biological control of CAS was repeated.

2021

A detailed look at how the Guam CAS invasion fit into the chronology and geography of CAS invasions throughout numerous countries was published (Marler et al. 2021). Enemy release occurs when an invasive species thrives within its invaded locations as a result of the lack of native biological control by enemies found within its native range (Heger et al. 2024). The long list of CAS invasions has revealed that the lack of natural enemies allowed CAS to kill its host plants until local biologists established biological control. Recommendations indicated that a dedicated search for fortuitous biological control organisms within newly invaded locations should be combined with the purposeful introduction of predators and parasitoids from other managed biocontrol programs which could provide advice and rapid responses.

2023

The results from another asexual propagation study were published which revealed that a CAS-infested plant may be killed by the added stress of transplantation or the take of stem cuttings for adventitious root formation (Marler 2023a). The findings indicated that salvage and propagation of CAS-damaged *C. micronesica* comprise an ill-informed conservation agenda and implementing sustainable biological control of CAS as recommended in 2005 remained the most important conservation agenda.

The fact that CAS herbivory reduces non-structural carbohydrates and this response to the herbivory decreases asexual propagation success was exploited to demonstrate that a visual starch stain technique could be useful for identifying CAS-damaged host trees that would yield the best chances of propagation success during salvage programs (Marler 2023b). The discussion of relevant conservation issues reiterated that "...species recovery would ensue without the need for expensive propagation and translocation rescue projects" if conservationists would stop spending funds on salvage and nursery projects and instead direct all available funds to establishing a multi-species classical biocontrol program.

The influence of the Guam CAS invasion on *C. micronesica* female tree behavior was studied following benchmarked pre-invasion data, and revealed reproductive effort and output remained constrained two decades after the invasion (Marler & Terry 2023). The outcomes revealed that, if adequate establishment of classical biocontrol of CAS were to be achieved, species recovery may require conservation practitioners to proactively manage population-level regeneration and recruitment behaviors. The implementation of a coalition of biological control organisms to stop the CAS-induced population damage was discussed as the most important conservation agenda.

The May 2023 Typhoon Mawar imposed the strongest tropical cyclone windspeeds on Guam since the 2003 CAS invasion. A coalition of CSG members responded to this stochastic event by discussing how the tropical cyclone caused damage to the in situ *C. micronesica* population & interacted with the history of funded conservation actions (Lindström et al. 2023). The recommendations indicated that "...a dedicated multi-step procedure for establishing classical biological control" remained the most important conservation action for saving *C. micronesica*, and that a serious response to the 2005 biocontrol recommendations (Moore et al. 2005; Tang et al. 2005; Terry & Marler 2005) would have likely preemptively mitigated the CAS threat such that *C. micronesica* would have never been ESA-listed.

2024

Disparities in biotic and abiotic stressors among the Guam and Rota habitats that were invaded by CAS from 2005-2010 were exploited to reveal the *C. micronesica* population response to nascent CAS damage was remarkably homogeneous (Marler & Cruz 2024). The results indicated that all co-occurring threats can be ignored by conservationists who should focus exclusively on establishing immediate classical biocontrol of CAS to remove the primary threat to species survival.

Summation of recommendations after ESA-listing

The general tone of the recommendations within CSG publications during the years following the ESA-listing was essentially a continuation of the decade of recommendations that were published prior to the ESA-listing. Funding from the U.S. military for *C. micronesica* conservation activities within Guam's forests was initiated in 2012, a project described by Marler & Cruz (2017). The amount of funding increased dramatically following the ESA-listing, resulting in the investment of more funds for cycad conservation than in any other location worldwide. These expensive projects were designed without any of the available public funds directed toward expanding biological control of CAS. Therefore, a new theme that began to define the CSG publications was the unfortunate misdirection of the millions of dollars of federal funding toward activities that were of no consequence to the primary threat of CAS herbivory.

Parallels

This devastating pest has steadily expanded its invasive range during the antecedent three decades. When CAS invaded Taiwan, the threat to the endemic *C. taitungensis* was immediate (Marler et al. 2021). Several years of CAS population expansion were required before CAS infested the in situ *C. taitungensis* localities, and the resulting plant mortality reached 62% by 10 years after the initial invasion (Liao et al. 2018). As a result, the status of this endemic island cycad was changed from Vulnerable to Endangered in 2010 (Bösenberg 2022b). The parallels to the Guam case study were striking, as the *C. micronesica* threat status was changed from Near Threatened to Endangered in 2006 (Bösenberg 2022a).

A remote ex situ germplasm collection of Guam's *C. micronesica* genotypes was constructed on the island of Tinian beginning 2006 and consisted of ≈ 1200 healthy plants in 2018 (Brooke et al. 2024). The Implementation Plan for managing this valuable germplasm exploited the concepts of "proactive biological control" (Hoddle et al. 2018). This biological control approach differs from classical biological control in that available natural enemies are pre-selected and permitted for introduction and release prior to an anticipated invasion of a target invasive pest. Development of proactive biological control programs are analogous to purchasing insurance, since the initial lag phases of classical biological control are avoided (Hoddle 2024). The scale predator *R. lophanthae* was established on the nearby island of Rota at the time, and collection, transport to Tinian, and release in Tinian had been pre-approved in the event that CAS invaded Tinian at some time in the future. The plan mandated the cessation of all management activities until immediate introduction of *R. lophanthae* to Tinian had been successful, a process that should have required no more than 24–48 h. Unfortunately, the military biologists responsible for managing this germplasm and the practitioners contracted to protect the germplasm did not follow the mandates of the plan, allowing the nascent CAS infestation to become firmly established. The lack of concern for following through with the proactive biological control plan caused 83% mortality of the germplasm within four years of the invasion (Brooke et al. 2024).

Recent invasions persist that threaten more iconic endemic *Cycas* species. For example, the 2006 predictions that an armoured scale invasion to India would threaten the endemic *Cycas circinalis* L. (Muniappan & Viraktamath 2006) have come to pass with the 2023 invasion of the closely related *Aulacaspis madiunensis* Zehnter (Joshi et al. 2023). Similarly, Amami-Oshima Island was invaded by CAS in 2022 (Takagi 2023), and the subsequent invasions of other Ryukyu Islands and initial mortality of the endemic *C. revoluta* populations have been alarming developments (Deloso et al. 2024).

The continuing expansion of the invasive range of CAS underscores the value of the lessons learned from Guam, where a native *Cycas* species was threatened by non-native armoured scale herbivory for the first time. These lessons call for resident scientists and conservation agents in newly invaded countries to embrace the recommendations from international experts and implement immediate adaptive management endeavors addressing every facet of biological control.

Contemporary observations and recommendations from Guam

Benchmarked permanent plots throughout Guam revealed 245 stems per ha were alive in some 2015 habitats when *C. micronesica* was federally listed (Marler & Krishnapillai 2020). These same plots revealed 157 stems per ha were alive in 2020 when the five-year species recovery status report was published (USFWS 2020), indicating 36% mortality of the 2015 population occurred during these five years of federal protection. All available evidence indicated that 100% of this mortality was a direct result of herbivory by CAS and the resulting increases in damage by native stressors such as *A. marianarum* (Marler 2013a) and tropical cyclone winds (Marler et al. 2016). These native stressors were not damaging to the cycad population prior to the plant damage imposed by the CAS invasion. Yet the USFWS reviewed the first five years of ESA protection (USFWS 2020) with no mention of any biological control efforts designed to address the CAS threat. Similarly, the contemporary Integrated Natural Resource Management Plan crafted to define the ongoing conservation actions of the U.S. military (DON 2022) failed to mention any plans to expand biocontrol of CAS on Guam. This Guam case study has unfolded to inform conservationists in other regions of the world that apathy toward recommendations of international specialists concerning the need for immediate biocontrol of CAS can rapidly impose irreversible damage to in situ *Cycas* populations and the ecosystem services that they provide.

What is needed to more fully understand the current status of *C. micronesica* population survival and desired species recovery? We recommend that biologists within federal funding and permitting agencies at least minimally begin to connect with knowledgeable input from international experts. The many mistakes made in the heavily funded conservation projects on Guam could have been avoided if the funding agencies had followed this recommendation. For example, the U.S. military has spent millions of U.S. dollars on *C. micronesica* conservation in the past decade, numbers that dwarf the amount of cycad conservation funding from all other sources worldwide, yet none of these funds have been devoted to expanding the coalition of predator and parasitoid species to enhance the control of CAS on Guam. Therefore, a fundamental shift in culture of the empowered conservation decision-makers will be required to enable a respect for the need to embed adaptive management research by qualified specialists within every conservation project. As early as 2008 this Guam case study was being highlighted as an example in which the lack of rapid establishment of biological control of a new herbivorous insect invasion could cause irreversible damage to ecosystems (Messing & Watson 2008), and yet today the lack of adequate CAS biological control continues to be the greatest threat to *C. micronesica* survival.

Parasites comprise an ancient life form that remains prevalent today (Poulin 2014). Parasitism is an integral component of ecosystem function (Hatcher et al. 2012). The exploitation of highly specific parasitoids as endoparasites to control damaging herbivore arthropods has been a successful component of managed biological control programs for decades (Eggleton & Belshaw 1992). We continue to believe that the managed construction of a coalition of biological control organisms that includes parasitoids will actively suppress Guam's CAS population and passively engineer the recovery of the *C. micronesica* population. The list of biocontrol organisms that are available to introduce to Guam is extensive (Cave et al. 2013; Tang & Cave 2016; Marler et al. 2021).

Numerous attempts to introduce the parasitoids *C. fulvus* and *Aphytis lingnanensis* Compere to Guam from Florida and Hawaii were unsuccessful (Marler & Lindström 2017). The reasons for the lack of success remain elusive, but there was no parasitoid specialist included in those Guam activities. We recommend the inclusion of a parasitoid specialist to oversee a repeat of these endeavors, as both parasitoid species are readily available within the U.S.A. Dedicated trips to rear parasitoids from CAS-infested *Cycas* leaves within the CAS native range has led to collection of parasitoids that have not been described (Marler & Lindström 2017). These organisms cannot be imported to Guam until a taxonomist places a binomial on the animal, which is a prerequisite to applying for mandatory import permits. We have recommended a multi-stage program within which these parasitoids are described and named by taxonomic experts as part of the initial funding (Marler & Lindström 2017; Lindström et al. 2023), and we repeat this recommendation here. These conservation actions could have been completed between the 2003 invasion and the 2015 ESA-listing with a fraction of the funds that have been spent on *C. micronesica* salvage, transplantation, and nursery endeavors.

The fortuitous improvement in health of Guam's in situ *C. micronesica* population has been reported in the past few years (Lindström et al. 2023; Marler & Terry 2023). Some contemporary trees exhibit healthy leaves with no signs of herbivory, which is something that has not occurred since 2005. These observations point to a pivotal time period in which conservationists need to identify why reduced CAS herbivory is fortuitously occurring. Numerous geographic regions are characterized by native *Cycas* species, native CAS, and native biocontrol organisms coexisting in harmony. In these settings, the host plants are typically infested with CAS but remain unthreatened (Marler & Lindström 2017). Some *C. micronesica* trees in various Guam habitats exhibit general appearance that mimics the *Cycas* trees in these regions where native CAS is controlled by native natural enemies. These observations indicate that the likely cause of the recent decrease in CAS herbivory on Guam is a fortuitous improvement in biological control of the resident CAS population. An experienced cycad biologist would possess the wherewithal to experimentally determine if currently unidentified biocontrol of CAS has developed in recent years on Guam. The team of deciders empowered to define future conservation actions on Guam should include at least one cycad specialist who has worked within habitats containing sympatric native *Cycas*, CAS, and CAS enemies, as these biologists understand the gestalt traits of the cycad and CAS populations under these sustainably controlled conditions.

We believe the CAS invasions of Guam in 2003, Rota in 2007, and Palau in 2008 were enabled by the frequent international flights in these three airports, flights from regions that contained *Cycas* populations that were heavily infested with CAS. We also believe that the infrequent flights to the Yap airport explain why Yap remains uninvaded by CAS to date, despite a thriving *C. micronesica* population. New investments of United States national security funding into Yap will likely ensure a CAS invasion of Yap in the near future. Indeed, an estimated US$37 million is being spent to expand the Yap Island airport (Island Times 2023) and an estimated US$3.3 billion will be spent on Yap and other FSM islands over next 20 years (Island Times 2024). The resulting increases in human travel to Yap indicate that *C. micronesica* conservationists need to be on the lookout for the probable Yap invasion by CAS in the near future.

The Guam and Rota cycad populations were decimated by the CAS invasions of those islands because of apathy toward the need to rapidly establish biocontrol. Yap's conservation community has an opportunity to be ready to construct the biological control program that will be required to save Yap's *C. micronesica* population from being decimated by CAS. Similarly, the conservation communities within the recently invaded *C. circinalis* and *C. revoluta* habitats have an opportunity to construct the biological control program that will be required to save these iconic cycad species. In so doing, they can emerge as the first location where successful conservation actions were implemented in compliance with evidence-based approaches based on the best available science as communicated by the CSG.

Global Biodiversity Targets

Lessons from every conservation case study are integral for informing the global biodiversity crisis. Legal instruments that create opportunities for international cooperation are useful for addressing declines in genetic diversity, compromises in ecosystem services, and the risk for localized ecosystem collapse. The Kunming-Montreal Global Biodiversity Framework (GBF) has been developed to operationalize global biodiversity targets (Convention on Biological Diversity 2022). Single species case studies in isolated island communities do not operate in isolation from global crises, and goals of the GBF will not be possible without commitments for compliance in these isolated biodiversity cases.

The GBF's four goals and 23 targets provide guidelines to mobilize resources to maintain the Earth's biodiversity. This Guam case study falls directly in line with species and ecosystem conservation in goal A, with attention on threats that are driven by human activities. Implementation of this goal requires local conservationists to identify the factors that are known to threaten each species as the baseline for progress. As outlined in this review, the threats to *C. micronesica* are clearly understood and have been pointedly communicated in the primary literature for decades. Recovery actions in target 4 for species that require urgency necessitates the identification of the root causes of human-induced extinction. The consistent and ongoing expansion of the invasive range for CAS (Marler et al. 2021) has emerged as one of the greatest threats to cycad conservation (Tang et al. 2005), and the root causes of the threats are unambiguously understood as transport of CAS-infested plant materials and phoresis of CAS crawlers through human travel. This Guam case study falls directly under target 6 which calls for combatting the consequences of invasive species. The demise of Guam's 2005 *C. micronesica* population when CAS began killing in situ plants has been documented with 96% mortality as of 2020 (Marler & Krishnapillai (2020), and as outlined herein the causes have been a failure to implement a classical multi-species biological control program to mitigate the CAS threat.

Goals and targets are integral parts of the international solution to guide biodiversity policy reforms. Successful implementation, however, cannot occur without learning from past programs which provide successful and unsuccessful case studies. Bureaucracy and politics have been identified as institutional barriers, and staff turnover and limited use of available knowledge have been identified as organizational barriers to successful recovery of endangered species (Guerrero et al. 2024). Guam's government agency bureaucracy, inter-agency politics, lack of collaboration with international experts, violation of human rights of Guam's indigenous peoples, rapid turnover of consequential decision-makers, failures to respect the value of adaptive management, and waste of resources on inconsequential projects have been discussed as barriers to conservation of *C. micronesica* and as root causes of environmental destruction (Marler 2014, 2019; Marler & Lindström 2014, 2017; Marler & Cruz 2017, 2024; Lindström et al. 2023; Brooke et al. 2024; Deloso et al. 2024). These barriers have been successfully exploited to marginalize international experts from having a seat at Guam's decision-making table. They have also generated public condemnation by the United Nations of the violations of human rights of Guam's indigenous peoples (United Nations Commission on Human Rights 2021) and an ongoing lawsuit from the Center for Biological Diversity for systemic violations of the tenets of the ESA (Center for Biological Diversity 2023). The contributions of this case study to future cycad conservation endeavors in particular and the GBF in general will require deciders in other countries which are invaded by CAS to avoid these same barriers.

SUMMATION

The 2003 CAS invasion of Guam created a case study that held the potential to develop mitigation protocols through biocontrol adaptive management research that could inform conservation planners in other nations where subsequent CAS invasions threatened native *Cycas* species. The deciders who hold power over the planning and funding of Guam's conservation actions have not exploited this opportunity. There is an urgent need to overhaul the manner in which Guam's policy and funding deciders view the input of relevant specialists. This Guam case study informs conservationists in other regions of the world that apathy toward inputs from international specialists concerning the need for immediate biocontrol of CAS can rapidly impose irreversible damage to in situ *Cycas* populations.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26163–26166

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9328.16.11.26163-26166

#9328 | Received 25 July 2024 | Final received 09 October 2024 | Finally accepted 11 November 2024

OPEN ACCESS

SHORT COMMUNICATION

Photographic record of Kashmir Gliding Squirrel *Eoglaucomys fimbriatus* (J.E. Gray, 1837) from the Gurez Valley, Jammu & Kashmir, India

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Abstract: Around 48 species of gliding squirrels are present in the world; found mainly in tropical regions of North America, Eurasia, and southeastern Asia. The Kashmir Gliding Squirrel *Eoglaucomys fimbriatus* is a rare species of rodent that belongs to the family Sciuridae. It is a nocturnal species, resting during the daytime in tree shelters and emerging at night to feed. This paper documents the direct sighting of the Kashmir Gliding Squirrel from the Gurrai Nallah, Gurez Valley of Jammu & Kashmir.

Keywords: Biodiversity, direct sighting, Gurrai Nallah, mammal, monotypic, nocturnal, rodent, Sciuridae, temperate forest, western Himalaya.

There are around 48 species of gliding squirrels recognized in the world (Corbet & Hill 1992; Hoffmann 1993; Thorington et al. 1996). They are found mainly in tropical regions of North America, Eurasia and southeastern Asia (Hoffmann 1993; Koprowski & Nandini 2008). Gliding squirrels have long intrigued mammalogists worldwide, triggering extensive research into their ecology, biology and population dynamics (Oshida et al. 2005; Dinets 2011; Qamar et al. 2012; Wassmer & Refinetti 2016). In India, the eastern Himalaya has high diversity of gliding squirrels than the western Himalaya. Only two species of gliding squirrels have been recorded from western Himalaya, namely, the Red Giant gliding Squirrel *Petaurista petaurista* and Kashmir Gliding Squirrel *Eoglaucomys fimbriatus* (Koli 2016). The Kashmir Gliding Squirrel is a rodent species that belongs to the family Sciuridae. It is a monotypic species within the genus *Eoglaucomys* and is found in Afghanistan, Pakistan, and India (Dar 1996; Nowak 1999; Sheikh & Molur 2005). In India, the species is distributed across the northwestern Himalaya from northern Punjab to Shimla, Himachal Pradesh, and Jammu & Kashmir (Corbett & Hill 1992). Pasha & Suhail (1997) collected a specimen from Ranikhet Kumao, Uttar Pradesh which extends its range about 300 km.

Primarily confined to the Himalayan moist temperate forest, it often coexists with the Red Giant Gliding Squirrel *Petaurista petaurista* (Corbet & Hill 1992; Roberts 1977). The Kashmir Gliding Squirrel is an arboreal and nocturnal rodent, remains inactive during daytime hours and rests in tree shelters, and becoming active at night when it feeds (Sperry et al. 2013). It exhibits gliding flight, facilitated by long flaps of loose skin and nests in a variety of deciduous and conifer trees such as Pine, Abies, and Spruce (Roberts 1977). The diet mainly consists of leaves, flowers, seeds, fruits, insects, and a variety of nuts (Ahmad et al. 2023). Gliding squirrels are immensely important as seed dispersers and pollinators in forest ecosystems.

Editor: Murali Krishna Chatakonda, Amity University, Noida, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Lone, G.M., B.A. Bhat, M.S. Hussain & A.N. Lone (2024). Photographic record of Kashmir Gliding Squirrel *Eoglaucomys fimbriatus* (J.E. Gray, 1837) from the Gurez Valley, Jammu & Kashmir, India. *Journal of Threatened Taxa* 16(11): 26163–26166. https://doi.org/10.11609/jott.9328.16.11.26163-26166

Funding: The Ministry of Tribal Affairs, Govt of India for financially supporting this work by providing grants under UGC (NFST) fellowship scheme.

Competing interests: The authors declare no competing interests.

Acknowledgements: We duly acknowledge and appreciate the contributions of all the authors who participated in data collection, manuscript writing and revision, and finalization.

The Kashmir Gliding Squirrel has a scattered population across the Himalaya. Their overly dependence on old-growth forests makes them vulnerable to habitat changes such as deforestation and habitat fragmentation both of which have contributed largely to their population decline (Sheikh & Molur 2005). Besides, their fascinating agility to glide through the air also renders them attractive targets for hunting (Umapathy & Kumar 2000; Kumara & Singh 2006). The population size and distribution of the Kashmir Gliding Squirrel is not known. Although listed as 'Least Concern' (IUCN 2016), the species is rare to find and has remained understudied due to its elusive and nocturnal nature, and inhabiting remote and inaccessible areas. This emphasizes the urgent need for conservation efforts to protect this unique species.

Study Area

The valley of Gurez lies in the Great Himalayan range of Jammu & Kashmir. It is situated at an altitude of 2,400–4,300 m. The vegetation of the region varies with the altitude and is characterized by pine, fir, and cedar at lower elevations to alpine flora such as juniper and rhododendron at higher reaches. The Gurez Valley is a treasure of a diverse array of wildlife species such as Himalayan Brown Bear, Himalayan Black Bear, Himalayan Marmot, Musk Deer, Common Leopard, Himalayan Ibex, and various species of birds (Dad & Khan 2011). The valley is crucial for its biodiversity and serves as a habitat for both resident and migratory bird species.

Results and Discussion

The two individuals of the Kashmir Gliding Squirrel were observed at 20:04 h on 25 May 2024, while walking in a dense forest near the Gurrai Nallah (34.593^{0}N 74.657^{0}E) at an elevation of 2,840 m (Image 1 & 2). The individuals displayed typical behaviours of squirrels such as gliding between trees and feeding on bark and tree foliage. Two gliding incidents were reported and these were performed between two spruce trees. The average gliding height was around 9 m and the distance between the gliding trees was 5 m. The identification of *E. fimbriatus* was confirmed on the basis of its distinctive morphological features which include a large dark brown

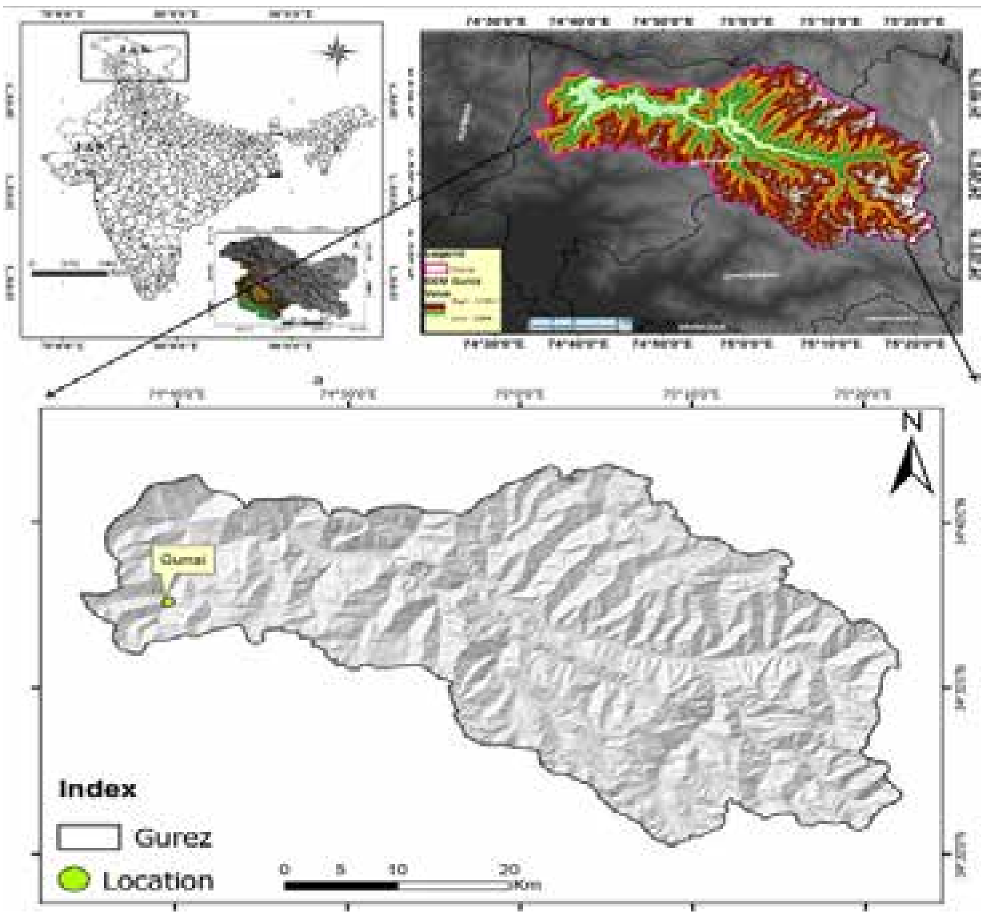

Image 1. Map of the Gurez valley showing sighting location of the Kashmir Gliding Squirrel.

Image 2. Photographic evidence of the Kashmir Gliding Squirrel in Gurrai Nallah, Gurez. © Gh Mustufa Lone.

Image 3. Habitat of the Kashmir Gliding Squirrel in Gurrai Nallah, Gurez. © Gh Mustufa Lone.

or black orbit, elongated skull, large and blunt ears, and long whiskers. The back fur is long and the belly hair is creamy in colour (Roberts 1977; Ahmad et al. 2023). The tail is slightly flat, bushy, and brown along with the black tip. The outer edge of the hindlimb has a broad clump of hairs (Jerdon 1867; Sterndale 1884; Pasha & Suhail 1997).

The habitat from where this species was recorded is a typical conifer dominated by *Abies pindrow, Picea smithiana,* and *Pinus wallichiana*, which are essential for the species' nesting and foraging activities (Image 3). The sighting of the Kashmir Gliding Squirrel from the Gurez Valley highlights that the valley's forest ecosystems provide suitable habitat conditions for the species. The presence of mature forests with ample tree cover and minimal human disturbance appears to be critical for the survival of *E. fimbriatus*. This finding emphasizes the importance of preserving these habitats from deforestation and other anthropogenic pressures to ensure species' long-term survival.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26167–26171
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.9118.16.11.26167-26171
#9118 | Received 04 May 2024 | Final received 25 October 2024 | Finally accepted 07 November 2024

SHORT COMMUNICATION

Winter population of raptor species in the Vellalore dump yard of Coimbatore City, India

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Abstract: A study from December 2020 to March 2021 in the Vellalore dump yard, Coimbatore City in southern India recorded 34 bird species, including seven raptors. Notably, two vulnerable species of winter migrant raptors, the Greater Spotted Eagle *Aquila clanga,* and the Tawny Eagle *Aquila rapax* were the most frequently observed. The presence of cow carcasses, poultry, and fish waste in the dump yard attracted scavenger birds, particularly raptors. The study utilized the systematic vantage point method to gather data and aims to establish a baseline understanding of the winter raptor population and other bird species in the garbage dump yard. This research serves as a foundation for long-term monitoring and conservation efforts.

Keywords: Abundance, Accipitriformes, city wastes, congregations, conservation raptors, habitat, migratory, scavenger, southern India, threats.

Birds of prey commonly known as "raptors" include kites, hawks, buzzards, falcons, eagles, harriers, and vultures in the order Accipitriformes and family Accipitridae consisting of a total number of 293–313 species worldwide (Naoroji 2006). Of the 63 species of raptors recorded in India, 59 are believed to migrate in at least part of their range (Ripley 1982; Ali & Ripley 1987; Zalles & Bildstein 2000). Raptor distributions are influenced by factors like the availability of nest sites and food (Thiollay 1989; Anderson 2001); density and reproductive success by prey abundance (Smith & Murphy 1979). Many species of avian groups have recently been shown to be able to colonize and even thrive in urban areas, by attraction to abundant prey supplies usually directly or indirectly promoted by human subsidies (Boal & Dykstra 2018). Dump yards have been shown to provide good feeding habitats to some migratory species of raptors (Garrido & Sarasa 1999; Garrido et al. 2002). The effects of garbage dumps on raptors are of great interest for conservation efforts (Sergio et al 2006) in areas that are or will be developed by humans. Raptors are efficient scavengers therefore their conservation is needed. The objectives of the study were to get baseline information on the winter population of raptors and other birds in the garbage dump yard which would lead to long-term monitoring and its conservation since no earlier study has been undertaken on the avifaunal diversity in the Vellalore dump yard, Coimbatore City.

Materials and Methods

Study Area

The study was conducted in the Vellalore dump yard from December 2020 to March 2021. The dump yard (10.9544^{0}N, 77.0098^{0}E) between Podanur and Chettipalayam is 14 km east of the central part of Coimbatore City (Figure 1). The dump yard area is about 260.21 ha. About 1,300 metric tonnes of garbage waste

Editor: H. Byju, Coimbatore, Tamil Nadu, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Balaji, V. & R. Venkitachalam (2024). Winter population of raptor species in the Vellalore dump yard of Coimbatore City, India. *Journal of Threatened Taxa* 16(11): 26167–26171. https://doi.org/10.11609/jott.9118.16.11.26167-26171

Funding: Self-funded.

Competing interests: The authors declare no competing interests.

Acknowledgements: We are extremely thankful to Kumaravel Pandian, I.A.S., Former Commissioner, Coimbatore City Municipal Corporation grant permission for undertake the survey in Vellalore dump yard.

generated daily from households in the city areas are transported and dumped at the Vellalore dump yard (Thyagarajan et al. 2021). The vegetation of the dump yard consists of *Prosopis juliflora*, *Albizia saman, Azadirachta indica*, *Calotropis giganteus,* and *Parthenium* spp. The dump yard is situated on the southern bank of the Noyyal River that irrigates agricultural lands in and around the city facing various threats due to urbanization.

The survey method was followed by point counts at select four vantage points covering the area of the dump yard (Image 1). The points for the survey were chosen in elevated locations with maximum visibility to detect the soaring raptors (Thiollay 1989; Nijman 2004; Eduardo et al. 2007). The study site was visited twice a month and a survey was conducted from 0900 h to 1800 h. The raptors were observed using binoculars (Nikon 15 X 70), and photographs were taken for identification using (Nikon P900), and done with the help of field guides (Ripley 1982; Ali & Ripley 1987; Grimmett 2011). Relative abundance was estimated using the index (percentage) of the total number of individual species divided by the total number of species population, multiplied by one hundred (Woffinden & Murphy 1977)

$$\text{Relative abundance} = \frac{\text{No. of individual of species}}{\text{No. of individual of all species}} \times 100$$

Results

About 34 species of birds including seven species of raptors were recorded during the study from the Vellalore dump yard of Coimbatore City (Tables 1 & 2). The highest occurrence percentages were recorded for four common raptor species throughout the season: Black Kite *Milvus migrans,* Booted Eagle *Hieraaetus pennatus*, Shikra *Accipiter badius*, and Greater Spotted Eagle *Aquila clanga* (Figure 2). While other resident raptor species were observed only sporadically over a few months, all seven raptor species were recorded in March. Black Kites and Booted Eagles particularly congregated abundantly at the dump yard. The winter migrant, the Greater Spotted Eagle, was observed throughout all months, meanwhile, Tawny Eagle *Aquila rapax* was recorded only in March. Tawny Eagle and Greater Spotted Eagle are 'Vulnerable' according to the IUCN Red List (IUCN 2024). Additionally, a total of 27 other bird species, including the passage migrant Rosy Starling *Pastor roseus,* were also recorded

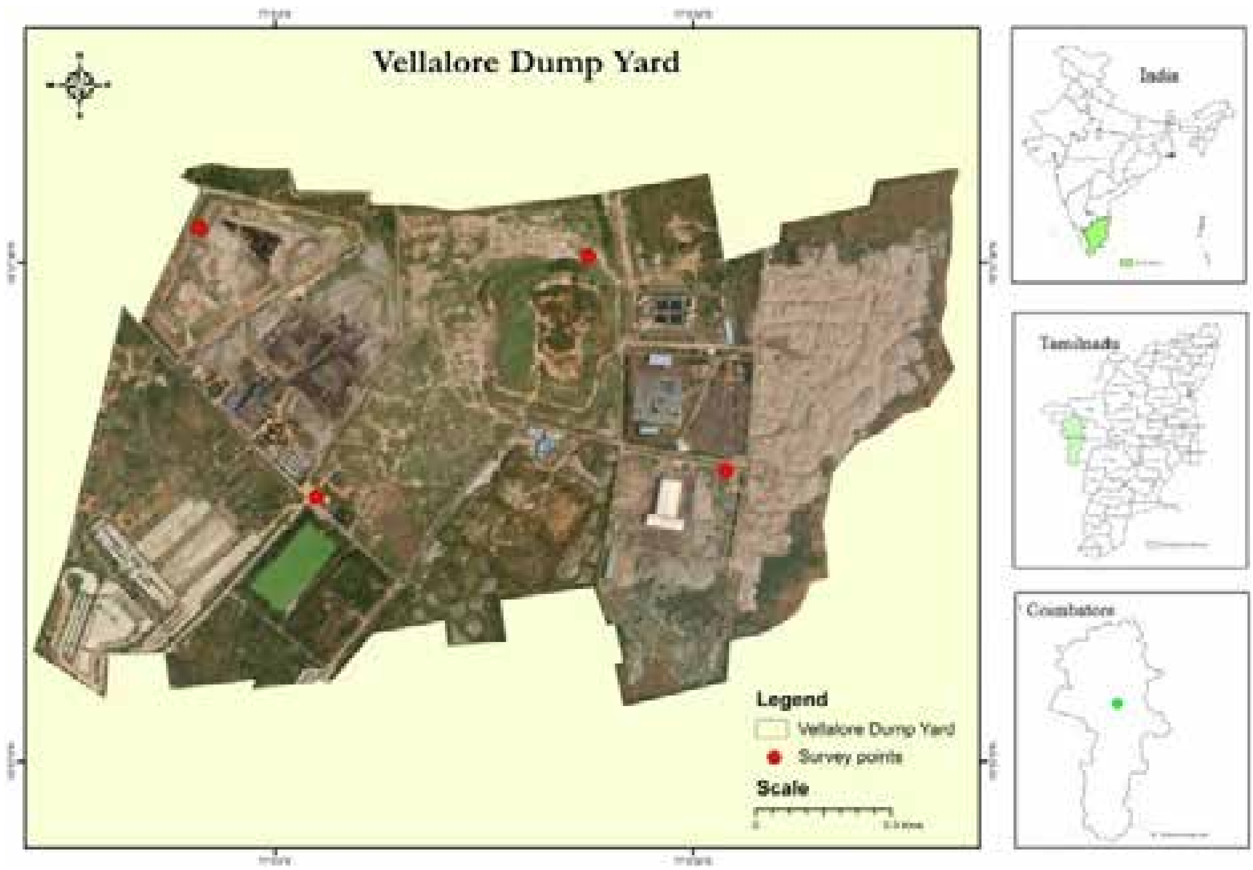

Figure 1. Map showing the study area - Vellalore dump yard.

Table 1. List of diurnal raptors at Vellalore dump yard during the study period.

	Common name	Scientific name	Migratory status	IUCN Red List status/ WPA 2022	RA
Order: Accipitriformes **Family:** Accipitridae	Black Kite	*Milvus migrans*	BR	LC/Sch-II	37.0
	Black-shouldered Kite	*Elanus caeruleus*	BR	LC/Sch-II	3.7
	Brahminy Kite	*Haliastur indus*	BR	LC/Sch-I	0.9
	Shikra	*Accipiter badius*	BR	LC/Sch-I	3.7
	Greater-spotted Eagle	*Aquila clanga*	W	Vu/Sch-I	6.5
	Tawny Eagle	*Aquila rapax*	W	Vu/Sch-I	0.9
	Booted Eagle	*Hieraaetus pennatus*	W	LC/Sch-I	47.2

W—Winter visitor | BR—Breeding Resident | LC—Least Concern | Sch—Schedule | Vu—Vulnerable | RA —Relative abundance | WPA—Wildlife Protection Act.

Table 2. Percentage relative abundance bird species (other than raptors) recorded in the study area (December 2020–March 2021).

	Species name	Scientific name	RA
1	Intermediate Egret	*Ardea intermedia*	31.38
2	Red-wattled Lapwing	*Vanellus indicus*	19.24
3	House Crow	*Corvus splendens*	16.73
4	Black Drongo	*Dicrurus macrocercus*	11.71
5	Red-rumped Swallow	*Cercopis daurica*	6.27
6	Barn Swallow	*Hirundo rustica*	2.51
7	Indian Peafowl	*Pavo cristatus*	2.09
8	Indian Pond Heron	*Ardeo lagrayii*	1.67
9	Ashy Prinia	*Prinia socialis*	1.46
10	Greater Coucal	*Centropus sinensis*	1.04
11	Purple-rumped Sunbird	*Leptocoma zeylonica*	0.83
12	Bay-backed Shrike	*Lanius vittatus*	0.83
13	Common Tailorbird	*Orthotomus sutorius*	0.83
14	Large Grey Babbler	*Turtoides malcolmi*	0.83
15	Pied Bushchat	*Saxicola caprata*	0.41
16	Common Myna	*Acredotheres tristis*	0.41
17	Grey Wagtail	*Motacilia cinerea*	0.41
18	Sykes's Warbler	*Iduna rama*	0.41
19	Plain Prinia	*Prinia inornata*	0.41
20	Purple sunbird	*Cinnyris asiaticus*	0.41
21	Ashy-crowned Sparrow-lark	*Eremopterix griseus*	0.41
22	Asian Koel	*Eudynamys scolopaceus*	0.20
23	Common Kingfisher	*Alcedo atthis*	0.20
24	Rosy Starling	*Pastor roseus*	0.20
25	Rufous Treepie	*Dendrocitta vagabunda*	0.20
26	Asian Palm Swift	*Cypsiurus balasiensis*	0.20
27	Zitting Cisticola	*Cisticola juncidis*	0.20

in the dump yard. Among other common bird species, the Intermediate Egret *Ardea intermedia* showed predominance, followed by the Red-wattled Lapwing *Vanellus indicus*, House Crow *Corvus splendens*, and Black Drongo *Dicrurus macrocercus*, and several others were found with less than 1% occurrence (Table 1). Stray dogs were observed feeding on various organic wastes including poultry in the Vellalore dump yard.

Discussion

This study is the first survey of birds in the Vellalore dump yard, Coimbatore district, and a total of 34 species of birds were recorded. The presence of the winter migratory birds especially raptors such as the Tawny Eagle and Greater Spotted Eagle with the highest relative abundance of Booted Eagle indicated that dumpsites provide sufficient feeding and roosting space for a large number of bird species thereby playing a key role in the conservation of birds (Tesfahunegny & Assefa 2023). The Greater Spotted Eagle record in the dump yard is the 25th site in Tamil Nadu (Santhakumar et al. 2016). Earlier records from the Coimbatore urban area were 135 bird species (Ramakantha et al. 2005), and 321 species (Balaji et al. 2016). The highest relative abundance of few common birds particularly raptor species showed that these birds mostly preferred dump yards for foraging and roosting because the presence of poultry wastes and availability of organic food sources in dumpsites is one of the most important factors influencing the survival and distribution of birds in urban areas (Mehra et al. 2017). Some harmful residues of the toxic substances that are present in the waste may cause bird deaths (Donázar 1993; Newton 1998). Therefore, an urgent need to evaluate the toxicological and health effects of harmful residue exposure of birds especially the globally threatened species for better conservation efforts (Tesfahunegny & Assefa 2023).

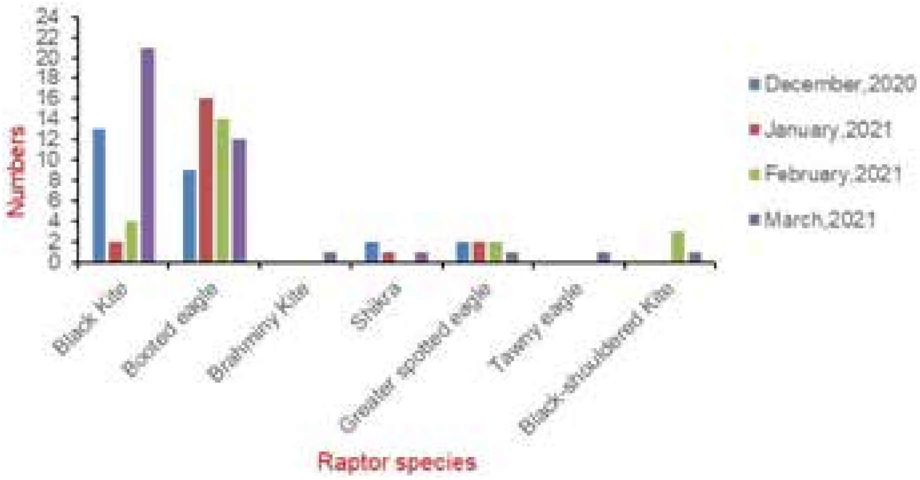

Figure 2. Monthly wise raptor species in the Vellalore dump yard.

Conclusion

This pivotal study on a population of raptors in the Vellalore dump yard offers the baseline data that aids in the long-term monitoring of wintering raptors and paves the way for conservation and preparing management policies as these dump sites also provide constant food for various bird species, particularly migratory raptor species.

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Appendix 1. 1—Booted Eagle | 2—Greater Spotted Eagle | 3—Indian Peafowl | 4—Red-vattled Lapwing | 5—Intermediate Egret.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26172–26175

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.8727.16.11.26172-26175

#8727 | Received 07 September 2023 | Final received 26 August 2024 | Finally accepted 03 October 2024

NOTE

Phenotypic variations in Mindoro Warty Pig *Sus oliveri* (Cetartiodactyla: Suidae)

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Four native wild pig species of the *Sus* genus have been identified in the Philippines. Very little information is available on the Mindoro Warty Pig *Sus oliveri*. Formerly, this species was treated as a subspecies of *S. philippensis* until it was recognized as distinct by Groves (1997) based on four skulls and one known skin collected from Mayapang, Rizal, Occidental Mindoro (Groves 2001). There are no recognized subspecies of *S. oliveri,* but it is closely related to two subspecies of *S. philippensis* (Groves 1997). Currently, *S. oliveri* is recognized as Vulnerable and Endangered by the IUCN Red List (Schütz 2016) and the Philippine Red List Committee through Department Administrative Order 2019-09, respectively.

According to Meijaard et al. (2011), the head skin

Editor: Anoymity requested.

Date of publication: 26 November 2024 (online & print)

Citation: Redeña-Santos, J.C., A.P.O. de Guia, N.H.A.Dagamac & F.G. Gil (2024). Phenotypic variations in Mindoro Warty Pig *Sus oliveri* (Cetartiodactyla: Suidae). *Journal of Threatened Taxa* 16(11): 26172–26175. https://doi.org/10.11609/jott.8727.16.11.26172-26175

Funding: This study is funded by the Department of Science and Technology (DOST)-Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD) through its Grants-In-Aid (GIA) program. JCRS received funding from IDEAWILD Foundation and DOST- Accelerated Science and Technology Human Resource Development Program (ASTHRDP) for the conduct of this survey. NHAD received support from RCNAS and DOST-Balik Scientist Program. FGG and D'ABOVILLE Foundation received funding from Zoologische Gesellschaft für Arten- und Populationsschutz (ZGAP), Association française des parcs zoologiques (AFdPZ), Tierpark Berlin, and Mandai Nature for their survey in Aruyan-Malati.

Competing interests: The authors declare no competing interests.

Acknowledgements: JCRS would like to thank the IDEAWILD Foundation for the equipment grant, and the DOST-ASTHRDP for the scholarship granted during the conduct of this research. NHAD would like to acknowledge the DOST-PCAARRD for the support as a Balik Scientist and funding of Project MATAPAT. We like to thank WWF for sharing their camera trap photos. We are also grateful for the Mt. Calavite Wildlife Sanctuary Protected Area Management Office (MCWS-PAMO) and the Iraya and Taobuid indigenous communities for their continued support.

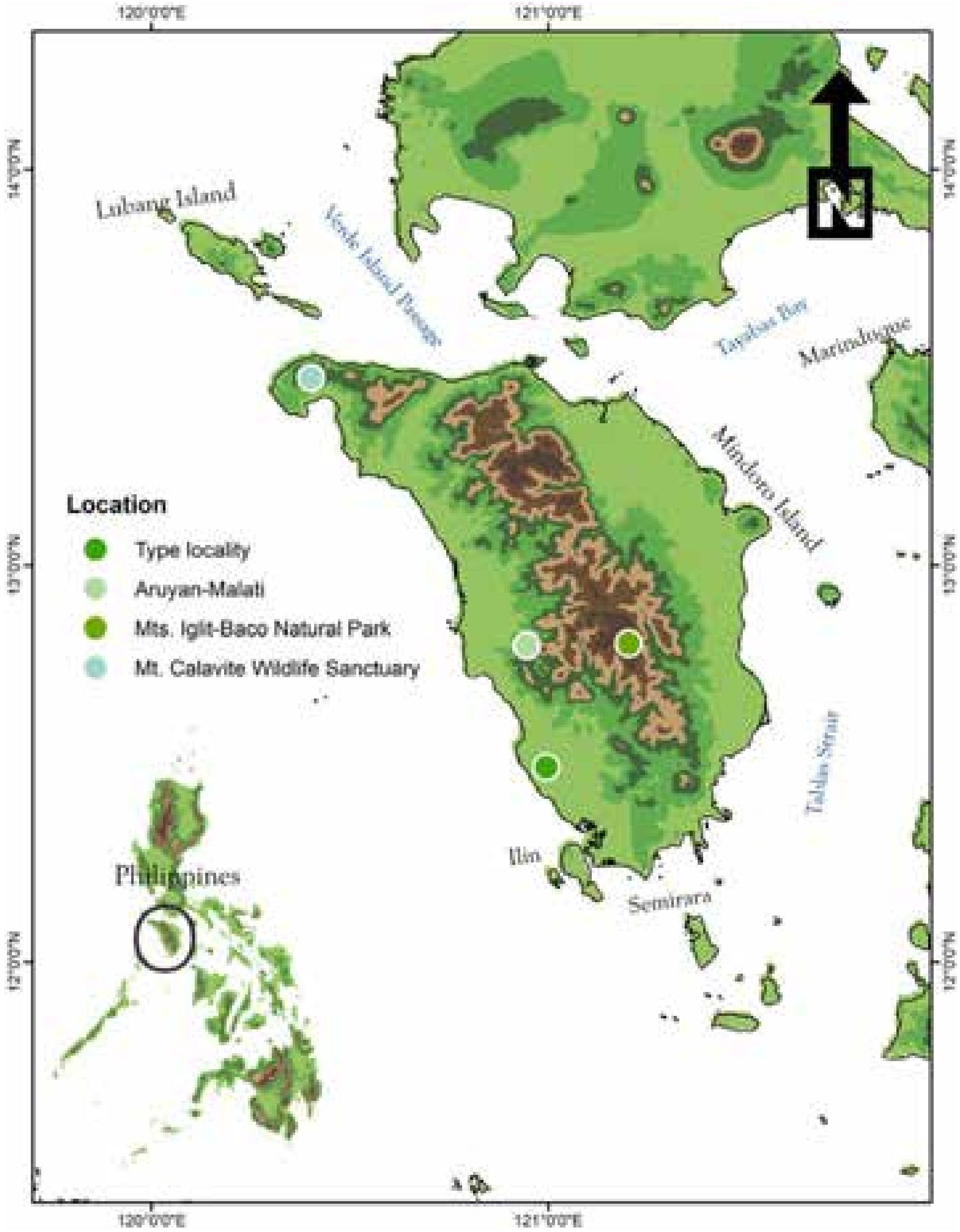

Figure 1. Map of Mindoro showing the area where the type locality and the camera trap photos of *Sus oliveri* were taken from.

of the holotype has sparse, dark brown or bristly black hair, usually longest along the spine and over the neck and back of the head, while the tusks and warts are conspicuous. Moreover, Groves (1997) described the species' head skin as having a black crown tuft mixed with straw-colored hairs, and no forward-directed components. The pre-ocular warts are well-developed with straw-colored gonial tufts. Recently, observations on camera trap photos of the Mindoro Warty Pig revealed a new phenotypic character between subpopulations of the species.

For this study, photos of *S. oliveri* were compiled from all camera trap surveys conducted in Occidental Mindoro, particularly in Mts. Iglit-Baco Natural Park (MIBNP), Mt. Calavite Wildlife Sanctuary (MCWS), and Aruyan-Malati (Figure 1). MIBNP and MCWS are both declared protected areas in Mindoro while Aruyan-Malati was proposed as a critical habitat under the Philippine laws. Warty pig photos in MCWS and Aruyan-Malati has been gathered from camera trap surveys conducted by the authors from 2020 to 2022. Notably, a total of 53 camera traps were installed in a ~650 ha plot in MCWS from December 2021 to May 2022 totaling 2,095 camera days, with cameras positioned at altitudes ranging 648–1,477 m. A maximum

Image 1. A—Camera trap photos of male *Sus oliveri* in MCWS with clear snout band (© MATAPAT Project) | B—two females in MCWS with clear snout band (© MATAPAT Project) | C—male in Aruyan-Malati without snout band (© DAF) | D—female in Aruyan-Malati with faint snout band (© DAF) | E—group of *S. oliveri* in MIBNP with clear snout band (© WWF Philippines).

of 20 and a minimum of 15 camera traps were deployed in Aruyan-Malati, covering a total of 894.4 ha with 140 camera placements from November 2020 to May 2022, with cameras positioned at altitudes ranging 149–590 m . The warty pig photos from MIBNP were provided by the World Wild Fund for Nature Philippines (WWF) from their camera trapping survey from 2013 to 2018. Originally, these camera trap surveys were intended to assess the distribution of medium- to large-sized mammals in all sites, particularly the 'Critically Endangered' Tamaraw *Bubalus mindorensis.*

Forty-six warty pig photos were collected with the animal presence in MIBNP, while our camera trap surveys in MCWS and Aruyan-Malati recorded 15 independent events (30 min intervals) in each site. For this study, only independent events where the facial appearance of the warty pig is observable were included. Five adult males, four adult females, two adult individuals of unknown sexes, and six juveniles were identified in MCWS. Two adult males and one adult female were identified in Aruyan-Malati. Meanwhile, eleven adults, two subadults, and four juveniles, all of the undetermined sexes, were identified in MIBNP. Based on photos, it has been observed that in both sexes, the faces of adult *S. oliveri* in MCWS and MIBNP (Image 1) are marked with a prominent whitish band in the snout. The white facial band is more conspicuous on adult individuals compared to subadults and juveniles. In contrast, this white band is absent in the adult males (Image 1) and faint in the females of Aruyan-Malati (Image 1), which was also absent in the holotype description by Groves (1997). According to personal communications with the "amayan" (elders) and "punong balayan" (tribe leaders) of Iraya-Mangyan tribes in MCWS, there are two types of warty pigs in the protected area. As such, they call the warty pig without the white band "baboy-laon" or forest warty pig. These wild pigs are commonly found in the lower elevations of the protected area and are seldom caught in traps they put in their croplands and swidden agriculture areas. In addition, these warty pigs are very common in forested habitats with huge and longstanding trees. On the other hand, they call the warty pig with the white band "baboy-isiw" or Bamboo Warty Pig. This pig is found mostly in the higher elevations. They are less common and more elusive than "baboy-laon" as they inhabit the thick bamboo forests or forested areas in proximity to bamboo habitats.

Very little information is available on the true appearance of the Mindoro Warty Pig since only one head skin was used in describing the species. Compared to all the native *Sus* species in the Philippines, the warty pig in Visayas *S. cebifrons* is the only wild pig with a well-marked whitish band in the snout. Wherein, this white band covers the bridge on the nose and continues to follow the jawline until the angle of the jaw. Although this band is generally less pronounced in females than males, their white stripe is one of the primary distinguishing characteristic that separates this species from other wild pigs in the Philippines (Species Husbandry Guidelines 2003, unpublished) . Thus, it is interesting to note that our observations on some *S. oliveri* individuals from camera trap photos found that this species also has a well-marked facial band. Unlike the white stripe in *S. cebifrons*, the white band of *S. oliveri* extends only from the bridge of the snout up to the end of the mouth and does not continue to cover the angle of the jaw. Moreover, it is also important to note that the holotype descriptions by Groves (1997) appear to be similar to the warty pig of Aruyan-Malati (low elevation) but the warty pigs photographed in MIBNP and MCWS (medium to high elevation) resemble more that of *S. cebifrons*.

The study has observed two distinct forms of *S. oliveri*, one variation matches the original holotypic descriptions while the other shows a prominent white snout-band. This difference can occur between the lowland and highland populations of the animals within Occidental Mindoro, as qualitative information through occasional interviews with local communities indicates, but further research is needed to verify this aspect. These different highland and lowland forms have been verified by the local communities but further research is needed to determine their degree of distinctiveness. In the case of MCWS, this difference likely indicates some kind of isolation between the two morphologies, either physical, behavioral, or reproductive. Although insights from indigenous people affirm that the two forms of *S. oliveri* are morphologically and ecologically distinct from each other, it is difficult to conclude the origin of these differences. Whether they are separate species, they have different adaptations to their environment, or some of their subpopulations are experiencing intense hybridization is currently unknown. The possibility of hybridization between *S. oliveri* and *S. scrofa* may also be considered, particularly in the lowlands and community forests where interbreeding between the two species is likely to occur (Oliver et al. 1995), and such may be the case in the warty pigs of Aruyan-Malati as they are both from lowland areas. Therefore, investigation of genotypes between the two distinct forms should be done to identify the origin of such phenotypic differences. Such efforts should also investigate the rate of introgression with *S. scrofa* to determine the genetic integrity of the species. This also calls for genetic studies to investigate the relationship of *S. oliveri* with other Philippine *Sus* species in order to construct a comprehensive evolutionary history. Overall, the above-mentioned recommendations will aid decision-makers in appropriately assessing the status of *S. oliveri* and formulating appropriate conservation strategies for the species.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26176–26179

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.9031.16.11.26176-26179

#9301 | Received 14 March 2024 | Final received 17 September 2024 | Finally accepted 28 October 2024

OPEN ACCESS

NOTE

First photographic evidence of the Chinese Pangolin *Manis pentadactyla* (Linnaeus, 1758) in Raimona National Park, Assam, India

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Chinese Pangolin *Manis pentadactyla* is a highly trafficked, elusive, solitary, nocturnal, burrowing mammal with scarce information on its distribution and current occurrence across its distributed range (Heinrich et al. 2017). Two species of pangolins—Indian Pangolin *Manis crassicaudata* and Chinese Pangolin—occur in India (D'Cruze et al. 2018). Considering the high extinction risks due to low population level and extensive hunting and poaching for illegal trafficking, the Chinese Pangolin was listed in Appendix-I of CITES (Anonymous 2022), as 'Critically Endangered' in the IUCN Red List (Challender et al. 2019) and in Schedule-I of the Indian Wildlife (Protection) Act, 1972 (Anonymous 2024). In India, despite stringent legal protections, pangolins are continuously hunted for meat, body parts and traditional medicinal purposes (Mitra 1998; Mohapatra et al. 2015). Although the global population of Chinese Pangolin is unknown, it is certainly declining, and the prevailing threats are anticipated to contribute to a rapid population loss along its distributional ranges (Challender et al. 2019).

Chinese Pangolin currently occurs in eastern, northern and southeastern Asian countries, spanning India, Bhutan, Bangladesh, Nepal, Myanmar, Thailand,

Editor: L.A.K. Singh, Bhubaneswar, Odisha, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Lahkar, D., M.F. Ahmed, B. Sinha, P. Talukdar, B. Basumatary, T. Basumatary, R.H. Begum, N. Medhi, N. Kalita & A. Harihar (2024). First photographic evidence of the Chinese Pangolin *Manis pentadactyla* (Linnaeus, 1758) in Raimona National Park, Assam, India. *Journal of Threatened Taxa* 16(11): 26176–26179. https://doi.org/10.11609/jott.9031.16.11.26176-26179

Funding: US FWS and Panthera.

Competing interests: The authors declare no competing interests.

Acknowledgements: We thank Mr. Anindya Swargowari, IFS (Retd. council head of the forests, Bodoland Territorial Council), Mr. R.P. Singh, IFS (Former council head of the forests, Bodoland Territorial Council), and Mr Suman Mohapatra, IFS (Council head of the forests, Bodoland Territorial Council) for supporting our research. We also thank Mr. RajualIslary, chairman, of United Forest Conservation Network (UFCN) and Green Forest Conservation, for his ground support during the implementation of the field work. We thank all frontline forest staff and NGO volunteers particularly Karuna Ranjan Brahma S(RO), Changma Narzari (RO), Mathias Basumatary, Manoj Brahma, Hayen Brahma, Pradip Gayari, Sukumar Roy, Ringkhang Narzary, Ajay Basumatary, Kamiya Narzary, Suniram Sonapaoria, Rohendra Basumatary, Pankaj Basumatary, Shobit Narzari, Aprajita Singh, Sansuma Narzari, Hiron Wary, Pabitra Sutradhar, Sudem Narzary, Dhonanjoy Islary, Bitunjoy Brahma, Gladwing Narzary for helping us during the field surveys. We also thank Mr. Arup K. Das of the Geo-spatial Technology and Application Division (GTAD), Aaranyak for preparing the map. Aaranyak is thankful to USFWS and Panthera for financial assistance to carry out the surveys.

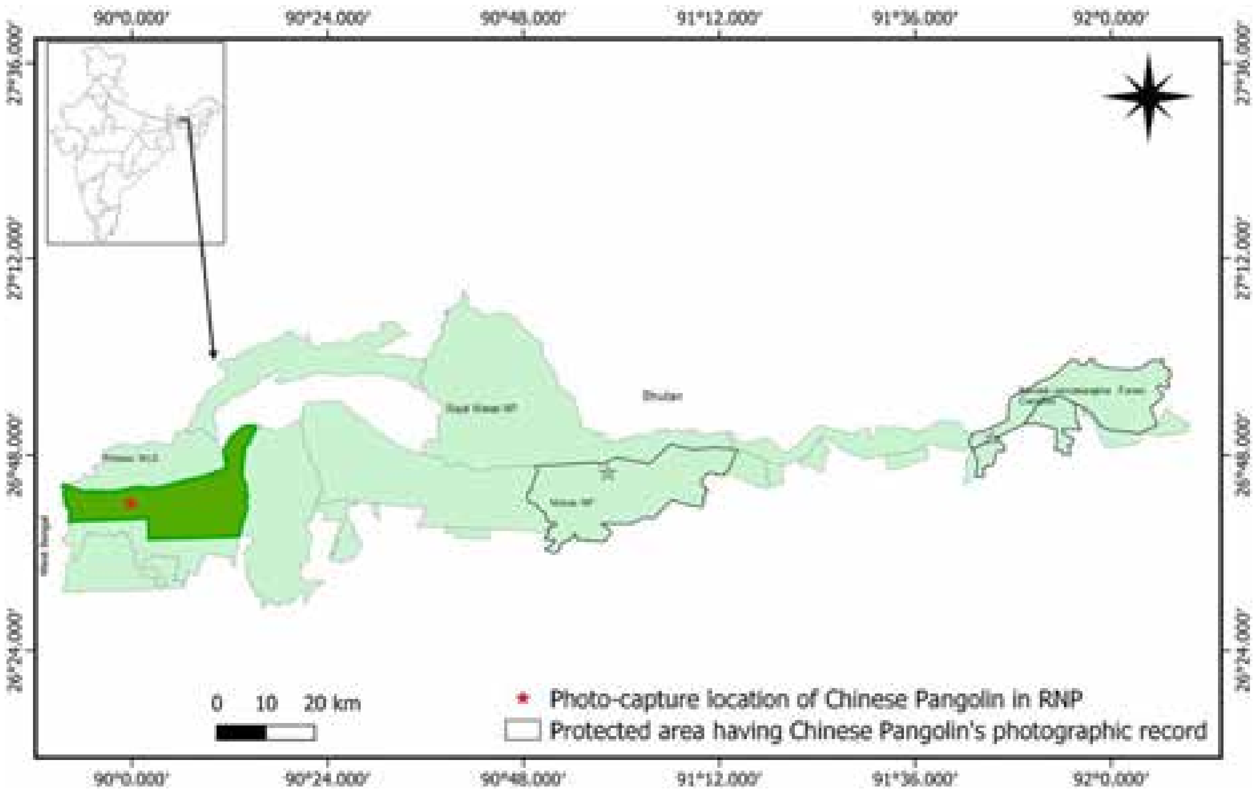

Figure 1. The map of the Transboundary Manas Conservation Area (TraMCA), depicts Raimona National Park in deep green fill, with the red star indicating the first photo-captured location of the Chinese Pangolin and the grey stars indicating past photo-captured locations in the TraMCA.

China, Hong Kong, Taiwan, Lao PDR, and Vietnam at elevations of 0–3,000 m (Challender et al. 2019). In India, the Chinese Pangolin is restricted to the foothills of the Himalaya, in the northern and across the northeastern region. The confirmed photo-capture records of Chinese Pangolin are from Valmiki Tiger Reserve of Bihar (Maurya et al. 2018), Neora Valley National Park of West Bengal (Mallick 2010), Kamlang Tiger Reserve (Jhala et al. 2020), and Pakke Tiger Reserve (Chandan Ri pers. comm. 08.iv.2024) of Arunachal Pradesh and Dampa Tiger Reserve (Sethy et al. 2021) of Mizoram. In Assam, apart from the sporadic media records of rescue, which source of the specimens is largely unknown leading to uncertain distributional predictions, whereas in situ camera-trapped records of Chinese Pangolin are extremely limited. Camera trapped record of Chinese Pangolin in Assam was documented at Manas National Park (Lahkar et al. 2018), Indo-Bhutan Barnadi-Jomotsangkha Forest Complex (Ahmed et al. 2019), and Dibru- Saikhowa National Park (Choudhury 1998). It is worth mentioning that some of the occurrences of Indian Pangolin from northeastern India are mere misidentifications of Chinese Pangolin as the species has not been recorded from the wild with certainty. Similarly, one of the records from Nagaon, Assam is based on a rescued animal, which perhaps, originated from illegal trade, although details are not available (Anwaruddin Choudhury pers. comm. 08.iv.2024)

Raimona National Park (RNP) is located along the foothills of the Himalaya and is contiguous with the Phibsoo Wildlife Sanctuary of Bhutan, the westernmost protected area within the India-Bhutan Transboundary Manas Conservation Area (6,500 km^2) (Figure 1). RNP is considered an important protected area in the complex that connects the forested areas of northern West Bengal, Bhutan, and Manas Tiger Reserve to the west, north, & east, respectively. RNP is a new protected area, established in 2021 with a total area of 422 km^2, under the administration of Kachugaon division, Bodoland Territorial Region, Assam. The area has had a long history of ethnopolitical conflicts, which have potentially affected the conservation mechanism.

Since 2019, the RNP authority, in collaboration with Aaranyak, has been conducting systematic annual camera trapping surveys to assess and monitor species assemblages and populations of terrestrial mammals. During the systematic surveys, with the camera trapping efforts of 1,470 trap-days in 2022, three independent

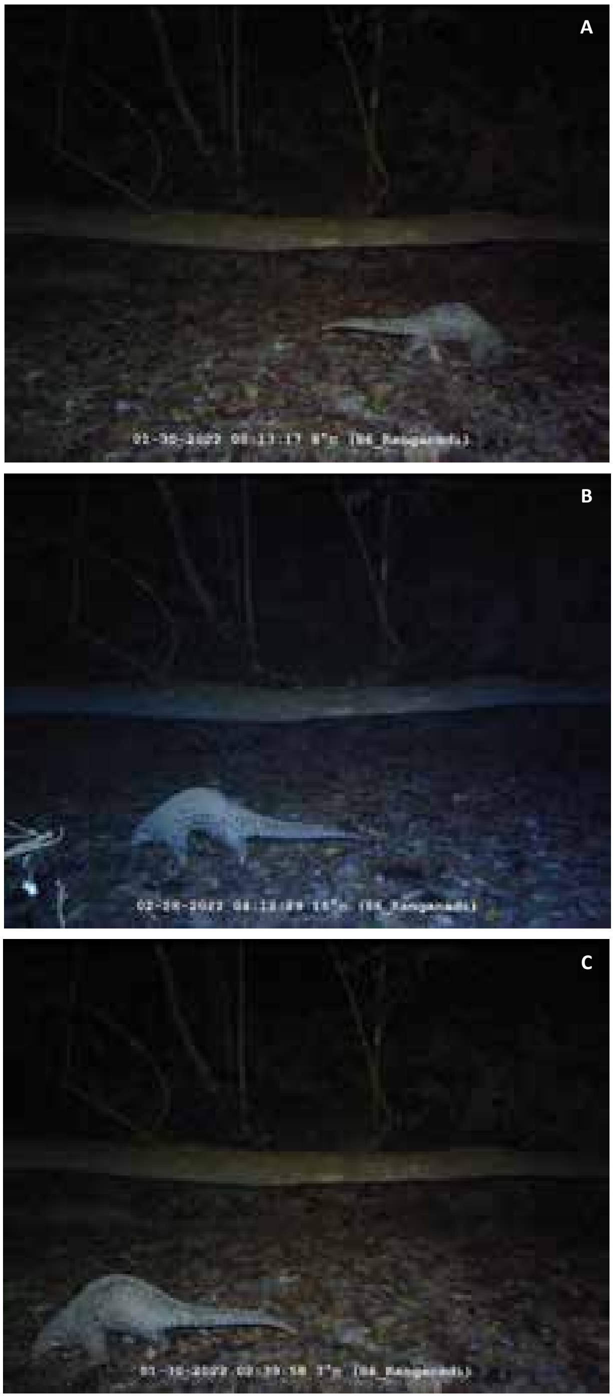

Image 1. A,B—Two independent photo-capture events of Chinese Pangolin *Manis pentadactyla* recorded on 30 January 2022 | C—28 February 2022 at an elevation of 166 m in the Raimona National Park, BTR, Assam, India.

Indian Pangolin
- Tail is long, thick, and dorsally flat.
- Face small and tubular mouth.
- Ear notch is not distinct.
- Scales are large and brown.

Chinese Pangolin
- Tail is relatively narrow and slender and the tip is necked.
- Face colour is clearer.
- Ear notch is visible.
- Scales are smaller and more greyish.

Image 2. Basic photographic identification description between Indian Pangolin (A) and Chinese Pangolin (B).

photo-capture of the Chinese Pangolin were obtained from the semi-evergreen forest on 30 January 2022 at 1213 h and 0239 h, and one more on 28 February 2022 at 0412 h from the Ranganadi area under the western range (Raimona) of the RNP (Images 1 & 2). The photograph was captured on a dry stream that joins with the river Ranganadi, covered with semi-deciduous forest dominated by Sal Trees *Shorea robusta.* The surface around the camera station was primarily blanketed by small to medium stones covered by the dry leaf litters. This is the first confirmed photographic evidence of the Chinese Pangolin in the RNP.

Chinese Pangolins were formerly distributed across protected and unprotected areas of Assam (Anwaruddin Choudhury pers. comm. 08.iv.2024). The species is now extremely rare and threatened across the region because of hunting and poaching to cater to the international illegal trade. The present photographic evidence of the Chinese Pangolin at the junction of the Indian states of West Bengal, Assam and Bhutan foothills presents critical evidence of its current occurrence in the region.

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Erratum

Citation: Kumar, N., D. Kumari, Dhani Arya & T.S. Rana (2024). *Impatiens devendrae* Pusalkar (Balsaminaceae): an addition to the flora of Jammu & Kashmir, India. *Journal of Threatened Taxa* 16(10): 26035–26039. https://doi.org/10.11609/jott.9277.16.10.26035-26039

Correct legend of Figure 1 on page 26036 is:
Figure 1. Distribution of *Impatiens devendrae*: a—Map of India | b—Ramban District, Jammu & Kashmir | c—Collection site.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26180–26184
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.9406.16.11.26180-26184
#9406 | Received 05 September 2024 | Final received 05 October 2024 | Finally accepted 31 October 2024
OPEN ACCESS
NOTE

Habenaria spencei (Orchidaceae): rediscovery other than its type locality and new distribution record to Karnataka, India

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Orchidaceae are one of the largest families in the world comprising about 26,000 species (Mabberley 2017). *Habenaria* is the genus, comprises about 898 species (Choudhury 2012; Mabberley 2017; POWO 2024). India contributes 64 species (Singh et al. 2019a,b; Prasad 2019; Prasad & Naik 2020). The flowering plants of Western Ghats, India represents 38 *Habenaria* (Nayar et al. 2014) where Karnataka has a record of 28 species (Lakshminarasimhan et al. 2019; Sringeshwara & Sanjappa 2019; Ravikumar et al. 2021). Orchids of Maharashtra reports 23 *Habenaria* species in which *Habneraia spencei* is not recorded (Jalal 2019). Total 40 species were recorded for Western Ghats of which 24 species are endemic. After reviewing recent available literature on *Habenaria* from India and abroad, it revealed that the genus was represented by 66 species with 30 endemic species to India (Dangat & Gurav 2015).

During our field survey in the Western Ghats of Karnataka, authors collected *Habenaria* from Shri Seetalayanagiri temple road, on the way to Mullayanagiri Peak, Chikkamagaluru district. After analysis of the specimen, is identified as *Habenaria spencei* Blatt. & McCann. The species which was never been collected since its type collection from Mahableshwar, Fitzgerald Ghat in dense jungle at 1,220m altitude in (McCann type: BLAT3026; Cotype: BLAT3027) on 28 August 1930 (Blatter & McCann 1932). May be this species is confused and has not been reported by any other authors from that location until now. So, after 94 years it has been rediscovered other than its type location with 10–15 individuals at one location at 1,624 m. This species has not been reported from Karnataka State until now (Image 2). *Habenaria spencei* Blatter and McCann has got a new distribution record to Karnataka, other than its type location Mahableshwar, Maharashtra after 94 years.

Taxonomic treatment

***Habenaria spencei* Blatt. & McCann,** J. Bombay Nat. Hist. Soc. 36: 17 (1932). *Habenaria gibsonii* var. *foliosa* (A. Rich.) Santapau & Kapadia J. Bomb. Nat. Hist. Soc. 56: 194 (1959); *Habenaria foliosa* A. Rich. Dangat & Gurav, Studies Genus Habenaria, 75. (2015).

Type: McCann type: BLAT 3026; cotype: BLAT 3027

Terrestrial, up to 20–60 cm tall. Tubers ellipsoid, 1–2, greyish-black. Leaves arranged 8–12 cm above ground. Leaves alternate forming leaf sheaths, oblong-lanceolate, acuminate apex, margin wavy, 3 main nerved, 8–12 × 3–4 cm. Flowers in lax raceme of 5–12 flowers, 1.5 × 1 cm, scape yellowish-green turns scarlet

Editor: V.B. Sreekumar, Kerala Forest Research Institute, Peechi, India. **Date of publication:** 26 November 2024 (online & print)

Citation: Betageri, S. & K. Kotresha (2024). *Habenaria spencei* (Orchidaceae): rediscovery other than its type locality and new distribution record to Karnataka, India. *Journal of Threatened Taxa* 16(11): 26180–26184. https://doi.org/10.11609/jott.9406.16.11.26180-26184

Funding: Karnatak University research fellowship (URS) for research programme.

Competing interests: The authors declare no competing interests.

Acknowledgements: Authors are thankful to Karnatak University's scholarship for University research scholarship. Authors are thankful to Prashant Kardhakatti during the field survey.

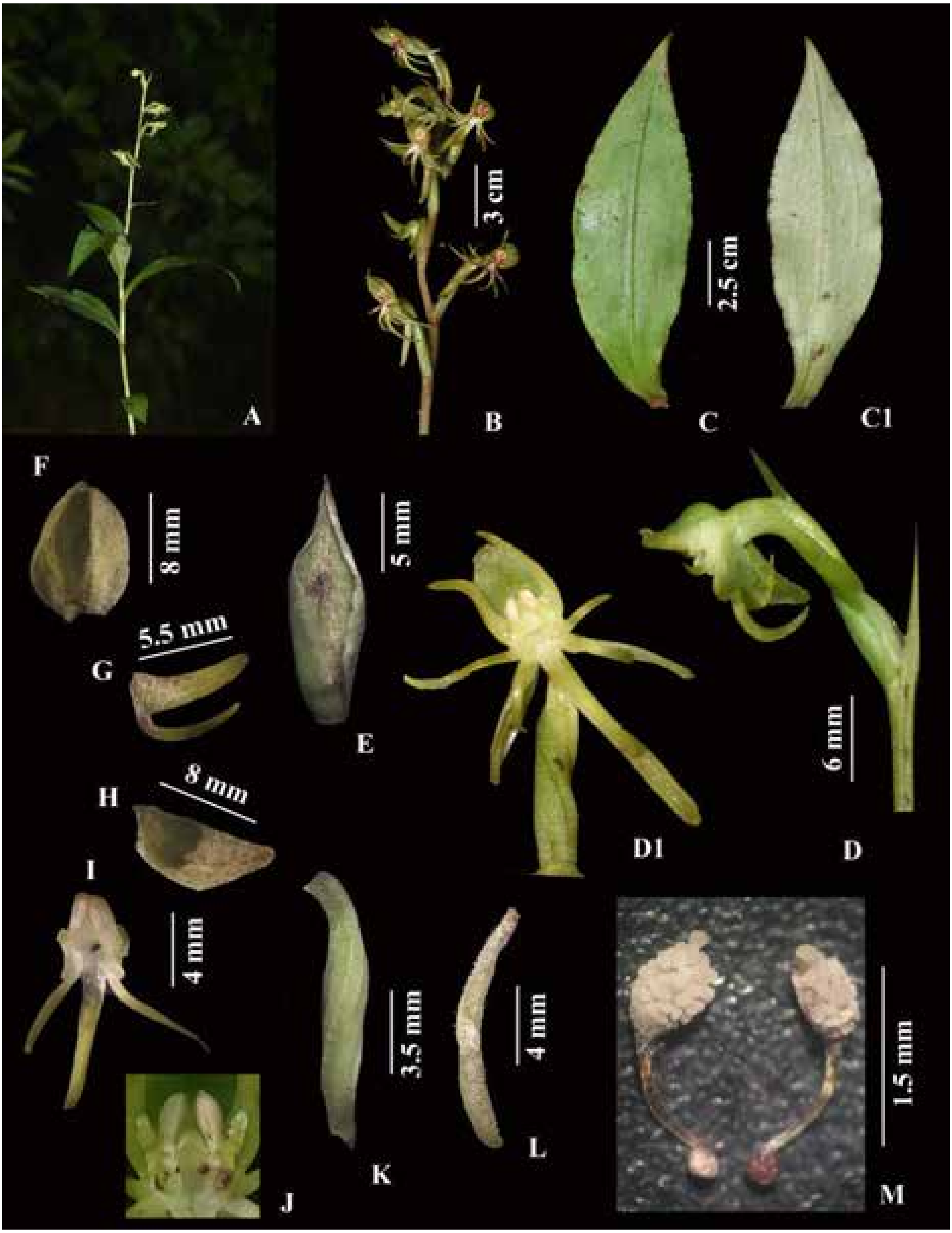

Image 1. ***Habenaria spencei*** **Blatter & McCann.: A—Habit | B—Inflorescence | C & C1—Adaxial & Abaxial leaf | D—Side view of Flower | D1—Front view of flower | E—Bract | F—Dorsal sepal | G—Petal | H—Lateral sepal | I—Labellum | J—Rostellum with stigmatic lobes | K—Ovary | L—Spur | M—Pollinarium. © Shreyas Betageri.**

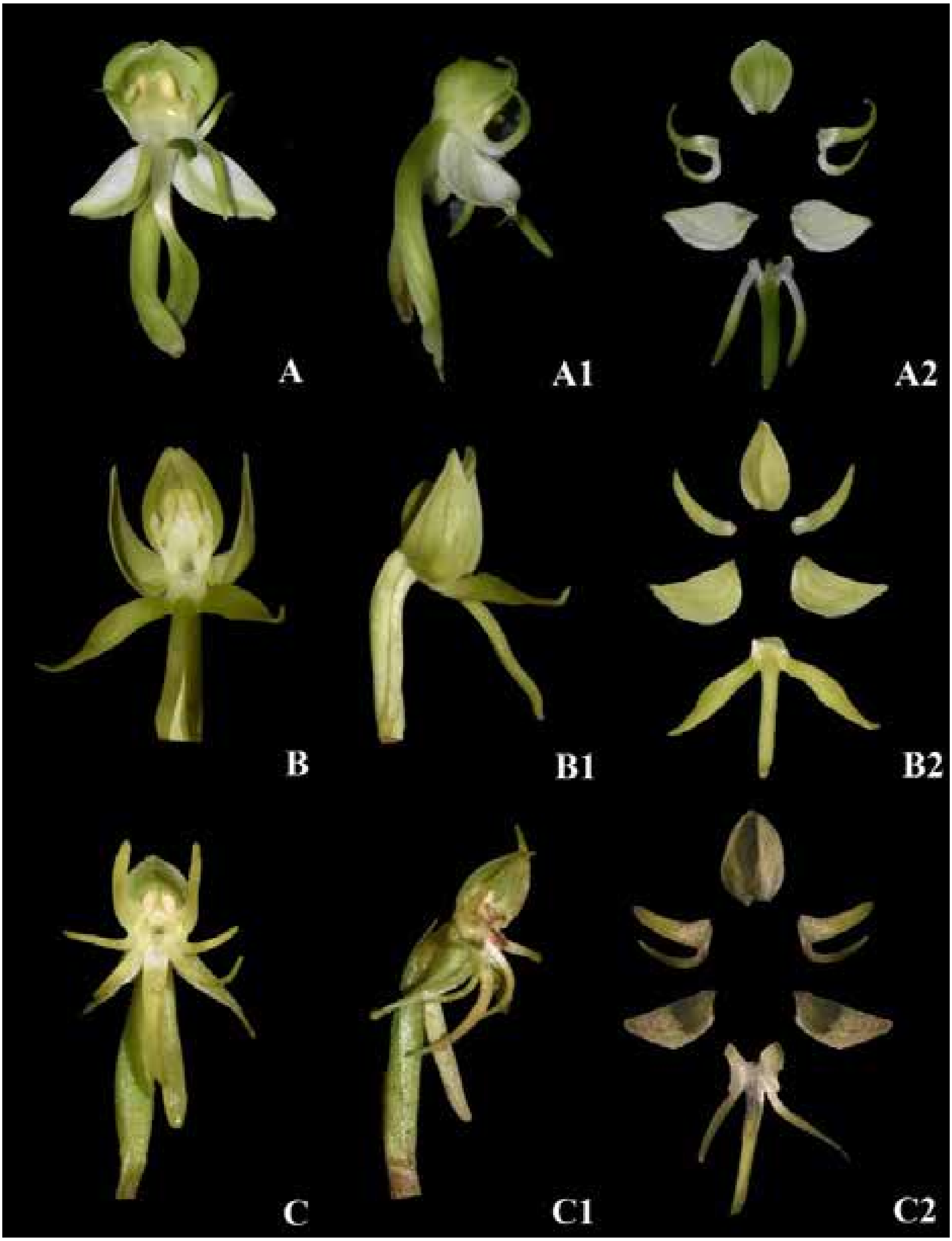

Image 2. Comparative account of congeneric species: front view of flower, side view of flower & dissected sepals, petals & labellum: A, A1, A2—*Habenaria gibsonii* | B, B1, B2—*Habenaria digitata* | C, C1, C2—*Habenaria spencei*. © Shreyas Betageri.

Table 1. A comparative account of congeneric species (*Habenaria digitata* Lindl.; *Habenaria gibsonii* Hook.f.) (Image 2).

Characters	*Habenaria digitata* Lindl.	*Habenaria gibsonii* Hook.f.	*Habenaria spencei* Blatt. & McCann
Habit	Terrestrial herbs, 20–40 cm tall. Leaves arranged 5 cm above ground.	Terrestrial herbs, 20–50 cm tall. Leaves arranged 6–8 cm above ground.	Terrestrial herbs, 20–60 cm tall. Leaves arranged 8–12 cm above ground.
Leaves	Leaves alternate, coriaceous 4–8 x 2–4 cm, elliptic-lanceolate, acute apex, entire, 3-nerved, pale green.	Leaves alternate, not-coriaceous, 3–5 x 1.2 cm, ovate- lanceolate, acute apex, wavy margins, 1-nerved, pale green.	Leaves alternate, 8–12 × 3–4 cm, oblong-lanceolate, acuminate apex, margin wavy, 3-nerved, dark green
Inflorescence	Densely arranged	Densely arranged	Laxly arranged
Flower	Yellowish-green, sweet-scented, 2.5 cm long	Whitish-green, foul-scented, 2 cm long	Yellowish-green, foul- scented, 2.5 cm long
Sepals	Ovate, 1 cm long, oblique 8 mm long.	Boat-shaped, 5 mm long, ovate, 5 mm long	Boat-shaped, 8 mm long, sickle-shaped, 8 mm long.
Petal	Linear, sub-falcate, 1 cm long	Petals are bi-partite, sickle-shaped, 4 mm long.	Petals bi-partite, recurved upwards, 5.5 mm long.
Labellum	Labellum 3 partite, yellowish-green, 1.2 cm long; mid lobe equal to sidelobes; sidelobes broad, acute.	Labellum 3-partite, greenish-white, 1 cm long; mid lobe larger than sidelobes; sidelobes linear, acute.	Labellum 3-partite, yellowish-green, 1 cm long, side lobes smaller than mid lobe and recurved backward
Spur	1.4 cm long, yellow- green, fully flat all along.	1.2 cm long, greenish-white, flat at the tip.	1 cm long, yellowish green, flat all along.

light pink after pollination of flowers. Bracts 1.5 cm long, linear-lanceolate, 1-nerved, covering width of half ovary. Flower pale green, faintly scented, ca. 2 × 1 cm. Dorsal sepal boat-shaped, 3-nerved, ovate-acuminate, ca. 8 mm long. Lateral sepals lanceolate-acute, 1-nerved, sickle shape, 8 mm long, reflexed backward. Labellum tri-partite, mid lobe straight linear, 10 mm long, side lobes smaller than mid lobe and recurved backward. Petals bi-partite, linear, recurved upwards, and 5.5 mm long. The ovary is twisted, 1.2 cm across, green, and brown when mature. The spur is attached to the labellum, flat, shorter than the ovary, and 1 cm long. Stigma clavate-oblong, appressed to the labellum. Pollinaria sac upcurved, rostellum shorter, obtusely triangular. Pollinarium is present in two sacks, one pair with a viscidium pad at the base, and 1.5 mm long. The fruit is brown with ridges (Image 1).

Ecology and phenology: prefers forest undergrowth and grows at more than 1,220 m altitude. Flowering starts in August followed by fruit.

Associations: *Habenaria spencei* is associated with *Malaxsis versicolor*, *Peristylus aristatus*, *Liparis wightiana*, *Lycopodium* sp., *Funaria* sp., and *Paspalum* sp.

Threats: As the location of species is not in protected area, may be in future it may go to extinct, as this species found its particular location with threats like small landslides, road widening and coffee plantations.

Geographic disjunction: As first type specimen is collected from Mahabaleshwar, Maharashtra at 4,000 m. Therefore, this species is very common at high altitudes under tree shades. This species has been collected from Kolaphur (Maharashtra), Gudehalli gudda (Karwar), Mullayanagiri peak (Chikkamagaluru district) and Pusphgiri Hills (Kodagu). The altitude mentioned above provides a clue for us to explore other unexplored places in the Western Ghats to learn more about this species.

Species examined: India, Karnataka, Shri Sitalayyanagiri Gudi road, on the way to Mullayanagiri peak, Chikkamagaluru district, 13.3873N, 75.7070E, 23 August 2024, Shreyas B. & K. Kotresha, Herbarium of Karnatak Science College, Dharwad (HKSCD) [20372] (Image 3).

IUCN Threat Assessment: After several investigations throughout the Shri Seetalayanagiri temple road, on the way to Mullayanagiri peak observed with 5–10 individuals in two spots. This species is not present on adjacent hills near Bababudangiri Hills, as this species has threats like small landslides, road widening, and coffee plantations. As per IUCN Red List status, this species is 'Data Deficient' (IUCN 2024).

Historical context: *Habenaria spencei* was first time discovered by Blatter & McCann in August 1930. Later Santapau & Kapadia collected the same species (1966) renamed as conspecific to *Habenaria gibsonii* var. *foliosa* and also reported it from Poona in Deccan, Karwar in N. Kanara (Type: BLAT: Kapadia 14, 622). Dangat & Gurav (2015) collected *H. spencei*, misconception with *Habenaria foliosa* A. Rich (Plate 2C,D) in his doctoral thesis work, Studies on Systematics of the Genus *Habenaria* Willd. (Orchidaceae) in Western Ghats. Patil & Lakshminarasimhan (2020) recorded six *Habenaria* species from Pushpgiri Wildlife Sanctuary, misconception *Habenaria spencei* Blatt. & McCann with *Habenaria digitata* Lindl. (Sameer Patil 207358; Plate 7H.) from Kodagu district, Karnataka.

Image 3. Herbarium specimen of *Habenaria spencei* Blatter & McCann. submitted to Herbarium of Karnatak Science College Dharwad. © Shreyas Betageri.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 November 2024 | 16(11): 26185–26186
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.9503.16.9.26185-26186
#9503 | Received 19 November 2024

BOOK REVIEW

Fairies of the day and angels of the night

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Edition 2024, 206 pages.
ISBN: 978-81-8171-636-1.
Published by Zoological Survey of India, Western Regional Centre, Pune.

After the publication of Edward Osborne Wilson's magnum opus 'Biodiversity' in 1988, the term biodiversity became the most-spelt word across the globe in relation to conservation of nature. Subsequently, Meyers's classification of biodiversity hotspots in 1988 was another eye opener raising concern over the protection of the crucially important natural treasure troves that housed unique flora and fauna in these hotspots. The most widely accepted definition of biodiversity hotspots, viz., according to The Critical Ecosystem Partnership Fund biodiversity hotspots is "areas on Earth that house a minimum of 1,500 species of vascular plants, known as 'endemic' species, and have experienced the loss of at least 70 per cent of their primary native vegetation". In India, the Western Ghats are one of the biodiversity hotspots of international reputation endowed with unique endemic flora and fauna besides their rich cultural values and immense ecological importance. Running parallel to India's western coast the Western Ghats traverse across the states of Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra, and Gujarat occupying an area of 1,40,000 km². Wilson's "little things that run the world" and "the silent majority" namely the insects, generally, are under appreciated and have received lesser attention as compared to the other flora and fauna in these regions. For example, as per the estimate from the United Nations Educational, Scientific, and Cultural Organisation, around 54% of plants, 65% of amphibians, 62% of reptiles, and 53% of fishes are categorized as endemic. The status of the invertebrates, particularly insects, remain to be studied extensively in the Western Ghats.

Lepidoptera (butterflies and moths), especially the butterflies, are admired for their beauty, colour, elegance, while the moths are not as popular as the butterflies. This may be attributed to their nocturnal habit and lesser appeal. In the recent days, moths are a volatile subject of study in the field of biology considering their significance and their role in the functioning of

Date of publication: 26 November 2024 (online & print)

Citation: Narayanasami, C. (2024). Fairies of the day and angels of the night. *Journal of Threatened Taxa* 16(11): 26185–26186. https://doi.org/10.11609/jott.9503.16.11.26185-26186

the different ecosystems. Apart from this, Lepidoptera gained importance in the field of agriculture as crop pests, pollinators, and are also commercially exploited, for instance, the variety of silkworms that provide the much favoured special fibre "the silk". In addition, Lepidoptera is part of the cuisines either raw or cooked in different parts of the world. They have captured the vivid imagination of innumerable poets also in different languages globally.

Previous recent publications like 'South Indian Butterflies' by K. Gunathilagaraj, T.N. Perumal, K. Jayaram, & M. Ganesh Kumar (2015) and 'A Guide to the Butterflies of Western Ghats' by Milind Bhakare & Hemant Ogale (2018) seized the minds of butterfly enthusiasts. As mentioned earlier, moths despite their enduring beauty and significance suffer step-motherly treatment in comparison to the butterflies. Thus, the publication of 'A Field Guide to Common Butterflies & Moths (Lepidoptera) of Western Ghats' is a progressive step in knowing the Lepidoptera of the Western Ghats. The authors are to be applauded for including the moths.

In the preface, the authors have indicated that this field guide is an extended part of DST-SERB-Accelerate Vigyan Scheme. The book encompasses 122 species of butterflies and 349 species of moths. In the introduction, the biology, common resting positions, collection & preservation of Lepidoptera, and the status of the butterfly diversity in the Western Ghats are provided. Next family-wise Lepidoptera are illustrated with mind-blowing and captivating photographs, with details of the distribution, status of protection as per the Wildlife (Protection) Act, 1972, larval host plants, and brief diagnostic characters and some special remarks for a few species. Diagnostic characters are provided only for the butterflies. The common "step-motherly syndrome of the moths" prevails in this book. If the diagnostic characters of the moths had also been included this beautiful field guide would be consummate. Fascinating and fabulous photography speak volumes on the beauty of the Lepidoptera and is the forte of this field guide.

Two species of moths, viz., *Maruca fuscalis* Yamanaka, 1998 (Crambidae) in p. 90 and *Ambulyx matti* (Jordan, 1928) in p.110 require further scrutiny on their identity. This may be new records for India if the identity is correct. Trivial inadequacies of the field guide are: host plant family names Leguminosae and Fabaceae are given instead of Fabaceae only as per the recent nomenclature of plant family names, older names of countries like Ceylon, Burma and their present names Sri Lanka and Myanmar are spilled across the guide without uniformity, the plant family names those given in brackets for example in p.110 it is written as (combretaceae) with the first letter of the plant family name in lower case, and in the title of the book "the" before the Western Ghats is missing which is generally given for a group of mountains, hills, islands, states. Trivial things make perfection but perfection is not trivial.

The authors are to be congratulated for their commendable efforts to document the Lepidoptera of the Western Ghats. Further, the cost of the book is reasonable, and this field guide should be a part of every nature lover's bookshelf.

Journal of Threatened Taxa is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64

Print copies of the Journal are available at cost. Write to:
The Managing Editor, JoTT,
c/o Wildlife Information Liaison Development Society,
3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore,
Tamil Nadu 641006, India
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Journal of Threatened Taxa

www.threatenedtaxa.org

OPEN ACCESS

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

November 2024 | Vol. 16 | No. 11 | Pages: 26063–26186
Date of Publication: 26 November 2024 (Online & Print)
DOI: 10.11609/jott.2024.16.11.26063-26186

Articles

Endemicity and diversity of birds of the Kuvempu University Campus, Shivamogga District, Karnataka: an updated checklist
– M.N. Harisha & B.B. Hosetti, Pp. 26063–26077

Unregulated wild orchid trade in Manipur: an analysis of the Imphal Valley markets from the Indo-Burma hotspot
– Kamei Kambuikhonlu Kabuini & Maibam Dhanaraj Meitei, Pp. 26078–26088

Watershed survey of streams in western Bhutan with macroinvertebrates, water chemistry, bacteria and DNA barcodes
– Juliann M. Battle, Bernard W. Sweeney, Bryan Currinder, Anthony Aufdenkampe, Beth A. Fisher & Naimul Islam, Pp. 26089–26103

Communications

Indian Leopard predation on the sub-adult Himalayan Griffon Vulture (Accipitridae: Accipitriformes)
– Soumya Sundar Chakraborty, Debal Ray, Apurba Sen, P.J. Harikrishnan, Nabi Kanta Jha & Rounaq Ghosh, Pp. 26104–26109

Diet composition and diet choice of Lesser Mouse-tailed Bat *Rhinopoma hardwickii* (Gray, 1831) (Rhinopomatidae: Chiroptera)
– Pawan Kumar Misra, Sayma Farheen, Shaktivardhan Singh & Vadamalai Elangovan, Pp. 26110–26115

DNA barcoding and distribution of *Osteobrama peninsularis* (Teleostei: Cyprinidae) in India
– Boni Amin Laskar, Asha Kiran Tudu, Shibananda Rath & Laishram Kosygin, Pp. 26116–26123

Diving into diversity: aquatic beetles of Sukhna Wildlife Sanctuary, Chandigarh, India
– Karmannye Om Chaudhary, Pp. 26124–26130

Review

An updated checklist of snakes (Reptilia: Squamata) in northeastern India derived from a review of recent literature
– Bijay Basfore, Manab Jyoti Kalita, Narayan Sharma & Ananda Ram Boro, Pp. 26131–26149

Viewpoint

Decades of IUCN recommendations for biocontrol of invasive pest on the Guam cycad: you can lead policy-makers to conservation proposals but you cannot make them follow
– Thomas E. Marler, Anders J. Lindström, L. Irene Terry & Benjamin E. Deloso, Pp. 26150–26162

Short Communications

Photographic record of Kashmir Gliding Squirrel *Eoglaucomys fimbriatus* (J.E. Gray, 1837) from the Gurez Valley, Jammu & Kashmir, India
– G. Mustufa Lone, Bilal A. Bhat, Mir Shabir Hussain & Arif Nabi Lone, Pp. 26163–26166

Winter population of raptor species in the Vellalore dump yard of Coimbatore City, India
– V. Balaji & R. Venkitachalam, Pp. 26167–26171

Notes

Phenotypic variations in Mindoro Warty Pig *Sus oliveri* (Cetartiodactyla: Suidae)
– John Carlo Redeña-Santos, Anna Pauline O. de Guia, Nikki Heherson A. Dagamac & Fernando García Gil, Pp. 26172–26175

First photographic evidence of the Chinese Pangolin *Manis pentadactyla* (Linnaeus, 1758) in Raimona National Park, Assam, India
– Dipankar Lahkar, M. Firoz Ahmed, Bhanu Sinha, Pranjal Talukdar, Biswajit Basumatary, Tunu Basumatary, Ramie H. Begum, Nibir Medhi, Nitul Kalita & Abishek Harihar, Pp. 26176–26179

***Habenaria spencei* (Orchidaceae): rediscovery other than its type locality and new distribution record to Karnataka, India**
– Shreyas Betageri & Katrahalli Kotresha, Pp. 26180–26184

Book Review

Fairies of the day and angels of the night
– Chitra Narayanasami, Pp. 26185–26186

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